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Drought resistance of mycorrhizal pepper plants independent of leaf P concentration – response in gas exchange and water relations

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Pepper (*Capsicum annuum* L.) plants with and without the VA-mycorrhizal fungus *Glomus deserticola* Trappe, Bloss and Menge (VAM and NVAM, respectively), were drought acclimated by four drought cycles (DA) or kept well watered (NDA). All plants were then subjected to an additional drought followed by a 3-day irrigation recovery period. Measurements of water relations, gas exchange and carbohydrates were made at selected intervals throughout the drought cycles and recovery. To equalize growth and avoid higher P in VAM plants, NVAM plants received higher P fertilization. Consequently, similar transpirational surface and shoot mass were achieved in all treatments, but NVAM had a higher tissue P concentration than VAM plants. Plants that were either VAM or DA, but especially the VAM-DA plants, tended to be high in net photosynthetic flux (A), A per unit of tissue P concentration (A/P), stomatal conductance (g) or leaf turgor (ψ_p) during high environmental stress or recovery from stress. During this time, NVAM-NDA plants had low A, A/P and leaf chlorophyll, but high soluble carbohydrate concentrations in their leaves. All VAM and DA plants had some osmotic adjustment compared to the NVAM-NDA plants, but VAM-DA plants had the most. Osmotic adjustment was not due to accumulation of soluble carbohydrate. The high turgor, A and g in the VAM-DA plants during and following environmental stress indicated superior drought resistance of these plants; however, osmotic adjustment was only apparent during recovery and cannot account for the observed drought resistance during environmental stress. Drought resistance of VAM-DA plants was not attributable to high leaf P concentration or confounded by differences in plant transpirational surface.

Key words – *Capsicum annuum*, drought acclimation, *Glomus deserticola*, phosphorus nutrition, photosynthesis, vesicular-arbuscular mycorrhizal fungi.

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Introduction

VA-mycorrhizae can mitigate plant response to water stress (Nelsen 1987). Possible mechanisms for improved drought resistance of VA-mycorrhizal plants include increased root hydraulic conductivity (Safir et al. 1972), stomatal regulation, perhaps by homones (Allen et al. 1982), enhanced water uptake due to extraradical hyphae (Hardie 1985), osmotic adjustment that promotes turgor maintenance even at low tissue water potential (Augé et al. 1986a) and cell wall elasticity changes (Augé et al. 1987b). Regardless of how drought resistance develops, it should increase plant hydration and turgor, which in turn should promote stomatal conductance (g) and photosynthetic flux (A) (Boyer 1976).

According to Fitter (1988), the influence of VA-mycorrhizae on plant water relations may be a secondary consequence of enhanced host P nutrition, although

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these effects are inconsistent. Increased tissue P concentration in mycorrhizal plants was associated with improved water relations in onion (Nelsen and Safir 1982) and citrus (Graham and Syvertsen 1984). Conversely, mycorrhizae improved water relations during drought for rose (Augé et al. 1986a) and geranium plants (Sweatt and Davies 1984) differentially fertilized to equalize tissue P concentrations. Hence, the involvement of P in drought resistance of mycorrhizal plants is controversial.

A major problem with many previous VA-mycorrhizal studies involving drought has been that mycorrhizal and nonmycorrhizal plants were of unequal size, with mycorrhizal plants generally having greater evaporative surfaces and a higher tissue P concentration. This is particularly a problem if available water is limited by a container, where a relatively large leaf area should lead to rapid depletion of soil moisture. More recently investigators have varied P fertilization in efforts to overcome this problem (Augé et al. 1986a, Bethlenfalvay et al. 1988, Fitter 1988). Studies that have equalized tissue P concentration and plant size between mycorrhizal and nonmycorrhizal plants generally have not included photosynthesis measurements, a key component of growth, or time course data of other parameters that should allow insights into timing of events during drought. Our objective was to determine the effects of VA-mycorrhizae on the time course of photosynthesis, stomatal conductance, plant water status and other related parameters of plants before, during and after exposure to repeated drought. Care was taken to equalize plant transpirational area and mass and to avoid having a relatively low P concentration in nonmycorrhizal plants.

Abbreviations – A, net photosynthetic flux; DA, drought-acclimated; g, stomatal conductance; LANS, Long Ashton Nutrient Solution; NDA, nondrought-acclimated; NVAM, nonmycorrhizal; VAM, inoculated with *Glomus deserticola*; WSD, % water saturation deficit; ψ_{leaf}, leaf water potential; ψ_p, leaf turgor potential; ψ_{soil}, soil water potential; saturated ψ_π, leaf osmotic potential at saturation.

Materials and methods

Plant inoculation, growing conditions and tissue elemental analyses

The mycorrhizal fungus *Glomus deserticola* (Trappe, Bloss and Menge) was obtained from NPI (Salt Lake City, UT, USA) and added to container growth medium at rates to give 20 000 spores l^{-1}. A water suspension of mycorrhizal inoculum was sieved through 11 μm using US Standard Sieve Series (Precision Scientific Co., Chicago, IL, USA) and Whatman Filter paper # 1 (Whatman Int. Ltd, Maidstone, UK) to remove VAM propagules, and the filtrate was added to controls to equalize the background microflora in all treatments. Inoculum was banded 4 cm below the surface in 1-l containers of pasteurized (60°C for 30 min) river sand (91% sand, 7% clay and 2% silt; Davies and Linderman 1991). A sandy medium was used to allow us to use the sand aggregation method for measuring mycorrhiza hyphal development and to better control P nutrition.

Three-week-old seedlings of pepper (*Capsicum annuum* L. cv. Early Bountiful) were transplanted, one plant per pot, into the containers and grown in a glasshouse for 4 weeks beginning 13 April at 28/15°C average day/night temperature with daylight supplemented for 16 h by a photosynthetic photon flux density (PPFD) of 600 μmol m^{-2} s^{-1} from 400 W high pressure sodium vapor lamps (GE, Circleville, OH, USA). Over the 7-week period following transplanting (inoculation), the solar photoperiod was about 14 h; solar PPFD in the glasshouse averaged over cloud-free shaded and brightly lit areas at solar noon was about 700 μmol m^{-2} s^{-1}. Beginning 13 April, the weekly averages for all wavelengths of solar irradiance at the Oregon State University Climatic Research Institute field location (11 km from our glasshouse) were 16, 24, 13, 17, 22 and 16 MJ m^{-2} day^{-1}. Data from the 4th week were unavailable. Until drought treatments began, containers were fertilized weekly with 200 ml Long Ashton Nutrient Solution (LANS; Hewitt 1966) modified to contain 22 μg P ml^{-1} for mycorrhizal (VAM) plants; nonmycorrhizal (NVAM) plants received the nomal 44 μg P ml^{-1} in an effort to equalize size and tissue P concentration between mycorrhizal and nonmycorrhizal plants. In addition, plants were irrigated with water every 2 or 3 days to prevent wilting.

After the initial 4-week establishment period (plants were then approximately 7 weeks old) half the plants were given drought acclimation (DA) treatments consisting of 4 drought cycles, each lasting 4 days and ending with irrigation to container capacity; LANS was applied during alternate irrigations. Nondrought acclimated (NDA) plants received the same nutrition as did the DA plants. At the end of the 4th day of drought in cycle 5 immediately before irrigation of plants, predawn ψ_{leaf} and ψ_{soil} of drought stressed plants were −1.1 and −0.7 MPa, respectively, whereas comparable predawn values 24 h after irrigation were −0.2 and −0.1 MPa, respectively. To determine ψ_{soil}, two soil psychrometers (Wescor PCT 55, Wescor Inc., Logan, UT, USA) were placed permanently into the soil (upper and lower halves of pot) of two containers per treatment, and the voltage was measured with a microvoltmeter (Wescor HR33T) in the dewpoint mode. Measurements of ψ_{soil} were made before dawn to minimize soil water concentration gradients and thermal gradients between the soil and air. As a result of equipment problems, we were unable to take ψ_{soil} measurements for each cycle and treatment as we had planned. Plants from all 4 combinations of treatments (with and without inoculation and drought acclimation; NVAM-NDA, NVAM-DA, VAM-NDA, VAM-DA) were then subjected to a final 4-day drought (cycle 5) followed by 3 successive days of irrigation (cycle 6), after which the experiment was terminated.

At the end of the experiment, roots were washed with water, then cut into 1-cm segments. Roots were cleared and stained (Phillips and Hayman 1970) and mycorrhizal colonization was determined by sampling 25 1-cm root segments from five plants (n = 125) and determining the percentage that contained vesicles and hyphae. Colonization levels of inoculated plants were 9.6 and 15.2% for NDA and DA plants, respectively; non-inoculated plants were not colonized (data not shown).

Leaf tissue P was analyzed on an inductively coupled plasma atomic emission spectrophotometer (3510ICP, W. R. Grace & Co., Fogelsville, PA, USA). Leaf area and shoot and root dry mass were measured immediately before the first drought cycle and at the end of the experiment; leaf chlorophyll (Arnon 1949) was measured at the end of the experiment.

Leaf carbohydrate analysis

Leaf discs (4.1 cm^2) from newly expanded, physiologically mature leaves (3 to 6 nodes from the shoot apex) were cut and dried at 70°C, weighed and used for starch determination (Potter and Breen 1980). Sampling time was just before dawn (0530 h, Pacific Daylight Time) and again at 1600 h. The supernatant fraction from the starch analysis was used for soluble carbohydrate determination with the phenol-sulfuric acid colometric method (Hodge and Hofreiter 1962). The standard for the soluble carbohydrate assay was the average A_{490} of individual solutions of sucrose, glucose and fructose. Because carbohydrate data normalized over area or mass varied similarly with treatment, only the normalized area data are presented.

Plant water measurements

Leaf water potential (ψ_{leaf}) was measured at the same time as for carbohydrate analysis on newly matured leaves using a pressure chamber (Scholander et al. 1965). Turgor (ψ_p) was determined by the difference between ψ_{leaf} and fresh weight osmotic potential. The latter was measured by isopiestic psychrometry (Boyer and Knipling 1965) using 4.1 cm^2 leaf discs excised from leaves as they were removed from the pressure chamber. Leaf discs were immediately sealed in parafilm envelopes, frozen at −60°C for at least 2 h and thawed. To determine osmotic adjustment, leaves used for the above measurements were allowed to fully hydrate by recutting petioles under water and keeping the petioles submerged for 2 h with the leaves in a darkened humid chamber. The ψ_π of fully hydrated leaves ($\psi_{leaf} \simeq -0.01$ MPa) was then determined as above. Percent leaf water saturation deficit (WSD) was determined as 100% (sw-fw)/(sw-dw), where sw = saturated weight, fw = fresh weight at the time of leaf excision and dw = dry weight (Kramer 1983). Fresh weights were determined on leaf discs taken from newly matured leaves at the time water potentials were measured. After fresh weight was determined, a disc was floated on water in a closed Petri dish for 2 h to achieve saturation without water accumulating in the intracellular spaces, and then the disc was dried at 70°C.

Gas exchange measurements

Measurements of A (per unit leaf area), PPFD and g were made with a portable photosynthesis system (Model 6000, LI-COR Inc., Lincoln, NE, USA) using the LI-COR quarter liter chamber, operating as a closed system. Measurements were made on newly matured leaves from 0900–1100 h and from 1330–1530 h in the glasshouse where plants were grown. All A and g measurements were made with the initial CO_2 concentration at about 360 $\mu l\ l^{-1}$ under 1 000 $\mu mol\ m^{-2}\ s^{-1}$ of PPFD from a 400 W high pressure sodium vapor lamp filtered through 5 cm of water enclosed in a Plexiglas box. Plants were removed from the randomized design immediately before each measurement, moved at most 3 m, and placed under the lamp. An opaque curtain was lowered to exclude over 99% of the external light, and a fan was used to disperse CO_2 and heat within the curtain. Boundary layer conductance within the photosynthetic chamber was 1.6 $mol\ m^{-2}\ s^{-1}$ based on preliminary determination of evaporation flux from a wet filter paper replica of a pepper leaf. The efficiency of A per unit of P (A/P) was calculated by dividing afternoon A (at the times given in the data) by leaf P concentration at the end of the experiment, except for the values of A/P in cycle 1, where the denominator was leaf P concentration before DA treatment began.

Statistical design

The 2 VAM × 2 DA factorial experiment was in a completely randomized design with each plant as an experimental unit. There were 40 plants per treatment, and plants were sampled at times indicated with the data. Treatment effects were determined by using ANOVA (SAS Institute, Inc. 1988) as indicated with the data. For phosphorus analyses, nonsenescent leaves of three plants were pooled for a single measurement. Initial measurements, taken immediately before DA treatment, were made on 3 pooled samples per treatment (n = 3), while final measurements, taken after cycle 6, were made on 5 (n = 5). All growth parameter measurements at experiment termination were made on 15 plants (n = 15). The following numbers (n) of samples per treatment were taken at each sampling time: (a) for gas analysis, n = 10 (2 leaves from each of 5 plants); (b) for water status determination, n = 6 (3 plants) except on cycle 6 (recovery period), when n = 10 (5 plants) for saturated ψ_π and (c) for carbohydrate analyses, n = 6 (3 plants). For carbohydrate and water status measurements, the same 3 randomly selected plants were used for both morning and afternoon of a given day and these plants were not reused. For A and g

Tab. 1. Leaf P and chlorophyll content and plant size at experiment termination of nonmycorrhizal (NVAM) and mycorrhizal (VAM) *Capsicum annuum* L. plants that were acclimated to drought (DA) or nonacclimated (NDA). Means followed by a common letter are not significantly different by Fisher's protected LSD test ($P \leq 0.05$); n = 15, except for P where n = 5 and chlorophyll where n = 10. Significance of ANOVA = NS, *, **; Nonsignificant or significant at 5% or 1% levels, respectively. Nonmycorrhizal plants received more P fertilizer than VAM plants.

Treatment	P mg (g DW)$^{-1}$	Leaf area (cm^2)	Shoot DW (g)	Root DW (g)	Leaf Chlorophyll A_{652}
NVAM-NDA	5.4a	538a	4.4a	3.1a	0.20c
NVAM-DA	3.8b	490a	4.3a	2.6b	0.27a
VAM-NDA	2.3c	518a	4.5a	2.6b	0.23b
VAM-DA	1.7d	508a	4.5a	2.4b	0.29a
Significance					
VAM	*	NS	NS	**	*
DA	*	NS	NS	**	**
Interaction	*	NS	NS	NS	NS

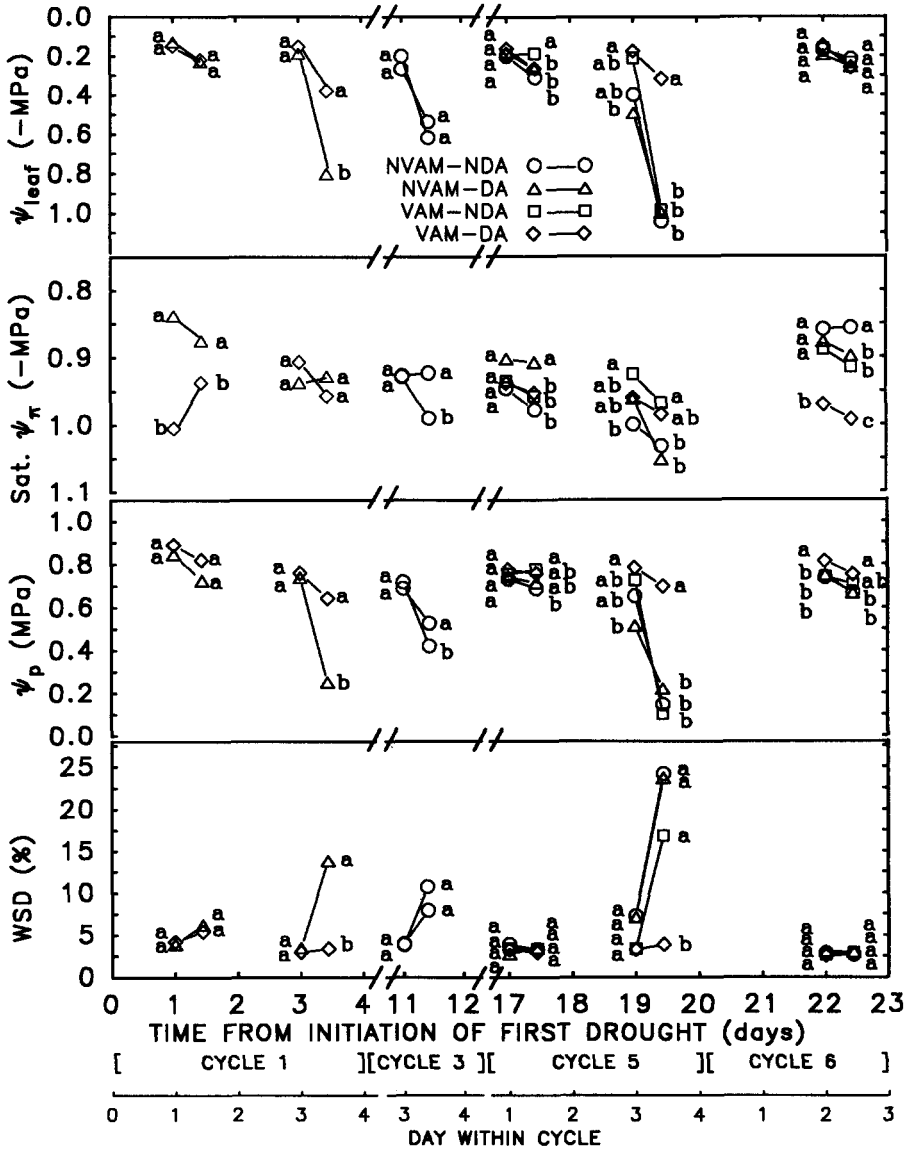

Fig. 1. Changes from predawn to afternoon in ψ_{leaf}, saturated ψ_π, ψ_p and WSD of VAM and NVAM pepper plants over the 5 drying cycles followed by recovery over the sixth cycle. Through cycle 4, only the DA plants were subject to drought acclimation treatment while NDA plants were not drought acclimated. During cycle 5, plants of all treatments were subject to drought. Measurements were taken from well hydrated plants during day 2 early in drought cycles 1 and 5 and day 3 of cycle 6 (recovery). Also, measurements were taken during day 4 late in drought cycles 1, 3 and 5. Points at a given time followed by the same letter are not significantly different by Fisher's protected LSD test ($P < 0.05$), n = 6 or 10.

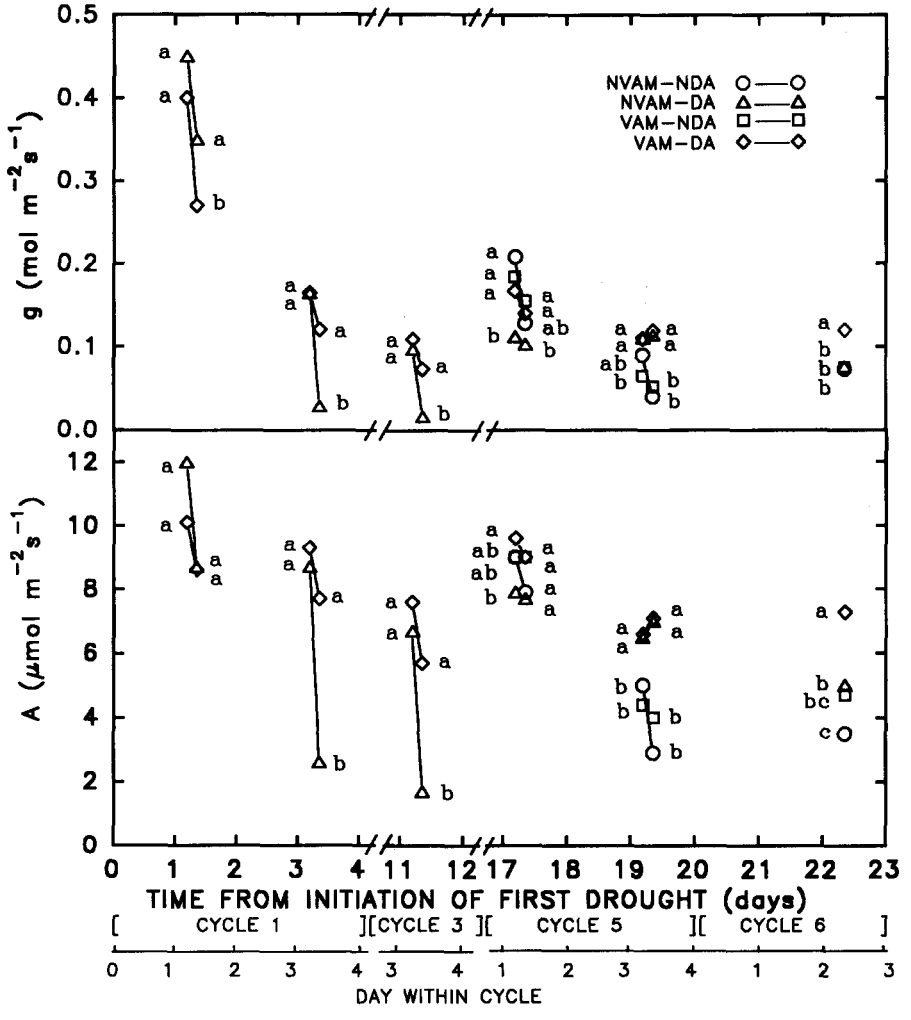

Fig. 2. Changes from morning to afternoon in g and A of VAM and NVAM pepper plants that were DA or NDA over the 5 drying cycles followed by recovery over the sixth cycle. Plant material and statistical analyses were as in Fig. 1, n = 6.

measurements on a given day, 5 plants were selected from the pool at random, used for both morning and afternoon measurements and returned to the pool. In preliminary trials, successive measurements did not cause stomatal closure.

Results

At the end of the experiment, leaf area and shoot dry mass did not differ significantly among treatments (Tab. 1). There was a greater root mass in NVAM-NDA plants; however root mass among other treatments was similar. At the beginning of the first drought cycle, for NVAM and VAM plants, leaf areas were 357 and 313 cm^2, shoot dry masses were 2.2 and 1.8 g and root dry masses were 1.0 and 1.1 g, respectively, and these were not significantly different ($P = 0.05$). Our aim was to ensure that VAM plants did not have a greater tissue P concentration than noninoculated plants. Prior to the initiation of DA, leaf P of NVAM was greater ($P = 0.05$) than that of VAM plants [3.3 vs 2.2 mg (g dry weight)$^{-1}$]. At the termination of the experiment, leaf P concentration remained higher in NVAM than VAM plants, and DA plants had lower P than NDA for comparable VAM treatments (Tab. 1). P was highest in NVAM-NDA (5.4 mg g^{-1}) and lowest in VAM-DA (1.7 mg g^{-1}) plants; in the commercial production of pepper, leaf P levels of 2.0 mg g^{-1} are considered sufficient, and 1.5 mg g^{-1} deficient (Lorenz and Maynard 1988).

During day 4 of cycles 1 and 3, there were substantial decreases in ψ_{leaf} and ψ_p for both NVAM-DA and VAM-DA plants, much greater than the decreases in these parameters for the well-watered plants on day 2 of cycles 1 and 5 (Fig. 1). The decreases in these parameters were measured at about 1600 h, but should continue for another 6 h until the end of the photoperiod. During the afternoon of day 4, cycle 5, only VAM-DA plants did not show a large decrease in ψ_{leaf} and ψ_p and a large increase in WSD. However, at the end of the photoperiod on day 4 of every drought cycle, many plants in all treatments that included drought visually appeared severely wilted.

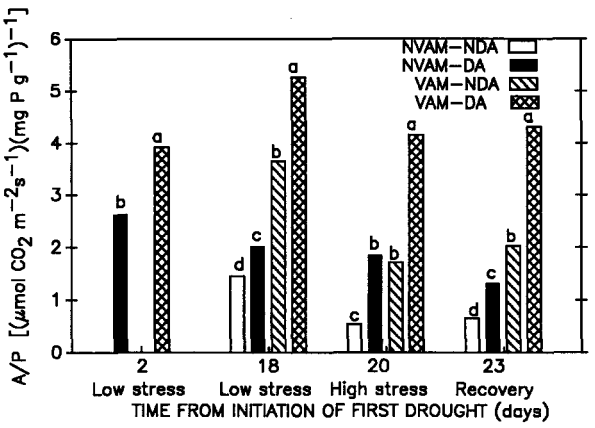

Fig. 3. Efficiency of A per unit of leaf tissue P concentration during low stress, high stress and recovery of VAM and NVAM pepper plants that were DA or NDA. Plant material and statistical analyses were as in Fig. 1.

The initial high g from well-hydrated plants before DA (day 2, cycle 1) was not maintained, even by well-watered plants never subjected to drought or by well-hydrated DA plants during the recovery period (Fig. 2); ANOVA significance was 0.0001 with mean separation across time by Duncan's multiple range test. During day 2, cycle 5, when all plants were well watered, g was lowest in NVAM-DA, and equal among other treatments, but by the afternoon of day 4, cycle 5, DA plants had higher g than NDA plants. During both the morning and afternoon of day 4, cycle 5, DA plants had higher A than NDA plants (Fig. 2).

During recovery (cycle 6), VAM-DA had the highest A, a value nearly as high as the initial afternoon value in cycle 1 (Fig. 2). In contrast, NVAM-NDA plants lost 60% of their initial A over the same time span (Fig. 2; ANOVA and mean separation as above) and had the lowest chlorophyll of all plants (Tab. 1). All NDA plants were lower in chlorophyll than DA plants at the end of the experiment.

Efficiency of A per unit of leaf tissue P concentration was greatest in VAM-DA and least in NVAM-NDA plants at any given time (Fig. 3). At the beginning of the

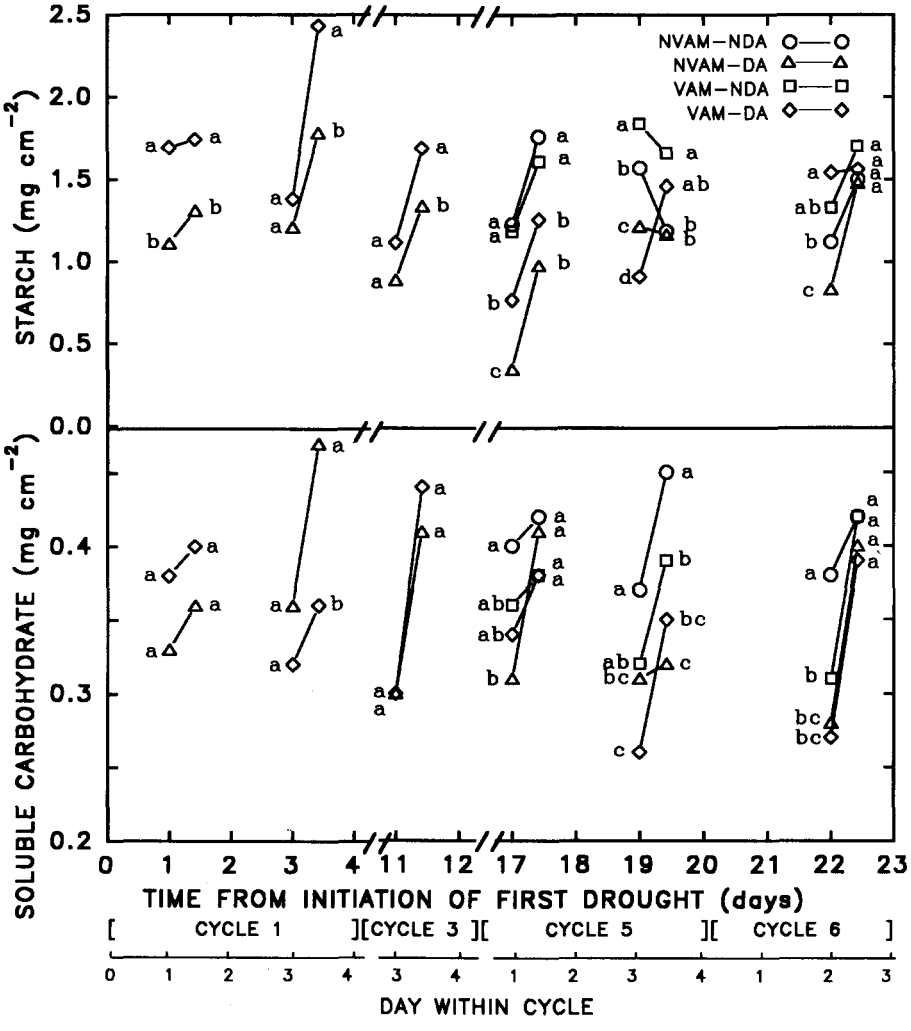

Fig. 4. Changes from predawn to afternoon in leaf starch and soluble carbohydrate concentration of VAM and NVAM pepper plants that were DA or NDA over the 5 drying cycles followed by recovery over the sixth cycle. Plant material and statistical analyses were as in Fig 1, n = 6.

DA treatments (day 2, cycle 1) and whenever plants were well watered, VAM had higher A/P than NVAM plants.

Differences in saturated ψ_π among treatments were inconsistent, but during the recovery period, VAM-DA plants clearly had the lowest (most negative) saturated ψ_π and generally had higher ψ_p than other treatments, even though ψ_{leaf} and WSD were equal among treatments (Fig. 1). During the recovery, NVAM-NDA had the highest saturated ψ_π.

Differences in saturated ψ_π during stress and recovery were not accounted for by leaf soluble carbohydrate concentrations; rather, soluble carbohydrates tended to be lower in VAM-DA compared to NVAM-NDA plants (Fig. 4). Generally, both starch and soluble carbohydrate concentrations increased from morning to afternoon, but during the high stress of day 4, cycle 5, only VAM-DA plants showed increased starch levels, while all others lost starch (Fig. 4).

Discussion

The strength of the present study is that it relates long-term and time course changes in water relations, stomatal behavior and photosynthesis of VAM and NVAM plants of comparable mass and evaporative surface, while avoiding low tissue P in NVAM plants. We were unable to equalize tissue P between VAM and NVAM plants; to achieve equalization in both mass and tissue P is extremely difficult (Bethlenfalvay et al. 1988, Fitter 1988). The higher P concentration of our NVAM plants indicated that the poor drought resistance (as measured by poor water relations or low gas exchange rates) of NVAM-NDA plants was not due to deficient P nutrition. Clearly a model that attributes improved drought resistance of mycorrhizal plants to a high P concentration (Nelsen 1987) does not account for our results.

In the present study, except for leaf tissue P concentration and A/P, there were no consistent differences between VAM and NVAM plants for any measured plant parameter before plants were first exposed to drought if both DA and NDA plants are considered. For an important parameter such as g, the present study agrees with the data of Koide (1985) and Fitter (1988) who reported that mycorrhizal colonization increased g of nondrought-treated plants only if the tissue P concentration was at the low end of the concentration ranges given in their studies. In a similar study Augé et al. (1986b) reported that mycorrhizal colonization increased g only if plants received low P fertilization, but unlike the studies of Koide (1985) and Fitter (1988), increasing P fertilization decreased g of mycorrhizal plants, had no effect on g of nonmycorrhizal plants and only increased leaf P concentration of nonmycorrhizal plants. Apparently, the relation between mycorrhizal colonization, tissue P concentration and g varies greatly depending on experimental conditions, and mycorrhizal colonization may not affect g unless plants are subjected to drought.

The present study also included response to drought by VAM and NVAM plants that were previously exposed to drought. The effects of VAM colonization and DA treatment together promoted overall drought resistance. In a study of rose plants that did not include photosynthesis measurements (Augé et al. 1986a), there was an additive effect on drought resistance when prolonged drought was combined with *Glomus desert-icola* but not with *G. intraradices*.

In studies such as ours with plants confined to containers, it is difficult to compare treatments if plants do not have equal leaf areas, because unequal leaf areas cause an unequal rate of soil water depletion, thereby causing unequal environmental stress. Unequal leaf area was not a significant factor in our study, and similar shoot mass and specific leaf weight (reciprocal of specific leaf area, data not presented) across all treatments indicated that these important parameters were unaffected by VAM or DA.

Neither DA treatment nor the development of drought resistance affected leaf area significantly at the end of the experiment. Plant stress was manifest only in the afternoon on the 4th day of a given drought cycle. Apparently the duration of drought was too brief and drought resistance developed too late in the experiment to affect final plant size.

If plants exposed to equal environments have equal leaf area and equal g, they should deplete soil moisture equally. During cycle 5, the g values of the NVAM-NDA and VAM-DA plants were not significantly different until mid-afternoon on day 4 at which time the NVAM-NDA plants were suffering from desiccation, had lost turgor and had closed their stomata. Based on equal leaf area and g values, we conclude that by mid-morning on day 4, NVAM-NDA and VAM-DA plants probably had depleted soil moisture equally. Apparently the NVAM-NDA plants were on the threshold of drought as defined by soil moisture low enough to cause water stress, and starting at this point, differences in water status, g or A reflect differences in drought resistance. Root mass was not the rate-limiting factor in soil water extraction, because NVAM-NDA plants with relatively large root masses had equal water loss rates compared to VAM-DA plants based on leaf area and g data.

The low g of the NVAM-DA plants during day 2, cycle 5, when water stress was low, could allow these plants to conserve water for the future. However, the water status of these plants 2 days later at high stress indicated that there was no significant saving of water, although both A and g of these plants at high stress were relatively high.

The parameter of A/P is the efficiency of photosynthetic flux per unit of leaf P concentration. It is relevant to studies of this nature because A is a key growth parameter, and P nutrition is considered by many to be

central to mycorrhizal enhancement of plant growth. Furthermore, it is well documented that P deficiency reduces A (Dietz and Foyer 1986). The high value of A/P for VAM plants at all sampling times other than the water stress period of cycle 5, was due more to low P concentration than to high A in the VAM plants. Although the A values of the VAM plants were not always highest, their higher A/P values under well-watered conditions emphasize that A of the VAM plants was not limited by P, despite a lower P concentration in the VAM plants. The relatively low A/P for VAM-NDA plants on day 4, cycle 5, probably was due to low g, which should decrease A independently of P. Values for A/P can be compared among treatments even though A was normalized over leaf area while leaf P was normalized over mass, because the specific leaf areas did not differ significantly with treatment ($P = 0.05$). High A/P values in mycorrhizal plants have been reported by others (Brown and Bethlenfalvay 1988).

The high A and g of the VAM-DA plants during recovery (cycle 6) agree with the data of Bildusas et al. (1986), and were probably in part a consequence of maintenance of favorable water relations during high stress in the previous cycle. Differences in A among treatments following drought are also a measure of drought resistance. Severe desiccation can reduce g long after leaves become rehydrated (Steuer et al. 1988). The low A of our NVAM-NDA plants during recovery apparently was not due to low g but rather to low chlorophyll. Also, the high A and g of our VAM-DA and NVAM-DA plants during high stress are consistent with the data of Bildusas et al. (1986).

The drought resistance of the VAM-DA plants during high environmental stress (day 4, cycle 5) cannot be attributed to osmotic adjustment because osmotic adjustment (as indicated by low saturated ψ_π) only occurred during recovery (cycle 6). This is in contrast to data from studies of Augé et al. (1986a) where osmotic adjustment enhanced drought resistance during drought, but in agreement with data of Henderson and Davies (1990); both studies used mycorrhizal rose plants. Pepper is capable of osmotic adjustment (Turner and Wellburn 1985), but in our experiment, low ψ_{leaf} developed abruptly and lasted only a short time before the next irrigation, due to the rapid decrease in ψ_{soil} as our sandy soil neared complete dryness late in each drought cycle. Osmotic adjustment develops to the greatest extent with gradually decreasing and prolonged low ψ_{leaf}, as opposed to short drought cycles (Morgan 1984).

Soluble carbohydrates did not account for differences in osmotic adjustment in our plants, a finding similar to that of Cutler and Rains (1978) with a nonmycorrhizal study of cotton. In our study, as in that of Augé et al. (1987a), mycorrhizae had a variable effect on leaf starch and soluble carbohydrate, probably due to a complex source-sink interaction (Harris and Paul 1987).

The drought resistance of the VAM-DA plants was associated with enhanced development of extraradical hyphae (measured by soil aggregation), as noted in a part of this study reported elsewhere (Davies et al. 1992). The favorable water relations of the VAM-DA plants at this time suggest that hyphae promoted soil water uptake.

There is some inconsistency in the time course data; A and g during the afternoon of day 4, cycle 5, were equal for all DA plants, but at this time the water status of the NVAM-DA and VAM-DA plants differed greatly. Among 160 plants ($n = 40$ per treatment) evaluated for visible wilting in the 4 treatments, 83% of NVAM-NDA plants were severely wilted compared to 15% of VAM-DA plants (Davies et al. 1992); this variation could contribute to inconsistency because the A and g values were the means of 5 plants, whereas the water relations values were the means of 3 plants. We have the most confidence in data that are based on the most observations. With the exception of NVAM-DA, the water relations and gas exchange data agree for the other 3 treatments.

Both NVAM and VAM plants were subjected to repeated drought during the first 4 cycles. The drought cycles caused more stress on day 4 than on day 2, indicating that plant stress was due to drought and not merely a transient midday stress in moist soil. Drought acclimation occurred in this experiment as indicated by the ability of VAM-DA plants to delay the onset of plant stress on day 4, cycle 5. Furthermore, VAM-DA plants responded to DA by developing a more extensive hyphal system (Davies et al. 1992).

In summary, drought resistance in VAM-DA plants in this long-term time course study was not attributable to leaf P concentration or confounded by plant size. More extraradical hyphae developed on VAM-DA plants, which could have facilitated water uptake during high stress (Davies et al. 1992). Osmotic adjustment developed by cycle 6 at the end of the study. The combination of these effects should promote drought resistance.

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