

Non-nutritional stress acclimation of mycorrhizal woody plants exposed to drought

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Summary Mycorrhizal enhancement of drought resistance of two woody plant species, loblolly pine (*Pinus taeda* L.) and rose (*Rosa hybrida* L. cv. Ferdy), occurred independently of phosphorus nutrition. Mycorrhizae tended to alter root morphology and carbon allocation patterns of shoots and roots. Increased drought resistance of mycorrhizal plants was in part attributed to drought-induced colonization by mycorrhizae and the ability of the mycorrhizal plants to maintain high transpiration rates as a result of greater lateral root formation and lower shoot mass (in ectomycorrhizal loblolly pine), and a higher root/shoot ratio and leaf abscission (in endomycorrhizal roses). Neither the endo- nor ectomycorrhizal symbionts affected osmotic adjustment of droughted plants.

Keywords: extraradical hyphae, gas exchange, *Pinus taeda*, phosphorus nutrition, *Rosa hybrida*.

Introduction

Mycorrhizal fungi form a major interface between the soil and plant. The extraradical hyphae serve as extensions of the root system and function as absorptive structures for water and mineral uptake (Bethlenfalvay and Linderman 1992). Because hyphae of endomycorrhizae and vesicular-arbuscular mycorrhizae (VAM) extend several centimeters from the root, they can effectively bridge the zone of nutrient depletion around roots and absorb immobile elements from the bulk soil. The effect may be more pronounced in ectomycorrhizae, because hyphae may extend several meters from the root. Soil hyphae also attract other microbes, and together they form water-soluble aggregates necessary for maintaining good soil structure (Tisdall 1991, 1994). The colonization of roots by mycorrhizal fungi, plus the enhanced water and nutrient uptake from the soil by the extraradical hyphae, results in physiological and morphological changes in the host plant. Very often, these

changes in the host plant cause mycorrhizal plants to grow and respond to environmental stresses (e.g., water and nutrient limitations) more successfully than nonmycorrhizal plants.

Mycorrhizae can increase the drought resistance of host plants, but it is not known if this is a secondary response to improved host nutrition—particularly of P (Nelsen 1987). Mycorrhizal colonization of roots has been reported to enhance plant water relations by influencing root hydraulic conductivity (Sands and Theodorou 1978, Sweatt and Davies 1984, Nelsen 1987, Newman and Davies 1988), leaf gas exchange (Hardie 1985, Davies et al. 1993), leaf expansion (Koide 1985), osmotic adjustment (Augé et al. 1986), extraradical hyphae development (Davies et al. 1992), phytohormone production (Gogala 1991, Duan et al. 1996), and by altering nonhydraulic root to shoot communication during soil drying (Augé and Duan 1991). Despite the extensive literature and the general tendency for higher transpiration rates in mycorrhizal plants than in nonmycorrhizal plants (Augé 1989), the role of mycorrhizae in plant water relations remains uncertain.

We have studied some of the physiological and morphological mechanisms by which the mycorrhizal associations in root systems of woody plants are reported to enhance drought resistance. To test the hypothesis that both ecto- and endomycorrhizal symbionts can enhance drought resistance of woody plants independently of tissue phosphorus concentrations, we examined drought resistance mechanisms in loblolly pine (*Pinus taeda* L.) seedlings colonized with the ectomycorrhizal fungi, *Pisolithus tinctorius* (Pers) Coker and Couch (Pt) and *Rosa hybrida* L. cv. Ferdy colonized with the endomycorrhizal fungi (VAM), *Glomus deserticola* Trappe, Bloss and Menge. Both woody plant species are highly mycorrhizal. Loblolly pine is the most important timber species in the southern USA. *Rosa hybrida* cv. Ferdy is one of a new generation of low-maintenance, drought-resistant field rose bushes used in horticulture–landscape systems.

Materials and methods

Drought resistance of loblolly pine seedlings inoculated with Pisolithus tinctorius

Plant culture and ectomycorrhizal inoculation A half-sibling family of *Pinus taeda* L. (8-76) from the coastal plain of North Carolina was selected because of its rapid growth and susceptibility to drought. Surface-sterilized and stratified seeds were sown, three per container, in 1-liter Marx Roottrainer™ containers (Spencer Lemaire, Alberta, Canada) filled with a fritted clay medium. Seedlings were grown in a greenhouse in a 16-h photoperiod with a maximum photon flux density (PFD) of 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$, consisting of natural light and incandescent light. Day/night temperatures were maintained at $30 \pm 3/22 \pm 3$ °C. Seedlings were fertilized five times weekly with 150 ml of Long-Ashton nutrient solution (LANS) (Hewitt 1966) at 60% of full strength for all nutrients.

Sixteen weeks after sowing, seedlings were thinned to one per pot, and half of the pots were band inoculated with 40 ml (1/25 v/v) of vegetative mycelium of *Pisolithus tinctorius* (Pers) Coker and Couch (Pt). Noninoculated seedlings received 40 ml of autoclaved inoculum. To ensure that Pt-inoculated seedlings did not have higher tissue P concentrations than noninoculated seedlings, the LANS was modified so that Pt-inoculated seedlings received 11 $\mu\text{g P ml}^{-1}$ and noninoculated seedlings received 44 $\mu\text{g P ml}^{-1}$.

Water deficit treatments Twenty-eight weeks after sowing, noninoculated and Pt-inoculated seedlings were subjected to three water-stress regimes, based on predawn plant water potentials (Ψ_{pd}): (1) well-watered treatment in which seedlings were watered daily to container capacity to maintain a Ψ_{pd} of -0.5 ± 0.1 MPa, as measured with a pressure chamber (Svenson et al. 1991); (2) cyclic water deficit treatment in which seedlings were subjected to cyclic, rapidly developing water deficits by watering to container capacity on 10-day cycles when Ψ_{pd} reached -1.8 ± 0.5 MPa; and (3) single water deficit treatment in which seedlings were subjected to a single, slowly developing water deficit by initially watering containers with 50 ml of water daily, and reducing the amount added by 5 ml every 5 days until seedlings reached a Ψ_{pd} of -1.8 ± 0.5 MPa. Rate of water-stress development of seedlings in the cyclic water deficit treatment averaged -0.18 MPa day^{-1} , compared to -0.036 MPa day^{-1} for seedlings in the single water deficit treatment.

Water relations measurements Peak predawn plant water deficits occurred on the last day of the fifth drying cycle in plants in the cyclic water deficit stress treatment (coinciding with the last day of the single water deficit treatment), and recovery occurred 20 h after the pots were rewatered to container capacity. Parameters measured included: midday transpiration (E), midday fascicle water potential (Ψ_{md}), midday relative water content (RWC_{md}), and Ψ_{pd} . All measurements were made on the most recently fully expanded intact fascicles (at least 12 cm long) of four seedlings from each water regime and Pt treatment combination.

An LI-1600 steady-state porometer (Li-Cor, Inc., Lincoln, NE) was used to measure E , and the location of the fascicle was marked for repeated measurements on successive days. Measured E values were corrected for surface area as recommended by the manufacturer. Surface area was estimated based on the unit-length of the fascicle (Svenson and Davies 1992). Fascicle water potentials were measured with a pressure chamber, and relative water content (RWC) was determined (Svenson et al. 1991).

After peak drought measurements, six seedlings from each water regime and Pt combination were rehydrated by placing the containers in warm water for 20 h in a dark laboratory at an ambient temperature of 23.3 ± 1.1 °C and a relative humidity of $45.2 \pm 2.3\%$. Two fascicles from each rehydrated seedling were used to measure Ψ with a pressure chamber. The fascicles were then frozen in liquid nitrogen before measurement of osmotic potential (Ψ_{π}) of the frozen-thawed tissue in a Wescor C-52 chamber attached to a Wescor HR33T microvoltmeter operating in the dewpoint mode (Wescor Inc., Logan, UT). Turgor potential (Ψ_{p}) was calculated as the difference between Ψ and Ψ_{π} .

Plant harvest and ectomycorrhizal analysis After final water status measurements, seedlings were harvested, and fascicle, root and total plant dry mass were recorded, and root/shoot ratio calculated. Percent of lateral roots colonized by Pt and by other ectomycorrhizae was recorded, and lateral roots colonized by Pt were estimated by a Pt index (Svenson et al. 1991). Samples of the most recently fully expanded fascicle from each treatment were subjected to dry-ash analysis and the elemental nutrients determined by inductively coupled plasma atomic emission spectrometry (3510 ICP, W.R. Grace and Co., Foggsville, PA).

Statistical analysis Water relations parameters were subjected to ANOVA based on a 3 water regime \times 2 Pt inoculation factorial arrangement in a completely randomized design with four replications and two nested subsamples. Harvest and nutrient data were similarly analyzed based on 15 and five replications, respectively. All data were processed by the SAS General Linear Models with type III sums of squares procedure (SAS Institute Inc. 1988, Cary, NC).

Drought resistance of Rosa hybrida rooted cuttings inoculated with Glomus deserticola

Plant culture and endomycorrhizal inoculation Rooted cuttings of *Rosa hybrida* L. cv. Ferdy were planted in 3.8-liter pots containing a 4/1 (v/v) mix of composted pine bark and sand, amended with 3.6 kg m^{-3} N,P,K (18,2.6,10) slow-release fertilizer, 3 kg m^{-3} gypsum, 3 kg m^{-3} dolomitic limestone, and 74.2 g m^{-3} fritted trace elements.

Half of the plants were inoculated with 2000 spores of the endomycorrhiza (VAM) *Glomus deserticola* Trappe, Bloss and Menge per 3.8 liters of medium. To reestablish the microflora associated with the mycorrhizal inoculum, noninoculated plants (NVAM) received inoculum extract sieved to 45 μm and filtered through Whatman No.1 filter paper. Plants were grown

for five months in a greenhouse in long days with a 2-h night interruption. Incandescent lights provided a maximum PFD of $900 \mu\text{mol m}^{-2} \text{s}^{-1}$ at plant height. The minimum/maximum temperature was 18/30 °C. To avoid possible nutritional effects on plant water relations because of higher P in tissues of mycorrhizal plants, mycorrhizal and nonmycorrhizal roses received LANS modified to contain 11.5 and 22 $\mu\text{g P ml}^{-1}$, respectively.

Water deficit treatments After establishment, water was withheld from one-third of the VAM and NVAM plants for four 3-day and three 4-day drought cycles; these plants were termed “acclimated.” All other plants were well watered during these cycles. After seven drought cycles, the acclimated plants and half of the well-watered (nonacclimated) plants received one 4-day drought cycle, hereafter termed the peak stress cycle. The remainder of the nonacclimated plants were watered daily throughout the study.

Water relations measurements Between 1300 and 1600 h on the first and fourth (peak stress) afternoons of the peak stress cycle, Ψ of two of the uppermost fully expanded leaves was measured on six plants per treatment with a pressure chamber ($n = 12$), with precautions to prevent water loss from leaves. Osmotic potentials (Ψ_{π}) were measured on 10 leaf samples per treatment collected between 1700 and 1800 h on the fourth afternoon of the stress cycle. Two terminal shoots with six leaves each were removed from each plant. The base of each shoot was recut under distilled water, and then supported with the base immersed in water and leaves exposed to moist air in a humidity chamber for 2 h, until full turgor was reached ($\Psi \approx -0.1 \text{ MPa}$). Leaves were then removed and immediately frozen at $-30 \text{ }^{\circ}\text{C}$ in a Revco ULT 1786 A-O-B freezer (Rheem Manufacturing Co., Asheville, NC). Leaves were later thawed for approximately 1 h in waterproof envelopes, and Ψ_{π} of expressed sap was measured psychrometrically with a C-52 chamber coupled to a PR-55 psychrometer microvoltmeter (Wescor Inc., Logan, UT).

Transpiration rate (E) was determined gravimetrically (Graham et al. 1987). On the first day of the final cycle, plants were irrigated, allowed to drain, and the containers were covered with polyethylene bags, secured around the plant crown ($n = 4$). Plants were weighed daily at 1000 and 1630 h. From these data and leaf areas measured at harvest, E was determined.

Plant harvest and endomycorrhizal analysis On completion of the study, plants were harvested, and leaf number, leaf area and root/shoot ratio were determined. For cuticle and epicuticular wax determination, six fully expanded leaves of equal age and location were taken from the newest growth area of the plants in each treatment (Reed and Tukey 1982). Four pooled leaf samples from each treatment ($n = 4$) were analyzed for elemental content as previously described. For endomycorrhizal analysis, root samples were cleared and stained according to the modified procedures of Phillips and Hayman (1970). Percent colonization was determined as described by Biermann and Linderman (1981).

Statistical analysis The 3 water regimes \times 2 VAM factorial experiment was in a split plot design that included 12 containers per treatment. The three watering treatments (acclimated, nonacclimated and exposed to a late drought cycle, and non-stressed) were main plot treatments, and the two endomycorrhizal treatments (with or without mycorrhiza) were the subplot treatments. An ANOVA was performed on all data, and LSD values were determined for significant main effects and interactions.

Results

*Drought response of loblolly pine seedlings inoculated with *Pisolithus tinctorius**

Growth, nutrition and colonization analysis Root and total plant dry mass were reduced by both the cyclic and single water deficit treatments, but fascicle dry mass was unaffected (Table 1). The Pt-inoculated seedlings had smaller fascicles and shoot dry mass than the noninoculated seedlings. Because inoculation with Pt did not affect root dry mass, Pt-inoculated seedlings had greater root/shoot ratios than noninoculated seedlings. Noninoculated seedlings in the cyclic and single water deficit treatments showed 34 and 36% reductions in total plant dry weight, respectively, compared to well-watered noninoculated seedlings, whereas Pt-inoculated seedlings showed growth reductions of only 24% (cyclic) and 26% (single) compared to well-watered Pt-inoculated seedlings.

The different P regimes delivered to the noninoculated and Pt-inoculated seedlings led to comparable fascicle P concentrations in the Pt-inoculated and noninoculated seedlings (Table 1); however, fascicle P concentration was reduced by the cyclic water deficit treatment (Table 1). Inoculation with Pt enhanced zinc uptake, regardless of the water deficit regime. There were no other differences in tissue elemental concentrations between noninoculated and Pt-inoculated seedlings, except for a decreased nitrogen concentration of Pt-inoculated seedlings in the single water deficit treatment (data not presented).

Inoculated seedlings were heavily colonized by Pt (Table 2). The total number of lateral roots was higher in Pt-inoculated seedlings than in noninoculated seedlings, but root dry mass was similar in the two treatments. Inoculation with Pt increased not only the Pt index but also the percent of lateral roots colonized by other ectomycorrhizae. Noninoculated seedlings were nearly 50% ectomycorrhizal, with an unidentified fungus forming thick, creamy-white mantles and fibrous white rhizomorphs; however, only a small fraction of the colonization was from Pt contamination. The color, branching habit, and the presence of epiphytic fruiting bodies on the stem at soil level suggested that the unidentified fungus was *Thelephora terrestris* (Echr.) Fr.

Plant water relations During peak drought, midday E , Ψ_{md} , and RWC_{md} of droughted seedlings were lower than those of well-watered seedlings (Table 3). However, droughted Pt-inoculated seedlings had higher E and Ψ_{md} than droughted non-

Table 1. Effects of water regime and inoculation with *Pisolithus tinctorius* (Pt) on needle, shoot, total plant dry weight, root biomass to shoot biomass ratio, and fascicle phosphorus (P) and zinc (Zn) concentrations of *Pinus taeda* seedlings. Seedlings were kept well watered, or subjected to a rapidly developing cyclic drought stress, or to a single long period of drought. Values presented are means \pm SE.

Type of water deficit	Pt inoculation	Fascicle dry weight ¹ (g)	Root dry weight ¹ (g)	Total plant dry weight ¹ (g)	Root/shoot ratio ¹	P ² (%)	Zn ² (mg kg ⁻¹)
None	No	18.1 \pm 2.9	15.2 \pm 0.9	46.0 \pm 3.7	0.54 \pm 0.05	0.11 \pm 0.02	22.4 \pm 1.7
	Yes	12.5 \pm 1.7	13.9 \pm 0.9	33.0 \pm 3.1	0.66 \pm 0.07	0.12 \pm 0.01	26.6 \pm 2.6
Cyclic	No	18.4 \pm 1.3	9.6 \pm 0.4	30.2 \pm 1.7	0.46 \pm 0.04	0.09 \pm 0.01	21.9 \pm 2.8
	Yes	10.2 \pm 0.4	9.3 \pm 0.7	25.0 \pm 0.2	0.57 \pm 0.04	0.09 \pm 0.01	34.5 \pm 4.9
Single	No	13.7 \pm 0.7	9.1 \pm 0.7	29.6 \pm 1.2	0.43 \pm 0.03	0.12 \pm 0.01	23.7 \pm 2.8
	Yes	10.8 \pm 1.1	8.6 \pm 0.6	24.3 \pm 1.9	0.48 \pm 0.04	0.13 \pm 0.01	23.7 \pm 1.3
<i>Significance</i> ³							
Water regime (W)		NS	**	**	NS	**	NS
Inoculation (I)		*	NS	*	NS	NS	**
W \times I		NS	NS	NS	NS	NS	NS

¹ $n = 15$.

² $n = 5$.

³ ANOVA, NS = nonsignificant, * = significant at 5% level, ** = significant at 1% level.

Table 2. Effects of water regime and inoculation with *Pisolithus tinctorius* (Pt) on total number of lateral roots, ectomycorrhizal colonization, and Pt index of *Pinus taeda* seedlings. Seedlings were kept well watered, or subjected to rapidly developing cyclic drought stress, or to a single long period of drought. Values presented are means \pm SE; $n = 15$.

Type of water deficit	Pt inoculation	Number of lateral roots per seedling	Percent lateral roots with ectomycorrhizae	Pt index ¹
None	No	27.3 \pm 2.3	49.4 \pm 2.9	3.5 \pm 0.8
	Yes	30.2 \pm 2.5	84.5 \pm 1.9	59.2 \pm 3.3
Cyclic	No	23.4 \pm 2.6	43.9 \pm 4.7	23.3 \pm 1.0
	Yes	33.2 \pm 3.1	84.9 \pm 2.4	59.1 \pm 4.3
Single	No	24.9 \pm 2.2	45.7 \pm 3.9	3.0 \pm 1.1
	Yes	30.7 \pm 2.2	87.7 \pm 1.7	64.0 \pm 3.7
<i>Significance</i> ²				
Water Regime (W)		NS	NS	NS
Inoculation (I)		**	**	**
W \times I		NS	NS	NS

¹ Pt index = $a(b/c)$, where a = percent of sampled roots with Pt, b = percent lateral roots with any ectomycorrhizae, and c = percent of lateral roots with Pt.

² ANOVA, NS = nonsignificant, ** = significant at 1% level.

inoculated plants. Stomatal conductance (data not presented) was lower in droughted seedlings than in well-watered seedlings and mirrored the E response. There was a significant interaction between the inoculation and drought treatments: when compared with the corresponding noninoculated seedlings, Pt-inoculated seedlings had a slightly lower RWC under well-watered conditions, a similar RWC in the cyclic water deficit treatment, and a higher RWC in the single water deficit treatment. The cyclic and single water deficit treatments had similar effects on both E and Ψ_{md} of noninoculated seedlings, whereas among Pt-inoculated seedlings, the effects of the water deficit treatments on E were similar, but Ψ_{md} was decreased more by the cyclic water deficit treatment than by the single water deficit treatment.

During recovery from drought, seedlings exposed to the cyclic and single water deficit treatments had lower Ψ_{md} than well-watered seedlings, even though both groups of seedlings were rehydrated to similar Yp values (Table 4). Seedlings inoculated with Pt had lower Yp than noninoculated seedlings, even though Pt-inoculated seedlings rehydrated to a greater Yp than noninoculated seedlings. Water deficits and Pt-inoculation both induced a shift in the osmotic potential at full turgor, suggesting that osmotic adjustment had occurred. However, osmotic adjustment did not account for the differences in water relations during peak drought, because droughted Pt-inoculated seedlings had a higher Ψ_{md} during the peak drought than droughted noninoculated seedlings.

Table 3. Effects of water regime and inoculation with *Pisolithus tinctorius* (Pt) at peak water deficit on midday transpiration rate (E), fascicle water potential (Ψ_{md}), and relative water content (RWC) of *Pinus taeda* seedlings. Seedlings were kept well watered, or subjected to a rapidly developing cyclic drought stress, or to a single long period of drought. Values are means \pm SE; $n = 8$.

Type of water deficit	Pt inoculation	E (mmol m ⁻² s ⁻¹)	Ψ_{md} (MPa)	RWC
None	No	2.20 \pm 0.22	-0.59 \pm 0.01	0.97 \pm 0.01
	Yes	2.69 \pm 0.25	-0.56 \pm 0.02	0.94 \pm 0.01
Cyclic	No	0.14 \pm 0.03	-1.91 \pm 0.02	0.79 \pm 0.02
	Yes	0.26 \pm 0.04	-1.83 \pm 0.02	0.81 \pm 0.01
Single	No	0.20 \pm 0.05	-1.98 \pm 0.07	0.74 \pm 0.02
	Yes	0.26 \pm 0.07	-1.75 \pm 0.03	0.80 \pm 0.02
<i>Significance</i> ¹				
Water regime (W)		*	**	**
Inoculation (I)		**	*	NS
W \times I		NS	**	**

¹ ANOVA, NS = nonsignificant, * = significant at 5% level, ** = significant at 1% level.

Table 4. Effects of water regime and inoculation with *Pisolithus tinctorius* (Pt) during rehydration recovery (at full turgor) on fascicle water potential (Ψ_{md}), osmotic potential (Ψ_{π}), and turgor pressure (Ψ_p) of *Pinus taeda* seedlings. Seedlings were subjected to rapidly developing cyclic drought stress, a single long deficit, or not subjected to drought. Values presented are means \pm SE; $n = 8$.

Type of water deficit	Pt inoculation	Ψ_{md} (MPa)	Ψ_{π} (MPa)	Ψ_p (MPa)
None	No	-0.21 \pm 0.01	-1.64 \pm 0.05	1.43 \pm 0.05
	Yes	-0.23 \pm 0.01	-1.77 \pm 0.06	1.54 \pm 0.05
Cyclic	No	-0.48 \pm 0.01	-1.87 \pm 0.06	1.39 \pm 0.06
	Yes	-0.51 \pm 0.01	-2.07 \pm 0.05	1.57 \pm 0.05
Single	No	-0.46 \pm 0.01	-1.87 \pm 0.04	1.41 \pm 0.04
	Yes	-0.49 \pm 0.02	-1.99 \pm 0.08	1.50 \pm 0.05
<i>Significance</i> ¹				
Water Regime (W)		**	**	NS
Inoculation (I)		**	**	*
W \times I		NS	NS	NS

¹ ANOVA, NS = nonsignificant, * = significant at 5% level, ** = significant at 1% level.

Drought response of Rosa hybrida rooted cuttings inoculated with Glomus deserticola

Growth, nutrition, and colonization analysis Plants acclimated to drought had fewer leaves and less leaf area than nonacclimated and nonstressed plants, but comparable root/shoot ratios (Table 5). Although VAM plants had fewer leaves than NVAM plants, leaf area (Table 5) and shoot and root dry weights (data not presented) were not significantly different. Acclimated VAM plants had a greater root/shoot ratio than acclimated NVAM plants. The watering regimes had no effect on wax or cuticle weight of the plants, but VAM plants had lower wax and cuticle weights than NVAM plants (Table 5). Among the treated plants, drought-acclimated VAM plants had the highest colonization rates (Table 6). There was no difference in leaf tissue P content among VAM or irrigation treatments (Table 6).

Plant water relations Nonstressed plants had the highest values of E and Ψ_{md} and nonacclimated plants had the lowest values (Table 7). Drought-acclimated plants had the lowest Ψ_{π} . Irrespective of watering regime, VAM plants had a higher E than NVAM plants. Inoculation with VAM did not affect Ψ_{md} or Ψ_{π} .

Discussion

Mycorrhizal effects occurred independently of phosphorus nutritional effects

We observed that, in loblolly pine and rose, respectively, the direct effects of ectomycorrhizal and endomycorrhizal symbiosis occurred independently of P nutritional effects. This was accomplished by eliminating the P effect in the drought studies by adjusting P-fertilization regimes so that inoculated and

Table 5. Plant growth, wax, and cuticle development of VAM and NVAM *Rosa hybrida* cv. Ferdy under three water regimes: non-stressed, non-acclimated and exposed to a late drought cycle, and acclimated to cyclic drought. Values presented are means of replicate determinations.

Endomycorrhizal treatment	Water regime	Leaf area ¹ (cm ²)	Leaf no. ¹	Root/shoot ratio ¹	Wax ² (µg mm ⁻²)	Cuticle ² (µg mm ⁻²)
NVAM	Non-stressed	1453	253	0.69	1.25	1.20
	Non-acclimated	1649	250	0.77	1.41	1.22
	Acclimated	1066	170	0.71	1.20	1.17
VAM	Non-stressed	1026	206	0.72	0.92	1.03
	Non-acclimated	1388	218	0.82	0.99	0.67
	Acclimated	1123	172	0.86	1.15	1.02
<i>Significance of main effects</i> ³						
Endomycorrhizae (VAM)		NS	23	0.10	0.11	0.20
Water regime (W)		356	48	NS	NS	NS

¹ n = 12.

² n = 6.

³ ANOVA, NS = nonsignificant or LSD at the 5% level.

Table 6. Mycorrhizal colonization and leaf phosphorus concentration of VAM and NVAM *Rosa hybrida* cv. Ferdy under three water regimes: non-stressed, non-acclimated and exposed to a late drought cycle, and acclimated to cyclic drought. Values are means of replicate determinations.

Endomycorrhizal treatment	Water regime	Colonization ¹ (%)	P ² (% dry wt)
NVAM	Non-stressed	0.8	0.16
	Non-acclimated	0.6	0.16
	Acclimated	0.6	0.17
VAM	Non-stressed	9.2	0.17
	Non-acclimated	8.0	0.15
	Acclimated	12.5	0.16
<i>Significance of main effects</i> ³			
Endomycorrhizae (VAM)		4.73	NS
Water regime (W)		NS	NS
<i>Significance of interactions</i> ³			
VAM means for same or different water regime		2.97	NS
Water regime means for same VAM treatment		2.92	NS

¹ n = 12.

² n = 4.

³ ANOVA, NS = nonsignificant or LSD at the 5% level.

non-inoculated loblolly pine and rose seedlings had similar tissue P concentrations.

Drought enhancement of the mycorrhizal symbiosis

Drought generally enhanced mycorrhizal colonization of the plant roots. In Pt-inoculated loblolly pine seedlings exposed to a single, slowly developing drought cycle, Pt colonization tended to increase, as indicated by the Pt index, and there was also an increase in number of lateral roots colonized with other ectomycorrhizae compared with well-watered Pt-inoculated plants. Rhizomorphs (specialized hyphal strands) and exten-

sive hyphal growth were observed in all Pt-inoculated seedlings, but were not quantified because of the difficulty in recovering mycelium from the fritted clay medium.

All Pt-inoculated pine seedlings had a greater Pt index and percent lateral roots colonized with ectomycorrhizae than non-inoculated seedlings. About 45% of the lateral roots of non-Pt-inoculated pine seedlings were naturally colonized with a non-Pt ectomycorrhiza that had the characteristics of *Thelephora terrestris*. Natural colonization of noninoculated *P. taeda* with *T. terrestris* and other ectomycorrhizae occurs in the southern USA after field outplanting, and its prevention (through soil fumigation and/or filtered air and water systems) is an anomaly under natural conditions, resulting in stunted growth and severe chlorosis (Marx et al. 1988, South et al. 1988). Despite contamination of noninoculated seedlings with *T. terrestris*, Pt-inoculated seedlings had better water relations than noninoculated seedlings. The Pt isolate #298 used for inoculation is an aggressive strain, reported to enhance drought resistance of outplanted pine species (D.H. Marx, personal communication).

In both woody and herbaceous plants, drought acclimation enhances the symbiosis of *Glomus deserticola* and the overall drought resistance of the host plant (Davies et al. 1992). Among *Rosa hybrida* shrubs inoculated with *G. deserticola*, drought-acclimated VAM plants had a higher VAM colonization than nonacclimated and well-watered VAM plants. In herbaceous pepper plants grown on a sandy loam medium, drought acclimation enhanced extraradical hyphae formation and colonization by *G. deserticola* (Davies et al. 1992).

Extraradical hyphae contribute to plant water uptake (Hardie 1985, and Davies et al. 1992). In addition to the role of hyphae in exploring soil, they can also bridge gaps between soil and roots, and bind soil particles to each other and to roots, which could be important as soil water decreases, causing soil shrinkage and the creation of gaps at the soil-root interface and between soil particles.

Table 7. Midday transpiration rate (E), leaf water potential (Ψ_{md}), and osmotic potential (Ψ_{π}) of VAM and NVAM *Rosa hybrida* cv. Ferdy under three water regimes: non-stressed, non-acclimated and exposed to a late drought cycle, and acclimated to cyclic drought. Means of measurements were taken during peak drought are presented.

Endomycorrhizal treatment	Water regime	Transpiration ¹ (mmol m ⁻¹ s ⁻¹)	Ψ_{md} ² (MPa)	Ψ_{π} ³ (MPa)
NVAM	Non-stressed	2.12	-0.61	-1.55
	Non-acclimated	0.63	-1.37	-1.53
	Acclimated	1.76	-1.03	-1.71
VAM	Non-stressed	2.38	-0.62	-1.52
	Non-acclimated	1.17	-1.45	-1.59
	Acclimated	1.86	-1.12	-1.66
<i>Significance of main effects</i> ⁴				
Endomycorrhizae (VAM)		0.29	NS	NS
Water regime (W)		0.34	0.07	0.07

¹ $n = 4$.

² $n = 12$.

³ $n = 10$.

⁴ ANOVA, NS = nonsignificant or LSD at the 5% level.

Mycorrhizal colonization altered root morphology and changed carbon allocation patterns of roots

Loblolly pine seedlings inoculated with Pt showed no increase in root dry mass but exhibited altered root morphology, i.e., they had a greater total number of lateral roots than noninoculated seedlings. Similarly, Alexander (1981) observed that the ectomycorrhizal fungus *Lactarius rufus* (Scop. ex Fr.) Fr. increased the number of short roots in spruce seedlings. Ectomycorrhizae are known to produce phytohormones such as gibberellic acid and cytokinins that can alter host root morphology and physiology (Reid 1984). Increased lateral roots would help the seedling obtain water from the rooting medium, thereby reducing shoot water deficits.

Reduced fascicle mass and a higher root/shoot ratio of Pt-inoculated seedlings compared with noninoculated seedlings would also tend to increase the Pt-seedling's ability to minimize plant water deficits. The reduced shoot mass of Pt-inoculated seedlings in the single water deficit treatment may be partially explained by decreased tissue N concentration (0.9 versus 1.2% N); however, there were no differences in nitrogen concentration of fascicles among well-watered and cyclic-droughted Pt-inoculated and noninoculated seedlings (data not shown). There may have been an increased carbon demand from Pt, resulting in a smaller shoot system. The carbon cost of endomycorrhizal colonization to 52-day-old *Citrus* accounted for 10 to 20% of net assimilate, resulting in a growth depression of the seedlings (Peng et al. 1993). Smaller shoot sizes have also been reported in ectomycorrhizal *Pinus radiata* D. Don (Sands and Theodorou 1978).

Loblolly pine seedlings inoculated with Pt had enhanced zinc uptake, regardless of the water deficit regime. It is well known that Zn uptake is enhanced by VAM, particularly in soils with low metal concentrations (Davies 1987, Kothari et al. 1990), and a similar explanation may account for the increased uptake of Zn by the Pt-inoculated loblolly pines seedlings in this study. We note that Marschner and Dell (1994) did

not report Zn enhancement with any ectomycorrhizae. Little is known about the potential role of Zn in drought resistance.

Because VAM plants had higher root/shoot ratios, which favor drought resistance (Kramer 1983), than NVAM plants, we conclude that VAM directly or indirectly contributed to changes in carbon allocation patterns of drought-acclimated rose plants. Drought-acclimated VAM rose plants tended to have the highest root/shoot ratios.

Mycorrhizal enhancement of plant drought stress

A model that attributes improved drought resistance of mycorrhizal plants to a high P concentration (Nelsen 1987, Fitter 1988) does not account for our results. Responses of endomycorrhizal plants to water deficits that are independent of P-nutritional status have been reported previously (Sweatt and Davies 1984, Augé et al. 1986, Davies et al. 1993).

In general, Pt-inoculated loblolly pine and VAM rose resisted drought by decreasing aboveground surface area, and either increasing root/shoot ratio or increasing lateral root development. Both droughted Pt-inoculated loblolly pine seedlings and VAM roses maintained higher transpiration rates (E) than noninoculated plants. In Pt-inoculated loblolly pine seedlings, higher turgor during the recovery from drought and higher E during peak drought were in part due to increased functional root area (more lateral roots and Pt extraradical hyphae) that facilitated water uptake. During peak drought, differences between noninoculated and Pt-inoculated seedlings were associated with drought avoidance mechanisms, including a decreased shoot system and an increased functional root area of the Pt-inoculated seedlings.

Drought avoidance mechanisms also helped to maintain increased E of droughted VAM roses. The VAM plants had enhanced leaf abscission, which potentially helped maintain a more favorable water status (i.e., higher E). Despite the higher E of VAM plants, RWC and Ψ_{md} tended to be lower, and this may have increased leaf abscission in the nonstressed and

nonacclimated plants. The observation that VAM increased leaf abscission in the laboratory is in agreement with our earlier findings that Ferdy roses abscise leaves when exposed to drought under field conditions. There was less epicuticular wax and cuticle on leaves of VAM than NVAM Ferdy roses, and this may have reduced barriers to cuticular water loss resulting in the increased E of VAM plants. Drought stress had no effect on cuticle and surface wax weights. The lack of increased wax during drought acclimation of Ferdy rose may be attributed to its tendency to abscise leaves when water is limiting.

In droughted loblolly pine seedlings, osmotic adjustment was only apparent during recovery and cannot alone account for the drought resistance during peak stress. Although osmotic adjustment has been reported previously in loblolly pine seedlings, the presence of ectomycorrhizae was not documented (Seiler and Johnson 1985). As in Pt-inoculated loblolly pines, VAM did not influence osmotic adjustment during peak drought of Ferdy roses. This finding contrasts with the studies of *Rosa hybrida* cv. Samantha where VAM increased osmotic adjustment (Augé et al. 1986), but agrees with studies of the landscape rose bush *R. hybrida* cv. Meieble (Bo 1992).

There is mounting evidence linking soil and root water status with the control of leaf growth and gas exchange (Augé and Stadola 1990, Augé and Duan 1991, Johnson et al. 1991). Increasing leaf water status has been correlated with decreasing ABA concentrations in xylem sap and declining ABA fluxes to leaves in mycorrhizal plants (Duan et al. 1996). Stomatal conductance of VAM cowpea is closely correlated with ABA concentration of xylem sap and ABA flux to leaves, suggesting that mycorrhizal fungi increase the ability of root systems to scavenge water at low Ψ_{soil} . Mycorrhizal hyphae also play an active role in water transport (Faber et al. 1991) by helping to exploit soil water when Ψ_{soil} decreases below the threshold accessible to nonmycorrhizal plants (Hardie and Leyton 1981).

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