

Diapause in the Boll Weevil (Coleoptera: Curculionidae): Seasonal Occurrence in Mississippi Populations

TERENCE L. WAGNER¹ AND ERIC J. VILLAVASO

USDA-ARS, P.O. Box 5367, Mississippi State, MS 39762

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ABSTRACT This study examines the diapause response among boll weevils collected as immatures in squares at different times of the summer and held under simulated field photoperiods and temperatures of northern Mississippi. The percentage of adults exhibiting prediapause increased seasonally, starting with the 1st generation in July and achieving a maximal rate of 98.7% late in the year. A greater proportion of males achieved diapause than females at any given time, except late in the season when convergence in the diapause response of both sexes occurred. These results suggest that individuals in a population have different thresholds of sensitivity to the environmental cues regulating the onset of diapause. Models described the percentage of males and females in prediapause as a function of Julian date of emergence and daylength on the day of emergence. The latter models have potential application over a wide region of the Cotton Belt. Information from this study was used to suggest August starting dates for diapause control applications in the mid-South—recommendations that subsequently were implemented in eradication programs in Alabama and Mississippi.

KEY WORDS *Anthonomus grandis grandis*, diapause, cotton, model

THE COTTON BOLL weevil, *Anthonomus grandis grandis* Boheman, was first detected in south Texas in the early 1890s. Movement of the insect 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 from this pest greatly curtailed cotton production in the southeast, an event that forced production to the southwestern United States (Frisbie et al. 1989). Adequate control of the insect was not possible until after World War II with the introduction of organochlorine 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 diapause in the boll weevil 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, especially those devoted to diapause induction and overwinter survival.

Despite several decades of research, diapause in the boll weevil remains a curiosity of great importance. In fact, some believe the insect may not diapause at all; rather, it overwinters in a state of quiescence (Guerra

et al. 1982, 1984; Spurgeon and Raulston 1996). Although well studied, the process is not well understood for several reasons, including the attitudes and approaches of researchers. Walker (1967) described the situation this way, "... The diapause phenomenon in the boll weevil is a complex subject that we have tended to regard in generalized terms."

Many studies dealing with boll weevil diapause involve the token stimuli responsible for its initiation and the expression of adult prediapause morphology that follows. Typically, this research has been observational in nature, not explanatory. It was often duplicated at several locations by different investigators, and when experimental designs differed, results were often contradictory and confusing. For example, different weevil strains, rearing conditions, and methods of diapause determination were used among investigators. Within studies, rearing conditions often varied between life stages. Most studies examined the progeny of colony weevils reared on artificial diets under static photoperiods and temperatures. These results were not correlated with conditions in the field. Tests were often unreplicated, and broad undocumented conclusions sometimes were drawn from poorly designed studies or from selective or preliminary data. Occasionally, relevant data were overlooked. In field studies, there was a lack of control over the environmental variables that influenced diapause, making replication difficult if not impossible.

These combined problems make it difficult to distinguish meaningful, scientifically sound results from those that are not. The problems promote a belief that

¹ USDA, Forest Service, P.O. Box 6124, Mississippi State, MS 39762.

investigators of diapause did not understand its phases, complexity, or dynamic nature (see Tauber et al. [1986] for an excellent review of seasonal adaptations in insects). To understand better the relationship between boll weevil diapause and the variables that influence it, we critique some of the prominent literature.

Effects of Photoperiod. 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 was that of Earle and Newsom (1964). Based on laboratory studies using colony weevils from Louisiana, they found that an 11-h fixed daylength induces diapause (59–83%) and a 13-h daylength suppresses it (6–10%). Generally, these findings have 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 the onset of diapause.

For example, Sterling (1966) reported that 8, 10, 11, 12, 14, and 15-h fixed daylengths induce diapause in colony weevils from Texas and that 12.5, 13, and 16-h daylengths suppress it (cited from Sterling [1972]). In a subsequent study, Sterling (1972) found increasing diapause in each life stage with decreasing daylengths of 15, 13, and 11 h. Because rates were so high under all conditions (46–92%), he concluded photoperiod was unable to override the dietary influences on the process, although no dietary tests were performed. Elsewhere, Lloyd et al. (1967) and Harris et al. (1967) concluded that exposure of immatures to a 11-h daylength induces diapause, despite rates that were typically low (<45%) and highly variable.

Earle and Newsom (1964) extended their observations on photoperiod by applying data from another test conducted under a 12-h daylength with that from the test described above. These tests were used to evaluate the critical photoperiod—the daylength eliciting diapause in 50% of a population (Tauber et al. 1986). Earle and Newsom (1964) concluded that the critical photoperiod falls between 12 and 13 h, but less than half the weevils exposed to a 12-h daylength went into diapause (39–45%), suggesting a shorter critical photoperiod.

In forming this conclusion, Earle and Newsom (1964) apparently overlooked differences in the design of the 2 tests involved. Immatures in the 12-h test were held under different light and temperature regimes than emerging adults (26°C and 12-h daylength for immatures versus 23°C and 8-h for adults). All stages in the 1st test were held at the same daylength (11 or 13 h) and temperature (21°C). Exposing immatures and adults to different temperatures was shown by Earle and Newsom (1964) to affect diapause, yet this information apparently was not taken into account in forming their conclusions on the critical photoperiod. Because daylength also varied between stages, their conclusion would be further compromised if adults were also sensitive to photoperiod. Although they concluded that adults are not sensitive,

the test examining this phenomenon was weak and the results apparently misinterpreted.

To test adult sensitivity to photoperiod, Earle and Newsom (1964) used weevils emerging from infested squares collected in October and November, when natural daylengths range between \approx 11.9 and 10.3 h. These insects were reared in darkness, and emerging adults were held under several light and temperature regimes. Between 68 and 79% of the weevils attained diapause, suggesting to Earle and Newsom (1964) that diapause is not inhibited by photoperiod in the adult stage if it is induced during the immature stages. From these tests they concluded that the early immature stages are sensitive to photoperiod but not the adults, when in fact they never tested sensitivity of late immature stages or adults to short daylengths.

These early results may have influenced others, even when the evidence suggested otherwise. For example, Cobb and Bass (1968) apparently overlooked adult sensitivity to photoperiod in their work. They collected punctured squares from Alabama in July and August when daylengths range between \approx 14.3 and 12.9 h, reared the weevils in cardboard cages (details not specified), and held emerging adults on squares or bolls under 2 fixed photoperiods. Greater diapause was reported under a 10-h (38–52%) than 14-h daylength (0–3%), suggesting that adults respond to short photoperiods. Mangum et al. (1968) also reported a higher incidence of diapause among adults held under an 11-h (49%) than under a 13-h daylength (17%), provided immatures were reared in darkness. In a well-designed study examining the effects of photoperiod on individual life stages, Sterling (1972) found that adults have a higher incidence of diapause when held under a 11-h or 15-h daylength compared with a 13-h daylength. If adult boll weevils are sensitive to photoperiod, as the data suggest (Lloyd et al. 1967, Cobb and Bass 1968, Mangum et al. 1968, Sterling 1972), then adult response to shortened daylengths would mask the sensitivity of immatures (Tauber et al. 1986), further compromising the Earle and Newsom (1964) test and their conclusion that the critical photoperiod falls between 12 and 13 h. It seems reasonable to conclude that the critical photoperiod in the Earle and Newsom (1964) study was between 11 and 12 h.

To define the critical photoperiod better, Mangum et al. (1968) examined the diapause response of males and females to 4 daylengths between 12 and 13 h. Just over half of the males (52–57%) achieved diapause under a 12.3-h daylength. Diapause never exceeded 48% among females, even under the shortest daylength. Percent diapause was greater in males than females, a finding supported by Earle and Newsom (1964) and Cobb and Bass (1968), but not by Carter and Phillips (1974). From these results, it appears that the critical photoperiod varies with gender, falling close to 12.4 h for males and 11.8 h for females (ignoring other effects). The results of Mangum et al. (1968) and Earle and Newsom (1964) are similar, which is not surprising given their work was con-

ducted on similar weevils from laboratory colonies in Louisiana.

To identify the sensitive life stage(s) to photoperiod, Earle and Newsom (1964) compared boll weevils held under an 11-h daylength for their entire life cycle with those initially held for 7 d under a 11-h daylength, followed by 13 h thereafter. Seventy-three percent of the weevils attained diapause in the 1st group and 40% in the 2nd. They estimated that the egg and 1st larval stages were present during the initial 7 d and that a maximal response would have occurred with an additional 7-d exposure to the short daylength. They concluded that "The embryo or the early instar larva receives the photoperiodic stimulus."

Assuming the critical photoperiod is ≈ 12 h, and only the early immature stages are sensitive to this stimulus, a cohort of adults emerging in late October and early November would potentially go into diapause at a rate of $\approx 50\%$. This calculation is based on a 12-h daylength occurring in late September at 30–33 N latitude, and ≈ 4 –5 wk of ensuing development after the early stages (Harris et al. 1966, Sterling and Adkisson 1971). Weevils would then require considerable time to feed before entering overwintering sites (Wagner and Villavaso 1999), pushing the entry date into late November. Fifty percent of the weevils entering diapause at such a late date is low, even with the late-maturing cotton varieties of the past. In fact, Harris et al. (1966) and Sterling and Adkisson (1971) found that few if any eggs laid after late September would contribute to the overwintering population in northern parts of the range, suggesting that conditions would never allow 50% of a cohort to achieve diapause.

Effects of Temperature. Early researchers believed that low temperatures during the fall were responsible for forcing weevils into hibernation (Hunter and Hinds 1904, Sanderson 1907). Although low temperatures may cause weevils to seek shelter and become quiescent, these early reports did not address diapause per se or the mechanisms responsible for its initiation. It is difficult to assess the effects of temperature on boll weevil diapause because of contradictions in results and the lack of studies comparing the responses of different life stages under a range of conditions. Most work was conducted on adults only.

For example, some studies showed an indirect relationship between percent diapause in adults and constant temperatures (e.g., higher temperatures suppressed diapause [Earle and Newsom 1964, Lloyd et al. 1967]). Daily variable temperatures also increased diapause frequency in adults relative to constant temperatures (Earle and Newsom 1964, Lloyd et al. 1967, Cobb and Bass 1968), but the lower range of the variable temperatures was never examined under constant conditions, and thus it is difficult to determine whether adults respond differently to constant versus variable temperatures. Nevertheless, Lloyd et al. (1967) concluded that low night temperatures induce diapause in adults.

Others failed to demonstrate a significant diapause-suppressing effect of high constant or variable temperatures on adults that otherwise were reared and

held under diapause-inducing environments (Wagner and Villavaso 1999, Wagner et al. 1999). The explanations for the variable results among studies is unknown, but they may have resulted from different treatments having been applied to early versus late life stages. If specific conditions do not occur at the proper time, then successful induction may not occur, and the occurrence or nonoccurrence of certain cues may alter or even reverse the induction process, causing results to vary under different treatments (Tauber et al. 1986).

Temperature is known to influence the rate of acquisition of the adult prediapausing phenotype, especially hypertrophy of the fat body. For example, Wagner and Villavaso (1999) showed that low temperatures prolong adult prediapausing development late in the year—a situation that could bias diapause determinations (based on dissection) of field-collected adults.

Effects of Diet/Host. Evidence supporting diet (nutrition) as a controlling factor in diapause induction is largely circumstantial, yet this explanation has been advanced by investigators in the absence of definitive data (Sterling 1972, Keeley et al. 1977, Hilliard and Keeley 1984a). Tests examined the influence of host condition (e.g., feeding on immature plants producing only squares or mature plants producing bolls), food type (e.g., feeding on squares or bolls), and food quantity. Assessing the influence of these variables on diapause is difficult for several reasons. At best, the variables only indirectly affect diapause (i.e., they are not causal), and because the underlying mechanisms are not known, tests have been difficult to control and results have been variable and inconclusive. In addition, dietary factors may regulate diapause induction per se, or they may simply influence the acquisition of characters associated with diapause (such as fat body development) or reproduction (such as egg production, Isely [1928, 1932], Hilliard and Keeley [1984a]). Without careful experimental design, it is difficult to determine which type of response (or both) is represented. Most tests were conducted on adults only, often without regard to the conditions under which the immatures had been held, or they were conducted on adults collected from the field without regard to other important field parameters (see discussion under *Results*). In field studies, measures of host condition were subjective and qualitative (e.g., plants growing vigorously versus nonvigorously, stressed versus nonstressed) and, at best, only indirectly related to diapause (i.e., they did not examine causal mechanisms). Field tests are difficult to interpret because of the uncontrolled variables influencing growing conditions in the field. For these reasons, results were often weak and contradictory.

For example, Earle and Newsom (1964) examined the effect of cotton "maturity" on diapause by feeding adults squares from actively fruiting plants with no bolls (immature cotton) or squares from plants with many bolls, producing few squares (mature). Weevils were reared on artificial diet under a 12-h daylength. No difference was observed between groups.

In contrast, Carter and Phillips (1973, 1974) examined the influence of early versus late-season cotton varieties and fruiting activity on diapause among weevils reared on these plants. They noted small differences among groups (typically <15%) and broadly concluded an association of diapause with reduced fruiting rates, retarded growth, induced maturation, amount of regrowth, and time of defoliation. Assessment of the fruiting activity was subjective, and because of the numerous uncontrolled variables potentially influencing the results, tests were more observational than scientifically valid.

Earle and Newsom (1964) noted a higher incidence of diapause among adults feeding on bolls (58%) than among those on squares and flowers (42%). No significant difference was observed in diapause among weevils feeding on squares (59%) versus squares and flowers (67%). Weevils were reared in squares or artificial diet. These investigators may have been aware of some of the problems evaluating this work, as they concluded that the "response to photoperiod was modified by temperature and food," an interesting statement suggesting that photoperiod alone induces diapause, and other stimuli act only to increase or diminish its effect.

In similar tests, Lloyd et al. (1967) reared colony weevils on artificial diet under continuous light, and held emerging adults on young plants bearing only squares or on mature plants bearing only bolls. Adults fed on squares had a lower incidence of diapause (0–15%) than those fed on bolls (38–58%), causing them to conclude that boll feeding as adults induces diapause. This conclusion neglects the possible link between host condition and diapause (see Wagner and Villavaso 1999). Lloyd et al. (1967) also reared larvae on artificial diet or bolls under continuous light, and fed emerging adults squares also held under continuous light. Eleven percent of the weevils reared on diet and 24% reared on bolls achieved diapause, prompting the conclusion that boll feeding as larvae induces diapause.

Cobb and Bass (1968) reported little difference in diapause among adults feeding on squares or bolls (favoring squares) in weevils reared from squares collected in Alabama during July and August. Adults feeding on artificial diet reduced the incidence of diapause compared with the other food types. In other incidental reports, Sterling (1971) found higher diapause among weevils reared on bolls relative to squares, but subsequently Sterling and Adkisson (1974) found no difference in diapause between the 2 larval food groups. Adults were fed on squares in all tests. Elsewhere, Carter and Phillips (1973) reported higher diapause among weevils reared on and fed squares relative to bolls.

Other investigators reported that weevils accumulate more lipids when feeding on bolls than on squares (Tingle and Lloyd 1969), even independent of their physiological status (Brazzel et al. 1957, Lambremont et al. 1964). The boll weevil consumes more on high carbohydrate, low nitrogen diets, and consumption is further stimulated by short days and cool tempera-

tures (Hilliard and Keeley 1984a, b). These events may explain the observations of enhanced fat among bollen weevils, but it is impossible to determine whether these attributes biased diapause determinations (based on dissection) in weevils.

Lloyd et al. (1967) showed an inverse relationship between food abundance during the adult stage and percent diapause, but these results are suspect for several reasons. One test was unreplicated, and diapause determinations apparently were based solely on the status of the adult reproductive system (possibly true for all tests). Poor or limited nutrition may prevent gametogenesis in reproductive weevils and fat body development in prediapausing adults and, thus, may bias diapause determinations. Percent diapause increased in all tests when weevil density per square increased, even when immatures and adults were held under continuous light—a condition that should prevent diapause. Even if the results represent a diapausing state (and not distress), the cause may be related to other factors and not food availability.

In a recent study, Spurgeon and Raulston (1998) tested adult sensitivity to different photoperiods, temperatures, and feeding regimes. Weevils were reared under a 13-h daylength at 29°C from infested squares collected in south Texas during mid-June and early July (~13.8 h daylength). Emerging adults were divided into 8 treatments of 11-h or 13-h daylengths, 29 or 29:10°C (L:D), and held as individuals fed 1 intact square per day or in groups of 25 fed 5 debracted squares per day. Percent diapause was influenced by feeding regime (significantly higher in grouped weevils) but, interestingly, not photoperiod or temperature.

Genetic Effects. Several studies reported differences in the propensity among boll weevil populations to diapause. For example, Earle and Newsom (1964) noted strong differences in the diapause response to photoperiod among strains from different geographical regions. A strain of weevils from Mexico (24° N latitude) showed little response relative to weevils from Louisiana (30° N). They also noted that weevils from Nicaragua (13° N) are capable of entering diapause, but not at rates as high as those from Louisiana. Sterling and Adkisson (1966) found similar variation among weevil populations in the state of Texas, with those from the High Plains showing a greater response to photoperiod than those from central Texas. Lloyd et al. (1967) reported that weevils originating in Mexico lost the ability to diapause after mass propagation for many generations, but Mississippi weevils held in the laboratory for about a year remained responsive. Diapause is under genetic control and can be influenced by laboratory propagation (McCoy et al. 1968).

An interesting contrasting observation proclaims that boll weevils do not diapause in subtropical and tropical regions (Guerra et al. 1982, 1984). Although the weevil no doubt uses quiescence to overcome periods of aseasonal adversity, as claimed, we believe the no-diapause hypothesis is not supported by the preponderance of evidence. The studies of Guerra et al. (1982, 1984) were not designed to test for diapause

per se in populations. They ignored the fact that populations comprise individuals in different physiological states at all times of the year, more pronounced at southern latitudes, and that selective sampling methods (e.g., traps or weevils in bolls) may bias the results relative to the overall population. They did not consider the length of time required for weevils to satisfy diapause requirements, nor the season in which these requirements are fulfilled (possibly in the fall and early winter), and the possibility that weevils may actually overwinter in a state of postdiapause quiescence. Evidence that the weevil satisfies diapause development relatively quickly, in fall or early winter, was available at the time of their work (Walker 1967, Walker and Bottrell 1970), as was other evidence that the insect exhibits diapausing characteristics in these locations (refer above, also Graham et al. [1978, 1979]). The speed at which the insect moved through the U.S. Cotton Belt (Burke et al. 1986) suggests a predisposition to diapause in its native range; but, regardless, the boll weevil could not survive at northern latitudes without an adaptation to endure harsh environments for long periods. It is solely dependent on commercial cotton at these latitudes, and cotton is effectively absent from the landscape for ≈ 6 mo of the year.

Objectives. We have had systematic studies underway to examine the environmental and physiological mechanisms regulating different phases of diapause in the boll weevil. Studies were designed to promote an understanding of diapause in the field in different parts of the insect's diverse range. Whereas past studies typically have been conducted under artificial conditions known to affect diapause (e.g., using laboratory weevils reared on artificial diets under static environments), this study evaluates diapause of wild weevils held under field and simulated field photoperiods and temperatures over the course of the growing season. Its purpose is to quantify and model the combined effects of these variables on diapause induction. This study is the result of earlier work to define methods for evaluating the effects of token stimuli on diapause within a more natural context.

Materials and Methods

Experimental Procedures. Boll weevils originated from populations oviposited in cotton flower buds (squares) from commercial fields in Union County, MS during July through September 1989, 1990, and 1992–1994. Fields were within 8 km of each other. In 1995, infested squares originated from Webster County, MS. To obtain a high percentage of early life stages among weevils, only green infested squares were removed from plants before flaring. Subsamples indicated that most weevils were eggs, 1st, and early 2nd instars at the time of collection, with few older larvae and no pupae.

Infested squares were brought to the laboratory immediately after collection and divided among clear plastic boxes (27 by 40 by 10 cm) that served as rearing containers. Squares were placed on hardware cloth

supported by damp sponges 4 cm above the bottom of the boxes. Screened holes at the ends of the containers allowed ventilation. High humidity was maintained to prevent square drying.

Rearing containers were placed in cabinets controlled by a DEC Pro-380 or DEC 3100 computer (Digital Equipment, Maynard, MA). The computer dynamically controlled the temperature and lights in the cabinets using the following instructions. A daily temperature profile was derived from 20-yr averages of daily maximal and minimal temperatures from Stoneville, MS (Hull et al. 1982), and the daily times of sunrise and sunset for Mississippi State, MS. Daily minimal and maximal temperatures in the cabinets were set at sunrise and 1500 hours, 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 1st function apportioned 75% of this decline between T1 and 2400 hours, and the 2nd function apportioned the remaining 25% between 2400 hours and sunrise. Cabinet temperatures were held at values derived from these equations at 15-min intervals. The computer adjusted temperature every 10 s by reading 2 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 (General Electric, Cleveland, OH) were turned on and off each day at sunrise and sunset (time resolution = 1 min). To simulate increasing and decreasing light intensity during the morning and evening, eight 20-W fluorescent light bulbs (4 Coolwhite and 4 Daylight [General Electric, Cleveland, OH]) were turned on and off ≈ 47 –71 min after sunrise and before sunset, respectively, depending on the Julian date (JD). The daily time delay between the incandescent and fluorescent lights simulated the relative position of the sun 15° above the horizon, as determined by a polynomial equation ($R^2 = 0.99$).

To characterize the seasonal pattern of diapause among boll weevils in Mississippi, infested squares were collected from the field on different dates throughout the diapause induction period and were placed in cabinets simulating an extension of the field environment from which they came. For example, cabinet environments varied within and among days, incremented from the date of collection (Table 1). In this manner, weevils experienced similar uninterrupted photoperiods and temperatures between the field and laboratory.

Boll weevils were collected from rearing containers each day of the emergence period and placed in clear plastic containers (15 cm diameter by 6 cm deep) of 100 weevils or less. Containers were provisioned with fresh, debracted, pristine squares or small bolls daily in excess of feeding demand. Some weevils were held as

Table 1. Percentage of prediapausing male and female boll weevils collected in the field as immatures in squares on different Julian dates and held in cabinets under dynamic photoperiods and temperatures until dissected

Year	Collect JD	Adult diet	Adult age ^a	Males				Females			
				N	n	Mean emerge JD	%	N	n	Mean emerge JD	%
89	208	S	15-22	61	60	219.6	36.7	72	71	219.6	9.9
89	230	S	16-21	94	93	243.7	54.8	92	92	243.5	34.8
89	249	S	16-21	136	136	264.5	91.9	140	139	264.7	89.9
90	212	S	14-16	192	192	223.4	49.0	167	165	223.2	26.7
90	229	S	19-21	57	57	242.5	77.2	51	51	241.8	47.1
92	213	S	14-22	86	86	225.5	32.6	83	81	225.3	21.0
92	255	S	21-25	50	50	272.6	94.0	39	39	272.6	92.3
93	230	S	30-43	40	40	243.0	80.0	146	145	242.3	33.8
93	252	S	8-24	18	18	268.0	94.4	139	108	267.2	81.5
93	252	B	8-22	40	40	268.5	97.5	34	30	266.9	96.7
93	263	S	14-15	47	47	281.4	93.6	69	65	281.6	98.5
93	263	B	14-16	46	46	284.2	97.8	36	36	284.6	97.2
94	200	S	17-35	166	166	211.1	17.5	171	155	211.0	5.8
94	231	S	14-18	203	203	247.2	90.6	173	172	246.8	69.8
95	215	S	4-15	105	105	226.7	24.8	132	130	226.5	5.4
95	227	S	16	70	70	240.8	72.9	82	80	241.0	53.7
95	241	S	4-15	71	71	255.0	100.0	94	94	254.9	95.7
95	241	B	4-15	61	61	255.2	98.4	74	70	254.8	95.7
95	255	B	5-21	22	22	269.2	95.4	27	24	269.0	95.8

S, squares and B, bolls. N, indeterminate, reproductive, and prediapausing adults used to calculate percentage of diapause. n, reproductive and prediapausing adults.

^a Age at dissection; the youngest males in the study were 10 d old.

individual mating pairs in clear plastic cubes (2.5 by 2.5 cm) and fed 1 square per day.

Physiological Status of Weevils. The physiological status of adults was determined by dissection typically 14-21 d after emergence, although weevil ages at dissection varied between 4-43 d (Table 1). Weevils were held in a phosphate-buffered saline solution during dissection (Wiygul et al. 1982). Observations were taken under 15-100 magnification on the volume of fat body, size and appearance of the testes, amount of sperm in the vas deferens and seminal vesicles, amount of food in the gut, physiological age of females (Grodowitz and Brewer 1987), minimal and maximal number of developing follicles, follicle appearance (health), presence of follicular relics, number of eggs in the lateral and common oviducts, and presence of sperm in the spermatheca. These characteristics were initially used to select reliable, minimal criteria for separating reproductive from diapausing states, and although they provided information on the general condition of the weevil, most were not needed for classification. Earlier work identified 2 characteristics as most useful in distinguishing the physiological status of boll weevils—sizes of the gonads and fat body (Brazzel and Newsom 1959, Earle and Newsom 1964).

Females. The age-grading index of Grodowitz and Brewer (1987) was useful in defining the physiological status of females; however, because of similarity between the ovaries of sexually immature females and those undergoing prediapause, this index alone could not be used in classifying diapause, especially in young adults. Prediapausing females have small ovaries with little or no follicular development, a condition like that of newly emerged females with N1 ovaries (Grodowitz and Brewer 1987). Depending on temperature, it takes females several days to acquire

the ovarian traits associated with reproduction (Isely 1932, Cole 1970). For this reason, fat body size was used in conjunction with physiological age of females. Fat body development was measured on a scale from 0 (no visible fat) to 6 (maximal fat), subdivided into equal parts.

A computer program classified females using the following set of rules. Females with parous ovarian development (P1-P4) were classified as reproductive regardless of fat body size. Fat body size in most reproductive females ranged between 2 and 3, although these females occasionally had enlarged fat bodies that were more pronounced when feeding on bolls. Females were also classified reproductive if they had an egg(s) in the lateral oviduct(s) and showed no evidence of follicular relics. Females with nulliparous ovarian development (N1-N3) and fat body size 5-6 were classified as prediapausing. (The exact status of diapause is unknown in the weevils in this study; therefore, we refer to them as prediapausing because data are plotted at the time of adult emergence.) These females all had small ovaries with little or no follicular development. The majority had N1 ovarioles with no follicles; but, when present, N2-N3 ovarioles had a small germarium and small vitellarium containing few, poorly developed follicles—conditions unlike those reported by Grodowitz and Brewer (1987) for prereproductive females. For example, an N3 ovariole in a female classified diapausing typically had 1 mature ovum followed by 1-3 small, undeveloped follicles. With their enlarged fat bodies, these females appeared to be in prediapause and were classified as such. Occasionally, follicular relics were present at the base of ovarioles that otherwise were classified nulliparous (N1 and N2). This condition suggested that follicular development and ovulation had occurred but then

stopped; thereafter, the ovary returned to a nulliparous state. Females exhibiting these traits were classified as prediapausing regardless of fat body size (which typically was >4). Lastly, females with nulliparous ovarian development (N1-N3) and fat body size 0-4 were classified as indeterminate. These females were either prereproductive at the time of dissection, or they were diapause inclined but lacked the characteristics typically associated with adult prediapausing.

Males. Two criteria were used to define the physiological status of males: testis size and evidence of sperm bundles in the testis lobes. Testis size was based on the combined diameters of both lobes of one testis relative to the length of the abdomen. A very small testis was $<3/8$, a small testis $3/8-1/2$, and a large testis $>1/2$ the abdomen length. Testis size was rarely $<1/4$ or >1 .

The computer program that classified males used the following rules. Males <10 d old were excluded from the study because, unlike females, gamete production is common among prediapausing males (Brazzel and Newsom 1959, Villavaso 1981). For this reason, it is difficult to distinguish prediapausing from prereproductive males at an early age (e.g., before testicular atrophy). To avoid bias, we selected only males old enough to express reproductive or diapausing characteristics, regardless of environment, by excluding those <10 d old. Males with very small or small testes were classified prediapausing regardless of other conditions. Most of these weevils had fat bodies >4 and showed evidence of sperm production. Males with large testes were classified reproductive, unless they lacked sperm bundles in the testes lobes, in which case they were classified indeterminate.

Analytical Procedures. To determine whether crowding of adults influenced their physiological status, the proportion of boll weevils classified as prediapausing was calculated for groups sorted by collection date, sex, and total number of adults per container among square-fed weevils (SAS Institute 1989). Linear regressions were fitted to the percentage of males and females in prediapausing versus the number of adults per container (density). Regressions were weighted by the total number of reproductive and prediapausing males or females in each density class. Regressions were examined for trend and significance. Weevils were grouped into 3 collection dates (JD 208-215, 227-230, and 241-263) to account for the seasonal variability in diapause and to increase the number of density classes and frequency of adults per density class.

To examine the seasonal incidence of diapause, mean emergence dates of weevils were calculated for groups sorted by year, collection date, adult diet, and sex. Frequency counts were obtained for indeterminate, reproductive, and prediapausing weevils in each group. The proportions of reproductive and prediapausing adults were calculated after excluding indeterminate weevils. Percentages of prediapausing males and females were plotted (dependent variable) at their respective mean emergence dates (independent variable) for each year of the study. The logistic

equation was used to describe this relationship among square-fed adults:

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

where $F(x)$ = the percentage of prediapausing boll weevils on a given emergence 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 midpoint of the curve along the x -axis (Julian date), and (k) the upper asymptote of the curve.

To examine the influence of photoperiod on diapause, the daylength (sunrise to sunset) associated with the Julian date of mean emergence was identified for prediapausing weevils in each group, and this independent variable was substituted for x in equation 1 (hereafter referred to as equation 2). The Weibull function was also used to describe this relationship:

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

where $F(x)$ = the percentage of prediapausing adults on a given emergence date with daylength x , and a and b 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 equations 2 and 3 have potential application in predicting diapause in areas outside of Mississippi. To explore this application, these equations were used to compare diapause of boll weevils from north Mississippi with those from the Lower Rio Grande Valley of Texas. A 3rd-degree polynomial (equation 4) was used to describe the relationship between daylength and Julian date for Mississippi State, 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 more utilitarian relationship of percent diapause versus Julian date.

Results and Discussion

Adults were held in groups of mixed sexes by emergence date or as individual mating pairs. An average of 36.5 weevils were held per container for those in groups, with actual numbers ranging from 3 to 100. A regression analysis was conducted to determine whether the number of adults in containers influenced the incidence of diapause through variable conditions of crowding or food. Linear regression trends of percent diapause versus adults per container were not significant for males or females (Fig. 1; Table 2). Thus, the conditions under which adults were held did not influence their physiological status, and weevil density was not considered in the seasonal evaluation of diapause.

Seasonal Diapause. Cotton grown in north Mississippi typically produces squares of the size preferred by boll weevils for oviposition (≈ 5 mm diameter or larger, Lincoln et al. [1963]) by mid-June. Adults emerge from these squares in July. Based on this chronology, results indicated that the percentage of prediapausing adults in a population increases progressively through the season starting with emergence of

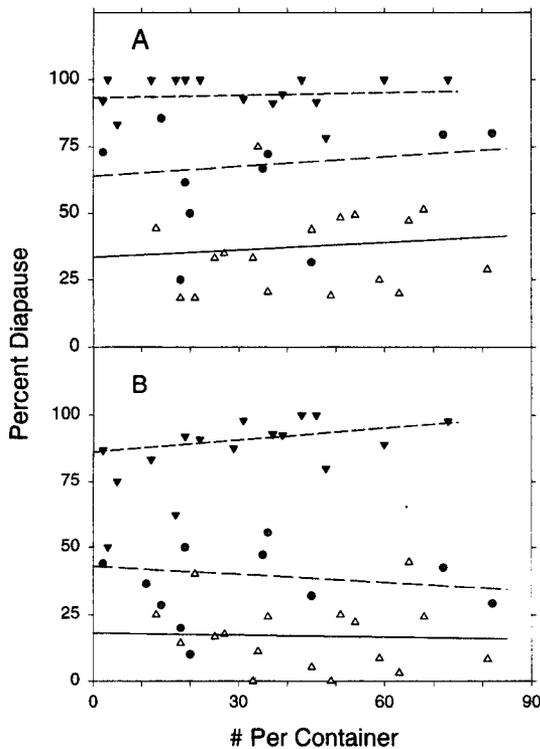


Fig. 1. Percentages of (A) male and (B) female boll weevils in prediapauses relative to the number of adults held per container for weevils collected between JD 208–215 (upright triangles), JD 227–230 (circles), and JD 241–263 (inverted triangles). Weighted linear regressions are shown as solid lines (early season), long-dashed lines (mid season), and short-dashed lines (late season).

the first generation (Fig. 2; Table 1). Illustrating the diapause response at the time of emergence is a meaningful way to represent the process because diapause occurs during the adult stage, and diapause control applications used in eradication are directed against prediapausing adults before they leave the field in search of overwintering sites. In the past, these applications have been initiated during the first 10 d of September (W. A. Dickerson, Technical Advisory Committee, Southeastern Boll Weevil Eradication Foundation, personal communication). Results from this study clearly advise an earlier starting date and, in fact, this information has been used in eradication programs in Alabama and Mississippi to initiate diapause control applications in August.

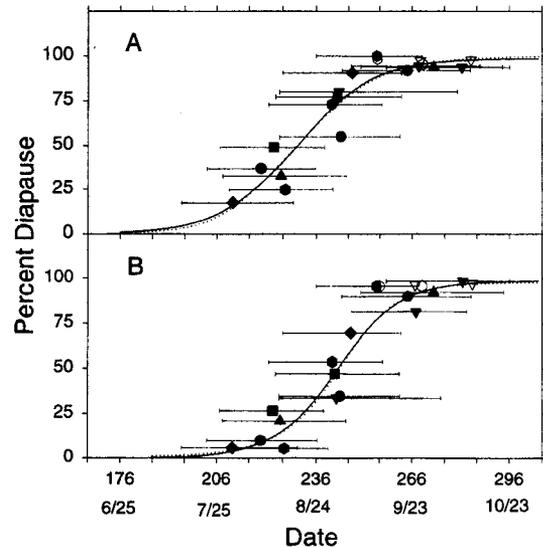


Fig. 2. Percentages of (A) male and (B) female boll weevils in prediapauses on the Julian/calendar date of adult emergence. Circles depict data from 1989, squares 1990, upright triangles 1992, inverted triangles 1993, diamonds 1994, and hexagons 1995. Horizontal bars through data depict the lifespan of weevils from approximate oviposition date (collection date minus 5 d) to mean dissection date. Solid lines depict equation 1 fitted to the closed data points (square fed); dotted lines depict predictions using equations 3 and 4 for males and 2 and 4 for females. Open data depict boll fed.

Equation 1 did a good job describing the percentage of square-fed adults in prediapauses as a function of emergence date, depicting a flattened “S-shaped” curve that tailed out at the beginning and end of the growing season (Fig. 2, solid lines; $R^2 = 0.88$ for males and 0.91 for females). Although the logistic equation predicted some adults in prediapauses at early dates, most of the response curve fell within a range of dates that are biologically meaningful. For example, 1 and 98% of the adults were in prediapauses on JD 177.2 and 287.0 (26 June and 14 October), respectively, for males and 197.8 and 290.5 (17 July and 17 October), respectively, for females. The upper asymptote of the curves is 98.7% (Table 3, parameter k), indicating that, on average, some members of a population will never initiate diapause, even late in the year. The model did not include results from boll-fed adults because of the scarcity of data (observed only late in the season), but these results were not unlike those obtained from square-fed adults (Fig. 2; Table 1).

Table 2. Linear regression equations describing the percentage of prediapausing male and female boll weevils (Y) versus the number of adults held per container (x) for weevils collected early (JD 208–215), mid (227–230), and late (241–263) in the season (test for slope $P > t$)

Collect date	Males				Females		
	Equation	R^2	$P > t$	Equation	R^2	$P > t$	
Early	$Y = 33.512 + 0.0955x$	0.014	0.651	$Y = 18.003 - 0.0242x$	0.001	0.895	
Mid	$Y = 63.906 + 0.1224x$	0.058	0.501	$Y = 42.869 - 0.1000x$	0.106	0.329	
Late	$Y = 93.311 + 0.0315x$	0.011	0.710	$Y = 86.109 + 0.1504x$	0.220	0.067	

Table 3. Parameter estimates and R^2 values for the logistic equation 1 describing the percentage of male and female boll weevils in prediapause versus Julian date of emergence, and the logistic equation 2, and Weibull function 3 describing the percentage prediapause versus daylength on the day of emergence

Sex	<i>a</i>	<i>b</i>	<i>k</i>	R^2
Diapause Versus Julian Date (equation 1)				
Male	0.0865	230.114	98.7141	0.884
Female	0.1023	242.553	98.7283	0.909
Diapause Versus Daylength (equation 2)				
Male	-2.8542	13.3123	97.8228	0.888
Female	-3.2546	12.9205	97.7309	0.914
Diapause Versus Daylength (equation 3)				
Male	13.5121	26.1629		0.880
Female	13.1207	25.3256		0.901

During most of the season, a greater proportion of males achieved prediapause than of females, although this gender difference decreased toward the end of the season, represented by a common upper asymptote of the curves (Fig. 2 A and B). According to equation 1, males emerging on JD 217.6, 230.4, and 243.4 (6, 18, and 31 August) attained prediapause at rates of 25, 50, and 75%, respectively. Predicted rates on these dates were 7.1, 22.1, and 51.5% for females. Viewed in another way, emerging females entered prediapause at rates of 25, 50, and 75% on 232.0, 242.8, and 253.8, respectively (20 and 31 August, and 11 September)—a 14.4-, 12.4-, and 10.4-d seasonal delay for females to attain rates equivalent to those of males. Earle and Newsom (1964), Cobb and Bass (1968), and Mangum et al. (1968) also reported a greater diapause response of males than females on a given date. The initial delay in the appearance of diapause in females (e.g., 21 d at 1%), and convergence in the diapause response between sexes at the end of the season (e.g., common *k* values), resulted in a greater rate of increase in the diapause response curve of females relative to males (Table 3, parameter *b*).

The preponderance of evidence from this and other studies (see introduction, also Wagner et al. 1999) supports photoperiod as a primary mechanism responsible for the seasonal increase in diapause among boll weevils in the northern parts of its range, although we believe this mechanism works in concert with other environmental cues to bring about the full diapause response in a population. Earle and Newsom (1964) came to a similar conclusion, "... diapause (in the boll weevil) has been demonstrated to be induced by photoperiod, and like many other species, the response to photoperiod may be modified by temperature and food." Daylengths decrease in north Mississippi during most of the time weevils are reproducing in the field (e.g., from ≈ 14.4 h on 20 June to 12 h on 27 September [Fig. 3A]). This decline is nearly linear after 24 July ($Y = -0.03x + 20.77$; $R^2 = 0.99$)—a date that marks the onset of daylengths below 14 h and pronounced increases in diapause. In contrast, average daily temperatures remained relatively stable and hot between mid-June and late August (Fig. 3B, generally ≥ 32 maximum and 20°C minimum), discounting a signifi-

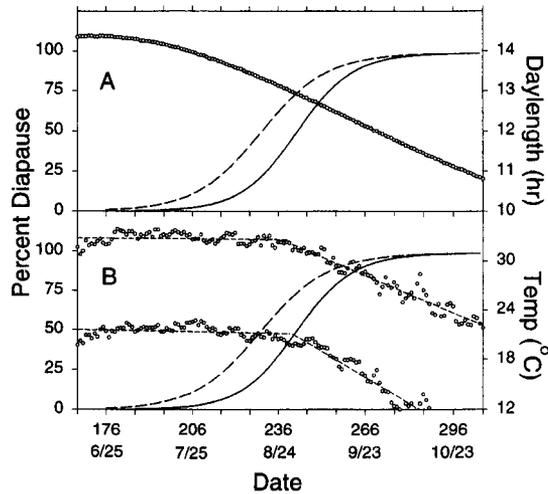


Fig. 3. Predictions of percent prediapause in male (dashed line) and female (solid line) boll weevils on the date of adult emergence generated by equation 1 compared with changes in (A) daylength and (B) average maximum/minimum temperatures (from Hull et al. 1982) for north Mississippi.

cant impact of temperature on diapause induction. High temperatures do not suppress diapause in the adult stage (Wagner et al. 1999), but they do influence the rate at which adults acquire diapausing characteristics (Wagner and Villavaso 1999).

Another possible mechanism for the seasonal increase in diapause among weevils involves exposure to a changing host. This study provides circumstantial evidence supporting this mechanism. For example, the percentage of adults in prediapause varied within and among seasons (Fig. 2). Diapause was lower than expected during the middle of 1989 but more typical early and late in the season (circles versus lines). The diapause response was steeper in 1995 than other years, starting lower and ending higher than normal (hexagons). The opposite response was evident in 1990 (squares). This variability was not the result of differences in photoperiod, because daylengths remain consistent from year to year at a given location. It probably was not the result of differences in temperatures among years, because immatures and adults were held under near identical temperatures in the laboratory. Except for 1995, all boll weevils originated from a small area in Mississippi, probably ruling out genetic differences among populations. Part of this variability may result from differences in weevil age at the time of dissection. For example, some weevils in 1993 were older (collection JD 230) or younger (JD 252) than normal at the time of dissection (Table 1), and females from these groups had lower than expected diapause (Fig. 2B, inverted triangles versus line). The incidence of diapause may have been suppressed in these groups because some older females may have terminated diapause before dissection (Walker 1967, Walker and Bottrell 1970) or some

younger prediapausing females may have been excluded from the analysis because they were too immature for classification. Nevertheless, evidence from the literature suggests that changes in host condition may contribute to the types of differences observed (e.g., changes in the slope of the diapause response curve among years).

Comparison With Field Data. Reports from the literature of diapause in the field were taken from boll weevils from several sources (e.g., adults collected from cotton plants and immatures collected from squares or bolls). Although these differences appear subtle, they have important implications for the results, sometimes overlooked by early investigators. The bulk of data originated from studies using hand-collected adults from cotton plants. Weevils were classified as reproductive or diapausing based on dissections typically performed immediately after collection. Studies were conducted in Louisiana (Brazzel and Newsom 1959), Texas (Brazzel and Hightower 1960, Sterling and Adkisson 1966, Sterling 1971, Sterling and Adkisson 1974, Graham et al. 1979, Cole and Adkisson 1983), Mississippi (Lloyd and Merkl 1961, Lloyd et al. 1964, Mitchell and Hardee 1974), Georgia (Beckham 1962, 1963), and North Carolina (Mitchell and Mistic 1965). Adults were often collected sequentially over the season, sometimes extending very late into the year, from experimental and commercial plots with variable planting dates, soil fertility, irrigation, fertilizer, and insecticide treatments. Not unexpectedly, diapause results were highly variable within and among fields and studies, even when objectives were similar (Fig. 4, closed data).

Diapause was first observed in field populations in July and August, ranging from collections made on 9 July (JD 190) in weevils fed squares before dissection to 24 September (JD 267) in weevils removed from a late-planted field containing few weevils. Initial values of diapause in some fields were higher than expected (e.g., >10%), suggesting that earlier diapause went unsampled or unnoticed. The number of adults used to evaluate populations was often small (<20 per sample), and, as noted by Sterling and Adkisson (1974), "due to the low incidence of diapause in July, fairly high numbers (of weevils) were needed to detect it." As noted by some investigators (Carter and Phillips 1974, Sterling and Adkisson 1974), 1st-generation weevils are capable of entering diapause. Once diapause began, it typically was observed in all subsequent samples, increasing among successive samples to a point after which it fluctuated for the remainder of the season. This deflection point was often the maximum observed for the season, ranging from 30 to 100%, and usually occurred in late August through October. In some studies, the seasonal pattern of diapause was bimodal, mistakenly interpreted by Lloyd and Merkl (1961) and Lloyd et al. (1964) to reflect the typical pattern of diapause among field populations.

Although investigators using hand-collected weevils recorded the general seasonal trend of diapause among adults in a field, these data are not reliable in

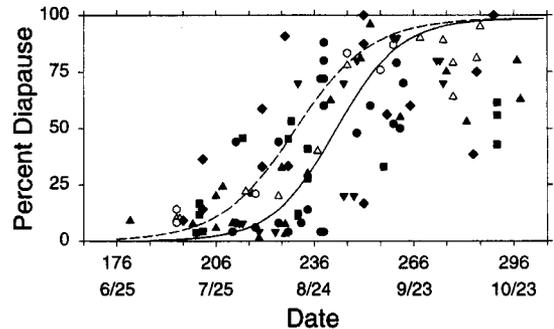


Fig. 4. Predictions of percent prediapausing in male (dashed line) and female (solid line) boll weevils on the date of adult emergence generated by equation 1 compared with literature reports of diapause in the field. Closed data depict prediapausing of hand-collected adults (plotted at the time of collection minus 10 d to estimate emergence times), and open data depict prediapausing of adults reared from infested squares or bolls and fed squares for ≈ 20 d (plotted at the time of emergence). Fields with multiple observations depict the initial observation and diapause at the deflection point (see text). Circles represent data from Mississippi (Lloyd and Merkl 1961, Lloyd et al. 1964, Mitchell and Hardee 1974), squares Louisiana (Brazzel and Newsom 1959), upright triangles Texas (Brazzel and Hightower 1960, Sterling and Adkisson 1966 [open data plotted 10 d after collection dates of squares to estimate emergence times], Sterling and Adkisson 1974, Sterling 1971, Cole and Adkisson 1983), inverted triangles Georgia (Beckham 1962), diamonds North Carolina (Mitchell and Mistic 1965), and hexagons Arkansas (Carter and Phillips 1973 [plotted 10 d after collection dates of squares]; 1974).

defining diapause among selected cohorts of adults emerging from fields. This fact was understood by Sterling and Adkisson (1966), who stated "... there may be inherent errors in this method of sampling since it does not take into account the age structure of the population or differences in behavior between reproductive and diapausing weevils." They also noted (1974) "the low incidence of diapause in field-collected weevils was attributed to the dilution of the population with old and young, newly emerged, reproductive weevils."

Information derived from hand-collected weevils is biased because there is no control over, or knowledge of, the age classes or origins of adults in the sampled population. These variables change during the season as a function of the overall age structure of a particular population and its variable rates of emigration and immigration relative to the stage of cotton. For example, if adults are collected during a period of high emergence, then a large portion of the sample may be too young to classify as reproductive or prediapausing. The classification of these weevils may be biased toward reproduction, especially if the fat body—a characteristic used in most studies—is used to assess diapause. If a sample is drawn between emerging generations, then it may contain a disproportionate number of older adults exposed to earlier environments, resulting in a different physiological makeup

than younger weevils exposed to later environments. At the same time, older weevils going into diapause leave fields in search of overwintering sites, selectively diminishing their numbers relative to reproductive weevils from the same fields. Older adults may even terminate diapause and become reproductive in the same season (Walker 1967, Walker and Bottrell 1970).

Field populations subject to emigration and immigration are likely to reveal an irregular pattern of diapause using hand-collected adults, a fact understood by Brazzel and Newsom (1959) who stated, "the percentage of diapausing weevils fluctuated with migration . . . during the remainder of the season." As cotton begins to mature in August, competition increases among females for fewer pristine oviposition sites (squares) (McGovern et al. 1987); coincidentally, reproductive weevils begin to disperse (Rummel et al. 1975). Emigration probably begins earlier from early-maturing fields, and late maturing fields become the foci of immigration (see Roach et al. 1984). Weevils undergoing prediapause are not likely to leave fields as long as edible fruit is available, and in many fields, some edible fruit will remain until crop termination (even after, with regrowth). If dispersing weevils are primarily reproductive, then emigration from early-maturing fields should bias percent diapause upwards in those fields, and immigration into late-maturing fields should bias it downwards. Dispersal to and from a field will have a weighted effect on diapause among resident adults depending on the density and age structure of the population. For example, the proportion of prediapausing adults may be underestimated during periods of low emergence and high immigration. Furthermore, prediapausing weevils continually and permanently leave the field in search of overwintering sites, continually diminishing their numbers from the emergent cohort to which they belong. Reproductive weevils disperse among fields in search of oviposition sites, yet late in the year they may accumulate in fields, biasing reproduction upward. As squares and bolls become scarce and temperatures decrease late in the year, the rate of development of reproductive and diapausing characteristics is slowed, biasing one or the other downward.

These collective problems compromised the conclusions drawn by many investigators who studied the seasonal pattern of diapause among adult weevils collected in fields. Investigators often observed diapause earlier and at higher levels in fields that matured earlier. From these observations, they concluded that diapause is related to the fruiting activity of the crop. Although this conclusion may be valid, it is not fully supported by the tests performed—especially when hand-collected adults were used to assess the proportion of weevils in diapause. Understanding the mechanisms responsible for diapause initiation in individuals and the expression of this characteristic in selected cohorts of a population are 2 different matters.

Estimates of diapause in the field using hand-collected adults probably are reasonable early in the season when dispersal is negligible (biased by the

proportion of overwintering to emerging adults and the age structure of emerging adults). These estimates compared favorably with predictions from equation 1 provided a time adjustment was made to correct for differences between the field data and the model (Fig. 4, closed data). Diapause of adults typically was recorded at the time of their collection, whereas the model expresses diapause at the time of adult emergence. Because the age of field adults is unknown, a 10-d reduction to the collection dates was imposed to approximate emergence times. Discrepancies between the observed and predicted percent diapause were greatest late in the season. Most notably, field observations were typically lower than the predictions that approached 100%. Possible reasons for this discrepancy are described above.

Recognizing the problems with hand-collected adults, several investigators recorded the seasonal incidence of diapause among weevils reared from infested squares or bolls that were collected from the field, held in an insectary during development, and fed squares as adults for a period of 7–20 d before dissection. Data originated from Texas (Sterling and Adkisson 1966, Sterling 1971, Sterling and Adkisson 1974, Cole and Adkisson 1983) and Arkansas (Carter and Phillips 1973, 1974). These results are not subject to the same criticisms applied to hand-collected weevils, and they generally compared favorably with the model's predictions (Fig. 4, open data).

Diapause Versus Daylength. Equation 1 is useful in describing the percentage of a population in prediapause throughout the growing season in north Mississippi, but it does not account for the mechanism(s) responsible for diapause induction and thus has limited value in predicting diapause outside of this specific region. Describing diapause in a population as a function of daylength is a meaningful relationship—photoperiod is a common environmental cue regulating diapause in most insects because of its stable seasonal relationship within a location. Decreasing daylengths are used by insects to warn of approaching environmental change harmful to survival. To describe this relationship, we regressed percent diapause versus daylength on the day of emergence. The logistic equation 2 did a good job describing the relationship for females (Fig. 5B, solid line; Table 3, $R^2 = 0.91$), but it predicted higher diapause among males early in the season relative to the Weibull function (Fig. 5A, dashed versus solid lines). For this reason, the Weibull function (equation 3) was chosen to represent the process for males (Table 3).

Regression analysis produced male prediapausing rates of 1, 25, 50, 75, and 95% at daylengths of 14.32, 13.68, 13.32, 12.88, and 12.06 h, respectively. Females attained the same rates at 14.32, 13.25, 12.91, 12.55, and 11.83 h of daylight at the time of emergence. It is difficult to compare these results with those from the literature because of procedural differences among studies. For example, daylengths eliciting 50% of a population to diapause were ≈ 1 h shorter when weevils were held under fixed daylengths (Mangum et al. 1968) compared with the dynamic daylengths re-

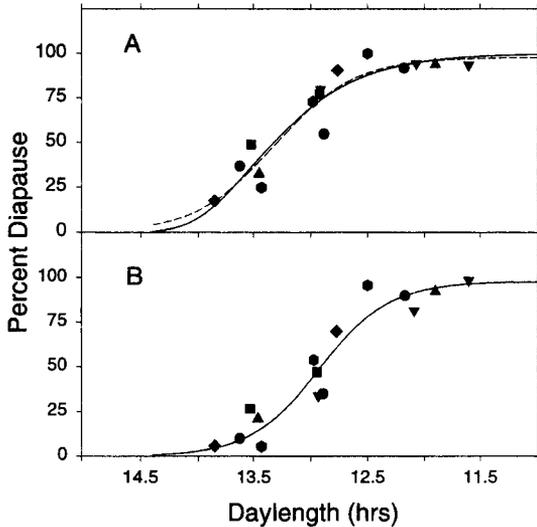


Fig. 5. Percentages of (A) male and (B) female boll weevils in prediapauses by daylength (hours between sunrise and sunset) on the date of adult emergence (symbols are the same as Fig. 1). Solid lines depict predictions using equations 3 for males and 2 for females; dashed line represents equation 2.

ported here. The implications of these longer critical photoperiods on the seasonal aspects of diapause were discussed in the introduction.

The seasonal dynamics of diapause can be reexamined by substituting equation 4 for x (daylength) in equations 2 and 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 a season. We used this approach to compare the expected diapause among boll weevils from north Mississippi and the Lower Rio Grande Valley of Texas. This area of Texas was chosen because it represents an extreme southern latitude in the U.S. Cotton Belt, and some reports suggest that boll weevils may not diapause at this location (Guerra et al. 1982, 1984). Daylengths were obtained for both areas (Fig. 6), and equation 4

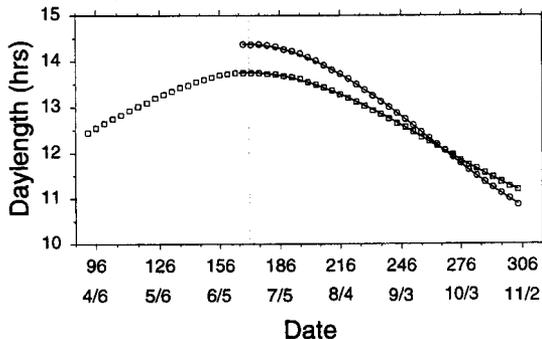


Fig. 6. Daylengths (hours between sunrise and sunset) for Starkville, MS (circles), and Brownsville, TX (squares), by Julian/calendar date. Solid lines depict equation 4 fitted to the data between JD 152-319 (MS) and 166-304 (TX).

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

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

was fitted primarily to the portions of the curves with decreasing daylengths (Table 4). We assumed that increasing daylengths do not induce diapause because this situation generally implies improving conditions for the growth of cotton and the boll weevil.

These analyses produced diapause response curves in Mississippi virtually identical to those given by equation 1 (Fig. 2 A and B, dotted versus solid lines). They also revealed a higher proportion of a population achieving diapause in Texas compared with Mississippi between late June and late September (Fig. 7). This condition resulted from the shorter daylengths observed in Texas during this period (Fig. 6). Decreasing daylengths occur only at the end of the cotton-growing season in south Texas, leaving developing weevils little time of exposure to conditions thought to induce diapause. The 25th of June 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 August. According to the model, males emerging between 25 June and 31 August will go into diapause at rates of 20-82%, and

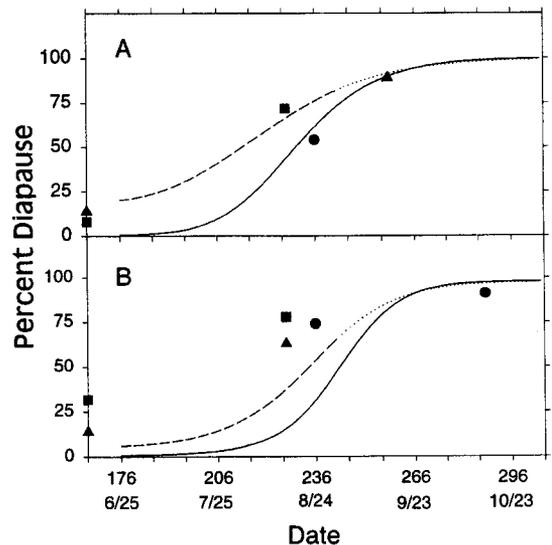


Fig. 7. Predictions of percent prediapauses in (A) male and (B) female boll weevils in north Mississippi (solid lines) and the Lower Rio Grande Valley of Texas (dashed and dotted lines) using equations 3 and 4 for males and equations 2 and 4 for females. Hand-collected weevils from the Lower Rio Grande Valley (Graham et al. 1979) are plotted in the middle of the sample interval for 1973 (circles), 1974 (squares), and 1975 (triangles).

females 6–66%. Data from Graham et al. (1979) indicate that these estimates are reasonable (Fig. 7).

Several assumptions are necessary when using the model, especially outside the mid-South region. For example, we assume that photoperiod is a primary mechanism responsible for diapause induction of the boll weevil, and that the weevil responds to this mechanism in a similar manner across the Cotton Belt. Based on the variability in the diapause response observed in virtually all studies, including this one (e.g., Fig. 2), we know that photoperiod is not the only mechanism involved. The preponderance of evidence supports it as a primary mechanism, but weevils may have adapted to a specific range of daylengths in their area. In this case, the model will have to be calibrated for different regions of the Cotton Belt. We also assume that increasing daylength does not induce diapause, as this situation generally suggests improving rather than deteriorating conditions for the host and insect. To the best of our knowledge, the influence of increasing daylengths on diapause induction has never been examined in the boll weevil. Like this assumption, the application of the model in other regions suggests that the rate of change in daylength during the life of the insect (before the onset of diapause) does not affect the diapause response per se. Longer maximal daylengths at the summer solstice and a more rapid decline in daylengths advancing toward the winter solstice occur at more northern latitudes of the Cotton Belt. These phenomena are clearly seen in Fig. 6, comparing the photoperiods of north Mississippi and south Texas. The rate of change in daylength does, in fact, influence the diapause response in some species (Tauber et al. 1986). Even if the rate of change in daylength does not influence diapause induction in the boll weevil, 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.

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