

Effects of Temperature, Relative Humidity, and Host Plant on Larval Survival of the Mexican Bean Beetle, *Epilachna varivestis* Mulsant^{1,2}

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ABSTRACT

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A comparison of mortality, developmental time, and pupal weight was made for Mexican bean beetle larvae reared on three stages of soybeans and reproductive stage lima beans in 12 constant temperature and relative humidity (RH) conditions. At high humidities and favorable temperatures (17, 22, and 27° C) there were no significant differences in survival to the pupal stage on the various host plants. On lima bean foliage, larval survival was not significantly influenced by moisture stress. However, survival decreased on all soybean hosts as temperature increased, or as RH decreased, and was inversely proportional to vapor pressure deficit. These stresses are also reflected in larger development times and lower pupal weights. Survival at 32° C in all cases was low. Female larvae appear to be more capable of enduring conditions of moisture stress than males. Stress due to adaptation to change in RH was also observed.

The sensitivity of all stages of the Mexican bean beetle, *Epilachna varivestis* Mulsant, to moderately high temperatures and low humidities has been well documented (Eddy and McAlister 1927, Sweetman and Fernald 1930, Marcovitch and Stanley 1930, Sweetman 1932). However, these studies were concerned with *Epilachna* on preferred host plants in the genus *Phaseolus* and did not consider other hosts of this oligophagous foliage feeder such as soybeans, *Glycine max* (Thomas). Recent expansion of soybean production in the Southeast has provided a disproportionate opportunity for population growth on this secondary host, and *E. varivestis* is presently one of the major insect pests of soybeans in North Carolina (Deitz et al. 1976). The irregular spatial and temporal distributions of this beetle on soybeans suggest that nutritional factors may exaggerate their susceptibility to regional weather stresses while feeding on a marginal host. Lockwood et al. (1979) and Kitayama et al. (1979) have characterized the dependence of adult Mexican bean beetle fecundity on temperature, relative humidity (RH), and soybean phenology. They found that, under constant conditions, egg production was limited to low temperatures and high RH conditions, compared with fecundities obtained in previous studies using *Phaseolus* hosts under similar conditions. Sprengel and Rabb (1981) have shown that slight moderation of temperature and RH extremes in the field can produce significant increases in larval survival on soybeans.

These studies corroborate observations by extension specialists and suggest that any predictive model of Mexican bean beetle population dynamics must include the influence of weather patterns on larval mortality and adult fecundity. Such a model should estimate: (1) the ability of the crop canopy and the leaf boundary layer to moderate ambient conditions, (2) the influence of these diurnally and seasonally varying conditions on mortality, fecundity, and lon-

gevity, and (3) the interaction of stresses due to weather and stresses due to host plant inadequacy. This study investigated the influence of temperature, RH, and host plant type on larval survival, development times, sex ratio, and resulting pupal weight.

Materials and Methods

Three stages of Bragg variety soybeans were obtained simultaneously in the field by using several planting dates and prolonging the day length in a 160-m² area with artificial lighting. At the beginning of the experiment the phenological stages of soybeans used were V3, R4, and R7, based on the terminology of Hanway and Thompson (1971). Reproductive-stage Henderson bush lima beans, *Phaseolus lunatus*, were used as a preferred-host comparison. Foliar samples were analyzed for macro- and micronutrients to provide a comparison of cultural or phenological differences. Plant petioles were cut underwater to maintain transpiration, and each was held in a separate test tube of water. Flexible rubber caps formed a seal between the stems and the test tubes to reduce direct evaporation. Three holes in the bottom of a clear plastic box (32 by 17 by 9 cm) each held a test tube containing a single petiole. Thirty newly hatched larvae were placed on the foliage in each plastic box, and this unit, covered with either a clear plastic top or polyester organdy, formed a replicate of a 4 × 3 × 3 completely randomized factorial design. At least two replicates were completed for each treatment.

Two RH levels (low and medium) were maintained at each of four temperatures (17, 22, 27, 32° C) in eight constant-environment chambers (Wilson 1979), at the Central Crops Research Station, Clayton, N.C. High humidity was obtained by using clear plastic lids on some plastic boxes. Water transpiring from the petioles or released from water-saturated cotton wicks maintained visible condensate patterns on the interior of these boxes, indicating the enclosed air was close to saturation for each temperature. The ambient temperatures and

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relative humidities were checked at least once a day. Humidity was measured by using lithium chloride probes (Hygrodynamics, Inc., type 15-3030E). Temperatures were maintained within $\pm 0.5^\circ\text{C}$, and RH varied most in the medium condition ($\pm 10\%$) compared with the stable low humidities ($\pm 5\%$) (Table 1). Photoperiod was held at 14L:10D for the duration of this experiment.

The foliage in each box was changed at least every other day, except in the low-temperature chambers, where it was occasionally left for 3 days. Each time the foliage was replaced, the numbers and stages of larvae found alive or dead was recorded. Live larvae were transferred to fresh foliage with a fine brush.

To study the relationship between larval size and mortality at low humidity, a similar experiment starting with newly molted 3rd-instar larvae was conducted. These larvae had been previously reared on mature soybeans in the insectary under high-humidity conditions.

Surviving pupae were collected, weighed, and allowed to emerge at room temperature. Percent emergence, sex, and dry weight of adults were also recorded.

Larvae used in this experiment were obtained from 160 field-collected females maintained in individual 1-quart (ca. 0.95-liter) paper containers on lima bean foliage. Eggs were kept in moist petri dishes in an open-air insectary until they hatched. The females producing the eggs were checked for microsporidian infections at the end of the experiment. Eggs from at least three females were used in each replicate, and their identities were recorded to permit retrospective analysis for differences in mortality or developmental time due to infection. Of the eggs used, 12% were from females infected with microsporidia. Natural population levels of this parasite are often high late in the growing season in North Carolina (Brooks et al. 1980). Inclusion of infected eggs may have increased the variance of the data presented but was thought to be representative of field populations.

Results

The effects of temperature and RH on survival of 1st-instar larvae to the pupal stage for all plant stages are summarized in Fig. 1. All main effects are highly significant as tested by a factorial analysis of variance.

At 32°C , survival was poor in all treatments. Desiccation was the probable cause of death at medium and low humidities, but at high humidity the extremely low survival was associated with signs of bacterial septicemia. Similar mortality has been previously noticed in attempts to rear larvae on moist filter paper in petri dishes at temperatures of 27°C or above.

Below 32°C , survival was essentially independent of temperature but directly proportional to RH when data for all plant types are combined. However, when the lima bean treatments are considered sep-

Table 1.—List of conditions maintained and equivalent VPD*

Temp. ($^\circ\text{C}$)	RH (%)			Equivalent mean VPD
	RH	Mean	Total range	
17	H	*	95-100	0.0
	M	73	70-90	5.4
	L	38	32-47	12.0
22	H	*	95-100	0.0
	M	70	68-80	7.9
	L	20	16-25	21.1
27	H	*	95-100	0.0
	M	48	35-60	18.2
	L	17	14-25	29.2
32	H	*	95-100	0.0
	M	47	35-55	24.3
	L	18	14-25	38.9

* H, M, L, High, medium and low RH, respectively.

^b Asterisk indicates range estimate for saturated air in sealed containers.

arately, no significant influence of RH is apparent (Fig. 2a).

Figure 2b and c shows the corresponding mean percent survival for larvae fed postbloom and prebloom soybean foliage, respectively. (Since no significant differences in survival were found for the two reproductive-stage soybean treatments, they were combined as a single, postbloom soybean category [0].) Larvae reared on the soybean hosts appear more vulnerable to low-humidity stress than those reared on the lima host in the same conditions.

The data in Fig. 2 a-c are replotted in Fig. 3 to reveal host-plant differences at each relative humidity. At the highest humidity (Fig. 3a) survival on the younger soybean foliage was significantly lower than the others (Duncan's multiple range test, $P < 0.05$). Survival on postbloom soybean foliage was comparable to that on lima bean foliage for all temperatures at high humidity, but at high temperatures and low humidity, the older soybean foliage was the least satisfactory host (Fig. 3c).

Atmospheric vapor pressure deficit (VPD) is a function of both temperature and relative humidity and is analogous to osmotic potential across a semi-

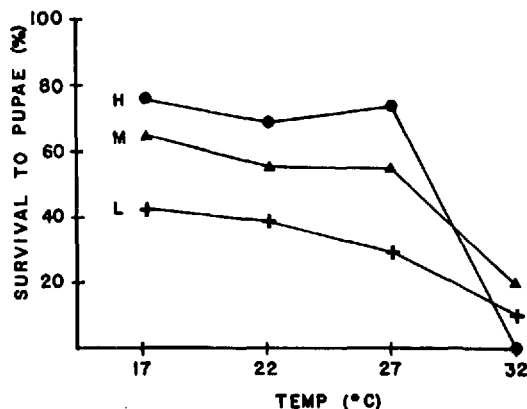


FIG. 1.—Percent survival to pupae from the 1st instar, averaged for all host plant types. (H, High; M, medium; L, low humidity).

permeable membrane. The linear relationship between survival and VPD is evident in Fig. 4. One weakness of using VPD alone is that at high humidities the VPD is low regardless of the temperature. This may obscure any direct thermal injury at high temperatures. (The 32° C, high-humidity treatments were left out of this analysis because of the high mortality due to bacterial septicemia in these conditions.) The relative suitability of prebloom and postbloom soybean foliage inverted as VPD increased. Under conditions of moisture stress, more larvae survived on prebloom foliage than on post-

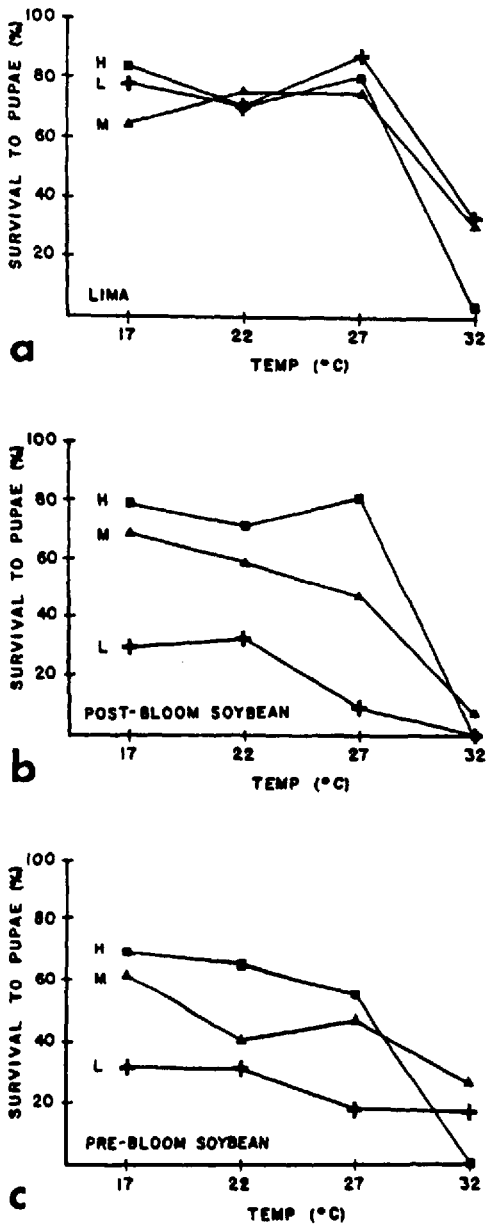


FIG. 2.—Treatment means for percent survival to pupation at high (H), medium (M), and low (L) humidities for each host. (a) Lima bean host; (b) postbloom soybean; (c) prebloom soybean.

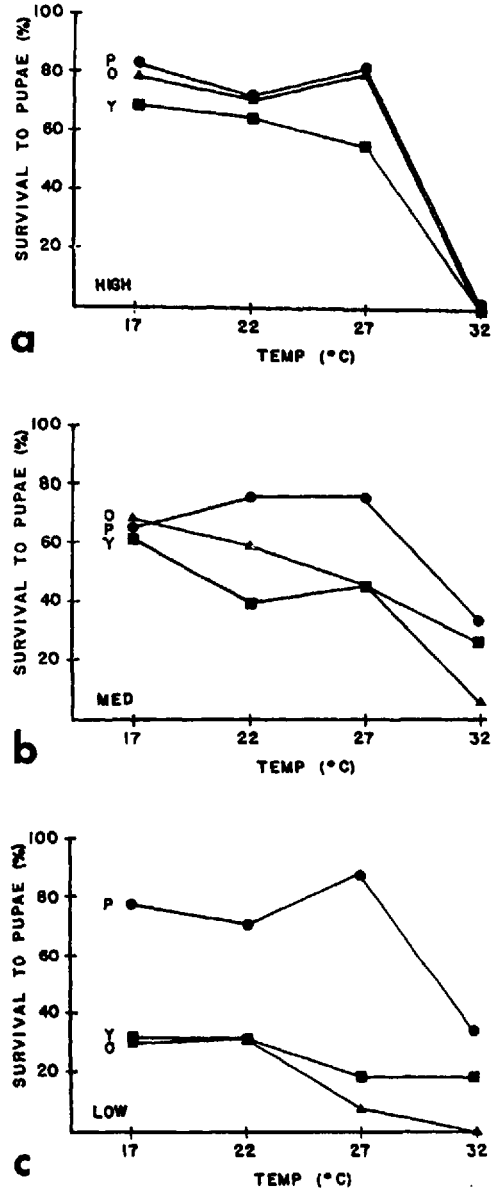


FIG. 3.—Treatment means from Fig. 2 plotted to reveal differences in survival on each host at each relative humidity. (P, lima; O, postbloom soybean; Y, prebloom soybean.) (a) High humidity; (b) medium humidity; (c) low humidity.

bloom foliage, but under favorable physical conditions survival was higher on postbloom foliage.

A visual comparison of the mean pupal weights for each treatment (Fig. 5) with the corresponding survival curves (Fig. 3) helps to substantiate the interactions between host plant suitability, RH, and temperature. A high correlation is expected in conditions where the differences in survival are substantial and where stresses are additive.

Table 2 lists estimates of days from hatch to the beginning of the prepupal period. Of the temperatures tested, 27° C appears closest to optimum. This

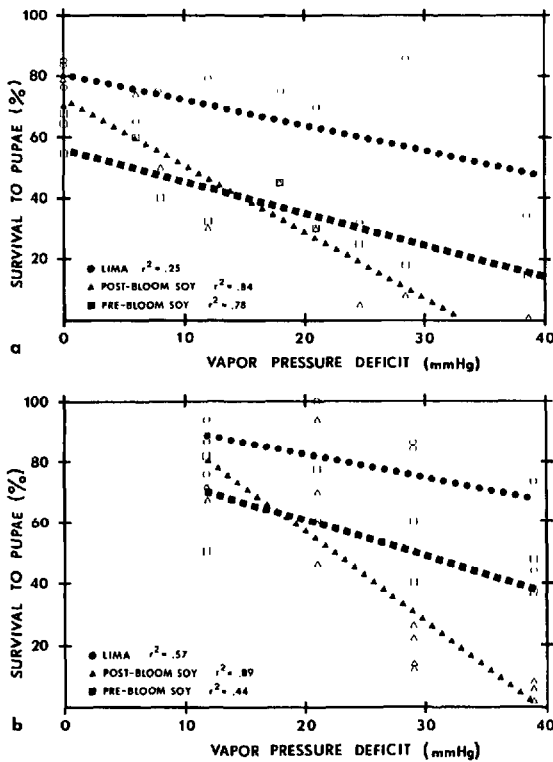


FIG. 4.—Survival to pupae vs. vapor pressure deficit. Symbols: ○, △, and □, treatment means for lima, post-bloom soybean, and prebloom soybean foliage treatments, respectively; ●, ▲, and ■, regression lines for lima, post-bloom, and prebloom foliage treatments, respectively. (a) Larvae started within 12 h of hatching; (b) larvae preconditioned at high humidity and moderate temperatures on mature soybean foliage for 1st and 2nd instars.

corresponds with previous work for the Mexican bean beetle (Sweetman and Fernald 1930, Marcovitch and Stanley 1930). Developmental times on lima bean foliage are shorter and are less influenced by RH than those of beetles on soybeans (Duncan's multiple range test, $P < 0.05$), but these differences diminish as the RH increases. Other species exhibit similar responses to moisture stress (Wigglesworth 1957) and nutritional stress (Isley 1935).

Although developmental times are generally longer at lower humidities, the time required to complete the 3rd and 4th instars is much shorter for larvae reared continuously in low-humidity conditions than for those acclimated to high humidity during the first two instars and then moved to the lower humidity

as newly molted 3rd instars (Table 3). This effect is highly significant and may reflect some extraordinary stress caused by behavioral or physiological adaptation to changing conditions.

A significantly greater proportion of females than males survived at high temperatures in the low-humidity conditions. The ratio of female to male survivors at 32° C and 20% RH was 3:1 and at 27° C was 1.5:1.

Foliar samples from each plant type were analyzed at the North Carolina Department of Agriculture Plant Analysis Laboratory in Raleigh for total dry-weight concentrations of nitrogen, potassium, phosphorus, sodium, calcium, zinc, magnesium, manganese, copper, and iron at three times during the experiment. Significant differences between plant types and sampling dates were found for all minerals tested. Lima bean foliage was consistently 10% lower in nitrogen than any stage of soybean except seedlings, whereas most other minerals were consistently higher in lima beans on a dry-weight basis.

Discussion

Mexican bean beetle larvae are known to be sensitive to moderately high temperatures and low humidities on the preferred host plants of this species in the genus *Phaseolus*. To effectively model the population dynamics of this beetle on soybeans, it is important to quantify the combined influences of these environmental stresses for larvae on this less preferred but more abundant host. This experiment demonstrates that, when temperature and RH conditions are favorable, survival on a soybean host is only slightly lower than on lima bean foliage, but that at higher constant temperatures, lower RH, or both, the inadequacy of this soybean host is striking. On soybean foliage, survival of this beetle varied inversely with vapor pressure deficit, whereas on lima bean foliage survival was high in all conditions below 32° C.

House (1972, 1977) found that larvae of the fly *Agria housei* developed faster at high temperatures on one type of artificial diet, whereas another diet with relatively high glucose levels provided the best growth rate at low temperatures. This inversion in food quality may be analogous to the findings reported here for an insect on a marginal natural host. Nutrient levels are much higher in postbloom soybean foliage, whereas water content is higher in younger foliage (Henderson and Kamprath 1970).

Table 2.—Treatment means of days ± 15 days to develop from hatch to the end of the 4th instar or the beginning of the prepupal stage^a

Plant	17°C			22°C			27°C			32°C		
	H	M	L	H	M	L	H	M	L	H	M	L
Lima bean	27.0	27.3	28.5	14.0	18.0	18.0	14.0	13.0	14.0	16.0	16.0	16.0
Postbloom soybean	27.0	31.0	38.7	15.0	21.2	28.2	13.6	18.0	25.0	—	22.0	—
Prebloom soybean	26.0	30.0	37.0	16.5	21.0	26.0	15.5	19.3	20.5	—	21.0	24.0

^a See footnote a to Table 1.

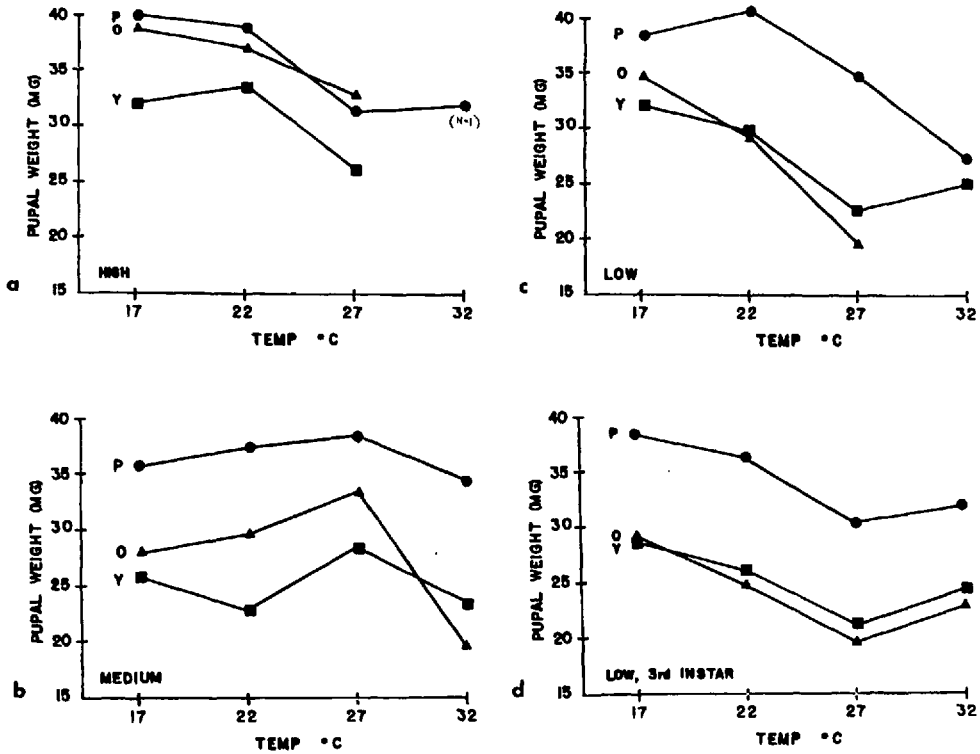


FIG. 5.—Average live pupal weight for each treatment. Symbols as in Fig. 3.

Care must be taken in applying these results to field conditions. For instance, nightly increases in relative humidity may permit the insects to recover daily water losses. Also, the apparent dependence of developmental rates on previous environmental conditions suggests that our understanding of transition processes is weak, even in the relatively simple case of changing from one set of constant conditions to another. If this is a real effect, then the impact of sudden shifts in field conditions may be difficult to assess. These data provide a means of estimating the relative mortality rates of field populations only if crop microclimatic data are available and only if an algorithm can be developed to apply constant temperature and RH data to varying field conditions. It may be possible to use the correlation between stress and pupal weight to help validate a model that attempts to make such estimates. Since pupal weight may in some ways be a reflection of accumulated stress on a given host plant, weights of

field-collected pupae might be used to corroborate mortality estimates derived from weather data and host plant information.

A myriad of behavioral, biochemical, and physical factors influence an insect's survival on a given host plant, and the relative importance of each is likely to change as the insect grows. The host plant factor most important in this case might be something as simple as leaf texture. That is, the pubescent surface of soybeans may interfere with the ability of these larvae to physically extract water and nutrients (Campbell 1958, Van Duyn et al. 1972). In addition to impeding access to the leaf surface, trichomes may also cause abrasion of cuticle waxes, which has been shown to increase the rate of water loss through some insect cuticles (Wigglesworth 1957).

An understanding of the relative importance of each of these factors in determining the ease of maintaining larval water balance on a given host may be necessary before a model of these physical stresses

Table 3.—Low-humidity treatment means of days to develop from the 3rd instar to pupation, for larvae starting as 1st instars compared with those reared at high humidity and transferred to treatment conditions as 3rd-instar larvae

Plant	Initial instar							
	17°C		22°C		27°C		32°C	
	1st	3rd	1st	3rd	1st	3rd	1st	3rd
Lima bean	15.0	20.0	9.0	12.2	6.0	12.0	7.7	7.0
Postbloom soybean	18.5	21.7	13.5	17.3	14.0	17.4	—	—
Prebloom soybean	30.0	21.0	13.5	14.5	10.0	16.0	12.7	12.0

can be generalized for use with other varieties of soybeans. The exaggerated sensitivity to physical factor extremes brought about by feeding on a marginal host may be important to consider when interpreting results of screening tests for potential resistant cultivars, both in the moderated climate of most laboratory tests and in the occasionally extreme conditions of field tests.

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