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Effects of drought on host and endophyte development in mycorrhizal soybeans in relation to water use and phosphate uptake

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Soybean [*Glycine max* (L.) Merr.] plants were grown in pot cultures and inoculated with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe or provided with P fertilizer (non-VAM plants). After an initial growth period (21 days), plants were exposed to cycles of severe, moderate or no drought stress over a subsequent 28-day period by rewatering at soil water potentials of −1.0, −0.3 or −0.05 MPa. Dry weights of VAM plants were greater at severe stress and smaller at no stress than those of non-VAM plants. Phosphorus fertilization was applied to produce VAM and non-VAM plants of the same size at moderate stress. Root and leaf P concentrations were higher in non-VAM plants at all stress levels. All plants were stressed to permanent wilting prior to harvest. VAM plants had lower soil moisture content at harvest than non-VAM plants. Colonization of roots by *G. mosseae* did not vary with stress, but the biomass and length of the extraradical mycelium was greater in severely stressed than in non-stressed plants. Growth enhancement of VAM plants relative to P-fertilized non-VAM plants under severe stress was attributed to increased uptake of water as well as to more efficient P uptake. The ability of VAM plants to deplete soil water to a greater extent than non-VAM plants suggests lower permanent wilting potentials for the former.

Key words – *Glomus mosseae*, *Glycine max*, permanent wilting potential, phosphorus nutrition, water use.

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Introduction

The growth response of plants to colonization by vesicular-arbuscular mycorrhizal (VAM) fungi as a function of soil water status is emerging as an area of interest and controversy (Fitter 1985, Nelsen 1987, Reid 1984, Safir and Nelsen 1981, Sieverding 1986). Interest is motivated by the problems of water-use efficiency in modern agriculture (Larson et al. 1981), while the controversy is related to the elucidation of the response mechanism. The availability of soil water and P to the plant are interdependent (Olsen et al. 1961) due to the linear relationship between the diffusion coefficient of phosphate and soil moisture (Viets 1972). Since VAM fungi are involved in the uptake of both soil water and phosphate (Safir and Nelsen 1981), the role in, and contribution to the growth response by each is of interest. The ability of VAM fungi to maintain adequate P nutrition in plants under drought stress has been postulated as a major factor in improved drought tolerance (Graham and Sylvertsen 1984, Kwapata and Hall 1985, Nelsen and Safir 1982, Sieverding 1986). Recently, evidence for water as an important factor has also been presented (Hardie 1985). We have indirect evidence suggesting that unavailable soil water (soil water at potentials below those associated with permanent wilting) may be involved in the VAM growth response (Dakessian et al. 1986). This prompted us to investigate further the rela-

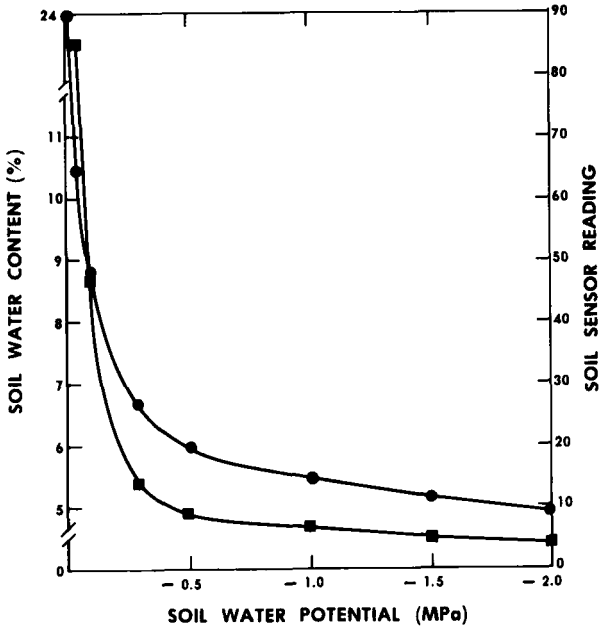

Fig. 1. Soil water content (●) and water potential relationships. Soil water potential determination and soil sensor calibration were accomplished simultaneously using a pressure plate apparatus. During the stress phase of the experiment, plants were watered when soil sensor readings (■) averaged −0.05, −0.3 or −1.0 MPa.

tionship between the development of the VAM symbionts and soil water status. The purpose of this experiment was to test further the hypothesis of P as the major factor in VAM response to drought, and to determine if a relationship existed between unavailable soil water and the response of the host plant to colonization by VAM fungi under drought stress.

Abbreviations – R/L, root/leaf; VAM, vesicular-arbuscular mycorrhizal.

Materials and methods

Experimental design

The experiment was designed as a 2 × 3 factorial consisting of 6 treatments with 6 replications for a total of 36 units. The factors were drought stress at 3 levels, and P nutrition mediated either by P fertilization or by colonization of plants by a VAM fungus.

Biological materials

Soybeans [*Glycine max* (L.) Merr. cv. Hobbit] were germinated for 2 days at 30°C, selected for uniformity, and planted in 1.5-l pots with or without an inoculum of the VAM fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe. The fungus had been collected at a desert site and recultured under controlled conditions on *Sorghum bicolor* L. The inoculum consisted of 60 ml soil containing ca 660 ± 50 sporocarps with 1–5 spores per sporocarp. It was mixed into the VAM-plant growth medium uniformly prior to planting. All plants received an inoculum wash free of VAM-fungal propagules to equalize the microbiota of the VAM and non-VAM treatments (Ames et al.1987).

Growth conditions

Soybean were grown in a walk-in type growth chamber at day/night regimes of 16/8 h, 27/21°C and 40/60% RH. Uniform illumination of 800 μmol m^{-2} s^{-1} was provided by 1500 mA cool white fluorescent and 100 W incandescent lamps. The growth medium was a Balcom series (Yolo County, CA, USA) heavy silt loam (Typic Xerorthent) of pH 7.7 (paste), with ammonium bicarbonate-extraxtable P and total N contents of 3.3 μg and 0.69 mg (g soil)$^{-1}$, respectively. The original soil had a sand/silt/clay content of 20.5/55.6/23.9% (v/v). It was mixed with fine sand (2:1, v/v, soil:sand) to avoid caking and cracking when dry, sterilized by autoclaving, potted, wetted from below, and allowed to stand 5 days prior to planting. Soil water content and water potential relationships of the soil-sand mix are shown in Fig. 1.

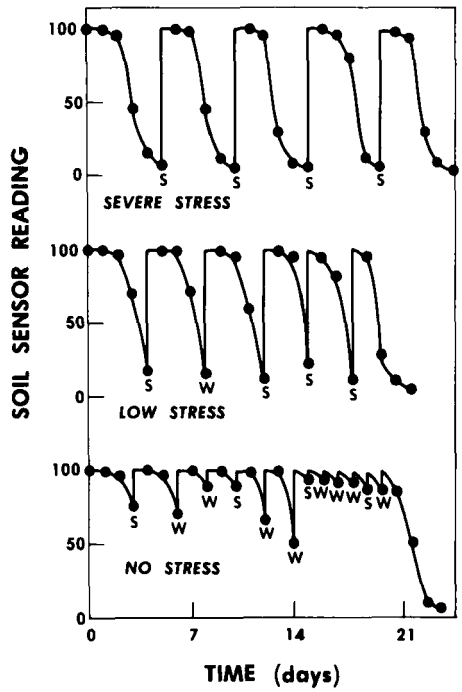

Fig. 2. Stress cycles. Plants (VAM-plant schedule shown only) were watered when soil sensor readings first reached or exceeded a range of readings corresponding to soil water potentials (ψ, MPa) at 3 stress levels: $-0.04 > \psi > -0.08$, field capacity; $-0.2 > \psi > -0.4$, moderate stress; $-0.5 > \psi > -1.5$, severe stress. To equalize nutrient inputs among stress treatments, all plants recieved nutrient solutions (S) with equal frequency. Plants at lower levels of stress also received water (W) to maintain their moisture regime between solution applications.

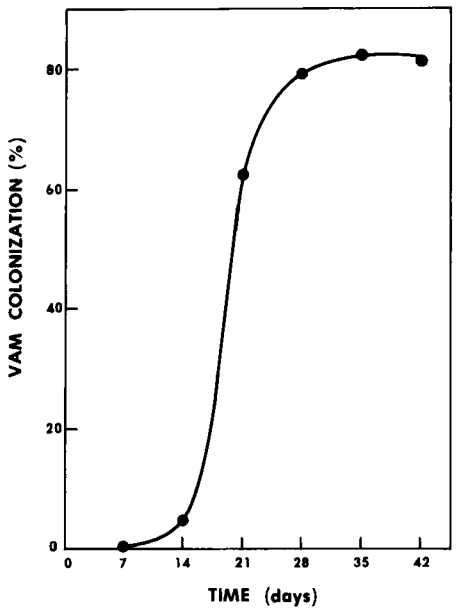

Fig. 3. Time course of VAM colonization. The progress of VAM colonization was monitored using extra plants. Experimental non-VAM plants recieved P amendments (1.2 m*M* KH_2PO_4) on days 21 and 27, after VAM colonization exceeded 60% of root length.

Plants were watered to saturation at 3-day intervals with a basal nutrient solution of 1.5 m*M* $CaCl_2$, 0.25 m*M* $MgSO_4$, 3.0 m*M* NH_4NO_3, 1.0 m*M* K_2SO_4 and micronutrients at one-quarter strength Johnson's solution (Bethlenfalvay et al. 1981) during the initial 21-day growth period. On day 21, when colonization of VAM-plant root length was 60% (Fig. 2), non-VAM plants received a nutrient solution amended with KH_2PO_4 (1.2 m*M*). This was repeated on day 27. At other times, all plants received the basal solution according to the watering schedule during the 28-day stress period (Fig. 3). During the stress period, plants kept at field capacity or rewatered at −0.3 MPa received nutrient solution as often as the plants rewatered at −1.0 MPa; at other times they received deionized water.

Assays

Soil water potential was monitored daily by use of individually calibrated (pressure plate technique, Fig. 1) soil moisture blocks (Soilmoisture Equipment Corp. Model 5201, Santa Barbara, CA, USA) placed in the pots at two-thirds (10 cm) depth. Treatments were rewatered when mean soil moisture values of the 6 replications were −0.05 MPa (unstressed), −0.3 MPa (intermediate stress) or −1.0 MPa (severe stress). Following the final watering and prior to harvest, all treatments were dried to permanent wilting (no recovery of turgor within 6 h on being placed in a chamber at 100% RH). In preliminary studies, this point coincided with a 90° droop of the stem at the growing tip, a complete recurving of the edges of the leaf distal to the youngest fully expanded leaf and with leaf conductance rates (Model LI-1600 Steady-State Porometer, LI-COR, Inc., Lincoln, NE, USA) of the last fully expanded leaf below 0.01 g m^{-2} s^{-1}. Experimental plants were continuously monitored after the last watering and harvested as soon as these conditions indicating permanent wilting appeared.

Soil water content at permanent wilting was determined by shaking the soil off the roots, sieving it rapidly though a 2 mm screen to eliminate root fragments, and weighing before and after drying at 110°C for 24 h. Root and leaf samples (three leaflets per plant) were taken from turgid (24 h after last watering), and stressed (at permanent wilting) plants of all treatments. Samples were frozen in liquid N_2 immediately upon excision, lyophilized, ground and analyzed for P (Allen 1940) and proline (Bates et al. 1973). Dry weights of roots, leaf blades and total plant matter were determined after drying at 70°C for 2 days. Biomass [μg (g soil)$^{-1}$] and hyphal length [m (g soil)$^{-1}$] of the extraradical VAM fungal mycelium were determined by the chitin assay and by microscopic investigation of soil samples (Bethlenfalvay and Ames 1987), respectively.

Colonization of VAM-plant root length was evaluated microscopically after staining (Bethlenfalvay et al. 1981). The development of VAM-fungal colonization was determined weekly on roots in soil cores from 4 extra plants (Fig. 3).

Comparisons of VAM and non-VAM treatment data were analyzed by Student's *t*-test, and those of drought levels by Duncan's multiple range test. Plant dry weight as a function of drought stress was evaluated by regression analysis for VAM and non-VAM plants.

Tab. 1. Stress effects on plant dry weight. Plants were harvested between days 44 and 47 after planting, following drought cycles of differing severity. Plants were watered at field capacity (−0.05 MPa), moderate stress (−0.3 MPa) and severe stress (−1.0 MPa). Plants were colonized by the VAM fungus *Glomus mosseae* (VAM) or fertilized with KH_2PO_4 (P-fed). Numbers are the means of 6 replications. Comparisons between P-nutrition methods (VAM *vs* P-fed) were evaluated for significance by Student's *t*-test (NS, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). The effects of stress were evaluated by Duncan's multiple range test, and were not significantly different ($P > 0.05$) where numbers are followed by the same letter.

Plant part	P nutrition	Dry weight (g)					
		−0.05 MPa		−0.30 MPa		−1.00 MPa	
Total plant	VAM	10.6***	a	8.3NS	b	7.2***	c
	P-fed	12.6	a	8.1	b	6.3	c
Root	VAM	3.1*	a	2.3***	b	1.8***	c
	P-fed	2.7	a	1.7	b	1.4	
Leaf	VAM	3.8***	a	3.2*	b	2.8*	c
	P-fed	5.2	a	3.5	b	2.7	c

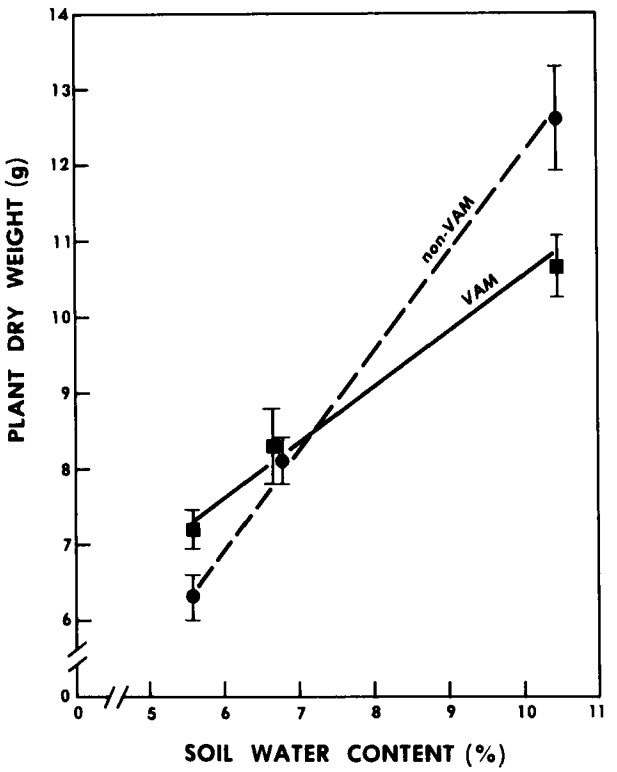

Fig. 4. Correlation of stress level and plant growth. Plant dry weight is shown as a function of soil water content corresponding to the average value of soil water potentials at rewatering during the stress phase (21 to 47 days). Evaluation of the data (six points at each level of soil water content) yielded highly significant linear correlations for each line (VAM, r = 0.95***; non-VAM, r = 0.98***), and a highly significant difference of the slopes ($P < 0.001$).

Results

Growth of both VAM and non-VAM plants decreased with increasing drought stress (Tab. 1). However, the effect of soil water status was more pronounced on non-VAM plants, whose dry weights were significantly greater without stress (field capacity) and smaller at severe stress (−1.0 MPa) than those of VAM plants (Fig. 4). At moderate stress (−0.3 MPa) there was no significant difference, as P nutrition of non-VAM plants was so regulated as to produce 'VAM-equivalent' comparison plants at this stress level. Statistical analysis of the dry-weight vs water-regime regression lines of VAM and non-VAM plants showed highly significant correlation coefficients and a highly significant difference between slopes (Fig. 4).

Phosphorus concentrations in roots and leaves of non-VAM plants were significantly higher at all stress levels than in VAM plants (Tab. 2), suggesting that P uptake was not a decisive factor in the growth enhancement of VAM plants at high stress. The soils of VAM plants, on the other hand, were significantly lower in water content at permanent wilting than those of non-VAM plants of all 3 stress treatments (Tab. 3). These 'permanent wilting percentages' of soil water content indicated an ability of the VAM root to take up soil water that was not available to the non-VAM root. Thus, the advantage of this ability of VAM plants may be expected to increase with decreasing availability of water. This may partly explain the larger size of VAM plants at severe stress. Higher transpiration rates generally observed in VAM plants on a leaf area basis (Allen and Boosalis 1983), together with a more thorough exploration of the soil by the larger root systems of our VAM plants could have intensified soil water depletion, resulting in greater water use.

Tab. 2. Stress effects on plant P concentration and total P content at harvest. Growth conditions and statistical analyses were as in Tab. 1.

Plant part	P nutrition	Stress level (MPa)		
		−0.05	−0.30	−1.00
		P concentration (mg g^{-1})		
Root	VAM	1.45***a	1.45***a	1.40***a
	P-fed	2.37 a	1.82 b	1.75 b
Leaf	VAM	0.82***a	1.02***b	1.10***c
	P-fed	1.88 a	2.07 a	1.95 a
		Total P content (g)		
Root	VAM	4.5** a	3.3NS b	2.5NS c
	P-fed	6.4 a	3.1 b	2.5 c
Leaf	VAM	3.1***a	3.3***a	3.1** a
	P-fed	9.8 a	7.2 b	5.3 c

Tab. 3. Soil water content of plants with or without a VAM fungus at permanent wilting. Percentage difference between soil water contents was calculated with the non-VAM plant as basis. The soils were rapidly processed prior to weighing before and after drying at 110°C for 24 h. Plant material and statistical analyses were as in Tab. 1.

Stress level (MPa)	Soil water (%)		Difference %
	VAM	P-fed	
−0.05	4.74* a	5.16 a	8.1
−0.30	4.71* a	5.06 a	6.0
−1.00	4.42**a	4.93 a	10.3

Tab. 4. Stress effects on root/leaf (R/L) ratios of dry weight and P concentration. Plant material and statistical analyses were as in Tab. 1.

R/L ratio	P nutrition	Stress level (MPa)		
		−0.05	−0.30	−1.00
Dry weight	VAM	0.81***a	0.72***b	0.63***c
	P-fed	0.53 a	0.48 a	0.50 a
P content	VAM	1.77***a	1.42***b	1.26***c
	P-fed	1.26 a	0.88 b	0.90 b

Tab. 5. Development of the VAM fungus. Intraradical VAM-fungal colonization (percentage of root length that contained fungal structures) was determined by staining and microscopic screening. Extraradical hyphae were assayed by the chitin test (biomass) and by microscopic determination of hyphal length. Extraradical mycelium data reflect only fungal structures contained in soil shaken from the roots at harvest. Hyphae adhering to roots were not recovered. The effects of stress were evaluated by Duncan's multiple range test and were not significantly different ($P > 0.05$) when numbers are followed by the same letter.

VAM-fungal mycelium	Parameter	Stress level (MPa)		
		−0.05	−0.30	−1.00
Intraradical	Colonization (%)	79.5 a	75.3 a	80.9 a
Extraradical	Hyphal length [m (g soil)$^{-1}$]	7.0 a	8.8 ab	10.4 b
	Biomass [µg (g soil)$^{-1}$]	14.8 a	21.6 b	25.1 b

A comparison of the dry-weight root/leaf (R/L) ratios, however, suggests that P nutrition was involved in the different growth responses (Tab. 4). Such ratios are influenced by the nutrient and water status of the soil (Wareing and Patrick 1975). For non-VAM plants in general, the greater the availability of water and nutrients, the smaller the R/L weight ratio tends to be. Thus, R/L weight ratios of VAM plants have been usually observed to be smaller than those of non-VAM plants grown in P deficient soil, due to the increased P uptake capability of the former. This is an expression of efficient resource allocation by the plant. In the present case, R/L weight ratios of non-VAM plants were invariant with stress, but were significantly smaller than those of VAM plants at all levels of stress. The R/L weight ratios of VAM plants, on the other hand, decreased significantly with increasing stress. The stress-invariant, low R/L weight ratios of non-VAM plants are construed to reflect the ample levels of P in root and leaf tissues and of P availability in the P-amended soil at all stress levels. The larger R/L weight ratios of VAM plants indicate the plant's need to increase its mineral uptake capability in a soil severely deficient in P. The decline in R/L weight ratios of VAM plants with increasing stress indicates a concomitant increase in their uptake capatility relative to non-stressed plants. This increased uptake capacity is reflected by the development of more extraradical VAM hyphae under stress. The decline in the R/L weight ratios was parallelled by a similar decline in the R/L phosphorus ratios (Tab. 4). This indicated that the efficiency of P uptake per unit root mass increased as total P uptake decreased with increasing stress (Tab. 2), permitting relatively more P to be translocated to the leaves.

Higher efficiency in water and P uptake was apparently related to the greater development of the extraradical VAM-fungal mycelium in the stressed plants compared to those grown at field capacity (Tab. 5). Such changes in fungal development related to soil water status were found by others (Reid and Bowen 1979, Sieverding 1984). However, these previous reports note a decline in fungal development at high and low levels of soil water content. Our results confirm the decline in extraradical mycelium development at a high but not at a low level of soil water. Colonization of the root (percentage of root length) did not vary with stress treatment. It appears likely that intra- and extraradical VAM-fungal development is influenced not only by soil water status but also by the differences in host, endophyte and soil combinations (Bethlenfalvay et al. 1985, Fitter 1985).

An important host contribution to enhanced water uptake is the regulation of plant water status in response to stress. This is of particular interest in VAM plants, if VAM roots are indeed capable of depleting soil water to a greater extent. To investigate this possibility, we determined the levels of proline, an osmoticum produced by leaves in response to stress (Paleg and Aspinall 1981), in leaves and roots of all treatments (Tab. 6). Proline levels in turgid plants (sampled 24 h after the last rewatering) showed a residual effect of cyclic water stress: concentrations were higher in plants exposed to more severe stress. This trend was inversely related to the decrease noted in the permanent wilting percentages of soil water (Tab. 3), suggesting a connection between drought conditioning and soil water depletion. Proline levels in the roots of wilted VAM plants (sampled at harvest), however, were not consistently higher than those of non-VAM plants. Thus, the implication of increased proline production in the leaf and transport to

Tab. 6. Stress effects on plant proline content. Proline contents were determined in turgid plants (24 h after last watering) and plants at permanent wilting. Plant materials and statistical analyses were as in Tab. 1. [1]Data for turgid roots not available.

Plant part	P nutrition	Proline content (mg g^{-1})					
		Turgid[1]			Wilted		
		−0.05 MPa	−0.30 MPa	−1.00 MPa	−0.05 MPa	−0.30 MPa	−1.00 MPa
Leaf	VAM	0.14* a	0.34* b	1.07* c	3.27* a	2.65* a	8.51* b
	P-fed	0.10 a	1.14 b	2.01 c	1.50 a	5.16 b	18.21 c
Root	VAM	–	–	–	1.63* a	1.48 a	2.38 b
	P-fed	–	–	–	0.71 a	1.49 b	2.26 c

the root as a mechanism facilitating the uptake of 'unavailable' water by VAM roots remains inconclusive. However, if proline accumulation provides a measure of the extent of stress experienced by plants (Paleg and Aspinall 1981), the low levels of proline in the leaves of stressed VAM plants relative to non-VAM plants indicate that VAM plants were suffering less stress.

Discussion

Our data confirm the findings of others (Ellis et al. 1985, Hardie and Leyton 1981, Nelsen and Safir 1982) that colonization by VAM fungi confers a growth advantage on the host plant under drought stress (Fig. 4). However, new findings presented here necessitate a reevaluation of the causes of the VAM growth effect. These findings demonstrate the ability of the VAM root to take up water that is not available to the root alone (Tab. 3). A suggestion of this phenomenon was first reported by Hardie and Leyton (1981), who calculated soil water potentials of VAM and non-VAM plants and found that VAM plants wilted at markedly lower potentials than non-VAM plants. They concluded that VAM plants are able to dry down the soil more effectively and ascribed this to the very much smaller root systems of their non-VAM plants. Later, Hardie (1985) was able to demonstrate the role of extraradical VAM-fungal hyphae in water uptake in an elegant experiment, in which root size as a factor in soil water depletion was eliminated, thus providing an excellent control for comparison.

We, too, feel that the question of a proper control is crucial to an evaluation of the VAM-drought growth response phenomenon. However, such controls are elusive, as P-supplemented non-VAM plants do not conform to the desired criteria of root and leaf comparability with VAM plants (Pacovsky et al. 1986). Thus, the choice of P supplementation must be made with a preconceived admission of compromise. In the present investigation, we have grown a non-VAM control at the intermediate stress level with the same total mass (larger leaves, smaller roots) as the VAM plant. As the nutritional state (tissue P concentration) of non-VAM comparison (control) plants grown with phosphate is an important criterion for deciding whether the growth effect observed is due primarily to P or water deficiency, the proper timing, form and amount of P application is of consequence. This is also elusive in view of the state of 'dynamic disequilibrium' applicable to the pool of labile P in soil (Helyar and Munns 1975).

The impact that the particulars of P application will have on results and interpretation is illustrated by a pioneering report by Nelsen and Safir (1982), whose P-supplemented non-VAM plants under drought stress were significantly smaller than stressed VAM plants. Since the P concentration of their non-VAM plants was also lower, they advanced the reasonable argument that the greater growth of the VAM plants could be attributed to their improved P nutrition. To quote Fitter (1985): 'It is highly significant that the nonmycorrhizal plants of Nelsen and Safir fared badly despite the addition of P to the soil, which was clearly unavailable to them in the dry condition'.

Our data, to the contrary, show less biomass and higher P concentrations in non-VAM plants under drought stress, and more biomass and less P in VAM plants. The higher levels of P in our non-VAM plants are easy to explain. We added P in the same form (KH_2PO_4) as used by Nelsen and Safir (1982), but timed its application to coincide with the end of the logarithmic growth phase of the fungus. Thus, the impact of the two methods of P input (by VAM fungi or fertilization) was simultaneous, and permitted less time for soil-P interaction and possible P fixation than is the case with P application at planting. The greater biomass of our P-deficient VAM plants, compared to non-VAM plants with P concentrations well above the critical P content for soybeans (De Mooy et al. 1973), suggests that under the conditions of our experiment, increased water uptake, in addition to more efficient P uptake, was a factor in bringing about the positive VAM growth response. Efficient P uptake by VAM plants in the dry soil was shown by the constancy of P uptake at all stress levels, while P uptake per unit root weight in the non-VAM plants declined with stress (Tab. 2). This is consistent with the concept that a main effect of VAM fungi in dry soil is on P uptake because of limited P diffusion.

The possibility of a relationship between the uptake of water unavailable to non-VAM plants, VAM fungi, and plant growth enhancement needs further testing with different host-endophyte combinations, soils of different water-holding capacities and diverse environmental and nutritional conditions. If substantiated by further evidence, applications in agriculture may be envisioned: VAM fungus-mediated access to unavailable soil water would be analogous to plant access to unavailable P, impacting on water-use efficiency and irrigation regimes. Physiologically, the mechanisms (probably by osmotic adjustment) of enhanced drought tolerance and water uptake by VAM plants need to be examined and clarified.

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