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Communication

Mycorrhizal Fungi and Nonhydraulic Root Signals of Soil Drying

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ABSTRACT

We propose that mycorrhizal colonization of roots alters nonhydraulic root to shoot communication of soil drying. Split-root rose (*Rosa hybrida* L. cv Samantha) plants—one side of the root system colonized by *Glomus intraradices* Schenck & Smith, the other side nonmycorrhizal—displayed different stomatal conductances upon partial drying, depending upon whether mycorrhizal or nonmycorrhizal roots were dried. No differences in leaf water status were observed among control plants and those whose mycorrhizal or nonmycorrhizal roots were dried.

VA[1] mycorrhizal symbiosis can modify relationships between stomatal conductance and both soil water content (6) and shoot water content (5). In some cases, the mycorrhizal effect is a result of phosphorus deficiency in nonmycorrhizal controls (9, 15). When nonmycorrhizal plants have similar or higher phosphorus content than mycorrhizal plants, however, mycorrhizal plants still frequently show higher stomatal conductance, whether plants are unstressed (3, 6, 12) or subjected to soil drying (6, 14). Various hypotheses unrelated to nutrition have been offered to explain how the symbiosis might influence host stomatal conductance, particularly during drought. These include increased water uptake via soil (extraradical) hyphae (8), altered radial or axial hydraulic conductivity of roots (13), altered hormonal relations (13), and altered root system architecture (16). Mycorrhizal changes in root hydraulic conductivity have been ascribed to changes in plant phosphorus status (2, 11). Hormonal studies with VA mycorrhizal plants have been inconclusive. The most popular notion is that soil hyphae greatly increase the absorptive area of root systems, acting as tiny conduits that move water into roots (8, 12).

The discovery of nonhydraulic root to shoot communication (7, 18, 20) reveals another possibility. Root dehydration in drying soil presumably triggers or elevates production of a chemical signal in roots that serves as a sensitive measure of soil moisture availability (20). One putative signal, a positive inhibitor (10) and likely ABA (20), moves via the transpiration stream (19) to leaves where it may reduce leaf growth rates (10, 18) and/or stomatal conductance (10, 20). Such reductions occur even in the absence of any change in leaf water status and even when other portions of the root system are adequately watered. VA mycorrhizal fungi penetrate roots, grow extensively between and within living cortical cells, and affect many aspects of root metabolism. It seems possible, therefore, that mycorrhizal fungi may affect root metabolic responses to environmental stress, *i.e.* that mycorrhizal fungi might alter the production of chemical "stress" signals in roots. Here we present evidence that stomata of turgid rose leaves respond differently to drying, mycorrhizal roots than to drying, nonmycorrhizal roots.

MATERIALS AND METHODS

Rooted stem cuttings of rose (*Rosa hybrida* L. cv Samantha) were placed in holes cut in the bend of L-shaped tubes (90° plumber's plastic elbows, PVC, 2.5 cm diameter) whose arm lengths were extended with PVC pipe into two 1.25 L plastic pots. Elbows and pots were filled with a mixture (1:1:1) of calcined montmorillonite clay (Turface, IMCore, Mundelein, IL), washed river sand and silica sand, pH at planting = 6.4. One pot of each plant was inoculated with 12 g of Nutrilink (NPI, Salt Lake City, UT), approximately 16,000 spores of *Glomus intraradices* Schenck & Smith affixed to particles of attapulgite clay. Roots in the other pot remained nonmycorrhizal and received an inoculum wash sieved free of mycorrhizal propagules, to establish a background bacterial population. Plants were grown in a greenhouse under natural light, with mycorrhizal and nonmycorrhizal pots receiving 0.0 and 3.0 mM P, respectively, weekly as KH_2PO_4. Mycorrhizal and nonmycorrhizal pots each received Peter's (Grace-Sierra, Fogelsville, PA) 15–0–15 (N-P-K) at 143 mM N with each irrigation and 143 μM Fe (as Sequestrene 138) monthly. Fertilization was discontinued when soil drying treatments began. Spent blooms with stems were pruned until soil drying commenced; at that time, all flowers and flower buds were removed and further pruning was suspended.

On January 3, 1991, when plants were 6.5 months old, they were randomly arranged on a greenhouse bench under 400 W mercury halide lamps. All plants were watered on this day and the following treatments begun: controls = both mycorrhizal and nonmycorrhizal halves of the root system watered daily; *mycor·H_2O/nonmycor·dry* = plants whose mycorrhizal

[1] Abbreviations: VA, vesicular-arbuscular; *mycor·H_2O/nonmycor·dry*, plants whose mycorrhizal roots were watered daily and whose nonmycorrhizal roots were allowed to dry after day 0; *mycor·dry/nonmycor·H_2O*, plants whose nonmycorrhizal roots were watered daily and whose mycorrhizal roots were allowed to dry after day 0; Ψ, water potential; RWC, relative water content.

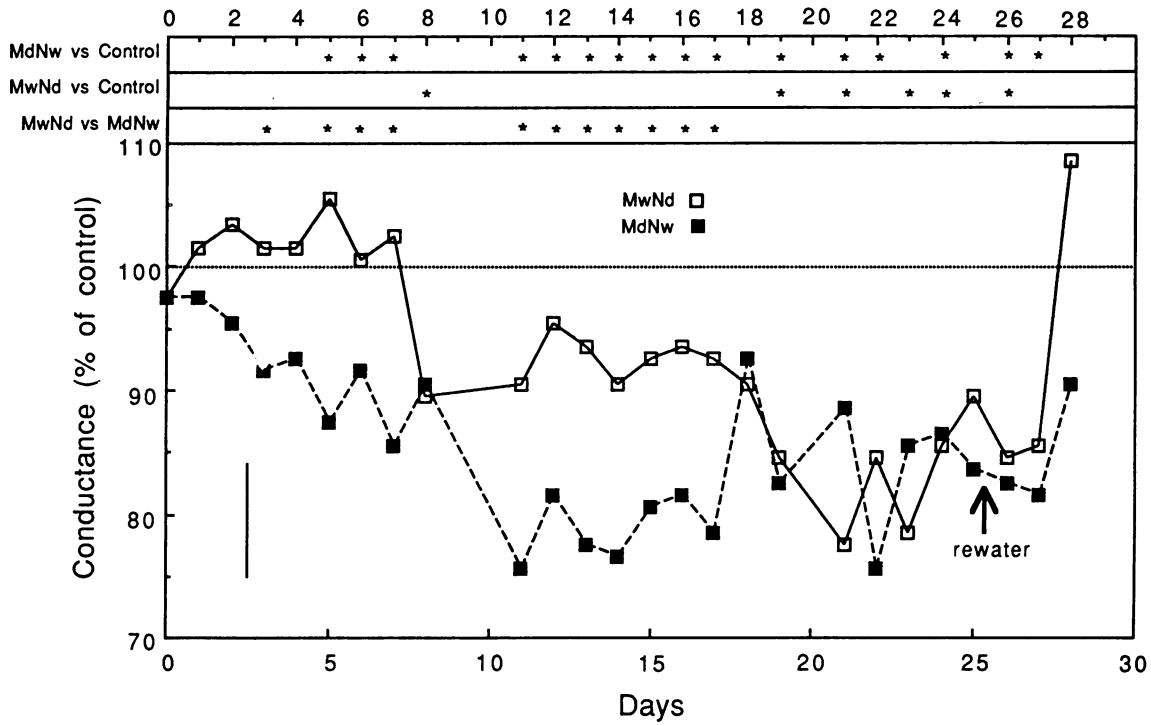

Figure 1. Stomatal conductance of rose plants grown with roots divided between two pots. Morning and afternoon stomatal conductances were averaged; $n = 50$ for control plants, $n = 40$ for *mycor·H_2O/nonmycor·dry* and *mycor·dry/nonmycor·H_2O* plants. Roots in one pot of each plant were inoculated with the VA mycorrhizal fungus *Glomus intraradices*, roots in the other pot were nonmycorrhizal. Both pots were well-watered until day 0, when water was subsequently withheld from either mycorrhizal or nonmycorrhizal roots. Controls = both mycorrhizal and nonmycorrhizal halves of the root system watered daily, MwNd = plants whose mycorrhizal roots were watered daily and whose nonmycorrhizal roots were allowed to dry after day 0 (abbreviated *mycor·H_2O/nonmycor·dry* in text), NwMd = plants whose nonmycorrhizal roots were watered daily and whose mycorrhizal roots were allowed to dry after day 0 (abbreviated *mycor·dry/nonmycor·H_2O* in text). Table at the top of the figure shows treatment differences on individual days, assessed by analysis of variance with linear contrasts; asterisk indicates means are significantly different, $P \leq 0.05$. Bar in the lower left of the figure represents 2× pooled standard error of the means, calculated by taking the square root of the error mean square from the analysis of variance and dividing it by the square root of the number of observations in a mean.

roots were watered daily and whose nonmycorrhizal roots were allowed to dry after day 0; *mycor·dry/nonmycor·H_2O* = plants whose nonmycorrhizal roots were watered daily and whose mycorrhizal roots were allowed to dry after day 0. At the beginning of soil drying, mycorrhizal roots had well-established colonizations (84% of root length, quantified as in ref. 3); uninoculated roots developed no colonizations.

Stomatal conductance of five recently matured, unshaded leaves, representing ≥3 main stems from each plant, was measured with a dynamic diffusion porometer (AP3, Delta-T Devices, Cambridge, England) each day between 0900 and 1030 h and again between 1330 and 1500 h. During these measurement periods, greenhouse humidity ranged from 30 to 50%, leaf temperature generally from 21 to 26°C, and PPFD reaching leaves from 500 to 800 μmol m^{-2} s^{-1}. Preliminary experiments with *Rosa* plants having all roots growing in one pot indicated that (a) stomatal conductance did not vary with PPFD in the above range; (b) diurnal variations were not evident during the above time periods; and (c) stomatal conductance did not vary with leaf age among the five most recently expanded leaves on a stem. Drying pots were not watered for 25 d; after stomatal conductance and leaf water status were measured on the afternoon of day 25, dried pots were watered. Between 25 and 28 d, both pots of all three treatments were watered daily.

Leaf Ψ and leaf RWC were measured around 1400 h at 20, 25, and 28 d after beginning soil drying. An unshaded, recently matured leaf was encased in thin polyethylene sheeting, excised immediately, and placed in a pressure chamber for estimation of Ψ. The leaf was immediately removed from the chamber, weighed, rehydrated 3 h in distilled H_2O in a dark, humid chamber at 2 to 4°C, blotted, reweighed, oven-dried, and reweighed for calculation of RWC. Preliminary tests revealed that wrapped leaves lost negligible amounts of water during Ψ determinations and that RWC values were not detectably affected by the preceding Ψ measurements.

RESULTS

Five days after withholding water from half the root system, stomatal conductance of *mycor·dry/nonmycor·H_2O* plants had declined to about 90% of control plants (Fig. 1). Stomatal conductance continued to decline in *mycor·dry/nonmycor·H_2O* plants, to about 80% of control plants by 11 d and through 18 d. Nearly 3 weeks were required for stomatal conductance of *mycor·H_2O/nonmycor·dry* plants to drop

Table I. *Leaf Water Potential (Ψ) and Leaf Relative Water Content (RWC)*

Rose plants were grown with roots divided between two pots. Roots in one pot of each plant were inoculated with VA mycorrhizal fungus *G. intraradices*, roots in the other pot were nonmycorrhizal. Control plants = both mycorrhizal and nonmycorrhizal pots watered daily; *mycor·H_2O/nonmycor·dry* = plants whose mycorrhizal roots were watered daily and whose nonmycorrhizal roots were allowed to dry after day 0; *mycor·dry/nonmycor·H_2O* = plants whose nonmycorrhizal roots were watered daily and whose mycorrhizal roots were allowed to dry after day 0. Analysis of variance with linear contrasts revealed no significant differences among soil drying treatments in either parameter on any day.

Time following Initiation of Soil Drying	Soil Drying Treatment					
	Controls[a]		*Mycor·H_2O/ nonmycor·dry*[b]		*Mycor·dry/ nonmycor·H_2O*[b]	
	Ψ	RWC	Ψ	RWC	Ψ	RWC
d	*MPa*	%	*MPa*	%	*MPa*	%
20	−0.52	95.7	−0.52	96.2	−0.54	96.3
25	−0.72	95.5	−0.67	95.6	−0.75	95.7
28[c]	−0.75	95.6	−0.65	96.1	−0.61	96.4

[a] $n = 5$. [b] $n = 4$. [c] Dried pots were rewatered on day 25, following afternoon measurement of conductance, leaf RWC, and leaf Ψ.

significantly below that of control plants. Stomatal conductance of *mycor·H_2O/nonmycor·dry* and *mycor·dry/nonmycor·H_2O* plants varied significantly on almost all days between 3 and 17 d of soil drying. After 17 d, stomatal conductance was similar in *mycor·H_2O/nonmycor·dry* and *mycor·dry/nonmycor·H_2O* plants, from 75 to 90% of controls, for the remainder of the drying period. Following rewatering, 3 d were required for stomatal conductance of *mycor·H_2O/nonmycor·dry* and *mycor·dry/nonmycor·H_2O* plants to return to levels of controls; stomatal conductance of *mycor·H_2O/nonmycor·dry* and *mycor·dry/nonmycor·H_2O* plants did not vary after rewatering. Leaf Ψ and leaf RWC of *mycor·H_2O/nonmycor·dry* and *mycor·dry/nonmycor·H_2O* plants remained at the same consistently high level as control plants throughout the 25 d drying period (Table I).

Mycorrhizal and nonmycorrhizal root systems developed similar dry mass in control plants (Table II). Watered and dried root systems did not differ statistically in dry weight at the end of the experiment ($P > 0.05$). Soil cores from dried pots at 15, 20, and 25 d contained live roots, and plants had mostly live roots at 28 d when roots were excavated.

DISCUSSION

No detectable differences were observed among treatments in leaf Ψ or leaf RWC during the drying period; therefore, stomata of half-dried plants were apparently responding to nonhydraulic, root-sourced signals rather than to loss of leaf hydration. The magnitude of the declines in stomatal conductance, to about 80% of controls, was similar to that noted previously for apple under comparable conditions (10). Root signals from partially dried root systems of other species have evidently resulted in larger declines in stomatal conductance (17, 20). In maize, for example, stomatal conductance dropped to 25% of fully watered controls (20), although in

Table II. *Root and Shoot Dry Weights*

Rose plants were grown with roots divided between two pots. Roots in one pot of each plant were inoculated with the VA mycorrhizal fungus *G. intraradices*, roots in the other pot were nonmycorrhizal. Control plants = both mycorrhizal and nonmycorrhizal pots watered daily; *mycor·H_2O/nonmycor·dry* = plants whose mycorrhizal roots were watered daily and whose nonmycorrhizal roots were allowed to dry after day 0; *mycor·dry/nonmycor·H_2O* = plants whose nonmycorrhizal roots were watered daily and whose mycorrhizal roots were allowed to dry after day 0. Watering of both pots of each plant was reinstated at 25 d. Plants were harvested at 28 d (see Fig. 1). Analysis of variance with linear contrasts revealed no significant differences among soil drying treatments in the weights listed below.

Soil Drying Treatment	Root Dry Weight			Shoot Dry Weight	Root/Shoot Ratio
	Mycorrhizal roots	Nonmycorrhizal roots	Total		
	g				*g/g*
Controls[a]	$4.4^{watered}$	$4.0^{watered}$	8.4	12.4	0.68
Mycor·H_2O/nonmycor·dry[b]	$6.5^{watered}$	3.9^{dried}	10.4	14.9	0.70
Mycor·dry/nonmycor·H_2O[b]	4.7^{dried}	$5.3^{watered}$	10.0	12.9	0.77

[a] $n = 5$. [b] $n = 4$.

other experiments with maize (18) no declines in stomatal conductance were observed with partial soil drying.

Stomata responded differently depending on whether dried (or watered) roots were mycorrhizal or nonmycorrhizal. In pots allowed to dry, mycorrhizal roots may have extracted soil moisture more rapidly than nonmycorrhizal roots, resulting in more rapid dehydration of soil and roots and, thus, more rapid production of the stress signal. Nonmycorrhizal and mycorrhizal root systems had similar mass when drying commenced, but most hyphae remain in soil during root excavation and are not included in measurements of root mass. There is evidence for (8, 12) and against (16) substantial hyphal contributions to water uptake. We sampled soil Ψ, but our sampling began after soil in the middle of pots, where rooting densities were greatest, had dried to below -1.5 MPa. Stomatal conductance of *Rosa* leaves typically begins to decline when soil Ψ reaches ≤ -0.5 MPa (RM Augé, unpublished observation). Additional work, including more frequent and thorough measurements of soil Ψ, is required to determine whether mycorrhizal and nonmycorrhizal root systems of similar size dehydrate soils at similar rates. Alternatively, increased production of the signal in mycorrhizal roots may have begun at higher soil Ψ than in nonmycorrhizal roots. Unstressed mycorrhizal *Rosa* roots have previously shown larger declines in bulk symplastic volume as root Ψ declined than nonmycorrhizal roots (4), and turgor has been lower in mycorrhizal than nonmycorrhizal roots of unstressed *Rosa* (4) and cowpea (RM Augé, unpublished observation). Root signal concentrations are presumably regulated by root turgor (7). Hence, it is possible that with soil drying, turgor in mycorrhizal roots declined sooner than in nonmycorrhizal roots to the "threshold" required for elevated signal production. It is also possible that mycorrhizae-induced changes in ABA levels of unstressed host leaves (1) modify leaf response to hormonal signals from roots in drying soil.

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