

Glycine-Glomus-Bradyrhizobium Symbiosis¹

X. Relationships between Leaf Gas Exchange and Plant and Soil Water Status in Nodulated, Mycorrhizal Soybean under Drought Stress

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ABSTRACT

Soybean (*Glycine max* [L.] Merr.) plants were colonized by the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe (VAM plants) or fertilized with KH_2PO_4 (nonVAM plants) and grown for 50 days under controlled conditions. Plants were harvested over a 4-day period during which the soil was permitted to dry slowly. The harvest was terminated when leaf gas exchange was no longer measurable due to drought stress. Significantly different effects in shoot water content, but not in shoot water potential, were found in VAM and nonVAM plants in response to drought stress. Leaf conductances of the two treatments showed similar response patterns to changes in soil water and shoot water potential but were significantly different in magnitude and trend relative to shoot water content. The relationships between transpiration, CO_2 exchange and water-use efficiency (WUE) were the same in VAM and nonVAM plants in response to decreasing soil water and shoot water potential. As a function of shoot water content, however, WUE showed different response patterns in VAM and nonVAM plants.

Mycorrhizal effects on plant water relations were first documented by Safir *et al.* (27) and are now well recognized in the rapidly expanding literature of plant symbioses (23). However, they have been slow in reaching general plant-science audiences (9, 29) and the specialized water-stress review literature (28). One reason for this lag may be the disagreement in the VAM² literature itself about the mechanisms of VAM effects during drought stress, especially as they relate to the role of VAM-mediated P nutrition in plant water relations (5, 7, 15). The role of P nutrition is well-established in the water stress physiology of nonVAM plants (1, 25, 26) but inconclusive in VAM plants (5, 15), suggesting a need for more information on VAM-mediated interactions between P nutrition and drought response to fit VAM effects into the overall structure of plant water relations (15).

Colonization of roots by VAM fungi affects, directly or

indirectly, the mechanisms which control plant water relations: root hydraulic conductivity (19, 23), leaf conductance (3, 5), leaf gas exchange (4, 16), leaf expansion (20), osmotic adjustment (6), and phytohormone production (2, 8, 13). Yet it is not possible to discern from the general literature of plant drought stress whether the information reported is based on VAM or nonVAM plants. Presumably, experiments under controlled conditions utilize sterilized (nonVAM) growth media, while the field work takes place in unsterilized (VAM) soil. Determination of the symbiotic status of plants and measurement of changes in their response to environmental conditions due to colonization by VAM fungi should make it easier to relate findings (21, 24) under controlled conditions to those in the field.

The purpose of this experiment was to determine if VAM and P-supplemented nonVAM plants showed different gas exchange characteristics relative to soil water status and to two measures of plant water status (shoot water potential and absolute water content) under the influence of increasing drought stress.

MATERIALS AND METHODS

Experimental Design

Twenty VAM and 20 nonVAM plants were grown in a completely random design. Plants were selected from this pool for evaluation over a 4-d harvest period, based on readings of soil moisture sensors. Harvests of individual plants were timed to determine plant water status and gas exchange over the range of available water in the slowly drying soil. The procedure produced two sets of data points, one for VAM and one for nonVAM plant parameters, which were evaluated by regression analysis within and between data sets. Differences between data sets were evaluated by the overlap of the confidence intervals ($P < 0.05$) of the regression coefficients. Differences in dry masses, nutrient status, and leaf areas due to P nutrition (by VAM fungi or P fertilization) were evaluated by Student's *t* test, averaging all VAM and nonVAM plants regardless of drought status.

Biological Materials and Growth Conditions

Soybean (*Glycine max* [L.] Merr. cv Hobbit) plants were grown in a walk-in type growth chamber for 50 d at day/night

¹ A contribution from the Plant-Soil Symbiosis Group, Plant Development-Productivity Research Unit.

² Abbreviations: VAM, vesicular-arbuscular mycorrhizal; CER, carbon dioxide exchange rate; WUE, photosynthetic water-use efficiency.

Table I. Plant Development and Nutrition and Root Colonization in Nodulated, VAM, and P-Fertilized (nonVAM) Soybean

Nutrient concentrations are those of the fifth fully expanded leaf. Evaluation of VAM and nonVAM plant parameters by Student's *t* test.

Treatment	Dry mass		Leaf Area cm ²	Nutrients		VAM Colonization %
	Root	Shoot		N	P	
	g			mg g ⁻¹		
VAM	1.29 NS ^a	3.98**	415**	3.49 NS	0.13**	67.5
nonVAM	1.31	4.71	540	3.46	0.17	

^a Not significant (NS), *P* > 0.05; **, *P* < 0.01

regimes of 16/8 h, 27°C/21°C, and 50/70% RH, and PPFD of 780 μE m⁻² s⁻¹. The soil (1.25 L per pot) was a Balcom series (Yolo County, CA) heavy silt loam (Typic Xerorthent) of pH 8 (Paste), NH₄HCO₃-extractable P of 3.2 μg g⁻¹, total N of 0.7 mg g⁻¹ and a sand:silt:clay content of 20.5:55.6:23.9%. This soil was mixed with fine sand (2:1, v:v, soil:sand), autoclaved, and initially soaked from below with deionized water.

A suspension of the diazotrophic bacterium *Bradyrhizobium japonicum*, strain Nitragin 61A118 (10 mL plant⁻¹, 10⁹ cells mL⁻¹) was added to all pots. A soil inoculum of the VAM fungus *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe, isolate WRRC-1 (60 mL of soil containing approximately 700 sporocarps with 1 to 5 spores per sporocarp), was mixed into the soil of each of the VAM plants prior to the planting of the pregerminated, surface-sterilized (70% ethanol) seeds. Seedlings were selected for uniformity. Both treatments (VAM and nonVAM) received a wash of the VAM inoculum, sieved free of VAM propagules to minimize differences in the soil microbiota. At d 7 and 14, until the establishment of VAM colonization (50% of total root length at 21 d), plants received one-quarter strength Johnson's solution (17) modified to contain low levels of P and N (0.2 mM

KH₂PO₄ and 1 mM Ca[NO₃]₂) to enhance nodulation without inhibiting VAM colonization. Thereafter, plants were watered twice a week, with VAM plants receiving no N or P, and non-VAM plants receiving no N and 0.4 mM KH₂PO₄ in their nutrient solutions.

Measurement of Soil and Plant Water Status

Watering was discontinued 50 d, after planting, before the roots became pot-bound. During the drying phase, soil water status was monitored by gypsum-block soil moisture sensors (Soilmoisture Equipment Corp, model 5201) embedded in each pot at two-third's depth. The sensors were calibrated by means of a chart which established a relationship between soil sensor readings, soil water potential as determined by pressure plate apparatus (Soilmoisture Equipment Corp. 15 Bar Ceramic Plate Extractor, Cat. No. 1500), and soil water content measured by weighing before and after drying at 110°C for 24 h. The range of available soil water was established by measuring soil water content at -0.1 MPa and at the permanent wilting point of plant leaves (determined by direct measurement of recovery from wilting). The water content of the soil of each pot was determined at harvest and expressed as a percentage of total available water.

Water loss from the soil was estimated by weighing the entire plant-soil unit at the beginning and the end of the drought period. When divided by the number of hours of light between these two times, soil water loss provided an expression for the rate of soil water depletion. Changes in biomass due to assimilation and respiration were disregarded as small relative to the change in water status.

Shoots were immediately weighed for fresh mass upon excision from the root at soil level, enclosed in plastic bags, and placed in a Scholander pressure cylinder for the determination of shoot water potential. Total plant leaf areas were measured with a LI-3000 area meter (LI-COR, Inc.). Roots

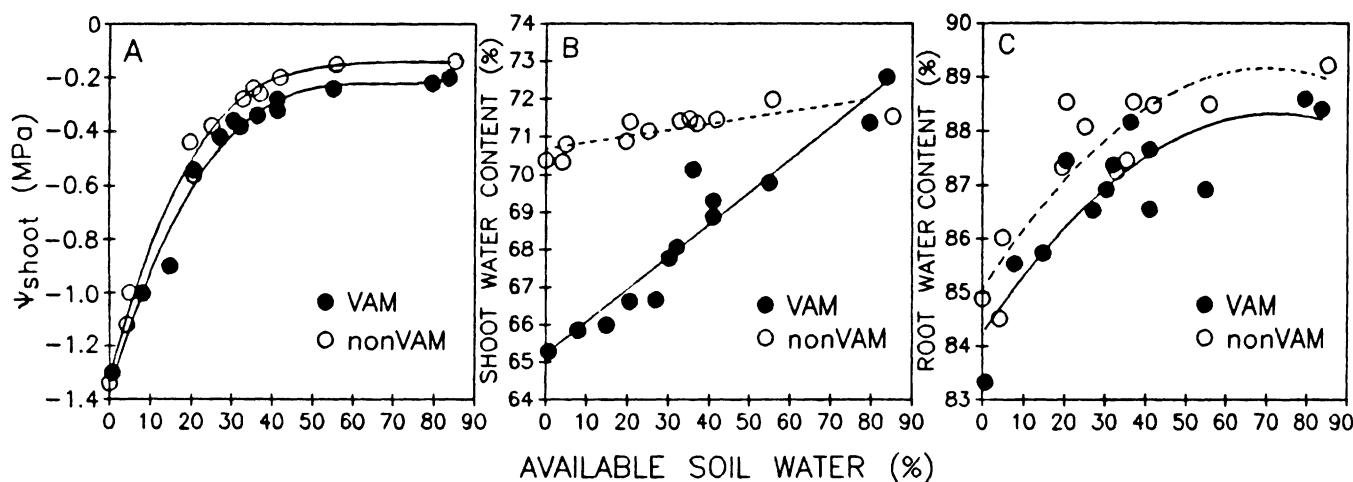


Figure 1. Shoot water potential (Ψ) and shoot and root water content as functions of available soil water in VAM and P-fertilized (nonVAM) soybean plants. A), VAM, $y = 1 - 0.43e^{-0.83x}$; nonVAM, $y = 1 - 0.56e^{-0.84x}$. B), VAM, $y = 0.09x + 65.2$, $r^2 = 0.91$, $P < 0.0001$; nonVAM, $y = 0.02x + 70.7$, $r^2 = 0.64$, $P < 0.01$; slopes significantly different ($P < 0.05$). C), VAM, $y = -7.9x^2 + 0.11x + 84.2$, $r^2 = 0.71$, $P < 0.01$; nonVAM, $y = -8.4x^2 + 0.12x + 85.5$, $r^2 = 0.79$, $P < 0.001$. Available soil water was determined between soil water potentials of 0.1 MPa and the permanent wilting point. Data points (one observation) are expressed as a percentage of this amount in the gradually drying soil. Plant water content was calculated as (fresh mass - dry mass)/fresh mass. Shoot water potential was determined with a Scholander pressure apparatus.

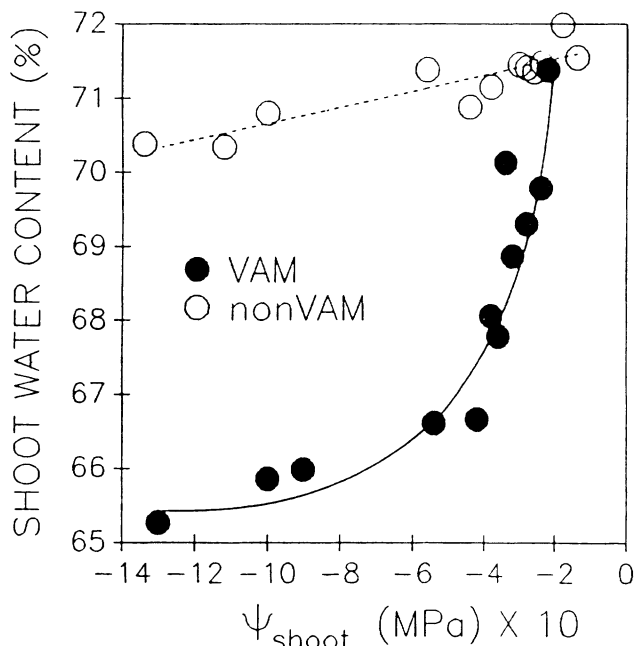


Figure 2. Relationship between shoot water potential (Ψ) and water content in VAM and P-fertilized (nonVAM) soybean plants in a gradually drying soil. VAM, $y = 65.6 + 0.006e^{0.6(x+14)}$; nonVAM, $y = 0.11x + 71.7$, $r^2 = 0.82$, $P < 0.001$.

were carefully washed free of soil, spread, blotted free of excess moisture between paper towels, and weighed for fresh mass. Dry masses of leaves and stems were determined separately after drying for 48 h at 70°C. Shoot and root water contents at harvest were calculated as (fresh mass-dry mass)/fresh mass. The N and P contents of ground samples of leaf blades were determined by Kjeldahl analysis and by Inductively-Coupled-Plasma/Atomic-Emission Spectrography, respectively. Root colonization by VAM fungi was measured by the grid-line intersect method.

Analysis of Leaf Gas Exchange

Transpiration and CER of entire shoots were determined in intact plants in a gas exchange system described elsewhere (11), utilizing an APPA-3 infrared gas analyzer (Anarad, Inc.) capable of simultaneous measurement of water vapor and CO₂ concentrations. Conditions during measurements in the fan-stirred plant chamber were PPFD of 750 $\mu\text{E m}^{-2} \text{s}^{-1}$, air temperature of $31.5 \pm 1.0^\circ\text{C}$, and entering air flow at 5 L min^{-1} containing 330 $\mu\text{L CO}_2 \text{L}^{-1}$ and humidified to 64% RH. Leaf temperatures were held at $32 \pm 1^\circ\text{C}$. Measurements were made from the third to the eleventh hours of the light period (between 8 AM and 4 PM), since previous studies have shown no diurnal fluctuations during this period. Leaf conductance was calculated as transpiration multiplied by the quotient of atmospheric pressure divided by the water vapor pressure difference between leaf interior and the chamber atmosphere (14). Since the measurements were made on whole shoots, air circulation varied with leaf size and position

along the shoot and from plant to plant. It was therefore not possible to distinguish between stomatal and boundary layer conductances.

RESULTS

When growing VAM plants and nonVAM plants supplemented with P for comparison, similarities in some plant parameters may be achieved, but only at the expense of dissimilarities in others (see ref. 9 and Table I). To avoid differences due to developmental and nutritional effects within VAM and nonVAM plants, we chose to base our comparative gas exchange measurements on entire shoots, rather than on individual leaves. Differences in P regimes produced plants similar ($P > 0.05$) in root mass and leaf N concentration but with greater shoot mass and leaf area and higher leaf P concentrations in the nonVAM plants (Table I).

The decrease in shoot water potential with the drying of soil was best expressed by an asymptotic curve and that in root water content by second order regression (Fig. 1, A and C). The VAM and nonVAM data sets did not differ significantly for either measure, but did so for shoot water content (Fig. 1B), which was a linear function of decreasing soil water. Absolute, rather than relative, water contents were measured because of the uncertainties inherent in the determination of saturation points needed for the latter (10). These uncertainties would be compounded by fluxes of apoplastic water contained in the stems if the entire excised shoot were to be rehydrated (30).

The relationship between shoot water potential and water content differed markedly between VAM and nonVAM plants (Fig. 2), although the relative development of succulent *versus* structural organs (leaf/stem ratio) was the same in both groups of plants (Fig. 3). The decline in shoot water content

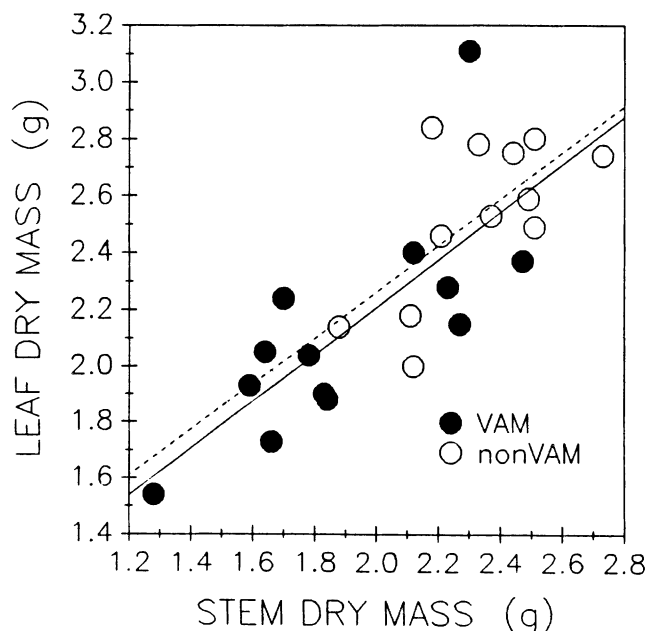


Figure 3. Relationship between leaf and stem dry mass in VAM and P-fertilized (nonVAM) plants. VAM, $y = 0.84x + 0.54$, $r^2 = 0.56$, $P < 0.05$; nonVAM, $y = 0.85x + 0.55$, $r^2 = 0.44$, $P < 0.05$.

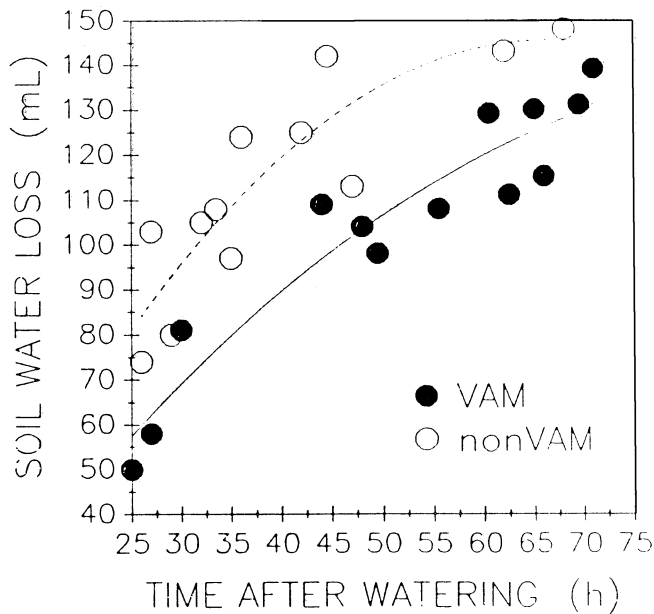


Figure 4. Water loss in the soils of VAM and P-fertilized nonVAM plants as a function of time in the light following the onset of the drought period. VAM, $y = -0.01x^2 + 3.3x - 14.2$, $r^2 = 0.90$, $P < 0.01$; $y = -0.04x^2 + 5.0x - 21.2$, $r^2 = 0.77$, $P < 0.01$.

with decreasing shoot water potential was exponential in the VAM plants, and this change was four times greater than the (linear) decline in the nonVAM plants (Fig. 2).

Soil water depletion was monitored by soil water sensors and permitted a more or less alternating pattern for the selection of VAM and nonVAM plants for harvest during the 4-d drought period. Soil water loss occurred at a higher rate in nonVAM than in VAM plants (Fig. 4) as a result of higher rates of leaf conductance (Fig. 5A) by the larger (Table I) nonVAM leaves.

The changes in leaf conductance with soil water were described by quadratic regression and those with shoot water potential by linear regression (Fig. 5, A and B). The changes were the same in VAM and nonVAM plants. The response of leaf conductance to shoot water content was different in VAM and nonVAM plants (Fig. 5C). Conductance declined steeply over a small (1.5%) change in shoot water content in nonVAM plants, while in VAM plants it declined more slowly over the entire drought range (7% loss in shoot water content). The relationship of transpiration and CER was linear for both treatments, and the responses of VAM and nonVAM plants were the same (Fig. 6).

The ratio of CER to transpiration, WUE, changed little at the initial stages of soil drying, but rapidly at lower levels of soil water (Fig. 7A), and decreased linearly with decreasing shoot water potential (Fig. 7B) for both VAM and nonVAM plants. Relative to shoot water content, however, VAM and nonVAM plant response in WUE was different. The VAM-plant response to shoot water content (Fig. 7C) was similar to that of available soil water (Fig. 7A), while WUE in nonVAM plants declined rapidly with shoot water content.

DISCUSSION

Leaf gas exchange and partitioning of dry matter to leaves and stems is closely related to P availability. The relationship between higher leaf conductance and better P nutrition sometimes observed in nonVAM plants (25) is also inconclusive in VAM plants (5, 15). Higher P availability has the same effect in both VAM plants and P-amended nonVAM plants on leaf development relative to stem development (20). The similarity in leaf/stem ratios of our VAM or P-amended nonVAM plants (Fig. 3) confirms Koide's observations (20) on P-mediated leaf development and indicates that the relative contributions of stem and leaf to the dry matter and (implicitly) to the water status of the two groups of plants were the same. The similarity in the leaf-conductance response pattern

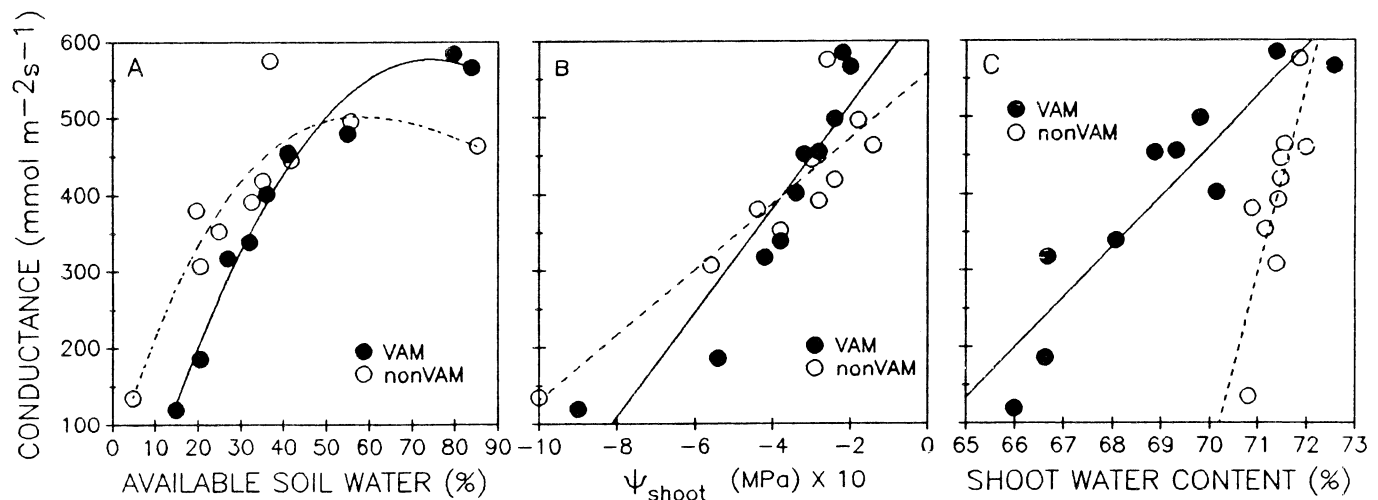


Figure 5. Leaf conductance as a function of available soil water (A), shoot water potential (B), and shoot water content (C) in VAM and P-fertilized (nonVAM) soybean plants. A), VAM, $y = -13x^2 + 19x - 130$; nonVAM, $y = -23x^2 + 19x - 50$. B), VAM, $y = 68x + 653$, $r^2 = 0.85$, $P < 0.01$; nonVAM, $y = 43x + 558$, $r^2 = 0.81$, $P < 0.01$; slopes not significantly different, $P > 0.05$. C), VAM, $y = 66x - 4135$, $r^2 = 0.96$, $P < 0.01$; nonVAM, $y = 245x - 17890$, $r^2 = 0.89$, $P < 0.01$; slopes significantly different, $P < 0.05$.

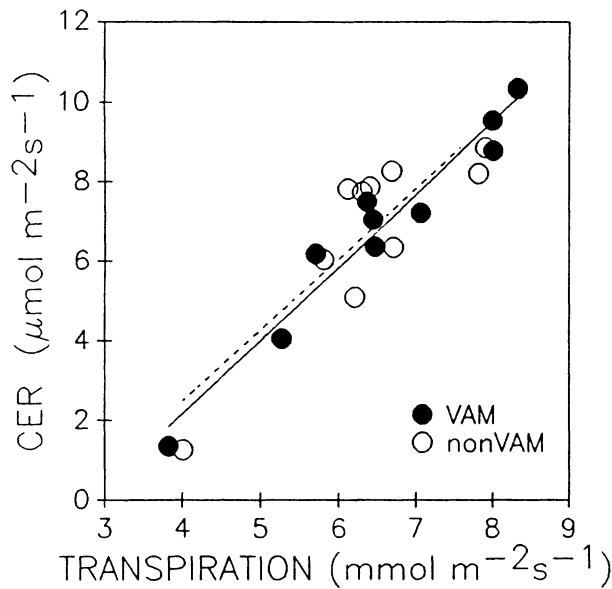


Figure 6. Relationship between transpiration and CER in VAM and P-fertilized (nonVAM) soybean plants. VAM, $y = 1.8x - 5.2$, $r^2 = 0.95$, $P < 0.01$; nonVAM, $y = 1.8x - 4.7$, $r^2 = 0.73$, $P < 0.05$; slopes not significantly different, $P > 0.05$.

of our VAM and nonVAM plants to changes in available soil water and shoot water potential indicates the absence of factors which fundamentally alter these relationships in the symbiotic plant. However, a fundamental difference is indicated between VAM and nonVAM plants in the relationship between shoot water content and leaf conductance.

The behavior of shoot water content, perhaps the most direct measure of tissue water status (10), was different in VAM and nonVAM plants as a function of available soil water (Fig. 1B) or of shoot water potential (Fig. 2) and as a determinant of conductance and WUE (Figs. 5C and 7C). Shoot water potentials, on the other hand, were statistically

invariant ($P > 0.05$) for the two treatments (VAM and nonVAM) relative to soil water content and the gas-exchange parameters. Although our present data do not permit a strict interpretation of this phenomenon, lower shoot water content in VAM than in nonVAM plants of the same shoot water potential at moderate to high stress (Fig. 2) do suggest different capacities for osmotic adjustment (22). Differences in osmotic adjustment between VAM and nonVAM plants have been observed by others (6) and are indicated here by the greater water loss required to lower the water potential of VAM shoots in comparison to nonVAM plants that attained the same low water potential with only a small reduction of water content. An interpretation of this phenomenon was offered by Turner *et al.* (31) who viewed the ability of a plant to maintain higher tissue water content at a particular tissue water potential (here nonVAM relative to VAM plants, Fig. 2) as an indication of greater drought resistance.

The relative stability of conductance with decreasing shoot water content (Fig. 5C) might indicate an adjustment to drought by tolerating greater tissue water loss. Similar (Figs. 7A and 6B) or higher (Fig. 7C) WUE in VAM plants at lower leaf P concentrations (Table I) confirms previous observations of higher P-use efficiency in VAM than in nonVAM plants (12). In view of the similar behavior of CER and transpiration in VAM and nonVAM plants (Fig. 5), higher WUE in VAM than in nonVAM plants as a function of water content (Fig. 7C) suggests a differential involvement of nonstomatal effects (18), *i.e.* higher CO_2 assimilation relative to transpiration in VAM than in nonVAM plants with increasing stress.

The drought responses of our VAM and nonVAM plants varied with the measure utilized in expressing plant water status. Absolute water content, rather than water potential, responded differently to soil water status in the shoots of VAM or nonVAM plants, and the effects of this difference were reflected in their leaf conductances and water-use efficiencies.

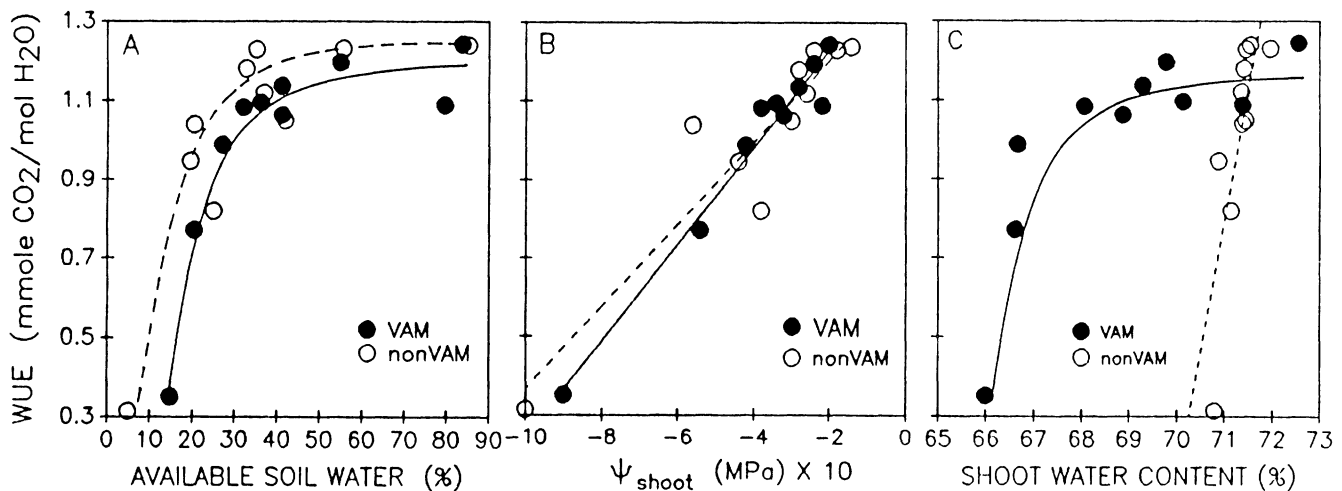


Figure 7. WUE as a function of (A) available soil water, (B) shoot water potential, and (C) shoot water content in VAM and P-fertilized (nonVAM) soybean plants. Statistical analyses: A), VAM, $y = 1.2 - 1.3e^{-0.07x}$; nonVAM, $y = 1.2 - 5.3e^{0.12x}$; asymptotic confidence intervals overlap, $P > 0.05$. B), VAM, $y = 0.12x + 1.47$, $r^2 = 0.96$, $P < 0.01$; nonVAM, $y = 0.10x + 1.41$, $r^2 = 0.86$, $P < 0.01$, slopes not significantly different, $P > 0.05$. C), VAM, $y = \log[(x - 65.977)/0.001]/7.294$; nonVAM, $y = 0.7x - 46.1$, $r^2 = 0.64$, $P < 0.05$.

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