

# Arbuscular mycorrhizae and soil/plant water relations

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Augé, R. M. 2004. **Arbuscular mycorrhizae and soil/plant water relations**. *Can. J. Soil Sci.* **84**: 373–381. The water relations of arbuscular mycorrhizal (AM) plants have been compared often. However, virtually nothing is known about the comparative water relations of AM and nonAM soils or about the relative influence of AM colonization of soil vs. AM colonization of plants on host water balance. In this review, I summarize findings that support the assertion that colonization of soil may play as important a role as colonization of roots regarding how AM symbiosis affects the water relations of host plants. We observed a slight but significant AM effect on the soil moisture characteristic curve of a Sequatchie fine sandy loam following 7 mo of mycorrhization by *Glomus intraradices*/Vigna unguiculata. In a separate study, few AM effects on either the wet or dry hysteric curves were discernible after 12 mo of mycorrhization by *G. intraradices* or *Gigaspora margarita* on roots of *Phaseolus vulgaris*. Using myc- bean mutants, we determined that about half of the considerable promotion of stomatal conductance by *G. intraradices* and *Gi. margarita* was attributable to soil colonization and about half to plant colonization. A path analysis modeling approach revealed that soil hyphal colonization had larger direct and total effects on dehydration tolerance of bean than did root hyphal colonization or several other soil or plant variables.

**Key words:** Mycorrhizal symbiosis, soil moisture characteristic, stomatal conductance, water relations

Augé, R. M. 2004. **Les mycorhizes à arbuscules et leurs liens avec la teneur en eau dans le sol ou la plante**. *Can. J. Soil Sci.* **84**: 373–381. On compare souvent les relations hydriques des plantes colonisées par les mycorhizes à arbuscules (MA). Toutefois, on ignore presque tout des relations hydriques des sols à MA et sans MA et de l'influence relative de la colonisation du sol par les MA sur le bilan hydrique de l'hôte par rapport à la colonisation des plantes par les MA. Dans cet article, l'auteur résume les constatations étayant l'affirmation que la colonisation du sol pourrait s'avérer aussi importante que la colonisation des racines quant à l'incidence des MA symbiotiques sur les rapports de la plante hôte avec l'eau. Après sept mois de colonisation par les mycorhizes de *Glomus intraradices*/Vigna unguiculata, on observe une légère mais significative incidence des MA sur la courbe des paramètres hydriques dans un fin loam sablonneux Sequatchie. Dans le cadre d'une autre étude, les MA ont eu peu d'effets discernables sur la courbe de l'hystérésis en sol sec ou humide après 12 mois de colonisation des racines de *Phaseolus vulgaris* par les mycorhizes de *G. intraradices* ou de *Gigaspora margarita*. L'utilisation de haricots mutants de type myc- révèle qu'on doit environ la moitié de la très forte promotion de la conductance des stomates par *G. intraradices* et *Gigaspora margarita* à la colonisation du sol et l'autre moitié à la colonisation de la plante. L'analyse causale par modélisation indique que la colonisation du sol par les hyphes a une plus grande incidence directe et globale sur la tolérance du haricot à la déshydratation que la colonisation des racines par les hyphes ou d'autres variables du sol ou de la plante.

**Mots clés:** Mycorhizes symbiotiques, paramètres de la teneur en eau du sol, conductance des stomates, relations hydriques

Arbuscular mycorrhizal (AM) fungi are commonly occurring soil microbes whose association with roots can have wide-ranging effects on growth of the host plant (Klironomos 2003). AM symbiosis can also alter plant water relations and responses to drought (Augé 2001). In many instances these AM-induced changes have been interpreted as improved drought resistance (e.g., Davies et al. 1993; Ruiz-Lozano and Azcón 1995; Subramanian and Charest 1998; Porcel et al. 2003).

Studies of mycorrhizal water relations have almost always focused on how AM fungi affect the plant. There is growing interest in the effects of AM fungi on the soil itself, and we have begun testing the possibility that "mycorrhizal soil" may somehow directly influence plant physiology, regardless of whether the plant itself is mycorrhizal. In this paper I summarize the plant-based mechanisms that have

been proposed or tested to explain AM influence. I suggest why it might prove worthwhile to examine the possibility that the effect of AM symbiosis on plant water balance could have as much to do with colonization of soil as of roots. And I summarize findings of experiments designed to: (1) determine if mycorrhizal colonization of soil can affect soil moisture retention properties, (2) compare the effects of AM soil colonization vs. AM root colonization on plant water relations, and (3) assess the relative contribution of soil colonization vs. root colonization to drought resistance and stomatal behavior.

## MYCORRHIZAE AND PLANT WATER RELATIONS

The most obvious explanation for AM-induced changes in plant water balance and drought resistance is probably indirect "big plant/little plant" effects associated with changes

**Abbreviations:** AM, arbuscular mycorrhizal (fungi);  $g_s$ , stomatal conductance; **myc+**, wild type plants; **myc-**, non-colonizing mutants;  $\theta$ , water content;  $\psi_s$ , water potential;  $\psi_m$ , matric potential

in plant size and phenology that occur via increased acquisition of phosphorus and sometimes other nutrients. The size of a plant can affect its water relations, and AM symbiosis often affects plant size. Larger plants with larger root systems may have access to more extensive soil water reserves (Fitter 1985; Koide 1993 for reviews), and so plant size effects are often particularly evident in drying soils. Mycorrhizal influence can be evident, however, even when nutrition is adjusted to control for plant size (Gupta 1991; Sánchez-Díaz and Honrubia 1994). Under these circumstances, several possibilities have been explored to explain the mycorrhizal influence.

When AM plants and nonAM controls have similar size and phosphorus concentrations, mycorrhizal plants often show higher stomatal conductance ( $g_s$ ) and transpiration, whether plants are adequately watered (Ebel et al. 1997; Green et al. 1998; Augé et al. 2004a; Fig. 1) or droughted (Augé et al. 1986; Duan et al. 1996). In terms of promoting gas exchange and drought resistance, the symbiosis can allow leaves to maintain more normal water balance (closer to responses of unstressed controls) and fix more carbon during drought stress (Augé et al. 1987a; Duan et al. 1996). During drought, mycorrhizal symbiosis can alter osmotic and elastic properties in leaves (Augé et al. 1986, 1987b; Goicoechea et al. 1997b) and roots (Augé and Stodola 1990), and accumulation of key solutes in leaves (Augé et al. 1987a) and roots (Augé et al. 1992b). Mycorrhizal plants often show physiological responses linked to increased drought resistance: e.g., the ability to maintain stomatal opening and hence carbon gain to lower soil water potential ( $\Psi$ ) (Augé et al. 1987a; Osonubi 1994; Duan et al. 1996) and leaf  $\Psi$  (Allen and Allen 1986; Augé et al. 1986) or water content ( $\theta$ ). Mycorrhizal plants also sometimes maintain leaf turgor to lower soil  $\Psi$  (e.g., Augé et al. 1986).

In addition to promoting stomatal opening during soil drying, AM fungi can also affect stomatal response when soil  $\Psi$  is lowered osmotically (Augé et al. 1992a). This suggests that AM root systems either scavenge water of low activity more effectively [a finding also implicated by Bethlenfalvay et al. (1988b)] or contribute to so-called non-hydraulic root-to-shoot communication (Davies et al. 1994) differently than nonAM root systems. Mycorrhizal symbiosis has modified host hormonal relations (Allen et al. 1982; Danneberg et al. 1992; Goicoechea et al. 1997a; Nikolaou et al. 2003), and we investigated the possibility that mycorrhizal fungi, which are confined to outer root tissues, were affecting distant organs like leaf stomata by changing the hormonal flow of information from roots to shoots in the transpiration stream. Working with maize, sorghum and cowpea, we did not observe much effect of mycorrhizal symbiosis on  $g_s$  or plant water relations under conditions where strictly nonhydraulic signaling of soil drying prevailed (Augé et al. 1994; Ebel et al. 1994; Augé et al. 1995).

Root hydraulic conductivity has often not been improved by AM symbiosis (e.g., Levy et al. 1983; Graham et al. 1987; Andersen et al. 1988), although Kyllö et al. (2003) recently reported significant, positive AM effects related to phylogeny and light, in understory shrub species. Water uptake by AM hyphae (Faber et al. 1991; Ruiz-Lozano and

Azcón 1995) and/or increased water uptake related to AM changes in root morphology (Kothari et al. 1990) or root fineness (Price et al. 1989) could conceivably allow mycorrhizal plants in drying soil to sustain higher  $g_s$  and leaf turgor by effectively providing roots access to more of the soil water reservoir. Recent comparisons among six AM species revealed significant contributions by extraradical hyphae to mycorrhizal root water uptake (Marulanda et al. 2003).

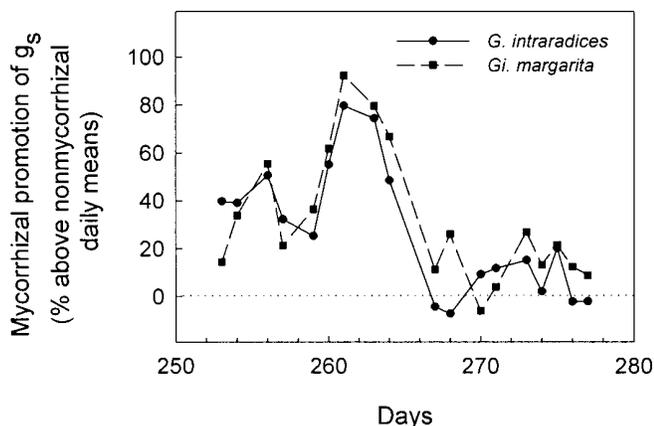
There is support for the idea that AM plants may have access to water that is unavailable to nonAM plants. Better growth of AM plants in drying soils may be related to improved exploitation of bound water, with mycorrhizae providing access to soil water below the permanent wilting  $\Psi$  of nonmycorrhizal plants (Dakessian et al. 1986; Bethlenfalvay et al. 1988b; Franson et al. 1991). Duan et al. (1996) concluded that AM fungi probably increased the capability of cowpea root systems to scavenge water in drier soil, resulting in less strain to roots and foliage and hence higher transpiration,  $g_s$  and shoot  $\Psi$ , and lower concentrations of ABA in xylem sap, at low soil  $\theta$ . This possibility is supported by a study with alfalfa (Goicoechea et al. 1997a), in which AM roots had lower ABA concentrations than nonAM roots at similar low soil  $\theta$ .

#### MYCORRHIZAE AND SOIL WATER RELATIONS

Many reports summarize the comparative water relations of AM and nonAM plants (Augé 2001). However, the influence of AM symbiosis on the water relations and moisture retention properties of soils remains largely unstudied.

Fungi may be the most effective soil organisms in stabilizing soil structure (McCalla 1946; Swaby 1949; Foster 1994), and AM fungi often comprise the largest portion of the soil microbial biomass (Hayman 1978). Fungal hyphae, especially those of AM fungi, grow into the soil matrix to create the skeletal structure that holds primary soil particles together via physical entanglement. They create conditions conducive to formation of microaggregates, and they chemically enmesh and stabilize microaggregates and smaller macroaggregates into macroaggregate structures (Miller and Jastrow 2000). As discussed in this volume (Rillig 2004), AM fungi produce copious amounts of the glycoprotein glomalin. Although soil aggregation is a complex hierarchical process (Tisdall and Oades 1982), concentration of glomalin is tightly correlated with aggregate stability across many soils (Wright and Upadhyaya 1998; Rillig 2004). AM symbiosis has been linked to changes in soil structure in both pot and field experiments (Schreiner and Bethlenfalvay 1995; Rillig 2004).

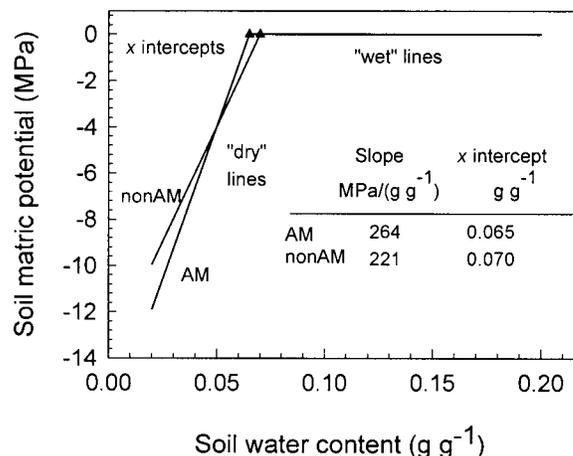
Soil structure refers to pore space as well as to aggregates, and the number and dimension of the pore spaces between soil particles are important in functional considerations of soil structure, especially from the standpoint of soil water relations (Hamblin 1985). The moisture characteristic of a soil depends on the size and distribution of its pores, or void space (Hamblin 1985). Because AM fungi affect soil structure, it seems logical to suspect that AM colonization of a soil might affect its moisture retention properties and, in turn, the behavior of plants growing in the soil, particularly when soil is relatively dry.



**Fig. 1.** Promotion of  $g_s$  by *Glomus intraradices* (*Gi*) and *Gigaspora margarita* (*Gm*), relative to nonAM plants. Mycorrhizal promotion of  $g_s$  was calculated as a percent: (absolute  $g_s$  of *Gi* or *Gm* plants – mean  $g_s$  of nonAM plants)/mean  $g_s$  of nonAM plants  $\times 100$ . A value of 0% promotion denotes that daily average  $g_s$  of *Gi* or *Gm* plants was similar to that of nonAM plants; nonAM plants are depicted by the dotted line. Symbols represent means of 30 replicates per treatment, three leaves per replicate ( $n = 90$ ). Pooled standard error of the means was 1.8% (smaller than the height of symbols). Redrawn with permission from *Mycorrhiza* (Augé et al. 2004a, Fig. 3), copyright held by Springer.

Well-structured soils contain more available water than poorly structured soils (e.g., Greacen and Williams 1983), and reductions in aggregate stability have been correlated with reduced soil  $\theta$  at particular soil  $\Psi$  (e.g., Fahad et al. 1982). More soil water at a particular low soil  $\Psi$  might explain how AM plants have wilted at lower soil  $\Psi$  (Hardie and Leyton 1981), maintained turgor to lower soil  $\Psi$  (Augé et al. 1986), developed lower soil  $\Psi$  at the permanent wilting point (Bethlenfalvay et al. 1988a, b) or maintained higher shoot water status at low soil  $\Psi$  or  $\theta$  (Osonubi 1994; Duan et al. 1996) than nonmycorrhizal plants. Correlative evidence has linked soil aggregation with increased drought resistance (Davies et al. 1992). Physiologically, mycorrhizae-mediated uptake of bound soil water may be analogous to the uptake of phosphorus, where tapping supplies not available to the nonAM plant results in a positive growth response (Bethlenfalvay and Schüepp 1994).

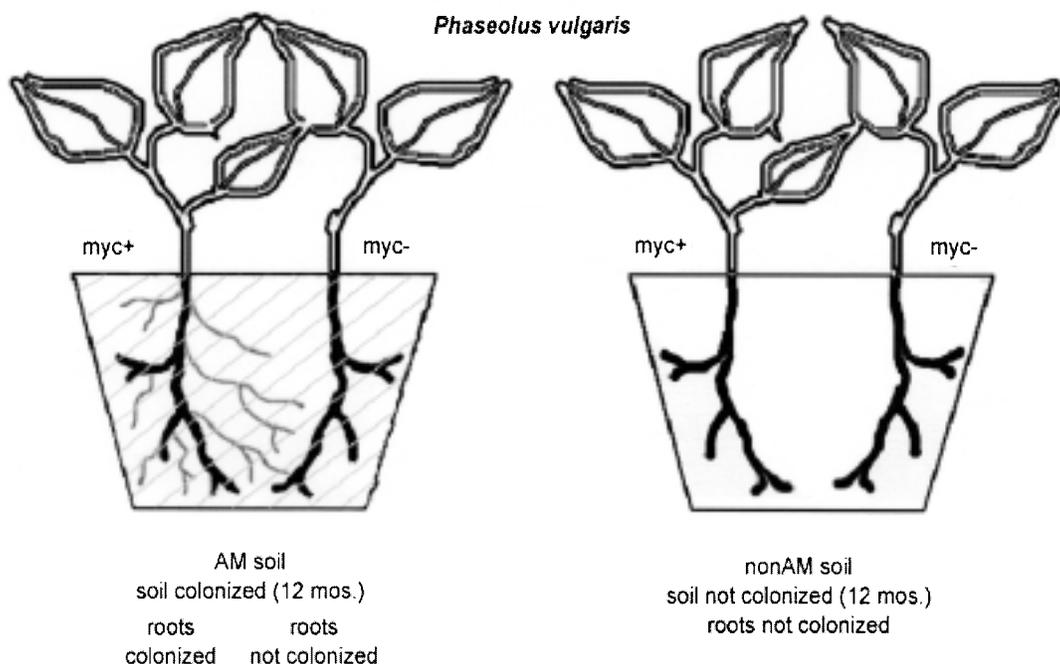
We examined the water retention properties of a Sequatchie fine sandy loam subjected to three treatments: 7 mo of root growth by (1) nonAM *Vigna unguiculata* (L.) Walp. given low phosphorus fertilization, (2) nonAM *V. unguiculata* given high phosphorus fertilization, (3) *V. unguiculata* colonized by *Glomus intraradices* Schenck and Smith and given low phosphorus fertilization (Augé et al. 2001). The AM soil had significantly more water stable aggregates and substantially higher extraradical hyphal densities than the nonAM soils. Importantly, in this study we were able to factor out the possibly confounding influence of differential root growth among AM and nonAM plants: root mass, root length, root surface area and root volume densities were each similar in AM and the two nonAM soils.



**Fig. 2.** Broken line regression analysis of soil moisture release plots, showing slight effect of AM colonization of soil on the  $\Psi_m/\theta$  relationship (from Augé et al. 2001). Plots were constructed for whole, undisturbed soils in pots using heat dissipation sensors to measure  $\Psi_m$ . AM = soils colonized by *Glomus intraradices*, nonAM = average of two nonmycorrhizal soils developed by plants fed low and high rates of phosphorus; the two nonAM soils were statistically similar and so plotted together.

The mycorrhizal soil lost more water before its matric potential ( $\Psi_m$ ) began to decline, indicated by the lower x intercept of the “wet line” and “dry line” portions of a soil moisture characteristic evaluated as broken line regression (Fig. 2). To compare soils at another biologically meaningful reference point, we calculated soil  $\theta$  at  $-1.5$  MPa [a number often cited as a typical soil  $\Psi$  at the permanent wilting point, e.g., Salisbury and Ross (1992)]. AM soils had a tendency ( $P = 0.06$ ) to dry more (to give up a larger percentage of their water) before reaching  $-1.5$  MPa. The slopes of the dry line portion of the curve also varied in AM and nonAM soils. Once soils lost sufficient water to cause  $\Psi_m$  to begin to decline, nonAM soils dried more (showed larger changes in  $\theta$ ) per unit change in  $\Psi_m$ . Insofar as soil  $\Psi_m$  determines water availability to roots, this suggests that a larger percentage of water would be available to roots at relatively high  $\Psi_m$  in the AM soil than in the nonAM soils, but that the nonAM soils gave up more water per unit decline in  $\Psi_m$  at very low  $\Psi_m$ .

Is it likely that this slight change in the soil moisture characteristic would have meaningful biological consequences? There was very little variation in the moisture characteristic among replicates of the control soils in this experiment, and it could be argued that any change in the moisture characteristic could conceivably have an impact on plants enduring a drought episode in that soil. But it is difficult to imagine an effect associated strictly with water availability. We calculated that the slight difference in  $\theta$  between the AM and the two nonAM soils would account for only a few extra hours of transpiration, at rates only a fraction of those typical for unstressed leaves. Alternately, if this extra water were absorbed but retained by the plant — the host in this



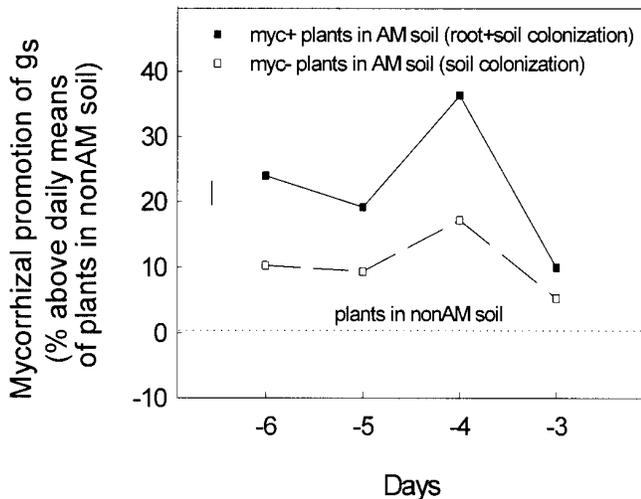
**Fig. 3.** Diagram illustrating experimental design. myc- and myc+ refer to non-mycorrhizing mutant and wildtype bean, respectively. One plant of each genotype was grown in each pot, in either AM soil (pot on left, *Gi* or *Gm* soil, extraradical AM hyphae in soil depicted as cross-hatching) or nonAM soil (pot on right, no cross-hatching). Colonization of roots by AM fungi, portrayed by thinner, dark lines in soil on lefthand pot, occurred only in myc+ plants in AM soils. myc+ plant in AM soil showed effect of root + soil colonization on plant behavior, relative to plants in nonAM soil (no colonization of roots or soil). myc- plant in AM soil showed effect of soil colonization alone on plant behavior, relative to plants in nonAM soil.

experiment was an extreme drought avoider, very proficient at retaining moisture — perhaps reaching the lethal  $\Psi$  during a drought episode would have been postponed somewhat, depending on plant size.

Even though slight, these preliminary findings are provocative. Their importance lies in the fact that a small but significant mycorrhizal effect on water retention properties was evident after only 7 mo of mycorrhizal colonization of soil and with similar root densities as nonAM soils. We postulated that mycorrhizal influences are apt to become more substantial over longer time periods and repeated the experiment, developing mycorrhizal soils for 12 mo (Augé et al. 2004b). As mycorrhizal fungal species can differ in their production of glomalin and extraradical hyphae, we added a second AM treatment, colonizing soil with *Gigaspora margarita* Gerdemann & Trappe as well as with *G. intraradices*. Despite the longer time allowed for hyphae to ramify and affect the soil, the moisture release plots were mostly similar among the two AM soils and the nonAM soil. The AM and nonAM soils did not differ in  $\theta$  near  $-1.5$  MPa, nor did the soils differ in  $\theta$  at  $-0.2$  MPa, a moderate stage of soil drought. AM soils did lose slightly but significantly more water than nonAM soil to reach the threshold/break-point at which soil  $\Psi_m$  first began to decline as soil dried. This stage of soil dryness coincided with the point of stomatal closure for the plants in this experiment (which was extended into drier soil for AM than for nonAM soils).

### PARTITIONING AM INFLUENCE INTO SOIL VS. ROOT COLONIZATION

We examined the possibility that mycorrhizal soil may directly influence plant water relations. Using wild type plants (myc+) and non-colonizing mutants (myc-) of *Phaseolus vulgaris* L. planted into previously produced mycorrhizal and nonmycorrhizal soils, we partitioned mycorrhizal influence on  $g_s$  and drought resistance into soil and root components, testing whether effects of mycorrhizal fungi occurred mostly via plant colonization, soil colonization, or both (Augé et al. 2004b). Using myc- mutants allowed the influence of mycorrhizal colonization of roots to be uncoupled from mycorrhizal colonization of the soil in which plant roots were growing. A loam/sand soil mixture was “mycorrhized” for 1 yr with either *G. intraradices* or *Gi. margarita* growing in association with roots of *Sorghum bicolor* (L.) Moench. Control, nonAM soils contained comparable root mass, but roots were not colonized by AM fungi. After removing sorghum shoots and keeping root and hyphal systems intact, pots were seeded with *P. vulgaris*. Each pot, containing either *G. intraradices*, *Gi. margarita* or nonAM soil, contained one myc- and one myc+ plant (Fig. 3). Myc+ plants in AM soil showed the effects of both soil and plants being colonized by AM fungi. Myc- plants in AM soil showed the effects of colonization of soil alone. Plants growing in nonAM soil remained unaffected by root or soil colonization by AM fungi.



**Fig. 4.** Mycorrhizal promotion of  $g_s$ , showing AM influence partitioned into soil and root components using wild type (myc+) and non-colonizing mutant (myc-) bean plants [see Augé et al. (2004b) for summary of experiment]. Mycorrhizal promotion of  $g_s$  was calculated as a percent for each bean genotype, relative to comparably sized nonAM plants of that genotype: (absolute  $g_s$  of plants in AM soils – mean  $g_s$  of nonAM plants)/mean  $g_s$  of nonAM plants  $\times$  100. A value of 0% promotion denotes that daily average  $g_s$  of AM plants was similar to that of nonAM plants; nonAM plants for each genotype are depicted by the dotted line. Plants in *G. intraradices* (*Gi*) soils and *Gi. margarita* (*Gm*) soils showed similar behavior, and so *Gi* and *Gm* were plotted together for each genotype. Symbols represent means of 24 replicates for each genotype, three leaves per replicate ( $n = 72$ ). Vertical line represents pooled standard error of the means. Each of the following linear contrasts was significant ( $P \leq 0.05$ ) over all 4 d: myc- AM soil vs. myc- nonAM soil (effect of AM soil colonization), myc+ AM soil vs. myc+ nonAM soil (effect of AM soil and root colonization), myc- AM soil vs. myc+ AM soil (effect of AM root colonization).

The myc- mutation itself had no effect on any water relations parameter: stomatal conductance before or during drought; pre-stress osmotic potential; leaf  $\Psi$ , leaf osmotic potential, soil  $\Psi$  or soil  $\theta$  at the lethal point; or osmotic adjustment. Colonization by *Gi. margarita* and *G. intraradices* had appreciable effects on lethal leaf  $\Psi$  and osmotic adjustment, relative to nonAM plants of comparable size (Augé et al. 2004b). Mycorrhizal effects on drought resistance were attributable to an effect on the plant itself rather than to an effect on soil colonization.

Figure 4 portrays the relative size of the AM effect on  $g_s$ . Mycorrhizal promotion of  $g_s$  (see figure legend for explanation) shows how large the mycorrhizal effect was on  $g_s$  over 4 d preceding the start of the drought treatment. A value of 0% promotion denotes that average  $g_s$  of AM plants was similar to that of nonAM plants [see Augé et al. (2004a) for other illustrations of mycorrhizal promotion of  $g_s$ ].

The myc+ line represents the influence of both root and soil colonization by AM fungi on  $g_s$ :  $g_s$  of myc+ plants in *Gi* and *Gm* soils relative to  $g_s$  of myc+ plants in nonAM soil. Myc+ plants in AM soils were exposed to colonization of

both roots and soil.  $g_s$  of these plants is expressed in Fig. 4 in relation to plants of the same genotype grown in nonAM soil, a situation in which both roots and soil were free of AM colonization.

The myc- line represents the influence of soil colonization alone:  $g_s$  of myc- plants in *Gi* and *Gm* soils relative to  $g_s$  of myc- plants in nonAM soil. Myc- plants in AM soils were exposed to soil colonized by AM hyphae but roots of these plants did not colonize.  $g_s$  of these plants is expressed in Fig. 4 in relation to plants of the same genotype grown in nonAM soil, a situation in which not only roots but also soil was free of AM colonization.

The influence of both root and soil colonization on  $g_s$  (solid line, closed symbols) was consistently twice that of soil colonization alone (dotted line, open symbols). This difference was significant across the 4 d ( $P = 0.002$ ). On each day, the average of myc+ plants in AM soil was about twice that of myc- plants in AM soil. Over the 4 d preceding soil drying, promotion of  $g_s$  by AM soil averaged 11%, significantly higher ( $P = 0.03$ ) than  $g_s$  of plants of the same genotype in the nonAM soil (represented by the dotted line). Over the 4 d preceding soil drying, promotion of  $g_s$  by mycorrhization of both roots and soil averaged 22%, significantly higher ( $P < 0.0001$ ) than  $g_s$  of plants of the same genotype in the nonAM soil. The difference between the solid and dotted lines in Fig. 4 represents the influence of root colonization alone.

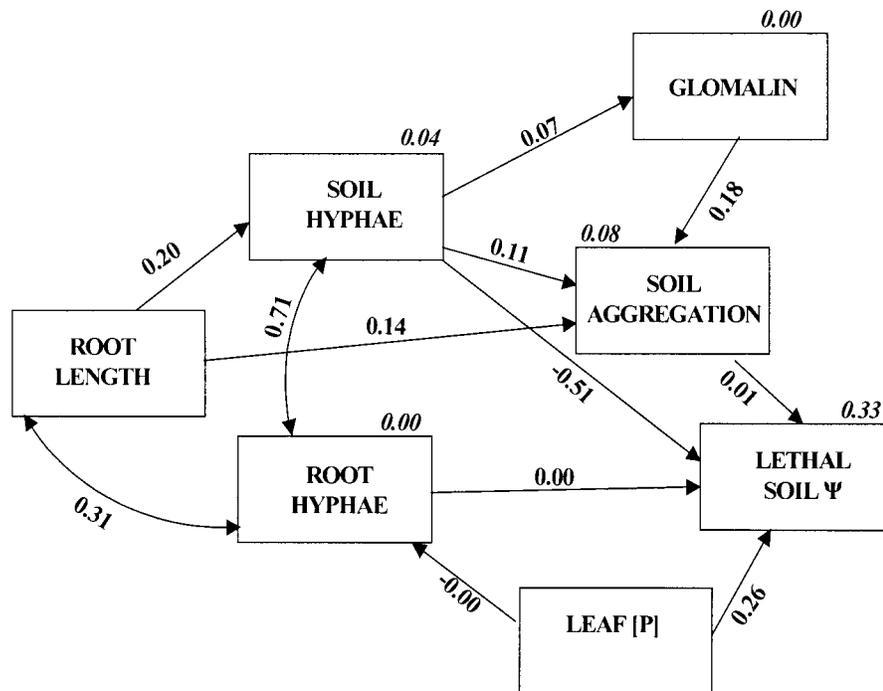
A full explanation of experimental details and findings is presented in Augé et al. (2004b).

About half of the considerable promotion of  $g_s$  by *G. intraradices* and *Gi. margarita* was attributable to soil colonization and about half to plant colonization. Surprisingly, merely growing in mycorrhizal soil resulted in promotion of  $g_s$  of nonmycorrhizal plants, in both amply watered and droughted plants. This is a key finding: mycorrhizal soil affected the physiology of nonmycorrhizal plants growing in that soil. Increased sink strength of AM roots has been suggested as a reason for the often observed mycorrhizal promotion of  $g_s$  (Augé 2000), yet sink strength was not a factor in this instance, as roots remained nonmycorrhizal.

#### TESTING CONTRIBUTIONS OF SOIL AND ROOT FACTORS TO AM EFFECTS

One AM symbiotic characteristic that has rarely been examined in relation to water relations but that could affect host plant behaviors during drought is extent of hyphal development in the soil. External (or “extraradical”) soil hyphae often comprise a large portion of AM fungal biomass (Miller and Jastrow 1994). Soil hyphae could conceivably impact plant water balance in drying soils by contributing to root water absorption (Faber et al. 1991; Ruiz-Lozano and Azcón 1995), by improving contact between roots and soil particles (Reid 1979), or by altering the soil moisture characteristic as described above. Davies et al. (1992) observed that development of extraradical hyphae in AM soils was associated with greater drought resistance of plants growing in those soils.

We tested the hypothesis that extent of colonization of soil by AM fungal hyphae would be significantly correlated



**Fig. 5.** Path model depicting the hypothesized causal relationships among independent and dependent variables, for lethal soil  $\Psi$  (from Augé et al. 2003a). Each single-headed arrow signifies a direct causal relationship in the direction of the arrow. Double-headed arrows indicate a correlation between two variables. Indirect causal effects occur if one variable is linked to another via other, intermediate variables. Numbers on arrows are path coefficients (standardized partial regression coefficients derived from the regression of each response variable on those variables directly linked to it) indicating the relative strength of each path leading to a given response variable. Italicized numbers above variable boxes are estimates of the proportion of total variance explained (squared multiple correlations) for each dependent variable. The model fit was significant (PGFI = 0.37,  $\chi^2 = 5.56$ ; df = 8;  $P = 0.70$ ). Redrawn with permission from Journal of Plant Physiology.

with measures of drought resistance in mycorrhizal plants (Augé et al. 2003). Physiological drought resistance of *P. vulgaris* was characterized by a plant-based measure and a soil-based measure of foliar response to drought: the leaf  $\Psi$  and the soil  $\Psi$  at which foliage died during a sustained drought (lethal  $\Psi$ ). We also tested the hypothesis that drought resistance of mycorrhizal plants would be better correlated with soil colonization than with root colonization. Effects of AM fungi on shoot behaviors in general have often not been closely linked to extent of AM colonization of roots (Fitter and Merryweather 1992; Smith and Read 1997), but such correlations have rarely been tested for host water relations responses. Additionally, we tested correlations of lethal  $\Psi$  with some other soil and plant characteristics that can be influenced by AM symbiosis and that may impact water relations. Plants were colonized by either *G. intraradices* or by a mix of AM fungi collected from a semi-arid grassland.

We used path analysis modeling to evaluate how colonization rates and other variables affected the lethal values. Of several plant and soil characteristics, variation in dehydration tolerance was best explained by soil hyphal density. Soil hyphal colonization had larger direct and total effects on both lethal soil  $\Psi$  (Fig. 5) and leaf  $\Psi$  (path diagram not shown) than did root hyphal colonization, root density, soil

aggregation, soil glomalin concentration, leaf phosphorus concentration or leaf osmotic potential. The findings support the assertion that external, soil hyphae may play an important role in mycorrhizal influence on the water relations of host plants. Bethlenfalvai and colleagues had suggested earlier that, theoretically, colonization of soil by hyphae might be expected to have as great (or greater) influence on host behavior during drought as colonization of roots (e.g., Bethlenfalvai and Linderman 1992). Others recently reported that, among six AM species tested, effectiveness in enhancing plant water uptake from soil appeared related to the amount of external mycelium produced by each AM fungus as well as to the frequency of root colonization in terms of live and active fungal structures (Marulanda et al. 2003).

The fact that ability to survive to lower soil hydration was associated with more soil hyphae in the bean study (Augé et al. 2003) implies that soil hyphae may somehow aid root systems in more thoroughly extracting water from drying soils. Others have suggested that, at similar bulk soil  $\Psi$  or bulk  $\theta$  in AM and nonAM soils, soil  $\Psi$  might actually be slightly higher in the rhizosphere of AM plants, if mycorrhizae more effectively ramify and dry out a particular volume of soil than do nonAM roots (Hardie and Leyton 1981; Gupta 1991; Duan et al. 1996). As noted, on several occa-

sions AM plants have been observed to deplete soil water more thoroughly than nonAM plants before achieving a similar shoot response. AM legumes developed lower soil  $\Psi$  before wilting (Hardie and Leyton 1981) or at the permanent wilting point (Bethlenfalvay et al. 1988a, b), relative to nonAM plants. AM symbiosis decreased lethal soil  $\Psi$  of *Artemisia tridentata* seedlings (Stahl et al. 1998). Soil  $\Psi$  at stomatal closure was 0.3 to 0.6 MPa lower in AM roses than in similarly-sized nonAM roses (Augé et al. 1986). AM sorghum was also able to maintain leaf  $\Psi$  to lower soil  $\Psi$  than similarly-sized nonAM plants (Osonubi 1994).

How might external, extraradical AM mycelia increase the efficacy of root water absorption in dry soil? In addition to the possibility of direct hyphal contributions to root water uptake (Hardie 1985; Faber et al. 1991; Ruiz-Lozano and Azcón 1995), soil hyphae may increase soil-to-root contact in drying soils (e.g., Reid 1979; Fitter 1985; Davies et al. 1992). Root and soil shrinkage creates gaps between the root and the soil, which can decrease water absorption (e.g., Nobel and Cui 1992). Root hairs can help prevent air gaps at the soil-root interface, as they grow into very small pores and effectively “glue” themselves to soil particles with exuded mucilages (Klepper 1990). AM soil hyphae might serve this same function, perhaps even more effectively than root hairs, because most hyphae can enter finer pores than can root hairs (Tisdall 1991). Better soil-root contact could translate into higher soil-to-root hydraulic conductance, and there is evidence that AM symbiosis can change soil-to-root hydraulic conductance (Allen et al. 1981, 1982; Bildusas et al. 1986).

### SUMMARY

Mycorrhizal effects on plant behavior may be at least partially mediated through the soil, regardless of whether host plants themselves actively associate with the fungi:

- Among a suite of plant and soil characters known to be affected by AM colonization and known or suspected to affect plant water relations, quantity of hyphae in soil had the strongest path to drought resistance of bean plants.
- Merely growing in a mycorrhizal soil had a promotive impact on stomatal conductance of nonmycorrhizal beans.
- Mycorrhization of soil for 7 and 12 mo induced a slight change in the soil's moisture retention properties.

Influences of AM symbiosis on plant water relations are often subtle and transient. Shifting our perspective to examine mycorrhizal soil as an experimental treatment, in addition to mycorrhizal roots, may improve our ability to predict mycorrhizal effects.

### ACKNOWLEDGMENTS

The author gratefully acknowledges the collaboration and support of the following investigators: Jenny Moore, Ann Stodola, Dr. Arnold Saxton, Dr. David Sylvia, Dr. Jean Stutz, Dr. Soon Park, Dr. Brian Buttery, Heather Toler. Much of the work cited above was supported by the US Department of Agriculture under Award No. 00-35100-9238 and by the Tennessee Agricultural Experiment Station.

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