*Ecological Entomology* (1990) **15**, 433–445

# Insect responses to plant water deficits. II. Effect of water deficits in soybean plants on the growth and survival of Mexican bean beetle larvae

GRANT. T. McQUATE and EDWARD F. CONNOR
Department of Environmental Sciences, Clark Hall, University of Virginia, Charlottesville, Virginia, and The Blandy Experimental Farm, Boyce, Virginia, U.S.A.

**Abstract.** 1. We examined the effect of water deficits in soybean, *Glycine max* [L.] Merr., on the growth and survival of Mexican bean beetle larvae, *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae).

2. Larvae were reared under growth chamber, glasshouse, and field conditions on foliage from plants that were either well-watered or subjected to water deficits.

3. Larval survival, growth rate and pupal weight were reduced, and development time was increased when larvae were reared on foliage from plants subjected to water deficits. These results are contrary to White's (1974) hypothesis that water deficits in plants cause increased growth and survival of herbivorous insects.

4. Changes in foliage chemistry caused by water deficits, possibly in the concentration of free amino acids, are the most likely causes of the observed effects on growth rate and development time. However, under glasshouse and field conditions, physical changes in foliage and/or the foliage's environment that accompany water deficits are also important.

**Key words.** Plant water deficits, soybean, Mexican bean beetle, water stress, growth, development, survival.

## Introduction

White (1969, 1974, 1976, 1978, 1984) argues that water deficits alter plant chemistry, improving their nutritional quality and consequently increasing the growth and survival of insects feeding upon them. In particular, he proposes that 'water stress' increases the concentration of nitrogenous compounds in plants (i.e. free amino acids) and may lead to improved survival of early larval instars. White (1974) proposes this as a general mechanism to explain insect outbreaks. Originally, this theory was based on sapsucking insects (White, 1969), but it was later expanded to include chewing insects (White, 1974) and herbivorous animals in general (White, 1978).

For herbivorous insects to benefit from any improvement in the nutritional quality of their host plant caused by water deficits, they must choose to feed on water-deficient foliage. In previous experiments (McQuate & Connor, 1990), we demonstrated that larvae of the Mexican bean beetle (*Epilachna varivestis*

Correspondence: Dr E. F. Connor, The Blandy Experimental Farm, P.O. Box 175, Boyce, VA 22620, U.S.A.

Mulsant) usually preferred to feed on foliage from well-watered soybean plants (*Glycine max* [L.] Merr.). However, larvae preferred to feed on rehydrated foliage that had previously been subjected to very mild water deficits, rather than foliage that had been well watered.

In order to determine if the feeding preference of *E.varivestis* larvae for water-deficient (from mildly to severely water-deficient) and well-watered foliage is correlated with performance, we reared larvae on foliage from well-watered and water-deficient soybean plants in controlled environment chambers, in a glasshouse, and in the field. We monitored survival, growth rate, weight gain, and development time from the first instar through pupation as indices of larval performance.

## Methods

*Overview of experimental design.* Experiments were performed to determine if water deficits in soybeans affect the growth or survival of Mexican bean beetles. Pots with soybean plants were grown in a glasshouse and randomly divided into a well-watered group (control) and groups which received limited water (treatments). Treatment and control pots were spatially intermingled within the glasshouse. The water status of plants was monitored regularly to document the magnitude of the experimentally imposed water deficit. In 1983 and 1984, larvae were reared on excised leaves in controlled environment chambers. In 1984, larvae were also reared on intact plants in a glasshouse and in the field. Larval survival and weight at each instar were recorded. Plants were subjected to less severe water deficits in 1984 because feeding preference tests had indicated that larvae avoided plants subjected to more severe water deficits (McQuate & Connor, 1990).

*Plant growth conditions.* In 1983, soybean (*Glycine max* [L.] Merr. cv. Williams) seeds were inoculated with *Rhizobium japonicum* and planted as described in McQuate & Connor (1990). A nitrogen-free nutrient solution was used to provide adequate mineral nutrition (Ahmad & Evans, 1960; McClure & Israel, 1979). In 1984, seeds of cv. Ransom were planted as above and fertilized with a nitrogen fertilizer as in McQuate & Connor (1990). In 1984, we switched to a nitrogen fertilizer, rather than bacterial nitrogen fixation, for a nitrogen source because Wilson & Stinner (1984) suggested that the growth and survival of Mexican bean beetle larvae might be improved. Soybeans were also planted in a field at the Blandy Experimental Farm, Clarke County, Virginia, U.S.A. (78° N, 39° W). Seeds were inoculated and planted in rows 1 m apart in 8 × 8 m plots.

*Water treatments.* In 1983, thirty pots each were assigned at random to control (well-watered) and treatment (water-deficient) groups. Soil moisture levels were monitored daily with electrical resistance blocks composed of electrodes embedded in gypsum (termed gypsum blocks; Hillel, 1980). After establishment, treatment pots were monitored daily and watered if soil water potential dropped below $-1.5$ MPa. In 1984, thirty-three pots of plants were divided among control, mild and moderate water deficit treatments. Leaf water potentials in the mild and moderate water deficit treatments were kept no more than 0.15 MPa and 0.5 MPa below those of control plants, respectively. Control pots were watered as needed to maintain soil water potential above $-0.06$ MPa.

Water deficits were achieved in the field by using one 3 × 3 m rain shelter. This shelter was open on all sides to a height of 1.2 m to minimize heat buildup. Gypsum blocks were embedded in the soil under and outside the shelter, and resistances were recorded weekly.

*Plant water status.* The water status of foliage was determined by measuring leaf diffusive resistances with a Li-Cor Li-1600 diffusive porometer and leaf water potential with a PMS pressure chamber. Diffusive resistance was measured between 15.00 and 17.00 hours prior to leaf excision for feeding experiments (1983). In 1984, both diffusive resistance and leaf water potential were measured between 13.30 and 16.30 hours, prior to leaf excision the following morning. Both of these parameters were measured every third day on plants used for rearing insects in the glasshouse, and weekly on field grown plants.

*Insect growth conditions.* A stock colony of Mexican bean beetles was maintained on lima bean foliage (*Phaseolus lunatus* L.) as described in McQuate & Connor (1990).

*Experiments using excised leaves.* In 1983, soybean leaves were excised 1 h after sunset, and in 1984 leaves were excised before dawn. Foliage of equal age (first, second, third or fourth trifoliate leaves) was provided to larvae in each treatment in order to remove within-plant variability in water status and foliage chemistry. This was achieved by using foliage from plants planted at different times. New leaves were provided at least every 48 h (1983) or every 24 h (1984). Growth studies were conducted in an environmental chamber at 23°C with a 14 h photoperiod. Immediately following excision, leaflets were placed in Petri plates on top of filter paper which was kept constantly moist. Newly emerged larvae were weighed and then placed individually on these leaves.

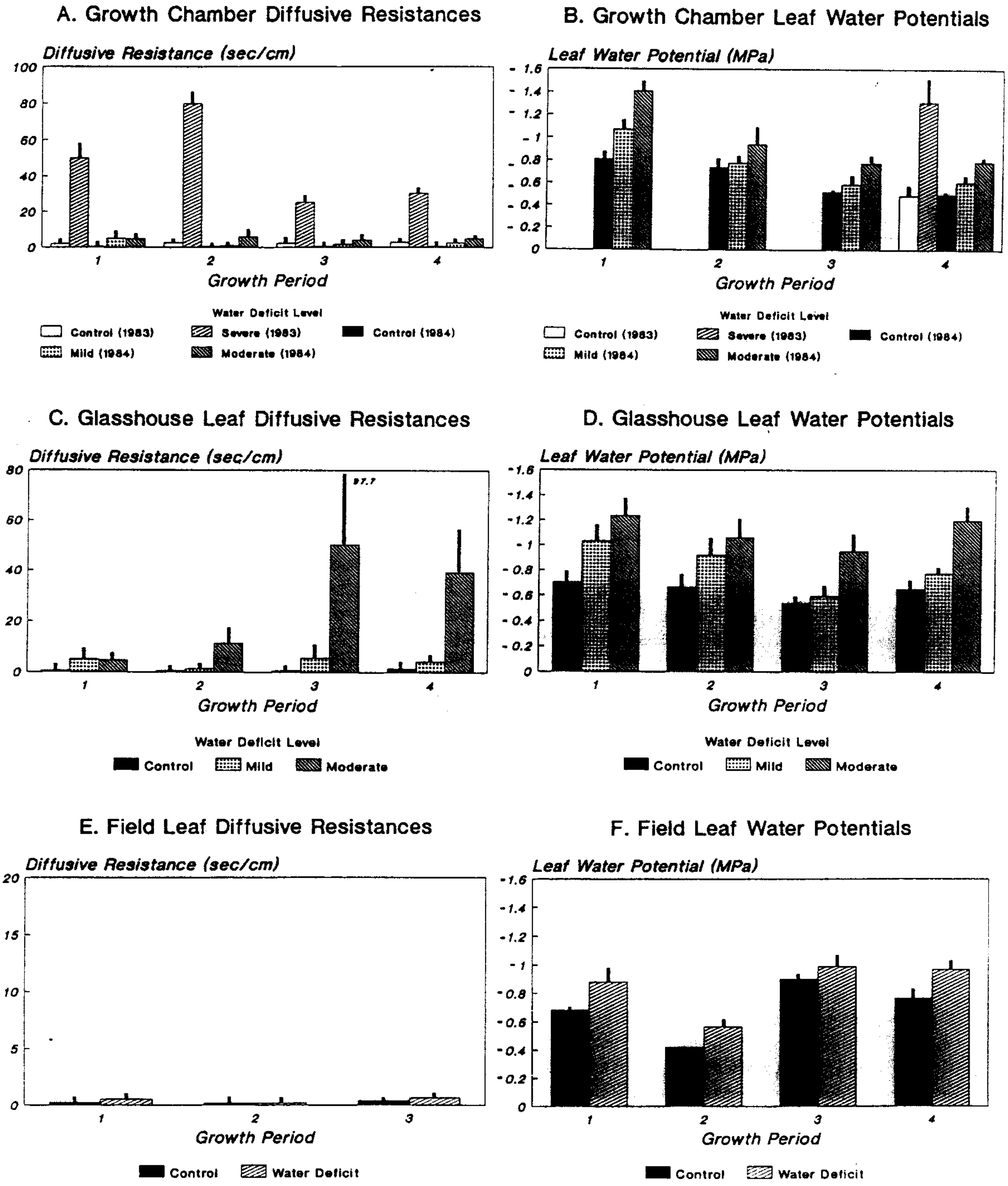
Adult *E. varivestis* are known to be sexually dimorphic in weight, and preliminary observations suggested that this was also true for larvae and pupae (McQuate, 1985). Therefore, individuals were reared separately and sexed upon transformation to the adult stage. This increased the statistical power of our test of treatment effects and allowed us to examine sex-specific differences in weight gain at each instar. In 1983, sixteen larvae from each of three egg clusters were distributed equally between control and treatment foliage so that treatment effects were assessed within full-sib families of *E. varivestis* (differences in larval response among egg clusters will be referred to as the dam effect). This amounted to a total of twenty-four larvae per treatment. In 1984, thirty larvae from each of two egg clusters were divided among the three treatment groups (control, mild and moderate water deficit). This amounted to twenty larvae per treatment. Sample size was chosen so that a 30% difference in mean larval weight between treatments could be detected with Type I and Type II error rates both equal to 5% (McQuate, 1985).

Larvae were checked daily and weighed 1 day after the moult preceding the second and the third instars, 2 days after the moult preceding the fourth instar, and 3 days after the outer skin of the fourth instar was pushed posteriorly in the course of pupation. These weighing times were selected because they represent points during development when there is minimal variance in larval weights (Kogan, 1972). In 1984, after transformation to the adult stage, each individual was sexed, killed by freezing, and oven-dried to constant weight. The dry weight of adults beetles was measured to ensure that treatment effects were not caused by differences in water content of the insects. Moulting times, time of pupation, and time of metamorphosis were recorded for each individual.

Growth was separated into weight gain between set points in the growth cycle, daily gain in weight (growth rate), and the time between instars (development time). Treatment effects on weight gain, growth rate, and development time were examined using separate four-way (sex, treatment, dam and developmental stage) repeated measures analyses of variance, each with developmental stage as the repeated factor. The data was analysed assuming multivariate normality and *P*-values were adjusted using the Huyndt-Feldt Epsilon to correct for deviation from compound symmetry of the variance–covariance matrices (Winer, 1971; O'Brien & Kaiser, 1985). The experimental design allowed us to estimate treatment effects free from the confounding influences of sexual dimorphism, as well as maternal effects on weight, growth rate, or development time.

*Experiments using intact plants.* Larvae were reared *in situ* in the glasshouse on both control and water-deficient plants. Three pots from each treatment were randomly chosen for these experiments. Larvae from three egg clusters with twenty-one, thirty-six and eighteen eggs, respectively, were divided equally among the plants from the control and the two treatment groups. Initially, first instar larvae were used for these experiments. However, because of high mortality on both treatment and control plants, early third instar larvae were used. Larvae were allowed to roam freely on all plants in a given pot. Immigration between pots was limited by keeping adjacent pots widely separated.

Larvae were also reared on field grown plants. Second and third instar larvae were released on field plants on three successive days. On each release (cohort), larvae were derived from a single egg cluster and divided equally between a treatment and a control plant. A total of twenty-eight, thirty and twenty-four individual larvae were released on each date. Dispersal was prevented by enclosing plants in a 0.5 mm mesh screen cage, 1.5 m high by 0.6 m in diameter. A total of three plants were used for each treatment. Although treatments were replicated across plants, since only one rain shelter was


A. Growth Chamber Diffusive Resistances

B. Growth Chamber Leaf Water Potentials

C. Glasshouse Leaf Diffusive Resistances

D. Glasshouse Leaf Water Potentials

E. Field Leaf Diffusive Resistances

F. Field Leaf Water Potentials

**Fig. 1.** Average diffusive resistance and leaf water potential of soybean foliage used in growth chamber, glasshouse and field experiments. Solid vertical lines depict one standard error. Plant growth periods correspond to Mexican bean beetle larval instars. No data on leaf water potentials were collected during the first three growth periods in 1983.

used to deprive field grown plants or water, this experiment could be considered 'pseudoreplication' (Hurlbert, 1984).

All larvae were allowed to pupate *in situ*. They were then collected, weighed and sexed upon transformation to the adult stage. Treatment effects were examined using the average pupal weights and average development times observed on each plant. For the glasshouse experiment, treatment effects were examined using Page's test for ordered alternatives (Hollander & Wolfe, 1973). For the field experiment treatment effects were examined using a paired *t*-test.

## Results

### *Experiments with excised leaves*

Diffusive resistance was greater and leaf water potential lower in leaves from plants grown under water deficits than in control plants (Fig. 1). The magnitude of the water deficit was not constant over the duration of the larval growth period, being more severe during the first and second instars. Also, the water deficit imposed in the 1983 experiments was more severe than those used in 1984 (henceforth termed the 'severe' water deficit). Both of the water deficits imposed in 1984 fall within the range of diffusive resistances and leaf water potentials upon which larvae prefer to feed ($<2.73$ s/cm and $<-1.13$ MPa, respectively; McQuate & Connor, 1990). Given the observed diffusive resistances and leaf water potentials, *E. varivestis* larvae normally would have avoided foliage from plants grown under the severe water deficit (McQuate & Connor, 1990).

When larvae were reared on foliage from plants subjected to the severe water deficit they grew more slowly ($F_{1,29}=19.02$, $P<0.001$), were smaller in mass ($F_{1,29}=29.9$, $P<0.001$), and took longer to complete development ($F_{1,29} = 13.4$, $P<0.001$) than siblings reared on foliage from well-watered control plants (Figs 2a–c). However, when reared on foliage from plants grown under the mild or moderate water deficit treatments, Mexican bean beetle larvae grew no better than siblings reared on well-watered control foliage (growth rate: $F_{2,31}=1.77$, $P>0.1$, weight gain: $F_{2,31}=0.29$, $P>0.7$, development time: $F_{2,31}=1.71$, $P>0.1$; and Figs 2d–f).

Larvae grew at different rates, gained different amounts of weight, and took different lengths of time to complete development in each instar. While males and females grew at similar rates, females gained more weight than males and were heavier at pupation. Significant variation among dams in the time needed for their offspring to complete development was also detected (ANOVA, $P<0.05$).

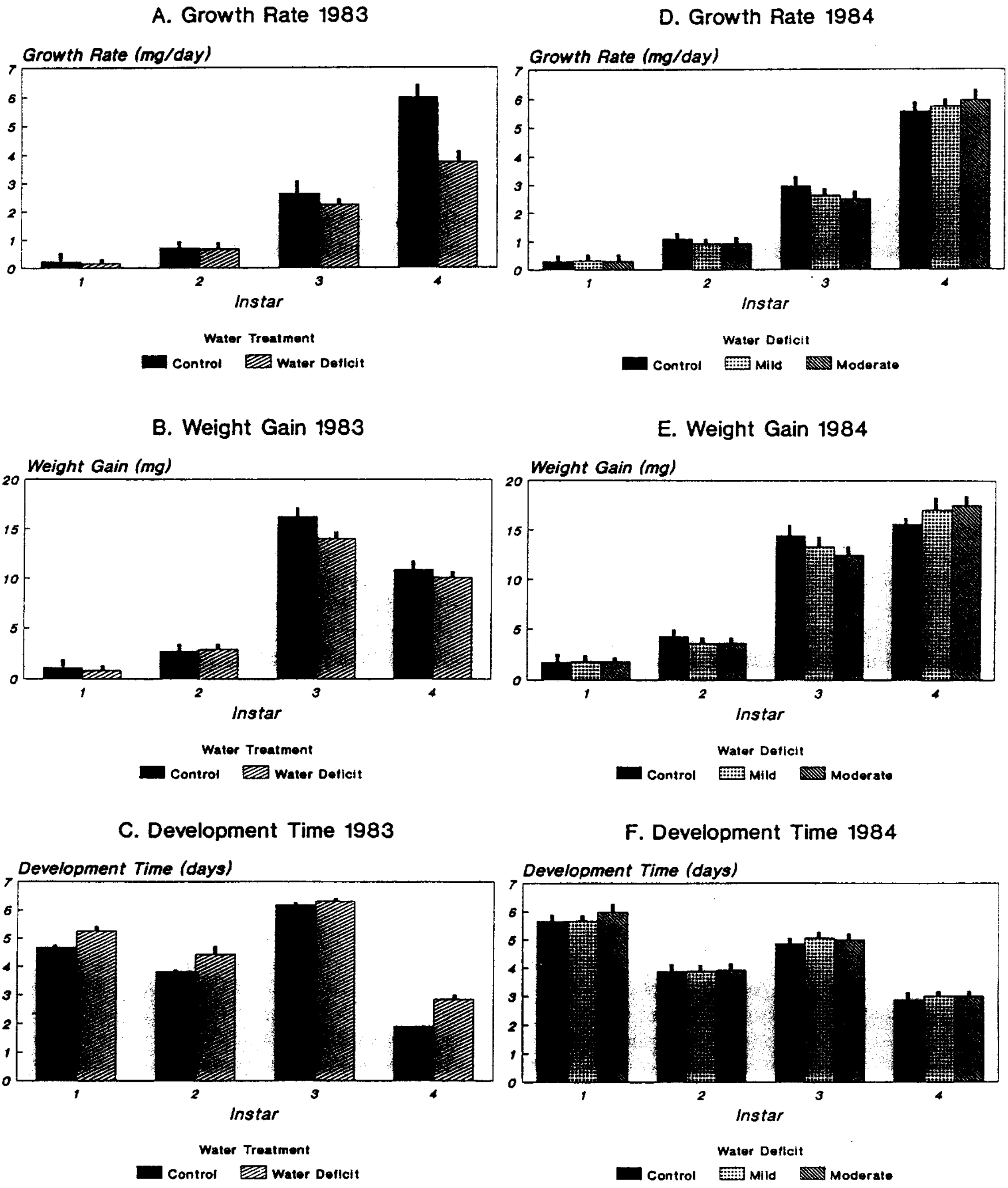
The average water content of the experimental insects after transforming to the adult stage was 84.5±0.13%. The water content of adults did not differ between treatments or sexes ($F_{2,36}=0.76$, $P=0.476$, and $F_{1,36}=1.83$, $P=0.185$, respectively). Therefore, the observed treatment effects on growth rate and weight gain are not due to differences in the water content of the insects.

No difference in survival rates were detected between larvae reared on well-watered excised foliage and larvae reared on foliage from the severe water deficit treatment in 1983 (paired $t=1.73$, df=2, $P=0.225$). Furthermore, no difference in survival was found between larvae reared on control or water-deficient foliage in 1984 (Friedman's test $\chi^2=0.25$, df=2, $P=0.8825$). While not statistically different, average survival rates were generally lower on water-deficient foliage (Fig. 3).

### *Experiments using intact plants*

Plants used for rearing larvae *in situ* in a glasshouse were subjected to water deficits of approximately equal severity as plants from which foliage was excised for the growth chamber experiments (Figs 1a–d). However, the survival of larvae reared on intact plants in a glasshouse was lower overall (Fig. 3). This is probably due to the lower relative humidity of the glasshouse. Growth chamber relative humidity ranged between 90% and 100%, while in the glasshouse it averaged 60%. In the glasshouse, larval survival was significantly lower on water-deficient plants than on control plants (repeated measures MANOVA $F_{2,4}=18.43$, $P=0.01$, with cohort as the repeated factor, and Fig. 3).

For those larvae that did survive in the glasshouse, pupal weights were lower and development times longer for those individuals reared on treatment foliage (Page's test $L=28$, $k=3$, $n=2$, $P=0.05$).


A. Growth Rate 1983

D. Growth Rate 1984

B. Weight Gain 1983

E. Weight Gain 1984

C. Development Time 1983

F. Development Time 1984

**Fig. 2.** Average growth rate, weight gain, and development time for Mexican bean beetle larvae at each instar when reared on well-watered and water-deficient soybean foliage under growth chamber conditions. Solid vertical lines depict one standard error.

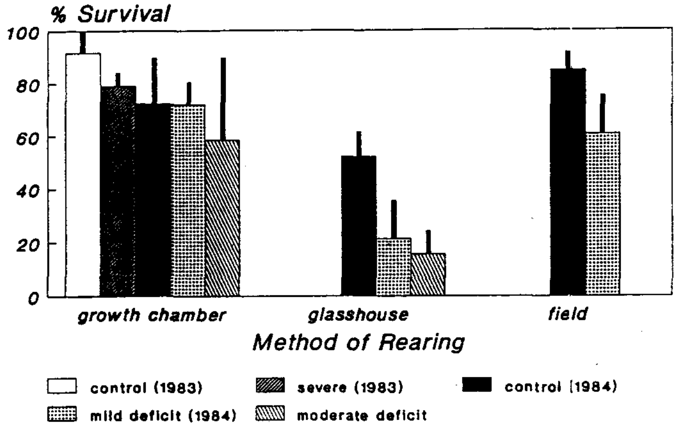


**Fig. 3.** Average survival of Mexican bean beetle larvae reared on well-watered and water-deficient soybean foliage under growth chamber, glasshouse and field conditions. Solid vertical lines depict one standard error.

Field-grown plants were subjected to water deficits comparable to the 'mild' water deficit used in the growth chamber and glasshouse experiments (Figs 1a–f). However, the diffusive resistances of field grown plants were much lower than glasshouse grown plants possibly because of the lower relative humidity of the glasshouse (Fig. 1e).

Larval survival in the field experiments appears higher than in both the growth chamber and the glasshouse experiments (Fig. 3). However, because the field experiments were initiated with later instar larvae than in all other experiments survival would be expected to be higher. Although no difference in the survival of larvae reared on control and water-deficient foliage in the field was detected (paired $t$=4.29, df=1, $P$=0.146), the average survival rate of larvae reared on water-deficient foliage was lower than for larvae reared on control foliage (Fig. 3).

Larvae reared on water-deficient foliage under field conditions tended to produce smaller pupae (paired $t$-test, $t$=1.51 df=2, $P$=0.136) and tended to take longer to develop (paired $t$-test, $t$=1.62, df=2, $P$=0.124) than siblings reared on control foliage, but the differences were not statistically significant.

### *Combined results: growth chamber, glasshouse and field experiments*

Fisher's method for combining probabilities was used to provide an overall assessment of the effects of water deficits on the growth and survival of *E. varivestis* (Sokal & Rohlf, 1981). Since each of these experiments provides an independent test of the hypothesis that growth rate, weight gain, development time, and survival are no different for larvae reared on well-watered and water-deficient foliage, the '$P$-values' from these tests can be combined to assess the affect of plant water deficits. This procedure can increase the statistical power of the tests of treatment effects. Table 1B shows that the overall performance of larvae was poorer on water-deficient foliage than on foliage from well-watered plants. Larvae reared on water-deficient foliage grew more slowly, took longer to complete development, were smaller, and experienced higher mortality than siblings reared on foliage from well-watered plants.

## Discussion

We present evidence of a consistent tendency under growth chamber, glasshouse and field conditions for water deficits in soybeans to worsen rather than improve the growth and survival of Mexican bean beetle larvae. Although some individual experiments yielded results that were not statistically significant ($P$>0.05), there was a consistent pattern of reduced growth and survival and extended developmental period for larvae reared on water-deficient foliage when compared to siblings reared on well-watered foliage (Figs 2 and 3).

**Table 1.** The probabilities of a Type I error for each test of the hypothesis that water-deficits effect the growth and survival of *E. varivestis*, and the probability of a Type I error for the combined test for each effect calculated using Fisher's method (Sokal & Rohlf, 1981).

A. Individual probabilities of a Type I error

| | Growth rate | Weight gain | Development time | Survival |
|---|---|---|---|---|
| Excised leaves (1983) | 0.001 | 0.001 | 0.001 | 0.225 |
| Excised leaves (1984) | 0.187 | 0.728 | 0.198 | 0.8825 |
| Glasshouse | –* | 0.884 | – | 0.01 |
| Field | – | 0.001 | 0.005 | 0.146 |

* Not possible to perform test in these instances.

B. Combined tests using Fisher's method

| Growth rate | Weight gain | Development time | Survival |
|---|---|---|---|
| $\chi^2=17.17$ | $\chi^2=28.51$ | $\chi^2=27.65$ | $\chi^2=16.29$ |
| df=4 | df=8 | df=6 | df=8 |
| $P<0.005$ | $P<0.001$ | $P<0.001$ | $P<0.05$ |

Even under the mildest water deficits that we could establish, levels approximating those where *E. varivestis* larvae had shown feeding preference over control plants (McQuate & Connor, 1990), larvae experienced no improvement in growth and survival. Furthermore, when this group of experiments is examined as a set of independent tests of the same null hypotheses, statistically significant reductions in growth and survival and a lengthening of developmental period are detected (Table 1, $P<0.05$).

### *Why do bean beetles grow and survive poorly on water-deficient foliage?*

Both physical and chemical changes in foliage accompany water deficits in plants, and either or both could account for the observed poorer performance of larvae on water-deficient foliage. Our growth chamber experiments were designed to minimize physical changes in foliage that accompany water deficits to determine if chemical changes alone could affect the growth and survival of *E. varivestis* larvae. To achieve this, we took advantage of normal diurnal variation in the relative water content (RWC) and toughness of leaves. After sunset and before dawn, previously water-deficient foliage rehydrates and regains its turgor. Dornhoff & Shibles (1970) show that the RWC of non-irrigated soybeans returned to near 90% within an hour after sunset. We observed the early morning RWC of both well-watered and rehydrated soybeans to be 97% (McQuate & Connor, 1990). Lewis (1982) found that leaf toughness in re-watered, previously water-deficient sunflower plants was no different from that of turgid controls, and we obtained similar results for soybeans (McQuate & Connor, 1990). Since water content and leaf toughness may be of dietary significance to herbivorous insects (Scriber, 1977, 1979; Lewis, 1982; Connor, 1988; McQuate & Connor, 1990), we chose to excise foliage between 1 h after sunset and before dawn to ensure that foliage from both control and water-deficit treatments had rehydrated prior to excision. Furthermore, by conducting experiments in growth chambers we could also control the microclimate experienced by each larva. We believe that these procedures were sufficient to eliminate or minimize most of the physical differences between the foliage excised from well-watered and water-deficient plants. However, our experimental design could not eliminate differences in cell size and in the proportion of cell wall material. Nevertheless, we conclude that the reduction in growth and survival observed in our growth chamber experiments is most likely caused by chemical changes in water-deficient foliage.

Growth and survival of *E.varivestis* larvae was reduced to a greater extent in the glasshouse and field experiments than in the growth chamber experiments (Figs 2 and 3). In both the glasshouse and the field experiments we could neither control nor eliminate the effects of the physical changes in foliage that accompany plant water deficits. Therefore, an additional effect of the physical differences between well-watered and water-deficient foliage and/or the foliage's environment appears to be responsible for the greater reduction in growth and survival observed when larvae were reared on intact plants. Whether this is due to differences in the water content or toughness, or to differences in the humidity or temperature near the leaf surface between well-watered and water-deficient foliage, cannot be determined from these experiments. However, the overall poorer survival of *E.varivestis* larvae in the glasshouse, where relative humidities were lowest, is consistent with Wilson *et al.*'s (1982) conclusions that Mexican bean beetle larvae are sensitive to low relative humidities.

### *What changes in chemical composition are responsible for the poorer performance of larvae on water-deficient foliage?*

The chemicals in soybean foliage of potential nutritional significance to insects that are known to change concentration under water deficits include sugars (Fukui & Ojima, 1957; Ford, 1984), pinitol, an O-methyl-inositol (Ford, 1984), and free amino acids (Fukutoku & Yamada, 1981a, b; McQuate & Connor, 1990). It is also possible that changes in secondary plant metabolites are responsible for the observed poor performance of *E.varivestis* on water-deficient soybean foliage (Gershenzon, 1984). Hart *et al.* (1983) have shown that ultraviolet radiation, and possibly mechanical damage and herbivory, can induce the production of phytoalexins in soybeans. They have also shown that fourth instar Mexican bean beetles avoid such foliage. However, it has not been demonstrated that similar chemical changes can be induced by water deficits.

Increases in sugar concentration have been reported for soybeans grown under water deficits (Fukui & Ojima, 1957), and sucrose concentration has been shown to affect the consumption rates and growth of Mexican bean beetle larvae, although not linearly (Kogan, 1971; Jones *et al.*, 1981). However, increased sugar concentrations are probably not important since we observed *E.varivestis* larvae to avoid water-deficient foliage (McQuate & Connor, 1990) and to grow and survive poorly when reared on such foliage, in opposition to the expected impact of elevated sugar levels.

Pinitol, a B-vitamin, increases 5-fold in concentration in soybean leaf tissue under very severe water deficits (leaf water potentials of $-6.2$ MPa; Ford, 1984). However, the concentration of pinitol has no effect on the growth and development of *Heliothis zea* (Boddie) or *Trichoplusia ni* (Hubner) (Gardner *et al.*, 1984). If we can assume that high concentrations of a B-vitamin will not serve as a feeding deterrent, then Ford's (1984) and Gardner *et al.*'s (1984) results combined with ours argues that pinitol is not responsible for the differences in larval performance we observed.

Concentrations of free amino acids increase in soybeans under water deficits (Fukutoku & Yamada, 1981a, b; Waldren & Teare, 1974; McQuate & Connor, 1990). The extent of the increase at a given water deficit varies among soybean varieties (Singh & Gupta, 1983). Dietary levels of amino acids, especially the essential amino acids, are important components of insect diets (Rock, 1972; Brodbeck & Strong, 1987). Water deficits alter the balance of free amino acids in foliage, including the proportion of essential amino acids (Fukutoku & Yamada, 1981a). If these changes have an adverse affect on the amino acid balance, amino acids in excess of balanced needs will be used as sources of amino groups, leaving surplus fragments. The disposal of these fragments may produce metabolic dysfunction which could be reflected in poorer growth (Dadd, 1973). It is also conceivable that increased levels of certain amino acids could serve as feeding inhibitors. We have previously shown that water deficits in soybeans increase free amino acid levels and avoidance of such foliage by Mexican bean beetle larvae (McQuate & Connor, 1990).

Of the chemical changes known to occur under water deficit conditions in soybeans, changes in free amino acid concentrations are the most likely to explain the observed effects on growth, survival, and development of Mexican bean beetle larvae. However, without additional experiments we cannot specifically implicate changes in the total or individual concentrations of amino acids as the cause of the observed reduction in larval performance. The fact that many correlated chemical changes occur in plants experiencing water deficits makes it very difficult to determine conclusively the exact chemical cause of any observed insect response to such plants. A more comprehensive understanding of the chemical changes that occur in plants experiencing water deficits and their consequences for herbivorous insects is necessary before a specific chemical cause for the reduction in growth and survival we observed can be proposed.

### *White's hypothesis*

The reduction in growth rate, weight gain, and survival and the increase in development time observed for larvae of *E. varivestis* reared on foliage subjected to water deficits clearly does not support White's (1969, 1974, 1976, 1978, 1984) hypothesis. We have documented that amino acid concentrations increase in soybeans subjected to water deficits (McQuate & Connor, 1990), but that instead of improving the growth and survival of *E. varivestis* larvae as predicted by White, growth and survival are impaired. Fig. 4 summarizes the relationship between the growth, development, survival, and

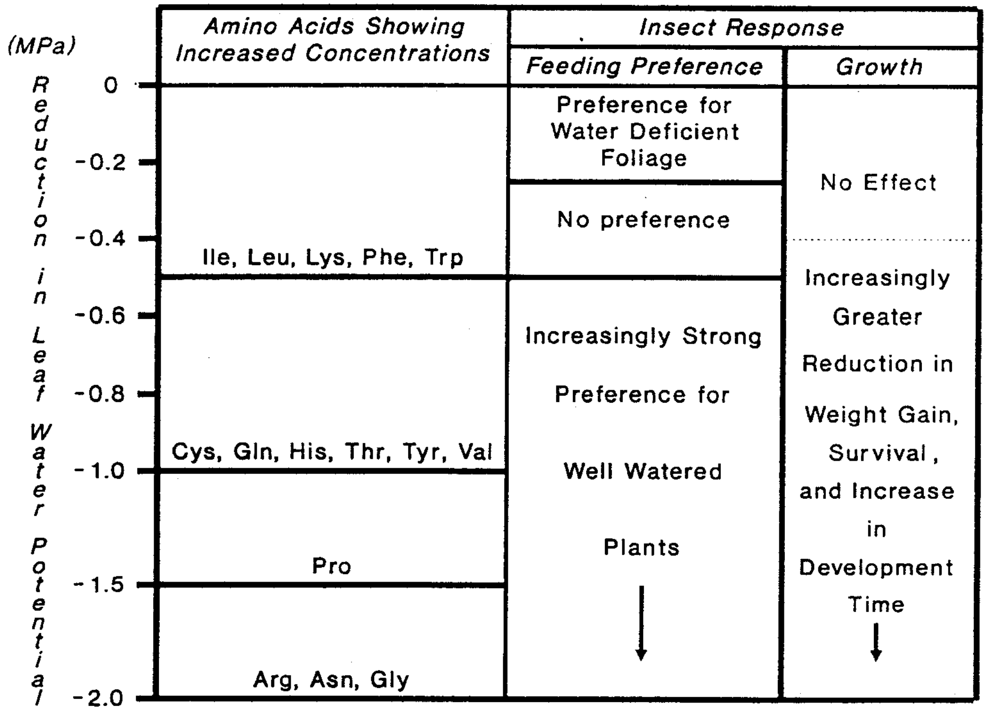


**Fig. 4.** Summary of the relationship between Mexican bean beetle feeding preference, growth, development and survival, and changes in free amino acid concentrations in soybean plants subjected to increasingly severe water deficits. Reduction in leaf water potential is the decrease observed in water-deficient plants compared to well-watered control plants. Glutamic acid, alanine, aspartic acid and serine do not change concentration in plants experiencing water deficits. Data on changes in free amino acid concentrations in soybeans experiencing water deficits are from Fukutoku & Yamada (1981b) and McQuate & Connor (1990). The abbreviations indicate which amino acids increase in concentration at particular levels of water deficit: Arg, arginine; Asn, asparagine; Cys, 1/1-cystine; Gln, glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Phe, phenylalanine; Pro, proline; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.

feeding preference (McQuate & Connor, 1990) of Mexican bean beetle larvae and changes in the concentration of amino acids found in soybeans experiencing water deficits.

Viewed in conjunction with other studies that have shown a wide variety of responses by herbivorous insects to plants experiencing water deficits (Kennedy *et al.*, 1958; Wearing, 1967; Wearing & van Emden, 1967; Sidhu & Kaur, 1976; Scriber, 1977, 1979; Gould, 1978; Chandler *et al.*, 1979; Arora & Sidhu, 1982; Brodbeck, 1982; Chadda & Arora, 1982; Lewis, 1982, 1984; Miles *et al.*, 1982a, b; Service & Lenski, 1982; Cates *et al.*, 1983; Mellors & Propts, 1983; Cockfield & Potter, 1986; Price & Clancy, 1986; Watt, 1986; Connor, 1988; McQuate & Connor, 1990), our results suggest that White's hypothesis is untenable as a general explanation for insect outbreaks. The experiments we performed in a controlled environment chamber with rehydrated previously water-deficient foliage were contrived to be a strong test of the hypothesized nutritional effects of water deficits on herbivorous insects. These experiments show that under the very conditions proposed by White to improve the survival of early larval instars (increased foliar amino acid concentrations induced by water deficits), larvae either avoided such foliage or experienced reduced growth and survival. Furthermore, when the physical changes in foliage that accompany water deficits are combined with increased amino acid concentrations, growth and survival were reduced even more.

While the number of studies that have explored the mechanisms that underlie insect responses to plant water deficits is small, none of the studies that suggest that water deficits benefit herbivorous insects have conclusively demonstrated that the observed benefit stems from an improvement in the nutritional quality of the host plant. On the other hand, Waring & Price (1990) have shown that for species of gall-forming Cecidomyidae that preferentially infest water-deficient creosote bush, the cause of the infestation is an increase in the number of oviposition sites brought about by water deficit-induced changes in host plant morphology, not improvement in nutritional quality. This result combined with ours argues that the mechanistic causes of responses by herbivorous insects to water deficits in plants will include changes in plant chemistry, the physical characteristics of plant tissues, the plant microenvironment, and plant morphology, among others (Mattson & Haack, 1987a, b). Without an understanding of how each of the possible consequences of water deficits in plants can impinge on the behaviour and ecology of associated herbivorous insects, it will be difficult to develop a general theory of how insects interact with plants experiencing water deficits. Studies that compare the effects of water deficits on the interactions of several insect species that feed in different ways (chewing, sucking, skeletonizing, galling or mining) on the same host plant, or that examine insects that feed in similar ways on plants with different physiological and morphological responses to water deficits may provide a basis for a general theory of the effects of water deficits in plants on herbivorous insects.

## Acknowledgments

We thank R. Stinner and K. Wilson for their cooperation and guidance in this research, and W. M. Brooks for providing the stock colony of *E. varivestis*. We thank J. McCormick, D. Israel and P. German for technical advice. This paper benefited from careful reading by M. Beck, M. Bowers, C. Sacchi, J. Dooley, M. Taverner, and an anonymous reviewer. This research was supported in part by NSF Grant 80-21779 to EFC, and by a Moore Research Award and an ARCS Fellowship to G.T.M.

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Accepted 24 November 1989