Planta (1994) 193:74–82

Planta

Nonhydraulic signalling of soil drying in mycorrhizal maize

Robert M. Augé, Xiangrong Duan, Robert C. Ebel, Ann J.W. Stodola

Institute of Agriculture, University of Tennessee, P.O. Box 1071, Knoxville, TN 37910–1071, USA

Received: 24 August 1993 / Accepted: 14 September 1993

Abstract. Our objectives were to (1) verify that nonhydraulic signalling of soil drying can reduce leaf growth of maize, (2) determine if a mycorrhizal influence on such signalling can occur independently of a mycorrhizal effect on leaf phosphorus concentration, plant size or soil drying rate, and (3) determine if leaf phosphorus concentration can affect response to the signalling process. Maize (*Zea mays* L. 'Pioneer 3147') seedlings were grown in a glasshouse with root systems split between two pots. The $2 \times 3 \times 2$ experimental design included two levels of mycorrhizal colonization (presence or absence of *Glomus intraradices* Schenck & Smith), three levels of phosphorus fertilization within each mycorrhizal treatment and two levels of water (both pots watered or one pot watered, one pot allowed to dry). Fully watered mycorrhizal and nonmycorrhizal control plants had similar total leaf lengths throughout the experiment, and similar final shoot dry weights, root dry weights and leaf length/root dry weight ratios. Leaf growth of mycorrhizal plants was not affected by partial soil drying, but final plant leaf length and shoot dry weight were reduced in half-dried nonmycorrhizal plants. At low P fertilization, effects of nonhydraulic signalling were not evident. At medium and high P fertilization, final total plant leaf length of nonmycorrhizal plants was reduced by 9% and 10%, respectively. These growth reductions preceded restriction of stomatal conductance by 7 d. This and the fact that leaf water potentials were unaffected by partial soil drying suggested that leaf growth reductions were nonhydraulically induced. Stomatal conductance of plants given low phosphorus was less influenced by nonhydraulic signalling of soil drying than plants given higher phosphorus. Soil drying was not affected by mycorrhizal colonization, and reductions in leaf growth were not related to soil drying rate (characterized by time required for soil matric potential to drop below control levels and by time roots were exposed to soil matric potential below typical leaf water potential). We conclude that mycorrhizal symbiosis acted independently of phosphorus nutrition, plant size or soil drying rate in eliminating leaf growth response to nonhydraulic root-to-shoot communication of soil drying.

Key words: Drought stress – Mycorrhizal symbiosis – Nonhydraulic root-to-shoot communication – Phosphorus nutrition – Stomatal conductance – *Zea* (leaf growth, nonhydraulic signalling)

Introduction

Partial drying of a root system can reduce leaf growth or stomatal conductance (Cs), even when the rest of the root system is moist enough to fully supply leaves with water (Davies and Zhang 1991). Most evidence for a nonhydraulic mechanism for root-to-shoot communication of reduced soil water availability supports the existence of a positive chemical message; the message is produced in dehydrating roots and moves via the transpiration stream to leaves where it modifies leaf behavior (Davies and Zhang 1991). The response of leaves to root signal(s) of soil drying varies considerably, from no response in Cs (Saab and Sharp 1989) to declines in Cs of 50% or more (Zhang and Davies 1989, 1990) and declines in leaf area of 15–18% after three weeks of drying (Saab and Sharp 1989; Zhang and Davies 1990). Leaf sensitivity to nonhydraulic signals may be dependent upon leaf water potential (Ψ) (Tardieu and Davies 1993), xylem pH and/or xylem ion concentration (Schurr et al. 1992). Certainly, stomatal environment, rates of soil drying, plant species and other factors peculiar to each study appear to affect the magnitude of leaf response to root signals.

Root biology might also affect the response, by directly affecting production and/or transport of the signal.

Abbreviations and symbols: ANOVA = analysis of variance; Cs = stomatal conductance(s); med = medium; P = probability; Ψ_s = matric potential(s); Ψ = water potential(s)

Correspondence to: R.M. Augé; FAX: 1 (615) 974 2765; Tel.: 1 (615) 974 2765

Root systems of native and agricultural plants commonly associate with vesicular-arbuscular mycorrhizal fungi, and many plants rely heavily on their fungal symbiont for nutrient absorption (Harley and Smith 1983). The studies upon which the theory of nonhydraulic root-to-shoot communication of soil drying is based have not indicated if experimental plants were mycorrhizal. As mycorrhizal symbiosis is known to affect general root metabolism and leaf growth (Harley and Smith 1983), Cs (Augé et al. 1987; Bethlenfalvay et al. 1987), leaf water status (Augé et al. 1987), root hydraulic conductivity (Andersen et al. 1988) and soil drying (Faber et al. 1991), the presence or absence of mycorrhizal fungi might influence the nonhydraulic signalling process.

Previous investigators have sometimes concluded that mycorrhizal modification of plant water relations or plant response to drought is the result of enhanced P nutrition of the mycorrhizal host (Nelsen and Safir 1982; Koide 1985; Fitter 1988), as the most consistent and dramatic consequence of mycorrhizal symbiosis is a greatly improved system for scavenging P from soil. We wanted to learn whether mycorrhizal symbiosis could alter leaf growth response to nonhydraulic signals of soil drying, independently of leaf P concentration, overall plant size or soil drying rate. The experiment was also designed to determine whether varied P nutrition played a role in nonhydraulic root-to-shoot signalling, at least within the range specified below. We chose maize because its response to nonhydraulic root signals has been documented (Saab and Sharp 1989; Davies and Zhang 1991) and because it associates readily with vesicular-arbuscular mycorrhizal fungi.

Materials and methods

Plant material and growing conditions. On 16 September 1992, seeds of *Zea mays* L. 'Pioneer 3147' were planted in 1.25-l pots into an autoclaved medium containing (2:1, v/v) silica sand and calcined montmorillonite clay (Turface; Industrial Materials Corp., Deerfield, Ill., USA). Before planting, fresh sorghum (*Sorghum bicolor* [L.] Moench) pot culture (live root pieces and soil) was incorporated into the medium at 5 medium: 1 pot culture, v/v. Pot-culture roots were either nonmycorrhizal or were heavily colonized by *Glomus intraradices* Schenck & Smith UT143. After 12 d, seedlings were transplanted into fresh medium (same mixture and inoculation rates as above) with roots divided about equally between two 1-l pots. In each pot, a 2.5- to 3.0-cm layer of sterile sand was placed above and below the inoculated mixture to deter cross-contamination. Plants were grown with root systems constrained to and fully ramifying small volumes, in an attempt to minimize differences in rate of soil water extraction between mycorrhizal and nonmycorrhizal plants. Before and after transplanting, and during the drying period, watered pots of all plants were fertilized at each irrigation with 14.3 mM N, 4.2 mM K and 3.6 mM Ca as 15–0–15 soluble fertilizer (Grace-Sierra, Milpitas, Cal., USA). Soluble trace elements (S.T.E.M.; Grace-Sierra) were applied once a month at 5 mg·liter^{-1}. Concentrations of Mg and S in the tap water used for irrigation were each 0.3 mM and no additional Mg or S fertilization was provided. Before transplanting, all plants were given 1.0 mM P as KH_2PO_4 once a week. The entire experiment was conducted in a glasshouse in Knoxville under ambient light. Usual day/night temperatures ranged from 24–32/20–22 C. All roots were watered daily until the drying treatment began.

Treatments, experimental design and statistical analysis. Treatments were applied in a $2 \times 2 \times 3$ design, with seven replicates of each treatment. There were two levels of mycorrhizal colonization (mycorrhizal fungi present or absent) and two levels of watering (both pots watered or one pot watered, one allowed to dry). Soil drying was begun 18 d after transplanting (16 October), and the one drying episode continued until the end of the experiment. Following transplanting, plants received three levels of P fertilization applied daily as KH_2PO_4, and these were different for mycorrhizal and nonmycorrhizal plants. Mycorrhizal plants received 0 mM P (low P), 0.6 mM P (med P) and 1.2 mM P (high P). Nonmycorrhizal plants received 1.2 mM P (low P), 2.4 mM P (med P) and 3.6 mM P (high P). Our aim was to produce mycorrhizal and nonmycorrhizal control plants of similar size, so that size would not confound our comparisons of colonization and watering treatments on shoot response. Within each P level (low, med and high), mycorrhizal and nonmycorrhizal controls were similar in size throughout the experiment (see *Results* section). Therefore, we analyzed all data by analysis of variance (ANOVA) as a $2 \times 2 \times 3$ factorial with P levels designated as "low", "med" and "high". Main effects and comparisons of individual treatments with linear and quadratic contrasts (Steel and Torrie 1980) were used to test three hypotheses: (1) nonhydraulic root-to-shoot signalling of soil drying would occur in maize, (2) mycorrhizal symbiosis would modify the influence of the nonhydraulic signal on shoot behavior, and (3) varying P fertilization would modify the influence of the nonhydraulic signal on shoot behavior. Pooled standard errors of the means were calculated by taking square roots of the error mean squares and dividing them by the square root of the number of observations in a mean (Steel and Torrie 1980).

Stomatal conductance. Abaxial Cs of two leaves of each plant of each treatment was measured every 2–3 d with a diffusion porometer (AP4; Delta T Devices, Cambridge, UK). Measurements were begun between 1100 and 1200 h and completed within 2.5–3 h. Preliminary tests indicated that Cs was fairly constant on sunny days during this time. Measurements were made near the center of laminae, adjacent to mid-veins of unshaded, growing leaves.

Leaf and root Ψ. Leaf and root Ψ were measured with thermocouple psychrometers (SC-10; Decagon Devices, Inc., Pullman, Wash., USA), calibrated before each use with a graded series of NaCl solutions. Psychrometer sample changers were connected to nanovoltmeter thermometers (NT-3; Decagon Devices), used to derive temperature and μV readings for conversion into Ψ values. Leaf Ψ was sampled from 11:00 a.m. to 12:30 p.m. and from 1:30 to 3:00 p.m., on strips cut from laminae adjacent and parallel to mid-veins and quickly placed inside psychrometer chambers with abaxial surfaces exposed to the center of sample cups. Plants from partially dried treatments were compared with control plants on 7 d during the drying period. Due to the large number of plants, not all replicates of all treatments were sampled each day. Full sampling of all half-dried replicates of all levels of the mycorrhizae and P factors was accomplished three times during the drying period (days 6–7, days 12–13, days 18–20), by sampling twice a day for 2–3 consecutive days. The ANOVA model included main effects and interactions of each factor for each of these three full samplings, as well as time of day and date (variation over consecutive days within each full sampling). Additional ANOVA compared control and half-dried plants on each of the seven measurement days. At each time period each day, equal numbers of half-dried mycorrhizal and nonmycorrhizal plants were sampled. Preliminary tests indicated that neither mycorrhizae nor P treatments affected leaf Ψ of fully-watered controls; hence, leaf Ψ was not measured for every control plant but for a random sample of control plants at each time period. Water potentials of samples of all droughted root systems were compared at the end of the experiment. Root samples were removed near soil matric-potential sensors and consisted of the terminal 2–3 cm (about 0.2 g fresh weight) of healthy, secondary roots. Leaf and root samples were allowed to equilibrate within the psychrom-

eter for a minimum of 2 h, as preliminary tests had shown this sufficient to attain thermal and water vapor equilibrium.

Soil matric potential. Soil matric potential (Ψ_τ) was estimated with heat-dissipation sensors (Soiltronics, Burlington, Wash., USA). At transplanting, each sterilized sensor was coated with a slurry of kaolinite (a non-swelling clay which improves hydraulic conductivity between sensor and soil) and placed vertically in the middle of each pot that was later to be dried. Sensors consisted of a thermocouple and a heating element (evanohm wire, 0.076 mm diameter) housed in a fixed, porous, ceramic cylinder (diameter 1.5 cm, length 3.0 cm). Rate of heat dissipation within the ceramic housing is correlated with soil Ψ_τ as follows (Phene et al. 1971). The temperature of the ceramic is measured at 1 s and 21 s during a 21-s heat pulse. The temperature rise in the ceramic resulting from the heat pulse is a function of its moisture content, or Ψ_τ: the drier the ceramic (the lower its Ψ_τ), the slower heat will dissipate. If the ceramic housing is in equilibrium with the soil surrounding it (if ceramic Ψ_τ = soil Ψ_τ), then the temperature rise of the ceramic is directly dependent on soil Ψ_τ. Estimates of Ψ_τ are independent of soil type and, at constant soil water content, do not vary with temperature between 0 and 40°C. Sensors were calibrated by the manufacturer by relating temperature rise to soil Ψ_τ in soil having few solutes (negligible osmotic pressure). Soil Ψ_τ between 0 and −0.02 MPa was measured with a hanging water column, soil Ψ_τ between −0.02 and −0.10 MPa with a pressure plate (Soilmoisture Equipment Corp., Santa Barbara, Cal., USA) and soil Ψ ($\approx \Psi_\tau$) between −0.10 and −20 MPa with a thermocouple psychrometer (SC-10; Decagon Devices).

Soil Ψ_τ within each of the 42 drying pots was measured every 2 h and integrated over each day throughout the experiment. Heat pulses were administered and temperatures were recorded with a datalogger (21X; Campbell Sci., Logan, UT, USA). Sensors were connected to the datalogger through multiplexers (AM32; Campbell Sci.). Curves of the soil moisture characteristics were constructed for eight samples of the sand/Turface medium, from sensor measurements of soil Ψ_τ and gravimetric measurements of soil water content (sensors were buried in unplanted pots).

Growth and other analyses. The length of each leaf on each plant was measured with a ruler every 3–4 d during the drying period. Shoot and root dry weights were measured at the end of the experiment. Phosphorus concentrations of the third-youngest leaves and of samples of droughted roots, oven-dried at 70°C for ≥ 24 h, were assayed spectrophotometrically using the vanadate-molybdate-yellow method on samples dry-ashed with magnesium nitrate at 750°C for 2 h and digested in 5.6 N nitric acid (Chapman and Pratt 1961). Total leaf calcium, magnesium and sulfur were assayed on an inductively-coupled plasma spectrophotometer (Model 61; Thermo Jarrell Ash, Inc., Franklin, Mass., USA) on samples dry-ashed at 500°C for 24 h and digested in 2 N HCl. Vesicular-arbuscular mycorrhizal colonization was characterized by assessing the presence or absence of hyphae, vesicles and arbuscules as recommended by McGonigle et al. (1990).

Results

Growth. In attempting to discover if mycorrhizal fungi alter host physiology in ways unrelated to an effect on host P status, it is desirable to produce mycorrhizal and nonmycorrhizal plants of comparable size and P nutrition, to exclude the possibility that observed mycorrhizal influences are simply size-alteration effects or effects due to alleviation of P limitation. Fully watered (control) mycorrhizal and nonmycorrhizal *Zea mays* plants had similar total leaf lengths through the experiment (MC vs. NC, Fig. 1). Moreover, at each level of P fertilization (low, med and high) mycorrhizal and nonmycorrhizal plants

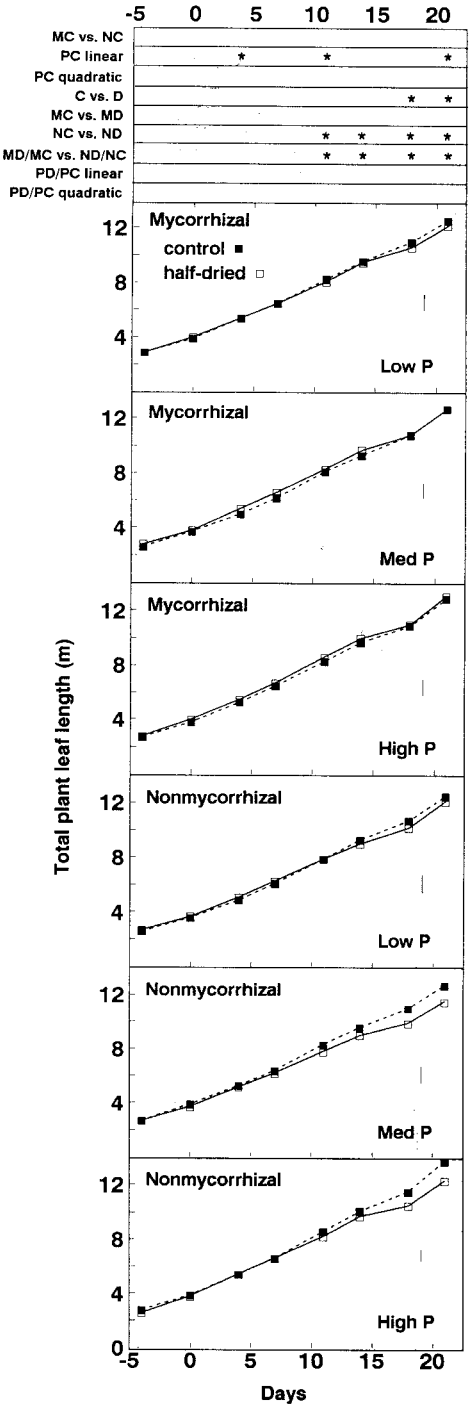

Fig. 1. Total leaf lengths of maize plants before and during the drying period, which was begun on day 0. $n = 7$. Linear and quadratic contrasts that compare mycorrhizal and nonmycorrhizal controls were computed for each day and are given at the top of the figure ("*" indicates significant at $P \leq 0.05$). *C*, control; *D*, half-dried; *M*, mycorrhizal; *N*, nonmycorrhizal; *P*, phosphorus. Low-, med- and high-P M plants received 0.0, 0.6 and 1.2 mM P daily, respectively. Low-, med- and high-P N plants received 1.2, 2.4 and 3.6 mM P daily, respectively. In D/C contrasts, D plants were analyzed as percentages of the mean of their respective controls within each level of the mycorrhizae and phosphorus factors, i.e. MD low P/MC low P, etc. The *vertical line* in each plot represents 2 × pooled SE

Table 1A, B. Probability ($P \leq 0.05$) of significance of linear and quadratic contrasts for maize plant characteristics at the end of the experiment. **A** Three-factor comparisons; **B** one- and two-factor comparisons. C, control; D, half-dried; M, mycorrhizal; N, nonmycorrhizal; P, phosphorus; DW, dry weight. "*" denotes significant

A) Contrasts	shoot dry weight	Total root dry weight	Leaf length/root DW	Leaf P concentration
MC vs. NC				*
PC linear	*			*
PC quadratic				
C vs. D			*	*
MD vs. MC		*	*	*
ND vs. NC	*		*	*
MD/MC vs. ND/NC				
PD/PC linear				
PD/PC quadratic			*	*

B) Contrasts	Watered root vs. dried root DW[a]	Root Ψ[a]	Root P concentration[b]	Colonization rate[c]		
				Hyphal	Arbuscular	Vesicular
M vs. N			*	–	–	–
P linear		*	*			*
P quadratic			*			

[a] Fully watered control plants were not involved in comparisons
[b] Half-dried plants were not measured
[c] Half-dried plants were not measured. All nonmycorrhizal plants were uncolonized so were not involved in comparisons

were not significantly different in total leaf length on each measurement day, except for low-P plants on day 0 (contrasts not shown). Mycorrhizal and nonmycorrhizal controls also had similar final shoot dry weights, root dry weights and leaf length/root dry weight ratios (Table 1A; Fig. 2). Final total leaf length and shoot dry weight of controls increased slightly with increasing P fertilization (PC linear, Fig. 1 and Table 1A). Root dry weight and leaf length/root dry weight ratio did not respond to varied P fertilization (Table 1A).

Half-dried mycorrhizal and nonmycorrhizal plants were each similar in total leaf length to their respective fully watered controls before the drying treatment began (MC vs. MD, NC vs. ND, Fig. 1). Control and half-dried mycorrhizal plants had similar total leaf lengths on all days of the experiment (MC vs. MD, Fig. 1) and similar final shoot dry weights (Table 1A, Fig. 2). However, by day 11 and each day thereafter, total leaf length of half-dried nonmycorrhizal plants was less than that of respective controls (NC vs. ND, Fig. 1). The MD/MC vs. ND/NC contrast, which compares half-dried mycorrhizal and nonmycorrhizal plants viewed as percentages of their respective controls, also signified that partial drying affected growth differently in mycorrhizal and nonmycorrhizal plants. Averaged over P treatments, final total leaf length of half-dried mycorrhizal plants was 99% that of watered controls, while total leaf length of nonmycorrhizal plants was 92% that of watered controls. Growth was not inhibited in low-P half-dried nonmycorrhizal plants. Final total leaf length of high-P and med-P half-dried nonmycorrhizal plants were 90% and 91% that of their respective watered controls. Previous experiments in our laboratory with maize and sorghum under similar conditions have shown that total plant leaf length declines of 10% translate roughly into area declines of about 20%. Shoot dry weight reflected the length differences; averaged over P treatments, final shoot dry weight of half-dried mycorrhizal plants was 103% that of watered controls, while shoot dry weight of nonmycorrhizal plants was 92% that of controls.

Plant nutrient concentration and colonization. Leaves of all treatments had ample P concentrations (above 2.5 $mg \cdot g^{-1}$; Jones 1985). Due chiefly to lower leaf P concentrations of low-P mycorrhizal plants, nonmycorrhizal controls had higher overall leaf P concentrations than mycorrhizal controls (Table 1A, Fig. 3), but there was also much overlap in mycorrhizal and nonmycorrhizal treatments. Medium- and high-P mycorrhizal plants had about the same leaf P concentrations as low- and med-P nonmycorrhizal plants. Phosphorus treatments had a linear effect in increasing leaf P in controls. The drying treatment lowered leaf P concentrations similarly in mycorrhizal and nonmycorrhizal plants (significant MC vs. MD and NC vs. MD, insignificant MD/MC vs. ND/NC). The effects of half-drying on leaf P were different at different levels of P fertilization (PD/PC quadratic). The P concentration of droughted roots was higher in nonmycorrhizal than in mycorrhizal plants (M vs. N) and increased with increasing P fertilization (P linear and quadratic, Table 1B).

The ANOVA indicated that there were no differences between mycorrhizal and nonmycorrhizal plants, or between med-P and high-P treatments, in final leaf concentrations of Ca, Mg or S (analysis not shown). Leaf Ca concentrations were significantly affected by watering treatment and averaged 0.16 and 0.19 $mg \cdot (g\ DW)^{-1}$ in watered and half-dried plants, respectively. Leaf concentrations of Mg and S, which averaged 0.12 and 0.10 $mg \cdot (g\ DW)^{-1}$, respectively, were not affected by the watering treatment. Low-P plants showed no nonhydraulically induced growth inhibition and so were not assayed for Ca, Mg or S.

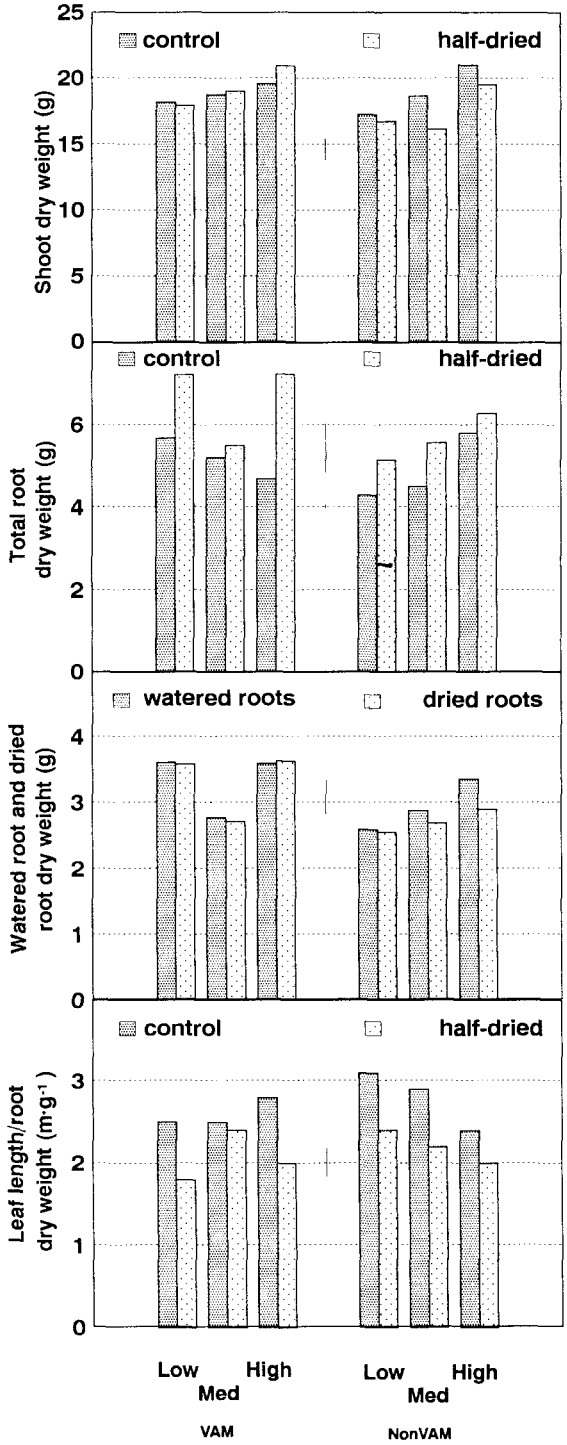

Fig. 2. Final size of maize plants: shoot dry weight of all plants, root dry weight of all plants, watered and dried root dry weight of half-dried plants, and total leaf length/total root dry weight ratio of all plants. $n = 7$. Low, med and high refer to low-, medium- and high-P fertilization treatments; *VAM*, mycorrhizal. The *vertical line* in each plot represents 2 × pooled SE

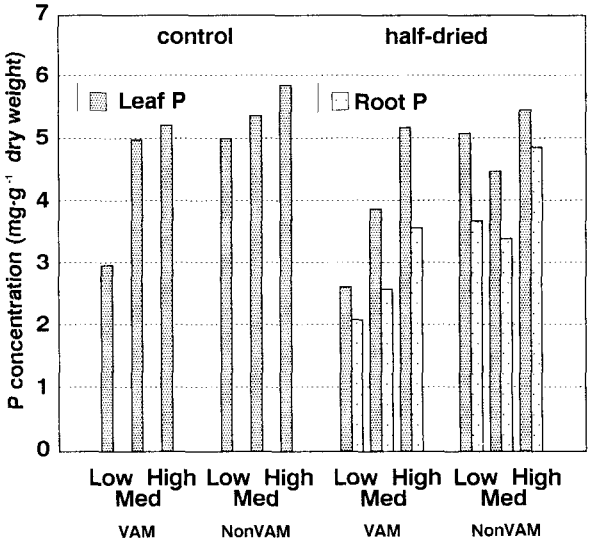

Fig. 3. Phosphorus concentrations of leaves ($n = 7$) and droughted roots ($n = 4$) of maize at the end of the experiment. Low, med and high refer to low-, medium- and high-phosphorus fertilization treatments; *VAM*, mycorrhizal. The *vertical line* in each plot represents 2 × pooled SE

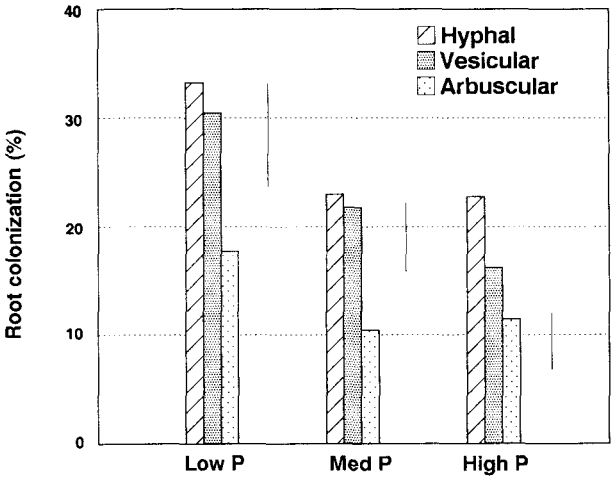

Fig. 4. Percentage of root system of fully watered maize plants colonized by hyphae, vesicles or arbuscules of *Glomus intraradices*. $n = 4$. *Vertical lines* represent 2 × pooled SEs for (left to right) hyphal, vesicular and arbuscular colonization

As usually occurs with most species of vesicular-arbuscular mycorrhizal fungi on most hosts (Harley and Smith 1983), increasing P fertilization decreased root colonization of maize by *Glomus intraradices* (Fig. 4, Table 1B). Vesicular colonization decreased in a linear manner ($r = 0.99$) with increased P, whereas hyphal and arbuscular colonization decreased from low to med P but were similar at med and high P.

Stomatal conductance. Stomatal conductance was similar in control and half-dried plants before drying began (C vs. D, Fig. 5). At 10, 18 and 20 d of drying, Cs of half-dried plants was lower than that of controls. Declines on day 10 (to 76% of controls) were observed only in half-dried mycorrhizal plants (MC vs. MD), plants which showed no growth reductions with half-drying. Declines on days 18 and 20 (to 76% and 60% of controls, respectively) were observed only in half-dried nonmycorrhizal plants (NC vs. ND), plants whose growth was diminished

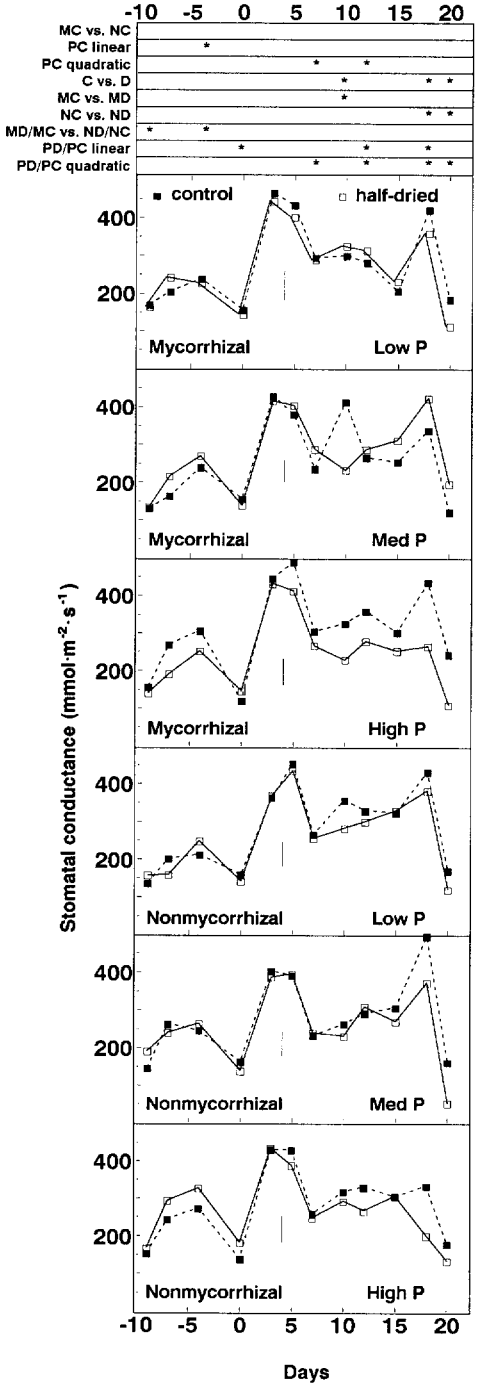

Fig. 5. Stomatal conductance of maize leaves before and during the drying period; water was withheld from half-dried plants beginning on day 0. $n = 7$. Linear and quadratic contrasts were computed for each day and are given at the top of the figure ("*" indicates significant at $P \leq 0.05$). *C*, control; *D*, half-dried; *M*, mycorrhizal; *N*, nonmycorrhizal. In D/C contrasts, D plants were analyzed as percentages of the mean of their respective controls within each level of the mycorrhizal and phosphorus factors, i.e. MD low P/MC low P, etc. The *vertical line* in each plot represents 2 × pooled SEs

by half-drying. Previous authors have ascribed Cs declines in maize of up to 50% or more to nonhydraulic signalling (Blackman and Davies 1985; Zhang and Davies 1989, 1990). Mycorrhizal and nonmycorrhizal

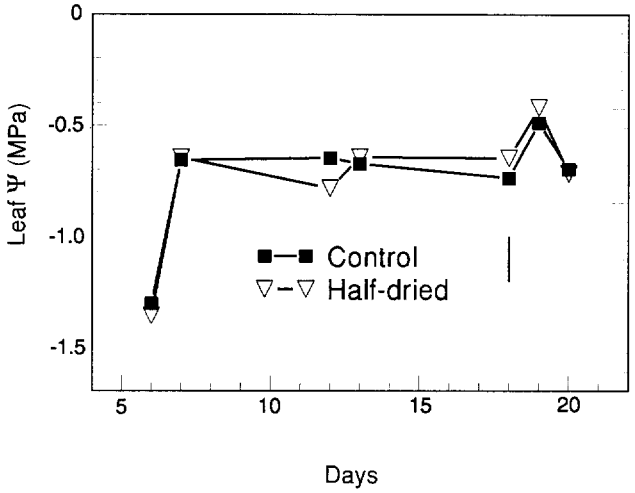

Fig. 6. Leaf water potential (Ψ) of control and half-dried plants (mycorrhizae and P factors combined); water was withheld beginning on day 0. None of the mycorrhizae, P or water treatments significantly affected leaf Ψ (analysis not shown). $n = 10$–24 for half-dried plants, $n = 3$–11 for control plants. The *vertical line* represents 2 × pooled SEs

controls did not differ in Cs on any day before or during drying. This is not an uncommon finding (Graham and Syvertsen 1984; Fitter 1988), although mycorrhizal plants have often had higher Cs than adequately nourished nonmycorrhizal plants (Augé et al. 1987; Bethlenfalvay et al. 1987; Wang et al. 1989). In a previous study involving *Rosa hybrida*, colonization of roots by *G. intraradices* changed response of Cs to partial soil drying (Augé and Duan 1991).

Significant effects of P treatments on Cs of control plants occurred occasionally (PC linear and PC quadratic). Phosphorus fertilization did influence the effect of half-drying on Cs for much of the drying period; PD/PC linear and/or quadratic effects were noted on days 7, 12, 18 and 20. The Cs of half-dried plants given high P appeared to be lower relative to controls than the Cs of half-dried plants given less P.

Leaf and root Ψ. Leaf Ψ (Fig. 6) of equal numbers of mycorrhizal and nonmycorrhizal plants were sampled on consecutive days, and full data sets for half-dried plants evaluated by ANOVA. Leaf Ψ was not affected by the watering, mycorrhizae or P treatments; the same contrasts listed at the top of Figs. 1 and 5 (excluding PC linear and PC quadratic) were performed for leaf Ψ and none were significant. Moreover, ANOVA showed no watering × phosphorus, watering × mycorrhizae, watering × date (variation over consecutive days) or watering × mycorrhizae × phosphorus interactions. As an additional test, we examined via ANOVA the effect of the watering factor on each of the seven sampling days, comparing control vs. half-dried plants for each day. Again, there was no significant watering effect or watering interaction on any day. Leaf Ψ was sampled at two time periods, 11:00 a.m.–12:30 p.m. and 1:30 p.m.–3:00 p.m., periods during which transpiration is typically high, the demand for water is great and Ψ of control leaves is most

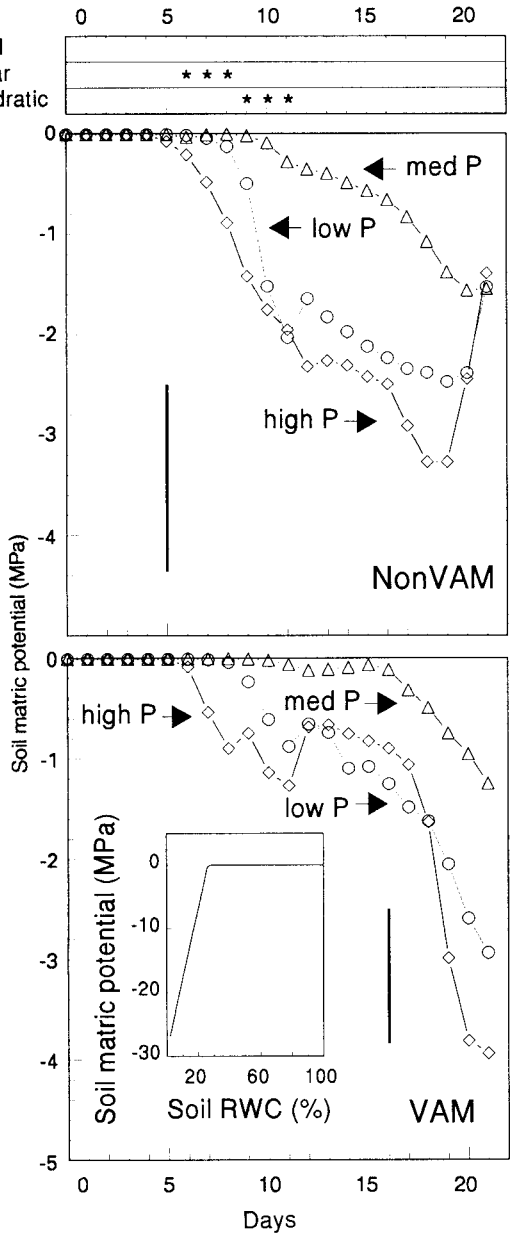

Fig. 7. Soil matric potential (Ψ_τ) in drying pots of half-dried nonmycorrhizal and mycorrhizal plants during the drying period; water was withheld beginning on day 0. $n = 7$. Soil Ψ_τ was measured every 2 h for each plant, integrated over each day and daily means compared ("*" indicates significant at $P \leq 0.05$). *M, VAM*, mycorrhizal; *N*, nonmycorrhizal. The *vertical line* in each plot represents 2 × pooled SEs. *Inset*, moisture-release plot of sand: Turface medium used in this study

likely to decline. There was no watering × time period interaction; at these times of day, leaf Ψ varied similarly in control and half-dried plants.

Final root Ψ of dried roots was affected by P treatment but not by mycorrhizae (Table 1B). Root Ψ was mostly not in equilibrium with the Ψ_τ of the surrounding soil. Mean root Ψ (and corresponding mean root Ψ – soil Ψ_τ difference at sampling) of half-dried plants was −2.47 (2.29) MPa for low-P mycorrhizal plants, −1.60 (0.78) MPa for med-P mycorrhizal plants, −0.77 (2.50) MPa for high-P mycorrhizal plants, −1.16 (0.88) MPa for low-P nonmycorrhizal plants, −1.21 MPa (0.15) for med-P nonmycorrhizal plants and −0.89 (0.86) MPa for high-P nonmycorrhizal plants.

Soil matric potential. The experiment was unique in that it enabled us to compare rates of soil drying in mycorrhizal and nonmycorrhizal plants under continued high (rather than progressively decreasing) transpiration. Rate of decline in soil Ψ_τ was not affected by mycorrhizae (Fig. 7). Phosphorus fertilization affected soil drying rates on days 6 through 11; for the second half of the drying period P had no effect on drying rate. There were large variations in soil drying rates among individuals within treatments, compounded by the rehydration of dried soil that occurred in several plants. Eighteen half-dried root systems rehydrated dried soil around the sensor back to essentially 0 MPa at least once during the drying period, eight of these having dropped to −4 MPa or below. We examined soil Ψ_τ frequently and were aware of the rehydration throughout the experiment. We assured that no water was inadvertently entering soil of drying pots from the top or bottom of pots. Presumably drying roots were being rehydrated by water movement from watered roots, and rehydration was substantial enough to remoisten the rhizosphere (Ψ_τ sensors were in close contact with roots). The root Ψ – soil Ψ_τ differences noted above further suggest that roots in dry pots received water from roots in watered pots. Others have noted water movement between watered and drying roots in split-root maize plants under similar circumstances (Saab and Sharp 1989) and in potted sorghum (Xu and Bland 1993).

Discussion

Nonhydraulic signalling in maize. The C vs. D linear contrast is a test of our first hypothesis – that nonhydraulic root-to-shoot signalling of soil drying would occur in maize – assuming that reasonable proof is provided showing that the drying treatment did not result in direct hydraulic limitation of leaf growth or stomatal behavior. We observed no differences in leaf Ψ between controls and half-dried plants on any of the seven measurement days. Growth declines occurred only in nonmycorrhizal plants; by day 11, half-dried nonmycorrhizal plants were smaller than their fully watered controls, yet there were no measured differences in Cs between half-dried and control nonmycorrhizal plants until 7 d later. That declines in leaf length preceded declines in Cs is also suggestive of a lack of leaf water deficit. Stomatal closure in droughted maize has previously been shown to be sensitive to very slight declines in soil Ψ (less than 0.02 MPa) with stomatal closure occurring before any changes in leaf Ψ were detectable (Davies et al. 1980).

Others have noted that declines in maize leaf growth preceded any change in Cs (Saab and Sharp 1989). Leaf area of maize was reduced in that study by 15% after 18 d of drying half the root system. This agrees with our average decrease of 8% in final total leaf length (which translates roughly into a 16% decrease in leaf area) in

nonmycorrhizal maize plants. In sorghum, which has a similar leaf length/area relation to maize, we have noted decreases of 10% and 18% in total leaf length and leaf area, respectively, in nonmycorrhizal plants when half of the roots were subjected to soil drying, also without observing any decline in Cs. Others, working under different conditions with a maize cultivar different from ours, have observed that Cs can be restricted by nonhydraulic signalling several days before leaf growth declines (Zhang and Davies 1990). Leaf area in that study was also reduced by 18% after 20 d of partial soil drying.

Mycorrhizal alteration of nonhydraulic signalling. Our second hypothesis was verified in maize, at least in terms of leaf growth: mycorrhizal symbiosis not only modified, but eliminated, leaf growth response to the nonhydraulic signal. We have observed a similar influence of mycorrhizal symbiosis on nonhydraulic root-to-shoot signalling in sorghum, where final total plant leaf area was reduced by 18% in half-dried nonmycorrhizal plants but was not reduced in half-dried mycorrhizal plants. Production or transport of the signal(s), and/or sensitivity of leaf elongation to the signal(s), must have been greater in nonmycorrhizal plants in these studies. Although mycorrhizal plants showed no signal effect in terms of final size, there was a decrease in leaf-extension rate (data and analysis not shown) during one time period toward the end of the experiment, allowing the possibility for an eventual influence of nonhydraulic signalling on mycorrhizal plants.

Others have observed that differences in drought response between similarly fertilized nonmycorrhizal and mycorrhizal plants were eliminated when nonmycorrhizal plants could be provided with enough additional P to also eliminate differences in plant size (Safir et al. 1972; Koide 1985; Fitter 1988). Since our nonmycorrhizal and mycorrhizal controls had similar total leaf lengths, shoot dry weights and root dry weights, the mycorrhizal effect was not related to a change in plant size. Even when overall plant biomass is similar, though, mycorrhizal plants can have different shoot/root ratios than nonmycorrhizal plants of the same species growing under similar conditions (e.g. Kothari et al. 1990), and shoot/root ratio can play a role in regulation of stomatal behavior of some species (Meinzer et al. 1991). Mycorrhizal and nonmycorrhizal controls had similar ratios of total leaf length to root dry weight (and hence similar ratios of leaf area to root dry weight, assuming mycorrhizal symbiosis did not alter leaf morphology), eliminating this possibility for the mycorrhizal effect.

As rate of decline in soil Ψ_τ in this study was not affected by mycorrhizal fungi, differences in soil drying rates do not appear to explain the difference between mycorrhizal and nonmycorrhizal plants. In plants that showed significant growth reductions with half-drying (nonmycorrhizal plants), there was no correlation ($r = 0.24^{NS}$) between final total leaf length and the time each was exposed to low soil Ψ_τ (days at or below -0.60 MPa, typical mean leaf Ψ); a previous study had noted that inhibition of leaf elongation began when soil Ψ_τ approached that of leaves (Saab and Sharp 1989). Nor were final growth reductions correlated with number of days required for soil Ψ_τ to drop below control levels ($r = 0.14^{NS}$).

Mobility and availability of P in soils is reduced by soil drying (Viets 1972), and root colonization by mycorrhizal fungi has previously been required to alleviate P limitation even in soils having relatively high P (Nelsen and Safir 1982; Sylvia et al. 1993). In our study, neither plant size nor leaf P concentration was preferentially reduced in nonmycorrhizal plants, so differences in P concentration among plants did not appear to explain the absence of a leaf growth response to the signal in mycorrhizal plants. Medium-P and high-P mycorrhizal plants had leaf P concentrations similar to those of low-P and med-P nonmycorrhizal plants, respectively, yet the two groups of mycorrhizal plants still showed no growth reductions with drying and the two groups of nonmycorrhizal plants still showed growth reductions (mean final total leaf length 93% of controls). Growth reductions were not correlated with leaf P concentration ($r = 0.16^{NS}$), root P concentration ($r = 0.09^{NS}$), drying-root dry weight ($r = 0.32^{NS}$), total root dry weight ($r = 0.14^{NS}$) or leaf length/drying-root dry weight ratio ($r = 0.14^{NS}$).

Leaf phosphorus concentration and nonhydraulic signalling. Our third hypothesis, tested by the PD/PC polynomial contrast, was validated: varying P fertilization modified nonhydraulic effects on shoot growth and on Cs. Varying soil phosphate availability could conceivably affect nonhydraulic signalling of reduced soil water availability in several ways. Previous studies have concluded that the ion composition of xylem sap probably modulates stomatal response to nonhydraulic root-to-shoot communication of soil drying (Gollan et al. 1992) and that there is a close connection between the effectiveness of abscisic acid as a root-to-shoot signal and the nutritional status of the plant (Schurr et al. 1992). Tiny cation/anion imbalances in xylem sap may also regulate the influence of the nonhydraulic signal (Hartung and Radin 1989). Leaf P status has been shown to affect Cs, at least in P-starved plants (e.g. Atkinson and Davison 1972; Nagarajah and Ratnasuriya 1978) and stomatal sensitivity to abscisic acid can vary as a function of phosphate status of the leaf (Radin 1984). Several investigators have suggested the likely importance of plant nutrition in determining or affecting the signalling process (Boyer 1989; Saab and Sharp 1989; Davies et al. 1990; Jones 1990).

In conclusion, we have demonstrated that mycorrhizal symbiosis can alter nonhydraulic root-to-shoot signalling of soil drying. This is important because live roots of most native and crop plants typically associate with mycorrhizal fungi for most of their life cycles. Unlike most past experiments in mycorrhizal drought physiology, the mycorrhizal influence was not related to an alteration of plant size, shoot/root ratio, leaf P concentration or soil drying rate. Regardless of mycorrhizae, nonhydraulically induced declines in stomatal conductance and leaf growth were influenced by phosphorus fertilization,

highlighting both the importance of mineral nutrition in regulating nonhydraulic response to drought and the caution required in planning and interpreting mycorrhizal drought research.

This work was supported by the U.S. Department of Agriculture grant No. 91-37100-6723 and a University of Tennessee Professional Development Research Award to R.M.A. We thank Angela Berry for the graphics.

References

Andersen, C.P., Markhart, A.H. III, Dixon, R.K., Sucoff, E.I. (1988) Root hydraulic conductivity of vesicular-arbuscular mycorrhizal green ash seedlings. New Phytol. **109**, 465–471

Atkinson, D., Davison, A.W. (1972) The influence of phosphorus deficiency on the transpiration of *Arctium minus* Bernh. New Phytol. **71**, 317–326

Augé, R.M., Duan, X. (1991) Mycorrhizal symbiosis and nonhydraulic root signals of soil drying. Plant Physiol. **97**, 821–824

Augé, R.M., Schekel, K.A., Wample, R.L. (1987) Leaf water and carbohydrate status of VA mycorrhizal rose exposed to water deficit stress. Plant Soil **99**, 291–302

Bethlenfalvay, G.J., Brown, M.S., Mihara, K.L., Stafford, A.E. (1987) *Glycine-Glomus-Rhizobium* symbiosis. V. Effects of mycorrhiza on nodule activity and transpiration in soybeans under drought stress. Plant Physiol. **85**, 115–119

Blackman, P.G., Davies, W.J. (1985) Root to shoot communication in maize plants of the effects of soil drying. J. Exp. Bot. **36**, 39–48

Boyer, J.S. (1989) Water potential and plant metabolism: comments on Dr. P. J. Kramer's article 'Changing concepts regarding plant water relations', Vol. 11, No. 7, pp. 565–568, and Dr. J. B. Passioura's Response pp. 569–571. Plant Cell Environ. **12**, 213–216

Chapman, H.D., Pratt, P.F. (1961) Methods of analysis for soils, plants and waters, p. 161–174. University of California, Riverside, CA, USA

Davies, W.J., Zhang, J. (1991) Root signals and the regulation of growth and development of plants in drying soil. Annu. Rev. Plant Physiol. **42**, 55–76

Davies, W.J., Mansfield, T.A., Wellburn, A.R. (1980) A role for abscisic acid in drought endurance and drought avoidance. In: Plant growth substances 1979, pp. 242–253, Skoog, F., ed. Springer, Berlin Heidelberg New York

Davies, W.J., Mansfield, T.A., Hetherington, A.M. (1990) Sensing of soil water status and the regulation of plant growth and development. Plant Cell Environ. **13**, 709–719

Faber, B.A., Zasoski, R.J., Munns, D.N., Shackel, K. (1991) A method for measuring hyphal nutrient and water uptake in mycorrhizal plants. Can. J. Bot. **69**, 87–94

Fitter, A.H. (1988) Water relations of red clover *Trifolium pratense* L. as affected by VA mycorrhizal infection and phosphorus supply before and during drought. J. Exp. Bot. **39**, 595–603

Gollan, T., Schurr, U., Schulze, E.-D. (1992) Stomatal response to drying soil in relation to changes in the xylem sap composition of *Helianthus annuus*. I. The concentration of cations, anions, amino acids in, and pH of, the xylem sap. Plant Cell Environ. **15**, 551–559

Graham, J.H., Syvertsen, J.P. (1984) Influence of vesicular-arbuscular mycorrhiza on the hydraulic conductivity of roots of two citrus rootstocks. New Phytol. **97**, 277–284

Harley, J.L., Smith, S.S. (1983) Mycorrhizal symbiosis. Academic Press, New York

Hartung, W., Radin, J.W. (1989) Abscisic acid in the mesophyll apoplast and in the root xylem sap of water-stressed plants: the significance of pH gradients. In: Current topics in plant biochemistry and physiology, vol. 8, pp. 110–124, Randall, D.D., Blevins, D.G., eds. University of Missouri, Columbia, USA

Jones, A.J. (1985) C4 grasses and cereals, p. 267. Wiley, New York Chichester Toronto

Jones, H.G. (1990) Control of growth and stomatal behavior at the whole plant level: effects of soil drying. In: Importance of root to shoot communication in the responses to environmental stress (Symp. Proc. Brit. Soc. Plant Growth Reg., monograph 21), pp. 81–93, Davies, W.J., Jeffcoat, B., eds. Parchments Ltd., Oxford, UK

Koide, R. (1985) The effect of VA mycorrhizal infection and phosphorus status on sunflower hydraulic and stomatal properties. J. Exp. Bot. **168**, 1087–1098

Kothari, S.K., Marschner, H., George, E. (1990) Effect of VA mycorrhizal fungi and rhizosphere microorganisms on root and shoot morphology, growth and water relations in maize. New Phytol. **116**, 303–311

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A. (1990) A new method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. New Phytol. **115**, 495–501

Meinzer, F.C., Grantz, D.A., Smit, B. (1991) Root signals mediate coordination of stomatal and hydraulic conductance in growing sugarcane. Aust. J. Plant Physiol. **18**, 329–338

Nagarajah, S., Ratnasuriya, G.B. (1978) The effect of phosphorus and potassium deficiencies on transpiration in tea (*Camellia sinensis*). Physiol. Plant. **42**, 103–108

Nelsen, C.E., Safir, G.R. (1982) Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. Planta **154**, 407–413

Phene, C.J., Hoffman, G.J., Rawlins, S.L. (1971) Measuring soil matric potential in situ by sensing heat dissipation within a porous body: I. Theory and sensor construction. Soil Sci. Soc. Am. Proc. **35**, 27–33

Radin, J.W. (1984) Stomatal responses to water stress and to abscisic acid in phosphorus-deficient cotton plants. Plant Physiol. **76**, 392–395

Saab, I.N., Sharp, R.E. (1989) Non-hydraulic signals from maize roots in drying soil: inhibition of leaf elongation but not stomatal conductance. Planta **179**, 466–474

Safir, G.R., Boyer, J.S., Gerdemann, J.W. (1972) Nutrient status and mycorrhizal enhancement of water transport in soybean. Plant Physiol. **49**, 700–703

Schurr, U., Gollan, T., Schulze, E.-D. (1992) Stomatal response to drying soil in relation to changes in the xylem sap composition of *Helianthus annuus*. II. Stomatal sensitivity to abscisic acid imported from the xylem sap. Plant Cell Environ. **15**, 561–567

Steel, R.G.D., Torrie, J.H. (1980) Principles and procedures of statistics, 2nd edn. McGraw-Hill, New York

Sylvia, D.M., Hammond, L.C., Bennett, J.M., Haas, J.H., Linda, S.B. (1993) Field response of maize to a VAM fungus and water management. Agron. J. **85**, 193–198

Tardieu, F., Davies, W.J. (1993) Integration of hydraulic and chemical signalling in the control of stomatal conductance and water status of droughted plants. Plant Cell Environ. **16**, 341–349

Viets, F.G. (1972) Water deficits and nutrient availability. In: Water deficits and plant growth, vol. 3., pp. 217–239, Kozlowski, T.T., ed. Academic Press, London New York Tokyo

Wang, G.M., Coleman, D.C., Freckman, D.W., Dyer, M.I., McNaughton, S.J., Acra, M.A., Goeschl, J.D. (1989) Carbon partitioning patterns of mycorrhizal versus non-mycorrhizal plants: real-time dynamic measurements using CO_2. New Phytol. **112**, 489–493

Xu, X., Bland, W.L. (1993) Reverse water flow in sorghum roots. Agron. J. **85**, 384–388

Zhang, J., Davies, W.J. (1989) Abscisic acid produced in dehydrating roots may enable the plant to measure the water status of the soil. Plant Cell Environ. **12**, 73–81

Zhang, J., Davies, W.J. (1990) Changes in the concentration of ABA in xylem sap as a function of changing soil water status can account for changes in leaf conductance and growth. Plant Cell Environ. **13**, 277–285