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Relating foliar dehydration tolerance of mycorrhizal *Phaseolus vulgaris* to soil and root colonization by hyphae

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Summary

Mycorrhizal symbiosis can modify plant response to drying soil, but little is known about the relative contribution of soil vs. root hyphal colonization to drought resistance of mycorrhizal plants. Foliar dehydration tolerance, characterized as leaf and soil water potential at the end of a lethal drying episode, was measured in bean plants (*Phaseolus vulgaris*) colonized by *Glomus intraradices* or by a mix of arbuscular mycorrhizal fungi collected from a semi-arid grassland. Path analysis modeling was used to evaluate how colonization rates and other variables affected these lethal values. Of several plant and soil characteristics tested, variation in dehydration tolerance was best explained by soil hyphal density. Soil hyphal colonization had larger direct and total effects on both lethal leaf water potential and soil water potential than did root hyphal colonization, root density, soil aggregation, soil glomalin concentration, leaf phosphorus concentration or leaf osmotic potential. Plants colonized by the semi-arid mix of mycorrhizal fungi had lower lethal leaf water potential and soil water potential than plants colonized by *G. intraradices*. Our findings support the assertion that external, soil hyphae may play an important role in mycorrhizal influence on the water relations of host plants.

Key words: arbuscular mycorrhizal symbiosis – drought resistance – lethal water potential – path analysis – *Phaseolus vulgaris* (bean) – root colonization – soil hyphal density

Abbreviations: AM = arbuscular mycorrhizal. – AZ = species assemblage of AM fungi from a semi-arid grassland in Arizona. – GI = *Glomus intraradices*. – Ψ_π^{100} = osmotic potential at full turgor. – PGFI = Parsimonious Goodness of Fit Index. – [P] = phosphorus concentration. – Ψ = water potential. – WSA = water-stable aggregation

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Introduction

Arbuscular mycorrhizal (AM) symbiosis can modify water relations and drought responses of host plants (Augé 2001). Reports are numerous, and almost all have involved comparisons between AM and nonAM plants. Fitter (1996) noted that investigators in mycorrhizal experiments place undue emphasis on nonAM controls as such plants are anomalous in nature, and it would be profitable to expand the focus of investigation beyond simple comparisons of nonAM vs. AM plants and ask other questions.

One AM symbiotic characteristic that has rarely been examined in relation to water relations but that may in theory affect host plant behavior during drought is extent of hyphal development in the soil. External (or "extraradical") soil hyphae can comprise a sizeable portion of AM fungal biomass (Miller and Jastrow 1994). Soil hyphae could conceivably impact host water balance in drying soils by contributing to root water absorption (Faber et al. 1991, Ruiz-Lozano and Azcón 1995) or by improving contact between roots and soil particles (Reid 1979). Alternately, AM soil hyphae, through their effects on soil aggregation and structure (Tisdall 1991, Jastrow et al. 1998), could conceivably modify soil water characteristics (Hamblin 1985). Colonization of soil by AM fungi has affected moisture retention and drainage properties, relative to nonAM soils (Augé et al. 2001 a, Bearden 2001). Davies et al. (1992) observed that extraradical hyphal development in AM soils was associated with greater drought resistance of plants growing in those soils.

Our principal objective was to test the hypothesis that extent of colonization of soil by AM fungal hyphae would be significantly correlated with measures of drought resistance in mycorrhizal plants. Physiological drought resistance was characterized by a plant-based measure and a soil-based measure of foliar response to drought: the leaf water potential (Ψ) and the soil Ψ at which foliage died during a sustained drought (lethal Ψ). 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 Ψ with some other soil and plant characteristics that can be influenced by AM symbiosis and that may impact water relations, such as soil aggregation, soil glomalin concentration, rooting density and leaf phosphorus concentration [P]. The overall goal of this and related experiments is to improve ability to anticipate how and when AM symbiosis will affect host water balance.

Materials and Methods

Plant materials and culture

Tests were conducted on *Phaseolus vulgaris* L. (bush bean, common bean), an important agronomic crop with considerable drought avoidance capability (Augé et al. 2001 b). In an effort to broaden the range of soil and root hyphal colonization among individuals and hence increase the generality and power of the modeling analysis, we produced an experimental system using two AM inoculum types and varied inoculation rates.

One inoculum (GI) was composed of *Glomus intraradices* Schenck & Smith isolate UT143 (INVAM, West Virginia University, Morgantown, WV, USA), previously shown to affect host and soil water balance (e.g. Augé et al. 1986, Duan et al. 1996, Augé et al. 2001 a). The GI inoculum consisted of pot culture, soil plus roots, of 4-month-old sorghum plants grown on the experimental Sequatchie/sand potting medium described below. A second inoculum (AZ) was a mixture of AM fungal species collected from a semi-arid riparian grassland along the Babocomari River at Lyle in southern Arizona, USA. The AZ inoculum consisted of pot culture, soil plus roots, started with soil collected from the rhizosphere of *Sporobolus wrightii* (big sacaton grass). Soil was mixed (2/1/1) with # 12 and # 20 steam-pasteurized silica sand, seeded with *Sorghum sudanese* (sudan grass) and grown for 4 months in a greenhouse. Species composition of the AZ inoculum, determined by spore extraction (Daniels and Skipper 1982), was as follows: *Glomus eburneum* Kennedy, Stutz & Morton, approximately 50 % of the spores; *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, approximately 25 %; other species in decreasing order of abundance, *Glomus spurcum* Pfeiffer, Walker & Bloss, *Glomus intraradices*, an undescribed *Glomus* species (AZ123), *Acaulospora delicata* Walker, Pfeiffer & Bloss, *Glomus microaggregatum* Koske, Gemma & Olexia and *Acaulospora rehmii* Sieverding & Toro. *G. intraradices* allocates substantial carbon into intraradical vesicles and might be expected to produce relatively less extraradical hyphae, whereas species such as *G. eburneum* (which does not form vesicles, Kennedy et al. 1999) and *G. spurcum* tend to allocate more carbon into extraradical hyphae.

Eighty plants of *P. vulgaris* cv. Blue Lake 274 were grown from seed in 2.8 L plastic pots, in an autoclaved soil/sand potting medium (v/v/v): 18 parts soil (Sequatchie, fine-loamy, siliceous, thermic Humic Hapudults, sieved at 4 mm before mixing)/35 parts silica sand (medium-to-coarse, mined, sieved)/1 part limestone sand. The limestone sand was used to adjust pH to 7.5. Forty plants were inoculated with GI and 40 plants with the AZ mix. At planting, 2.0 L of autoclaved medium (2× in 24 h at 121 °C, 30 min each time) was placed into each pot. AZ or GI inoculum was then placed into pots at rates of 25, 50, 75, 100 or 125 mL per pot (8 pots of each rate for each inoculum), mixed with an additional 200 mL of fresh, autoclaved medium. A 2–3 cm layer of sterile medium was placed at the top of the pots to retard cross-contamination.

With each watering, all plants received a liquid macro- and micronutrient fertilizer at 10.7 mmol/L N and 3.2 mmol/L K (Champion 15N-0P-15K Alkaline Plus, Chilean Nitrate Co., Norfolk, VA, USA). All plants received 0.8 mmol/L P weekly as KH_2PO_4. The experiment was conducted under ambient light in a glasshouse in Knoxville, TN, USA, and plants were adequately watered until the drying treatment began. Glasshouse temperatures, measured each s during the experiment using shaded thermocouples placed near the canopy, averaged 22.7 ± 0.1 during the day and 19.4 ± 0.2 °C during the night. PPFD, measured each s during the drying period with an unshaded quantum sen-

sor (LiCor, Lincoln, NE, USA) averaged 131 ± 9.6 mol m^{-2} d^{-1}, with daily maxima (integrated hourly) averaging 314 ± 34 μmol m^{-2} s^{-1}.

Drought treatment and water relations measurements

Twelve weeks after germination, before soil volume was fully ramified by roots, 5 plants of each inoculation rate and each AM treatment (50 plants total) were watered to field capacity and then subjected to a continuous soil drying episode by withholding water from pots. On the last day plants were watered (subsequently referred to as day 0), one recently expanded, unshaded leaf from each plant was collected between 0830 and 1030 h EST for measurement of initial, pre-drought osmotic potential at full turgor (Ψ_π^{100}) using vapor pressure osmometry as described before (Augé et al. 2001 b).

Foliar dehydration tolerance has been operationally defined as the Ψ (called the lethal Ψ) of the last surviving leaves on a plant subjected to a slow, continuous soil drying episode (Ludlow 1989). Each plant was checked daily after beginning the drying episode and lethal measurements began when fewer than five live leaves with minimal necrotic areas (less than 25 % of total leaf area) remained. Tests were conducted in an earlier experiment to determine the visible signs of the lethal drought point (Augé et al. 2001 b). Ψ of two leaves was measured for each plant at the lethal point using thermocouple psychrometers (TruPsi, Decagon Devices, Inc., Pullman, WA, USA) calibrated daily with a graded series of NaCl solutions. Measurements were made on strips cut from leaflet laminae adjacent and parallel to mid-veins and placed inside the psychrometer chamber with abaxial sides exposed to the center of sample cups. Preliminary tests indicated that leaf samples of each species generally reached thermal and water vapor equilibrium in the psychrometer chamber within 3–4 h; all samples were allowed to equilibrate for 4 h before measurements were made. Sampling was performed between 0830 and 1000 h EST.

Immediately after loading psychrometers with lethal Ψ samples, one leaflet from each plant at the lethal point was collected for lethal leaf Ψ_π^{100}. Soil Ψ at the lethal point was characterized using a a dew-point potentiameter (WP4, Decagon Devices, Pullman, WA, USA), on two ~2.5 g samples excavated from near the center of each pot at the time of sampling for lethal leaf Ψ.

Other plant and soil measurements

At the start of the drought episode, [P] of one of the most recently expanded leaflets of plants to be droughted was 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). The remaining three replicates of each treatment were sacrificed at this time for measurement of shoot dry weight.

On the last day of the drought episode for each plant (the lethal point), soil was removed from the pot, placed in a tray and gently but thoroughly mixed. Two subsamples of the homogenous soil mass were sealed immediately in plastic bags and refrigerated, for several root and soil measurements. The remainder of the soil mass was allowed to air dry completely on the greenhouse bench, for measurement of aggregation. Hyphal, arbuscular and vesicular colonization of roots was determined on each of the dried plants, on one grid intersection on each of 100 ~ 0.5-cm root pieces from each plant, after clearing with 10 % KOH in an autoclave at 121 °C for 15 min, staining with trypan blue for 1 h, and destaining.

Soil hyphal density was measured based on the protocols of Bethlenfalvay and Ames (1987) and Miller et al. (1995). After thoroughly mixing soil from each pot, a 10 g sample was removed and suspended in a glycerol/HCl destaining solution. The suspension was sieved (45 μm) and remaining material brought to 200 mL with distilled water. After stirring slowly, 10 mL of suspension was placed on a membrane filter (GN6, 0.45 μm, grid line interval 3 mm, Gelman Sci., Ann Arbor MI, USA) which was sprayed first with 10 % ethanol and attached to a vacuum apparatus. After vacuuming, the filter was covered with a trypan blue staining solution for 10 min. After rinsing stain, hyphae were resuspended in water to ensure a homegenous distribution on the membrane. Water was filtered off and the membrane placed in a covered foil weighing pan with ~ 1 mL of water (to prevent dessication of hyphae). Hyphal segments were quantified using a dissecting microscope and hyphal density calculated as described by Ambler and Young (1977). Gravimetric measurements of soil water content were made at the same time that soil was sampled, so that soil hyphal density could be computed on a soil dry weight basis. Hyphal density of the fresh soil : sand medium before inoculation and plant growth averaged 12 cm g^{-1} dry soil.

Roots were carefully excavated from another 25 g of soil of each replicate, for measurement of root length, using scanning equipment and imaging software (WinRhizo, Regent Instruments Inc., Quebec City, Canada).

Water-stable aggregation (WSA) of air-dried soil was determined as described before (Schreiner and Bethlenfalvay 1997, Augé et al. 2001 a). A 200 mL sample of soil was 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 mm, 1 mm, 0.5 mm, 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 oven-dried water-stable fraction by the original sample weight.

Easily extractable glomalin was obtained and quantified according to the procedure of Wright and Upadhyaya (1999). Well-mixed, 0.25-g soil samples were placed in 18-mm flat bottom glass vials and 2 mL of 20 mmol/L 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.

Hypotheses, experimental design and statistical analysis

We tested two hypotheses: (1) lethal leaf or lethal soil Ψ of mycorrhizal plants will be significantly correlated with quantity of AM hyphae in soil, and (2) lethal leaf or lethal soil Ψ of mycorrhizal plants will be better correlated with quantity of hyphae in soil than with quantity of hyphae in roots.

The experiment was arranged as a 2 × 5 factorial, completely randomized design, with two fungal and five inoculation rate treatments. There were five replicates of each treatment combination (total of 50 plants) for the lethal data and an additional three replicates of each treatment combination (total of 30 plants) for the plant size data. For leaf and soil Ψ, individual replicates were sub-sampled twice. Univar-

iate analyses of variance with Fisher's LSD were run on all data to partition the variance into main effects and interactions. Correlations among water relations parameters and the root and soil characters were tested by computing Pearson correlation coefficients.

Path analysis was used to test relationships among variables hypothesized to be involved in determining lethal soil and leaf Ψ. Although correlation and regression analyses were also conducted, the advantages of path analysis are in examining relationships among explanatory variables and allowing intermediate dependent variables to act as explanatory variables for later steps in the hypothesized path (e.g. Mitchell 1992, Jastrow et al. 1998, Rillig et al. 2002). Information provided by path analysis measures the ability to explain the ultimate variables of interest, such as lethal soil and leaf Ψ. In addition, direct and indirect paths connecting all variables in the model provide a basis for deeper understanding of the entire system. Path modeling techniques do not allow determination or testing of causality among variables. Rather, *a priori* knowledge of the system or theoretical reasoning are used to construct a conceptual model (path diagram) of the causal and noncausal relationships among the measured variables. These data are then used to evaluate the model (Jastrow et al. 1998).

Separate path diagrams for lethal soil and leaf Ψ were initially proposed based on previous knowledge. Variables involved were checked for normality and path analysis run. Preliminary analyses resulted in minor modifications to the path diagrams. Parsimonious Goodness of Fit Index (PGFI, Mulaik et al. 1989) and chi-square (χ^2) were used to assess model fit, and residuals were examined.

Path coefficients, indirect effects, standard errors and coefficients of determination were calculated with Proc Calis in SAS (SAS Institute Inc. 1993).

Results and Discussion

Drought resistance of *Phaseolus vulgaris*

Drought resistance, whether based on avoidance or tolerance mechanisms (Levitt 1980), can be characterized by how much soil drying a plant can withstand before its foliage dies: soil Ψ at the lethal point. *P. vulgaris* is a relatively drought resistant crop, as evidenced by low lethal soil Ψ (Augé et al. 2001 b and current study, Table 1). Plants achieve this resistance via extreme drought avoidance, as foliage is relatively intolerant of dehydration (very high lethal leaf Ψ, Ludlow 1989). Drought avoidance, the ability of plants to postpone or avoid foliar dehydration as soil dries, can be quantified as the difference between lethal leaf Ψ and lethal soil Ψ (Augé et al. 2001 b). *P. vulgaris* exhibited considerable avoidance in this experiment, with leaf Ψ – soil Ψ of about 4 to 5 MPa (Table 1). This is consistent with that previously observed for *P. vulgaris* and exceeds that previously shown by cowpea (about 3.5 MPa), soybean (1 to 1.5 MPa) and *Ocimum basilicum* (1 MPa or less) (Augé et al. 2001 a, b). We measured initial and final Ψ_π^{100} to evaluate osmotic adjustment using path analysis, in the event adjustment occurred. As is typical of many drought avoider plants (Ludlow 1989), *P. vulgaris* did not osmotically adjust during the lethal drought episode (Table 1).

Species comparisons

AZ plants survived to about 1 MPa lower soil Ψ than GI plants (Table 1), indicating that they conferred greater overall drought resistance to plants. The AZ fungi also affected foliar dehydration tolerance, with leaves of AZ plants surviving to Ψ about 0.3 MPa lower than those that killed leaves of GI plants. In addition to greater drought resistance and dehydration tolerance, AZ plants also showed greater ability to avoid drought: leaf Ψ – soil Ψ at the lethal point was about 0.7 MPa larger in AZ than in GI plants (Table 1). Length of the lethal drying episode did not differ between the two AM treatments, nor did Ψ_π^{100} before drought or at the lethal point.

These findings are consistent with the idea that AM fungi from semi-arid conditions may promote greater drought resistance in hosts than fungi reared in moister climes. Like the

Table 1. Water relations variables: ranges and means. Within rows, means followed by *, ** or *** indicate that GI and AZ treatments differed significantly at $P = \leq 0.05$, 0.01 or 0.001, respectively (Fisher's LSD). Variables were averaged across inoculation rates, $n = 25$. Day 0 refers to last day plants were watered.

	Glomus intraradices (GI)	Arizona mix (AZ)
Lethal leaf Ψ (MPa)	−1.44 to −2.65	−1.64 to −3.13
	−1.95**	−2.22**
Lethal soil Ψ (MPa)	−4.21 to −7.95	−5.77 to −8.71
	−6.08***	−7.01***
Leaf Ψ – soil Ψ at lethal point (MPa)	2.76 to 6.09	3.84 to 6.62
	4.13**	4.82**
Length of lethal drought episode (d)	13 to 21	12 to 21
	17.7	18.1
Full-turgor leaf Ψ_π on day 0 (MPa)	−0.69 to −1.09	−0.61 to −1.33
	−0.89	−0.90
Full-turgor Ψ_π at lethal point (MPa)	−0.42 to −1.46	−0.45 to −1.27
	−0.75	−0.83

Table 2. Plant and soil variables: ranges and means. Within rows, means followed by *, ** or *** indicate that GI and AZ treatments differed significantly at $P = \leq 0.05$, 0.01 or 0.001, respectively (Fisher's LSD). Variables were averaged across inoculation rates, $n = 25$ ($n = 15$ for shoot dry weight, which was determined for 3 additional, undried replicates of each of the five inoculation rates at the start of the drought episode). WSA was determined for the 0.25–0.5, 0.5–1.0, and 1.0–2.0 mm size classes and presented here in total (0.25 to 2.0 mm).

	Glomus intraradices (GI)	Arizona mix (AZ)
Shoot dry weight (g)	15.7	14.4
Leaf [P] (mg g^{-1})	2.09 to 4.58	2.10 to 3.85
	2.90	2.95
Hyphal root colonization (%)	23 to 57	32 to 82
	38***	66***
Arbuscular root colonization (%)	0 to 8.0	0 to 8.0
	3.2	2.4
Vesicular root colonization (%)	36 to 70	3 to 34
	57***	15***
Soil hyphal density (cm g^{-1} dry soil)	90 to 230	420 to 1011
	152***	667***
Root length density (cm g^{-1} dry soil)	2.2 to 34.3	10.6 to 38.5
	14.4***	19.8***
Soil hypha/root length ratio (cm cm^{-1})	4 to 52	13 to 91
	12.5***	37.8***
Water-stable aggregation (mg g^{-1} soil DW)	63 to 72	57 to 77
	66.6	68.0
Soil [glomalin] (mg g^{-1})	1.0 to 1.7	1.0 to 1.9
	1.39	1.43

AZ fungi, *G. intraradices* isolate UT143 was originally isolated from a semi-arid location (personal communication, Dr. Joe Morton). Our GI isolate, however, has been cultured for several years under mesic greenhouse conditions. Alternately, AZ plants may have had greater physiological drought resistance than GI plants because there were several species of fungi in the AZ inoculum; host roots had a greater "choice" of fungal symbionts in the AZ inoculum.

Type of AM inoculant did not affect shoot dry weight or leaf [P], but AZ and GI plants did differ in root and soil colonization (Table 2). Hyphal colonization rates in AZ roots were nearly twice those in GI roots. Arbuscular colonization rates by each fungal inoculant were fairly low. Many more vesicles formed in GI roots than in AZ roots, consistent with the fact that *G. intraradices* sporulates inside roots. Amount of hyphae in soil was also greater in AZ pots, with soil hyphal density over four times higher in AZ than in GI soil. More root length formed in AZ soils, although average root surface area densities were similar in AZ and GI soils, due to larger average diameter of GI roots. Neither water-stable aggregation nor glomalin concentrations differed in AZ and GI soils.

Rate of inoculation had no effect on water relations variables or other plant and soil characteristics.

Path analysis

We used the path analysis modeling approach to compare the effects of soil hyphae and root hyphae on three measures of plant response to severe drought: drought resistance (lethal soil Ψ), dehydration tolerance (lethal leaf Ψ) and drought avoidance (lethal leaf Ψ – lethal soil Ψ). Water relations variables are summarized in Table 1. Our conceptual model for evaluating the contribution of soil and root hyphae to variations in lethal Ψ included several other soil and plant characteristics that are often affected by AM symbiosis and that have been postulated to affect plant or soil water relations in a direct or indirect way (Figs. 1 and 2). In the path diagrams, a straight single-headed arrow indicates a direct causal path. Indirect causal effects are connoted by a variable being linked to a given dependent variable via one or more intermediary variables. The path coefficients (numbers on lines) indicate the relative magnitudes of the direct effects of the independent variables linked to each dependent variable. The total causal effect of one variable on another is the sum of the direct and indirect effects. Proportion of total variance explained by the model was computed for each dependent variable (italicized numbers above boxes).

Drought resistance/lethal soil Ψ. Extent of root colonization by AM hyphae was included in the model because it has previously been correlated with plant response to soil drying (Bethlenfalvay et al. 1988 b). We selected hyphal rather than arbuscular or vesicular colonization rates for the model because amount of intraradical hyphae can be expected to play a more important role in water movement than vesicles, and because arbuscular colonization rates were so low and offered a much narrower range of values than the much higher hyphal colonization rates. Water-stable aggregation was in-

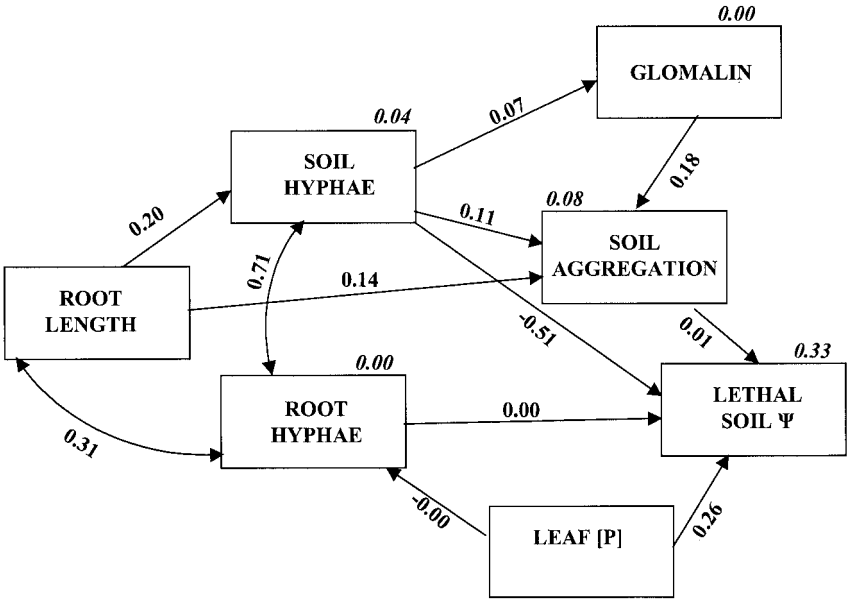

Figure 1. Path model depicting the hypothesized causal relationships among independent and dependent variables, for lethal soil water potential (Ψ). Each single-headed arrow signifies a direct causal relationship in the direction of the arrow. Double-headed arrows indicate a correlation between two variables. 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. Full variable names and their units are given in Tables 1 and 2. The model fit was significant (PGFI = 0.37, $\chi^2 = 5.56$; df = 8; $P = 0.70$).

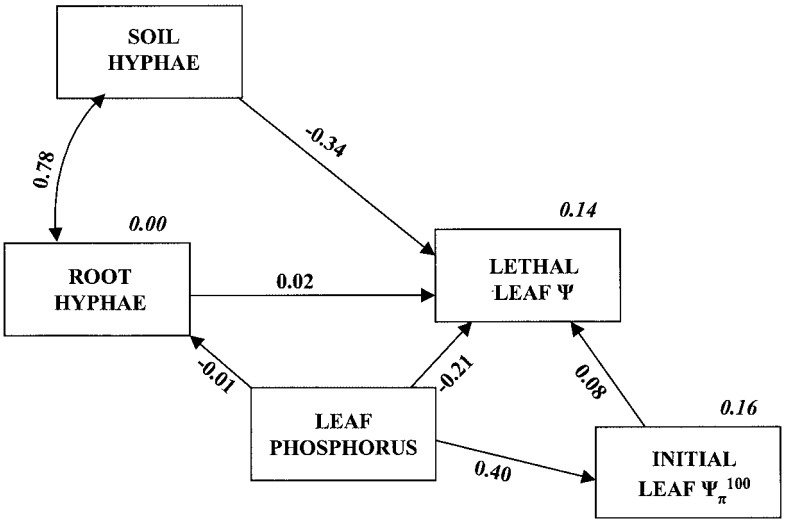

Figure 2. Path model depicting the hypothesized causal relationships among independent and dependent variables, for lethal leaf water potential (Ψ). See Figure 1 legend for explanation of diagram components. The model fit was significant (PGFI = 0.30, $\chi^2 = 0.34$; df = 3; $P = 0.95$).

cluded in the model as a measure of soil structure. Water relations of drying soils, in particular the quantity of water at particular Ψ, is affected by the structure of soil. Well-structured soils contain more available water than poorly structured soils (Greacen and Williams 1983), and reductions in aggregate stability have been correlated with reduced soil water contents at particular soil Ψ (Fahad et al. 1982). Total WSA (0.25 to 2.0 mg g^{-1} soil DW) provided a stronger path to lethal leaf and soil Ψ than did any one of the three size classes and so was used in the path analysis. Several of the variables depicted in the path diagram in Figure 1 can contribute to soil structure. AM and other fungal hyphae grow into the soil matrix to create the skeletal structure that holds primary soil particles together. They create conditions conducive to formation of microaggregates, and they enmesh and stabilize microaggregates and small macroaggregates into macroaggregate structures (Oades and Waters 1991, Tisdall 1991). Soil aggregate stability has also been correlated with root bio-

mass and root length density (Thomas et al. 1986, Jastrow et al. 1998). Glomalin, a protein produced exclusively by AM hyphae and excreted into soil in relatively copious amounts, can have as large or larger effect on soil aggregation as soil hyphae (Rillig et al. 2002). Leaf [P] was included in the model because it is often affected by AM symbiosis, and leaf nutrition could conceivably influence the ability of leaves to withstand dehydration and a steep Ψ gradient against drying soil.

Among all measured variables, soil hyphae had the strongest path (largest direct effect) on lethal soil Ψ (Fig. 1). The effect was negative; more drought resistance (lower lethal soil Ψ) was correlated with more soil hyphae. Water-stable aggregation had no effect on lethal soil Ψ, nor did extent to which roots were colonized by AM hyphae. Interestingly, leaf [P] had a relatively strong positive effect on lethal soil Ψ; ability to survive to lower soil Ψ was correlated with lower leaf [P]. Soil hyphal density and root colonization by hyphae were strongly correlated, and root length and root hyphal colonization were also positively correlated.

Dehydration tolerance/lethal leaf Ψ. The ability of leaves to survive water loss can be affected by their concentrations of osmotically active solutes (Tyree and Jarvis 1982), measured here as Ψ_π^{100} on the day the drought episode began. Dehydration tolerance could also conceivably be affected by leaf [P], as phosphorus nutrition sometimes affects plant water relations (Augé 2001). Both leaf Ψ_π and [P] can be altered by AM symbiosis (e.g. Augé et al. 1986). Ability of leaves to withstand drying might also be affected by other biochemical changes arising from the symbiosis, through hyphal invasion of living root tissues, through transport of substances from live soil hyphae back to the plant, or less directly through alteration of the rhizosphere soil solution. Quantities of hyphae in roots and soils were therefore also included in the lethal leaf Ψ model.

Among the variables depicted in Figure 2, soil hyphae had the strongest path to lethal leaf Ψ, as it did for lethal soil Ψ (Fig. 1). Again, the effect was negative; greater ability to withstand dehydration (lower lethal leaf Ψ) was correlated with more soil hyphae. Leaf [P] also had a substantive direct effect on lethal leaf Ψ, while root hyphal colonization and initial Ψ_π^{100} had little or no direct effect. The direct path coefficient for leaf [P] was negative for lethal leaf Ψ (Fig. 2) but positive for lethal soil Ψ (Fig. 1). This inconsistency in relationship, as well as lack of significance of the Pearson correlation between lethal leaf Ψ and leaf [P] (Table 3), indicates that leaf [P] probably did not exert a meaningful influence on plant response to drought in this experiment.

Drought avoidance/lethal leaf Ψ *– lethal soil* Ψ. The path analysis for lethal leaf Ψ – lethal soil Ψ yielded almost identical path and correlation coefficients as the analysis for lethal soil Ψ. Therefore, the path diagram and its discussion is not reproduced for leaf Ψ – lethal soil Ψ.

All models. Table 3 summarizes correlation analysis among experimental variables. Lethal soil and leaf Ψ were significantly correlated; ability to survive in drier soils was linked with ability to survive more leaf dehydration. Soil hyphal density was the only measured variable having a significant Pearson correlation with both lethal soil Ψ and lethal leaf Ψ. Among all variables, soil hyphal density also had the highest correlation coefficients with both lethal soil Ψ and lethal leaf Ψ, as well as the largest total effects on lethal Ψ (Table 4). The negative correlation in both cases indicates that more hyphae in soil was linked to greater ability to withstand drought and tissue dehydration. Root hyphal colonization was significantly correlated with lethal soil Ψ. Root length density and WSA were each significantly, negatively correlated with lethal leaf Ψ.

There were little or no indirect effects in the path diagrams for the lethal variables that described plant drought response (Table 4). The interpretation is that the various independent variables operated directly on lethal Ψ and not indirectly through other measured variables in the path.

The path models accounted for about one third of the of the variation in lethal soil Ψ and leaf Ψ – lethal soil Ψ, and only 14 % of the variation in lethal leaf Ψ. Due to typically high inherent biological variation in plant water relations characteris-

Table 3. Pearson's product-moment correlations (r) between variables included in the path models (Figs. 1 and 2). *, ** and *** indicate that correlations were significant at $P \leq 0.05$, 0.01 or 0.001, respectively; significant correlations also in bold. Correlation coefficients not followed by asterisk(s) indicate correlation was not significant. + and – signify positive or negative correlation.

	Lethal leaf Ψ	Lethal soil Ψ	Initial leaf Ψ_π^{100}	Leaf [P]	Soil hyphae	Root length	Root hyphae	WSA	Soil [glomalin]
Lethal leaf Ψ	1.00								
Lethal soil Ψ	**0.47*****	1.00							
Initial leaf full-turgor Ψ_π	−0.00	**0.32***	1.00						
Leaf [P]	−0.15	**0.29***	**0.40****	1.00					
Soil hyphal density	**−0.33***	**−0.54*****	−0.05	−0.08	1.00				
Root length density	**−0.30***	0.21	0.04	−0.04	0.27	1.00			
Root hyphal colonization	−0.24	**−0.42****	0.05	−0.07	**0.78*****	**0.34***	1.00		
Water-stable aggregation	**−0.29***	−0.14	0.15	0.16	0.18	0.21	0.17	1.00	
Soil [glomalin]	0.01	0.05	0.08	0.16	0.02	−0.05	0.10	0.14	1.00

Table 4. Decomposition of correlations into direct, indirect and total effects. Direct effects are simple paths and are equal to the path coefficients in Figs. 1 and 2. Indirect effects are the sum of the products of the chain of path coefficients for all compound paths for which the independent variable is connected to the dependent variable while maintaining the causal direction of the arrows. Total effects are the sum of indirect and direct effects. Dashes refer to relationships not created by the path model.

	Direct	Indirect	Total
Effects on lethal soil Ψ			
Soil hyphal density	−0.51	0.00	−0.51
Root hyphal colonization	0.00	–	0.00
Root length density	–	−0.10	−0.10
Leaf [P]	0.26	0.00	0.26
Soil aggregation	0.01	–	0.01
Soil [glomalin]	–	0.00	0.00
Effects on lethal leaf Ψ			
Soil hyphal density	−0.34	–	−0.34
Root hyphal colonization	0.02	–	0.02
Leaf [P]	−0.21	0.03	−0.18
Initial full-turgor Ψ_π	0.08	–	0.08

tics and the limitations in equipment and sample size involved in measuring them, statistical models often do not account for a great deal of variation in variables such as plant or soil Ψ associated with drying and dying plants.

Evaluating a system having roots and soil colonized by different AM fungi had the advantage of broadening the range of values modeled, as lethal Ψ, root hyphae and soil hyphae differed in AZ and GI plants (Tables 1 and 2). This greater range of values improved the strength of the path analysis. It is possible that effects of AZ and GI groups on lethal Ψ differed in ways that were not accounted for by the variables portrayed in Figs. 1 and 2 (variables not associated with the hypotheses being tested). Analysis of a single fungal group accounted for less variation in lethal Ψ than did the combined analysis.

Mycorrhizal colonization and plant water relations

Correlation (or lack thereof) with root colonization has been reported only rarely for water relations variables. We have tested the relationship in several previous studies in our lab and have generally not found a significant correlation between changes in plant water relations variables and extent of root colonization. However, in one of the most compelling reports of an AM-induced change in host drought resistance, soil moisture content at permanent wilting of individual plants was closely inversely correlated with the extent of root colonization (Bethlenfalvay et al. 1988 b).

Based on subsequent examinations of external hyphae, Bethlenfalvay and colleagues suggested that colonization of soil by hyphae may be expected to have as great (or greater) influence on host behavior during drought as colonization of roots (e.g. Bethlenfalvay and Linderman 1992). Several possible plant-based mechanisms for AM effects on host water relations have been explored using similarly-sized AM and nonAM plants, and none have emerged having compelling support as a primary mode of AM influence (Augé 2001). Mycorrhizal effects on soils have not been nearly as well investigated as possible explanations for AM effects on plant water relations.

The fact that ability to survive to lower soil hydration was associated with more soil hyphae in the current study 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 Ψ or bulk water content in AM and nonAM soils, soil Ψ 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). On several occasions, 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 Ψ before wilting (Hardie and Leyton 1981) or at the permanent wilting point (Bethlenfalvay et al. 1988 a, b), relative to nonAM plants. Soil Ψ at stomatal closure was 0.3 to 0.6 MPa lower in AM roses than in similarly-sized nonAM roses (Augé et al. 1986). Soils of AM cowpeas had to lose more water than soils of similarly-sized nonAM plants, before evoking similar stomatal conductance, shoot Ψ, transpiration and abscisic acid in xylem near stomatal closure (Duan et al. 1996). AM sorghum was also able to maintain leaf Ψ to lower soil Ψ than similarly-sized nonAM plants (Osonubi 1994). Dakessian et al. (1986), Bethlenfalvay et al. (1988 b) and Franson et al. (1991) have provided evidence that AM plants apparently have access to soil water below the permanent wilting Ψ of nonAM plants.

How might external, extraradical AM mycelia increase the efficacy of root water absorption in dry soil? As suggested by several authors (e.g. Reid 1979, Fitter 1985, Davies et al. 1992), soil hyphae may increase soil-to-root contact in drying soils. Perirhizal resistance – resistance to water flow across the soil-root interface – results from draw-down resistance, diurnally imposed by the rapid loss of water from the soil immediately adjacent to the root, and from contact resistance, which increases as the surface of the root has less contact with rhizosphere water (Tinker 1976, Klepper 1990). Contact resistance increases as water retreats from large pores into smaller and smaller capillary areas in the soil and decreases the amount of root length actually wetted (Herkelrath et al. 1977). 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). Further, extraradical hyphal development and soil aggregation by AM plants have been greater under drought conditions (Davies et al. 1992). Enhanced 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, Allen 1982, Bildusas et al. 1986).

Others have suggested that extraradical hyphae may also contribute directly to water absorption by root systems (Hardie 1985, Davies et al. 1992). AM hyphae were reported to enhance water uptake in sunflower and cowpea (Faber et al. 1991) and lettuce (Ruiz-Lozano and Azcón 1995) but not in clover or couchgrass (George et al. 1992) or wheat (Tarafdar 1995). When calculated rather than measured, hyphal water transport rates have generally been considered negligible (Graham and Syvertsen 1984, Fitter 1985, George et al. 1992, Koide 1993). However, Read and Boyd (1986) suggested that some experiments and computations may have involved unrealistically low numbers of hyphal entry points, and that hyphal contributions to water uptake may be significant.

Alternately, soil hyphae might indirectly influence host water relations by changing soil water relations. Fungal hyphae and exudates, especially those of AM fungi, improve soil structure through both the physical and chemical binding of soil aggregates (Tisdall 1991, Jastrow et al. 1998). Improved soil structure generally has positive impacts on soil moisture retention properties (Hamblin 1985). Colonization of soil by AM fungi has recently been shown to change soil moisture retention properties, in concert with changes in soil hyphal density and associated soil characters (Augé et al. 2001a, Bearden 2001).

This experiment was not an attempt to identify a mechanism of AM influence on host drought resistance. Rather, it was a test of whether colonization of soil or colonization of plants by AM fungi would contribute a stronger path to drought resistance. The findings support the contention of Bethlenfalvay (personal communication) that soil hyphal development plays an important role in mycorrhiza-induced changes in plant capacity for drought resistance. We evaluated a system composed of two AM groups, and it would be worthwhile in future investigations to conduct the analysis across several functional AM groups colonizing a particular host. It will also be instructive to learn if amount of soil hyphae contributes a strong path to other, sub-lethal water relations variables (such as stomatal behavior) or to lethal Ψ values in other host species.

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