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Insect responses to plant water deficits. I. Effect of water deficits in soybean plants on the feeding preference of Mexican bean beetle larvae

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Abstract. 1. We examined the preference of larvae of the Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae), for foliage of soybean (*Glycine max* [L.] Merr.) grown under several levels of water deficit. Third instar larvae were exposed simultaneously to excised foliage from plants that were either well-watered (control) or had experienced water deficits (treatment).

2. Water-deficient plants were re-watered 12 h prior to initiating feeding trials to eliminate physical differences between control and treatment foliage such as leaf water potential, diffusive resistance, relative water content, and foliage toughness.

3. Examination of the free amino acid contents of re-watered and excised foliage indicated that amino acid concentrations increased markedly in foliage grown under water deficits, and that this increase persisted during the preference tests.

4. Larvae preferred control foliage, but shifted preference to treatment foliage under mild water deficits. When the leaf water potential of water-deficient treatment foliage was lower than −1.13 MPa or when it was more than 0.5 MPa lower than that of control foliage, larvae preferred to feed on foliage from well-watered control plants.

5. The expression of preference for well-watered control foliage was coincident with increases in the concentrations of total free amino acids and individual free amino acids in the water-deficient treatment foliage.

6. These results are inconsistent with White's (1974) hypothesis because Mexican bean beetle larvae avoid plants grown under water deficits that have increased concentrations of free amino acids.

Key words. Plant water deficits, free amino acids, preference tests, water 'stress', soybean, Mexican bean beetle.

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Introduction

One theory, recently proposed as an explanation for insect outbreaks, suggests that water deficits lead to the mobilization of nitrogen making plants more nutritious for herbivorous insects. The improved nutritional quality of the plant leads to increased survival of juvenile insects whose active growth requires higher dietary nitrogen levels than adults. The increased survival of juveniles ultimately leads to marked increases in abundance (White, 1969, 1974, 1976, 1978, 1984). Initial support for this theory was based only on correlations between insect abundance and rainfall anomalies. A mechanistic foundation for White's theory is provided by studies that have documented changes in the concentration of nitrogenous compounds in plants in response to water deficits (Levitt, 1980; Stewart & Larher, 1980; Hanson & Hitz, 1982), along with studies that show that the survival and growth of herbivorous insects depends on the form and concentration of nitrogen in their diet (McNeil & Southwood, 1978; Mattson, 1980).

Subsequent experiments have illustrated that White's theory is tenable for some, but not all, insect–plant interactions. Some studies have shown that the performance of insects and other arthropods improves when reared on plants grown under water deficits (Wearing, 1967; Wearing & van Emden, 1967; Chandler *et al.*, 1979; Brodbeck, 1982; Lewis, 1982, 1984; Cates *et al.*, 1983), while others have shown no effect, a reduction in performance, or avoidance of so-called 'water-stressed' plants (Kennedy *et al.*, 1958; Wearing, 1967; Wearing & Van Emden, 1967; Sidhu & Kaur, 1976; Gould, 1978; Arora & Sidhu, 1982; Brodbeck, 1982; Chadda & Arora, 1982; Miles *et al.*, 1982a, b; Mellors & Propts, 1983; Service & Lenski, 1982; Cockfield & Potter, 1986; Price & Clancy, 1986; Watt, 1986; Connor, 1988). However, only a few of these studies measured concentrations of nitrogenous compounds in conjunction with arthropod growth or preference.

The diversity of responses by insects to plants experiencing water deficits raises questions concerning the generality of White's theory. Water deficits produce a number of changes in plants, in addition to chemical changes, that may affect insect–plant interactions including: a reduction in leaf-water content (Scriber, 1977, 1979), an increase in leaf toughness (Lewis, 1982), and changes in leaf microclimate (Wilson *et al.*, 1982). In order to relate insect preference or performance to changes in leaf chemistry these potentially confounding factors must first be controlled or eliminated. Thus far, most experiments designed to examine the impact of water deficits in plants on herbivorous arthropods, and their putative chemical causes, have failed to rule out these confounding physical factors. This could account in part for the observed diversity of herbivore responses to plants grown under water deficits.

Previous tests of White's theory also suffer from the lack of standardized information on the water status of the host plant. In most of the experiments cited above, water deficits are established by the reduction or cessation of watering, but without direct measures of plant water status, so the severity of the water deficit is not known. This makes it difficult to compare the results of different studies, and to know what chemical or physical changes in foliage were likely to have accompanied the level of water deprivation established in each.

Physiological studies illustrate that a wide variety of plants show similar chemical responses to water deficits (Hsiao *et al.*, 1976), but that the severity of the water deficit determines which chemicals change concentration and the magnitude of the change (e.g. Fukutoku & Yamada, 1981a). Understanding the causes of arthropod responses to water deficits in plants requires that experiments be designed to control either the physical or chemical characteristics of foliage, that levels of water deficits be chosen cognizant of the attendant changes in plant physiology and biochemistry, that the water status of the host plant be monitored directly, and that the chemistry of the foliage be examined.

White's theory requires that insects do not avoid foliage experiencing water deficits. If insects actually prefer such foliage, then chances will be increased that they or their offspring will benefit from whatever nutritional improvement occurs in such foliage. Both Lewis (1982, 1984) and Connor (1988) illustrate that some insects have the behavioural and sensory capability to alter preference for foliage depending on its water status. House (1969) and Lewis (1984) also show that insects can perform better when reared on the foliage they prefer, although this is not always true (Thompson, 1988). Therefore, we suggest that feeding preference tests should be performed preliminary to experiments designed to determine if growth or survival are affected by plant water deficits. Preference tests can also be used for choosing levels of water deficits for subsequent experiments to determine effects on growth, survival, and fecundity.

In order to test White's theory, we examined the feeding preference of third instar Mexican bean beetle larvae (*Epilachna varivestis* Mulsant) for foliage from soybean plants (*Glycine max* [L.] Merr.) grown under well-watered and water deficit conditions. Our experimental protocol was designed to remove physical differences between control and treatment foliage so that effects arising because of chemical differences could be assessed. Plant water status, relative water content, leaf toughness, and free amino acid concentrations were measured on control and treatment plants.

Materials and Methods

Overview of experimental design. Sixty pots with soybean plants were grown in a glasshouse. Treatments were assigned at random to individual pots, so that control and treatment plants were spatially intermingled within the glasshouse. One group was well-watered (control) while water was withheld for varying periods of time from plants in the second group (treatment) to achieve a range of water deficits. Replicates of the preference test were run on consecutive days using foliage excised from plants in different pots on each day. On the afternoon prior to initiating a replicate preference test, one pot with treatment plants was re-watered at 17.00 hours to ensure that the physical characteristics (e.g. leaf water potential, diffusive resistance, relative water content (RWC), and leaf toughness) of the treatment plants would be comparable to that of the control plants by the following morning. This enabled us to remove physical differences between control and treatment foliage so that the cues that determined feeding preference would be largely chemical. Leaves of equal age were excised the following morning from one plant each in the control pot and the rehydrated treatment pot for use in a feeding preference test, to assess plant water status, and for amino acid analysis. Leaf toughness was measured using a separate group of control and treatment plants. To determine feeding preference, paired leaflets from control and rehydrated treatment plants were presented to third instar larvae in choice tests under controlled environmental conditions. A more detailed description of all methods can be found in McQuate (1985).

Growth conditions. Plants. Soybean (*Glycine max* [Merr.] cv. Ransom) seeds were inoculated with *Rhizobium japonicum* and planted four or five to a pot (16 cm × 20 cm) in a soil mix of 2:1 coarse sand and vermiculite. Peter's Hydro-sol fertilizer mixed to provide 50:48:210 ppm N:P:K was added weekly in equal amounts to each pot. $CaCl_2-2H_2O$ was added as a source of calcium. Pots were randomly assigned to control or treatment groups. Water was withheld from the treatment pots beginning between 21 and 32 days after planting until a pot was selected for use in a replicate of the feeding preference test. Plants had not bloomed at the beginning of the treatment period (growth stages v4–v7; Fehr *et al.*, 1971). The soil surface in pots with control plants was kept constantly moist by saturation watering every 5 days.

Insects. Mexican bean beetles (*Epilachna varivestis* Mulsant) were obtained from a microsporidian-free colony maintained at North Carolina State University (Raleigh, N.C., U.S.A.). Upon eclosion, larvae were transferred to a Petri plate containing lima bean leaflets (*Phaseolus lunatus* L.) and moist filter paper. Leaflets were replaced as needed. Larvae were reared in a constant environment chamber at 23°C with a 14:10 day:night photoperiod until the third instar.

Plant water status measurements. Water status measurements were made on each plant to quantify the severity of the water deficit. Measurements were made between 13.00 and 15.00 hours prior to re-watering treatment plants, and at dawn the following morning, just prior to leaf excision. Leaf diffusive resistance was measured using a Li-Cor Li-1600 null balance diffusive porometer. Resistances are expressed in sec/cm with higher positive values indicating more severe water deficits (Beardsell *et al.*, 1972). Leaf water potential was measured using a PMS pressure chamber. Water potentials are expressed as negative numbers in megapascals (MPa), with higher absolute value indicating more severe water deficits. Relative water content (RWC), the proportion of saturated water content, was determined using three 0.86 cm^2 punched leaf discs per leaf. Leaf discs were collected into a sample bottle that had been stored in a desiccator. Further procedures for determining RWC follow those used by Turner *et al.* (1978).

Foliage chemistry. The concentrations of free amino acids in foliage were examined for six control and six treatment plants selected from twelve different pots. Leaves of equal age were excised at dawn on the morning following re-watering treatment plants. A standardized excision time was necessary since free amino acids vary in concentration diurnally in soybeans (Fukutoku & Yamada, 1981b). Leaves were collected into sealed plastic bags over dry ice and stored in a −70°C freezer. Leaves were prepared for amino acid analysis by thawing for 1 h and then removing the midribs. The leaf cell sap was expressed with a plant press. 0.15 ml of cell sap was filtered through a 0.2 μm filter with distilled water added. The filtrate was eluted from a 6 × 1.1 cm Dowex H^+ (200–400 mesh) column with 50 ml of 10% NH_4OH (specific gravity = 0.902). 1.0 ml of the eluent was then evaporated to dryness over gentle heat, under nitrogen. Each sample was then frozen, but analysed within 36 h. After freezing, samples were reconstituted in 0.25 ml of HCl buffer (pH 2.2) and injected in an American Research Products Corporation (ARPCO) HPLC A-52000 amino acid analyser with an ARPCO Fluoro-Tec fluorometric detector. Only the fluorometrically detectable fractions of the total free amino acids were examined. Amino acid concentrations were determined based on peak areas of standards measured with a Hewlett-Packard 3392A integrator. Samples from control and treatment plants paired for preference tests were analysed consecutively.

Leaf toughness measurements. Foliage toughness was measured on control and treatment plants both before and after treatment plants were re-watered to determine if re-watering eliminated differences in toughness between control and treatment plants. Foliage toughness was estimated as the force necessary to penetrate non-vascular tissue with a no. 3 insect pin, the relative yield point force (RYPF). Measurements were made using apparatus fabricated by the authors. Leaves of equal age were selected and excised from eight control and seven treatment plants. RYPF was estimated as the average of three trials on a single leaf. RYPF was measured between 13.00 and 15.00 hours prior to re-watering treatment plants, and on the same plants at 09.00 hours on the following morning.

Feeding preference tests. Soybean leaves of equal age were excised at dawn from eighteen pairs of control and treatment plants from thirty-six different pots. Leaves were placed in moist, sealed, plastic bags for transport to the growth chamber. Leaflets (three per leaf) were transferred to three separate Petri plates. Paired leaflets (one control and one treatment) were placed on filter paper which was kept constantly moist. Six to eight full-sib third instar larvae were added to each Petri-plate, and placed in an environmental chamber with a constant 23°C temperature and a 14:10 day:night photoperiod. 24 h later, larvae were removed and leaflets were dried in a plant press. After drying, the total area consumed from each leaflet was measured using the average of two trials with a Li-Cor Li-3000 leaf area meter. An index of preference was determined for each trial using the following formula:

$$\frac{\text{(control leaf consumption − treatment leaf consumption)}}{\text{(control leaf consumption + treatment leaf consumption)}}$$

Positive values indicate preference for control leaflets and negative values indicate preference for treatment foliage. Data from the three leaflets from each pair of leaves were averaged to produce a single estimate of preference.

Results

Water relations and foliage chemistry

The controlled cessation of watering yielded treatment plants with lower leaf water potentials, lower relative water content, higher diffusive resistances, and greater foliage toughness than control plants (Table 1A). Each of these differences is consistent with the conclusion that the treatment plants were experiencing more severe water deficits than were the control plants. After re-watering the treatment plants, however, no differences between control and treatment foliage in leaf water potentials, relative water content, or toughness were detectable (Table 1B). Because of the high intercorrelation

Table 1. Comparison of the physical characteristics of control and treatment foliage.

A. Prior to re-watering treatment plants

	Control		Treatment		*t*-Tests*		
Character	Mean (SE)	Range	Mean (SE)	Range	*t*	df	*P*
Leaf water potential (MPa)	−0.69 (0.37)	−0.38 to −0.86	−1.19 (0.71)	−0.53 to −1.93	7.89	19	<0.0001
Relative water content (%)	86.6 (1.14)	75.1−91.7	75.4 (3.18)	39.9−96.8	3.56	19	<0.002
Diffusive resistance (s/cm)	0.41 (0.02)	0.29−0.62	8.74 (2.33)	0.42−40.9	3.56	19	<0.002
Leaf toughness (g)	66.3 (0.75)	63.0−70.0	68.6 (0.49)	67.2−78.9	2.59	13	<0.025

B. 12 h after re-watering treatment foliage, just prior to beginning preference tests

	Control		Treatment		*t*-Tests*		
Character	Mean (SE)	Range	Mean (SE)	Range	*t*	df	*P*
Leaf water potential (MPa)	−0.28 (0.21)	−0.14 to −0.44	−0.31 (0.24)	−0.12 to −0.49	1.04	17	>0.3
Relative water content (%)	97.3 (0.40)	92.9−100.0	97.4 (0.32)	94.1−99.4	0.14	18	>0.9
Leaf toughness (g)	76.0 (0.63)	74.3−79.8	75.1 (0.44)	74.2−77.5	1.14	13	>0.1

* All *t*-tests are paired *t*-tests except for differences in leaf toughness.

between the different measures of leaf water status, diffusive resistances were not measured after re-watering.

Concentrations of the eleven amino acids detected were higher in foliage from treatment plants than in foliage from control plants even after re-watering the treatment plants (Table 2). The total concentration of amino acids in foliage from re-watered treatment plants was 26.0% higher on average than in control plants, and the concentrations of individual amino acids increased by between 0.5% and 650.9% in treatment plants. However, because the treatment foliage was subjected to a range of water deficits including very slight water deficits, the observed increases in concentration were not statistically significant (Table 2). Regression analyses indicated that a significant portion of the variation in the total amino acid concentration in foliage was attributable to either the water potential, the diffusive resistance, or the relative water content of the foliage prior to re-watering (Table 3). When the amino acids were examined individually, the concentrations of five out of eleven were inversely correlated with leaf water potential and seven out of eleven were inversely correlated with relative water content, both water status indices being measured prior to re-watering. These results suggest that, although re-watering removed differences in the physical characteristics of treatment and control foliage, chemical differences persisted.

Feeding preference

The feeding preference of third instar Mexican bean beetles was closely related to the difference in leaf water potential, leaf diffusive resistance, and relative water content of control and treatment foliage as measured prior to re-watering treatment plants. Fig. 1 shows that, as the difference between control and treatment foliage in leaf water potential, diffusive resistance, and relative water content increased, an increasing proportion of feeding occurred on control foliage. In other words, larvae preferred to feed on control foliage except when treatment foliage had been exposed to mild water deficits. Preference was positively and linearly correlated with the difference between control and treatment leaf water potential ($r=0.56$, $F_{1,16}=7.43$, $P=0.015$) and the difference in relative water content ($r=0.63$, $F_{1,16}=10.46$, $P=0.0052$). Preference was also negatively and curvilinearly related to the difference between control and treatment leaf diffusive resistance (multiple $r=0.74$, $F_{2,15}=9.00$, $P=0.0027$).

Larvae preferred to feed on treatment foliage when its water potential, prior to re-watering, was greater (less negative) than −1.13 MPa or

Table 2. Comparison of amino acid concentrations in control and re-watered treatment foliage (μmol/ml).

Amino acid	N	Control mean (SE)	Treatment mean (SE)	Increase (%)	t-Tests* t	df	P
Total	6	5.1672 (0.378)	7.0296 (1.027)	36.0	1.93	5	0.056
'A'[†]	5	0.4040 (0.021)	0.5722 (0.122)	41.6	1.43	4	0.114
Aspartic acid	6	2.8805 (0.391)	3.5398 (0.504)	22.9	1.92	5	0.056
Serine	5	0.2924 (0.042)	0.3458 (0.025)	18.3	1.43	4	0.114
Glutamic acid	5	0.0416 (0.007)	0.0418 (0.012)	0.5	0.02	4	0.494
'B'[‡]	6	0.6778 (0.034)	0.8492 (0.076)	25.3	2.22	5	0.039
Valine	6	0.1892 (0.014)	0.4215 (0.180)	122.8	1.29	5	0.127
Methionine	4	0.0055 (0.004)	0.0413 (0.023)	650.9	1.48	4	0.113
Isoleucine	6	0.0750 (0.012)	0.3663 (0.221)	388.4	1.35	5	0.118
Leucine	6	0.2500 (0.009)	0.5813 (0.253)	132.5	1.25	5	0.267
Tyrosine	3	0.1177 (0.007)	0.2830 (0.156)	140.4	1.11	2	0.191
Lysine	6	0.2613 (0.040)	0.2730 (0.021)	4.5	0.22	5	0.417

* All t-tests are paired t-tests.

[†] Amino acid 'A' represents a composite of threonine and asparagine.

[‡] Amino acid 'B' represents a composite of glycine and alanine.

when it was no more than 0.5 MPa lower than that of control foliage. When the water potential of treatment foliage, prior to re-watering, decreased below −1.13 MPa or was more than 0.5 MPa lower than that of control foliage, preference shifted to well-watered control foliage. Feeding preference was not related to any measure of leaf water status taken at the time of excision because no differences in any measure of water status remained (Table 1 and McQuate, 1985).

A strong preference for control foliage was displayed when the total free amino acid concentration in rehydrated treatment foliage substantially exceeded that of control foliage. Linear and second order polynomial regressions accounted for 65.9% and 97.7% respectively, of the variation in preference as a function of

Table 3. Correlations between amino acid concentrations and water status measurements.

A. *r* prior to re-watering treatments plants

Amino acid	*N*	Leaf water potential	Relative water content	Diffusive resistance
Total	12	−0.79**	−0.85***	0.63*
'A'†	11	−0.76**	−0.66*	0.73*
Aspartic acid	12	−0.45	−0.59*	0.61*
Serine	11	−0.43	−0.32	0.35
Glutamic acid	11	−0.28	−0.29	−0.15
'B'‡	12	−0.55	−0.38	0.08
Valine	12	−0.76**	−0.77**	0.39
Methionine	10	−0.83**	−0.67*	0.27
Isoleucine	12	−0.77**	−0.82***	0.47
Leucine	12	−0.76**	−0.78**	0.39
Tyrosine	8	−0.50	−0.76*	0.62
Lysine	12	−0.37	−0.36	0.13

B. *r* 12 h after re-watering treatment plants, just prior to beginning preference tests

Amino acid	*N*	Leaf water potential	Relative water content	Diffusive resistance§
Total	12	−0.008	−0.225	–
'A'†	11	0.424	−0.482	–
Aspartic acid	12	−0.091	−0.519	–
Serine	11	0.333	−0.482	–
Glutamic acid	11	−0.407	0.412	–
'B'‡	12	0.149	0.637*	–
Valine	12	−0.047	0.112	–
Methionine	10	0.133	0.319	–
Isoleucine	12	−0.065	0.047	–
Leucine	12	−0.037	0.101	–
Tyrosine	8	0.081	0.216	–
Lysine	12	0.044	−0.081	–

*$P<0.05$; **$P<0.01$; ***$P<0.001$

† Amino acid 'A' represents a composite of threonine and asparagine.

‡ Amino acid 'B' represents a composite of glycine and alanine.

§ Diffusive resistances were not measured after re-watering.

differences between control and rehydrated treatment foliage in total amino acid concentration ($F_{1,4}=7.73$, $P<0.05$ and $F_{2,3}=64.05$, $P<0.005$, respectively, and Fig. 2). Larvae preferred to feed on rehydrated treatment foliage only when the total free amino acid concentration was no more than 0.5 μmol/ml greater than control foliage.

Feeding preference was negatively correlated with the difference between control and rehydrated treatment foliage in the concentration of seven out of eleven individual amino acids. Aspartic acid and amino acid 'A', a combination of threonine and asparagine, displayed the highest correlations with feeding preference

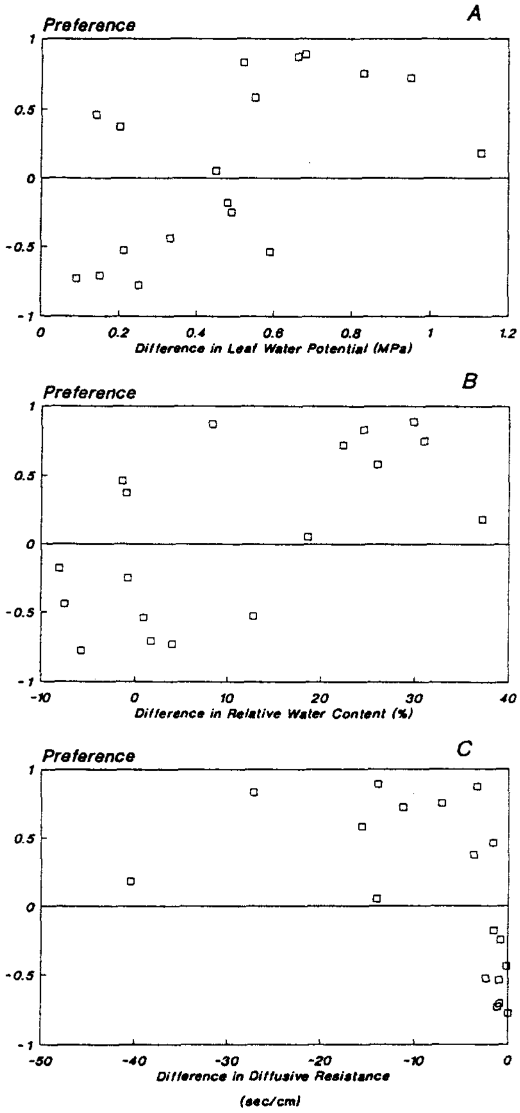

Fig. 1. Relationship between feeding preference and the difference between control and treatment foliage (control minus treatment) in three measures of water status prior to re-watering treatment plants. Positive values of preference indicate that a higher proportion of feeding occurred on control foliage, and negative values indicate that a higher proportion of feeding occurred on treatment foliage. (A) Difference in leaf water potential – higher positive values of difference in leaf water potential indicate that treatment plants were grown under increasingly more severe water deficits when compared to control plants. (B) Relative water content – higher positive values of difference in relative water content indicate that treatment plants were grown under increasingly more severe water deficits when compared with control plants. (C) Diffusive resistance – more negative values of difference in diffusive resistance indicate treatment plants were grown under increasingly more severe water deficits when compared to control plants. Note that preference shifts to control foliage as the severity of the water deficit to which treatment foliage was subjected increases.

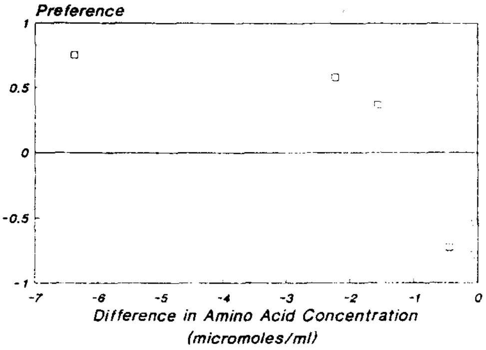

Fig. 2. Relationship between feeding preference and the difference between control and rehydrated treatment plants (control minus treatment) in the total concentration of free amino acids. Positive values of preference indicate that a greater proportion of feeding occurred on control foliage, and negative values of preference indicate that a greater proportion of feeding occurred on treatment foliage. Negative values indicate that the concentration of amino acids in treatment foliage was greater than in control foliage. Note that preference shifts to control foliage when the concentration of amino acids in treatment foliage exceeds that of control foliage by more than 0.5 μmol/ml.

(r=0.79, n=6, P=0.064 and r=0.83, n=5, P=0.085, respectively).

Discussion

Mexican bean beetle larvae discriminate between foliage from plants grown under well-watered conditions and plants grown under water deficits. When treatment foliage had been subjected to very mild water deficits (leaf water potentials greater than −1.13 MPa or less than 0.5 MPa below control plants) larvae preferred to feed on treatment foliage. However, when treatment foliage had been subjected to more severe water deficits, larvae preferred to feed on well-watered control foliage.

The protocol of re-watering treatment plants 12 h prior to initiating preference tests was sufficient to remove differences between control and treatment foliage in leaf water potential, relative water content, and leaf toughness. At the time foliage was presented to larvae, no relationship between preference and any estimate of plant water status was detected. Feeding preference was correlated with the leaf water potential, diffusive resistance, and the relative water content of foliage on the day preceding the preference test, prior to re-watering treatment foliage. Therefore, we conclude that physical differences between control and treatment foliage were not the proximal cues causing the observed pattern of feeding preference. However, our experiment could not eliminate possible differences in cell size, the proportion of cell wall material, or the density of trichomes that may arise in foliage grown under water deficits. Hsiao *et al.* (1976) report that the foliage of plants grown under water deficits displays reduced cell growth and a decrease in the synthesis of cell wall material.

On the other hand, chemical differences between control and treatment foliage were detected at the time the preference tests were begun. Treatment foliage had higher concentrations of the eleven amino acids detected and a higher total free amino acid concentration. The total concentration of free amino acids was correlated with each estimate of plant water status made prior to re-watering treatment foliage, but was not related to water status at the time the preference tests were begun. Several individual amino acids were also correlated with estimates of plant water status prior to rewatering treatment foliage, but not at the time the preference tests were begun. We interpret these results to indicate that the increased concentration of free amino acids in treatment foliage was due to the experimental imposition of water deficits.

Feeding preference was correlated with the difference in the total free amino acid concentration between control and rehydrated treatment foliage. When the total amino acid concentration in treatment foliage exceeded that of control foliage by 0.5 μmol/ml or more, larvae preferred to feed on control foliage. The concentrations of only two individual amino acids of eleven showed strong correlations with feeding preference. While we cannot conclude that the total concentration of free amino acids or the concentration of individual amino acids is the proximal cue leading to discrimination between control and treatment foliage, we do believe that discrimination is based on chemical differences in foliage caused by water deficits, and that the physical characteristics of foliage play no role. However, under natural conditions where larvae must select foliage that differs both in physical and chemical characteristics, physical differences may also be important in foliage selection.

The use of excised foliage in this experiment requires that one assume that the chemical changes that occur in foliage after excision are not a function of the water status of the foliage prior to excision. Otherwise, the results presented here could be an artefact of excision. Put another way, whatever chemical changes occur in foliage after excision must occur equally in the control and rehydrated treatment foliage. While we have no chemical data to support this assumption, we believe that the protocol of watering treatment plants 12 h prior to excision should minimize the potential for an interaction between excision and water status.

Exactly which chemicals serve as the proximal cues leading to discrimination between well-watered and water-deficient foliage is not known. Many different chemical changes may occur in soybeans in response to water deficits (Fukui & Ojima, 1957; Hsiao *et al.*, 1976; Jones *et al.*, 1980; Fukutoku & Yamada, 1981a, b; Ford, 1984; McQuate, 1985; McQuate *et al.*, 1986; Brodbeck & Strong, 1987; Mattson & Haack, 1987a, b). The results presented here show that increases in the concentrations of free amino acids accompany a shift in feeding preference from water-deficient foliage to well-watered control foliage. Since a diet which supports optimal growth must provide proper proportions and quantities of both essential and non-essential amino acids (Rock, 1972), the observed increases in free amino acid concentration in water-deficient foliage could inhibit feeding or lead to a nutritional imbalance if feeding continues. Hughes (1988) has shown that *E.varivestis* prefer to feed on foliage exposed to SO_2 pollution because of an increased concentration of glutathione, a combination of glycine, glutamic acid and cystine. Of these three amino acids, only glycine was observed in higher concentration in water deficient foliage. Further study, employing artificial diets or chemicals applied to leaf discs, will be necessary to determine if the concentration or composition of free amino acids play a causal role in feeding preference.

Epilachna varivestis prefers rehydrated foliage grown under water deficits only when the water deficit is very mild (leaf water potential greater than −1.13 MPa). This preference is difficult to attribute to increases in the concentrations of free amino acids in treatment foliage since at this level of water deficit the maximum increase in the total concentration of free amino acids that we observed is only 0.5 μmol/ml. However, because leaf enlargement is inhibited at higher leaf water potentials than is photosynthesis, there may be photosynthate accumulation under conditions of mild to moderate water deficits (Boyer, 1970). This would probably include an increase in sucrose concentration, since sucrose is the predominant compound used to translocate photoassimilated carbon from sites of photosynthesis to sink organs in soybeans (Kouchi & Yoneyama, 1984).

The presence of sucrose has been shown to increase consumption rates of adult Mexican bean beetles (Jones *et al.*, 1981), and Kogan (1971) has shown that the growth rates of fourth instar larvae are affected by sucrose concentration. An increase in sugar concentration has been reported for soybeans grown under water deficits, but the magnitude of the deficit associated with the increased sugar concentration was not reported (Fukui & Ojima, 1957). However, sucrose concentration does not increase significantly in soybeans grown under severe water deficits (Ford, 1984).

The photosynthate accumulated under water deficits may also be shunted into the production of secondary compounds (Gershenzon, 1984). If the concentration of secondary compounds increases in plants experiencing water deficits then insects may chose not to feed on water-deficient foliage. Therefore, an increase in the concentration of secondary compounds may be the cause of the pattern of feeding preference displayed by *E.varivestis*. Further experiments are necessary to identify the chemical changes in water-deficient foliage that are responsible for *E.varivestis*' pattern of feeding preference.

The fact that larvae of *Epilachna varivestis* discriminate between foliage from well-watered and water-deficient plants is consistent with experiments performed by Lewis (1982, 1984) with *Melanoplus differentialis* (Thomas) (Orthoptera: Acrididae) and by Connor (1988) on *Corythucha arcuata* (Say) (Heteroptera: Tingidae). *Melanoplus differentialis* prefers to feed on previously wilted foliage of sunflower (*Helianthus annuus* L.) whether or not it has been rehydrated. The level of water deficit that Lewis (1982) used to achieve wilting appears to be comparable to the most severe water deficits used in our study. Therefore, her results show that *M.differentialis* prefers to feed on foliage grown under more severe water deficits than *E.varivestis* prefers. *Corythucha arcuata* shows no preference at all when presented foliage from well-watered *Quercus alba* L. and foliage rehydrated following removal from plants grown under water deficits. However, *C.arcuata* does prefer to feed on well-watered foliage and foliage grown under very mild water deficits when foliage is not rehydrated. These results illustrate that some insects have the ability to perceive and respond to differences in foliage caused by growth under conditions of water deprivation, but that the insect's response and the proximal cues used in foliage selection vary among insect species.

The implications of these results for White's (1974) theory are twofold. First, while preference for foliage grown under water deficits is not a necessary component of White's (1974) theory, its presence increases the likelihood that plant water deficits can benefit insects nutritionally. We have demonstrated that amino acid concentrations increase in foliage grown under water deficits, but that these increased concentrations are associated with avoidance of drought grown foliage by Mexican bean beetle larvae. This is inconsistent with White's (1974) theory, since he implicated the mobilization of nitrogen in the form of free amino acids as the likely cause of the hypothesized nutritional benefit. Second, Mexican bean beetle larvae do prefer rehydrated foliage that had been grown under mild water deficits. Thus, it remains conceivable that a nutritional benefit could be obtained from feeding on foliage grown under mild water deficits. McQuate & Connor (1990) present experiments designed to determine if larvae reared on foliage grown under mild water deficits grow faster, grow larger, or survive better than larvae reared on well-watered foliage.

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