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Stomatal response of mycorrhizal cowpea and soybean to short-term osmotic stress*

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SUMMARY

Cowpea [*Vigna unguiculata* (L.) Walp.] and soybean [*Glycine max* (L.) Merr.] plants were grown in pots and either inoculated with the vesicular-arbuscular (VA) mycorrhizal fungi, *Glomus intraradices* Schenck and Smith (cowpea) and *G. mosseae* (Nicol & Gerd.) Gerd. and Trappe (soybean), or provided with regular P fertilization (non-VA mycorrhizal plants). When plants were six to ten weeks old, roots were exposed to osmotic stress and stomatal behaviour monitored for several hours. Leaves of VA mycorrhizal cowpea had higher stomatal conductance (g_s) than those of non-mycorrhizal cowpea before and after lowering soil water potential (Ψ) to $-0{\cdot}7$ MPa with either sorbitol or macronutrient solutions. Pre-stress g_s and the initial decline in g_s after exposure to $-0{\cdot}5$ MPa sorbitol were similar in mycorrhizal and non-mycorrhizal soybean leaves. Stomatal conductance was higher in the latter after 2 h but higher in the former after 21 h. CO_2 exchange rates and leaf water relations were similar in VA mycorrhizal and non-mycorrhizal soybean before and after soil Ψ was lowered. Higher g_s at equal soil Ψ suggests that mycorrhizal root systems either scavenged water of low activity more effectively or influenced nonhydraulic root-to-shoot communication differently from that in non-infected root systems.

Key words: *Glomus intraradices*, *Glomus mosseae*, photosynthesis, roots, stomatal conductance, water stress.

INTRODUCTION

Vesicular–arbuscular mycorrhizal symbiosis can affect stomatal behaviour of host leaves (Augé & Duan, 1991; Bethlenfalvay *et al.*, 1987). In some situations, effects have been observed only in relation to phosphorus-deficient non-mycorrhizal controls (Safir, Gerdemann & Boyer, 1972; Koide, 1985; Fitter, 1988). Other data show that stomatal conductance (g_s) of both woody and herbaceous plants having vesicular–arbuscular mycorrhizas is often higher than g_s of adequately nourished non-mycorrhizal plants, whether soils are moist (Augé, Schekel & Wample, 1986; Bethlenfalvay *et al.*, 1987; Wang *et al.*, 1989) or dry (Allen & Allen, 1986; Bildusas *et al.*, 1986; Augé, Schekel & Wample, 1987; Ibrahim *et al.*, 1990). One explanation is that mycorrhizal root systems are capable of more water uptake than non-mycorrhizal root systems of similar size, particularly in dry soils, owing to altered root morphology (Kotari, Marschner & George, 1990) or contributions by soil hyphae (Faber *et al.*, 1991). Either mechanism would presumably allow plants to explore a given soil volume more thoroughly and deplete it of readily available (i.e. high energy) water. In other words, increased soil ramification by roots and hyphae would expose a plant to relatively more soil water at high water potential (Ψ). Our objective, however, was to determine if mycorrhizal plants can sustain higher g_s than non-mycorrhizal plants even when root systems are exposed to water of equal Ψ. One simple way to test this is to lower soil Ψ with an osmoticum. Various osmotica have been used to lower soil Ψ quickly and simulate water deficit, e.g., sorbitol (Bowman, 1988), polyethylene glycol (Zekri & Parsons, 1990) and solutions of macronutrients (Termaat & Munns, 1986).

This paper reports the results of two experiments with cowpea and one experiment with soybean. We examined cowpea because of its stomatal sensitivity to declines in soil Ψ (Shackel & Hall 1983; Ludlow, 1989) and its propensity for extensive mycorrhizal colonization, and soybean because mycorrhizal fungi have formerly proved to alter its g_s in some way unrelated to phosphorus nutrition (Bethlenfalvay *et al.*, 1987).

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MATERIALS AND METHODS

Cowpea

Plant culture and fungal inoculation. Seeds of cowpea [*Vigna unguiculata* (L.) Walp. cv. California Blackeye] were planted, two plants per pot, on 16 November 1989, in 1·25 l of a mixture of calcined montmorillonite clay (Turface; IMCore, Mundelein, IL):washed river sand:silica sand (1:1:1, v:v:v). This mixture had a pH of 6·4, no organic matter and mineral concentration (mg kg^{-1}) as follows: 0·5 P, < 1 N, 41 K, 97 Ca, 20 Mg. Each plant in the mycorrhizal treatment received at planting 12 g of Nutrilink (NPI, Salt Lake City, UT), consisting of approximately 16000 spores of *Glomus intraradices* Schenck & Smith affixed to particles of attapulgite clay. Non-mycorrhizal treatments received an inoculum wash sieved free (25 μm openings) of mycorrhizal propagules. Plants were grown in a greenhouse in Knoxville, TN, supplemented with 400 W mercury halide lamps, lit from 06.00 to 23.00 h. Until osmotic stress treatments began, plants were well-watered and received liquid fertilization with each irrigation: Nutriculture 15-0-15 Plus Ca^{+2} (Plant Marvel Laboratories, Chicago Heights, IL) at a concentration of 10 mM N and 3 mM K, plus $MgSO_4$ at 4 mM Mg. Plants were watered monthly with Sequestrene 138 at 18 μM Fe. Non-mycorrhizal plants received 3 mM P weekly, as KH_2PO_4; mycorrhizal plants received one 3 mM P irrigation 7 weeks after planting.

Osmotic stress. Soil Ψ was lowered by irrigating pots with two 400 ml washes of solutions of sorbitol or of macronutrients (Termaat & Munns, 1986) adjusted to $-0{\cdot}7$ MPa. The macronutrient solution was composed of 40 mM $MgSO_4$, 90 mM $Ca(NO_3)_2$, 1·6 mM KH_2PO_4, 62 mM KNO_3 and 19 mM NH_4NO_3. Soil Ψ, measured on soil cores from several test pots with a SC-10 thermocouple psychrometer (Decagon Devices, Pullman, WA, USA) neared $-0{\cdot}7$ MPa within 30 min of application and remained steady throughout the 6 h measurement periods, for both mycorrhizal and non-mycorrhizal treatments. This degree of osmotic stress was chosen because in preliminary tests it was shown to cause significant declines in g_s without closing the stomata completely. Each osmoticum at $-0{\cdot}7$ MPa generally caused foliar wilting of both treatments within 15 to 30 min of application. Recovery of mature foliage generally occurred by the end of the 6 h measurement period, and recovery of immature foliage by the following morning.

Stomatal response. For measurement of stress effects, plants from a larger population were selected on the basis of health and uniformity. Stomatal conductance was examined before and after application of osmotica, in plants eight weeks old (macronutrients) or ten weeks old (sorbitol). A preliminary screening showed that non-mycorrhizal treatments were not infected one week prior to beginning osmotic stress experiments, yet some mycorrhizal contamination had occurred by the following week. Plants in mycorrhizal and non-mycorrhizal treatments had colonization levels of $77 \pm 4\%$ and $11 \pm 4\%$, respectively.

Plants were brought into the laboratory on the day before they were to be examined so that osmotica could be applied and g_s monitored under controlled conditions. Plants were watered shortly before g_s measurements commenced. Each day, g_s of four plants (one pot each of mycorrhizal and non-mycorrhizal treatments) was monitored before and after exposure to the macronutrient osmoticum. This routine was repeated for four consecutive days, giving eight replicates of each treatment. The experiment was then repeated with the sorbitol osmoticum. In each experiment, mature, healthy, unshaded leaves of similar age were examined under similar PPFD, ranging from 490 to 680 μmol m^{-2} s^{-1} and provided by a 400 W high-pressure sodium lamp. Air temperatures during porometry measurements ranged from 22 to 24 °C, and relative humidity remained near 40%. Stomatal conductance was measured on abaxial leaf surfaces with a dynamic diffusion porometer (AP3, Delta-T Devices, Cambridge, England), calibrated daily and corrected for changes in relative humidity, leaf temperature and porometer sensor head/leaf temperature gradients. Stomatal conductance measurements were begun 2 h into the light period and continued every few minutes for at least one hour before osmotica were applied. Preliminary tests indicated that variation among leaflets within a leaf was random, but that within leaflets, g_s was generally highest on that portion of the lamina closest to the midvein. In all experiments, therefore, the porometer sensor was placed parallel with and adjacent to the midvein. Leaves were tagged at the beginning of each experiment, and the highest g_s of the three leaflets of tagged leaves recorded at each time interval: the same leaf, and the highest g_s within that leaf at any given time, was monitored throughout.

Root water potential components. To determine if root water status responded to exposure to osmoticum, root Ψ components were estimated by pressure-volume analysis (Tyree & Jarvis, 1982) before and 24 h after each osmoticum was applied. SC-10 thermocouple psychrometers with sample changers connected to NT-3 nanovoltmeter thermometers (Decagon Devices, Pullman, WA, USA) were used to determine root Ψ as described previously (Augé, Hickok & Stodola, 1989). Roots were excavated, rinsed and rehydrated to saturation for 15 min in H_2O at room temperature, and terminal portions (about 2 cm long and about 0·2 g fresh weight)

blotted dry, weighed and placed in a psychrometer chamber. All manipulations were performed quickly in an enclosed humid chamber to minimize evaporative loss from root tissue. Estimates of Ψ were obtained at several states of root hydration between about 60 and 100% root relative water content (RWC).

Root P content and mycorrhizal colonization levels. Root pieces from soil cores were cleared in 10% KOH, bleached in H_2O_2, infiltrated with HCl and stained with trypan blue for quantification of mycorrhizal colonization (Augé *et al.*, 1986). All plants were harvested following measurement of the final replicates for determination of dry weights and leaf areas. Phosphorus contents of oven-dried (70 °C, 24 hours) leaves and roots were assayed spectrophotometrically with the vanadate-molybdate-yellow method on samples dry-ashed with magnesium nitrate at 750 °C for 2 h and digested in nitric acid (Chapman & Pratt, 1961).

Soybean

Plant culture and fungal inoculation. Soybean [*Glycine max* (L.) Merr. cv. Hobbit] plants were grown singly in 1·25 l pots in an autoclaved, loamy sandy soil of pH 7·7, 0·2% organic matter and mineral concentration (mg kg^{-1}) as follows: 4·8 N, 5·7 P, 51 K, 313 Ca, 13·4 Zn, 13·5 Fe. Half the plants were non-mycorrhizal, half were inoculated with 50 g pot culture (fresh root pieces plus soil) of *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe grown on *Sorghum bicolor* L. Plants were started 1 March 1990, and grown in a greenhouse in Albany, California, supplemented with 1000 W metal halide lamps for 8 h d^{-1}. Maximum greenhouse PPFD during this time reached approximately 1850 μmol m^{-2} s^{-1}. Day/night temperatures were typically near 28/18 °C and daytime RH ranged from 30 to 50%. Beginning on 15 March, all plants received daily irrigations of a nutrient solution equivalent to one quarter strength Hoagland's solution, which was 0·2 mM in P (KH_2PO_4); P was withheld from mycorrhizal plants after 30 March.

Osmotic stress. Osmotic stress treatments were begun when plants were six weeks old. Plants received an extra irrigation during the late afternoon which preceded osmoticum application and measurements, to assure a well-watered condition the following morning. At time 0, soil Ψ was lowered by irrigation with two 200 ml volumes of a $-0{\cdot}5$ MPa sorbitol solution. Net gas exchange rates were measured at 15 min intervals for 3 h, and RWC and Ψ components at 20 min intervals for 2 h, after exposure to osmoticum. A final set of measurements was made at 21 h. Soil Ψ, measured with an SC-10 thermocouple psychrometer on soil cores from each pot, measured $-0{\cdot}55 \pm 0{\cdot}02$ MPa in mycorrhizal pots and $-0{\cdot}51 \pm 0{\cdot}03$ MPa in non-mycorrhizal pots at 3 h and $-0{\cdot}60 \pm 0{\cdot}04$ MPa in mycorrhizal pots and $-0{\cdot}47 \pm 0{\cdot}03$ MPa in non-mycorrhizal pots after 21 h. This degree of osmotic stress did not cause wilting of either mature or immature foliage of either kind of plants.

Gas exchange measurements. CO_2 exchange rate (CER) and g_s were measured with a LI-COR 6200 portable photosynthesis system with a 1 l chamber. Plants were irradiated with a 1000 W metal halide lamp and shielded from the light source by a water bath. PPFD at the leaf surface within the chamber was about 750 μmol m^{-2} s^{-1}. Each day, the CER and g_s of one plant was monitored continuously before and after application of osmoticum. Mycorrhizal and non-mycorrhizal plants were alternated day by day until four replicates of each were stressed and measured. The youngest matured leaf was placed in the chamber, gas exchange was allowed to acclimate (60 min) and measurements were initiated when CO_2 concentration of air entering the chamber reached 320 μl l^{-1}. Mean leaf temperature and chamber RH, $26{\cdot}9 \pm 0{\cdot}1$ C and $66{\cdot}0 \pm 0{\cdot}5$%, respectively, did not differ between infected and non-infected measurements. Leaves remained outside the chamber overnight, were replaced the following morning and one more set of measurements were made, again after 60 min acclimation to chamber conditions. Tests with unstressed plants of both kinds indicated that excision of leaflets from neighbouring leaves (for water relations measurements) did not perturb the baseline g_s of the leaf in the chamber.

Leaf water relations. At 20 min intervals, RWC, Ψ, osmotic potential (Ψ_π) and, by difference, turgor potential (Ψ_p) were determined on the same leaflet. A leaflet was excised and two strips (about 1×3 cm) were cut from either side of the midrib. One was immediately placed into an SC-10 thermocouple psychrometer and its Ψ measured. The other was sealed immediately in a piece of Tygon tubing, frozen in liquid N_2 and the Ψ ($\simeq \Psi_\pi$) of thawed strips determined. This Ψ value was corrected for apoplastic water dilution using estimates of apoplastic water fraction derived as described below. Apoplastic water was assumed to be solute-free. The psychrometer was calibrated daily at ambient laboratory temperature with a graded series of NaCl solutions. Preliminary experiments with leaves of varying Ψ indicated that Ψ readings stabilized (i.e. were repeatable within 0·02 MPa over several subsequent 20 min intervals) after 80 min of sample equilibration within the psychrometer, and that Ψ agreed consistently within 0·08 MPa both among leaflets of a single leaf and between lamina on either side of the midrib within individual leaflets. Preliminary tests also indicated that leaf Ψ did not vary

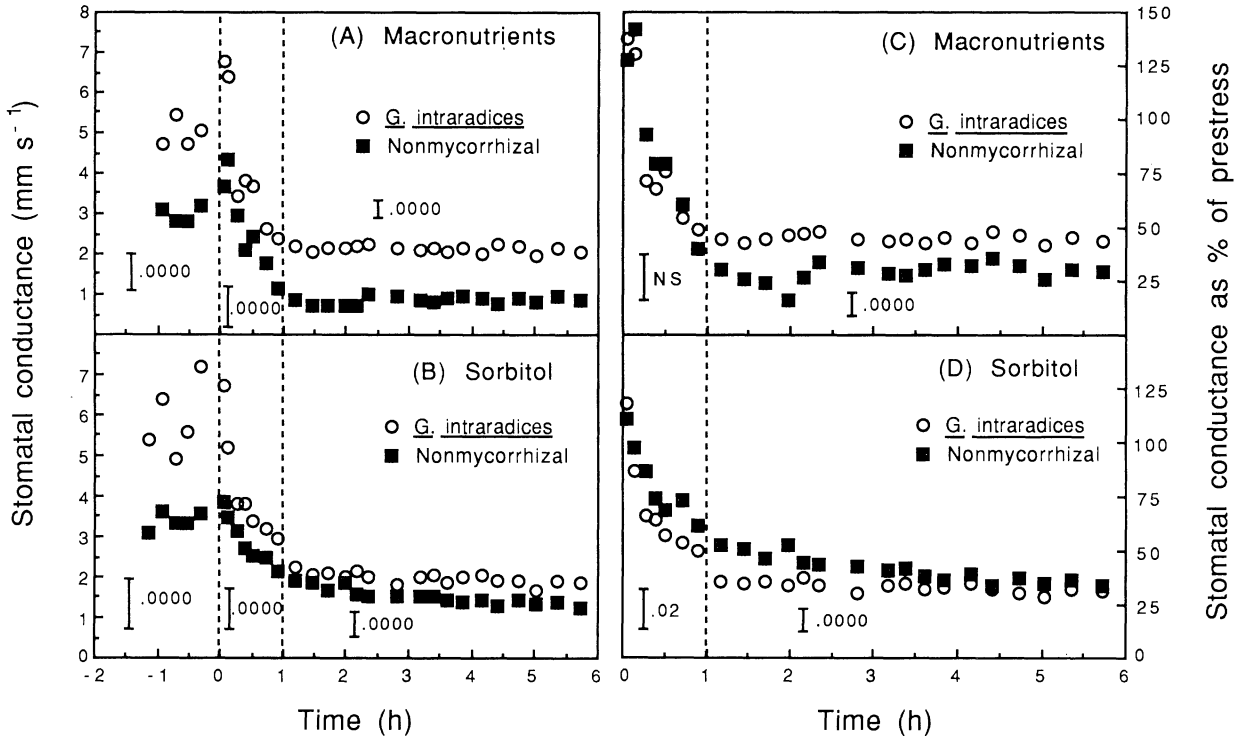

Figure 1. Stomatal conductance of mycorrhizal (*Glomus intraradices*) and non-mycorrhizal *Vigna unguiculata* plants, before and after roots were exposed to −0·7 MPa solutions of macronutrients (A, C) or sorbitol (B, D). (A) and (B) give actual g_s before and after exposure to osmotica, (C) and (D) give g_s after exposure as a percentage of each treatment's mean pre-stress g_s. Osmoticum was applied at time = 0 h. Each point is the mean of eight replicate plants, whose measurements were gathered over four consecutive days. Each plot was analysed as three segments: pre-stress, −1 to 0 h; initial decline during the first hour after lowering of soil Ψ; stabilization, 1 to 5·75 h. Solid vertical lines represent 2 × pooled standard errors of the means within each time segment. Numbers beside each line give probability of significance ($P \leqslant 0{\cdot}05$), determined by ANOVA; NS = non-significant ($P > 0{\cdot}05$).

in a consistent manner with the leaves being studied (the four that had most recently become fully expanded). Leaf RWC was ascertained by measuring the averaged fresh and rehydrated (overnight at 4 °C on distilled water) and dry (80 °C for 3 d) weights of six leaf segments (approx. 10 cm^2 total) cut from the above leaflet's remaining lamina. Relative *in situ* bulk protoplast volume of leaves (Santakumari & Berkowitz, 1990) was calculated at each RWC. Bulk leaf apoplastic water fractions were estimated by comparing symplastic Ψ_π of live, turgorless tissue (Ψ_π^{sym}) and Ψ_π of frozen/thawed tissue (Ψ_π^{mixed}) at equivalent RWC. Leaves were allowed to dehydrate on the bench until turgor was lost; at this point a psychrometric estimate of leaf Ψ is an estimate of symplastic Ψ_π ($\Psi \simeq \Psi_\pi$ when $\Psi_p = 0$). Ψ determination of frozen/thawed tissue indicates Ψ_π of mixed symplastic and apoplastic water. The proportion of apoplastic water in the sample is then computed as: apoplastic fraction $= 1 - \Psi_\pi^{mixed}/\Psi_\pi^{sym}$. Each leaf sample was simultaneously subsampled for Ψ_π^{sym} and Ψ_π^{mixed} after turgor loss. Turgor loss points were estimated from pressure-volume curves (Tyree & Jarvis, 1982). Mean leaf apoplastic water percentage was 9 ± 1 %, $n = 8$.

Tissue elemental content and colonization levels. Mycorrhizal colonization of root length (68 ± 8 %) was by the grid-line intersect method (Giovannetti & Mosse 1980). Nutrient analyses were performed by the Research Extension Analytical Laboratory, Ohio State University, Wooster, OH, USA.

Experimental design and statistical analyses

Each experiment consisted of two treatments: plants inoculated with *Glomus* and plants not inoculated but fed additional P. Each cowpea experiment involved eight (stomatal response) or four (pressure-volume analysis of roots) replicates of each treatment. Soybean experiments involved four replicates for both gas exchange and water relations. Plants were arranged in completely randomized designs. Univariate analyses of variance were used to evaluate treatment effects. Standard errors of the means were calculated by taking the square root of the error mean square from the analysis of variance and dividing it by the square root of the number of observations in a mean (Steel & Torrie, 1980).

RESULTS

Cowpea

Before application of osmotica to the root system, g_s of control plants was 59 % that of mycorrhizal plants in each cowpea experiment (Fig. 1A and B). Stomatal conductance increased 1–2 mm s^{-1} in both mycor-

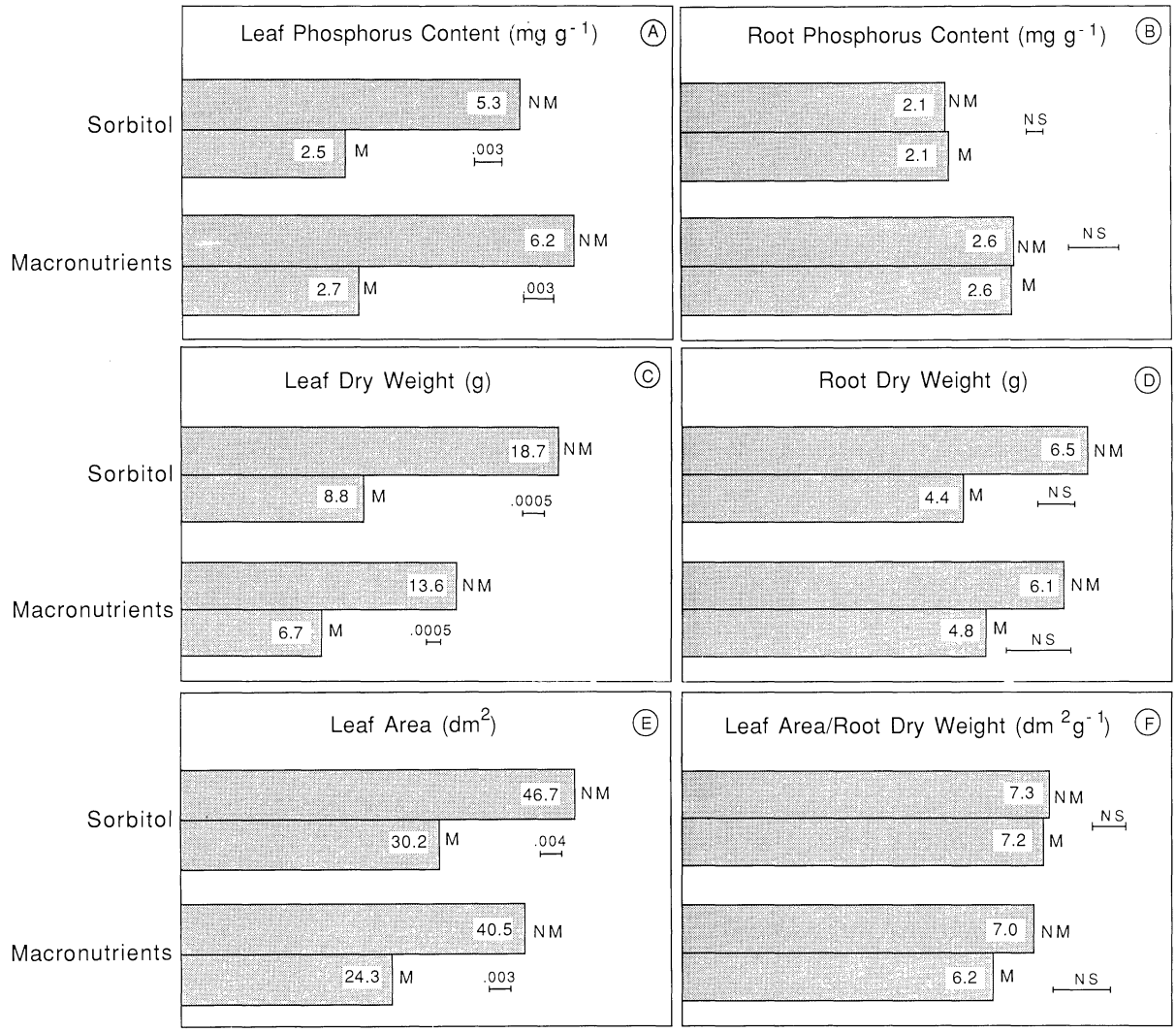

Figure 2. Effect of mycorrhizal colonization on leaf and root phosphorus contents, dry weights, leaf areas and leaf area/root dry weight ratios for the *Vigna unguiculata* plants whose stomatal conductances are depicted in Fig. 1. Each shaded bar represents the mean of eight replicate plants, M, mycorrhizal (*Glomus intraradices*); NM, non-mycorrhizal. Lines represent 2 × pooled standard errors of the means. Numbers above each line give probability of significance ($P \leqslant 0{\cdot}05$), determined by ANOVA; NS, non-significant ($P > 0{\cdot}05$).

rhizal and non-mycorrhizal plants within minutes of application of the macronutrients osmoticum, an effect observed previously in triticale (Morant-Avice, Ferard & Coudret, 1989) and in rose (Augé, unpublished). Stomatal conductance then declined steadily during the first hour. The decline levelled off in both kinds of plant 1 h after exposure to either osmoticum and remained constant for the next 4·75 h. Whether soil Ψ was lowered with macronutrients or sorbitol, g_s was higher in mycorrhizal cowpea. Stomatal conductance of plants stressed with the macronutrient osmoticum stabilized at about 2·1 and 0·8 mm s^{-1} for mycorrhizal and non-mycorrhizal treatments, respectively. Similarly, stomatal conductance of plants stressed with the sorbitol osmoticum stabilized at about 2·0 and 1·5 mm s^{-1}, respectively. When soil Ψ was lowered with the macronutrient solution, the percentage decline in g_s was similar in both treatments during the initial decline phase (0 to 1 h) but g_s of mycorrhizal plants stabilized at about 45 % of their pre-stress g_s, whereas g_s of controls stabilized near 30 % of their pre-stress g_s (Fig. 1C). When soil Ψ was lowered with the sorbitol solution, percentage declines in g_s were initially larger in mycorrhizal plants (Fig. 1D). By about 3·5 h after stressing, g_s of both inoculated and non-inoculated plants had stabilized near 35 % of their pre-stress g_s.

Phosphorus concentrations in the roots of both kinds of plant were similar, but mycorrhizal P concentrations of leaves were markedly lower (Fig. 2A and B). The roots of both treatments had similar dry weights. Leaf dry weight of mycorrhizal plants was about half that of controls and leaf area was 60 to 65 % (Fig. 2C–E). Leaf area/root dry weight ratios were not statistically different (Fig. 2F).

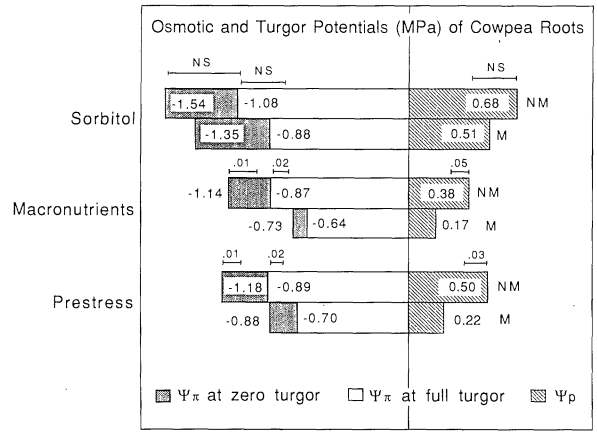

Figure 3. Osmotic and turgor potentials at full turgor and osmotic potential at zero turgor (turgor loss point) of rehydrated roots of *Vigna unguiculata* before and 24 h after exposure to −0·7 MPa solutions of either macronutrients or sorbitol. Numbers within bars are means of four replicate plants and give actual values of each water potential component. Lines above bars represent 2× pooled standard errors of the means. Numbers above each line give probability of significance ($P \leqslant 0{\cdot}05$), determined by ANOVA; NS, non-significant ($P > 0{\cdot}05$). M, mycorrhizal (*Glomus intraradices*); NM, non-mycorrhizal.

Osmotic adjustment of roots may have occurred after 24 h exposure to sorbitol, indicated by differences in full turgor Ψ_π before and after stress (Fig. 3). Exposure to the macronutrient osmoticum for 24 h did not result in lowered root Ψ_π. Pre-stress root Ψ_π at full and zero turgor were 0·19 and 0·30 MPa lower, respectively, in non-mycorrhizal than in mycorrhizal roots (Fig. 3). Turgor of unstressed rehydrated roots of cowpea was about 0·3 MPa higher in the former. After 24 h exposure to the macronutrient osmoticum, Ψ_π were still lower and Ψ_p higher in non-mycorrhizal roots.

Soybean

Stomatal conductance was similar in both treatments before exposure to stress and during the initial decline phase (0 to 2 h) after exposure to −0·5 MPa sorbitol. Stomatal conductance stabilized in both kinds of plant after 2 h of exposure to sorbitol, with g_s in leaves of mycorrhizal plants at about 80 % that of controls (Fig. 4A). Final declines were, however, larger in the latter: after 21 h of exposure, g_s of non-mycorrhizal soybean leaves was 60 % that of mycorrhizal leaves. CER was similar between treatments before and throughout the exposure period (Fig. 4B). Water potential components, relative water content and protoplastic volume were unchanged from 0 to 2 h and after 21 h exposure to osmoticum, remaining essentially at pre-stress values, and did not differ between treatments (Fig. 4C & D). When expressed as a percentage of each treatment's mean pre-stress values, g_s and CER

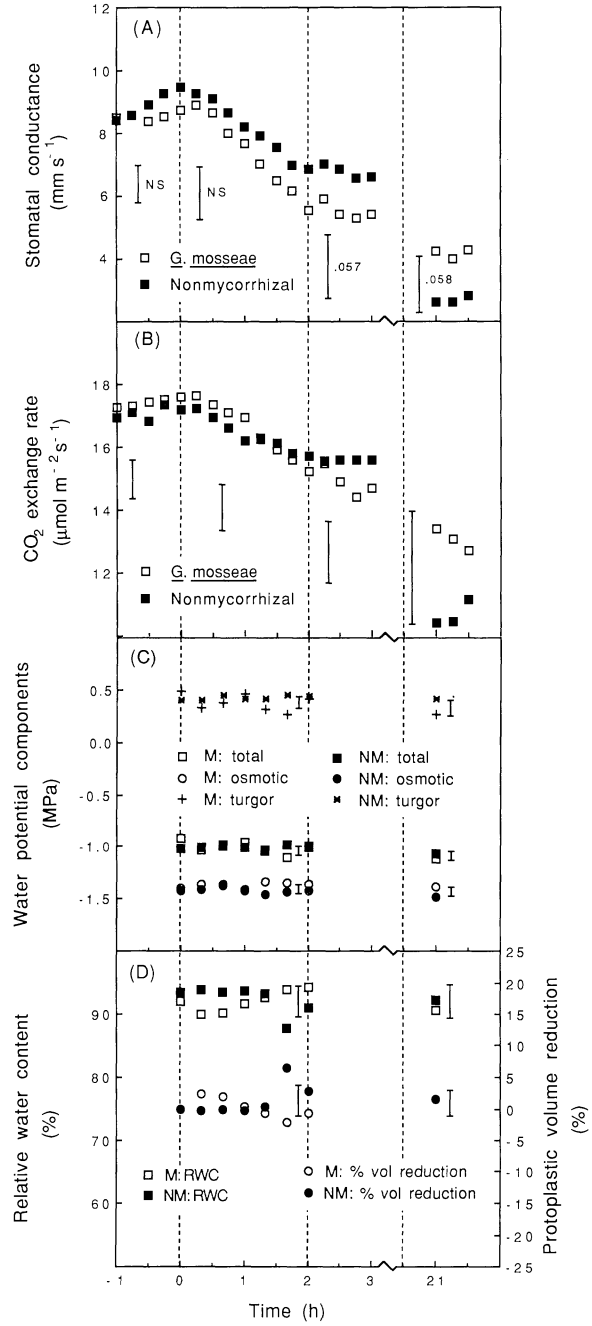

Figure 4. Net carbon exchange rate, stomatal conductance and water relations of leaves of mycorrhizal (*Glomus mosseae*) and non-mycorrhizal *Glycine max* plants. For measurement of gas exchange, leaves were placed in the cuvette at time = −2 h. Soil Ψ was lowered to −0·5 MPa with a sorbitol solution at time = 0 h. Each point is the mean of four replicate plants; measurements were gathered over eight consecutive days, one plant per day. Each plot was analysed as four segments: pre-stress, −1 to 0 h; immediate decline after lowering of soil Ψ, 0 to 2 h; stabilization, 2 to 3 h; and 1 d after application, 21 h. Solid vertical lines represent 2× pooled standard errors of the means. Numbers beside each line give probability of significance ($P \leqslant 0{\cdot}05$), determined by ANOVA; NS or no number, non-significant ($P > 0{\cdot}05$).

declined at similar rates from 0 to 2 h in leaves from both kinds of plant (Fig. 5A & B). After 2 h, g_s stabilized and remained near 75 % (mycorrhizal) and

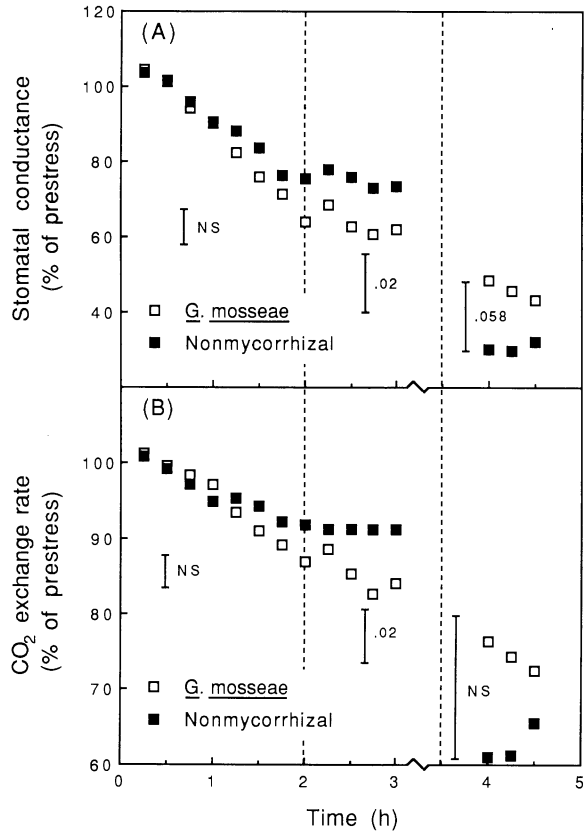

Figure 5. Stomatal conductance (A) and net carbon exchange rate (B) of the leaves of the mycorrhizal (*Glomus mosseae*) and non-mycorrhizal *Glycine max* plants illustrated in Fig. 4, expressed as a percentage of each treatment's mean pre-stress values. Solid vertical lines represent 2× pooled standard errors of the means. Numbers beside each line give probability of significance ($P \leqslant 0{\cdot}05$), determined by ANOVA; NS, non-significant ($P > 0{\cdot}05$).

60% (non-mycorrhizal), of each treatment's pre-stress g_s for the next h (Fig. 5A). After 21 h, g_s of mycorrhizal plants was about 45% of initial g_s, and g_s of controls about 30% of initial g_s. Expressed as a percentage of pre-stress values, CER were lower in mycorrhizal than in non-mycorrhizal plants after stabilization (2 to 3 h) and statistically similar in both at 21 h (Fig. 5B).

Mycorrhizal and non-mycorrhizal soybeans were similar in size; there were no significant differences between treatments in leaf area, leaf dry weight, root dry weight or leaf area/root dry weight ratios (Fig. 6A–D). Leaf and root P contents were also similar in both treatments (Table 1). Non-mycorrhizal roots had nearly twice as much K as mycorrhizal roots. Mycorrhizal roots had slightly more Ca and mycorrhizal leaves slightly less Ca than non-infected plants. Mn content was much higher in the latter, over 3 times higher in leaves and over 2 times higher in roots. Non-mycorrhizal leaves also had about two-thirds more Fe than mycorrhizal leaves. Mycorrhizal roots had over twice as much Na as controls.

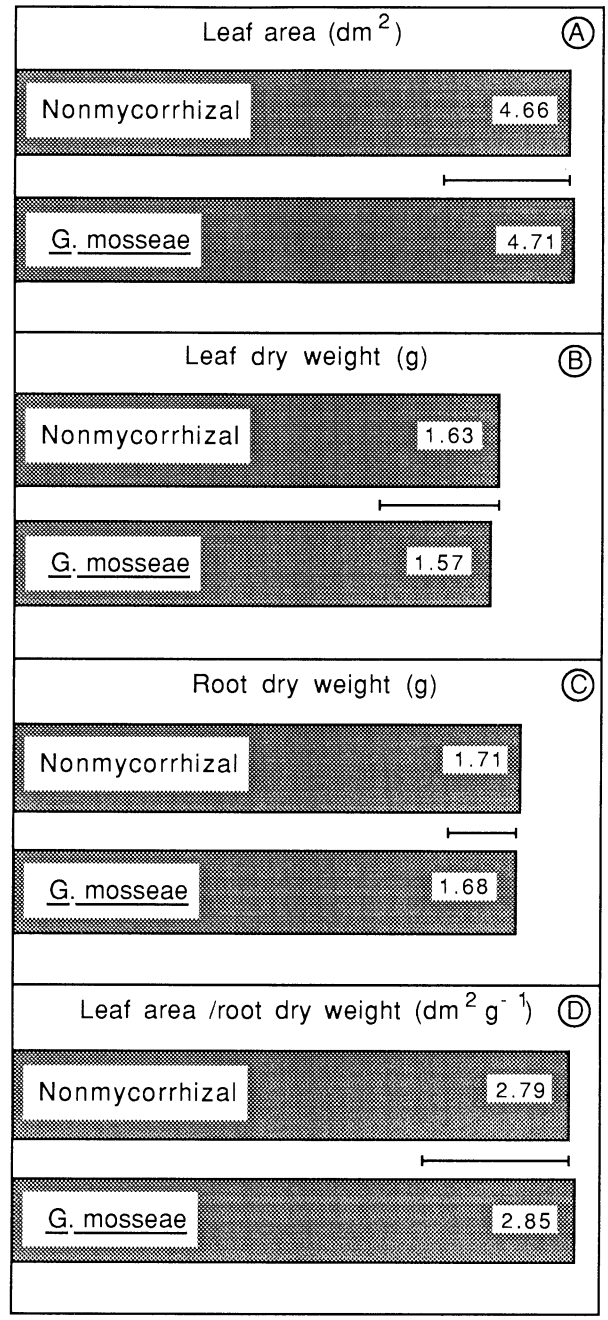

Figure 6. Leaf areas, leaf and root dry weights, and leaf area/root dry weight ratios for the *Glycine max* plants whose gas exchange and water relations are depicted in Figs. 4 and 5. Each shaded bar represents the mean of four replicate plants. Lines represent 2× pooled standard errors of the means. Analysis of variance showed no differences between mycorrhizal and non-mycorrhizal plants.

DISCUSSION

Substantial hyphal contributions to water uptake (Faber *et al.*, 1991) and/or increased water uptake related to mycorrhizal changes in root morphology (Kotari *et al.*, 1990) could allow mycorrhizal plants in *drying* soil to sustain higher g_s, by depleting soil water more thoroughly and lowering Ψ of a larger percentage of a given soil volume. When soil is flooded with osmoticum, however, Ψ is lowered

Table 1. *Elemental composition of leaves and roots of mycorrhizal and non-mycorrhizal* Glycine max

	Mycorrhizal		Non-mycorrhizal		ANOVA[a] Mycorrhizal vs. non-mycorrhizal	
Nutrient	Leaves	Roots	Leaves	Roots	Leaves	Roots
			(mg g^{-1} dry wt)			
P	1·4[b]	1·5	1·5	1·2	NS	NS
K	17·2	10·0	16·2	18·7	NS	0·0060
Ca	6·8	5·3	8·1	3·8	0·02	0·0007
Mg	3·7	7·5	4·0	7·4	NS	NS
			(μg^{-1} dry wt)			
Mn	76	351	245	820	0·0002	0·0005
Fe	87	2200	150	2167	0·002	NS
B	31	11	33	14	NS	NS
Cu	7	28	7	21	NS	0·05
Zn	49	51	45	40	NS	0·02
Al	22	3136	23	3298	NS	NS
Na	17	1124	16	480	NS	0·004

[a] Number indicates significance, $P \leqslant 0{\cdot}05$; NS, non-significance, $P > 0{\cdot}05$.
[b] Each value is the mean of four replicates.

rapidly and evenly throughout the soil volume, both at the rhizoplane and in bulk soil, eliminating the possibility that shoots are provided access to an effectively larger reservoir of high energy water. If g_s remains higher in mycorrhizal plants under these conditions, then the mycorrhizal root is either more capable of absorbing water at low Ψ than the non-mycorrhizal root, or the differences in stomatal behaviour are a consequence of nonhydraulic rather than hydraulic differences between root systems (Augé & Duan, 1991). Although the root mass available for water absorption and supply to any given leaf area may affect the rate of water loss by that leaf area when soil moisture is limiting, examination of stomatal responses of cowpeas of varying leaf area/root mass ratios has revealed no dependence of g_s upon leaf area/root mass ratio (Augé, unpublished). Plants with larger leaf area/root mass ratios sustained the same g_s as plants with lower leaf area/root mass ratios under soil Ψ of $-0{\cdot}7$ MPa imposed by sorbitol treatment. Others have also failed to detect a relationship between shoot/root ratio and rate of water loss per unit leaf area (Eavis & Taylor, 1979), although such links sometimes occur (Meinzer, Grantz & Smit, 1991).

It is difficult to produce mycorrhizal and non-mycorrhizal plants of equal P concentrations and shoot and root sizes. Since P-deficiency affects g_s of some species (e.g., Fitter, 1988; Saneoka, Fujita & Ogata, 1990), most investigators try to assure that non-mycorrhizal controls have as much or more P than mycorrhizal plants. Although P concentrations of non-mycorrhizal and mycorrhizal cowpeas in our study were not equal ($P \leqslant 0{\cdot}05$), other experiments have failed to detect an influence of leaf P concentration on g_s of cowpea during osmotic stress (Duan & Augé, 1992).

One difficulty in evaluating drought effects lies in quantifying and evenly applying the drought treatment, both spatially and temporally within and among pots. The problem is compounded by differences in organ sizes and surface areas that can occur among mycorrhizal treatments; even when fertilization schemes are adjusted to produce mycorrhizal plants and non-mycorrhizal controls of similar phosphorus status, shoot:root ratios often differ (Kotari *et al.* 1990). Moreover, the application and quantitation of drought treatments are typically very time-consuming processes. Advantages of osmotica include speed, consistency and repeatability in exposing root systems of varying size and/or morphology to essentially identical declines in soil Ψ, the magnitudes of which are easily controlled. This provides a relatively quick test for short-term soil influences on stomatal response, under highly controlled conditions, before attempts are made to discern such influences under the more time-demanding and variable conditions of actual drought treatment.

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