Impact of Drought Stress on the Expression of Resistance to *Verticillium albo-atrum* in Alfalfa

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ABSTRACT

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Drought stress alters plant metabolism, causing changes in growth, hormone levels, photosynthesis, and nutrient uptake. *Verticillium albo-atrum* reduces growth and stomatal conductance in resistant alfalfa clones in the absence of abiotic stress. The additional effect of drought stress on the growth of resistant alfalfa infected with *V. albo-atrum* is unknown; consequently, experiments were conducted to assess the stability of resistance to *V. albo-atrum* in alfalfa grown under the stress of drought. Two resistant alfalfa clones were grown in the greenhouse in 0.03-m^3 cylinders that allowed the development of a gradually increasing drought stress. Plants were stubble-inoculated with *V. albo-atrum* and grew for 6 wk before drought stress was imposed. Effect of the combined stresses was measured over the next two 6-wk growing periods, with measurements taken at harvest during the second growth period and weekly during the third growth period. The experiment was repeated. Plant height, leaf and stem dry weight, and aerial biomass were significantly reduced by drought during both the second and third growth periods. *V. albo-atrum*-infected plants were shorter and had more stems, fewer internodes, and reduced stem and aerial biomass at the second harvest. Only height and flowering were reduced in infected plants during the third growth period. Significant pathogen × clone interactions indicated that the two clones differed in sensitivity to *V. albo-atrum*. Significant pathogen × water interactions in disease rating and stem dry weight demonstrated that stem dry weight was less affected by the pathogen, and fewer symptoms were present under drought stress than under nondrought-stressed conditions. *V. albo-atrum* had no consistent significant effect on stomatal conductance but did alter leaf water potential. The absence of pathogen × water interactions for most of the growth parameters and the decreased effect of the pathogen on stem dry weight under drought stress indicated that resistance to *V. albo-atrum* in alfalfa is stable under drought stress.

Additional keywords: lucerne, *Medicago sativa*, vascular wilt.

Pathogen-induced water deficit has been implicated in vascular wilt diseases caused by fungi (7–10,19,41) and is undoubtedly responsible for some of the symptoms associated with these diseases. Epinasty, leaflet rolling, desiccation, and leaf drop are symptoms common to both abiotically-induced drought stress and vascular wilt diseases (39). Increased hydraulic resistance was noted in tomato infected with *Fusarium oxysporum* f. sp. *lycopersici* (8,9), and reduced transpiration occurred in response to infection by *Verticillium albo-atrum* Reinke & Berthier (34). A reduction in vascular flow was detected in tomato infected with *V. albo-atrum* (38), confirming the earlier hypothesis (34) that reduced transpiration was due to reduced water supply to the leaves.

Abiotically-induced drought stress reduces net photosynthesis and alters plant water relations, reducing stomatal conductance and leaf water potential (ψ) (3,14). The reduced stomatal conductance noted in cotton (41) and chrysanthemum (19) infected with *V. dahliae*, in tomato infected with *F. o. lycopersici* (10), and in alfalfa infected with *V. albo-atrum* (32) was evidence of biotically-induced drought stress. In addition, inhibition of vessel element development, which would increase hydraulic resistance, was noted in susceptible alfalfa infected with *V. albo-atrum* (30).

Although water deficit stress has been demonstrated in plants infected with vascular wilt fungi, little is known about the effect of the additional stress of abiotically-induced drought on the host/pathogen system. Environmental factors could alter resistance (35), and the combined effects of biotic and abiotic drought stress may be additive and result in permanent alteration of the host (2). *V. dahliae* had a reduced effect on transpiration and stomatal conductance when potato plants were drought stressed (13), but *Verticillium* was more aggressive in drought-stressed yellow poplar (22). Resistant wheat plants became susceptible to *Fusarium roseum* f. sp. *cerealis* when their ψ dropped below −3.5 MPa (24). Vascular wilt fungi have the ability to grow at greatly reduced ψ with substantial growth of *V. albo-atrum* being reported at ψ as low as −9.0 MPa (4).

Although biotically-induced drought stress occurs in susceptible plants infected with *Verticillium* (19,41), the situation is less clear in infected resistant plants. Wilhelm et al (45) noted that true immunity to *V. dahliae* does not exist in cotton and felt that resistance to *V. dahliae* could break down in an environment that favored the pathogen rather than the host. Resistant alfalfa plants are not immune to *V. albo-atrum* (31). Growth suppression and transient reduction in stomatal conductance was noted in resistant, infected alfalfa (32), but no reduction in net photosynthesis was detected in infected young leaves (28). Thus, there is evidence that resistant alfalfa infected with *V. albo-atrum* experiences some biotically-induced drought stress. Little is known, however, of alfalfa's response to *V. albo-atrum* under the additional stress of abiotically-induced drought. Dry soil does

not inhibit infection of alfalfa by *V. albo-atrum* (17), and Panton (23) observed greater symptom expression in *V. albo-atrum*-infected alfalfa following a period of limited precipitation.

The objective of this study was to assess the stability of resistance to *V. albo-atrum* in alfalfa subjected to abiotically-induced drought stress. Drought stress develops gradually in the field, allowing plants an opportunity to acclimate to the stress (16). A gradually intensifying abiotic drought stress was imposed during this study to allow the plants to express drought acclimation mechanisms that might influence plant response to the combined biotic and abiotic stresses.

MATERIALS AND METHODS

Plant material, growth containers, and inoculation method. A portion of this study was previously reported, and the plant material, growth containers, method of imposing drought stress, and inoculation method were described (32,33). Two resistant alfalfa (*Medicago sativa* L.) clones (clone 1079 and clone WL-5) were used in this study to minimize the variation associated with the genetic heterogeneity of alfalfa plants grown from seed (32). Six-week-old plants were inoculated with *V. albo-atrum* by placing a 20-μl drop of spore suspension (3.65×10^6 spores/ml) on the end of each cut stub. Plants were cut immediately before inoculation to assure similarly aged infection courts and were kept in a mist chamber for 24 h after inoculation and before being placed in the greenhouse. Control plants were treated similarly with water but were not put in the mist chamber to minimize the possibility of cross-contamination. Seven plants of an extremely susceptible clone (selected from cultivar DK-131) were inoculated, solely to verify the pathogenicity of the *V. albo-atrum* isolate. All plants grew for 6 wk following inoculation, before being cut to 4 cm in height and subjected to drought stress.

The effect of drought stress on the growth of infected, resistant clones was assessed in the greenhouse in polyvinyl chloride growth containers, 20 cm in diameter and 90 cm in height, that allowed development of large root systems and gradually intensifying drought stress. Plants were subjected to two drought episodes, one during the second 6-wk growth period and the other during the third 6-wk growth period. Watering was based on tensiometer readings. All nondrought-stressed plants were watered when the first tensiometer located 30 cm from the soil surface reached −0.04 MPa, whereas drought-stressed plants were not watered until the first tensiometer located 67 cm from the soil surface reached −0.08 MPa.

The potting mix was formulated to induce drought stress within the working range (−0.01 to −0.08 MPa) of soil tensiometers. The potting mix in each 0.03-m^3 container was amended with 160 g of 13%N-13%P-13%K slow-release fertilizer (Osmocote, Mallinckrodt, Inc., St. Louis, MO), 90 g of gypsum, and 67 g of Esmigran slow-release micronutrients (Sierra Chemical Co., Milpitas, CA) to ensure adequate mineral nutrition for long-term growth and to minimize the effect of drought on nutrient availability. The plants were fertilized with nitrogen rather than inoculated with *Rhizobium meliloti* to eliminate the effect of drought on nitrogen fixation and to insure a uniform level of nitrogen fertility among experimental units.

The experiment was conducted during the winter months (from November to March) to avoid the confounding influence of high-temperature stress on both drought and the expression of resistance to *V. albo-atrum* (18). Day length was extended to 14 h with 400-watt metal halide lamps. The lamps supplied an additional mean of 116 (range 60–155) μmol $m^{-2}s^{-1}$ of photosynthetically active radiation (PAR) at soil level and a mean of 1,842 (range 590–3,370) μmol $m^{-2}s^{-1}$ of PAR at 100 cm above soil level. Quantum flux was monitored with LICOR Line Quantum Sensors (LICOR, Lincoln, NE).

The experiment was conducted in 1988 and was repeated in 1989. In 1988, temperatures (monitored with a hygrothermograph) ranged between 19 and 25 C for 54.5% (424 of 778 h) of the second growth period and 52% (414 of 790 h) of the third growth period. In 1989, temperatures were in this range for 50% (386 of 772 h) and 44% (354 of 798 h) of the respective growth periods and were above 25 C for 6.5 and 9% of the two growth periods.

Plant measurements and pathogen isolations. The plants were cut to a height of 4 cm every 6 wk following inoculation for a total of three harvests. Isolations for *V. albo-atrum* were conducted at the first harvest, as previously described (32). The first drought episode was imposed during the second growth period, and the effect of the combined stresses of drought and *V. albo-atrum* was assessed at harvest. Data collected for each plant included disease rating (1 = no symptoms, 2 = 1 or 2 chlorotic leaflets, 3 = leaflets on more than one shoot chlorotic, 4 = most of the leaflets chlorotic, and 5 = dead) (12); height of the tallest stem; number of internodes on the tallest stem; number of stems; presence or absence of flowering; and leaf, stem, and total aerial (leaf + stem) dry weight after drying for 48 h at 70 C.

A second drought episode was imposed during growth period three. Growth and disease ratings were assessed weekly during the third growth period on one plant removed from each growth container each week. The plants were severed below the crown to prevent regrowth. One plant remained in each container at the end of the experiment, insuring a measure of plant competition throughout the experiment.

V. albo-atrum was isolated from a subsample of stems selected from each plant in two replications on a weekly basis during drought episode two (growth period three). The stems selected for colonization analysis were diagrammed on paper, surface-sterilized in 0.525% sodium hypochlorite for 5 min, drained on paper towels, and aseptically divided into serial, 15-mm-long pieces. The stem pieces were placed on 2% water agar, incubated for 10 days at room temperature (approximately 25 C), and then examined for conidiophores of *V. albo-atrum*. Stem sections from which the pathogen was isolated were noted on the respective stem diagrams.

Isolation procedures prevented inclusion of a subsample of stems in dry weight determinations. However, the following protocol allowed analysis of whole-plant growth. The plant minus the subsample will be termed the crop growth analysis sample (CGA). The fresh weight of the CGA sample was determined, and leaves and stems were separated, dried at 70 C for 48 h, and weighed. Subsample fresh weight was determined, stems were used for isolations, and leaves were dried and weighed. The dry weight of the entire sample was then determined mathematically by solving the equation:

$$\text{total dry weight} = (A \times B) \div C$$

where A = CGA leaf dry weight + CGA stem dry weight, B = CGA fresh weight + subsample fresh weight, and C = CGA fresh weight. Total leaf dry weight was determined by adding the dry weight of the CGA leaves to that of the subsample leaves. Total stem dry weight was found by subtracting total leaf dry weight from total plant dry weight.

Stomatal conductance was measured with a LI-1600 Steady State Porometer (LICOR, Lincoln, NE). Variation due to stomatal response to quantum flux was reduced by measuring similarly aged leaves with similar orientation to the sun. Stomatal conductance was measured in 1989, in weeks 3 and 4 of growth period two, and six times a day on a weekly basis during growth period three. Stomatal conductance was measured less frequently in 1988 due to the limited availability of the porometer.

A pressure bomb (PMS, Corvallis, OR) was used to measure leaf water potential (ψ). Leaves were encased in cellophane tape before removal from the plant to prevent the immediate loss of moisture that occurs when the petiole is severed (21). Leaf ψ was measured twice during drought episode one (weeks 3 and 4) at seven intervals between 0600 and 2200 h to determine diurnal leaf ψ curves. Leaf ψ was also measured between 1300 and 1500 h in weeks 3–6 of the second drought episode in growth period three.

The level of drought stress was monitored during both drought episodes by daily measurement of growth-medium water

potentials and weekly stomatal conductance and leaf ψ measurements.

Experimental design and statistical analysis. The influence of drought stress on resistance to *V. albo-atrum* was tested in a $2 \times 2 \times 2$ factorial experiment arranged in a randomized complete block. The factors were: pathogen (inoculated and noninoculated), water (drought stress and no drought stress), and two resistant clones. There were five replications of the eight treatments, giving a total of 40 experimental units. Each growth container was an experimental unit and contained seven plants.

Growth data and disease ratings from each year were pooled over years because error variances were homogeneous. The three factors were grouped under the single variable, treatment, and subjected to an analysis of variance, using the general linear models (GLM) program of SAS (SAS Institute Inc., Cary, NC), to test for year $\times$ treatment interactions. In addition, orthogonal contrasts were used to evaluate the effects of pathogen, water, and clone and all interactions of these factors. All the factorial effects, including interactions, were evaluated over weeks, using orthogonal polynomials to determine the best model for generating predicted response curves. The predicted response curves were generated using the least squares means when the orthogonal polynomials indicated significance.

When the year $\times$ treatment interaction was significant, an additional analysis of variance was done using all single-degree-of-freedom contrasts to determine the factor or factors causing the year $\times$ treatment interaction. Data from the physiological tests were not combined over years, because of the limited amount of data available from the first experiment. Physiological data were subjected to an analysis of variance. The diurnal leaf ψ curves were subjected to analysis of variance with orthogonal polynomials.

When interactions were detected, the mean square of the interaction was compared to the mean square of the main effect. When the main effect mean square was greater than the interaction mean square, indication of an interaction of degree rather than direction, both interaction and main effects data are presented.

RESULTS

Isolations. The highly susceptible clone of alfalfa, inoculated to check pathogenicity of the *V. albo-atrum* isolate, showed severe symptoms of Verticillium wilt after 2 wk. All plants in the factorial experiment were cultured for *V. albo-atrum* at the first harvest to determine the efficiency of the inoculation. *V. albo-atrum* was isolated from 84% (118/140) of the plants inoculated in 1988 and from 83% (116/140) of the inoculated plants in 1989. The pathogen was isolated from inoculated plants in each experimental unit and was not isolated from any noninoculated plants.

TABLE 1. Results of the ANOVA of data pooled over years showing the main-effect means of pathogen (*V. albo-atrum* and no *V. albo-atrum*), water (drought stress and no drought stress), and clone (clones 1079 and WL-5) on the growth of resistant alfalfa at harvest 2, following drought episode one

	Treatments[y]					
	Pathogen		Water		Clone	
Plant[x]	No Vaa	Vaa	Wet	Dry	1079	WL-5
Disease rating	1.1	1.5 a	1.2	1.3 a	1.5	1.1 a
Height (cm)	83.5	77.2 a	86.3	74.5 a	80.8	79.9
Stem dry wt (g)	2.3	2.1 d	2.5	1.9 a	2.2	2.1
Leaf dry wt (g)	1.4	1.3	1.5	1.2 a	1.3	1.4
Aerial biomass (g)	3.6	3.3 d	4.0	3.0 a	3.5	3.4
Stem number	8.3	8.9 c	8.7	8.5	10.6	6.6 a
Internode number	13.1	12.8 d	13.0	12.8 d	12.9	12.9
Flowering (%)[z]	70.9	58.1 b	61.3	67.7	88.9	40.0 a

[x]Means are the average of 40 values.
[y]a = Treatment significant at $P = 0.0001$; b = treatment significant at $P = 0.005$; c = treatment significant at $P = 0.01$; d = treatment significant at $P = 0.05$.
[z]Percentage of total number of plants.

Serial isolations conducted during drought episode two (growth period three) (Fig. 1) show a flush of colonization beginning at week 5 in the nondrought-stressed clone 1079 but not in clone WL-5. Colonization began 1 wk earlier in the drought-stressed inoculated clone 1079. The pathogen was recovered from clone WL-5 sporadically throughout the study.

Growth analysis: Drought episode one. Growth data and disease ratings were collected at harvest and were analyzed over years. A pathogen $\times$ clone interaction ($P = 0.0001$) was detected in disease rating. The significant interaction was due to clone 1079 having a disease rating of 1.8, whereas clone WL-5 had a disease rating of 1.1. In both cases, the disease ratings were well within the range (<3.0) for alfalfa considered resistant to *V. albo-atrum*. No other interactions were detected.

Plants subjected to drought stress exhibited suppressed growth as did *V. albo-atrum*-infected plants (Table 1). Clonal differences were significant for stem number, flowering, and disease rating.

Growth analysis: Drought episode two. Growth data and disease ratings were collected weekly during growth period three and were analyzed over weeks as well as years. Significant year $\times$ clone (Fig. 2) and year $\times$ water interactions (Fig. 3) were detected, but *V. albo-atrum* affected plant growth similarly in both years.

Pathogen $\times$ water interactions were detected in disease rating and stem dry weight. Disease rating was lower when the plants were under drought stress (Fig. 4A) but was in the range considered resistant even under nondrought-stressed conditions. *V. albo-atrum* caused significantly greater suppression of stem dry weight under nondrought-stress conditions than under drought-stress conditions (Fig. 4B).

The two clones of alfalfa, although both resistant to *V. albo-atrum* on the basis of disease rating, showed different responses to the pathogen. Significant clone $\times$ pathogen interactions were detected in disease rating, height, and stem number and in the percent of plants flowering (Fig. 5A–D). As was the case at harvest two (drought episode 1), clone 1079 had a higher disease rating than did clone WL-5 (Fig. 5A). *V. albo-atrum* caused greater suppression of height in clone WL-5 than in clone 1079 (Fig. 5B), whereas stem number was increased in the clone 1079 and not affected in clone WL-5 in response to the pathogen (Fig. 5C). Infected plants of the early flowering clone 1079 showed less suppression of flowering than did plants of the later flowering clone WL-5 (Fig. 5D).

Drought stress significantly reduced disease rating and suppressed height (Fig. 6A and B) and stem, leaf, and aerial biomass (Fig. 7A–C) over the 6-wk growth period. Clonal differences were also detected (Fig. 6 and 7). *V. albo-atrum*-infected plants had a significantly higher disease rating, reduced height, and a lower percentage of plants flowering than did the noninoculated plants (Fig. 6A–C).

Physiological parameters. Stomatal conductance (data not shown) of plants in the drought-stress treatment was significantly less than that of the nondrought-stressed plants in weeks 3, 5, and 6, irrespective of growth period, and reflected the decreasing soil ψ (Fig. 8). Differences in stomatal conductance due to *V. albo-atrum* were detected inconsistently, and may have been obscured by the large suppressive effect of drought on stomatal conductance.

Analysis of diurnal leaf ψ curves from the first drought episode (growth period two) showed a significant pathogen $\times$ water interaction during week 3 (Fig. 9A). The infected drought-stressed plants had higher leaf ψ throughout the day than did the noninfected drought-stressed plants. Conversely, the leaf ψ of the infected nondrought-stressed plants was lower than that of the noninfected plants in the morning but higher in the afternoon. Significant pathogen $\times$ water $\times$ clone interactions were also detected during week 3 and week 4 (Fig. 9B and C). The drought stress was severe enough by week 3 to cause significant differences in leaf ψ between the water treatments. Additionally, the two clones responded differently to the additional stress of *V. albo-atrum* depending on the water treatment (Fig. 9B). Under nondrought-stressed conditions, both inoculated clones developed lower leaf ψ than the respective nondrought-stressed control plants

during the morning and higher leaf ψ than the controls during the afternoon. The pathogen × water × clone interaction was caused by failure of inoculated, drought-stressed clone 1079 to follow the pattern established under nondrought-stressed conditions. Rather than developing a lower leaf ψ than the control as did inoculated, drought-stressed WL-5 during the morning, clone 1079 had a higher leaf ψ than the noninoculated, drought-stressed control plant until 1600 h.

The influence of the drought-stress treatment was very evident in leaf ψ by week 4 (Fig. 9C). The effect of *V. albo-atrum* was apparent in the nondrought-stressed, infected plants, with both clones having higher leaf ψ throughout the day than the noninfected plants. In the presence of drought stress, however, clone WL-5 infected with *V. albo-atrum* had lower morning leaf ψ than the control plants, whereas clone 1079 maintained the pattern of leaf ψ seen in the nondrought-stressed treatment.

Leaf ψ was measured weekly between 1300 and 1500 h during drought episode two (growth period three) of the second experiment. Significant differences in leaf ψ between drought-stressed and nondrought-stressed plants were present in weeks 3–6 (Fig. 10), but no pathogen × water interactions were detected. The lack of pathogen × water interactions may have been due to the decreased statistical precision associated with analyzing data collected weekly vs. seven times per day as in drought episode one. The significant differences in leaf ψ as well as the soil ψ values (Fig. 8), however, are evidence of the presence of drought stress during growth period three when crop growth analysis was conducted.

DISCUSSION

The effect of combined stresses on plant growth is not well understood, and this is particularly true for combined biotic and abiotic stresses (2). Vascular wilt fungi can grow at very low water potentials (4,5), a characteristic that should enhance the ability to cause disease under drought conditions. The limited saprophytic ability of both *Verticillium* sp. and *F. oxysporum* (15,26), however, reduces pathogen exposure to low soil water potentials and undoubtedly contributes to the current belief that the main effect of drought stress on vascular wilt diseases occurs after infection (7,35).

Vascular wilt fungi spread in vivo by spore translocation (7,26,29). Drought stress, which reduces stomatal conductance (3,14) and, therefore, transpiration, should slow translocation of pathogen propagules (5). Isolation data presented in this study support that theory. Plants under drought stress were watered on day 28 and the isolation pattern for clone 1079 shows a corresponding surge in colonization. Clone WL-5 had less

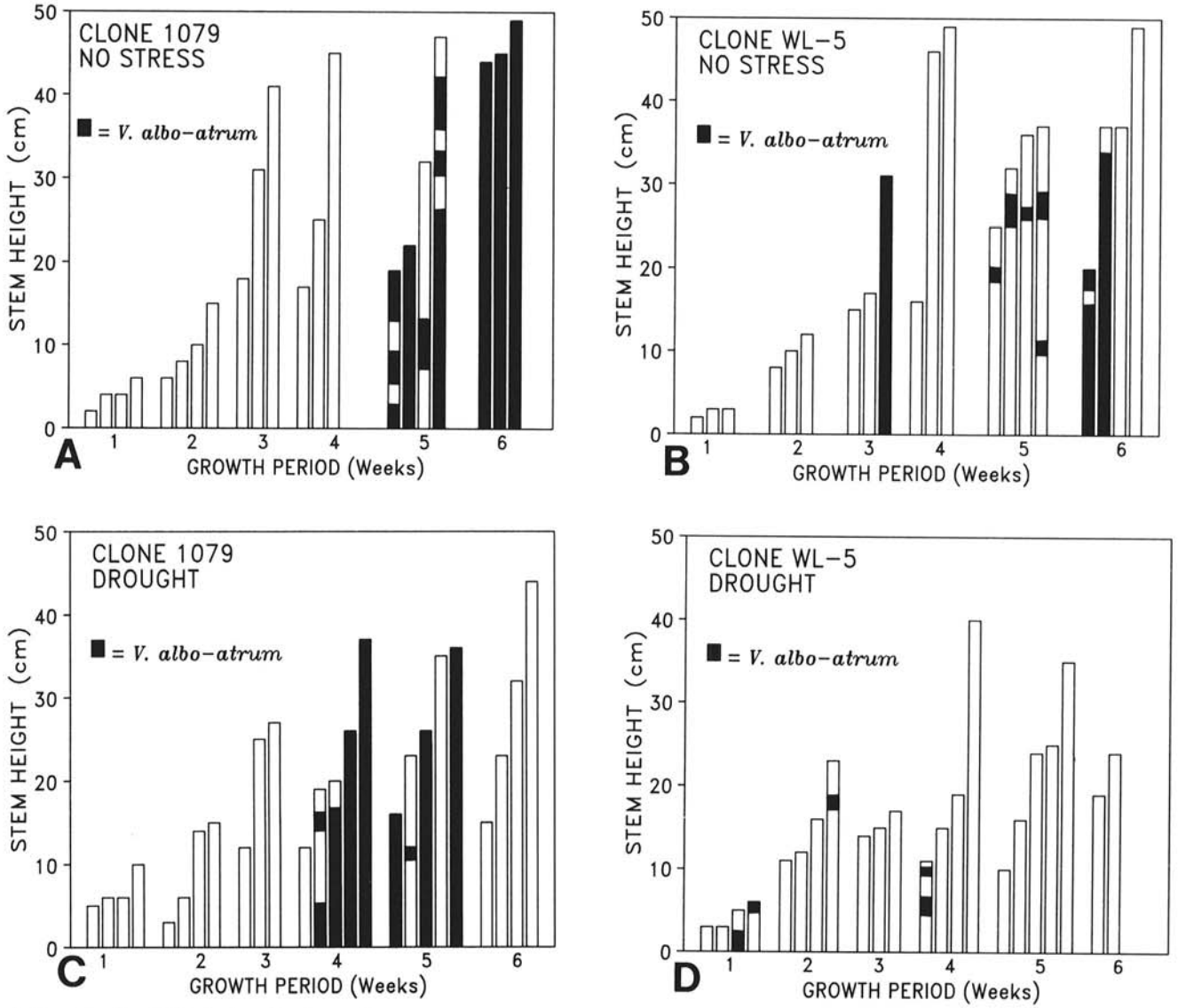


Fig. 1. Results of serial isolations for *Verticillium albo-atrum* conducted at weekly intervals during drought episode two (growth period three). Each bar represents a single stem selected from the plant sampled for growth analysis.

colonization by *V. albo-atrum* than clone 1079 and failed to show increased colonization following watering. Although the colonization data are limited, they may indicate different resistance mechanisms in the two clones. Clone 1079 prevented spore translocation until late in the growth period, whereas spore translocation occurred sporadically in clone WL-5 throughout the growth period.

The watering regime used in this study caused a moderate degree of drought stress as evidenced by the previously reported suppressed growth and reduced stomatal conductance of drought-stressed control plants (33). The turgor loss point for herbaceous, field-grown crops is in the leaf ψ range of -1.3 to -1.6 MPa (3). Plants under our drought treatment reached a leaf ψ of -1.3 to -1.5 MPa during the first drought episode and -1.7 MPa during the second episode without wilting. The lack of wilting at leaf ψ comparable to those causing wilt in the field may reflect a species difference and/or drought acclimation. Analysis of the noninoculated plants detected -0.12 MPa of osmotic adjustment at full hydration in the drought-stressed plants, which may have contributed to the lack of turgor loss (33). The relatively gradual development of drought stress allowed the plants to acclimate and thus mimicked drought stress under field conditions (16).

V. albo-atrum caused growth alterations during this study similar to those previously reported (32). Inconsistencies in growth parameters affected when only nondrought-stressed plants were analyzed (32), compared to those affected when data from all treatments were analyzed, were probably due to experimental variation introduced by the large suppressive effect of drought on growth. The alfalfa clones were resistant to *V. albo-atrum* in the absence of drought stress; therefore, alteration of the host/pathogen interaction by abiotically-induced drought stress would be indicated statistically as a pathogen × water interaction. No pathogen × water interactions were detected in the first drought episode, but two such interactions occurred during the second drought episode. Disease rating was lower under drought stress than under nondrought-stressed conditions, and drought stress reduced the suppressive effect of *V. albo-atrum* on stem dry weight. The abiotically-induced drought stress apparently altered some facet of the host/pathogen interaction in favor of the host. Detection of pathogen × water interactions in the second drought episode but not the first may reflect the increased statistical precision associated with analyzing data over weeks rather than at harvest only.

Abiotically-induced drought stress causes concentration changes in several growth hormones and stimulates the production of ethylene (3,6,35). Ethylene also accumulates in plants during

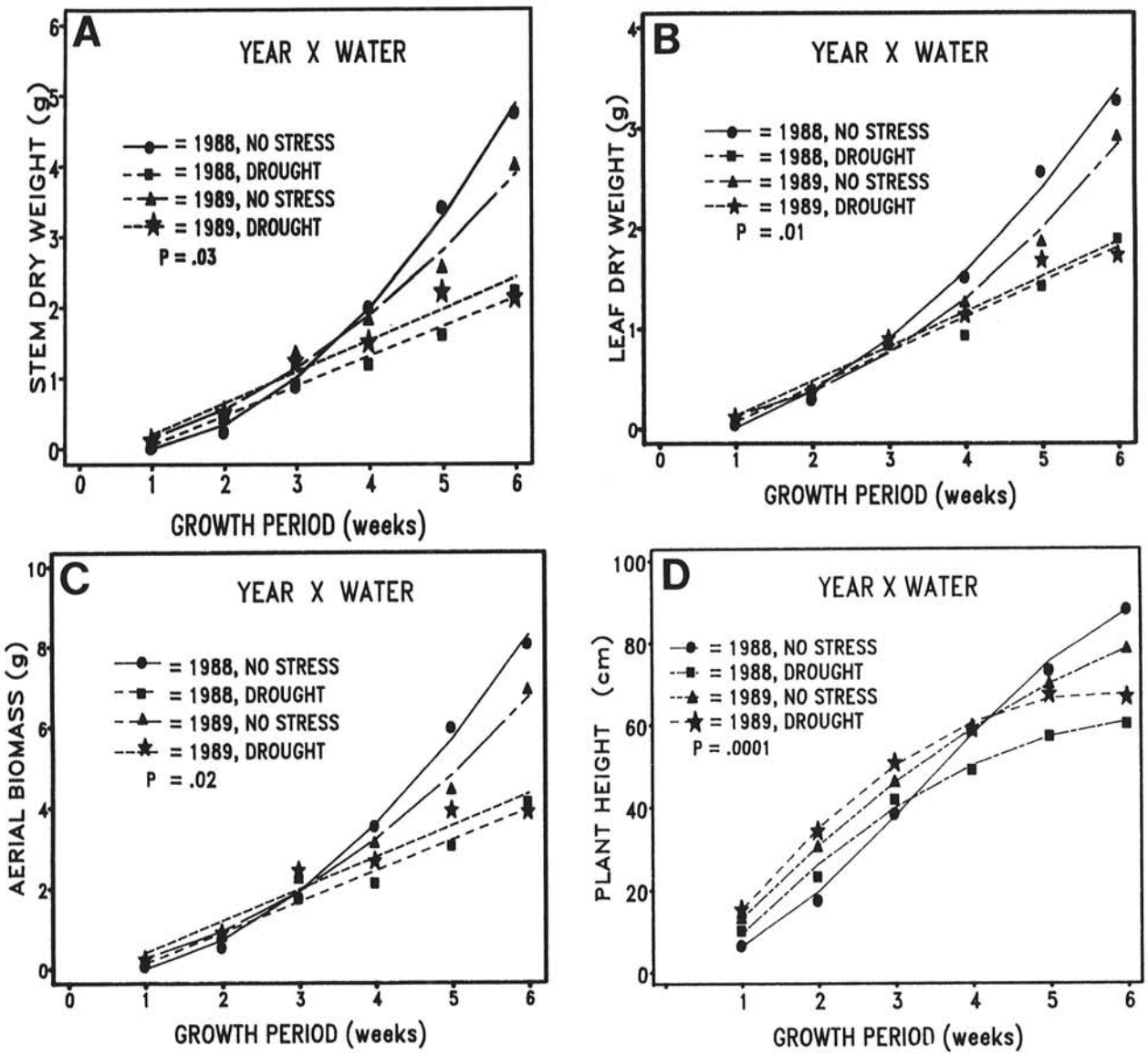
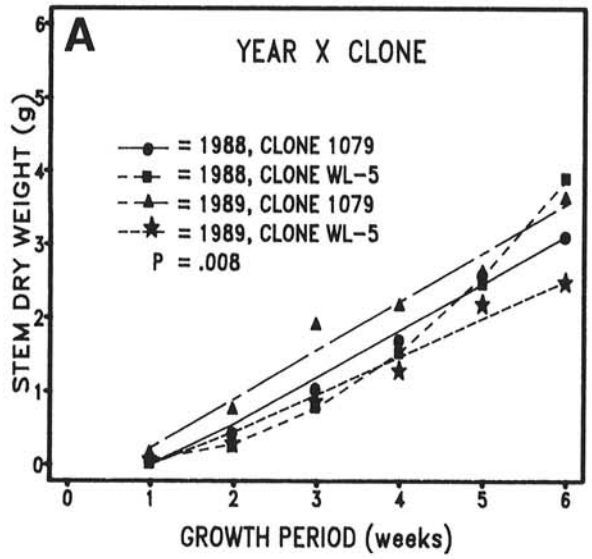
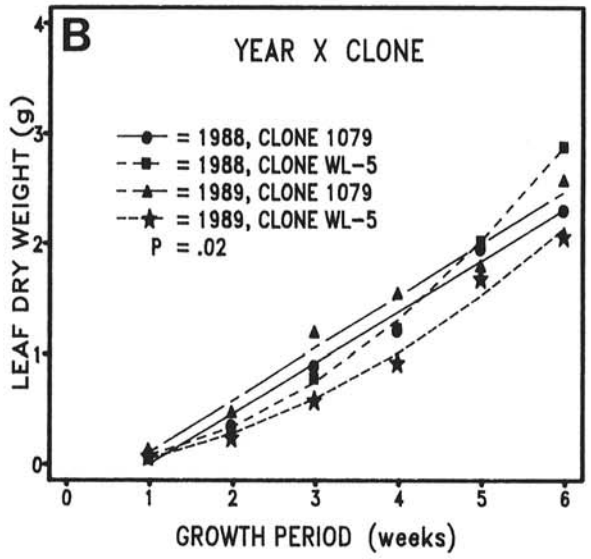


Fig. 2. Year × water interactions in stem dry weight, **A**, leaf dry weight, **B**, aerial biomass, **C**, and height, **D**. Each symbol represents the mean of 20 values. The *P*-value on each graph indicates the significance of the interaction, as determined by regression analysis using orthogonal polynomials. Note that the year × water interaction was due to the increased growth response of the nondrought-stressed plants in 1988.





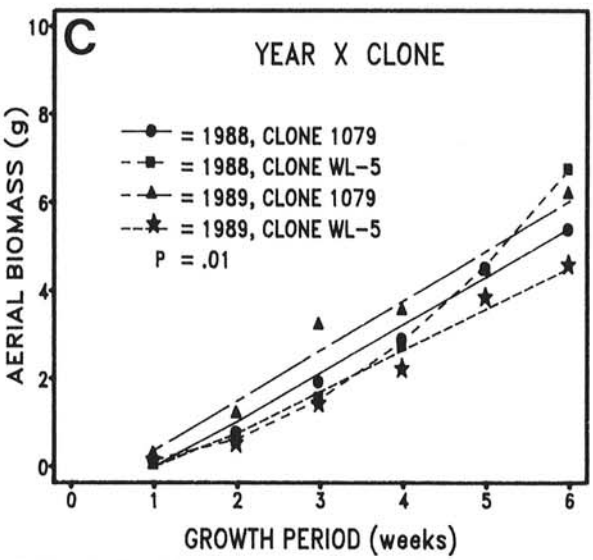
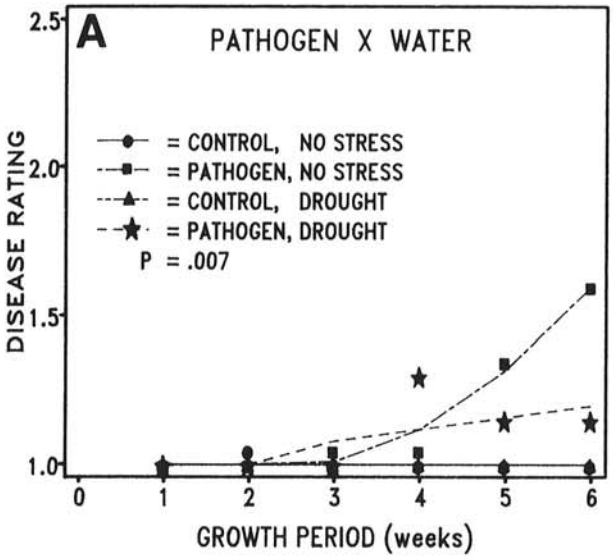


Fig. 3. Year × clone interactions in stem dry weight, **A**, leaf dry weight, **B**, and aerial biomass, **C**. Each symbol represents the mean of 20 values. The *P*-value on each graph indicates the significance of the interaction, as determined by regression analysis using orthogonal polynomials. Note that the year × clone interactions were due to the growth suppression experienced by clone WL-5 during 1989.

vascular wilt disease (26) and is involved in vascular gelation reactions triggered by the pectolytic activity of polyglacturonase from *F. o. cubense* (43). Vascular gel formation was found in susceptible alfalfa infected with *V. albo-atrum* (30) and is a common host response to vascular wilt fungi (42). Ethylene found in cotton infected with *V. dahliae* is of host, not pathogen, origin (40,41). Ethylene associated with abiotic drought stress might enhance vascular gelation in plants infected with vascular wilt fungi. Increased vascular gelation would slow pathogen spread via spore translocation. The surge of colonization after irrigation of the drought-stressed plants that we noted may reflect the ephemeral nature of such gels and their dispersal in the transpiration stream (26).

In addition to the involvement of ethylene in vascular gel formation, ethylene from abiotically-induced drought stress might increase resistance to *V. albo-atrum* in other ways. An increase in production of xylem vessels in tomato infected with *V. albo-atrum* followed exogenous application of ethylene (25). Fewer vessels contained hyphae, and increased resistance was attributed to an increased number of pathogen-free vessels (25). In addition,



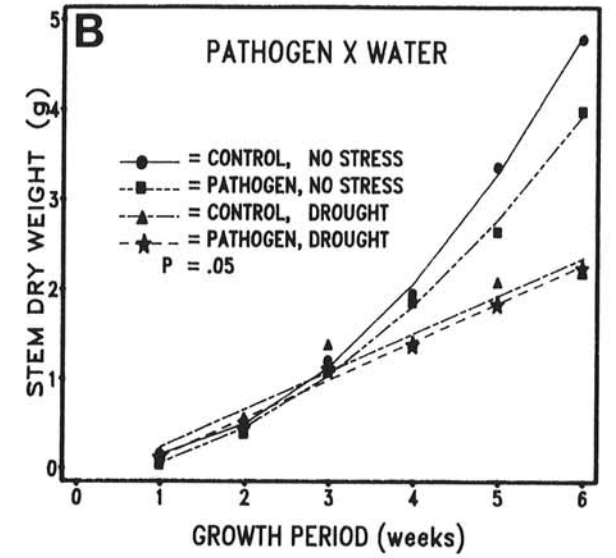


Fig. 4. Pathogen × water interactions in disease rating, **A**, and stem dry weight, **B**. Each symbol represents the mean of 20 values. Lines for the noninfected plants, **A**, are superimposed on the bottom of the graph. The *P*-value on each graph indicates the significance of the interaction, as determined by regression analysis using orthogonal polynomials.

ethylene interacts with many metabolic processes (3,6) and induces the formation of chitinase (44). Increased levels of 1,3-β-glucanase and chitinase and a negative correlation between increased levels of the glycosidases and mycelial colonization occurred in tomato infected with *V. albo-atrum* (27). Ethylene-induced chitinase may slow colonization of drought-stressed alfalfa by *V. albo-atrum*.

The leaf ψ in the two infected clones responded differently to drought stress. A pathogen × water × clone interaction was detected in weeks 3 and 4 of drought episode one. Drought-stressed, noninoculated clone 1079 developed a lower leaf ψ than did noninoculated clone WL-5, a good indication that stomatal conductance was greater in clone 1079. Increased stomatal conductance increases the amount of water lost through transpiration, thus lowering leaf ψ. Stomates of infected plants often lose the ability to respond to the environment (1). Infection by *V. albo-atrum* apparently altered the ability of clone 1079 to maintain stomatal conductance under favorable conditions, as evidenced by the higher leaf ψ of leaves of inoculated clone 1079. The effect was not as pronounced in the infected leaves of clone WL-5 and is perhaps another indication that different resistance mechanisms may operate in the two clones.

Pathogen × clone interactions were detected during the second drought episode (growth period three) in disease rating, height, stem number, and percent of plants flowering. In general, these interactions indicated that clone WL-5 showed fewer symptoms of Verticillium wilt than did clone 1079 but had greater growth suppression due to the presence of the pathogen. Defense reactions exact an energy cost from the plant (37). The greater growth suppression experienced by clone WL-5 than by clone 1079 may reflect the energy cost of superior symptom suppression.

The interaction expressed in percent of plants flowering may demonstrate the effect of timing on the sensitivity of flowering to suppression by biotic stress. Plants are particularly sensitive to water imbalances at germination, flowering, and fruit-set (11), and floral initiation is particularly sensitive to drought stress (36). Clone WL-5 flowers later than clone 1079 and may have encountered pathogen-induced drought stress.

Additional interactions were detected for clone × year and for water × year. Temperatures differed between the two experiments. The optimum daytime temperature for alfalfa growth is 15–25 C (20). The nondrought-stressed plants grew better in 1988, reflecting the more favorable temperatures for growth. The drought-stressed plants grew similarly in both years due to the dominant effect of water deficit on expansive growth (14). Alfalfa genotypes vary widely in response to temperature (20), which explains the clone × year interactions we detected.

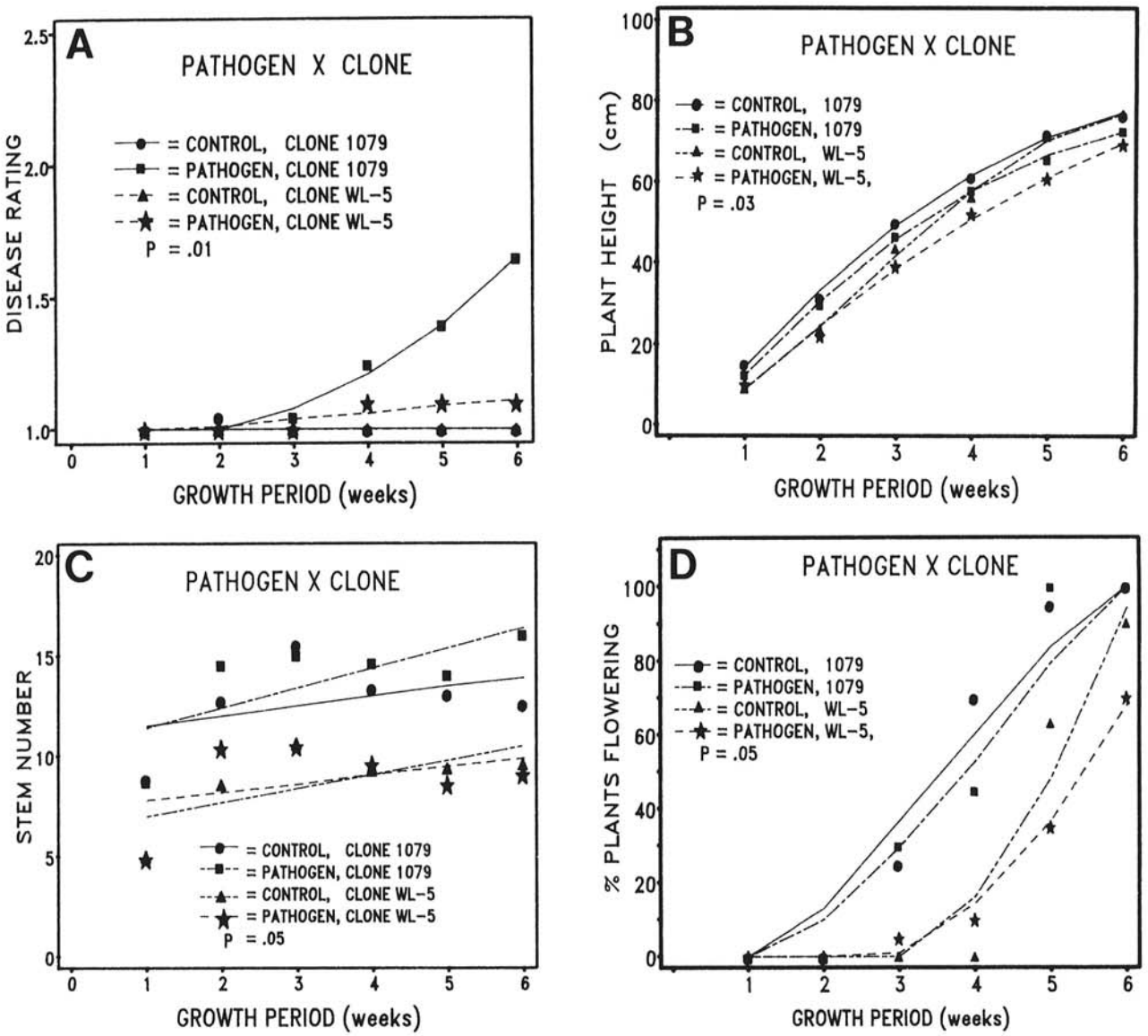


Fig. 5. *Verticillium albo-atrum* × clone interactions reflected in disease rating, **A**, height, **B**, stem number, **C**, and percent plants flowering, **D**. Each symbol represents the mean of 20 values. The *P*-value on each graph indicates the significance of the interaction, as determined by regression analysis using orthogonal polynomials.

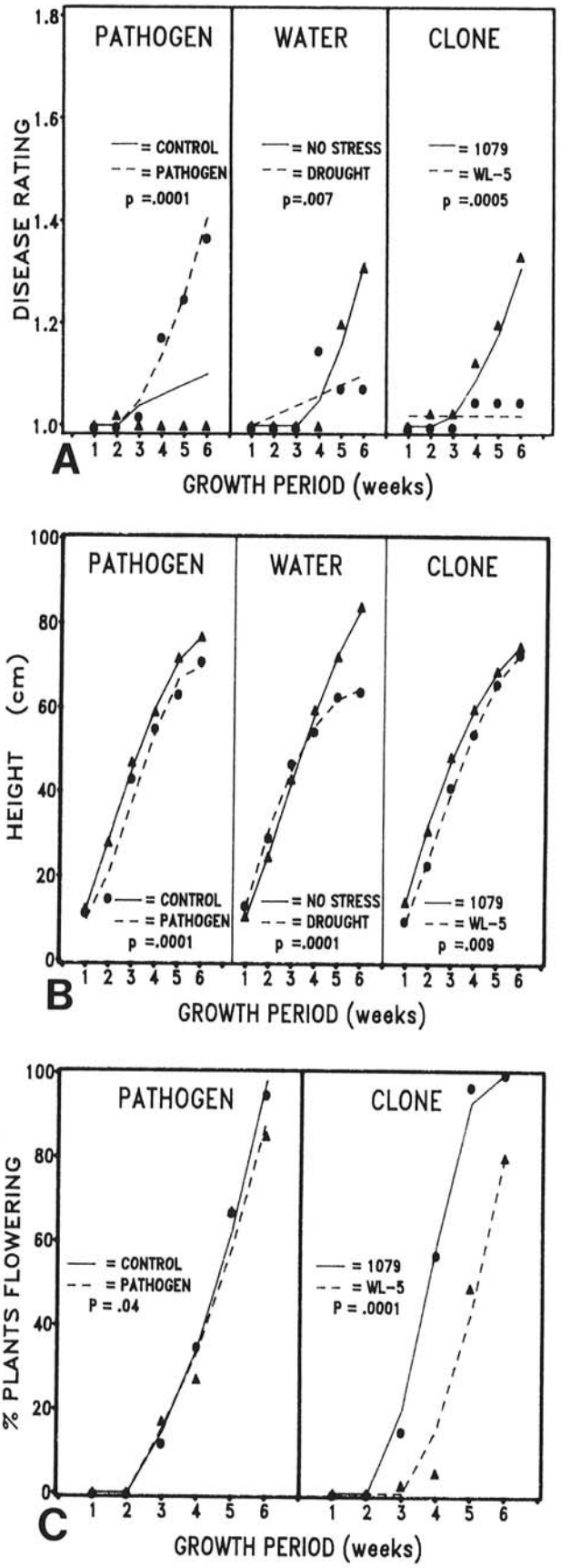


Fig. 6. The effect of *Verticillium albo-atrum*, drought, and clone on the growth of alfalfa as reflected in disease rating, **A**, height, **B**, and percent plants flowering, **C**. Each symbol represents the mean of 40 values. Lines are significantly different at the levels indicated on the graphs, as determined by regression analysis using orthogonal polynomials.

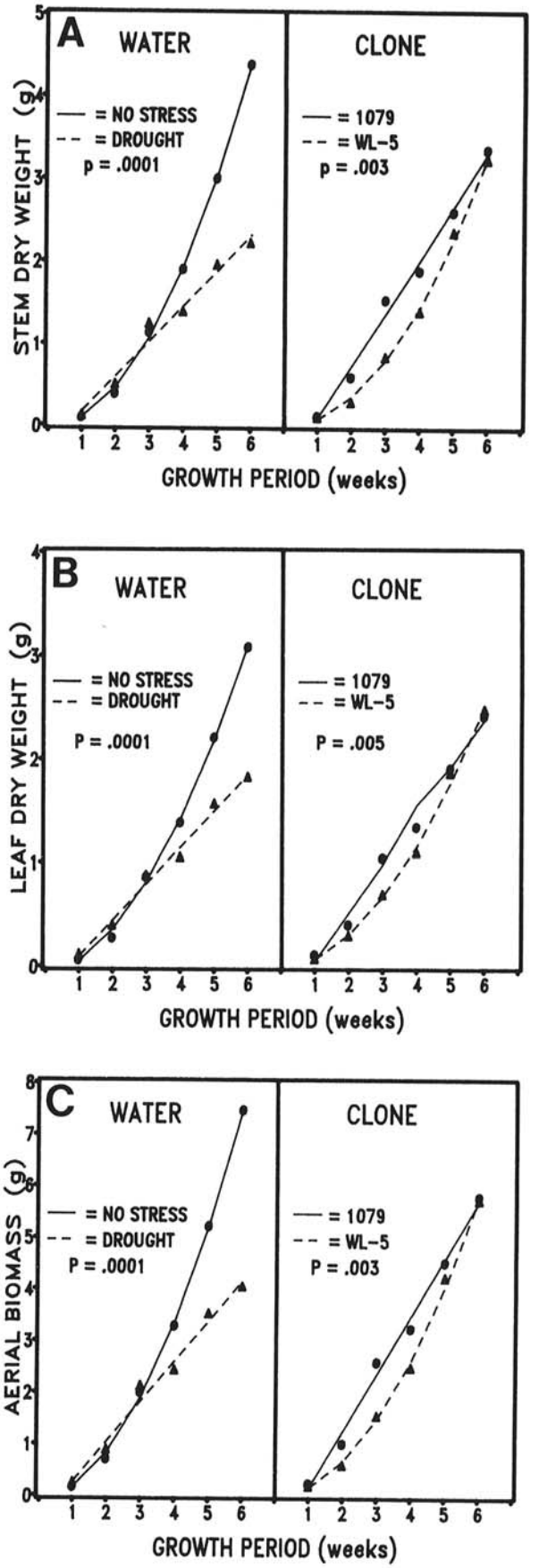


Fig. 7. Water and clone affected the growth of alfalfa as shown for stem dry weight, **A**, leaf dry weight, **B**, and aerial biomass, **C**. In every case drought stress reduced the growth of alfalfa. Each symbol represents the mean of 40 values. Lines are significantly different at the levels indicated on the graphs, as determined by regression analysis using orthogonal polynomials.

The detection of pathogen × water interactions indicated that drought stress altered the host/pathogen interaction. However, rather than reduce resistance to *Verticillium* as noted in yellow poplar (22), drought stress actually reduced symptom expression and the suppressive effect of the pathogen on stem dry weight. Evidence of improved resistance as expressed in stem dry weight and disease rating coupled with the lack of pathogen × water interactions for any other parameters leads to the conclusion that resistance to *V. albo-atrum* in alfalfa is stable under drought stress.

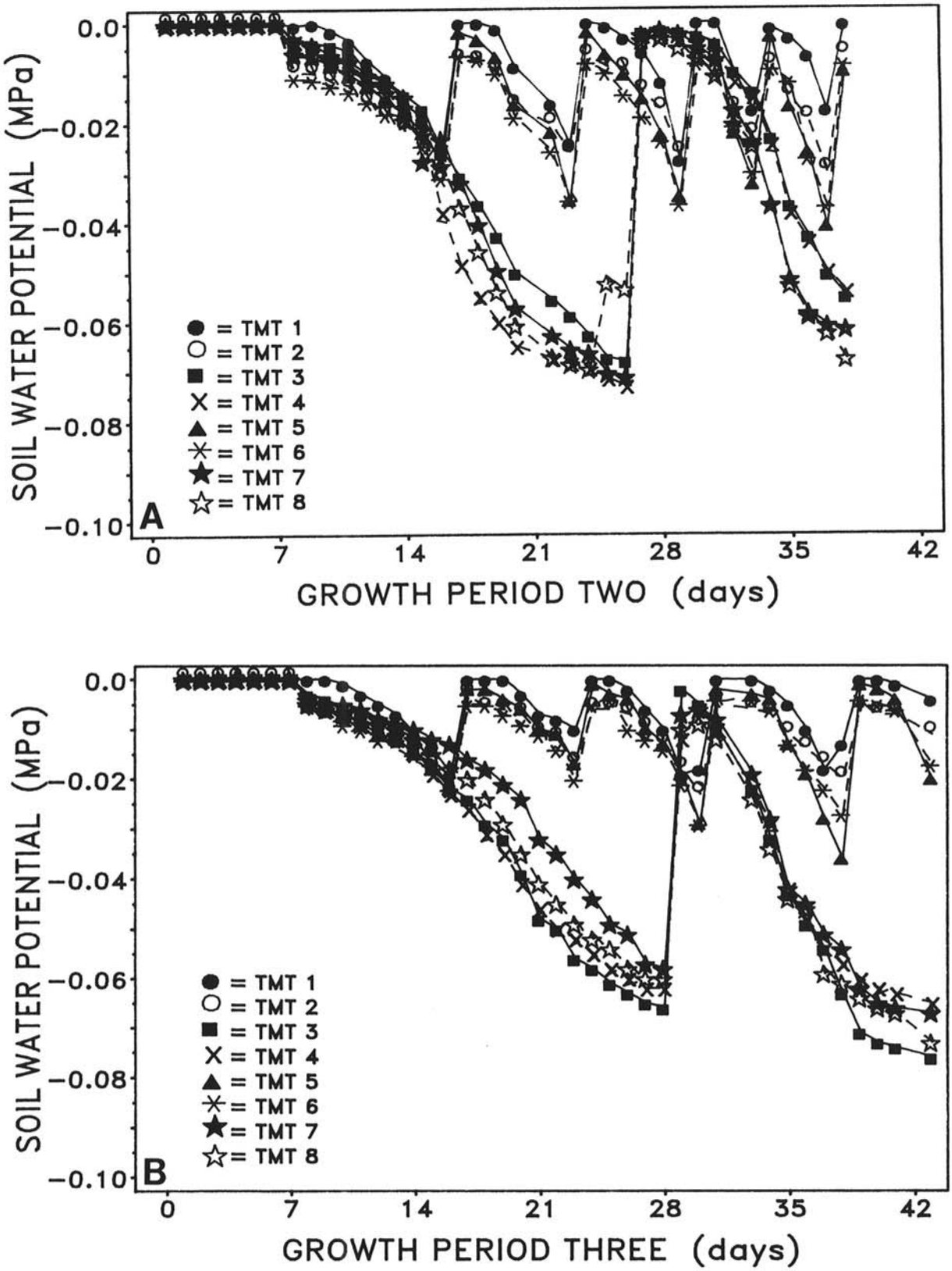
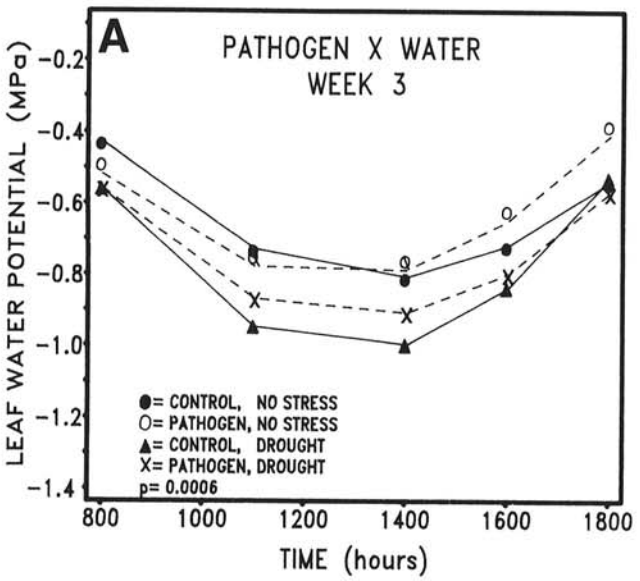
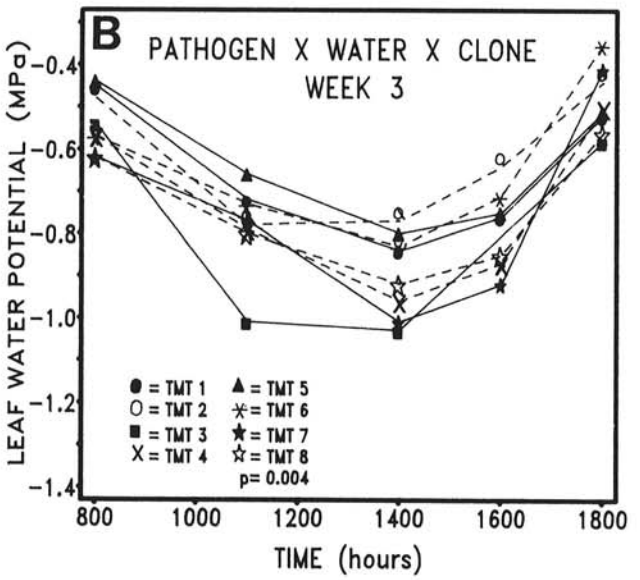


Fig. 8. Soil water potentials, measured at a depth of 30 cm, for drought episode one, **A** (growth period two), and drought episode two, **B** (growth period three), in 1988. Similar curves were obtained in 1989. Each point represents the average of four values. Plants in treatments 1, 2, 3, and 4 were clone 1079 and plants in treatments 5, 6, 7, and 8 were clone WL-5. Plants in the even-numbered treatments were inoculated with *Verticillium albo-atrum* and plants in the odd-numbered treatments were noninoculated. Plants in treatments 1, 2, 5, and 6 were not drought-stressed, whereas plants in treatments 3, 4, 7, and 8 were drought-stressed.





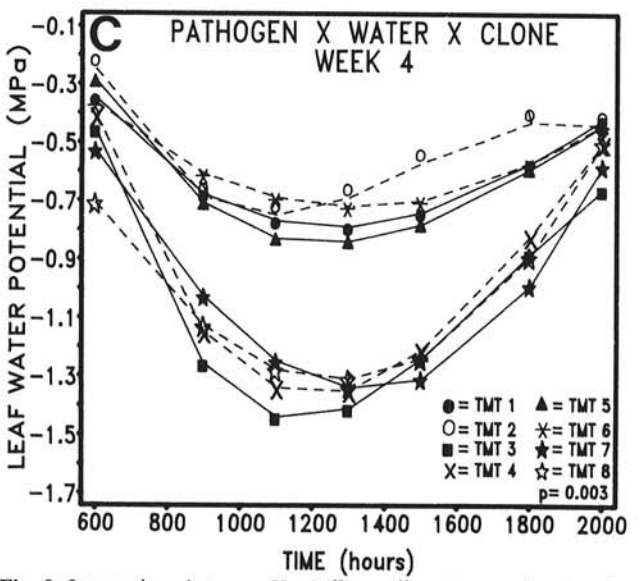


Fig. 9. Interactions between *Verticillium albo-atrum* and water, **A**, and *V. albo-atrum* and water and clone, **B** and **C**, during drought episode one (growth period two), as shown by predicted diurnal leaf ψ curves. Each symbol in **A** represents the mean of four values. Symbols in **B** and **C** represent the mean of two values. The lines are significantly different at the levels indicated on the graphs, as determined by regression analysis using orthogonal polynomials. Treatments are identified in Figure 8.

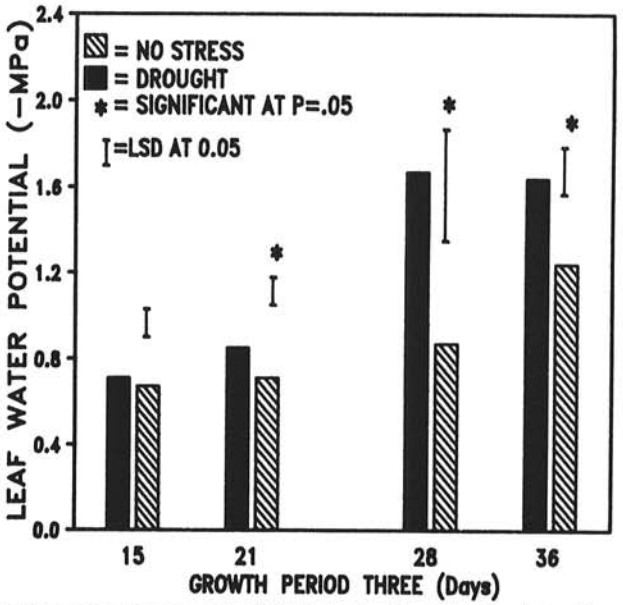


Fig. 10. Leaf water potential of alfalfa during drought episode two (growth period three), showing the effect of the increasing drought stress. Note that drought stress was present at week 3 and throughout the remainder of the growth period. Bars represent the mean of 12 values. Measurements were taken between 1300 and 1500 h.

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