Implications of Larval Mortality at Low Temperatures and High Soil Moistures for Establishment of Pink Bollworm (Lepidoptera: Gelechiidae) in Southeastern United States Cotton

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ABSTRACT The pink bollworm, *Pectinophora gossypiella* (Saunders), remains a significant pest of cotton (*Gossypium* spp.) in the southwestern United States, but is not known to be established in the primary cotton production areas of the southeastern United States. Absence of *P. gossypiella* may be the result of federal regulatory action (e.g., monitoring, quarantine, and eradication), climate, or other ecological factors. The objectives of this study were to determine how low temperatures and high soil moisture common to the southeastern United States might affect mortality of diapausing, preconditioned, and nondiapausing larvae of *P. gossypiella.* In constant temperature incubators set between 22 and 5°C (0% moisture, 0:24 [L:D] h), nondiapausing prepupal (fourth or fifth instar) larvae died more quickly at lower temperatures. At 5°C, 90% of the cohort was dead after 12 d. Similarly, prepupal larvae that had been reared under diapause inducing conditions (20°C, 10:14 [L:D] h) since neonate stage also died more quickly at lower temperatures. A separate developmental assay indicated that the larvae were not in diapause. In this case, 26 d at 5°C were required to achieve 90% mortality. For diapausing, prepupal larvae collected from the field, mortality was greater at 5°C than at any other temperature tested, but larvae could withstand 5°C for 60 d before 90% of the cohort died. In response to moisture, as soils at 10°C became saturated (>195% gravimetric soil moisture), most diapausing larvae (≈60%) died within the first 10 d of the experiment. These studies suggest that diapausing, late instar larvae of *P. gossypiella* are more resilient to the effects of low temperature than nondiapausing individuals and are able to tolerate high soil moisture for moderate lengths of time. Temperatures and soil moistures in the southeastern United States are not sufficiently cold or wet to completely preclude establishment of *P. gossypiella.*

KEY WORDS *Pectinophora gossypiella*, diapause, biological invasions, demography, risk

THE PINK BOLLWORM, *Pectinophora gossypiella* (Saunders), is a significant pest of cotton (*Gossypium* spp.) worldwide. Where *P. gossypiella* is established, larvae may reduce lint yields by as much as 60% (Fry et al. 1978). However, damage in commercial fields is typically limited to ≤10% because of management activities (Hutchison 1999). To limit the spread of *P. gossypiella* in the United States, federal quarantine prohibits the transport of cotton plants (and other hosts), cottonseed, cotton refuse, and harvesting equipment from infested states (Arizona, New Mexico, Oklahoma, Texas, and southern California) to other cotton producing regions of the country (USDA 1997).

In the southwestern United States, *P. gossypiella* may complete four to six generations per year (Noble 1969). Adult females will deposit eggs on any part of a cotton plant, but bolls (>15 d old) are preferred. Larvae then complete four or five instars. Larvae hatching on squares or bolls quickly enter those plant parts and remain until development is complete. Fully developed fourth- or fifth-instar larvae drop to the ground to pupate. Diapause is not obligatory but is triggered primarily by shortened photoperiod. Most overwintering larvae in diapause will remain in cotton seeds, but some individuals will drop to the soil. In soil cracks or litter, nondiapausing larvae will form a cocoon and pupate, whereas diapausing larvae will form a cocoon and remain quiescent until warm temperatures and adequate soil moisture stimulate pupation the following spring.

Regulatory action may have prevented the establishment of *P. gossypiella* in midsouthern and southeastern United States cotton. From 1917 through the late 1960s, the presence of *P. gossypiella* in historically uninfested states such as Louisiana, Missouri, Georgia, and Florida triggered a series of eradication programs by the U.S. Department of Agriculture–Animal and Plant Health Inspection Service (USDA-APHIS; Noble 1969). Monitoring, quarantine, and eradication efforts were required as recently as 1990-1999 with the detection of the pest in southeastern Missouri, north-

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eastern Arkansas, and western Tennessee (NAPIS 1999). However, regulatory action may be unnecessary if climate in the eastern half of the Cotton Belt is not adequate to sustain reproductive populations of *P. gossypiella.* For example, the outbreak of the pest in Georgia from 1932 to 1936 ended after an eradication effort (Noble 1969). Yet, historical records indicate that 1932-1936 was warmer than average in Georgia (ClimProb 3.1 climatological software, Meyer et al. 1996). Had no eradication been attempted, would populations have persisted through cooler years?

Like many other invertebrates, population dynamics of *P. gossypiella* are strongly influenced by environmental conditions, particularly temperature and moisture (Tauber et al. 1998). Estimates of the temperature at which *P. gossypiella* can develop and reproduce vary from 6.9°C (Venette and Hutchison 1999) to 15.6°C (Sevacherian et al. 1977). High temperatures (>35°C) reduce survivorship of larvae (Pinter and Jackson 1976), longevity of adults (Graham et al. 1967), viability of eggs (Fry and Surber 1971, Henneberry et al. 1977), and overall population growth rate (Philipp and Watson 1971). Both dry soils (less than ≈10% moisture) and wet soils (more than ≈20% moisture) lower survivorship of larvae and pupae (Chapman and Cavitt 1934, Clayton and Henneberry 1982).

A recent risk assessment of the potential establishment of *P. gossypiella* in the southeastern United States suggests that climate is a critical determinant of the geographic range of this pest (Venette and Hutchison 1999). Cold winters that increase overall mortality or cool springs that provide inadequate degree-days for the pest to complete physiological development in the northern United States are likely to confine populations to more southern climes. Excessive moisture may also increase population mortality in the eastern half of the United States. However, most of the Cotton Belt is likely to provide suitable habitat for local reproduction and population maintenance of *P. gossypiella* (Venette and Hutchison 1999).

The risk assessment by Venette and Hutchison (1999) emphasized the importance of measuring survival of *P. gossypiella* under temperature and moisture conditions common to southeastern United States cotton. Published reports do not indicate a consistent impact of temperature or moisture on the demography of *P. gossypiella,* prompting Venette and Hutchison (1999) to conclude that this basic information remains a significant knowledge gap. The objective of the current study was to investigate the effects of low temperatures and wet soils on the survivorship of late instar larvae. Based on our previous risk assessment, we hypothesized that larval mortality should be greater at temperatures below 6.5°C than at 20°C; mortality should also be greater at moistures >65% of soil moisture holding capacity than at 0%. We then used this information to refine estimates of where establishment of *P. gossypiella* might be precluded in the southeastern United States.

Materials and Methods

Effect of Temperature on Nondiapausing Larvae. *Pectinophora gossypiella* larvae were obtained from a colony maintained at the USDA-ARS Laboratory in Phoenix, AZ. This colony has been under continuous culture on artificial diet since 1972 without any introductions of feral stock (Bartlett and Wolf 1985; T. J. Henneberry, personal communication). In preparation for this experiment, rearing containers with developing larvae were held at ≈25°C and a photoperiod of 14:10 (L:D) h until prepupal larvae (fourth or fifth instar) emerged (i.e., "cut out"). Fifty larvae were placed in a waxed paper cup (6 by 9.5 cm diameter) filled about half full with small, dry Styrofoam beads (≈3 mm diameter). The Styrofoam provided a medium in which the larvae could readily burrow and spin cocoons in preparation for pupation. A total of 16 cups was prepared for each of three upright incubators at 5, 10, and 22 ± 0.5°C and a photoperiod of 0:24 (L:D) h, respectively. After 1 d and at weekly intervals thereafter, two arbitrarily selected cups (i.e., replicates) were removed from each incubator and destructively sampled for live and dead larvae, pupae and adults. The entire experiment consisted of 2,400 larvae in 48 cups.

Effect of Temperature on Preconditioned Larvae. In preparation for this experiment, we exposed neonate larvae in rearing containers to diapause inducing conditions (20°C at 10:14 [L:D] h) as per Gutierrez et al. (1981). Prepupal larvae were collected as they emerged from rearing containers. Diapause was evaluated by placing 200 larvae at 27°C (16:8 [L:D] h) and monitoring for pupation (Wellso and Adkisson 1964). Because all larvae pupated within 60 d (indicating that developmental diapause was not induced), we considered the larvae to be preconditioned. Within 24 h after emerging from rearing containers, prepupal, preconditioned larvae were placed in Styrofoam-filled containers (as above). A total of 32 cups was placed in each of four incubators at 5, 10, 15, and 20 ± 0.5°C (0:24 [L:D] h), respectively. After 1 d of exposure and at weekly intervals thereafter, four arbitrarily selected cups were removed from each incubator and destructively sampled to examine insect status. The entire experiment consisted of 6,400 larvae in 128 cups.

Effect of Temperature on Diapausing Larvae. Diapausing *P. gossypiella* larvae (fourth or fifth instar) were obtained from cotton bolls harvested from Blythe, CA, in late September 1998. Bolls were held in an outdoor, screened insectary subject to ambient temperatures and photoperiod. After emerging from bolls (October–November 1998), prepupal larvae were placed in plastic petri dishes and held in an incubator at 20°C (10:14 [L:D] h). Diapause was verified by exposing 200 putatively diapausing larvae under long day conditions (16:8 [L:D] h) at 27°C. Approximately 5% pupation occurred by 60 d under these conditions.

When an adequate number of prepupal larvae were obtained, 50 individuals were placed in a waxed paper cup filled about half full with a potting soil mix. The

soil mix was a blend of commercial potting soil (Perma-Gro, Tempe, AZ), sand, peat moss, and perlite with an overall composition of ≈32% organic matter. The soil mix was sterilized by autoclave and dried to 0% gravimetric moisture before use. Larvae were positioned on the soil surface and allowed to burrow for 24 h at room temperature (≈25°C). A total of 18 cups was then placed in each of three incubators at 5, 10, 15, and 20 ± 0.5°C (0:24 [L:D] h), respectively. To allow more direct comparisons to experiments with nondiapausing and preconditioned larvae, an additional treatment consisting of dry styrofoam beads rather than soil was prepared and placed at 10°C. Approximately every 10 d, three arbitrarily selected cups were removed from each treatment and destructively sampled. The entire experiment consisted of 4,500 larvae in 90 cups.

Effect of Soil Moisture on Diapausing Larvae. For this experiment, cotton bolls from the Blythe, CA, collection (see above) were cut open, and diapausing, prepupal larvae were removed. Individuals were placed in plastic petri dishes and used immediately without any storage in an incubator. As before, 50 larvae were placed in waxed paper cups filled half full with the sterilized soil mix. Larvae were positioned on the soil surface and allowed to burrow for 24 h at room temperature (≈25°C) before adding deionized water to achieve soil moisture levels of 0, 50, 100, 150, and 200% moisture (by weight). Previous soil analysis (Soil Characterization Laboratory, University of Minnesota, St Paul, MN) indicated that the soil mix became saturated at ≈195% moisture by weight. A total of 18 cups was prepared for each soil moisture level, and all cups were held at 10°C (0:24 [L:D] h). Three arbitrarily selected cups per moisture level were destructively sampled about every 10 d. At the time of sampling, remaining cups were weighed and adjusted for any moisture loss caused by evaporation. The entire experiment consisted of 4,500 larvae in 90 cups.

Data Analysis. Mortality data were analyzed in two ways. We first used probit analysis (PROC PROBIT; SAS Institute 1995) to estimate the amount of time (±95% fiducial limits) necessary to achieve 90% mortality (i.e., LT_{90}) in a specific treatment. Because the source and pretreatment of larvae differed for each experiment, each experiment was analyzed separately, and only treatment comparisons within an experiment are appropriate.

We then employed logistic regression (PROC LOGISTIC; SAS Insitute 1995) to determine the rate of larval mortality under a specific set of environmental conditions. Logistic regression provides the likelihood of an event (i.e., the probable mortality of an individual) given explanatory information (Hosmer and Lemeshow 1989, Venette and Hutchison 1999). For our analysis, the primary explanatory (i.e., independent) variable was days since exposure to a given set of environmental conditions. The dependent variable, proportionate mortality (p_t) at time t, was appropriately transformed, and the resulting linear model followed the form:

$$\ln\left(\frac{p_t}{1-p_t}\right) = mt + b, \qquad [1]$$

where m and b are coefficients describing the slope (i.e., mortality rate) and intercept (i.e., initial mortality) of the line, and t is time in days. Coefficients are determined through maximum likelihood estimation. When back-transformed, the equation follows:

$$\hat{p}_t = \frac{1}{1 + e^{-(mt+b)}},$$

where $\hat{p}_t$ is the estimated proportionate mortality at time t. This line applies to one experimental treatment only.

To compare between treatments within an experiment, we added a series of binary variables (i.e., equal 0 or 1) that code for each treatment (Neter et al. 1990). In general, for an experiment with c treatments, c-1 binary variables were needed. For example, the experiment with nondiapausing larvae had three treatments: 22, 10, and 5°C. We set the new variables X_1 = 1 when then temperature was 5°C or = 0 when the temperature was 22 or 10°C, and X_2 = 1 when the temperature was 10°C or 0 when the temperature was 5 or 22°C. By multiplying each new variable by time and including the four variables in our logistic regression, we could estimate parameters for the different experimental conditions simultaneously. Specifically,

$$\ln\left(\frac{p_t}{1-p_t}\right) = b_0 + b_1X_1 + b_2X_2 + m_0t + m_1(X_1t) + m_2(X_2t). \qquad [2]$$

The coefficients b_0, b_1, and b_2 are regression parameters that are independent of time (i.e., intercepts), and m_0, m_1, and m_2 are dependent on time (i.e., slopes). Because X_1 and X_2 only assume values of 0 or 1, equation 2 simplifies to the following set of three equations

$$\ln\left(\frac{p_t}{1-p_t}\right) = \begin{cases} m_0t + b_0, \text{ when} \\ X_1 = 0, X_2 = 0, (\text{temp} = 22°\text{C}). \\ \\ (m_0 + m_2)t + (b_0 + b_2), \text{ when} \\ X_1 = 0, X_2 = 1, (\text{temp} = 10°\text{C}) \\ \\ (m_0 + m_1)t + (b_0 + b_1), \text{ when} \\ X_1 = 1, X_2 = 0, (\text{temp} = 5°\text{C}). \end{cases} \qquad [3]$$

If parameter m_2 were significantly differently from zero (as determined by Wald's chi-square), larvae at 10°C would die at a different rate than larvae at 22°C. Likewise, if m_1 were significantly different from zero, larvae at 5°C would die at a different rate than larvae at 22°C. If b_2 or b_1 were different from zero, the intercept (i.e., the initial degree of mortality) at 10 or 5°C would be different from the intercept at 22°C. This particular analysis did not allow for statistical comparison of 5 and 10°C. Consequently, data were recoded and the logistic regression rerun until all possible treatment comparisons were complete. Each experiment was analyzed separately. Because experiments were conducted at different times with larvae collected from different rearing conditions, statistical

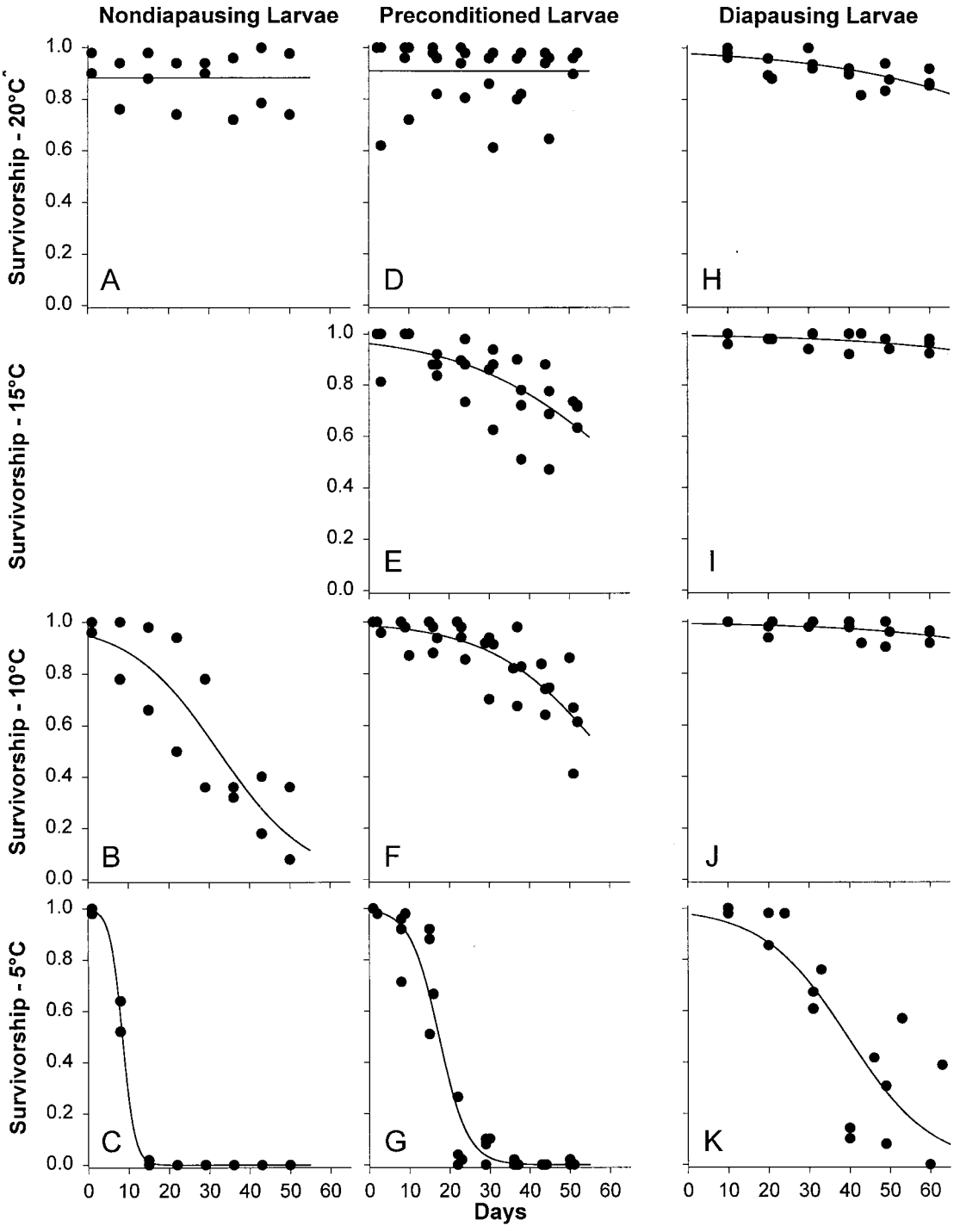

Fig. 1. Average proportional survivorship of *Pectinophora gossypiella* as affected by time and temperature. Circles are observed values, and lines are expected values from logistic regression. *, Experiment with nondiapausing larvae at 20°C; experiments with preconditioned and diapausing larvae at 22°C.

comparisons between experiments are not appropriate.

We then used ClimProb 3.1 (Meyer et al. 1996) to determine the median number of days per year with a maximum air temperature <5°C in southeastern cotton-producing states. Analyses were conducted for a total of 188 locations in Missouri, Arkansas, Tennessee, Louisiana, Mississippi, Alabama, Georgia, Florida,

Table 1. Parameter estimates (±SEM) for logistic regression models relating proportionate mortality of nondiapausing, preconditioned, or diapausing *P. gossypiella* larvae to time at different, constant temperatures

Temp, °C	Intercept (b)	Slope (m)
	Nondiapausing larvae	
5	−5.61 ± 0.85a	0.66 ± 0.10a
10	−2.92 ± 0.21b	0.09 ± 0.01b
22	−2.28 ± 0.22c	0.01 ± 0.01c
	Preconditioned larvae	
5	−4.87 ± 0.29a	0.28 ± 0.02a
10	−4.20 ± 0.25a	0.07 ± 0.01b
15	−3.25 ± 0.19b	0.05 ± 0.005c
20	−2.38 ± 0.17c	0.002 ± 0.01d
	Diapausing larvae	
5	−4.05 ± 0.27ab	0.10 ± 0.01a
10	−5.27 ± 0.66a	0.04 ± 0.01b
15	−4.48 ± 0.57a	0.02 ± 0.01b
20	−3.36 ± 0.33b	0.03 ± 0.01b

The model follows the form: $\ln(p_t/[1 - p_t]) = b + (m)$ days, where p_t is the proportion of individuals dying by day, t. For a given experiment, parameters within a column followed by the same letter are not significantly different ($P > 0.05$). For experiments with nondiapausing larvae, prepupal larvae were collected from a laboratory colony maintained at 25°C and a photoperiod of 14:10 (L:D) h; for preconditioned larvae, from a colony exposed to 20°C and a photoperiod of 10:14 (L:D) h; and for diapausing larvae, from cotton bolls gathered from Blythe, CA, in late September 1998.

South Carolina, North Carolina, and Virginia. Geographically referenced results were exported to the geographic information system ArcView 3.1 (Environmental Systems Research Institute, Redlands, CA) to generate isopleths between points.

Results

Effect of Temperature on Nondiapausing Larvae. Mortality of nondiapausing larvae at 22°C was independent of time over the course of the experiment (Fig. 1A); average mortality was 12 ± 1% (mean ± SEM). All survivors completed pupation and emerged as adults before the experiment ended at 49 d. At 10°C, initial mortality was less, but the rate of mortality was greater, than at 22°C (Table 1). An average of 22 ± 20% of the larvae at 10°C remained alive at the end of the experiment. None of the survivors pupated, and 69 ± 28% had not formed a cocoon. At 5°C, initial mortality was less, but the rate of mortality was greater, than at either 10 or 22°C (Table 1). At 5°C, no larvae were alive nor observed within cocoons at the end of the experiment.

Survivorship of *P. gossypiella* declined as temperatures were reduced (Fig. 1 A–C). Correspondingly, the amount of time necessary to achieve 90% mortality was lower at cooler temperatures (Fig. 2). At 5°C, 90% mortality was achieved after 12 d exposure.

Effect of Temperature on Preconditioned Larvae. For larvae that had been reared at 20°C under short-day conditions, mortality at 20°C was independent of time over the course of the experiment (Fig. 1D); average mortality was 9 ± 2%. Of the survivors, 99 ± 1% emerged as adults by the end of the experiment at 50 d. At 15°C, initial mortality was less, and mortality rate was greater than at 20°C (Table 1). An average of 70 ± 2% of the initial cohort at 15°C was alive at the end of the experiment (Fig. 1E). Of the survivors, 91 ± 2% were pupae; no adults were present.

At 10°C, initial mortality was less, but mortality rate was greater, than at 15 or 20°C (Table 1). At the end of the experiment, 64 ± 9% of the initial cohort at 10°C were alive (Fig. 1F). Of the survivors, none pupated, and 70 ± 4% had not formed a cocoon. At 5°C, initial mortality was not different from 10°C; the rate of mortality was greater than at 20, 15, or 10°C (Fig. 1G; Table 1). At the end of the experiment, one larva at 5°C was found alive without a cocoon. LT_{90} was substantially less at 5°C than at any other temperature (Fig. 2).

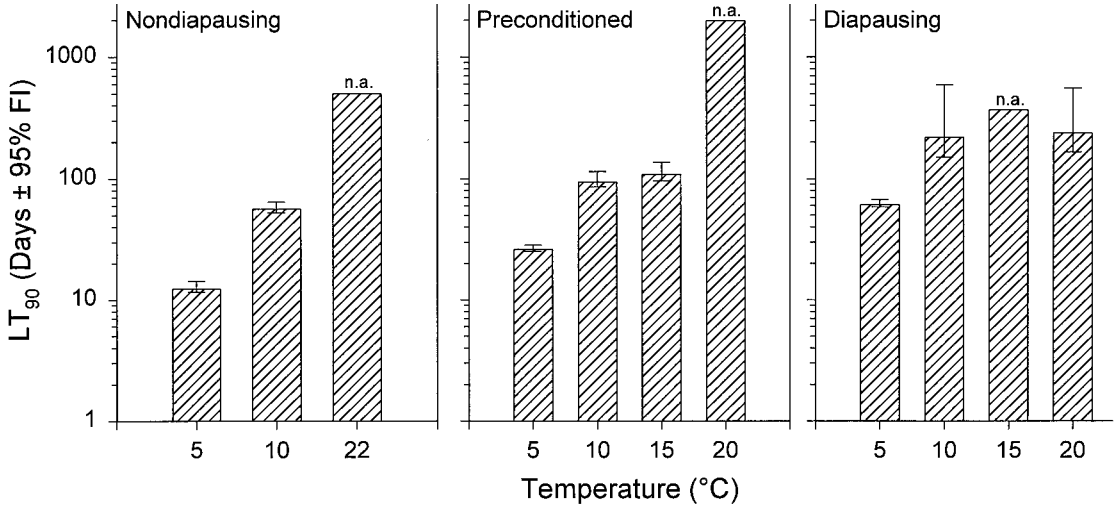

Fig. 2. Time required to achieve 90% mortality (i.e., LT_{90} ± 95% fiducial interval) of *P. gossypiella* larvae as affected by temperature. Within an experiment, treatments with overlapping fiducial intervals are not significantly different. n.a., 95% fiducial interval is not available because 90% mortality occurs well beyond observed data.

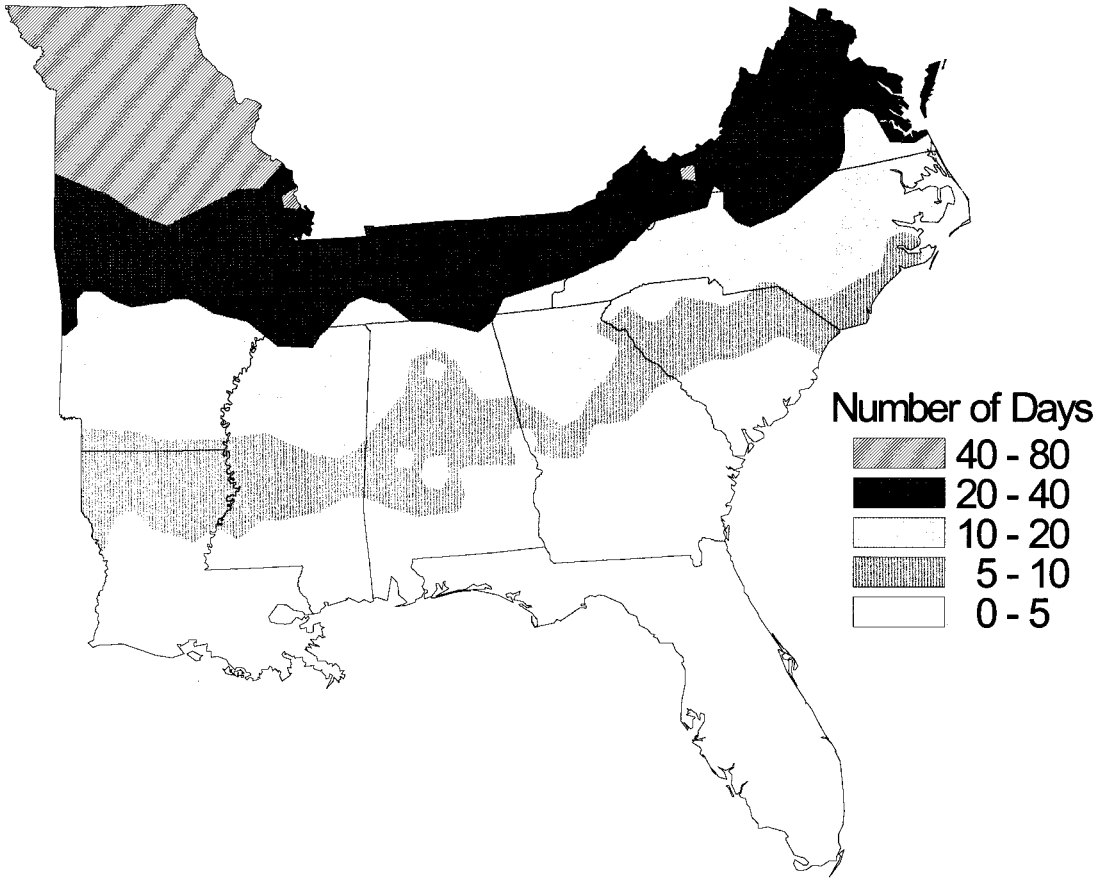

Fig. 3. Median number of days with a maximum air temperature <5°C in the midsouthern and southeastern portions of the United States' Cotton Belt.

Approximately 26 d at 5°C were required for 90% mortality. The LT_{90} for 10 and 15°C were less that the LT_{90} for 20°C but were not different from one another (Fig. 2).

Effect of Temperature on Diapausing Larvae. Mortality of diapausing, field-collected larvae at 20°C increased with time (Table 1). At the end of the experiment, 94 ± 4% of the initial cohort was alive (Fig. 1H). All survivors were larvae. Initial mortality at 10 and 15°C were less than at 20°C but were not different from each other. Rates of mortality at 10, 15, and 20°C were not different from one another (Table 1). Of the initial cohort at 15°C, 95 ± 2% were still alive at the end of the experiment (Fig. 1I). None of the survivors had pupated, but 77 ± 5% of the larvae had formed a cocoon.

At 10°C, 95 ± 1% of the initial cohort were still alive at the end of the experiment (Fig. 1J). All survivors were larvae, and 67 ± 12% of these survivors were found within a cocoon. At 5°C, 13 ± 13% of the larvae were still alive at the end of the experiment (Fig. 1K). Survivors only occurred in one replicate. Approximately one-half of these survivors had formed a cocoon, but none had pupated. For diapausing larvae, only the LT_{90} for 5°C was different from any other treatment (Fig. 2). Larvae could withstand 5°C for ≈60 d before 90% mortality occurred.

Substrate did not affect mortality rate. Mortality rate in dry soil at 10°C was not different from mortality rate in dry styrofoam at the same temperature (did not satisfy $\alpha = 0.05$ criterion for entry into regression model). However, initial mortality was less in soil than in styrofoam (1.1% versus 2.0%; Wald $\chi^2 = 4.745$; $P = 0.03$).

Median number of days per year with a maximum air temperature <5°C was greatest in northern Missouri and declined with distance to the south (Fig. 3). We selected 60 consecutive days at 5°C, the LT_{90} for diapausing larvae, as a critical time interval. In all states examined except Missouri, this critical period was never exceeded. In the gulf states (e.g., Louisiana, Mississippi, Alabama, and Florida), the number of days <5°C (not necessarily consecutive) ranged from 0 to 21. In northern Missouri, 60-76 d may have a maximum air temperature <5°C; however, in the past 80 yr, a cold spell with maximum temperatures <5°C has never lasted ≥60 consecutive days. Cold spells lasting ≥40 d occur once every 11–20 yr.

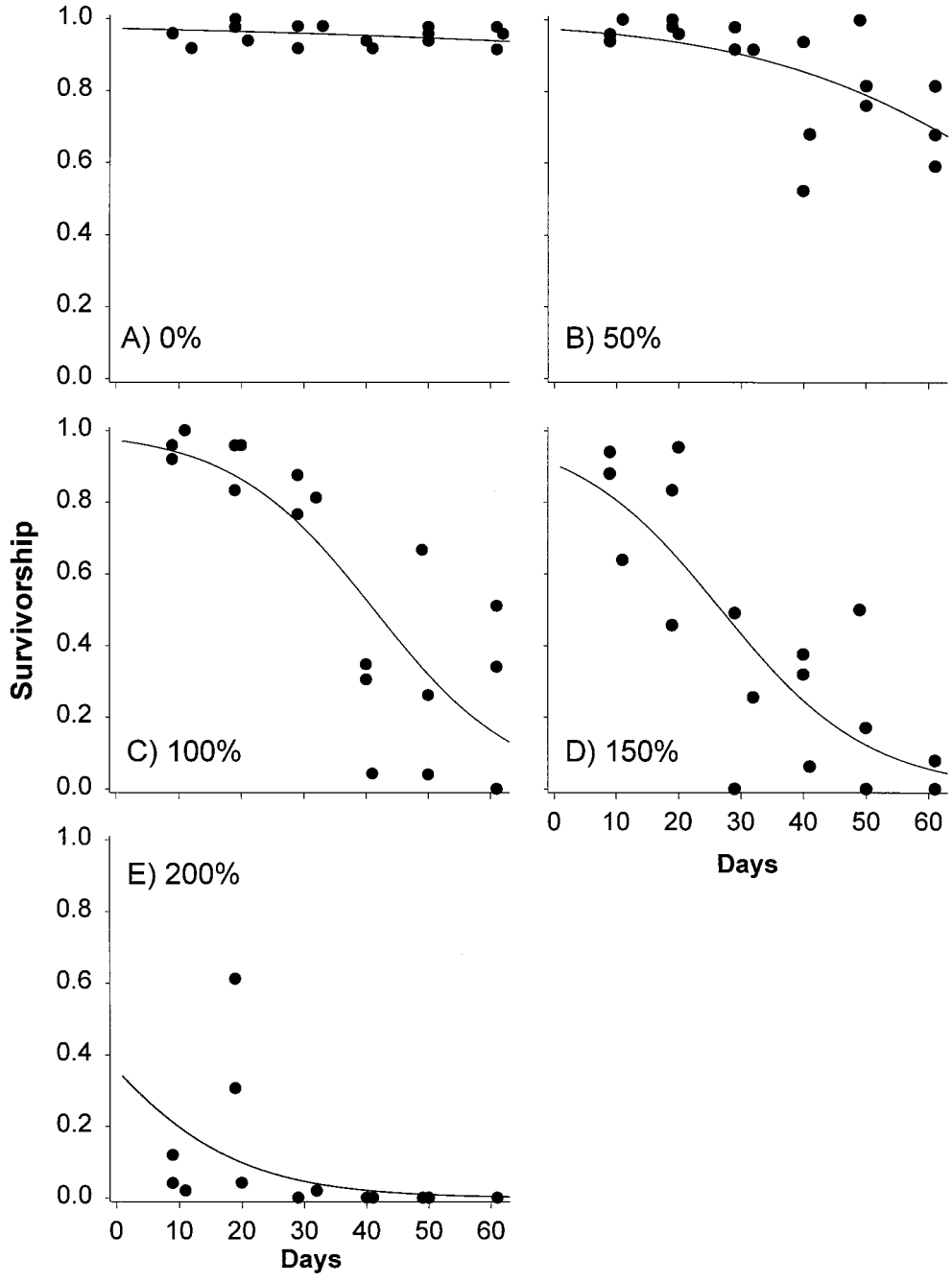

Fig. 4. Average proportional survivorship of diapausing *P. gossypiella* larvae as affected by time and gravimetric soil moisture (wt water: wt soil × 100). Circles are observed values, and lines are expected values from logistic regression.

Effect of Soil Moisture on Diapausing Larvae. Mortality of field-collected diapausing larvae was greater as soil moisture increased. At the end of the 60-d experiment, 95 ± 2% of the larvae were still alive at 0% moisture (Fig. 4A). At 50% moisture, initial mortality was not different, but mortality rate was greater, than at 0% moisture (Table 2). At 50% moisture, 70 ± 9% of the larvae were alive at the end of the experiment (Fig. 4B), 77 ± 3% of which had formed a cocoon.

At 100% moisture, initial mortality was not different, but rate of mortality was greater, than at 0 or 50% moisture (Table 2). Although only 28 ± 7% of the larvae were alive at the end of the experiment (Fig. 4C), 64 ± 20% of the initial cohort had formed a

Table 2. Parameter estimates (±SEM) for logistic regression models relating proportionate mortality of *P. gossypiella* larvae to time at different, constant soil moistures

% moisture	Intercept (b)	Slope (m)
0	−3.11 ± 0.36a	0.003 ± 0.01a
50	−3.91 ± 0.32a	0.05 ± 0.01b
100	−3.59 ± 0.25a	0.09 ± 0.01c
150	−2.27 ± 0.20b	0.08 ± 0.01c
200	0.58 ± 0.27c	0.08 ± 0.01c

The model follows the form: $\ln(p_t/[1 - p_t]) = b + (m)$ days, where p_t is the proportion of individuals dying by day, t. Parameters within a column followed by the same letter are not significantly different ($P > 0.05$). Experiment was conducted with diapausing larvae from cotton bolls gathered from Blythe, CA, in late September 1998.

cocoon. No pupae nor adults were present. When soil moisture was raised to 150 or 200%, mortality rate did not differ from the rate of mortality at 100% moisture (Table 2). At 150% moisture, only 3 ± 3% of the larvae were alive after 60 d (Fig. 4D), and at 200% moisture, no larvae were alive at the end of the experiment (Fig. 4E). The predicted amount of time required for 90% mortality declined as soil moisture increased (Fig. 5).

Discussion

Invasion of southeastern United States cotton by *P. gossypiella* follows four stages: arrival, establishment, integration, and spread (Venette and Carey 1998). Although dispersal of adult moths into the Southeast is possible, the arrival of larvae is more likely. Larvae are likely to be transported to noninfested areas in lint, trash, or harvesting equipment. Arriving larvae are likely to be in various stages of diapause, depending on when infested fields were harvested. If fields were harvested late (i.e., October–November), >50% of the larvae that are present are likely to be in diapause (Miller et al. 1993).

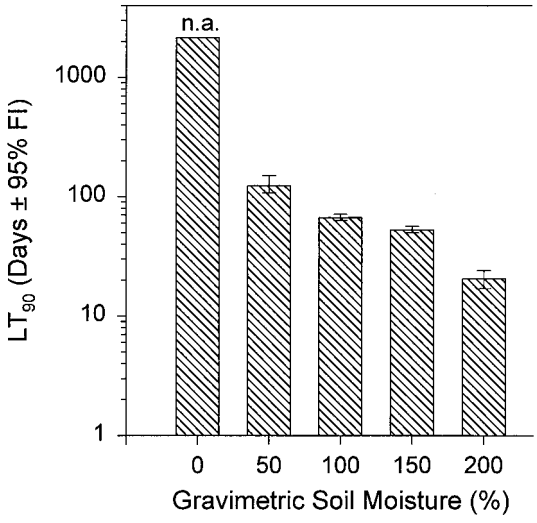

Fig. 5. Time required to achieve 90% mortality (i.e., LT_{90} ± 95% fiducial interval) of diapausing *P. gossypiella* larvae as affected by gravimetric soil moisture (wt water: wt soil × 100) at 10°C. Experiment was initiated with diapausing larvae. Within an experiment, treatments with overlapping fiducial intervals are not significantly different. n.a., 95% fiducial interval is not available because 90% mortality occurs well beyond observed data.

Once present in the southeastern United States, larvae face the challenge of finding a suitable host and climate to complete development and begin reproducing. If hosts are unavailable or climate is inappropriate, larvae must persist until the environment becomes suitable. Our studies explicitly compare the likelihood that nondiapausing, preconditioned, or diapausing larvae will withstand adverse conditions in the Southeast until establishment is possible. Alternatively, if larvae are introduced into an environment where development and reproduction are successful, resulting offspring, probably in diapause, must overwinter. This study also addresses the probability of larval survivorship through southeastern United States winters which is necessary for establishment of the pest.

Our results suggest that the introduction of diapausing larvae into the southeastern United States would increase the risk of *P. gossypiella* becoming established more than the introduction of nondiapausing larvae. In southeastern United States cotton, cold spells are neither sufficiently severe nor long to ensure >90% mortality of diapausing larvae (Fig. 3). Consequently, newly arriving larvae or their progeny are not likely to be eliminated by low temperatures if insects are in diapause.

These experiments clarify the findings of the risk assessment for *P. gossypiella* establishment in southeastern United States cotton (Venette and Hutchison 1999). In the risk assessment, different methods led to markedly different estimates of lethal temperatures and moisture. We hypothesized that *P. gossypiella* could withstand temperatures down to 6.5 or 0.8°C (Venette and Hutchison 1999). In the current study, the times required for >90% mortality at the various temperatures (Fig. 2) indicate that a 0.8°C threshold is too low. Also, applying parameters reported in Venette and Hutchison (1999) to soil properties for the current study, we predicted that *P. gossypiella* could survive at gravimetric soil moistures up to 125 or 234%. Because a significant fraction of the population can survive long periods (>40 d) at a gravimetric water content of 150% (Fig. 4), the 125% threshold seems too low. These results are consistent with qualitative observations by Slosser and Watson (1972) and Watson (1980) who suggested that winter irrigation (i.e., saturating soils) might reduce overwintering populations of *P. gossypiella.* Our studies suggest that late instar larvae of *P. gossypiella* are moderately resilient to the effects of low temperature and moisture.

The midsouthern and southeastern region of United States is cooler and more rainy than the southwestern United States where *P. gossypiella* is a perennial pest (Venette and Hutchison 1999). However, these obvious climatic differences are not so great as to eliminate the possibility of permanent establishment of *P. gos-*

sypiella in the eastern half of the United States' Cotton Belt. Only northern Missouri has maximum air temperatures <5°C for enough time to significantly affect numbers of diapausing larvae.

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