Mycorrhiza (1996) 6:119–127

ORIGINAL PAPER

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Arbuscular mycorrhizal symbiosis and nonhydraulic signaling of soil drying in *Vigna unguiculata* (L.) Walp.

Accepted: 4 August 1995

Abstract We examined the influence of *Glomus intraradices* on nonhydraulic signaling of soil drying, in a drought-avoiding plant having stomates that are extremely sensitive to changes in soil moisture. Cowpea [*Vigna unguiculata* (L.) Walp. 'White Acre'] seedlings were grown in a greenhouse 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 UT143), 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). Stomatal conductance was mostly similar in fully watered mycorrhizal and nonmycorrhizal controls. However, g_s of half-dried, nonmycorrhizal plants was reduced on fewer days and to a lesser extent than g_s of half-dried, mycorrhizal plants, perhaps related to quicker soil drying in mycorrhizal pots. The partial soil drying treatment had little effect on leaf relative water content or osmotic potential, indicating that declines in g_s and leaf growth were induced by some nonhydraulic factor. Leaf growth was inhibited only in nonmycorrhizal plants, evidently due to a difference in phosphorus nutrition between mycorrhizal and nonmycorrhizal plants. The mycorrhizal effect on g_s was not associated with phosphorus nutrition. Inhibition of g_s was directly related to extent of soil drying, while inhibition of leaf growth was inversely related to extent of soil drying.

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Key words Cowpea · Drought · *Glomus intraradices* · Phosphorus nutrition · Root signals

Introduction

Colonization of root systems by arbuscular mycorrhizal (AM) fungi can affect the stomatal behavior of host plants under well-watered conditions (Levy and Krikun 1980; Nelsen and Safir 1982; Augé et al. 1986) and during drought (Allen et al. 1981; Augé et al. 1987; Bethlenfalvay et al. 1990). Mycorrhizal effects on stomatal conductance (g_s) have often been associated with an improvement of host phosphorus nutrition (Safir et al. 1972; Graham and Syvertsen 1984; Koide 1985; Fitter 1988). Other experiments have shown that a mycorrhizal influence on g_s can occur independently of changes in leaf P concentration or plant size (Allen and Boosalis 1983; Augé et al. 1986, 1987; Bethlenfalvay et al. 1990). Possible explanations, unrelated to host nutrition, for a mycorrhizal effect on g_s include altered root or whole plant hydraulic conductivity (Allen et al. 1981), increased scavenging of soil water by extraradical hyphae (Allen 1982; Hardie 1985; Faber et al. 1991) or altered rate of hormone movement from roots to leaves (Levy and Krikun 1980; Allen et al. 1982). Recent findings concerning hormonal control of stomata of drying plants (Davies et al. 1994) has renewed our interest in the latter idea.

Stomatal closure can occur in plants with partially dried root systems, apparently in response to a nonhydraulic signal(s) moving from dehydrating roots to leaves (Davies et al. 1994). We have tested the idea that mycorrhizal influence on leaf growth and g_s of partially dried plants may be due in part to some change in the nonhydraulic root to shoot signaling process. With the species and growing conditions in our previous experiments, leaf growth was more sensitive than g_s to nonhydraulic signaling. In *Zea mays,* reductions in leaf growth appeared earlier and at higher soil moisture levels than reductions in g_s (Augé et al. 1994). In *Sorghum*

bicolor, leaf growth was inhibited in the absence of any change in g_s (Ebel et al. 1994). The relative insensitivity of stomata in those experiments to the nonhydraulic signals hindered our ability to discern mycorrhizal influences on stomatal response to the signals.

Here we examined the influence of AM symbiosis on g_s and leaf growth of *Vigna unguiculata*, an extreme drought avoider having stomates that are quite sensitive to changes in soil moisture relative to other plants (Shackel and Hall 1983; Ludlow 1989). Roots of *V. unguiculata* readily associate with mycorrhizal fungi, which have been shown previously to alter g_s of this host when whole root systems were exposed to a particular low soil Ψ (Augé et al. 1992).

Materials and methods

Plant materials and culture

On 6 April 1993, cowpea [*Vigna unguiculata* (L.) Walp. 'White Acre'] seeds were planted in 1.25-l pots, 6 seeds per pot. The planting medium was (2:1, v:v) autoclaved silica sand:calcined montmorillonite clay (Turface; Industrial Materials Corp., Deerfield, Ill.) that was mixed with fresh nonmycorrhizal or mycorrhizal (*Glomus intraradices* Schenck & Smith UT143) pot culture at 6:1, v:v, autoclaved medium:pot culture. Pot cultures were nonmycorrhizal or mycorrhizal *Sorghum bicolor* (L.) Moench growing in the sand:Turface medium described above. At germination and later at transplanting (see below), a 2- to 3-cm layer of sterile sand was placed at the top and bottom of pots to prevent propagule movement between pots. Plants were watered and fertilized every 3 days with soluble fertilizers at 12.0 mM N, 3.5 mM K and 3.0 mM Ca (15–0–15, Grace-Sierra, Milpitas, Calif.), 100 μM Fe as Sequestrine 138, and a soluble trace elements mix at 15 μM Mn (STEM, Grace-Sierra).

Seedlings were transplanted on 23 and 24 April 1993 (17 and 18 days after planting), with roots of each plant divided between two 1-l square pots containing the medium described above mixed with fresh nonmycorrhizal or mycorrhizal pot culture (4:1, v:v, autoclaved medium:pot culture). Both halves of a root system were either mycorrhizal or nonmycorrhizal. Both pots of each plant were fertilized twice per week for the first 3 weeks and then daily until soil drying began, with the fertilizer solution described above. All plants were given P, as KH_2PO_4, once before transplanting (18 April 1993) and once per week after transplanting, with mycorrhizal plants receiving either 0.3, 0.6 or 1.2 mM P and nonmycorrhizal plants receiving either 1.2, 2.4 or 3.6 mM P. Phosphorus fertilization was varied to obtain some overlap in size between mycorrhizal and nonmycorrhizal plants and thus control for effect of plant size, when evaluating effects of colonization and watering treatments on shoot response. Plants were grown in a glass greenhouse, shaded with 47% transmission shade cloth to assist in temperature control. Air temperatures ranged from 20–35 °C during the day and remained near 20 °C at night. Maximum daily photosynthetic photon flux density, measured every 10 min with a quantum sensor (LiCor, Lincoln, Neb.), ranged from 50 to 600 μmol m^{-2} s^{-1} during soil drying.

Experimental design and statistical analysis

Treatments were applied in a 2×3×2 factorial design with eight replicates per treatment (96 total plants): two colonization treatments (mycorrhizal, nonmycorrhizal), three phosphorus fertilization treatments, and two watering treatments (control: both pots watered; half-dried: one pot watered, one pot allowed to dry). Plants were allowed to become established for 3 weeks after transplanting before initiating watering treatments on 14 May 1993, hereafter referred to as day 0. Both pots of all plants were watered on day 0. Subsequently, one pot of "half-dried plants" and both pots of control plants were watered daily, while the second pot of half-dried plants received no further water for the duration of the experiment. Plants were harvested 26–28 days after day 0. Because of the difference in phosphorus fertilization for the two colonization treatments, data were analyzed by ANOVA with colonization treatments nested within phosphorus treatments. Main effects and linear contrasts were computed to help test three hypotheses: (1) g_s and shoot growth of cowpea can be suppressed by nonhydraulic signals of soil drying, (2) suppression of shoot behavior due to nonhydraulic signaling of soil drying will differ in mycorrhizal and nonmycorrhizal plants, (3) suppression of shoot behavior due to nonhydraulic signaling of soil drying will differ in plants fertilized by different amounts of P. 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.

Plant and soil measurements

On day 0, shoots were excised from root systems of five extra mycorrhizal and five extra nonmycorrhizal plants. One pot of each of these plants was used to check root colonization, and roots in the other pot of each plant were left in pots and allowed to dry on the greenhouse bench next to remaining experimental plants. During the drying period, soil matric potential ($\Psi\tau$) was estimated with heat dissipation sensors (Soiltronics, Burlington, Wash.) as described previously (Augé et al. 1994; Ebel et al. 1994). At harvest, gravimetric measurements of soil water content (θ) were made on samples removed near $\Psi\tau$ sensors, from each unwatered pot of the 48 half-dried experimental plants as well as from samples from the above 10 pots lacking shoots. Water potential (Ψ) of soil samples from each of the 10 pots lacking shoots was also measured, with thermocouple psychrometers (SC-10, Decagon, Pullman, Wash.). Abaxial g_s of the second and third youngest, unshaded lateral leaflets (midway between midrib and margin) of each plant was measured every 2–3 days with a diffusion porometer (AP4, Delta-T Devices, Cambridge, England), calibrated immediately before each sampling. Measurements were made at ambient CO_2 and vapor pressure deficit between 1100 and 1400 h, a time period during which previous tests had revealed no consistent, significant diurnal changes in g_s. Relative water content (RWC) and osmotic potential ($\Psi\pi$) of leaves were measured twice weekly, between 1100 and 1400 h. Fresh weight, weight after rehydration and dry weight were recorded for strips (about 2×1 cm) excised from the margins of lateral leaflets. After excising samples for RWC measurements, the remainder of each leaflet was immediately frozen in liquid N_2, sealed in a 1-ml syringe and stored at –20 °C until analysis of $\Psi\pi$. Osmotic potential of leaf sap, expressed from syringes after thawing 15–20 min, was measured with a vapor pressure osmometer (5500, Wescor, Logan, Utah), calibrated daily with a graded series of NaCl solutions.

At harvest (days 26–28), shoots were cut at the base, quickly weighed and xylem sap collected as described previously (Zhang and Davies 1990b). Four 2-cm sections of stem were cut from the bottom of the shoot, and the basal end of each stem segment was suspended and sealed above the bottom of a 1.5-ml microcentrifuge tube with parafilm. Tubes were spun at 1500 rpm for 15 min, removing 25–70 μl xylem sap per stem segment, resulting in 100–200 μl per plant. Each tube was then capped, frozen in liquid N_2, stored at –20 °C and lyophilized, pending abscisic acid (ABA) analysis using indirect ELISA. ELISA procedures, generation of the standard curves and calculation of ABA concentrations in samples were as described by Walker-Simmons (1987). Monoclonal antibody was obtained from Idetek (San Bruno, Calif.), and ABA conjugate was made according to Quarrie and Galfre (1985), except that the conjugate was dialyzed three times in 5 l of buffer. Lyophilized samples were extracted in 80% methanol con-

taining 100 mg l^{-1} of butylated hydroxytoluene for 2 h at 4 °C in the dark and centrifuged for 30 min at 10 000 *g*. The supernatant was dried and redissolved in 100 µl of methanol and 900 µl of water. Serial dilutions of this extract were assayed to ensure that the sample would fall within the range of the standard curve. Three separate aliquots were measured from each plant replicate. Flat-bottom, 96-well, disposable, microtiter plates (Immulon 4, Dynatech Laboratories, Alexandria, Va.) were used because they consistently provided high binding of the ABA-BSA conjugate compared to other plates tested. The outer most rows and columns were not used, to improve uniformity. Plates were incubated at 25 °C in the dark for about 1 h until the wells with no ABA gave an absorbance of approximately 1.0 at 405 nm. The absorbance was read using a microplate reader (E-max, Molecular Devices, Menlo Park, Calif.). Triplicate ABA standards were assayed for each plate. Validation of the ELISA assay for use with unpurified xylem exudate was confirmed by a dilution/spike recovery test for nonspecific interference. Either 22.5 or 25.0 µl of 10% xylem sap, diluted with distilled water, were added to four concentrations of (±) ABA standard and assayed. Plots of ABA added versus ABA detected produced lines parallel to the ABA standards, demonstrating the absence of nonspecific interference.

Areas of all terminal leaflets were measured on day 0 with a portable leaf area meter (LI-3000A, LiCor). Total plant leaf areas, shoot and root fresh, and dry weights were determined at harvest. Phosphorus concentration of the sixth oldest leaf of each plant was determined spectrophotometrically using the vanadate-molybdate-yellow method on samples dry-ashed with magnesium nitrate at 750 °C for 2 h and digested in nitric acid (Chapman and Pratt 1961). Hyphal, arbuscular and vesicular colonization of roots was determined on 100 1-cm root pieces from each control plant (McGonigle et al. 1990) after clearing with 10% KOH in an autoclave at 121 °C for 20 min, staining with trypan blue for 1 h, and destaining. Root samples of all other plants were checked for the presence or absence of mycorrhizal structures.

Results

For Figures 1–4 and 6, ANOVA main effects and linear contrasts were computed for each measurement day and probability values <0.05 given at the top of the Figure.

Water relations

Mycorrhizal colonization had little effect on g_s of fully watered control plants (Fig. 1a). Average g_s of controls ranged from 150–650 mmol m^{-2} s^{-1} during the drying period, and mycorrhizal and nonmycorrhizal controls differed on only one of the 10 measurement days. In an analysis of variance of all days and treatments (analysis not shown), there was a significant main effect ($P<0.05$) of mycorrhizae on g_s, as well as a significant main effect of day and a significant mycorrhizae × day interaction. Partial drying of the root system resulted in inhibition of g_s (by 82% across treatments) by day 8, and g_s was lower in half-dried plants than in controls on all days thereafter except the final day (Fig. 1b). Stomatal conductance of half-dried, nonmycorrhizal plants was reduced on fewer days and was reduced to a lesser extent than g_s of half-dried, mycorrhizal plants. Larger inhibition of mycorrhizal g_s may have been related to quicker soil drying in mycorrhizal pots (Fig. 2). Soil $\Psi\tau$

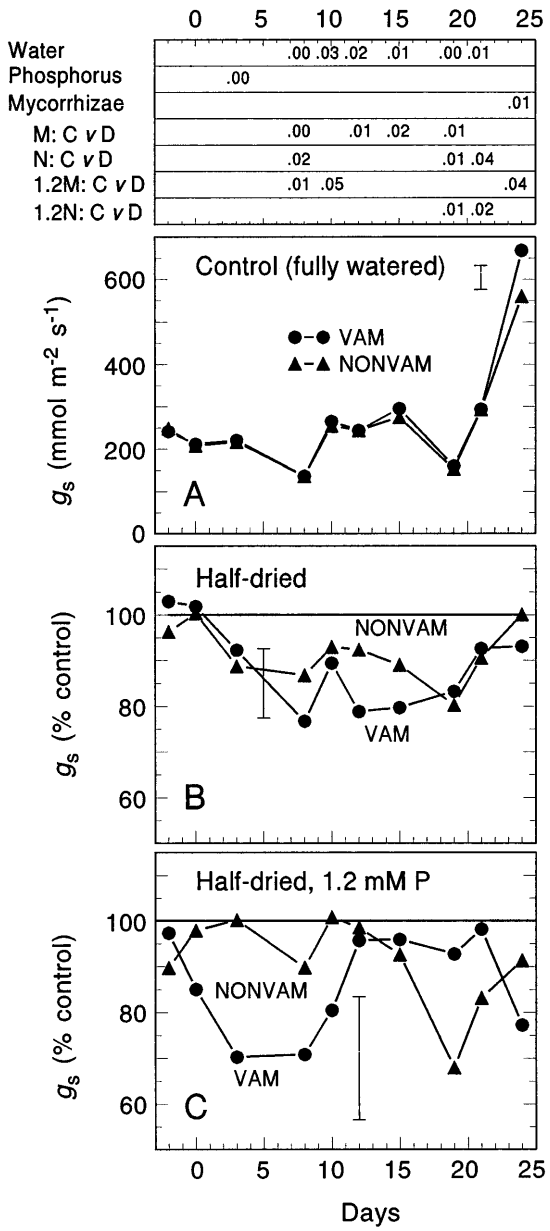

Fig. 1A–C Stomatal conductance of cowpea plants before and during the drying period. Water was withheld from half-dried plants beginning day 0. **A** Control plants, $n=24$. **B** Half-dried, mycorrhizal and nonmycorrhizal plants, averaged over P treatments, $n=24$. **C** Half-dried, mycorrhizal and nonmycorrhizal plants fertilized with 1.2 mM P, $n=8$. Half-dried plants are expressed as percentages of the means of their respective control plants within each mycorrhiza and P treatment. ANOVA main effects and linear contrasts were computed for each measurement day and probability values <0.05 given in the table at top of the figure (*.00* refers to probability values <0.005). *Vertical line* in each plot represents 2× standard error of the means. (*C* Control, *D* half-dried, *M, VAM* mycorrhizal, *N, NONVAM* nonmycorrhizal)

was lower in drying pots of mycorrhizal plants than nonmycorrhizal plants on all but one day after day 15 (due to the large variability in soil drying rate among replicates of each treatment, significance was viewed using probability values <0.09). Stomatal conductance

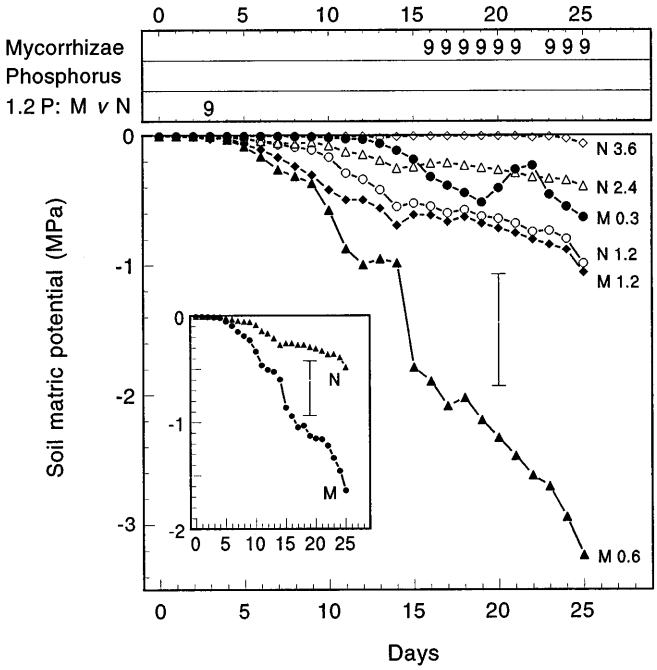

Fig. 2 Soil $\Psi\tau$ in drying pots of plants of each mycorrhiza and P treatment during the drying period; water was withheld beginning day 0, $n=8$. *Inset* soil $\Psi\tau$ of mycorrhizal (*M*) and nonmycorrhizal (*N*) plants, averaged over P treatments, $n=24$. Soil $\Psi\tau$ of each plant was measured every 2 h and daily averages plotted. The *9* in the ANOVA table at top of figure indicates significance at the $P<0.09$ level; there was considerable variation in soil drying rates among replicates within each treatment, so we gave probability values to 0.09. *0.3, 0.6, 1.2, 2.4, 3.6* fertilization in mM P. The *vertical bar* in each plot represents 2× standard error of the means in that plot

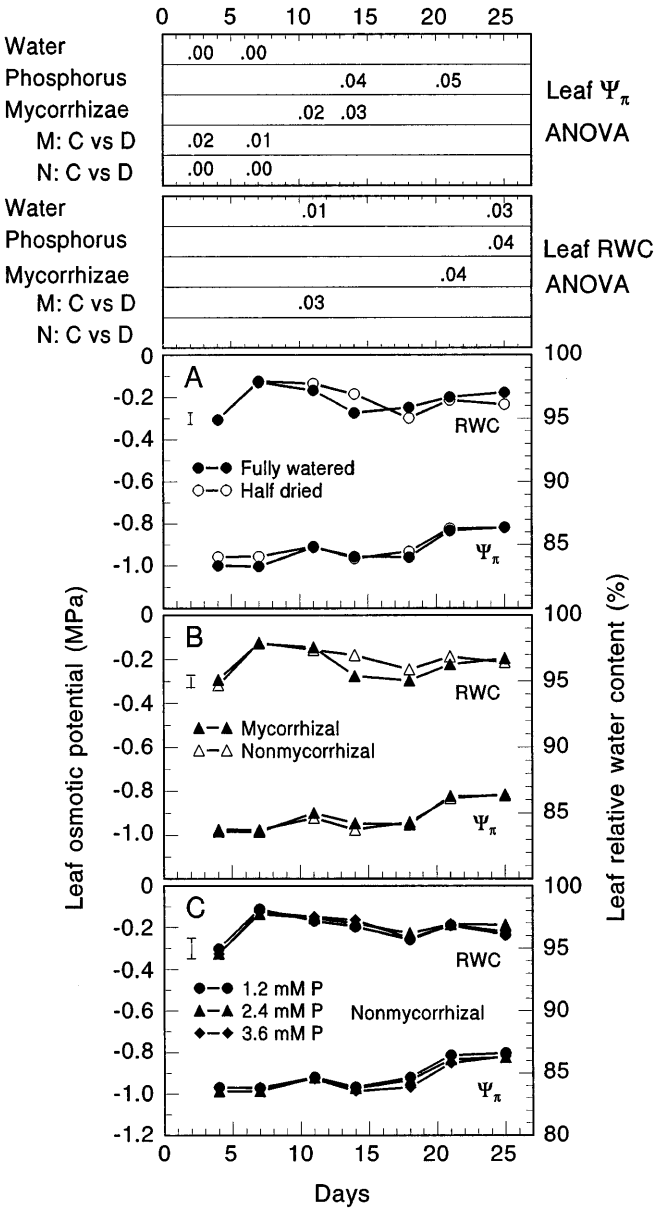

Fig. 3A–C Influence of the water, mycorrhiza and P treatments on leaf osmotic potential ($\Psi\pi$) and relative water content (RWC) during drying. **A** Control and half-dried plants, averaged across mycorrhiza and P treatments, $n=48$. **B** Mycorrhizal and nonmycorrhizal plants, averaged across phosphorus and water treatments, $n=48$. **C** Nonmycorrhizal plants of 1.2, 2.4 or 3.6 mM P treatments, averaged across water treatments, $n=16$; data for mycorrhizal plants were similar. Abbreviations, ANOVA tables and SE bars as defined in Figs. 1, 2. SE bars for $\Psi\pi$ were less than height of symbols on all three plots

was affected by the P treatments on only one of the ten measurement days (Fig. 1, ANOVA table), perhaps related to the fact that P treatments did not affect rate of soil drying (Fig. 2).

Leaf $\Psi\pi$ and RWC were less sensitive than g_s to varying greenhouse conditions (Fig. 3). Treatment averages of leaf $\Psi\pi$ ranged from –0.80 to –1.00 MPa and of leaf RWC from 95 to 98% during the drying period. Leaf water relations were mostly similar in half-dried and control plants (Fig. 3a). On days 4 and 7, leaf $\Psi\pi$ (averaged over colonization and P treatments) was actually slightly higher in half-dried plants than in controls. Leaf RWC varied slightly but statistically significantly between half-dried and control plants on two of the seven measurement days. On day 11, leaf RWC of control and half-dried plants were 97.2% and 97.7%, respectively. On day 25, leaf RWC of control and half-dried plants were 97.0% and 96.1%, respectively. Neither colonization nor P treatments had much effect on leaf RWC or $\Psi\pi$ (Fig. 3b,c).

By the end of the experiment, soil $\Psi\tau$ of several drying pots had not dropped below –0.01 MPa ($\Psi\tau$ of pots watered daily). We measured final RWC of samples of soil from all unwatered pots, to verify that these soils did dry; final soil RWC within individual pots ranged from 17 to 47%. A soil moisture-release plot of the 2 sand:1 Turface medium used in this study has been published before (Augé et al. 1994), indicating that soil $\Psi\tau$ first began to decline below –0.01 MPa at a soil RWC of about 26% (θ of this medium at field capacity was 23.5%). Hence, soil in unwatered pots dried considerably, but not enough in several pots to lower $\Psi\tau$ below control levels.

We also measured RWC and Ψ of soil in 10 other pots containing roots that had been severed from shoots on day 0. Comparing final RWC and Ψ of soil from these pots with final RWC and $\Psi\tau$ values for dried soil from the 48 pots having roots still attached to shoots (and to the watered half of the root system) indicated that water moved in intact plants from watered roots to unwatered roots during the experiment; soil in unwatered pots having intact roots was moister than soil in unwatered pots having detached roots. Final Ψ of soil from the 10 detached pots ranged from –4.1 to –17.8 MPa (mean = –12.4 ± 1.5 MPa), whereas final $\Psi\tau$ of the 48 dried, intact pots ranged from –0.01 to –8.8 MPa (mean = –1.08 ± 0.33 MPa). Final RWC of soil from the 10 detached pots ranged from 11 to 27% (mean = 17 ± 2%), whereas final RWC of the 48 dried, intact pots ranged from 17 to 48% (mean = 32 ± 1%). Rehydration of drying soil via another, watered portion of the root system has been observed previously (Xu and Bland 1993; Augé et al. 1994; Ebel et al. 1994). Averaged over P treatments, final soil RWC was 32.8% ± 1.7% in dried mycorrhizal pots and 32.1 ± 1.8% in dried nonmycorrhizal pots. Final mean soil $\Psi\tau$ averaged –1.64 ± 0.57 MPa in dried mycorrhizal pots and –0.52 ± 0.30 MPa in dried nonmycorrhizal pots.

Growth, leaf P, root colonization, xylem ABA

Colonization of cowpea roots by *G. intraradices* did not result in the typical growth promotion often seen in mycorrhizal plants, probably due to the low phosphorus fertilization and carbon drain as the fungus colonized the plant. Averaged over P treatments, fully watered mycorrhizal controls had smaller final leaf areas (Fig. 4a), shoot dry weights (Fig. 4b) and leaf P (Fig. 4e) than fully watered nonmycorrhizal controls. Increasing P fertilization resulted in increased leaf P, leaf areas, shoot dry weights and root dry weights. Control plants given 1.2 mM P were similar in size and leaf P, regardless of colonization treatment.

At the end of the experiment, leaf area of half-dried plants as a whole was 88 ± 3% that of respective controls (Fig. 4a). Averaged over P treatments, leaf area was reduced by 17 ± 4% in half-dried, nonmycorrhizal cowpeas but was similar in control and half-dried, mycorrhizal cowpeas. With the exception of mycorrhizal plants given 0.3 mM P, soil drying rate was negatively correlated with plant size: within each mycorrhizae treatment, plants given lower P dried soil more quickly (Fig. 2). Final shoot dry weight of half-dried plants as a whole was 85 ± 3% that of respective controls (Fig. 4b). Averaged over P treatments, shoot dry weight was reduced by 19 ± 4% in half-dried, nonmycorrhizal plants but was similar in control and in half-dried, mycorrhizal plants. Root dry weight was not affected by the water or colonization treatments (Fig. 4c). Reductions in leaf areas and in shoot and root dry weights were more pronounced in plants given more P fertilization (Fig. 4a–c);

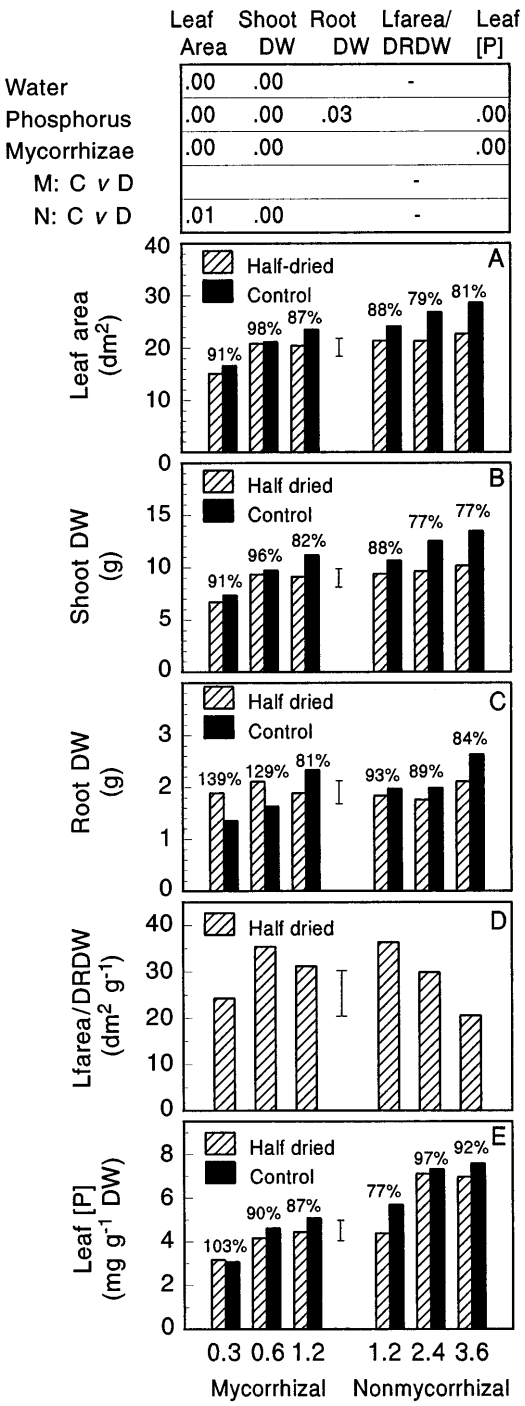

Fig. 4 **A** Final leaf area (*Lfarea*), **B** shoot and **C** root dry weight (DW), **D** leaf area/dried root dry weight (*DRDW*) ratio and **E** leaf phosphorus concentration (*[P]*) of control and half-dried plants of each mycorrhizae and P treatment, $n = 8$. Abbreviations, ANOVA tables and SE bars as defined in Figs. 1, 2. *Numbers* above histogram bar pairs give values for half-dried plants as percentages of their respective controls

the percentages given above bars in Figure 4 indicate values of half-dried plants as a percentage of their respective fully watered controls. Neither colonization nor P treatment influenced leaf area/dried root dry weight ratios of half-dried plants.

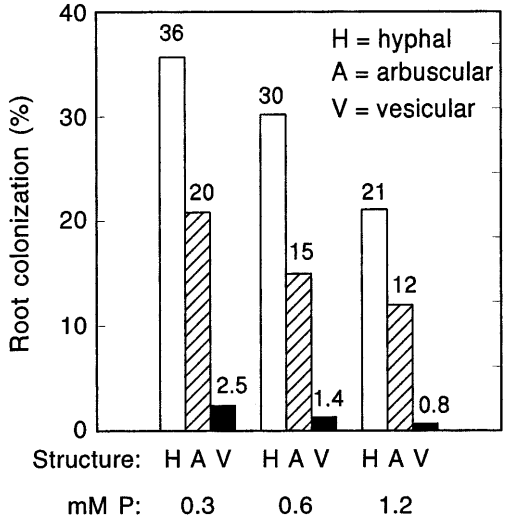

Fig. 5 Percentage of root system of mycorrhizal control plants colonized by hyphae, arbuscules or vesicles of *Glomus intraradices*, $n=8$. Means are also given numerically above histogram bars

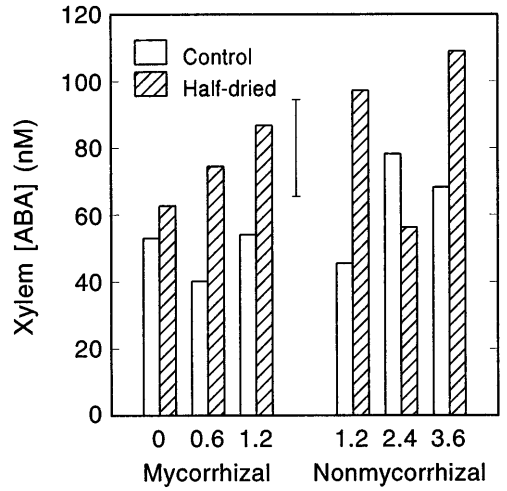

Fig. 6 Abscisic acid concentrations (*[ABA]*) in stem exudate from mycorrhizal and nonmycorrhizal cowpea plants at the end of the experiment (days 26–28), $n=6$–8. The *vertical line* represents 2× standard error of the means. The water main effect (C vD) was significant ($P=0.03$). Other main effects, interactions, and the following linear contrasts were not significant ($P\geq 0.10$): M: C v D, N: C v D, M1.2 v N1.2. Abbreviations as defined in Figs. 1, 2

Hyphal, arbuscular and vesicular colonization of mycorrhizal controls appeared to decrease with P fertilization, but probability of significance of the P main effect on colonization was above 0.10 for each fungal structure (Fig. 5). Root samples of all mycorrhizal, half-dried plants and all nonmycorrhizal plants were also checked for presence or absence of mycorrhizal structures; all mycorrhizal replicates were colonized, and no mycorrhizal structures were observed in any of the nonmycorrhizal roots.

Concentrations of ABA in xylem sap at harvest were increased somewhat by the partial soil drying treatment (Fig. 6). Averaged over colonization and P treatments, final xylem [ABA] was 57 nM in control plants and 81 nM in half-dried plants. Except for nonmycorrhizal plants given 2.4 mM P, xylem [ABA] of half-dried plants tended to increase with increased P fertilization. Averaged over water and P treatments, final xylem [ABA] was 62 nM in mycorrhizal plants and 76 nM in nonmycorrhizal plants (significantly different means at $P\leq 0.06$). Xylem [ABA] was not correlated with soil $\Psi\tau$ or soil RWC at time of harvest (analysis not shown).

Discussion

Nonhydraulic signaling

The effect of soil drying on g_s and leaf growth was probably not hydraulic since RWC was not affected by the soil drying treatment except on the last measurement day. However, also indicative of a nonhydraulic rather than hydraulic effect was the lack of any observed decline in leaf $\Psi\pi$ in half-dried plants compared to the controls. It is possible that measurements of leaf $\Psi\pi$ may not always indicate changes in leaf water status as sensitively as measurements of total leaf Ψ, if small declines in water content result in larger leaf Ψ declines than $\Psi\pi$ declines. Leaf $\Psi\pi$, though, has been as sensitive or a more sensitive indicator of changes in soil and leaf water status than leaf $\Psi\pi$ in some studies (e.g. Khalil and Grace 1993; Gallardo et al. 1994). Additionally, measuring $\Psi\pi$ allowed us to sample all 96 plants within a relatively short time (1.25 h or less), which decreased the influence of temporal variations in climate. Nevertheless, it is possible that soil drying affected plant water status which was not detectable by the measurement methods.

Reductions in g_s and leaf growth with partial root drying and without changing leaf water status are consistent with the existence of a nonhydraulic root-to-shoot signal (Davies and Zhang 1991). The maximum reduction in g_s, to about 70% of controls, is within the range of previous findings. Under similar conditions, g_s has been reduced nonhydraulically to about 75% of controls in *Rosa hybrida* (Augé and Duan 1991), to about 65% in sorghum (R.M. Augé and A.J.W. Stodola, unpublished results) and below 50% in maize (Augé et al. 1994). Others have reported nonhydraulic-related declines in g_s of 50% or more in maize (Zhang and Davies 1989, 1990a), rice (Bano et al. 1993) and sycamore (Khalil and Grace 1993). Total plant leaf areas of half-dried, nonmycorrhizal cowpeas after 25 days of partial drying were 88% of controls, similar to declines observed under similar conditions after 21–25 days of partial drying in other species: 82% in sorghum (Ebel et al. 1994) and about 85% in maize (Augé et al. 1994). Inhibition of g_s appeared to be directly related to ex-

tent of soil drying (characterized in Fig. 7a as final mean soil $\Psi\tau$ of drying pots in each treatment). The extent of growth inhibition, however, was inversely related to extent of soil drying (Fig. 7b). The different response of g_s and growth to soil drying may be due to different sensitivities to the same signal or g_s and growth may be responding to separate signals (Ludlow et al. 1989).

[ABA] in half-dried, nonmycorrhizal plants (excepting plants given 2.4 mM P) is consistent with previously observed links between leaf [P] and [ABA] (Danneberg et al. 1993). There was no relationship between xylem [ABA] and soil θ, perhaps because root systems were only partially droughted; we have observed a close relationship between xylem [ABA] and soil θ when entire root systems were dried (R.M. Augé and R.C. Ebel, unpublished results). Xylem [ABA] of cowpea were similar to values reported previously for a number of species (Neales et al. 1989; Wartinger et al. 1990; Bano et al. 1993; Gowing et al. 1993).

That nonhydraulic-related stomatal closure occurred at higher soil $\Psi\tau$ (in the one drying pot) in cowpea relative to maize (Fig. 8) suggests that greater sensitivity to nonhydraulic signaling of soil drying may contribute to the extreme drought avoidance character previously reported for cowpea (Shackel and Hall 1983; Ludlow 1989). In many instances, g_s of half-dried cowpea declined below control levels at soil θ higher than that required to affect soil $\Psi\tau$; note the abundance of g_s below 100% falling on the right y axis, at essentially 0 MPa in Fig. 8. Stomatal conductance of cowpea mostly remained inhibited between 0 and –1 MPa, while g_s of maize was frequently not inhibited until soil $\Psi\tau$ fell below –1 MPa. The scatter in the plot is to be expected; only a portion of the root system was drying, and many factors besides nonhydraulic signals may affect g_s (day-to-day changes in greenhouse vapor pressure, irradiance, temperature, etc., as well as possibly the varying P fertilization treatments).

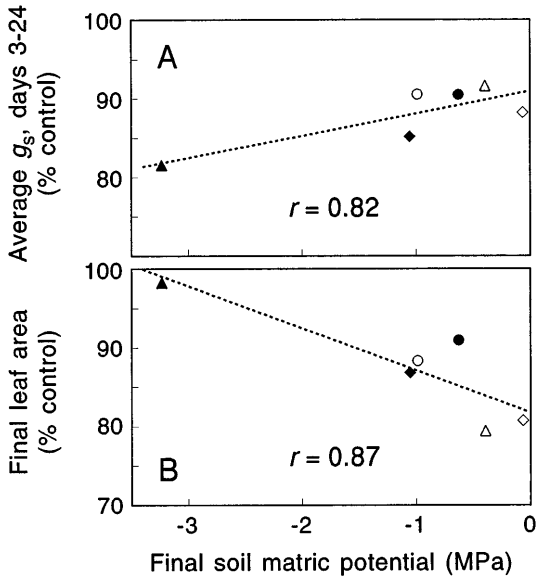

Fig. 7 Relationship of **A** average g_s during the drying period and **B** final leaf area with final soil $\Psi\tau$ of drying pots. Each point represents the mean of eight plants within each mycorrhizal and P treatment. The r values are correlation coefficients

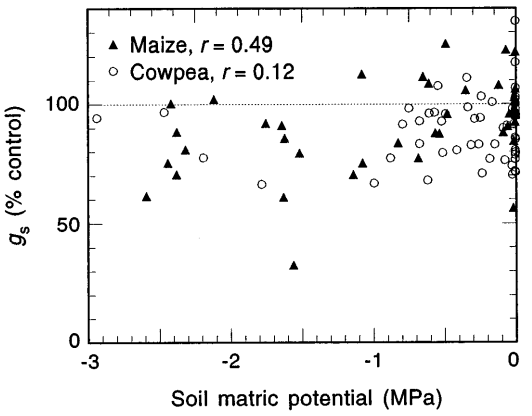

Fig. 8 Relationship between g_s and soil $\Psi\tau$ of drying pots for half-dried, split-root cowpea and maize plants. Half-dried plants are expressed as percentages of the means of their respective control plants within each mycorrhizae and P treatment. Maize data from Augé et al. (1994)

Mycorrhizal symbiosis

More frequent and larger declines in g_s as a consequence of mycorrhizal symbiosis, seen here in cowpea, were also observed in *Rosa hybrida* (Augé and Duan 1991). Soil water potential was not measured in that prior work, but we found in cowpea that mycorrhizal plants, in spite of their smaller size, tended to dry soil in their unwatered pots more quickly than nonmycorrhizal plants. Due to variability in individual drying rates, probable statistical differences in average soil $\Psi\tau$ between plants of mycorrhizal and nonmycorrhizal treatments did not occur until 16 days after withholding water, but by 5 or 6 days of drying, more mycorrhizal than nonmycorrhizal plants had dried the soil in their unwatered pots below –0.01 MPa (control values), and this trend was evident throughout the experiment. So it is possible that the larger inhibition of g_s in mycorrhizal plants was related to more extensive soil drying by half of the root system. However, half-dried, mycorrhizal and nonmycorrhizal plants of the same size (plants given 1.2 mM P) also differed in g_s, despite their removing water from soil at remarkably similar rates.

Differences between mycorrhizal and nonmycorrhizal cowpea plants in nonhydraulic inhibition of leaf growth disappear when comparing plants of similar size. Reductions in final leaf area of half-dried plants were twice as large in nonmycorrhizal compared to mycorrhizal plants, viewed across P treatments. However, mycorrhizal and nonmycorrhizal controls that were given 1.2 mM P were similar in size, and declines in leaf area in half-dried plants given 1.2 mM P were also sim-

ilar: 87% and 88% in mycorrhizal and nonmycorrhizal plants, respectively. In this experiment, then, unlike prior experiments with other species (Augé et al. 1994; Ebel et al. 1994), the mycorrhizal-induced alleviation of growth suppression appeared related to P nutrition. Smaller cowpea plants were less affected by nonhydraulic signaling of soil drying, whereas plants of similar size suffered similar growth suppression, regardless of mycorrhizal symbiosis. In a previous experiment with sorghum, plant size at onset of drying had as much or more influence than mycorrhizal symbiosis on nonhydraulic growth inhibition (R.M. Augé and A.J.W. Stodola, unpublished results). We have also found that plants given higher rates of P fertilization consistently tend to be more sensitive to nonhydraulic effects of soil drying. Total plant leaf length (Augé et al. 1994) and shoot dry weight (R.M. Augé and A.J.W. Stodola, unpublished results) were reduced in nonmycorrhizal maize and sorghum, respectively, by partial soil drying in plants given relatively high rates of P fertilization, while plants given a lower rate of P remained unaffected by the drying treatment.

The slight (and probably physiologically insignificant) difference in xylem [ABA] between mycorrhizal and nonmycorrhizal plants also disappears when comparing plants of similar size. Plants given 1.2 mM P had similar final xylem [ABA], regardless of colonization. Mycorrhizal fungi have previously been shown to alter root and leaf [ABA] (Allen et al. 1982; Danneberg et al. 1993), possibly due to mycorrhizal effects on plant size or tissue [P].

The experiment enabled us to compare rates of soil drying in mycorrhizal and nonmycorrhizal plants under continued high (rather than progressively decreasing) transpiration. The quicker drying of soil around mycorrhizal roots observed here with cowpea has been observed before in sorghum under similar conditions (Ebel et al. 1994). This suggests that, as soil $\Psi\tau$ declined, resistance to water flow in drying roots increased more quickly in nonmycorrhizal roots than in mycorrhizal roots. Conversely, water may have moved more readily from wet to dry roots in the nonmycorrhizal root system, resulting in some rehydration of drying roots and soil as has been noted previously in potted sorghum and maize (Xu and Bland, 1993; Augé et al. 1994).

Acknowledgements We thank Ann Stodola and Xiangrong Duan for assistance with the experiment. This research was supported by US Department of Agriculture Competitive Research Grant 91–37100–6723 (R.M.A.) and a University of Tennessee Professional Development Research Award (R.M.A.).

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