Partitioning mycorrhizal influence on water relations of *Phaseolus vulgaris* into soil and plant components

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Abstract: There is growing appreciation of arbuscular mycorrhizal effects on soil properties and their potential consequences on plant behavior. We examined the possibility that mycorrhizal soil may directly influence plant water relations. Using wild-type and noncolonizing bean mutants planted into soils previously produced using mycorrhizal or nonmycorrhizal sorghum plants, we partitioned mycorrhizal influence on stomatal conductance and drought resistance into soil and root components, testing whether effects of mycorrhizal fungi occurred mostly via mycorrhization of roots, mycorrhization of soil, or both. The mutation itself had no effect on any water relations parameter. Colonization by *Gigaspora margarita* Gerdemann & Trappe and *Glomus intraradices* Schenck & Smith had appreciable effects on leaf water potential at the lethal point and on osmotic adjustment, relative to nonmycorrhizal plants of comparable size. Mycorrhizal effects on drought resistance were attributable to an effect on the plant itself rather than to an effect of mycorrhizal soil. Mycorrhizal effects on stomatal conductance were attributable to mycorrhization of both roots and soil, as well as to mycorrhization of roots alone. Surprisingly, merely growing in a mycorrhizal soil resulted in promotion of stomatal conductance of nonmycorrhizal plants in both amply watered and droughted plants. Mycorrhizal effects on droughted plants did not appear to be related to altered soil water retention properties, as *Gigaspora margarita* and *Glomus intraradices* altered the soil's moisture characteristic curve only slightly.

Key words: arbuscular mycorrhizal symbiosis, bean, drought, *Gigaspora margarita*, *Glomus intraradices*, stomatal conductance.

Résumé : On reconnaît de plus en plus les effets des mycorhizes arbusculaires sur les propriétés du sol et leurs conséquences potentielles sur le comportement de la plante. Les auteurs ont examiné la possibilité que le sol mycorhizé puisse influencer directement les relations hydriques de la plante. À cette fin, ils ont utilisé une race sauvage de haricot ainsi qu'un mutant non colonisant, plantés dans des sols préalablement produits avec des plants de sorgho mycorhizés ou non mycorhizés. Ils ont ensuite réparti l'influence des mycorhizes sur la conductance stomatale et la résistance à la sécheresse entre les composantes sol et racines, la mycorhization du sol, ou les deux. La mutation elle-même est sans effet sur l'ensemble des paramètres hydriques. La colonisation par le *Gigaspora margarita* Gerdemann & Trappe et le *Glomus intraradices* Schenck & Smith a des effets manifestes sur le potentiel hydrique foliaire au point létal, et sur l'ajustement osmotique, par rapport à des plants non mycorhizés de grosseur comparable. On attribue les effets de la mycorhization sur la résistance à la sécheresse à une action sur la plante elle même plutôt qu'à un effet du sol mycorhizé. Dans le cas de la conductance stomatale, les effets des mycorhizes sont attribuables à la mycorhization à la fois des racines et du sol, aussi bien qu'à la mycorhization des racines prises séparément. Il est surprenant de constater que le seul fait de croître dans un sol mycorhizé arrive à promouvoir la conductance stomatale des plants non mycorhizés, chez les plantes bien pourvues en eau aussi bien que chez les plantes asséchées. Chez les plantes soumises à la sécheresse, les effets des mycorhizes ne semblent pas reliés à une modification des propriétés de rétention de l' eau par le sol, puisque le *Gigaspora margarita* et le *Glomus intraradices* ne modifient que légèrement les caractéristiques de l'humidité du sol.

Mots clés : symbiose mycorhizienne arbusculaire, haricot, sécheresse, *Gigaspora margarita*, *Glomus intraradices*, conductance stomatale.

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Introduction

Arbuscular mycorrhizal (AM) symbiosis has been shown upon hundreds of occasions to change the water relations of host plants (Augé 2001). Effects vary and have been sporadic, and scientists have not learned exactly how the symbiosis exercises its influence or under which conditions the influence is most likely to occur. Most investigations have focused upon the symbiosis in the plant: whether colonization of roots by AM fungi affects characteristics such as stomatal conductance (g_s), leaf or shoot water potential (Ψ) or osmotic potential (Ψ_π), soil drying rates, or root or plant hydraulic conductance. The possibility of a direct contribution of soil in which AM mycorrhizas and extradical hyphae have grown (AM soil) to AM effects on plant water relations has rarely been the focus of these studies, perhaps as AM effects on soil may be slower to develop than direct effects of AM colonization on plants.

AM symbiosis can affect the structure, chemistry, and biology of soils (Tisdall 1991; Miller and Jastrow 1999). Bethlenfalvay and colleagues (e.g., Bethlenfalvay and Linderman 1992) have suggested that AM modification of soils may be as or more important than direct AM modification of plants, in terms of the mechanisms responsible for AM effects on plant behavior in native and agricultural ecosystems. In particular, as soil structure affects its moisture retention properties (Hamblin 1985), it appears likely that AM symbiosis may influence soil moisture retention properties (Augé et al. 2001*b*; Bearden 2001), with the potential for consequent effects on water relations of plants growing in those soils.

Our goal was to uncouple the influence of mycorrhization of roots and soils on plant water relations. To determine if an AM soil could influence plant water relations in the absence of AM colonization of the plant, we examined a noncolonizing mutant of common bean grown in soils that were comparable in texture and rooting density, but had been maintained as mycorrhizal or nonmycorrhizal for 1 year. We also compared wild-type (myc^+) and noncolonizing mutant bean plants (myc^-) grown in these soils, to assess the influence of mycorrhization of roots and soil versus mycorrhization of soil alone. We tested three hypotheses: (*i*) mycorrhization of roots and soil would affect stomatal behavior or drought resistance of the host plant, (*ii*) plant growth in mycorrhizal soil in the absence of root colonization would affect stomatal behavior or drought resistance of the host plant, and (*iii*) mycorrhization of roots alone would affect stomatal behavior or drought resistance of the host plant, independently of AM effects on soil.

Materials and methods

Production of AM and nonAM soils

AM and nonAM soil treatments were established for 12 months prior to planting the experimental myc^- and myc^+ beans: 10 months of active root growth in soils was followed by a 2-month resting period. Thirty-six 1-L plastic pots were seeded with *Sorghum bicolor* L. 'DeKalb DK40Y' on 23 April 2001, with approximately 70 seeds per pot. The potting medium was composed of two parts autoclaved silica sand (medium to coarse, mined, sieved) and one part soil (Sequatchie, fine-loamy, siliceous, thermic Humic Hapudults, pH 7.5). A soil–sand mix was used to improve plant growth and to increase the propensity for AM ramification of soil to change soil structure. Twelve pots were inoculated with pot culture of *Glomus intraradices* Schenck & Smith INVAM isolate WV114 (*Gi*), 12 pots with pot culture of *Gigaspora margarita* Gerdemann & Trappe INVAM isolate 215 (*Gm*), and 12 pots with nonAM pot culture, at a rate of 60 mL pot culture per pot. *Gm* pot cultures contained roots and soil of 3-month-old *Zea mays* L. plants grown in a sandy, low-P soil stored for 6 months at 4 °C prior to use. *Gi* and nonAM pot cultures contained roots and soil from 11-month-old *S. bicolor* plants grown on the sand–soil medium described previously. NonAM pot cultures, grown in the same greenhouse as *Gi* pot cultures and given inoculum filtrates when intiated, were used to encourage similar soil microflora among treatments and to maintain similar soil water retention properties. All sorghum plants were transplanted 7 weeks later into 5.8-L pots filled with the sand–soil mix.

All plants were grown in a greenhouse in Knoxville, Tenn., under natural light supplemented for 12 $h \cdot d^{-1}$ with high-pressure sodium lamps. With each watering, plants received a liquid macro- and micro-nutrient fertilizer at 10.7 $mmol \cdot L^{-1}$ nitrogen (N) (Champion 15N–0 P–15K Alkaline Plus, Chilean Nitrate Co., Norfolk, Va.). Phosphorus (P) was applied weekly as 0.8 $mmol \cdot L^{-1}$ KH_2PO_4 to *Gi* plants and *Gm* plants and as 1.6 $mmol \cdot L^{-1}$ KH_2PO_4 to plants in nonAM soils. Luxury fertilization levels for N and other nutrients were provided to assure that plants of each treatment were adequately nourished. Plants were watered regularly during the soil preparation phase. Plants were sheared back to crowns on 20 August 2001 and 5 October 2001 to regenerate shoot growth. The final shearing occurred on 22 February 2002, and crowns were removed to prevent resprouting. Soils were then allowed to dry in the greenhouse for 2 months before planting with the experimental beans.

Myc⁻ and wild-type *Phaseolus vulgaris*

On 23 April 2002, after 1 year of developing the AM and nonAM soils, each pot was seeded with two bean plants: wild-type *Phaseolus vulgaris* L. 'OAC Rico' (myc^+) and its nod^+/fix^- mutant *Phaseolus vulgaris* 'R69' (myc^-). 'R69' was developed as a nonfunctional (ineffective) mutant with tumour-like pseudo-nodules, by ethyl-methane sulphonate (EMS) treatment, with 'OAC Rico' as a wild type (Park and Buttery 1992). This characteristic is controlled by the root (Buttery and Park 1993). 'OAC Rico' develops normal AM infections, while 'R69' typically does not develop AM infection (Shirtliffe and Vessey 1996).

'OAC Rico' and 'R69' were grown on opposite sides of the pot. Myc^+ and myc^- plants were grown together in pots to maintain active mycorrhization of soil around myc^- roots during the experiment and to expose myc^- and myc^+ plants to identical soil conditions. In this way, if myc^+ root systems continued to produce an AM effect on soil beyond that already developed by mycorrhizal sorghum roots during the soil mycorrhization phase of the experiment, both myc^- and myc^+ plants were exposed to it. Bean plants received the same fertilization as sorghum plants (described previously).

Drought treatment and measurements of foliar dehydration tolerance

Eight weeks after planting bean seeds (17 June 2002, subsequently referred to as day 0), all pots were watered to field capacity and subsequently subjected to a continuous soil drying period by withholding water from pots. Two leaflets from separate, recently expanded leaves from each plant were collected on day 0 between 0930 and 1100 for measurement of initial (predrought) osmotic potential at full turgor (Ψ_π^{100}). Leaflets were rehydrated overnight, with leaf bases submerged in a covered beaker of distilled water in a refrigerator (4 °C). The following morning, leaves were blotted and placed in a syringe, frozen in liquid N_2, and placed in a freezer at –80 °C, pending analysis. Syringes were thawed until samples reached room temperature, and the Ψ_π of expressed sap was measured with a vapor pressure osmometer (model 5500XR, Wescor, Inc. Logan, Utah) calibrated with a graded series of NaCl solutions.

Foliar dehydration tolerance was characterized by measuring lethal leaf Ψ as described previously for *P. vulgaris* (Augé et al. 2001*a*, 2004). Each plant was checked daily after beginning the drying period, and lethal measurements were begun when fewer than six live leaflets remained; this was defined as the "lethal point" for that plant. Sampling of lethal values was performed between 0830 and 1000. Lethal leaf Ψ was determined on leaflets from two leaves per plant with thermocouple psychrometers (TruPsi, Decagon Devices, Inc., Pullman, Wash.) calibrated daily with a graded series of NaCl solutions.

Lethal, postdrought Ψ_π^{100} was measured as for predrought samples on leaflets from two leaves per plant. Leaf osmotic adjustment during the drying period was assessed as predrought Ψ_π^{100} – postdrought Ψ_π^{100}. This procedure for estimating osmotic adjustment integrates both phenologically induced and drought-induced solute changes.

Soil matric potential (Ψ_m) was measured and recorded every 6 h throughout the drying period using heat dissipation sensors (SoilTronics, Burlington, Wash.; now available as 229-L, Campbell Sci., Logan Utah) as described before (Augé et al. 1994, 2001*b*). Sensors were placed in soil longitudinally midway between the top and bottom of the pot and radially midway between the pot center and the pot edge. Heat pulses were administered and temperatures were recorded with a datalogger (CR10, Campbell Sci.); sensors were connected to the datalogger through multiplexers (AM32, Campbell Sci.). Sensors were dipped in a kaolinite slurry to improve sensor–soil contact and buried in the center of each pot. Sensors were individually calibrated using a dewpoint potentiameter (WP4, Decagon Devices, Pullman, Wash.), which measures total Ψ by the chilled mirror dewpoint technique (Campbell et al. 1973). At the lethal point for each plant, a vertical core of soil (12–14 cm length by 2.5 cm diameter) was removed from a point midway between the plant base and the pot edge and immediately sealed into a plastic bag. The soil was mixed, and soil water content (θ) was measured gravimetrically on an approximately 2.5-mL subsample. Remaining soil was kept in the sealed bag and placed in a freezer for further analysis of soil, root, and fungal characters (described below).

Stomatal conductance

On 4 d during the week prior to initiating the lethal drought period, g_s of three recently matured leaves from each of the 72 plants was measured with a diffusion porometer (AP4, Delta-T Devices, Cambridge, UK) between 1400 and 1600. To minimize effects of environmental and diurnal changes on treatment averages, one replicate (both the myc^+ and myc^- plant) of each of the three soil treatments was measured, then the second replicate of each treatment, until all measurements were completed. Plants were watered early in the morning on each day that g_s was measured. Stomatal conductance was also measured on day 4 of the drought period.

Soil moisture characteristic curves

Wet hysteretic curves

Protocols for soil moisture characteristic curves often involve sieving soil, which changes soil structure and therefore probably soil water retention properties (sieving effects on structure are roughly equivalent to tilling soil; Hamblin 1985). To best discern mycorrhizal influence on the soil moisture characteristic curve, we measured Ψ_m and θ of undisturbed soils, while soils were still in place in pots, using heat dissipation and gravimetric procedures as described before (Augé et al. 2001*b*). Ψ_m and θ were measured on most days for the next 12 weeks, enabling sampling of Ψ_m at several values of θ (40–50 measurements of Ψ_m per θ data pair for most replicates).

Dry hysteretic curves

After wet hysteretic curves were constructed, soil was removed from each pot, spread on a tray, and allowed to air dry on a laboratory bench. Soils were sieved (4 mm), and 100-g samples were placed in a plastic bottle. Then, 1.5 g of distilled H_2O was added to each sample, bottles were capped, and soil was gently shaken for 5 s. The bottle was then opened, and the soil was gently stirred for 5 s, then caps were replaced and the soils were allowed to stand on the laboratory bench overnight. A 2.5-mL sample was then removed from each bottle, and its Ψ was measured with a Decagon WP4 dewpoint potentiameter. Each sample was weighed immediately following Ψ measurement. Samples were then oven-dried to constant mass for calculation of θ. More distilled H_2O was added to the bottles (increasingly smaller amounts as curves progressed) and mixed as described. The mixture was allowed to stand overnight, and Ψ was measured again the following day. In this way, about 20 measurements of Ψ per θ data pair were obtained for four replicates of each treatment.

Root, soil, and shoot characteristics

For each plant, leaves were collected as they died both before and during drying, and stems and remaining leaves were excised at the lethal point, for determination of shoot dry mass. On day 0, a recently matured leaflet was excised from each plant for measurement of [P], determined spectrophotometrically using the vanadate–molybdate–yellow method on samples dry-ashed with magnesium nitrate at 700 °C for 2 h and digested in nitric acid (Chapman and Pratt 1961). Potassium (K) concentration of myc^+ leaves was determined using

inductively coupled plasma spectrophotometry (A&L Labs, Memphis, Tenn.).

Root and soil fungal colonization were quantified on subsamples from the soil core removed at the lethal point for each pot (described previously) after soil was mixed thoroughly. Hyphal, arbuscular, and vesicular colonization of roots were determined on 100 microscope fields over several 1-cm root pieces, after clearing with 10% KOH in an autoclave at 121 °C for 15 min, staining with Trypan blue for 1 h, and destaining. A separate test was conducted to confirm that myc⁻ root systems did not develop intraradical colonization. Sixteen bean plants (four plants of each fungus per bean genotype combination) were inoculated and grown as described above, and colonization levels were measured when plants were 8 weeks old.

Soil hyphal density was measured as described before (Augé et al. 2001*b*, 2003) on 10-g subsamples from the soil cores removed from each pot at the lethal point. At the same time as these samples were removed from the sealed, refrigerated bag, additional subsamples were removed for measurement of soil θ, for computing soil hyphal density on a dry-mass basis. Bean roots (sorghum roots had decayed) were carefully excavated from another 25-g subsample of each replicate's soil core for measurement of root length using scanning equipment and imaging software (WinRhizo, Regent Instruments Inc., Québec, Que.). Root decay and turnover were not measured.

Water-stable aggregation (WSA) of air-dried soil was determined as described before (Augé et al. 2001*b*, 2003) on a 200-mL sample sieved through a 4-mm sieve by hand-shaking at a uniform stroke length 30 times. A 40-g sample of soil that passed the sieve was spread evenly over the top of a nest of sieves (2, 1, 0.5, and 0.25 mm) and wet-sieved for 10 min in an automatic wet-sieving apparatus fashioned after Angers and Mehuys (1993). The percentage of water-stable aggregates was calculated by dividing the mass of the ovendried water-stable fraction by the original sample mass.

Easily extractable glomalin was obtained and quantified according to the procedure of Wright and Upadhyaya (1999). Well-mixed, 0.25-g soil subsamples were placed in 18-mm flat-bottom glass vials, and 2 mL of 20 mmol·L^{-1} citrate, pH 7.0, was added to each vial. These were autoclaved for 30 min at 121 °C, transferred to 2-mL microcentrifuge tubes, and centrifuged at 10 000*g* for 5 min to remove insoluble materials. The Bradford protein assay was used to determine protein contents of the supernatants.

Concentrations of available P and K in each soil, extracted using the Mehlich I procedure, were determined using atomic absorption spectrophotometry for K and color spectroscopy (molybdenum blue) for P (University of Tennessee Soil Test Lab, Nashville, Tenn.).

Experimental design and statistical analysis

Pots were arranged in a completely randomized block design, blocked over greenhouse benches, with 12 replicates for each soil treatment and genotype combination. A split plot was used, with one myc⁻ and one myc⁺ plant in each pot. ANOVA was performed using the general linear model procedure with linear contrasts (SAS Institute Inc., Cary, N.C.). Mean separation using Fisher's protected LSD was also performed.

For evaluating soil moisture characteristic curves, various models were tested for predicting Ψ_m from θ, and the following power exponential model best fit the data:

$$\Psi_m = \alpha \exp(\beta\theta^{\delta})$$

where α is the *y* intercept, β is the rate at which the curve approaches the asymptote, and δ is the sharpness of the curve. The model was fit to individual plants using nonlinear regression (SAS). ANOVA of the parameter estimates (α, β, and δ) was then used to test for treatment differences.

Results

Linear contrasts

The ANOVA linear contrasts were designed to partition mycorrhizal influence into soil and root components, to compare the two AM species, and to test the effect of the myc⁻ mutation on the various host variables summarized in Tables 1, 4, and 5. There were effectively six plant treatments, and five linear contrasts were employed. Contrast 1 denotes whether myc⁻ and myc⁺ plants differed in a particular behavior, independently of any effect of AM symbiosis. This contrast is important to the experimental objectives, as it establishes whether myc⁻ versus myc⁺ differences were directly attributable to AM colonization rather than possibly to an artifact of the genetic mutation. Contrast 2 indicates if mycorrhization of roots alone affected host variables, independently of AM effects on soil. Myc⁻ plants, which did not develop intraradical AM colonization, and myc⁺ plants, which did develop extensive intraradical colonization, were compared while growing together in soils that had been colonized for 12 months by *Gi* or *Gm*. Because plants were growing in the same pot, soil hyphal densities were similar at seeding for myc⁻ and myc⁺ plants and remained similar around myc⁻ and myc⁺ root systems during the predrought- and drought-period measurements. Contrast 3 indicates whether mycorrhization of soil alone affected host variables, independently of direct AM effects on the plant via presence of live hyphae in roots. Myc⁻ plants in AM soil, plants which did not develop intraradical AM colonization but were growing in soils colonized by either by *Gi* or *Gm*, were compared with other plants that similarly did not develop intraradical AM colonization and were growing in nonAM soil; nonmycorrhizal plants were compared after growth in AM versus nonAM soil. To test the influence of mycorrhization of both roots and soil by AM fungi on host variables (contrast 4), colonized plants in colonized soil were compared with uncolonized plants in uncolonized soil. Contrast 5 tests whether *Gi* and *Gm* differed in their influence on the host.

Differences among the three soil treatments on soil variables summarized in Tables 2, 3, and 6 were tested using two linear contrasts, to compare AM and nonAM soil and to compare *Gi* and *Gm* soil.

Plant, soil, and fungus attributes

The genetic mutation that inhibited mycorrhizal colonization by roots did not affect shoot size; myc⁺ and myc⁻ plants growing in nonAM soil had similar shoot dry masses (Table 1, contrast 1). Uncolonized plants grown in AM soils were smaller than uncolonized plants grown in nonAM soils

Table 1. Shoot dry mass and leaf phosphorus (P) concentration of myc⁻ (noncolonizing mutant) and myc⁺ (wild-type) *Phaseolus vulgaris* plants grown in arbuscular mycorrhizal (AM) soil or nonAM soil.

(A) Effect of mutation and mycorrhizal treatments.

Treatment	Shoot dry mass (g)	Leaf [P] ($mg \cdot g^{-1}$)
Glomus intraradices soil		
Myc⁻ plants	4.5	4.6
Myc⁺ plants	10.2	5.0
Gigaspora margarita soil		
Myc⁻ plants	6.8	4.8
Myc⁺ plants	5.1	5.4
NonAM soil		
Myc⁻ plants	7.9	5.2
Myc⁺ plants	7.3	5.8
Fisher's protected LSD	1.6	0.8

(B) Linear contrasts.

		Significance	
Contrast	Effect of	Shoot dry mass	Leaf [P]
(1) Myc⁻ vs. myc⁺ plants in nonAM soil	Mutation	ns	ns
(2) Myc⁻ vs. myc⁺ plants in AM soil	Mycorrhization of roots	***	*
(3) Myc⁻ plants in AM soil vs. myc⁺ and myc⁻ plants in nonAM soil	Mycorrhization of soil	***	**
(4) Myc⁺ plants in AM soil vs. myc⁺ and myc⁻ plants in nonAM soil	Mycorrhization of roots and soil	ns	ns
(5) *Gi* vs. *Gm* plants	Fungal taxon	*	ns

Note: Values are means of 12 replicates. For linear contrasts, AM indicates that data were combined for fungal types. ns, nonsignificant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. "Effect of" column denotes experimental implication of each linear contrast. *Gi*, *Glomus intraradices*; *Gm*, *Gigaspora margarita*.

Table 2. Soil and root characters at the conclusion of the experiment with arbuscular mycorrhizal (AM) and nonAM soil.

(A) Effect of mycorrhizal treatments.

Treatment	Root length density ($cm \cdot g\ soil^{-1}$)	Soil hyphal density ($m \cdot g\ soil^{-1}$)	Average root diameter (mm)	Soil [P] ($mg \cdot kg^{-1}$)	Root hyphae (%)
Glomus intraradices soil	27	1.33	0.38	13	55
Gigaspora margarita soil	31	2.73	0.40	14	52
NonAM soil	37	0.04	0.37	36	0
Fisher's protected LSD	6	0.49	0.01	2	21

(B) Linear contrasts.

	Significance				
Contrast	Root length density	Soil hyphal density	Soil hyphal density	Soil [P]	Root hyphae
AM vs. nonAM soil	**	***	***	***	***
Gi vs. *Gm* soil	ns	***	***	ns	ns

Note: Values are means of eight replicates for each variable. For linear contrasts, AM signifies data were combined for fungal types. ns, nonsignificant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. *Gi*, *Glomus intraradices*; *Gm*, *Gigaspora margarita*.

(Table 1, contrast 3), probably owing to the larger P content of nonAM soils (Table 2). Colonized plants (myc⁺ plants in AM soils) were similar in size to uncolonized plants in nonAM soils (Table 1, contrast 4). The relative size of myc⁻ and myc⁺ plants differed in *Gi* and *Gm* soils; overall, *Gi* plants were slightly larger than *Gm* plants (Table 1, contrast 5). It is possible that these size variations may have contributed to or obscured treatment-induced changes in plant behavior.

Leaf [P] was quite adequate in all plants but somewhat higher in plants grown in the nonAM soil than in the AM soils. Although leaf [P] was similar among treatments, total P uptake was higher in plants colonized by *Gi*, owing to the larger size of myc⁺ plants in *Gi* soil. Leaf [K] was measured in myc⁺ plants to determine if the slightly higher K fertilization to nonAM soils had an effect on foliar [K] of plants in those soils, but no effect was detected. Leaf [K] was similar in plants grown in the three soils (treatment averages ranging from 2.6% to 2.8%), despite higher [K] of nonAM soils (56 $mg \cdot kg^{-1}$ in nonAM soil vs. average of 40 $mg \cdot kg^{-1}$ in AM soils).

Plants in nonAM soil remained uncolonized by AM fungi (Table 2). Myc⁻ plants in the two AM soils appeared to remain functionally nonmycorrhizal. We attempted to tease

Table 3. Water-stable aggregates of arbuscular mycorrhizal (AM) and nonAM soil at the conclusion of the experiment.

(A) Effects of soil treatments.				
	Water-stable aggregates (% total sample dry mass)			
Treatment	0.25–0.5 mm	0.5–1.0 mm	1.0–2.0 mm	2.0–4.0 mm
Glomus intraradices soil	23	26	16	11
Gigaspora margarita soil	21	25	16	12
NonAM soil	22	24	14	9
Fisher's protected LSD	2	2	1	2
(B) Linear contrasts.				
	Significance			
Contrast	0.25–0.5 mm	0.5–1.0 mm	1.0–2.0 mm	2.0–4.0 mm
AM vs. nonAM soil	ns	ns	**	**
Gi vs. *Gm* soil	ns	ns	ns	ns

Note: Values are means of eight replicates, with two subsamples per replicate. For linear contrasts, AM indicates that data were combined for fungal types. ns, nonsignificant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. *Gi*, *Glomus intraradices*; *Gm*, *Gigaspora margarita*.

Table 4. Stomatal conductance, leaf Ψ_π^{100}, and osmotic adjustment of myc$^-$ (noncolonizing mutant) and myc$^+$ (wild-type) *Phaseolus vulgaris* plants grown in arbuscular mycorrhizal (AM) soil or nonAM soil.

(A) Effect of mutation and mycorrhizal treatments.					
	Stomatal conductance ($mmol \cdot m^{-2} \cdot s^{-1}$)		Leaf Ψ_π^{100} (MPa)		
Treatment	Days –6 to –3[a]	Day 4	Day 0	Lethal point	Osmotic adjustment (MPa)
Glomus intraradices soil					
Myc$^-$ plants	418	35	–0.78	–1.16	0.37
Myc$^+$ plants	458	105	–0.81	–1.55	0.78
Gigaspora margarita soil					
Myc$^-$ plants	417	133	–0.78	–1.32	0.54
Myc$^+$ plants	457	103	–0.76	–1.54	0.79
NonAM soil					
Myc$^-$ plants	376	16	–0.76	–1.20	0.47
Myc$^+$ plants	368	33	–0.72	–1.11	0.43
Fisher's protected LSD	44	83	0.07	0.31	0.33

(B) Linear contrasts.							
		Significance					
		Stomatal conductance		Leaf Ψ_π^{100}		
Contrast	Effect of	Days –6 to –3	Day 4	Day 0	Lethal point	Osmotic adjustment
(1) Myc$^-$ vs. myc$^+$ plants in nonAM soil	Mutation	ns	ns	ns	ns	ns
(2) Myc$^-$ vs. myc$^+$ plants in AM soil	Mycorrhization of roots	**	ns	ns	**	**
(3) Myc$^-$ plants in AM soil vs. myc$^+$ and myc$^-$ plants in nonAM soil	Mycorrhization of soil	***	*	ns	ns	ns
(4) Myc$^+$ plants in AM soil vs. myc$^+$ and myc$^-$ plants in nonAM soil	Mycorrhization of roots and soil	***	**	ns	***	**
(5) *Gi* vs. *Gm* plants	Fungal taxon	ns	ns	ns	ns	ns

Note: Values are means for 12 replicate plants (for stomatal conductance, three leaves per plant for each of the 4 d, $n = 144$). For linear contrasts, AM indicates that data were combined for fungal types. ns, nonsignificant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. "Effect of" column denotes experimental implication of each linear contrast. *Gi*, *Glomus intraradices*; *Gm*, *Gigaspora margarita*.

[a]Day designations are in relation to the last day that plants were watered (day 0); plants were watered each morning prior to day 0.

Table 5. Leaf and soil Ψ and soil θ at the lethal point, and length of the drought period (time required to reach the lethal point), of myc⁻ (noncolonizing mutant) and myc⁺ (wild-type) *Phaseolus vulgaris* plants grown in arbuscular mycorrhizal (AM) soil or nonAM soil.

(A) Effect of mutation and mycorrhizal treatments.

Treatment	Leaf ψ at lethal point (MPa)	Soil ψ_m at lethal point (MPa)	Soil θ at lethal point ($mg \cdot g^{-1}$)	Lethal leaf ψ – lethal soil ψ (MPa)	Drought period (d)
Glomus intraradices soil					
Myc⁻ plants	–1.83	–5.88	16.6	5.1	13.1
Myc⁺ plants	–2.22	–6.60	15.2	4.8	14.1
Gigaspora margarita soil					
Myc⁻ plants	–2.19	–7.29	16.2	4.6	13.6
Myc⁺ plants	–2.27	–6.68	12.6	4.4	13.2
NonAM soil					
Myc⁻ plants	–1.87	–6.73	13.9	4.4	12.9
Myc⁺ plants	–2.03	–6.68	14.2	4.1	12.4
Fisher's protected LSD	0.28	1.36	3.9	1	1.1

(A) Linear contrasts.

		Significance				
Contrast	Effect of	Leaf Ψ at lethal point	Soil Ψ_m at lethal point	Soil θ at lethal point	Lethal leaf Ψ – lethal soil Ψ	Drought period
(1) Myc⁻ vs. myc⁺ plants in nonAM soil	Mutation	ns	ns	ns	ns	ns
(2) Myc⁻ vs. myc⁺ plants in AM soil	Mycorrhization of roots	*	ns	ns	ns	ns
(3) Myc⁻ plants in AM soil vs. myc⁺ and myc⁻ plants in nonAM soil	Mycorrhization of soil	ns	ns	ns	**	ns[a]
(4) Myc⁺ plants in AM soil vs. myc⁺ and myc⁻ plants in nonAM soil	Mycorrhization of roots and soil	**	ns	ns	ns	*
(5) *Gi* vs. *Gm* plants	Fungal taxon	*	ns	ns	ns	ns

Note: Values are means for 12 replicate plants. For linear contrasts, AM signifies data were combined for fungal types. ns, nonsignificant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. "Effect of" column denotes implication of each linear contrast. *Gi*, *Glomus intraradices*; *Gm*, *Gigaspora margarita*.
[a] $p < 0.1$ for this case.

apart root systems of myc⁻ and myc⁺ plants to isolate myc⁺ and myc⁻ roots for sampling for colonization, but because of the high root densities we were unable to always confidently distinguish myc⁻ and myc⁺ roots. Therefore, we conducted a separate experiment to test whether roots of myc⁻ plants could become colonized by *Gi* or *Gm*. Roots of myc⁺ plants developed extensive colonization when inoculated by *Gi* or *Gm* (80% total colonization level averaged over both fungi), whereas roots of myc⁻ did not develop any internal mycorrhizal structures in this test. Numerous hyphae were observed growing along the outside surface of some myc⁻ roots, but at no point did hyphae penetrate the epidermis and enter roots: myc⁻ plants remained nonmycorrhizal. Nodules were observed on roots of myc⁺ plants but not myc⁻ plants. A similar phenomenon was observed with the ineffective bean mutant 'R69' when it was inoculated with *Rhizobium leguminosarum* bv. *Phaseoli* strains. Vestigial, tumour-like white nodules formed in myc⁻ plants, but no N fixation activity was detected (Buttery and Park 1993; Shirtliffe et al. 1995), probably because of lack of penetration by rhizobial bacteria (Shirtliffe and Vessey 1996). Root hyphal colonization levels shown in Table 2 represent both myc⁺ and myc⁻ roots. Arbuscular colonization levels were low in myc⁺ plants in AM soil, 4% and 7%, respectively, for *Gi* and *Gm* plants. Vesicular colonization levels were 11% and 0%, respectively, for myc⁺ plants in *Gi* and *Gm* soil.

AM soil had slightly lower root length densities and markedly higher soil hyphal densities than nonAM soil (Table 2). Soil hyphal density was about twice as high in *Gm* than in *Gi* soils. Root fineness, indicated by average root diameter, was very slightly but significantly higher in nonAM soils and in *Gi* relative to *Gm* soils. Soil [P] levels were higher in nonAM than in AM soils. Glomalin concentrations were similar among the three soils, with treatment means ranging from 1.43 to 1.61 $mg \cdot g^{-1}$ (data not shown). The AM soil treatments did not affect the smaller WSA classes (Table 3), but WSA was higher in the two AM soils in the 1–2 mm and 2–4 mm WSA size classes. Signs of root decay and turnover were not obvious during coring for aggregation, density, and root colonization measurements.

Stomatal conductance

Stomatal conductance of all plants was measured under amply watered conditions on 4 d during the week preceding the start of the drying period and 4 d after initiating drought. The myc⁻ mutation did not affect g_s (Table 4, contrast 1). Myc⁺ and myc⁻ plants growing in the AM soils had higher g_s than corresponding plants growing in nonAM soil.

Table 6. Means for parameters describing arbuscular mycorrhizal (AM) and nonAM soil moisture release curves (wet hysteretics).

(A) Effect of soil treatments.

	Nonlinear regression					Linear regression		
Treatment	α^a (MPa)	β	δ	θ at –0.02 MPa^b (g water·g soil^{-1})	θ at –0.2 MPa (g water·g soil^{-1})	θ at –1.5 MPa (g water·g soil^{-1})	Slope, "dry line" (MPa/(g·g^{-1}))	*x* intercept, θ (g water·g soil^{-1})	*y* intercept, Ψ_m (MPa)
Glomus intraradices soil	–57	–1783	1.83	0.074	0.044	0.032	631	0.035	–21.4
Gigaspora margarita soil	–48	–1877	1.93	0.069	0.045	0.035	623	0.038	–23.1
NonAM soil	–23	–1803	1.88	0.078	0.046	0.031	493	0.033	–16.4
Fisher's protected LSD	–41	540	0.24	0.008	0.005	0.004	185	0.005	5.8

(B) Linear contrasts.

	Significance								
	Nonlinear regression						Linear regression		
Contrast	α	β	δ	θ at –0.02 MPa	θ at –0.2 MPa	θ at –1.5 MPa	Slope, "dry line"	*x* intercept, θ	*y* intercept, Ψ_m
AM vs. nonAM soil	ns	ns	ns	*	ns	ns	ns^c	ns	*
Gi vs. *Gm* soil	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: Values are means of eight replicate soils. For linear contrasts, AM signifies data were combined for fungal types: ns, nonsignificant, or significant at the 5% (*), 1% (**), or 0.1% (***) level. The same nonlinear and linear regression parameters were evaluated for dry line hysteretic curves (means not shown), contrasts were not significant.

[a] α, β, and δ refer to parameters in the power exponential equation (the function that best fit the data): $\Psi_m = \alpha \exp(\beta\theta^{\delta})$, where α is the *y* intercept, β is the rate at which the curve approaches the asymptote, and δ is the sharpness of the curve.

[b] Mean θ at soil Ψ_m of –0.02, –0.2, and –1.5 MPa were computed from the power exponential functions fitted to the data for each replicate of each treatment.

[c] $p < 0.1$ for this entry.

The myc$^-$ versus myc$^+$ in AM soil linear contrast represents the influence of root colonization on g_s (Table 4, contrast 2): myc$^-$ and myc$^+$ plants were tested side by side in AM soils, with only the myc$^+$ plants developing colonization of roots. Root colonization did promote higher g_s before drought. Comparing myc$^-$ plants in AM soil with plants growing in nonAM soil (Table 4, contrast 3) was a test of the influence of mycorrhization of soil alone. As seen with mycorrhization of roots, mycorrhization of soil alone also elevated g_s of bean plants before drought as well as after 4 d of drought. Contrast 4 of Table 4 represents the comparison between bean plants grown with no exposure to AM fungi with plants having both colonized roots and exposure to AM soil: myc$^+$ plants in AM soil versus plants in nonAM soil. Mycorrhization of both roots and soil led to increased g_s.

The ratio of AM promotion of g_s (myc$^+$ plants/myc$^-$ plants) was 2.3, 2.1, 2.1, and 1.9 for days –6, –5, –4, and –3, respectively. This indicates that the influence of AM soil and root colonization on g_s was consistently twice that of AM soil alone. Over the 4 d preceding soil drying and across both AM soil types (*Gi* and *Gm*), promotion of g_s by AM soil averaged 11% (relative to g_s of myc$^-$ plants in the nonAM soil; $p = 0.03$). Over the 4 d preceding soil drying and across both AM soil types, promotion of g_s by mycorrhization of both roots and soil averaged 22% (relative to g_s of myc$^+$ plants in nonAM soil; $p < 0.0001$). The difference between the 11% and 22% values represents the influence of root colonization alone. When averaged over the 4 d, g_s of myc$^+$ plants in the *Gi* and *Gm* soils relative to g_s of myc$^+$ plants in the nonAM soil was significantly higher than g_s of myc$^-$ plants in the *Gi* and *Gm* soils relative to g_s of myc$^-$ plants in the nonAM soil ($p = 0.002$).

Stomatal conductance was also measured on day 4 of the drying period, as plants neared stomatal closure. There was no effect of root colonization alone on g_s on day 4 (Table 4, contrast 2), but mycorrhization of soil did promote higher g_s (contrast 3). Mycorrhization of both roots and soil had a substantial effect on g_s, with g_s of myc$^+$ plants in AM soil over 4× higher than g_s of plants in nonAM soil.

Gi and *Gm* promoted g_s to a similar extent. Over the 4 d preceding drought, promotion by *Gi* and *Gm* averaged 10% and 11%, respectively, in myc$^-$ plants, and 24% and 20%, respectively, in myc$^+$ plants.

Foliar dehydration tolerance

Leaf Ψ_π^{100} was similar among plants of all treatments when the drought period began (day 0; Table 4). Leaf Ψ_π^{100} at the lethal point was about 0.3 MPa lower in myc$^+$ than in myc$^-$ plants in the AM soils, indicating more osmotic adjustment in myc$^+$ than in myc$^-$ plants in each AM soil. This means that mycorrhization of roots alone by either *Gi* or *Gm* did affect foliar osmotic responses to the severe drought (Table 4, contrast 2). Similarly, AM colonization of both roots and soil significantly affected leaf Ψ_π^{100} at the lethal point and osmotic adjustment during the drying period (Table 4, contrast 4). Mycorrhization of soil alone, by either fungus, did not affect foliar osmotic responses to drought (Table 4, contrast 3). Leaf Ψ at the lethal point was also affected by root colonization as well as by mycorrhization of both roots and soil. Leaf Ψ was 0.22–0.38 MPa lower in myc$^+$ than in myc$^-$ plants growing in AM soils (Table 5, contrast 2) and about

0.44 MPa lower in myc$^+$ plants in AM soil than in plants in nonAM soils (Table 5, contrast 4). Mycorrhization of soil alone, by either fungus, did not affect lethal leaf Ψ (Table 5, contrast 3). AM colonization, whether of roots, soil, or both roots and soil, had no effect on soil Ψ_m or soil θ at the lethal point (Table 5). Mycorrhization of soil alone reduced average lethal leaf Ψ – soil Ψ values by about 0.5 MPa (Table 5, contrast 3). Averaged over the two fungal species, AM colonization of roots and soil allowed plants to survive about 1 d longer than plants in nonAM soil before being killed by drought (Table 5, contrast 4).

The myc$^-$ mutation had no effect on any leaf or soil measure of plant dehydration tolerance; osmotic variables, and values of leaf and soil water status at the lethal point, were each similar in myc$^-$ and myc$^+$ plants (Tables 4 and 5, contrast 1). AM species type had no effect on osmotic relations (Table 4, contrast 5), nor on soil water Ψ_m or θ at the lethal point (Table 5, contrast 5). *Gm* plants had lower lethal leaf Ψ values than *Gi* plants (Table 5).

NonAM soils dried more quickly than AM soils (Fig. 1). Even at high soil moistures, when Ψ_m was near 0 MPa, Ψ_m was very slightly lower in nonAM than in AM soils. On days –1, 0, and 2, mean Ψ_m was about –0.006 MPa for AM soils and about –0.007 MPa for nonAM soils (SE of the means was 0.0002 MPa). Soil drying times did not differ between *Gi* and *Gm* soils.

Soil moisture characteristic curves

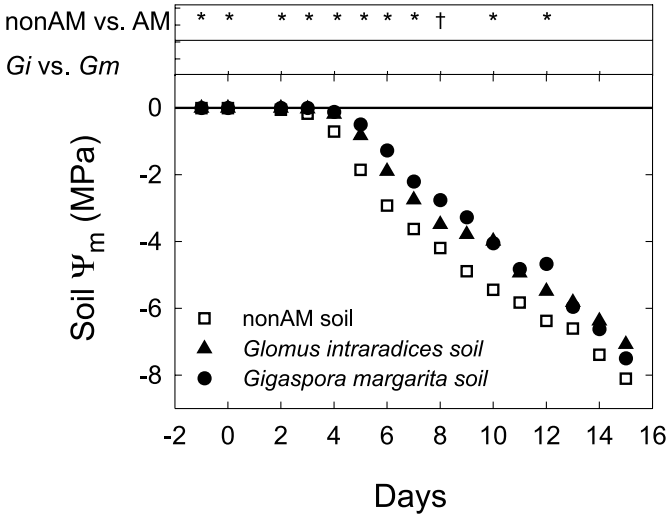
Soil moisture release plots were evaluated as in Augé et al. (2001*b*), with the addition of analysis of dry hysteretic plots. Data for all replicates are depicted in Fig. 2. As previously described for this substrate type (Augé et al. 2001*b*), power exponential functions gave the best fit to the data among several nonlinear regressions tested; r^2 averaged 0.98 ± 0.005.

Mycorrhization of soil had little effect on its moisture characteristic curve. For wet hysteretic curves, AM and nonAM curves tended to break at similar θ, with similar descent of the curves as θ declined further. These tendencies are described by the plot parameters, for which treatment comparisons are summarized in Table 6. α, β, and δ were similar in *Gi*, *Gm*, and nonAM curves, for both wet hysteretics (Table 6) and dry hysteretics (data not shown).

To further characterize and compare treatment effects, we computed θ at some representative and biologically meaningful Ψ_m for each replicate from its wet hysteretic characteristic curve. Treatment averages for θ at –0.02, –0.2, and –1.5 MPa are shown in Table 6. AM and nonAM soils had similar θ at –0.2 and –1.5 MPa. AM soils did need to dry more before their Ψ_m began to decline from their amply watered plateau levels; θ at –0.02 MPa was lower in AM than in nonAM soil.

Soil moisture characteristic curves can also be statistically analyzed as broken line regressions (e.g., Fuller and Gallant 1974). The linear "wet line" is horizontal with a slope of zero: that portion of the plot in which Ψ_m has not yet declined from fully watered values (approx. –0.01 MPa). The "dry line" is the portion of the plot developing after Ψ_m begins to decline linearly (below about –3 MPa for this substrate). Dry line slopes in Table 6 represent linear regressions of wet hysteretic curves within each treatment of all

Fig. 1. Soil Ψ_m during the drying period. Symbols represent means of eight replicates for each soil treatment. Linear contrasts indicate significance of treatment differences for each day (* and † denote $p \leq 0.05$ and 0.10, respectively). Pooled standard error of the means was 0.28 MPa (smaller than the height of the symbols).



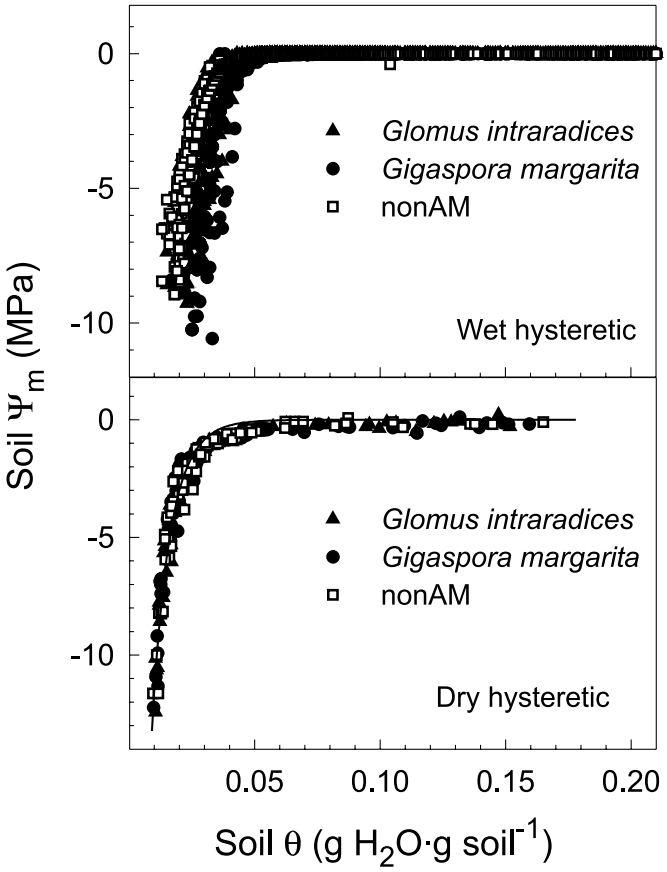
Ψ_m per θ data pair of $\Psi_m \leq -3$ MPa. AM soils tended to have larger dry line slopes than the nonAM soil. Average *x*-intercepts were similar in AM and nonAM soils, and *y*-intercepts occurred at Ψ that were about 7 MPa lower in AM than in nonAM soil.

Discussion

Almost all investigations of AM influence on plant water balance and drought resistance have focused on how AM fungi affect the plant. There is growing interest in the effects of AM fungi on the soil itself, as well as the possibility that AM soil may somehow directly influence plant physiology (Bethlenfalvay and Linderman 1992; Augé et al. 2003), regardless of whether the plant itself is mycorrhizal. We used myc$^-$ mutants and produced AM and nonAM soils to partition AM influence into soil and root components. Results of hypotheses tests were as follows: hypothesis 1: mycorrhization of both roots and soil affected stomatal behavior and drought resistance of the host plant; hypothesis 2: mycorrhization of soil alone affected stomatal behavior but not drought resistance of the host plant; and hypothesis 3: mycorrhization of roots alone affected both stomatal behavior and drought resistance of the host plant.

Mycorrhization of soil is more than the spread of hyphae in soil and refers more broadly to any AM-induced changes. We attempted to control for microbial populations using the conventional water filtrates of inocula. As a further method to incline nonAM and AM pots toward similar soil microbes and fauna, we applied nonAM pot cultures to nonAM plants, cultures grown in the same soils and in the same greenhouse as *Gi* pot cultures. Root density differed slightly between AM and nonAM soils, as did water-stable aggregation in two size classes. Root systems of plants of all treatments were constrained to limited soil volumes in pots for a relatively long period of time, allowing roots to fully explore

Fig. 2. Soil moisture characteristic curves. Wet hysteretics were constructed on whole, undisturbed soils in pots using heat dissipation sensors to measure Ψ_m; each data pair was measured on the same soil sample (all soil in the pot). Dry hysteretics were constructed on sieved soil samples using the chilled mirror dewpoint technique for measuring total Ψ; data pairs were each measured on separate samples (2.5 mL). Shown are all data for all replicates of each treatment. See Table 6 for statistical comparisons.



pots, an experimental procedure that may have tended to discourage large differences in root density among treatments, while allowing large hyphal densities to develop. The minor differences in leaf [P] among treatments probably did not have much effect on plant water relations, as at 4.6 $mg \cdot g^{-1}$ or above, leaf [P] was more than adequate for all treatments. The most obvious (though still quite small) differences in leaf [P] occurred between myc^- plants and myc^+ plants, yet there were no differences between myc^- and myc^+ plants in any measured water relations parameter. Amount of hyphae in soil and in roots differed dramatically between nonAM and AM soils, a matter of absence or near absence versus substantial presence of these two characters. Hence, the AM treatment most markedly affected hyphal growth in roots and soil, much more than the relatively slight variations in root density, root diameter, and soil aggregation that were associated with the AM treatments. Although the chief effects of mycorrhization among the many characters measured was presence or near absence of hyphae, it is possible that other unmeasured AM effects on soil may have occurred, for instance, AM effects on root turnover and carbon delivery to soil microbes.

Foliar drought tolerance has been characterized by ability to withstand dessication and to osmotically adjust during a lethal drying episode: by the extent to which leaf Ψ and leaf Ψ_π^{100} decline before leaves die (Ludlow 1989). In the present study, *Gm* had a greater effect than *Gi* on lethal leaf Ψ, while the two AM species had similar effects on osmotic adjustment. The AM effect on lethal leaf Ψ appeared to be due to an effect on the plant itself rather than an effect on mycorrhization of soil. This is reasonable, as a treatment effect on the degree of dehydration a leaf can endure before it dies represents a fundamental change in leaf physiology. Similarly, the AM effect on osmotic adjustment was also associated with an effect on the plant itself rather than with an effect on soil. Concurrent, AM-induced changes in lethal leaf Ψ and osmotic adjustment suggest that lethal leaf Ψ was lowered via an increase in osmotically active solutes.

Colonization by *Gi* has not previously altered ability of common bean, cowpea, soybean, or basil foliage to withstand lethal dehydration, relative to nonAM plants (Augé et al. 2001*a*; Kubikova et al. 2001). However, foliar drought tolerance was increased in plants colonized by a semiarid mix of AM fungi, relative to plants colonized by *Gi* (Augé et al. 2003). In a few prior instances, leaves of AM plants have shown more osmotic adjustment than nonAM plants when exposed to similar, nonlethal drought pressure (e.g., Allen and Boosalis 1983; Augé et al. 1986; Davies et al. 1993), as well as to lethal drought (Kubikova et al. 2001). Several studies suggest that arbuscular mycorrhizae can extract water from soils to lower soil Ψ than nonmycorrhizal roots, allowing leaves of AM plants to remain slightly better hydrated than leaves of nonmycorrhizal plants at these low soil Ψ (Hardie and Leyton 1981; Dakessian et al. 1986; Bethlenfalvay et al. 1988; Franson et al. 1991; Duan et al. 1996; Marulanda et al. 2003). Lethal leaf Ψ, lethal soil Ψ, and lethal leaf Ψ – lethal soil Ψ values were similar to those observed before for this host species (Augé et al. 2001*b*). Foliage showed considerably more osmotic adjustment in the current study than in that previous work.

Growing in soil that had previously hosted AM fungi for several months resulted in a promotion of g_s of nonAM plants in both amply watered and droughted plants. This is a key finding of the present work: AM soil affected the physiology of nonmycorrhizal plants grown in that soil. Increased sink strength of AM roots has been suggested as a reason for the frequently observed mycorrhizal promotion of g_s (Augé 2000), yet sink strength was not a factor in this instance, as roots remained nonmycorrhizal. We might have anticipated some AM soil effect in dry soils, as AM symbiosis has previously been implicated in altering soil moisture retention properties (Augé et al. 2001*b*; Bearden 2001). Stomatal conductance did remain higher during the first days of the drying episode in nonAM plants in AM soil compared with those in nonAM soil. It is noteworthy that functional soil–root hyphal connections and root colonization by AM hyphae did not affect stomatal behavior during soil drying, while colonization of the soil itself did prolong the drying time required to reach stomatal closure.

The higher g_s of plants in AM versus nonAM soil on day 4 of drying (Table 4) coincided with moister AM soil on that day (Fig. 1); Ψ_m on day 4 was near the break point for soils of the various treatments and was just starting to de-

cline. To reach the same Ψ near the break point (–0.02 MPa), AM soils had to dry more (achieve lower θ), an indication of possibly differing moisture retention properties of AM and nonAM soils.

We constructed soil moisture release plots to explore the possibility that the strictly soil-based influence of AM symbiosis on water relations of droughted host plants was associated with an AM-induced change in soil water relations. We hypothesized that mycorrhizing soils for a year may have an impact on their retention curves more than that previously observed after 7 months (Augé et al. 2001*b*), allowing more time for soil hyphal production, glomalin exudation, and changes in aggregation. However, the moisture release plots were mostly similar among *Gi*, *Gm*, and nonAM soils. The AM and nonAM soils did not differ in θ near the Ψ_m characteristically considered the wilting point for many species (–1.5 MPa), nor did the soils differ in θ at –0.2 MPa, a moderate stage of soil drought. AM soils did lose significantly more water than nonAM soil to reach the threshold or breakpoint at which soil Ψ_m first began to decline as soil dried. This stage of soil dryness coincides to some extent with the point of stomatal closure (which was extended into drier soil for AM than for nonAM soils).

We anticipated that if there were some direct effect of AM soil on plant water relations, it would most likely be observed under conditions of soil water limitation and probably less so or not at all under amply watered conditions. However, there was a marked, consistent effect of AM soil on stomatal opening of unstressed plants watered just prior to measurements of g_s. Soil Ψ_m was slightly higher in AM soils than in nonAM soils under amply watered conditions. The difference was very small, however, and though statistically significant, was probably not biologically significant. Another possibility is a nonhydraulic basis for the AM influence. Possibilities for both hydraulic and nonhydraulic effects on g_s have been tested for colonization of roots by AM fungi (Augé et al. 1994; Ebel et al. 1994, 1997; Duan et al. 1996). By comparison, how AM-induced changes in soil hydraulic properties might impact g_s of the host plant has been the subject of very little research, and no studies have been published regarding nonhydraulic possibilities for how AM soil might affect stomatal behavior, independently of plant colonization. Because of its relatively long-lived, abundant, and unique ecological presence in the rhizosphere, a zone of high microbial activity, AM symbiosis can exert a controlling influence on other soil microbes (Linderman 1992; Gryndler 2000; Suresh and Bagyaraj 2002). Soil organisms that interact with AM fungi include P solubilizers; free-living and symbiotic N fixers; producers of antibiotics, siderophores, and plant growth hormones; saprophytes; plant pathogens; predators; and parasites (Sun et al. 1999). It is possible that biochemical effects on soil deriving from AM interactions with other soil organisms may contribute to effects of AM soil on host g_s.

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