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ORIGINAL PAPER

The influence of arbuscular mycorrhizal colonization on soil–root hydraulic conductance in *Agrostis stolonifera* L. under two water regimes

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Abstract The hypothesis that mycorrhizal colonization improves the soil–root conductance in plants was experimentally tested in a growth chamber using pot cultures of *Agrostis stolonifera* L. colonized by *Glomus intraradices*. Plants were grown in 50-l pots filled with autoclaved sand/silt soil (1:1), with and without the mycorrhizal fungus. Within the mycorrhizal treatment, half of the pots remained well watered, while the other half was subjected to a progressive water deficit. Soil water potential (estimated as plant water potential measured at the end of the dark period), xylem water potential measured at the tiller base, transpiration rate, and soil water content were monitored throughout the experiment. Soil–root hydraulic conductance was estimated as the ratio between the instantaneous transpiration rate and the soil and xylem water potential difference. To obtain cultures with similar nutritional status, the P in the modified Hoagland's nutrient solution was withheld from the inoculated pots and applied only once a month. Even though there were no differences on growth or nutrient status for the mycorrhizal treatments, water transport was enhanced by the inoculum presence. Transpiration rate was maintained at lower xylem water potential values in the presence of mycorrhizae. The analysis of the relationship between soil–root hydraulic resistance and soil water content showed that mycorrhizal colonization increased soil–root hydraulic conductance as the soil dried. For these growing conditions, this effect was ascribed to the range of 6–10%.

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Introduction

In the past, the presence of mycorrhizae has been examined by researchers as an exception to "normal" conditions of root system. Indeed, it is well known that 80% of terrestrial plants are colonized by some arbuscular mycorrhizae (Koide 1993). Because the extent and degree of colonization is not known with precision, the possibility of adding manufactured inoculum to the soil opens up new opportunities for enhancing productivity in some situations, like in agricultural systems (Baar 2008). It is important to have an in-depth knowledge of the interactions between the host and the fungi and its effect on growth, water relations, and nutrition (Augé 2001).

The effects of mycorrhizae on plant growth have been traditionally ascribed to nutrition, mainly P nutrition. Mycorrhizal colonization increases the ability of crops to take up soil P (Price et al. 1989; Bolan 1991; Koide 1993; Jia et al. 2004) and other nutrients (Sharma and Srivastava 1991; Marschner and Dell 1994; Subramanian and Charest 1999; Caravaca et al. 2005). As a result, plant growth is enhanced, and consequently, water needs increase relative to nonmycorrhizal plants (Safir et al. 1971, 1972; Nelsen and Safir 1982; Caravaca et al. 2005).

There is some controversy in the existing literature concerning the effects of mycorrhizae on plant water relations. Some authors have found an effect (Allen 1982; Osonubi et al. 1991), while others did not find any (Graham and Syvertsen 1984; Bryla and Duniway 1997a, b; for a review, see Augé 2001). Gemma et al. (1997) stated that inoculation of creeping bentgrass with *Glomus intraradices*

provided significant avoidance capabilities under drought situations.

The physiological mechanisms that are involved in host water relations are unclear and still subject of active investigations. Some studies have observed an indirect effect on water uptake (Safir et al. 1971, 1972; Nelsen and Safir 1982; Caravaca et al. 2005) via enhanced mineral nutrition. The increase in absorptive surface by mycorrhizal extraradical hyphae enhances the ability of the plant to explore more soil volume (Hardie and Leyton 1981). Owing to its own structure, hyphae can grow in micropores with a diameter of less than 2 μm, where there is little root access; thus, the importance of mycorrhizae on nutrient uptake capacity is increased, depending on root architecture. Also, a change in soil hydrodynamic properties has been described (Augé et al. 2001).

Independently on these "indirect" effects linked to growth and mineral nutrition, other studies have shown a "direct" effect of mycorrhizae on host water relations (Graham et al. 1987). Stomatal conductance, transpiration rate, and water potential are generally enhanced in mycorrhizal plants in water-limited situations, as water uptake is enhanced (Augé et al. 1987; Subramanian and Charest 1995; Duan et al. 1996; Caravaca et al. 2003). Caravaca et al. (2003) demonstrated that, although stomatal conductance and photosynthetic rate were increased by mycorrhizae, the intrinsic water use efficiency was not altered by arbuscular mycorrhizal (AM) colonization, or even decreased, depending on species. The mechanisms underlying these effects are not well identified. Different hypotheses have been formulated; some authors highlight the modification of the biochemistry of water relations by hormonal signaling (Ebel et al. 1997; Goicoechea et al. 1997), while others found an induction of osmoregulation in the host (Augé et al. 1986; Wu and Xia 2006).

Some studies have shown an increase in root conductivity in the presence of mycorrhizae (Cui and Nobel 1992; Bogeat-Triboulot et al. 2004). On the contrary, other studies, such as that of Graham et al. (1987), showed a decrease in root conductivity when inoculated. Safir et al. (1972) ascribed the observed effect on root resistance to the enhanced nutrient status of mycorrhizal plants.

Water scarcity is becoming an important limitation to crop production and any factor that enhances plant response to water deficits (D) deserves attention. It is then important to characterize the effect of mycorrhizae on water transport and to understand the factors that may influence soil–root conductance and that may be the cause of the discrepancies found in the literature, such as different methodologies, differences in soil water content and in plant size. *Agrostis stolonifera* L. is a grass that is commonly used in golf courses. This species has been selected because this sector uses substantial amounts of irrigation water in areas of limited supplies and which is under close scrutiny (Rodriguez Diaz et al. 2007). Among golf course managers, attention has focused on strategies to reduce water consumption (Gemma et al. 1997). The aim of this study was to analyze the effects of inoculation of *A. stolonifera* L. with *G. intraradices* on transpiration, biomass, and hydraulic conductance.

Materials and methods

Plant culture and treatments

Cultures of *A. stolonifera* L. were sown in 16 pots (50 l each) and placed in a growth chamber with a constant temperature of 23°C and 14 h of photoperiod. The photosynthetic photon flux density was about 300 μmol $m^{-2}s^{-1}$ at the plant level. The substrate used was an autoclaved sand/silt soil mixture (1:1), a light texture soil. Estimated values of soil water content at field capacity and permanent wilting point were 22% and 6%, respectively. Half of the pots were inoculated with *G. intraradices* by placing a layer of commercial Mycosym Tri-ton (Mycosym®) inoculum 5 cm below the seeds (AM), while the other half remained noninoculated (nonmycorrhizal, NM). To ensure colonization, the inoculum was also added to the remaining soil volume up to the surface at a rate of 5% (*v*/*v*). According to the most probable number analysis (Porter 1979) made by Mycosym-Triton, there are 650 infective mycorrhizal propagules/g and around 600 g of the product was applied. Two drying cycles were applied on the same culture, managed by cuttings (at 6 cm stubble height) made before and after each cycle. The first drying cycle did not start until both the culture and the inoculum were well established (2 months after inoculation). Each drying cycle lasted about 50 days and gave almost identical results; the results from the last cycle will be reported. Four treatments were imposed, combining well-watered (W) and deficit (D) and AM and NM cultures. Watering treatments started 5 days after clipping to bypass the effect of defoliation on the nutrition dynamic (Richards 1993). The water supply for D treatments was reduced progressively by applying 80–60–40–25% of the weight loss in W.

Each treatment was replicated three times (12 pots). Four other pots (two AM and two NM) were prepared to be sampled on day 0 of the first drying cycle. For these pots, fresh and dry weights were determined for aboveground biomass and roots. Fresh samples of roots were washed to determine root length density. Calgon (sodium hexametaphosphate and sodium bicarbonate 20% solution) was added as dispersant. Roots were stained with Congo Red, sieved (1 mm mesh), and settled on filter paper. Root density was determined with a modification of the line intersection method (Newman 1966).

At the end of the drying cycles, leaf phosphorus and nitrogen content were determined in every replication by a colorimetric determination and Kjeldahl procedure (Nelson and Sommers 1973), respectively.

Once a week, a modified Hoagland's nutrient solution, as described by Feldmann and Idczak (1992), was added to ensure nutrient status. For mycorrhizal pots, phosphorus was subtracted from the nutrient solution and applied just once a month.

Data collection

Plant water status was followed by measuring Ψ_x for the whole tiller at the base in two tillers per pot with a pressure chamber (Soil Moisture Equipment, Santa Barbara, CA, USA). During the experiment, the root hydraulic conductance was determined after the Ohm's law analogy. From Eq. 1,

$$k_r = \frac{T_i}{(\Psi_s - \Psi_x)} \quad (1)$$

Where kr is the soil-root hydraulic conductance (g/MPa/h), Ti is the instantaneous transpiration rate (g/h) and Ψ_s and Ψ_x are the soil and plant water potential (MPa), respectively. T_i was determined gravimetrically by weighing the pots and calculated as the difference between two measurements, one taken 30 min after the lamps were switched on (to ensure transpiration steady-state conditions) and the other, 5 h later. Previously, the pot weight was recorded every hour during an interval of 5 h to ensure the maintenance of a constant transpiration rate (required to consider this measurement as representative of an instantaneous transpiration rate). The soil surface of the pots was completely covered by the *Agrostis* culture, so direct evaporation from the soil could be considered negligible. No drainage was allowed during the experiments.

To estimate Ψ_s, plant water potential was measured before the lamps were switched on. It was felt that because transpiration stops during the night and Ψ_x approaches equilibrium with Ψ_s, such an estimate provided an integrated value of Ψ_s which would be more accurate than point measurements inside the pot taken with a Ψ_s sensor.

Soil water content was measured with a time-domain reflectometer (Soil Moisture Equipment, Santa Barbara, CA, USA) on the same day that k_r was estimated.

At the end of the second drying cycle, all pots were harvested to determine fresh and dry weight (shoots and roots) and root length density.

The frequency of mycorrhizal colonization was estimated under optical microscope of fungal colonization after staining according to Phillips and Hayman (1970).

Statistical analysis

Statistical analyses were performed using SPSS® software (Analytical Software). Mean separations were ensured with the generalized linear model procedure using the least significant difference test when a significant t test result was found ($P<0.05$). Model used was a two-way ANOVA with four treatments and three replicates (containers) per treatment.

Results

Mycorrhizal colonization was assessed just before and after the experimental period. Similar values were found at the beginning and the end of the experiment. No colonization was ensured in NM treatments. AM treatments displayed an average frequency of 88%, with no difference concerning watering treatment.

Soil water content showed a similar trend for both D treatments, with a starting value around 14% and a final value of 6% (Fig. 1). From day 18 until 25 after cutting, soil water content in W treatments fell below 12%, so water supply was rapidly increased and it was maintained constant over 17%. AM/W started a mean value of 20%, significantly higher than the other treatments, even though the depletion rate was similar than NM/W.

Values of Ψ_x in W treatments were maintained close to −1 MPa during the whole experiment, except during the period when soil water content decreased and before it was corrected on day 25 (Fig. 2). For D treatments, values were similar until day 26 after cutting, achieving a mean value of −2.2 MPa. From day 26 to day 37 after cutting, Ψ_x stayed the same in NM/D, while in AM/D, it continued diminishing and reached −2.9 MPa. On day 41, both treatments displayed a similar Ψ_x value until the end of the experiment.

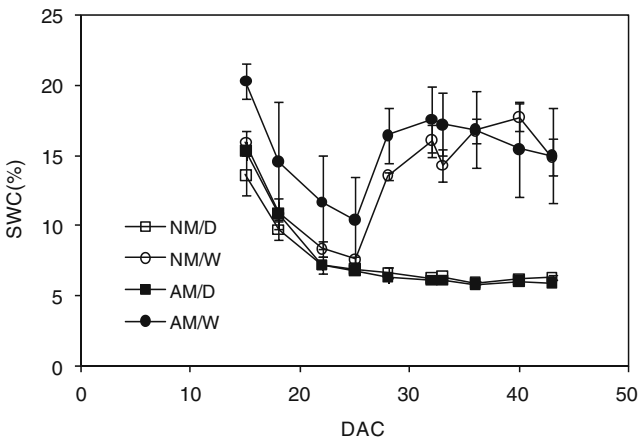

Fig. 1 Evolution of soil water content (*SWC*; %) for the four treatments. *Vertical bars* showed the standard error (n=3)

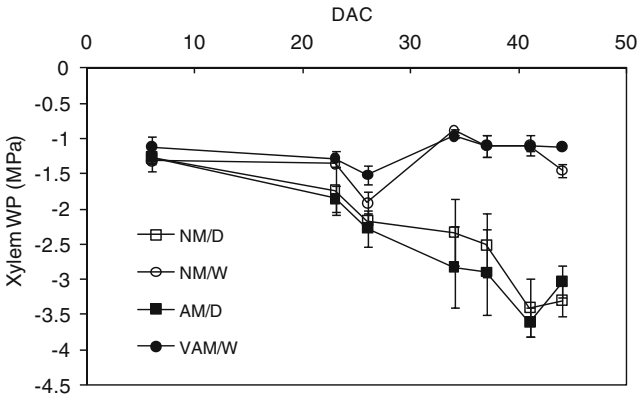

Fig. 2 Time course of xylem water potential (megapascal) measured at the base of the tiller 5 h after the lamps were switched on. *Vertical bars* showed the standard error (n=3)

The transpiration rate for W treatments increased during the whole experiment from 21 g h^{-1} to values above 50 g h^{-1} at the end of the experiment (Fig. 3). The only significant difference between both AM/W and NM/W was found on day 25, and it was associated with the depletion of soil water content, with a marked effect on NM/W. For D treatments, AM/D displayed a higher transpiration rate (19 g h^{-1}) than NM/D (12 g h^{-1}) on day 22. After that time, no differences were found between these two treatments. As arbuscular mycorrhizae increase phosphorus uptake, care was taken to maintain a similar nutrition level for both mycorrhizal treatments. At the end of the drying cycle, results obtained for N and P content in aboveground biomass showed no difference between treatments (2.5% and 0.17%, respectively).

No difference was found between AM treatments on dry matter accumulation during the experiments. At the end of the drying cycle (on day 53), a 6-cm stubble-height cut was made in order to evaluate the accumulation of dry matter during the experiment (Table 1). Differences were found

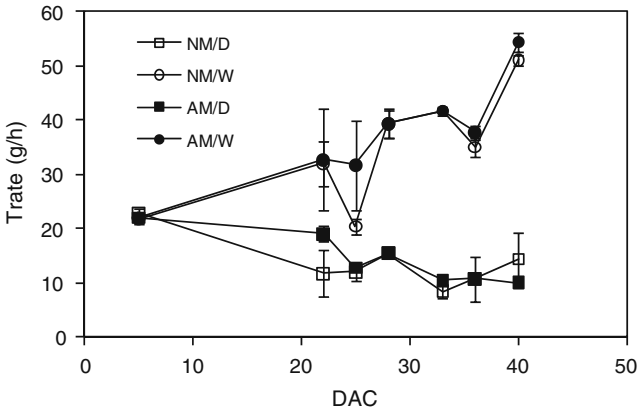

Fig. 3 Transpiration rate (grams per hour) measured gravimetrically for every treatment throughout the experiment. *Vertical bars* showed the standard error (n=3)

Table 1 Aboveground dry weight (grams per square meter), root dry weight (grams per square meter), and specific root length (micrograms per centimeter) for all treatments

Treatment	Aboveground DW (gm^{-2})	Root DW (gm^{-2})	Specific root weight (μgcm^{-1})
AM/W	149.8 a	2.12 b	0.183 b
AM/D	67.7 b	2.84 b	0.272 b
NM/W	147.3 a	6.40 a	0.637 a
NM/D	66.5 b	2.71 b	0.385 b

For each variable, values followed by the same letter are not statistically different (P>0.05)

only between watering treatments, with a mean value of 148 and 67 g m^{-2} for W and D, respectively.

Root dry weight was also measured at the end of the drying cycle. Differences were found only for NM/W with a mean value of 6.4 g m^{-2}, while the three other treatments had a similar value about 2.5 g m^{-2}. From the specific root weight (micrograms per centimeter; Table 1), it can be deduced that mycorrhizal roots were thinner compared to noninoculated treatments. Measurements made on pots harvested on day 0 indicated that there was no difference in root dry matter and root length density when treatments began (data not shown).

Tendency of soil–root hydraulic conductance (k_r) for AM/D treatment was to an increased transpiration rate for the same difference in water potential (Fig. 4a), especially for values of Ψ_s–Ψ_x larger than 0.5 MPa (see the figure inbox). These differences disappeared in W treatments (Fig. 4b). For Fig. 4a, b, measurements made on both drying cycles are considered. It must be stressed that k_r includes the path from the soil matrix to the root and within the root until the tiller base.

The evolution of k_r for D treatments displayed a similar tendency than the transpiration rate, i.e., a higher k_r during the first part of the experiment (Fig. 5a). For W treatments, NM/W showed a strong diminution between days 22 and 28, related to the water shortage during that period. Differences between mycorrhizal treatments diminished when hydraulic conductance was reported to root length, i.e., the hydraulic conductivity (Fig. 5b).

Soil–root hydraulic resistance ($1/k_r$, the reciprocal of soil–root hydraulic conductance) was strongly related to soil water content (Fig. 6). For values of soil water content up to 10%, there was no difference between AM and NM plants. Nevertheless, for values ranging between 6% and 10%, there was a clear increase in soil–root resistance in the NM plants, indicating that water transport was enhanced for AM plants, related to NM. In the graph inserted into Fig. 6, a zoom was made to the range of 6–10%, and a regression using a power function was adjusted.

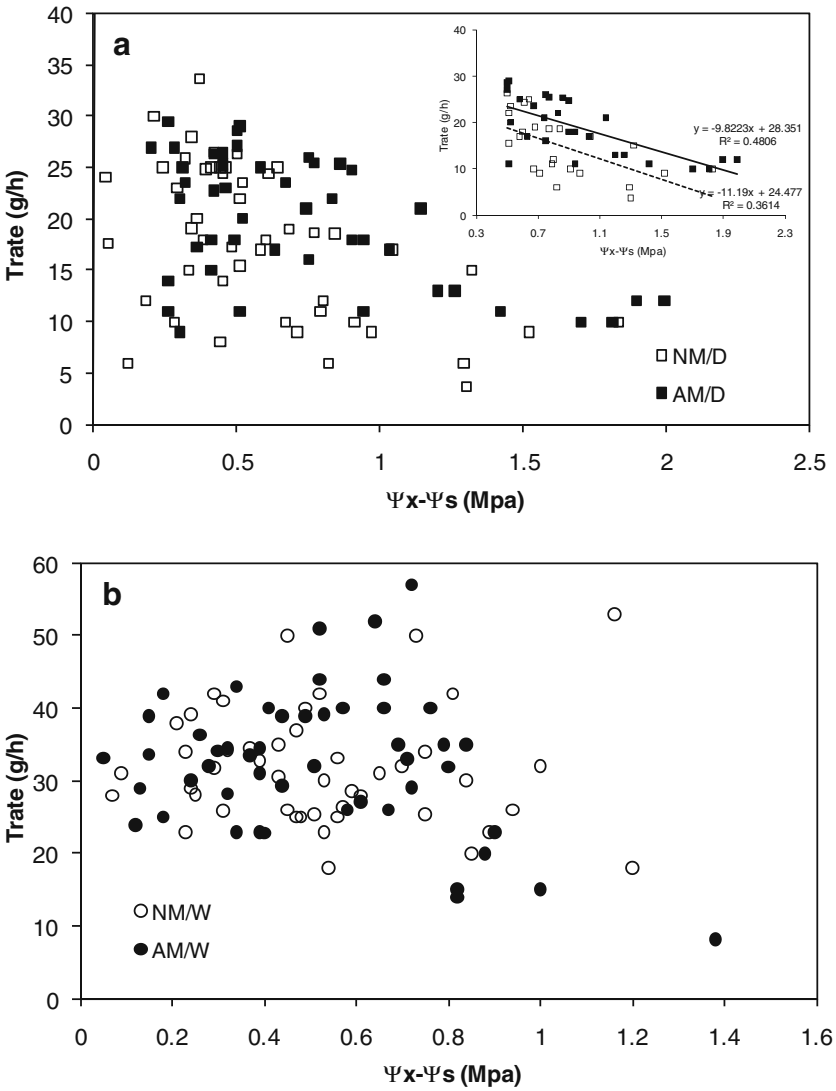

Fig. 4 Relationship between the transpiration rate (grams per hour) and the difference between soil (Ψ_s) and xylem water potential (Ψ_x; megapascal) for D treatments (**a**) and W treatments (**b**). For these figures, results from the two experimental drying cycles are considered. *Figure inserted* into **a** represents the linear regression for AM/D and NM/D for points with a Ψ_s–Ψ_x above 0.5 MPa

Discussion

The effect of mycorrhizal colonization on host water relations and especially on water conductivity has been analyzed by many researches, with contrasted results (Graham et al. 1987; Cui and Nobel 1992; Bogeat-Triboulot et al. 2004). From this study, it can be concluded that soil–root hydraulic conductance was increased by the mycorrhizal colonization, especially when the soil became drier. It must be stressed that no filtrate of the commercial AM inoculum was made, so any additive or microorganism contained in the formulation might influence the results obtained in this study.

It is generally accepted that root colonization is increased during drought episodes under field conditions (Augé 2001). In potted plants, the results are contradictory. Some authors found an increase in root colonization, while others described a decrease. For a complete review, see Augé (2001). In this study, there was no effect of watering treatments on the colonization frequency. The higher level of AM colonization observed since the beginning of the study and the reduced soil volume within each pot should be the cause for the maintenance of a constant value.

The magnitude of the differences in soil–root hydraulic conductance between AM and NM plants varied with time and was strongly related to soil water content. Indeed, for values of soil water content higher than 10% (under these growing conditions), both AM and NM displayed a similar soil–root hydraulic resistance. According to this fact, the differences encountered for W treatments, where soil water content never fell below 12%, should not be ascribed to mycorrhizal colonization but rather to differences in soil water content. For values ranged between 6% and 10%,

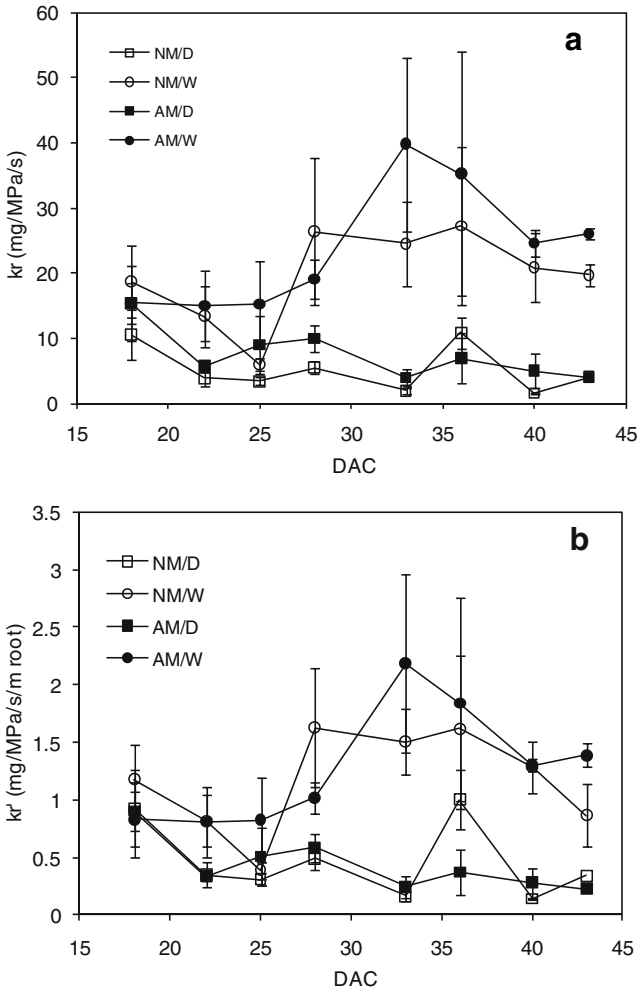

Fig. 5 Time course of soil−root hydraulic conductance (**a**; milligrams per megapascal per second) and soil–root hydraulic conductivity (**b**; milligrams per megapascal per second per meter root). *Vertical bars* showed the standard error (n=3)

AM plants displayed a smaller resistance, when compared to NM plants. The importance of this range of values can be assessed by the relationship between the soil water content and the xylem water potential (Fig. 7). Plant water potential was maintained over −1 MPa until soil water content diminished to 10%. It decreased afterward, indicating that, for this experiment and these growing conditions, water constraint started from this value of soil water content. As the soil became drier, resistance increased almost linearly for both treatments. This threshold should be variable, according to the characteristic soil moisture retention curve for a given soil.

Does this effect account for a part of the variability that has been encountered in previous studies? This evidence points out not only the relevance of the intensity of water stress but also the influence of soil type. For roots growing in sandy soil, which are prone to dry rapidly, sudden drying could induce a self-amplifying shrinkage of the cortex, which could precipitate a loss of hydraulic continuity between root and soil (Passioura 1988; Stirzaker and Passioura 1996). Even though mycorrhizal hyphae may serve to bind the soil to the root, preventing the loss of hydraulic conductivity (Fitter 1985) and reducing this gap between soil and roots, the rapid drying in sandy soil disables the monitoring of intermediate ranges. Although this experiment has been conducted on a relatively light soil (sandy loam texture), the progressive water stress that was imposed led us to observe fairly well the relationship of k_r with soil water depletion. We should hypothesize whether in heavier soils (with a higher clay content) the effects of AM on water relations should be amplified relative to our results. It is interesting to note that most of the studies that did not find any effect of arbuscular mycorrhizae have been carried out without any water constraint and on sandy soils (Graham and Syvertsen 1984; Bryla and Duniway 1997a, b) or with a fast drying rate of the soil (Levy and Krikun 1980) so that any effect on intermediate ranges of water stress may

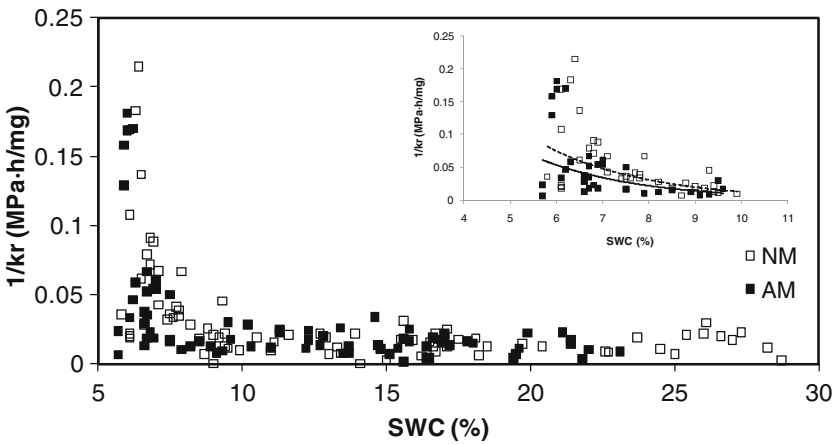

Fig. 6 Relationship between soil water content (*SWC*; %) and soil–root hydraulic resistance ($1/k_r$, megapascal second per milligrams) for AM and NM (W and D confounded). For this figure, results from the two experimental drying cycles are considered. *Figure inserted* focuses on the range below 10% of soil water content. Regression is plotted for AM and NM

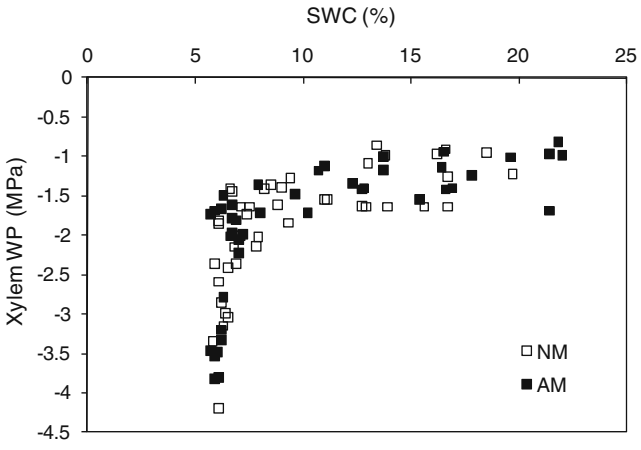

Fig. 7 Relationship between soil water content (*SWC*; %) and midday xylem water potential (megapascal) for AM and N (W and D confounded)

have been masked. Augé et al. (2001) have demonstrated that mycorrhizal colonization changes soil water retention properties.

The effect of mycorrhizal colonization on host water relations has been analyzed by many researchers (Augé 2001). The enhancement of growth and nutrient uptake, mainly P, resulting from mycorrhizal colonization leads to a modification of host water relations (Safir et al. 1972). Koide (1993) highlighted the importance of comparing mycorrhizal and nonmycorrhizal plants with a similar size to assess accurately the effect on water transport. Within this study, there was no difference in growth and mineral nutrition, so any improvement in water status was not ascribed to a better nutritional status.

Leaf and/or xylem water potential is often higher for mycorrhizal plants compared to noninoculated ones (Subramanian and Charest 1995; Morte et al. 2001; Porcel and Ruiz-Lozano 2004; Sánchez-Blanco et al. 2004), resulting in a higher capacity to transport water. In this case, xylem water potential was lower compared to NM plants, but with similar results, i.e., a higher capacity to transport water for the same difference between soil and xylem water potential. Transpiration rate was similar for mycorrhizal treatments, even though AM displayed a diminished water potential. The soil–root hydraulic conductance was increased for AM plants, related to NM. It is in agreement with other works (Hardie and Leyton 1981; Bogeat-Triboulot et al. 2004; Sánchez-Blanco et al. 2004).

It is generally stated that root hydraulic conductance must be stressed in terms of root surface (Koide 1993). In our study, soil–root hydraulic conductivity reflected a smaller effect of mycorrhizal colonization than soil–root hydraulic conductance. There are two points that must be considered regarding this issue: First, for the system considered, the soil–root system, the importance of root architecture is enhanced, as the distance to proximal root determines the path that water must flow in the soil until it reaches the root surface. On the other hand, as it is indicated by Nardini et al. (2000), the normalization by root surface area can be quite misleading because we rarely know what surface to use. This problem is not only ascribed to mycorrhizal studies but rather to every study concerning root absorption surface. Most of the water is collected by the active thinner roots, which are normally washed away during the root processing (Pierret et al. 2005). Moreover, the washed roots contained active, semi-active, and dead roots. This does not mean that measurements of root system must not be taken into account, rather considered in relative terms.

For this study, we have considered the conductance of soil–root system. We cannot conclude any result regarding the influence of AM on each soil and root component. It is well known that as the soil dries, soil hydraulic conductivity diminished considerably (Passioura 1988). This fact, in addition to the known effect of mycorrhizal hyphae to bind the soil and root surface and the relationship that was found between soil water content and k_r, leads us to hypothesize that the soil may play a significant role in the overall conductance determined here. For the root hydraulic conductivity, Cui and Nobel (1992) demonstrated, for three desert succulents, that mycorrhizal colonization increased radial conductivity but did not alter axial pathway within the vessels in roots. Increases in radial conductivity could result from the hyphae providing a low-resistance pathway across the cortex, as well as from changes in the apoplastic or the symplastic pathways (Cui and Nobel 1992).

Conclusions

The colonization with *G. intraradices* altered water relations in *A. stolonifera*, independently on nutrition or growth. Mycorrhizal plants transpired similarly to a decreased water potential. Within all the parameters involved in water relations, this research was focused on soil–root hydraulic conductance, as it is closely involved in the enhancement of water transport in mycorrhizal plants during drought. The main effect of mycorrhizal colonization on soil–root hydraulic conductance occurred as the soil became dryer and is strongly related to the soil water content. Therefore, large differences might be expected in the responses of the soil–root conductance in colonized plants for different soil types. For intermittent water shortages periods, the enhancement of water relations related with the mycorrhizal colonization would partially alleviate the negative effect of water stress.

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