Effects of Temperature and Adult Diet on Development of Hypertrophied Fat Body in Prediapausing Boll Weevil (Coleoptera: Curculionidae)

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Ann. Entomol. Soc. Am. 92(3): 403–413 (1999)

ABSTRACT This study examines the effects of temperature and adult diet on the development of hypertrophied fat bodies in prediapausing adult boll weevils, *Anthonomus grandis grandis* Boheman. Simulation models derived from this work are used to estimate the minimal ages at which male and female boll weevils exhibit diapause morphology, based on conditions in north Mississippi. The models assume that temperature is the principal independent variable regulating fat body development, that food is not limited, and, once the internal characteristics of diapause are expressed, that the weevil will leave the field in search of overwintering quarters. The information derived from the simulations was used to propose spray intervals for diapause control.

KEY WORDS *Anthonomus grandis grandis*, adult prediapause development, cotton, simulation model

THE BOLL WEEVIL eradication program has effectively eliminated this pest from several eastern cotton-producing states, notably Virginia, North and South Carolina, Georgia, Florida, and Alabama. Eradication efforts are underway in Mississippi, Tennessee, Louisiana, Arkansas, and Texas. The elimination of this pest has increased cotton production dramatically and decreased pest control costs in the southeast (Bradley 1994, Lambert et al. 1994, Roof 1994, Bacheler and Mott 1995), and the long-term benefits of this program have greatly outweighed the short-term costs.

During the 1st yr of eradication, a series of late season, ultra-low-volume malathion sprays are applied to all cotton fields in the operational zone. The sprays target prediapausing adults in the process of developing the diapause syndrome. The goal is to eliminate most of these weevils before they leave cotton in search of overwintering sites. The following spring, pheromone traps are used to identify the fields with emerging weevils, and, where found, malathion is applied at the pinhead square stage of cotton to prevent weevil reproduction. Unfortunately, overwintering weevils may emerge after pinhead square (Leggett et al. 1988), and these weevils will avoid the pinhead square applications. This situation is more pronounced in areas with higher weevil numbers, and populations can become established in fields where this occurs. To prevent weevil buildup, some fields may require additional early- and midseason sprays, both of which destroy beneficial insects and may exacerbate secondary pest problems. This is an unacceptable situation that, in combination with the multiple sprays the preceding year, can have devastating effects on an eradication program (Layton 1994, Ruberson et al. 1994, Smith 1994). The recission and later failure of eradication referenda in Mississippi during 1996 resulted primarily from perceptions that malathion caused severe outbreaks of tobacco budworms, *Heliothis virescens* (F.), in 1995 (Conner 1996, Layton et al. 1996).

To prevent additional in-season sprays during the 2nd yr of the program, it is essential that weevil numbers be greatly reduced the previous summer and fall. This understanding places importance on diapause control and, with it, knowledge of the number of sprays required to eliminate populations effectively before they overwinter. To satisfy this end, specific information on appropriate starting and stopping dates and spray intervals must be determined based on a thorough understanding of diapause induction in the weevil. Our research has focused on these issues.

This article describes the effects of temperature and adult food on the development time of hypertrophied fat bodies in prediapausing male and female boll weevils. Data derived from this study are used to develop simulation models that predict the minimal ages of adults acquiring the diapause morphology under field temperatures. The models are used to propose improved spray intervals for diapause control throughout the induction period spanning late summer and early fall (see Wagner and Villavaso 1999).

Examination of internal morphology through dissection has been the primary assay for distinguishing reproductive from diapausing boll weevils. Atrophied reproductive organs and hypertrophied fat body have traditionally been used to classify diapausing insects. Although the expression of this condition is generally recognized to be time, temperature, and food-dependent, little research has been conducted to quantify the effects of these independent variables on the process. It is generally accepted that the boll weevil re-

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quires ≈14 d to develop the morphology associated with diapause, yet this belief is poorly supported by research. The age of adults at the time of classification (dissection) varied among studies. For example, in many studies adults were dissected immediately after collection from the field, making their ages unknown (Brazzel and Newsom 1959, Brazzel and Hightower 1960, Lloyd and Merkl 1961, Beckham 1962, Lloyd et al. 1964, Mitchell and Mistric 1965, Walker and Bottrell 1970, Graham et al. 1979). Reports of percent diapause within and among these studies often varied, partly because of the lack of age assessments among weevils. In other studies, adults were fed for a period after collection from the field, varying from 14 to 21 d (Carter and Phillips 1973; Cole and Adkisson 1983; Sterling and Adkisson 1966, 1974). Elsewhere, laboratory-reared weevils were dissected from 7 to 21 d after emergence (Earle and Newsom 1964; Sterling and Adkisson 1966, 1974; Lloyd et al. 1967; Mangum et al. 1968; Carter and Phillips 1973, 1974; Cole and Adkisson 1983). Brazzel et al. (1957) found that the process of fat accumulation among square-reared and adult fed weevils took 10 d at 26.7°C. This process took somewhat longer (10–15 d), and more fat was accumulated, in weevils reared on bolls or fed bolls as adults. Unfortunately, there was no attempt to identify the physiological status of the adults in their study. Tingle and Lloyd (1969) reported similar results—7–14 d to acquire maximal fat at 26.7°C among adults fed bolls. The process was delayed in weevils held under variable 21.1/10°C temperatures, and in adults fed squares. Supporting observations were made by Walker (1967), who found that diapause-induced weevils fed vigorously on squares for the first 10–12 d at 23.9°C and declined thereafter.

Materials and Methods

Experimental Procedures. Boll weevils originated from populations oviposited in cotton flower buds (squares) from commercial fields in Webster and Oktibbeha Counties (1996), Carroll, Sunflower, and Washington Counties (1997), MS. Infested squares were collected on 9 sample dates between 25 July and 24 September. To obtain a high percentage of early life stages among weevils, only green infested squares were removed from plants before flaring.

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 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 the 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 incandescent and florescent lights simulated the relative position of the sun 15° above the horizon, determined by a polynomial equation (R^2 = 0.99).

Boll weevils developing in squares were held in cabinets under simulated field temperatures and photoperiods of north Mississippi. Cabinet environments varied within and among days, incremented from dates beginning in late August to mid-September to induce diapause in the majority of weevils regardless of their collection dates. For example, weevils collected in July were placed in starting environments advanced by 30 or 40 d, those collected in mid-August were advanced by 20 or 30 d, and late August by 12 d (Table 1). Weevils collected in September were either moved back in time 14 d or experienced similar uninterrupted temperatures and photoperiods 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) provisioned with fresh, debracted, pristine squares or small bolls daily in excess of feeding demand. Daily cohorts of adults were transferred to other cabinets set to promote diapause under the same (uninterrupted) dynamic photoperiods but constant temperatures. Nearly 6,000 weevils were held under 13 constant temperatures from 14.9 to 36.0°C (Table 1). Temperatures were maintained in containers of adults (as described above) at a constant set point, averaged over the duration of each experiment to establish the rate versus temperature relationship.

Physiological Status of Weevils. Weevils were dissected to determine their physiological status (e.g.,

Table 1. Mean and median days to acquire hypertrophied fat bodies in prediapausing male and female boll weevils collected in the field as immatures in squares on different Julian dates, reared in cabinets under dynamic photoperiods and temperatures from different starting dates, and fed squares (S) or bolls (B) as adults under constant temperatures

Collect JD	Start JD	Temp, °C	Diet	Males				Females			
				N	*n*	Mean ± SD	Median	*N*	*n*	Mean ± SD	Median
226	256	14.9	S	297	266	27.4 ± 4.73	27.308	278	269	26.6 ± 4.99	26.177
261	261	17.3	S	172	166	13.8 ± 2.08	13.704	161	160	13.0 ± 1.77	12.857
236	248	19.6	S	282	275	11.8 ± 2.09	12.063	275	268	10.9 ± 1.75	10.846
227	247	19.8	B	261	259	11.3 ± 2.07	11.000	292	290	10.5 ± 1.91	10.520
206	236	25.0	B	220	194	7.6 ± 1.61	7.333	189	162	7.0 ± 1.59	6.667
208,262	248,248	25.1	S	303	237	7.6 ± 1.44	7.452	262	195	7.0 ± 1.55	6.692
262	262	27.6	S	266	255	7.0 ± 1.86	6.645	280	255	6.7 ± 1.61	6.375
262	262	27.7	B	252	231	7.3 ± 1.95	6.750	254	234	6.8 ± 1.59	6.350
208,262	248,248	29.9	S	280	218	6.8 ± 1.30	6.704	263	183	6.1 ± 1.30	5.744
206	236	30.2	B	226	163	6.4 ± 1.44	6.107	260	155	6.0 ± 1.57	5.708
226,236	256,248	32.1	S	144	135	6.0 ± 1.08	5.943	130	119	5.1 ± 0.99	5.159
236	248	33.9	S	122	114	5.9 ± 0.96	5.868	115	104	5.5 ± 0.94	5.488
268	254	36.0	S	172	164	7.8 ± 1.62	7.800	186	165	7.2 ± 1.87	7.250

N, total number of adults reared. *n*, number of adults classified prediapausing.

reproductive or prediapausing) and the frequency of prediapausing adults with hypertrophied fat bodies. Weevils were held in a phosphate-buffered saline solution during dissection (Wiygul et al. 1982). Groups of adults were dissected daily from the time hypertrophied fat bodies were first expected until all prediapausing adults were classified as fat (see below), except at 14.9°C, where observations were made every other day. Development of the fat body was used as an indicator of how long prediapausing adults would remain in the field before searching for overwintering quarters. The fat body is a good indicator of the status of diapause induction for several reasons. This organ increases progressively in size during prediapause development, and its size is easily measured. Of all the morphological characteristics associated with diapause, the process of storing fat reserves compels the insect to feed for a period after emergence. It is reasonable to infer that hypertrophy of this organ coincides with the culmination of feeding and the initiation of dispersal from fields in search of overwintering sites. These events should mark the approximate end of prediapause in the boll weevil (Tauber et al. 1986). Fat body size was measured on a scale from 0 (no visible fat) to 6 (maximal fat), and weevils classified as 6 were considered capable of leaving the field at any time.

Females. The age-grading index of Grodowitz and Brewer (1987) was used to define 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 typically have small ovaries with little or no follicular development, a condition similar in 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 the physiological age of females.

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 and, on average, was somewhat larger in boll-fed than in square-fed weevils. Females were also classified reproductive if they had an egg(s) in the lateral oviduct(s) but no evidence of follicular relics. Females with nulliparous ovarian development (N1–N3) and fat body size 5–6 were classified as prediapausing. These females had N1–N2 ovaries with little or no follicular development. Because some weevils were dissected at a very young age, we also considered females with nulliparous ovarian development and fat body size 4 as prediapausing (deviating from the designation of Wagner and Villavaso [1999]). Occasionally, follicular relics were present at the base of the 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). Finally, females with nulliparous ovarian development (N1–N3) and fat body size 0–3 were classified as indeterminate. These females were either prereproductive at the time of dissection, or they were diapause induced but lacked the characteristics typically associated with adult prediapause.

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 1 testis relative to the length of the abdomen. A very small testis was <⅜ the abdomen length, a small testis was ⅜-½ the abdomen length, and a large >½. Testis size was rarely <¼ or >1.

The computer program that classified males used the following set of rules. 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. Prediapausing weevils were grouped by temperature, adult diet, sex, age, and amount of fat at dissection. The number of adults with hypertrophied fat bodies (fat = 6) relative to the total number of prediapausing adults in each age group represented the cumulative proportion of weevils exhibiting diapausing characteristics by age. These cumulative daily probabilities were converted to simple proportions and treated as frequencies in distributions of times to acquire the diapause morphology. The number of weevils representing each age class ranged from 11 to 81 (mean = 32.2).

Model Development. Standard statistics were derived from these distributions, as was all information used to construct the models. For example, the mean and median times for weevils to develop hypertrophied fat bodies were calculated from each distribution, as were the times at 1, 5, 10,. . . , 95, 99, and 100% of the cumulative distribution. The resulting 22 times were divided by the median time (time at 50%), yielding a cumulative distribution of normalized times at each temperature. A single normalized time was calculated at each of the 22 ordinate (Y) locations along the common curves for males and females (calculated as the weighted mean normalized time at each location). This calculation resulted in a single, temperature-independent distribution of normalized times for males and females. Using procedures of Wagner et al. (1984a), a three-parameter cumulative Weibull function was fitted to each curve. The distribution has the form:

$$F(x) = 1 - \exp(-[(x - \gamma)/\eta]^{\beta}), \qquad [1]$$

where $F(x)$ = the probability of adults with fully developed fat bodies at normalized time x, and eta (η), beta (β), and gamma (γ) are parameters to be estimated.

Reciprocals of median times were used to represent the rates of fat body development. The Sharpe and DeMichele (1977) model was fitted to the rate versus temperature data for males and females using procedures of Wagner et al. (1984b). The model has the form:

$$r(T) = \frac{RHO25 \frac{T}{298.15} \exp\left[\frac{HA}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right]}{1 + \exp\left[\frac{HL}{R}\left(\frac{1}{TL} - \frac{1}{T}\right)\right] + \exp\left[\frac{HH}{R}\left(\frac{1}{TH} - \frac{1}{T}\right)\right]}, \qquad [2]$$

where $r(T)$ = median fat body development rate at temperature T (°K), R = universal gas constant (1.987 cal deg^{-1} mole^{-1}), RHO25 = development rate at 25°C (298.15°K) assuming no enzyme inactivation; HA, enthalpy of activation of the reaction that is catalyzed by a rate-controlling enzyme; TL, Kelvin temperature at which the rate-controlling enzyme is ½ active and ½ low-temperature inactive; HL, change in enthalpy associated with low-temperature inactivation of the enzyme; TH, Kelvin temperature at which the rate-controlling enzyme is ½ active and ½ high-temperature inactive; and HH, change in enthalpy associated with high-temperature inactivation of the enzyme.

Simulation Model. The approach of Wagner et al. (1985) was used to simulate the length of time required for portions of a weevil population to acquire hypertrophied fat bodies and, thus, the minimal times that prediapausing adults are likely to spend in the field feeding. The approach uses equation 2 to predict median developmental rates under variable field temperatures. The rates are summed with each time step of the simulation, and the accumulated rates serve as the independent variable in equation 1, which apportions the population completing the process of fat body development through time. A modified SAS code (SAS Institute 1989) similar to that presented in Wagner et al. (1985) was used to execute the simulations.

The effect of temperature on the acquisition of adult prediapause morphology was analyzed by initiating simulations on the 1st and 16th of August, September, and October, using 96 daily temperatures averaged over a 15-yr period. Temperatures were obtained from a class A weather station located at Mississippi State, MS. Thermocouple readings were taken every 15 s, averaged over 15-min periods from August to December 1982–1996. Simulations were also conducted using temperatures from each of the 15 yr to identify the fastest and slowest development times, generally associated with the warmest and coldest years, respectively. To adjust the weather station temperatures to those of a cotton field located ≈200 m away, 96 daily temperatures were obtained from thermocouples touching the underside of 6 cotton leaves over a 45-d period starting 2 August 1994. These daily leaf temperatures were averaged over the period and paired with comparable average air temperatures from the weather station. The proportions of leaf to air temperatures were calculated for each 15-min period, and air temperatures used to drive the model were multiplied by these proportions to approximate conditions experienced by weevils in the field. Only August and early September simulations were rerun using transformed temperatures, to account for temperatures in a full cotton canopy during this time.

Results and Discussion

Wagner et al. (1999) demonstrated that the percentage of early-season boll weevils attaining diapause increased by exposing immatures to advanced photoperiods and temperatures. They also showed that high temperatures during the adult stage did not suppress diapause in weevils held under diapause-inducing photoperiods. This work formed the basis for conducting the experiments described in the current study. As expected, exposing adults to high constant temperatures did not prevent the development of adult prediapause morphology (Table 1). Sixty to 99%

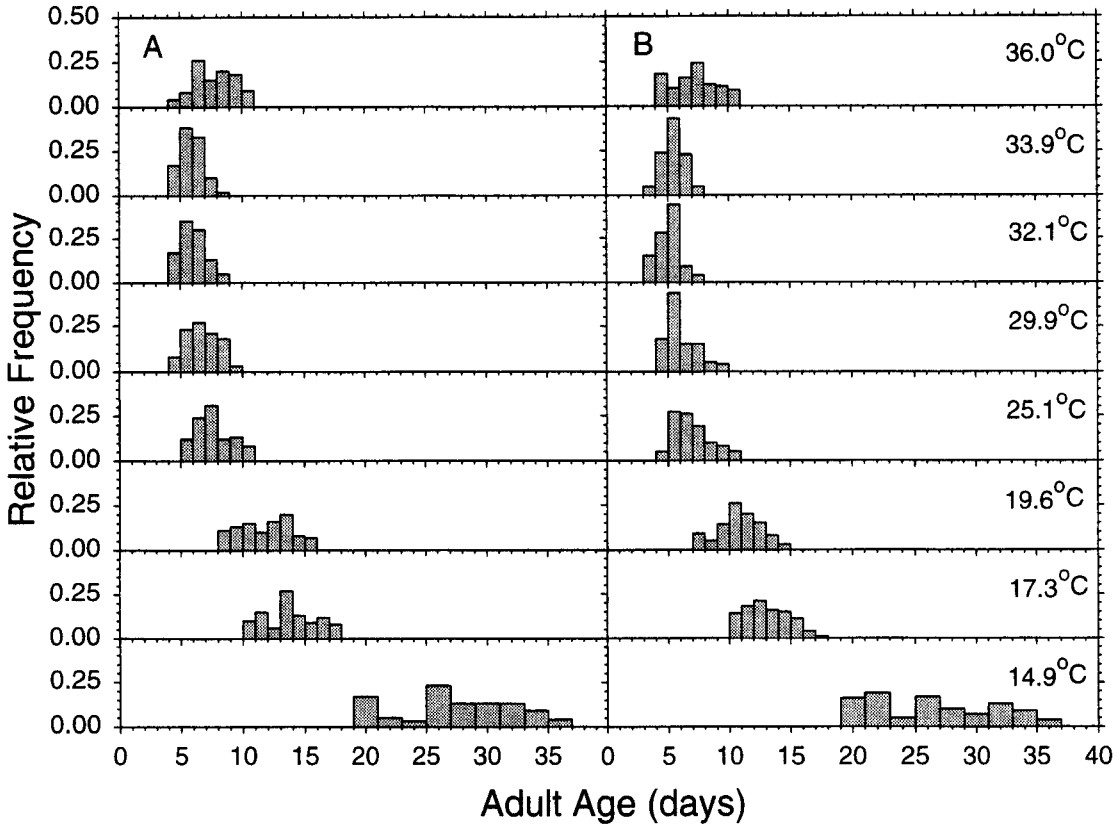

Fig. 1. Proportion of prediapausing (A) male and (B) female boll weevils by age with hypertrophied fat bodies (fat = 6) at different selected constant temperatures.

of all weevils were diapause-induced regardless of temperature. The lowest percentage was found in weevils collected the earliest (e.g., in July).

Temperature influenced the time to develop hypertrophied fat bodies in prediapausing adults (Fig. 1). Mean development times varied from ≈5 to 27 d over the range of temperatures observed, and it took males ≈0.6 d longer than females to accumulate maximal fat reserves (Table 1). The reason for this slight gender difference is unknown, but prediapausing males are known to produce sperm (Brazzel and Newsom 1959, Villavaso 1981), and the allocation of energy to gamete production may slow fat accumulation in males relative to females. Of the 2,677 prediapausing males observed in this study, all but 15 had sperm in the seminal vesicles. Prediapausing males may also have slightly higher respiration rates than females, although the data supporting this view are weak (Lambremont 1961).

Development of hypertrophied fat bodies was not influenced by adult diet (Table 1), and consequently the rate of fat development was very similar among square and boll-fed adults (Fig. 2). For this reason, a component describing the effects of diet on fat development of prediapausing males and females was not needed in the model. The model could be temperature-driven only—a fortunate event because it would be difficult to determine adult diet in the field.

Frequency distributions of development times (Fig. 1) provided all the information needed to model the effects of temperature on fat body development in prediapausing adults. For example, the inverse of median times derived from these distributions described the rates of fat body development (Fig. 2). Temperature influenced the rates in a manner similar to that observed for insect life-stage development (see Wagner et al. 1991), and detrimental effects were observed at low and high temperatures. Prediapause development proceeded over a broad range of temperatures that extended to surprisingly high limits before slowing. For example, the six-parameter Sharpe and DeMichele (1977) model predicted rates of 0.171 (5.8 d) and 0.189 (5.3 d) at optimal temperatures of 33.6 and 32.8°C for males and females, respectively. Although males took slightly longer to develop prediapausing morphology (Table 1), they have a higher optimal temperature associated with this development.

The regression curves also illustrated sensitivity to low temperatures as an increased downward deflection from linearity (e.g., reduction) in rates below ≈20°C (Fig. 2). Predicted rates fell to near zero at 10°C

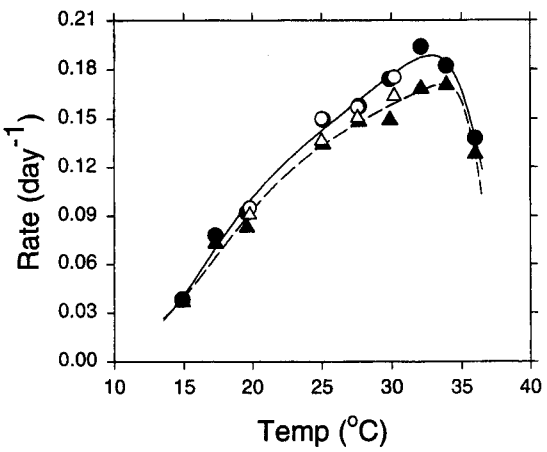

Fig. 2. Median rates to acquire hypertrophied fat bodies in prediapausing male (triangles) and female (dots) boll weevils feeding on squares (closed) and bolls (open) as adults at different constant temperatures, and the Sharpe and DeMichele (1977) model fitted to the male (dashed line) and female data (solid line).

(e.g., 0.0089 for males and 0.0063 for females). This temperature probably represents the threshold for feeding and is similar to other behavioral thresholds for the weevil. For example, thresholds of ≈2–10°C have been recorded for walking and 14–20°C for flying (Jones and Sterling 1979). Some movement has been observed at lower field temperatures [e.g., trap captures on days ranging between 2.0 and 3.5°C (Guerra et al. 1982)], suggesting the use of basking to warm body temperatures and allow movement at subthreshold air temperatures. Equation 2 describes the rate versus temperature relationship well for males and females ($R^2 = 0.99$). Parameter estimates for the models are given in Table 2.

Although temperature influenced the rate of prediapause development in the boll weevil, it apparently did not influence the progression of the induction process itself. For example, high temperatures did not halt or reverse the process of diapause induction in adults, which is important given the variability in temperatures in the southern United States during the induction period and the impending end to the growing season at crop termination or frost. Provided the insect can find food, adult prediapause development can occur very quickly during periods of warm autumn weather.

The variability in times to acquire hypertrophied fat bodies among individuals (Fig. 1) was modeled using the approach of Wagner et al. (1984a). The three-parameter Weibull function well described the cumulative distributions of normalized times (Fig. 3, $R^2 = 0.99$), with little difference in the form of these models with gender (Table 2). When used in simulations, accumulated rates derived from the rate equation serve as the independent variable in the distribution function, which apportions males or females completing the process of fat accumulation through time.

Simulations were initiated on 6 dates during the active period of diapause induction in north Mississippi (Fig. 4). The duration of the simulations was influenced by the prevailing temperatures of each period. For example, warmer temperatures generally decreased the age at which weevils acquired hypertrophied fat bodies and cooler temperatures increased the age. Most simulations produced smooth response curves, but short periods of cool weather, especially in October, intermittently protracted the simulations causing an irregular pattern in the cumulative curves. These patterns were most prevalent when temperatures fell below ≈15°C for several consecutive days.

Using average daily weather station temperatures over a 15-yr period to drive the model, 50% of prediapausing males emerging on 1 August completed fat body development in 7.4 d (Table 3). Females completed the process in 6.8 d. These times ranged from 7.0 to 8.1 d for males and 6.4 to 7.2 d for females, depending on the temperatures of a given year. During years with average to above-average temperatures, adults emerging on 16 August completed the process slightly faster than those emerging on 1 August; however, when cooler than average temperatures prevailed during the 2nd half of August, the process was slowed relative to the 1st period. The process of fat accumulation slowed somewhat in September as daily temperatures became more variable. For example, half the males emerging on 1 and 16 September completed prediapause development in 8.1 and 10.1 d, whereas females required 7.4 and 9.2 d. Temperature extremes among years resulted in a range in times of 2.9 and ≈4.6 d on 1 and 16 September, respectively. Temperatures became cooler and more variable in October, further slowing development and increasing the difference in times between the fast and slow simulations.

A striking result of the simulations is the potential speed at which prediapausing adults acquire the diapause phenotype, regardless of time of year. Fat accumulation in weevils emerging in September occurred very quickly in years with above-average temperatures, and the speed of these simulations was

Table 2. Parameter estimates for (A) the Sharpe and DeMichele model (equation 2) describing median development rates to acquire hypertrophied fat bodies versus constant temperature and (B) the Weibull function (equation 1) describing the standardized cumulative distributions of normalized times for male and female boll weevils undergoing prediapause

Sex	Rate equation						Distribution function		
	RHO25	HA	TL	HL	TH	HH	Eta	Beta	Gamma
M	0.14248	3,735.4	289.88	−55,627	309.79	246,133	0.49649	2.1108	0.58740
F	0.14616	6,515.1	288.87	−68,908	309.75	146,877	0.47205	1.9690	0.60926

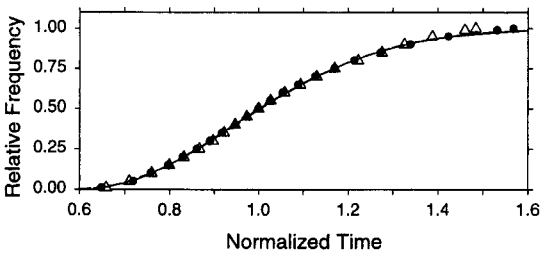

Fig. 3. Cumulative distributions of normalized times for prediapausing male (open triangles) and female (closed dots) boll weevils to acquire hypertrophied fat bodies, and the Weibull function fitted to the male (dashed line) and female data (solid line).

not unlike those of the previous month (Table 3). For example, half the males emerging on 16 September completed fat body development in 7.6 d when held under warm temperatures, compared with ≈7.4 d under average conditions of the previous month. Equivalent times for females were 7.1 and ≈6.8 d. Warm weather in October also allowed rapid development of the fat body, and by chance, the fastest simulations from 16 October outpaced those of 1 October. Late October simulations using above-average temperatures ran only ≈2.5 d slower (through the 3rd quartile of the population) than simulations generating the fastest times from 16 August.

Temperatures from the class A weather station were somewhat higher than those from beneath cotton leaves during most of the day in August and early September (maximum 2.7°C higher at 1815 hours; Fig. 5). Only between 0630 and 1200 hours were air temperatures slightly cooler than leaf temperatures (maximum 0.5°C lower at 0945 hours). To determine the effect of these differences on fat body development, weather station temperatures were multiplied by the proportions of leaf to air temperatures (Fig. 5), and simulations were rerun using the transformed temperatures. Altering weather station temperatures to those of cotton had little effect on the simulations, increasing average development times only 5.5, 2.8, and 5.6% under average, hottest (fast), and coolest (slow) conditions (Table 3).

Application to Diapause Control. The allocation of boll weevil diapause control sprays over the diapause-induction period is a matter of great importance to eradication officials in the mid-South. Sprays are particularly important during the 1st yr of the program, when there is a high expectation of eliminating most weevils before they overwinter. Coincidentally, there

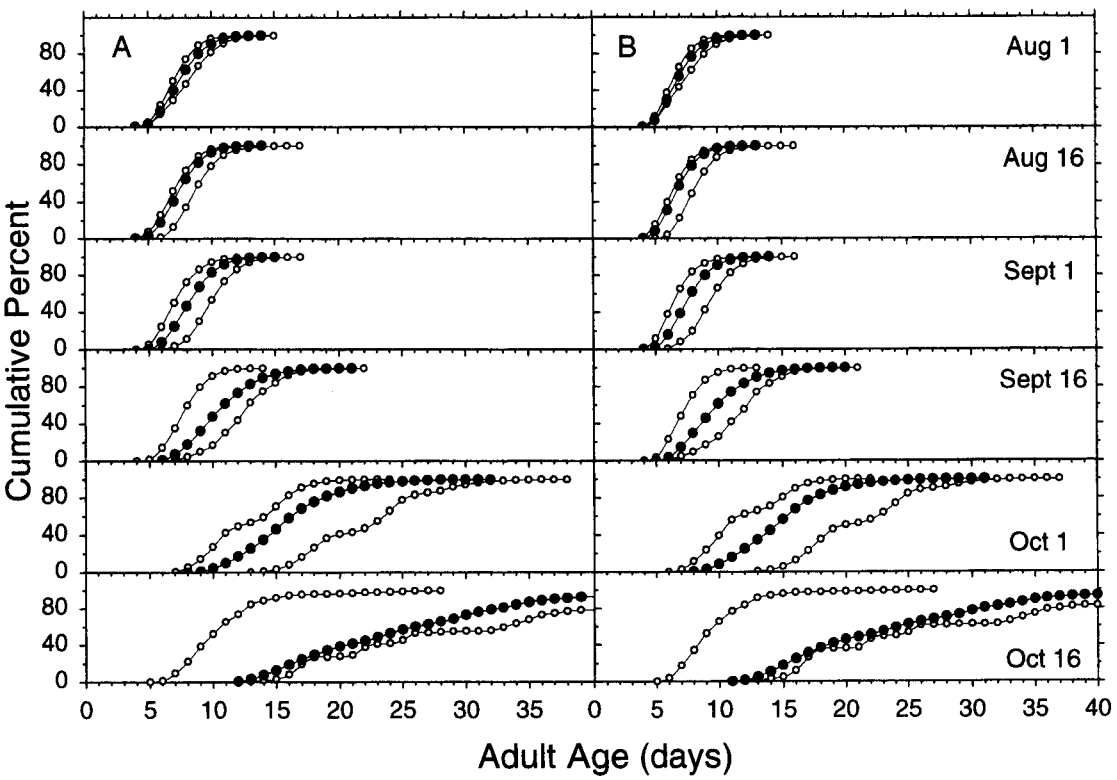

Fig. 4. Predicted cumulative percentage of prediapausing (A) males and (B) females acquiring hypertrophied fat bodies with age after emergence, generated from simulations using 15-yr average daily temperatures from a weather station (solid dots) and temperatures from years resulting in the fastest and slowest times (open dots) initiated on 6 dates during the diapause induction period in north Mississippi.

Table 3. Days from emergence to acquire hypertrophied fat bodies for 1, 25, 50, 75, and 99% of prediapausing male and female boll weevils, from simulations using 15-yr average daily temperatures from a weather station (Ave WS), average daily temperatures transformed to a cotton field (Ave COT), and temperatures from years (Yr) resulting in the fastest (Fast) and slowest (Slow) times, initiated on 6 dates during the diapause induction period

Type	Year	Males					Females				
		1%	25%	50%	75%	99%	1%	25%	50%	75%	99%
					1 Aug.						
Ave WS	—	4.7	6.3	7.4	8.6	12.0	4.4	5.9	6.8	7.9	11.2
Ave COT		5.0	6.7	7.8	9.1	12.6	4.7	6.1	7.1	8.3	11.8
Fast WS	87	4.5	6.0	7.0	8.0	11.1	4.2	5.5	6.4	7.4	10.2
Fast COT		4.7	6.2	7.2	8.3	11.4	4.4	5.8	6.6	7.7	10.6
Slow WS	90	4.8	6.7	8.1	9.4	13.0	4.4	6.0	7.2	8.7	12.2
Slow COT		5.0	7.1	8.5	10.0	13.6	4.7	6.3	7.9	9.1	12.8
					16 Aug.						
Ave WS	—	4.7	6.3	7.3	8.5	11.8	4.3	5.8	6.7	7.8	10.9
Ave COT		4.9	6.6	7.7	8.9	12.3	4.6	6.0	7.0	8.1	11.4
Fast WS	93	4.3	6.0	6.9	8.1	11.1	4.0	5.4	6.3	7.4	10.3
Fast COT		4.4	6.1	7.1	8.3	11.5	4.1	5.6	6.5	7.7	10.7
Slow WS	92	5.9	7.6	8.6	9.8	14.1	5.6	7.1	8.0	9.1	13.1
Slow COT		6.2	8.0	9.0	10.3	15.1	6.0	7.4	8.4	9.6	14.1
					1 Sept.						
Ave WS	—	5.2	7.0	8.1	9.4	13.1	4.9	6.4	7.4	8.7	12.2
Ave COT		5.4	7.3	8.6	10.0	13.9	5.1	6.8	7.9	9.1	12.9
Fast WS	90	4.5	6.0	7.0	8.1	11.6	4.3	5.5	6.4	7.4	10.8
Fast COT		4.6	6.1	7.1	8.3	12.0	4.3	5.6	6.5	7.6	11.1
Slow WS	88	6.3	8.7	9.9	11.1	14.5	6.0	8.2	9.3	10.5	13.9
Slow COT		6.9	9.2	10.3	11.8	15.3	6.4	8.7	9.8	11.0	14.5
					16 Sept.						
Ave WS	—	6.0	8.4	10.1	12.1	17.4	5.5	7.7	9.2	11.1	16.4
Fast WS	86	4.9	6.5	7.6	8.7	11.9	4.7	6.1	7.1	8.0	11.2
Slow WS	94	6.5	10.4	12.2	14.0	18.5	6.2	10.0	11.6	13.1	17.5
					1 Oct.						
Ave WS	—	9.0	13.0	15.2	17.9	26.9	8.3	12.0	14.2	16.8	25.6
Fast WS	83	7.1	9.8	12.1	15.2	19.7	6.6	9.1	10.7	14.3	18.9
Slow WS	87	14.0	17.9	22.1	24.7	33.2	13.1	17.1	19.9	24.1	32.2
					16 Oct.						
Ave WS	—	12.1	17.1	23.1	30.2	65.5	11.9	16.0	21.8	29.2	77+
Fast WS	85	5.9	8.1	9.9	12.1	24.7	5.5	7.5	8.9	10.6	22.2
Slow WS	95	13.1	17.4	25.5	37.1	58.6	12.1	16.9	24.0	35.1	58.3

is pressure to minimize the economic and environmental costs of the program, primarily by reducing the total number of sprays. In an efficient program, the number of sprays will be reduced annually, typically over 5 yr.

Achieving eradication success at the least cost places program officials in somewhat of a dilemma because the primary tool of eradication (insecticide) must be used conservatively. To achieve this objective, chemical applications must be properly timed, which requires optimal starting and stopping dates and appropriate spray intervals. Several factors influence efficacious spray schedules, including the seasonal incidence of diapause in weevil populations, the seasonal availability of squares and bolls used as food by prediapausing adults, the amount of time prediapausing adults remain in the field before leaving in search of overwintering sites (influenced by temperature on the acquisition of adult prediapause morphology and the availability of food needed to develop this morphology), efficacy rates of ultra-low-volume malathion against the weevil in cotton, and so on.

Seasonal availability of green squares and bolls (Reddy et al. 1985) required for adult prediapause development is inversely related to diapause-induction rates in north Mississippi (Wagner and Villavaso 1999). For example, when food is abundant in early August, few emerging weevils are diapause-induced; and when food is limited in late September, many emerging weevils are diapause-induced. Presumably, plentiful food early will enable most of the uncommon prediapausing adults to develop the diapause syndrome, whereas scarce food late may prevent most of the common prediapausing adults from developing the syndrome. Weevils unable to develop enlarged fat bodies probably are at a disadvantage to overwinter successfully in the northern parts of their range because of deficient metabolic reserves (Lambremont et al. 1964). Altering the acquisition and full expression of the diapause syndrome may affect the depth of the ensuing diapause and, thus, shorten its duration, or may even reverse the process of induction and intensification thereby preventing diapause altogether (Tauber et al. 1986). Notwithstanding, the multivoltine boll weevil often produces high population numbers late in the year, and some green squares and bolls will inevitably be available because of late harvest and regrowth. To prevent large numbers of weevils from

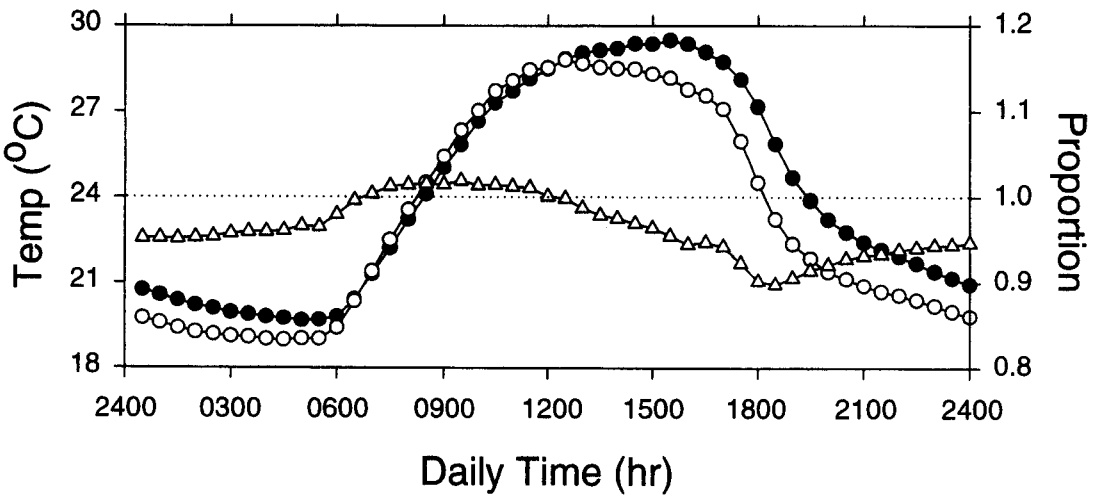

Fig. 5. Average daily temperatures from a class A weather station (solid dots) and beneath cotton leaves in a field (open dots) during a 45-d period from 2 August 1994, and the proportion of leaf to weather station temperatures (triangles).

achieving diapause late in the year, populations must be at low levels late or sprays must be continued in fields where food is available.

Assuming good kill rates of emerging weevils from ultra-low-volume malathion for 3 d (Villavaso et al. 1996, Mulrooney et al. 1997; E.J.V., unpublished data), sprays applied every 7 d from ≈1 August should prevent most oviposition and prediapause development after that date. In the absence of rain, this schedule will leave ≈4 d of unprotected cotton between sprays—hardly enough time for newly emerged weevils to develop mature follicles (Harris et al. 1966, Cole 1970, Sterling and Adkisson 1971) or hypertrophied fat bodies (Table 3) before reapplication. Some dispersing weevils locating cotton during unprotected days may oviposit, but few of these weevils will survive to the end of August. Extending the schedule from 7 to 10 d in mid-September should also provide good protection because decreasing temperatures in north Mississippi will prolong prediapause development (Table 3). Reproduction is not a concern by then because few weevils or oviposition sites will remain. Any offspring will not complete development before late October (Harris et al. 1966, Sterling and Adkisson 1971), too late to begin prediapause development. If all fields under eradication are sprayed as planned, and if they are resprayed if rain occurs within 2 d of the spray date (Villavaso et al. 1996), this schedule should virtually eliminate populations by the end of September and permit the termination of sprays in most fields at the time of defoliation.

In summary, a suggested generic plan for diapause control with application in northern parts of the mid-South consists of ≈10 sprays the 1st yr of the program—7 at 7-d intervals beginning the 1st wk of August, followed by ≈3 at 10-d intervals. During years with good weather and an early harvest, numerous fields probably would require fewer than 10 sprays, a few might require more. This spray schedule was adopted by the Technical Advisory Committee, Mississippi Boll Weevil Eradication Management Corporation during the 1st yr of the program in 1997. It was modified slightly to include an 4 August starting date with 3 sprays at 5-d intervals, 5 sprays at 7-d intervals, and the remaining sprays as needed at 10-d intervals (Fig. 6). The initial interval was shortened for reasons other than diapause control (e.g., to offer added weevil control as an incentive for farmer support, to help break the weevil's reproductive cycle, and to diminish the impending between-field dispersal that typically begins in early mid-August).

This generic recommendation was evaluated at the end of 1997 using the model to predict the spray intervals based on the actual temperatures recorded at Mississippi State. The simulation experiment was conducted using the assumptions described above. The goal was to adjust the spray interval to a period short enough to prevent 99% of all emerging prediapausing

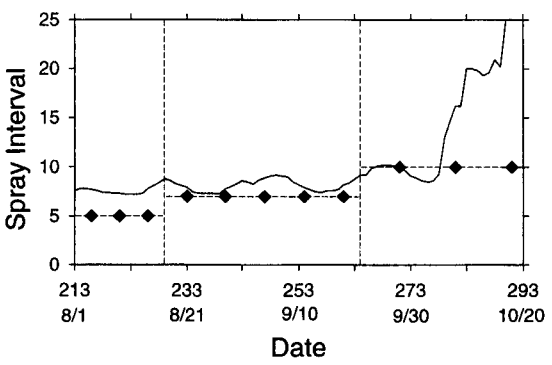

Fig. 6. Diapause control schedule (spray dates and intervals) recommended by the Mississippi Technical Advisory Committee before the program began (triangles) compared with predicted daily spray intervals based on 1997 temperatures recorded at Mississippi State, MS (solid line).

females that were not killed by the last application from developing an enlarged fat body. The interval was determined by adding 3 d to each date under consideration (to account for malathion kill) and then initiating the model to predict the time when 1% of the female population completed fat body development. The time to develop an enlarged fat body (slightly shorter in females than males) was assumed to be the minimal time required for feeding before a weevil would leave the field. The predicted time plus 3 d determined the appropriate spray interval.

The generic spray schedule provided a good guideline to kill prediapausing weevils except in October when intervals could have been lengthened (Fig. 5). By this time, however, few if any fields were still being treated. As noted, the 5-d spray interval in early August was shorter than necessary, but it was proposed for reasons other than diapause control. Because temperatures and rainfall varies annually, other plans may be equally or more effective within a given location. Ideally, the spray intervals should be determined as the season unfolds using the present conditions to drive the model.

Acknowledgment

This research was sponsored in part through a grant from Cotton Incorporated, Agreement No. 96-298MS.

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Received for publication 8 June 1998; accepted 5 February 1999.