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REGULAR ARTICLE

Quantification of mycorrhizal water uptake via high-resolution on-line water content sensors

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Abstract The benefits of mycorrhizas for host plants are well known for a large number of species. However, experimental evaluations of the hyphal contribution to the total water uptake and the assessment of the bulk flow velocity in the hyphae are so far contradictory. Barley (*Hordeum vulgaris* L. Scarlet) with the inoculum *Glomus intraradices* was grown in a split plant-hyphal chamber with a 5 mm air gap. During the preparation of the chambers with a loamy-silt soil, water content sensors were inserted in each of the plant and the hyphal compartments. These sensors allow non-destructive measurements with high resolution. In total, 8 drying periods with a length of several days were applied with repeated watering following each drying period. A clear decline in water content in the hyphal compartment during each drying period supports the ability of hyphae to transfer water into the plant compartment. The difference between the decline in the hyphal compartment with and without arbuscular mycorrhyzal fungi is significant at the $p < 0.000001$ level. The direct and indirect hyphal contribution to the total water uptake was estimated to be about 20%. The application of capacitance sensors for water content determination with a special geometry adapted to the plant-hyphal chambers allows the evaluation of the hyphal water flow with high accuracy.

Keywords Barley · Drought · *Glomus intraradices* · Stress · Plant-hyphal chamber · Compartmented pots · Water flow

Introduction

There is no doubt about the importance of mycorrhizas for plant development. The symbiosis between fungi and plants is widespread in nature and investigations have been conducted on a large variety of host plants and fungal species (Augé 2004). Two benefits of the symbiotic relationship are of particular importance for the plant: an improved nutrient uptake, especially of phosphorus, and an improved ability to acquire water for growth which may also be regarded as a secondary effect. The widely accepted mechanism for the increased drought resistance, often found for mycorrhizal plants, derives from the considerably smaller

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diameters of fungal hyphae compared to the roots of plants, enabling an improved access to water in even the smallest soil pores to the benefit of the host plant.

Variables including water uptake rate, transpiration, stomatal conductance, and drought resistance have all been investigated across a number of plant species (e.g. onion, (Rhodes and Gerdemann 1978), maize (Subramanian et al. 1995)), with most studies indeed demonstrating an increased water uptake rate of plants with mycorrhizas in comparison to the same plants without mycorrhizas. This finding, however, is not universal and the experimental results of all investigations cover a large range of outcomes. In this context, one must differentiate between investigations of the total water uptake of plants and more specific investigations which allow a separate assessment of water uptake by hyphae. Whereas some authors state only a negligible contribution of the hyphae to the total water uptake (Cooper and Tinker 1981; Fitter 1985; George et al. 1992; Koide 1993), others estimate that much of the water is taken up by the hyphae (Allen 1982, Ruiz-Lozano and Azcon 1995). Quantitative measurements also vary greatly, ranging from 0.1 μl/h for each hyphal entry point (Allen 1982) to 0.76 μl/h and 0.37 μl/h for each hypha passing the air gap between the two compartments in two separate experiments (Faber et al. 1991). For the velocity of the bulk flow inside the hyphae, values between $6{\cdot}10^{-4}$ and $3.6{\cdot}10^{-2}$ cm s^{-1} have been reported (Sanders and Tinker 1973; Allen 1982).

To some degree, the differences in conclusions stem from the difficulty in clearly interpreting the experimental results, which, in turn, derives from the nature of symbiosis itself. Because it is not possible to differentiate between effects of the root alone or the mycorrhiza alone from that of the two through cooperation, it is difficult to attribute any observed effect to any single parameter (Augé et al. 2001). Moreover, it is crucial to differentiate between primary and secondary effects. For example, it has been argued that any advantageous effect on the water supply of the host plants may instead be a side benefit deriving from the increased phosphorus uptake provided by the mycorrhiza (Graham and Syvertsen 1984). Similarly, the apparent benefit may derive from the comparatively increased weight of shoots and a larger leaf surface area that has been measured for plants with mycorrhizas compared with non-mycorrhizal controls (Augé et al. 2001).

Often compounding these fundamental problems is the experimental approach used to elucidate the action of the mycorrhiza, which traditionally involves comparing plants with and without mycorrhizas. The two treatment groups are typically obtained by growing plants in autoclaved soil as a control and in natural or inoculated soil as the test case (Allen 1982). In addition, only few attempts have been made to estimate the relative contribution of mycorrhizas to the total water uptake of the plant and the bulk flow velocity within the hyphae.

To improve the differentiation between root and mycorrhizal action, plant-hyphal chambers with at least two compartments have been introduced. The first compartment contains the plant with the roots (plant compartment) and it is separated from the second (hyphal) compartment by a screen or a net with holes and meshes with an inner diameter ranging from 35 μm to 260 μm (Rhodes and Gerdemann 1978; Cooper and Tinker 1981, Ames et al. 1983). This construction allows mycorrhizal fungi alone to grow through the holes from the plant compartment into the hyphal compartment. A further improvement of this design was obtained by using two screens with an air gap of several mm between the screens. Whereas the air gap inhibits a direct mass flow of water and nutrients between the two compartments (Faber et al. 1991), it does not inhibit fungal growth to the hyphal compartment because fungi can pass aerial locations in natural soil (Unestam and Sun 1995). This special split plant-hyphal chamber allows water content measurements in soil with roots and fungi (plant compartment) and in a second separated compartment measurements in soil with fungi alone (hyphal compartment). Furthermore, the number of hyphae spanning between the compartments can also be counted at the end of the measurement (Faber et al. 1991).

Even with this improved experimental setup, large variations in the relative contribution of the hyphae to the total water uptake have been reported. This value ranges from zero (no contribution) (e.g. George et al. 1992) up to 36% obtained from measurements with a plant-hyphal chamber (Faber et al. 1991). The direct measurement of the water flow in excavated roots yields an increase in hydraulic conductivity up to 24% with increasing rate of mycorrhizal infection of the root (Cui and Nobel 1992). Other authors report a considerable water uptake by hyphae but without

quantification (Ruiz-Lozano and Azcon 1995). On-line water potential measurements using tensiometers provide only an indirect determination of the water content (George et al. 1992).

A further step to potentially resolving the discrepancies in results and conclusions lies with the on-line and non-destructive monitoring of the water content in the soil of a plant-hyphal chamber. Such an experimental setup would enable not only the evaluation of water content at the end of the experiment (as in traditional studies), but also continuous measurements during plant development and during several wetting-drying-cycles. Water content sensors of the capacitance type provide the advantage to have a high sensitivity to water content variations and the possibility to adapt the sensitive volume of the sensor to the requirements of the root-hyphal-chamber. In addition, information can be obtained about the reverse flow in the hyphae from the plant compartment into the hyphal compartment during watering the plants. This is of importance for the understanding of phenomena such as hydraulic redistribution (Warren et al. 2008) and common mycorrhizal networks (Egerton-Warburton et al. 2007). Therefore, the objective of this paper is to quantify the water flow between the two compartments in a plant-hyphal chamber attributable to the hyphae through the use of sensitive physical water content sensors.

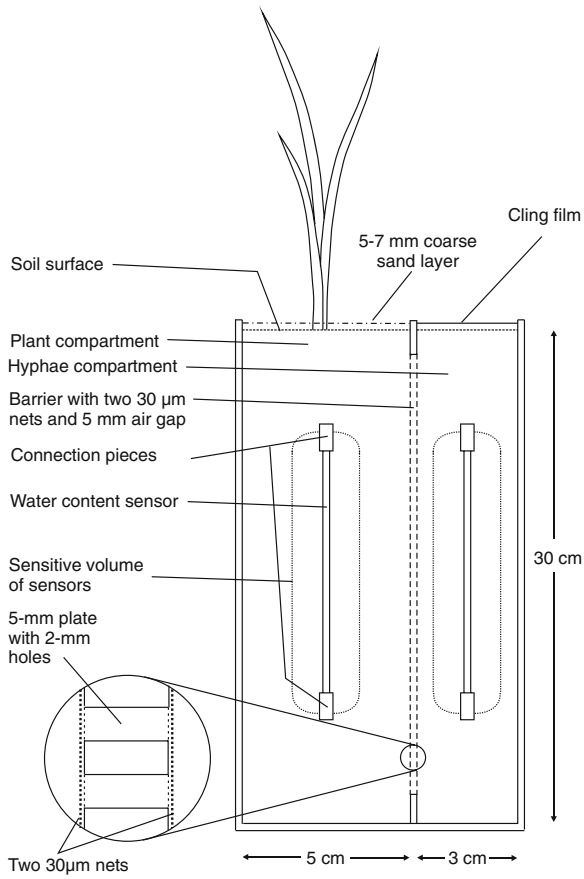

Fig. 1 Cross-section of the plant-hyphal chamber indicating the plant- and hyphal compartment and the positions of the water content sensors together with their sensitive volume for quantifying the acquisition of water through the action of arbuscular mycorrhizal fungi (AMF). The insert illustrates the barrier between the plant- and hyphal compartment, especially the relationship between the plate thickness and the hole diameter

Materials and methods

Specifications of the plant-hyphal chamber

In our experiment, the plant-hyphal chambers were constructed from non-transparent PVC-plates and consisted of the hyphal compartment H × L × W: 30 × 19 × 3 cm; V_{HC}=1,710 cm³) and the plant compartment (30 × 19 × 5 cm; V_{PC}=2,850 cm³), similar to that introduced by Faber et al. 1991. Both compartments had a common wall consisting of three layers: Two nylon nets with a pore size of 30 µm were separated by a 5 mm thick plastic plate having 2 mm square holes and 2 mm bars between the holes (see Fig. 1). The relationship between the thickness of the plate and the hole diameter, illustrated by the insert in Fig. 1, ensured that there was no contact between the two nylon nets, even if the soil was packed from both sides and that the 5 mm air gap was maintained. The pore size of 30 µm allowed only mycorrhizal fungus to pass this barrier and not the plant roots (Li et al. 1991). Furthermore, the bars prevented an exchange of the air in the air gap with the atmosphere. The three components of the barrier were fixed in a frame as common part of the two compartments.

Water content sensors

Water content sensors provide information about the water content, θ, by evaluating the dielectric number of the soil in comparison with the extreme values for pure water (~80) and for dry soil (between 3 and 5). In preliminary investigations, TDR-probes with a

small sensitive volume turned out to have a poor sensitivity to water content variation. For that reason, other water content sensors had been used which operate as capacitance sensors (Thomas 1966; Dean et al. 1987).

The geometry of the capacitance sensors for water content determination has been adapted to the requirements of the root-hyphal chambers. The special design results in a flat sensitive volume as defined by the area covered by the electrode rods (144 mm × 93 mm) and by the thickness (20 mm) as determined by the separation of the electrode rods. A side view of the sensors in the plant- and hyphal compartments together with an indication of the sensitive volume is provided in Fig. 1. The flat sensitive volume allows the determination of the specific water content in one compartment without any influence of the water content in the neighbouring compartment. As the two connection pieces between the electrode rods have a horizontal position and the sensors are connected to the exterior electronics by two horizontal wires, no artificial connection exists to the soil surface (see Fig. 1) and no preferential flow can be induced in this upper part of the soil contained in the compartments. Due to the restricted number of water content sensors, we used four plant-hyphal chambers and therefore eight water content sensors because in each of the plant and hyphal compartments one water content sensor was installed. The high sampling rate of six measurements per hour ensures a large number of measurements and therefore an analysis with a decreased statistical error. Thus, any differences in the decline of water content can be observed with a higher significance level. Further detailed information about the technique of the water content measurement is provided elsewhere (Ruth 1999; Ruth and Munch 2005; Ruth et al. 2008).

Soil, plants, arbuscular mycorrhizal fungus, and preparing the plant-hyphal chambers for measurements

Loamy-silt soil from the soil horizon (0–15 cm) was taken from a field of the Dürnast research station (Technische Universität München). The soil consisted of 23% clay, 48% silt, and 29% sand. Further specifications were 1.7% organic matter, C/N-ratio 0.14, 22 mg P_2O_5 per 100 g soil, 1.5 mg NH_4-N per 100 g soil, and pH 6.7. The soil was ground and passed through a 5-mm mesh screen. Before the soil was filled into either compartment, it was sterilized by autoclaving at 120°C and 1.3 bar pressure for 20 min. The initial gravimetric soil water content of the soil (0.23 g g^{-1} on dry soil basis) was achieved by adding distilled water to the soil and mixing thoroughly. During the filling of this soil into the four plant-hyphal chambers, the water content sensors in each compartment were fixed in the positions indicated in Fig. 1 and the soil was filled such that the bulk soil density was adjusted to 1.21 g cm^{-3}. From the soil texture and the bulk soil density the saturation water content of 0.37 g g^{-1} was calculated applying a pedo transfer function.

After preliminary investigations applying three arbuscular mycorrhizal fungi (AMF), we selected *Glomus intraradices* as the best one for our investigations (Khalvati et al. 2005). 25 g of pre-cultivated inoculum in a fluid medium (Institute of Pathology, University Hannover, Germany) showed the optimum results for root colonization. As verified by microscopy, 68% of the selected root segments were mycorrhized by the AMF. This inoculum was mixed into the soil of two plant compartments 2–3 cm below the surface (AMF treatment, two replicates). The other two plant compartments were left without inoculums and serve as controls (non-AMF treatment, two replicates).

Seeds of barley (*Hordeum vulgaris* L. Scarlet) were surface sterilized using a 0.5% NaClO solution for 15 min and then washed three times in sterile water before being germinated in Petri dishes. Seven days after they were transplanted into the plant-hyphal chambers, the number of plants per compartment was reduced to six.

To avoid water loss by evaporation over the course of the entire experiment, the soil surface of the hyphal compartment was covered with a watertight cling film, and that of the plant compartment by a 5–7 mm layer of coarse sand as indicated in Fig. 1.

Experimental and measuring procedures

Plants were grown in a controlled growth chamber under a 14-hour photoperiod with a photosynthetic photon flux density of 450 μmol m^{-2} s^{-1}. The air temperature was 20/18°C (day/night) with 65% humidity during germination and 15/15°C with 70% humidity for the entire subsequent period of plant growth.

During the initial plant development (up to day 14 after sowing), the chambers were weighed every 2 days and the volumes of water required to maintain the initial 0.23 g g^{-1} soil water content were added. Because of a slight nitrogen deficiency, indicated by visual symptoms, all plant compartments were fertilized at days 24, 42, and 63 with a 0.2 g l^{-1} solution of NH_4NO_3. The amount of nitrogen added was corrected for the volume added to apply comparable amounts to individual plant chambers. All other nutrients were adequately available based on initial nutrient analysis.

Drought treatments were accomplished by withholding irrigation to the plants over periods ranging from several days to 1 week. The drying cycles started on days 16, 24, 29, 35, 42, 50, 63, and 69. As measured by the water content sensors, the drought treatments reduced the soil water content in the plant compartments down to 0.07–0.12 g g^{-1}. Following each drying cycle, water was added to the plant compartment until the initial weight of the plant-hyphal chamber was achieved. To avoid preferential water flow especially along the compartment walls, the water was added only to the middle of the plant compartment and the necessary quantity of water was divided into four fractions that were added separately over the course of 1 day. Furthermore, a preferential flow along the compartment wall seems to be improbable because the water content to be achieved (0.23 g g^{-1}) is much lower than the saturation water content (0.37 g g^{-1}). This method to restore the total mass of the plant-hyphal chamber does not consider the increasing plant mass during plant development and it does not differentiate between the water content in the hyphal- and plant compartments. The re-watering method was chosen because of its simplicity and to avoid uncertainties due to the assessment of plant weight. During all drying periods, the water content was monitored by the water content sensors.

Final measurements

Following the last drying cycle, the plant-hyphal chambers were disassembled for inspection and further measurements on the soil, plants and fungi. For the evaluation of the dry weight of the shoots and the roots in the plant compartment, the shoots were cut off and the roots were carefully washed to remove all soil particles. Dry weight was determined after drying shoots and roots in an oven at 70°C for at least 48 h. In addition, the number of hyphae which had passed the air gap was estimated by cutting six pieces each with an area of 1 cm^2 out of the nylon net on the hyphal compartment side at different depths below the soil surface (approximately 6, 12 and 18 cm). Hyphae were stained using 0.2 g/l trypan blue (Kormanik and McGraw 1982) and the number of hyphae was observed and recorded using a high resolution digital zoom camera system (KAPPA® DX-30) and the KAPPA image analysis system (Hedwig Pfarrherr, Vertrieb Mikroskope Zubehör –System, Germany).

Statistical analysis

The main results are given by the drying rates in all compartments. For the statistical analysis, 2D-ANOVA (analysis of variance with two indices) was applied, using index A for characterizing the AMF— and non-AMF treatments, and index B for the differentiation between the two replicates.

Results

Fig. 2 shows the daily mean values of the water content in each compartment of the plant-hyphal

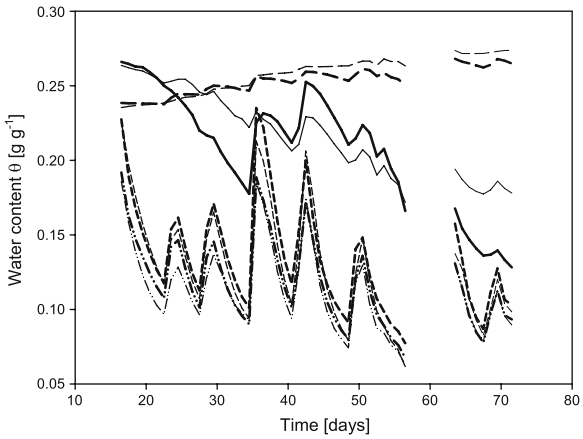

Fig. 2 Daily mean values of the water content θ during the drying periods and intermediate watering periods. Solid lines: hyphal compartment AMF treatment, long dashed lines: hyphal compartment non-AMF treatment, short dashed lines: plant compartment AMF treatment, dashed double-dot lines: plant compartment non-AMF treatment. Thin and thick lines indicate each of the two replicates per treatment. Between the days 57 and 62, no measurements were carried out

chamber. The most obvious difference occurs between the AMF and non-AMF treatments in the hyphal compartment. During the 8 drying periods starting on days 16, 24, 29, 35, 42, 50, 63, and 69, a clear decline in the water content of the hyphal compartment for the AMF treatment can be observed (solid lines). Although a decline at these times is also apparent in the hyphal compartments without AMF treatment (control), it is clearly smaller (long dashed lines).

To quantify the water flow from the hyphal into the plant compartment during the drying periods, the corresponding daily mean values in a single drying period were approximated using simple regression. This regression line includes all information about the water loss in one drying period and its value is less dependent on the values of the starting and end point. The slope of this line scales the water loss as drying rate M in the given drying period. Parameters of other possible functions, such as exponential functions, yield considerably higher variations because of the greater number of parameters underlying them. The mean drying rate results from the 16 single values obtained from the 8 drying periods in the two replicates. In the AMF treatment, the mean drying rate in the hyphal compartment was calculated as 0.0076 g g^{-1} day^{-1}. For the non-AMF control treatment, the analogous value was 0.0010 g g^{-1} day^{-1} (Table 1). Inspection of the drying rate values in each of the individual drying periods reveals a large degree of variation and no clear pattern. However, it appears that the drying rate in the hyphal compartment with AMF treatment obtains its maximum in the drying period after day 50.

Fig. 2 shows also that the water loss in the plant compartments is much larger than that in the hyphal compartments (short dashed lines, dashed-double-dot-lines). Again, using regression, the drying rates for the plant compartment were calculated to 0.0159 g g^{-1} day^{-1} for the AMF treatment and 0.0130 g g^{-1} day^{-1} for the non-AMF treatment. Inspection of the individual drying rates during the drying periods again did not reveal any significant time course and no apparent maximum value was observed after day 50 as for the hyphal compartment with AMF.

Although the differences in the drying rates between AMF—and non-AMF treatment in the hyphal compartments illustrated in Fig. 2 are apparent, the significance level will be provided to compare it with investigations in literature. 2D-ANOVA resulted in a significance level $p<0.000001$ for the difference in the drying rates for the AMF and non-AMF treatment in the hyphal compartment. In addition, there is no significance for the difference between the replicates and for the interaction of treatment and replicate. Further analysis for the plant compartments yielded a significance level $p<0.05$ for the difference between the drying rates for the AMF and non-AMF treatment, but again no significance for a difference between the replicates and a treatment-replicate interaction.

After the end of the last drying cycle, the chambers were dissembled. Dry weights of shoots and roots were as follows: AMF 11.9 g shoots and 11.3 g roots; non-AMF 6.2 g shoots and 9.4 g roots. The corresponding sums yield the AMF/non-AMF ratio of 1.49 for the dry weights. Mycorrhizal root colonization was determined in the AMF-treatment as 63.9%. Colonization by mycorrhizal fungi was not observed in non-AMF treatments, however unidentified other fungi and Fusarium sp. fungi were detected in the uninoculated non-AMF-treatment. Further, no roots were found in the hyphal compartments. The 35 μm nets were inspected and showed no indication for a direct contact of the soils in the hyphal and plant compartment. Estimates of the hyphae number per unit area passing through the nets and the air gap between the compartments were 64.4±12.3 cm^{-2} and 9±5 cm^{-2} for the AMF and non-AMF treatments, respectively. Similar to the inspection of the roots, AMF were identified for the AMF treatments and unknown fungi for the non-AMF treatments.

Table 1 Drying rates M and water flow F in the hyphal and plant compartments, together with the flux density into the plant compartment from the hyphal compartment (definitions in the text)

Compartment	Treatment	Drying rate M [g g^{-1} day^{-1}]	Mean water flow F [g day^{-1}]	Flux density across the barrier [μl cm^{-2} day^{-1}]
Hyphal	AMF	0.0076±0.0014	15.8	22.8
Hyphal	non-AMF	0.0010±0.0005	2.1	3.5
Plant	AMF	0.0159±0.0004	55.0	-
Plant	non-AMF	0.0130±0.0002	45.0	-

Mean water flows (F) out of each of the plant and hyphal compartments was calculated as the product of the soil mass in each compartment (m_C; 3,460 g and 2,074 g for plant and hyphal compartment, respectively) and the drying rates (M); values for the AMF—and non-AMF treatments are given in Table 1. With the assumption that all precautions (cling film, air gap) were sufficient to confine further possibilities of the water loss in the HC to a negligible amount, the mean water flow values F represent the flow conducted by the hyphae crossing the air gap. For the non-AMF treatment, the flow out of the hyphal compartment (2.1 g day^{-1}) is attributed to unidentified hyphae as suggested by the number of hyphae (9±5 cm^{-2}) in the non-AMF case. Provided that similar hyphae also exist in the AMF treatment case and that they also contribute to the flow (15.8 g day^{-1}), it is appropriate to regard the difference of 13.7 g day^{-1} as quantification of the water which is directly conducted by the AMF over the air gap. Furthermore, the consideration of the flow in the non-AMF case provides a conservative estimation of the hyphal contribution.

When the contact area between the two compartments (20 cm × 30 cm=600 cm^2) is taken into account, this water flow from HC to PC corresponds to a flux density of 22.8 μl cm^{-2} day^{-1} across the barrier. Dividing this flux density value by the hyphae number per unit area (64.4 cm^{-2}) yields the estimate of the water flow of 0.35 μl day^{-1} in the form of plasma flow in a single AMF-hypha. Considering the typical diameter for the central lumen of one hypha of 10 μm (Sanders and Tinker 1973), this translates to a bulk flow velocity on the order of 0.31 cm min^{-1}.

All these values are mean values over all drying periods without consideration of the state of the plant- and hyphal development. We note that the direction of this flow is from the wet hyphal compartment into the dry plant compartment and therefore corresponds to the gradient in the water potential.

With the assumption that the plant receives all the water conducted by the AMF and that all the water flow out of the plant compartment is conveyed only by the plants, the total water uptake of the plants equivalent to the delivery into the atmosphere in the AMF treatment amounts to the sum of the outflow of the plant compartment (55.0 g day^{-1}) and the inflow from the hyphal compartment (13.7 g day^{-1}) resulting in 68.7 g day^{-1}. These values provide an estimation of the relative contribution of the hyphae to the total direct and indirect water uptake of 20%.

There is also a clear difference in the water uptake of the plants between the AMF and non-AMF treatments, with the total consumption of the plants in the non-AMF treatment of 47.1 g day^{-1} when the hyphal flow is also taken into account (see Table 1). This yields an AMF/non-AMF ratio of 1.46 for the water uptake.

Discussion

The difference in water flow out of the hyphal compartment is apparent in Fig. 2 and is highly significant between the AMF and non-AMF treatments ($p<0.000001$) and much more so than comparable values in the literature ($p<0.05$, Faber et al. 1991; $p<0.10$, Cui and Nobel 1992). The higher significance level obtained in our measurements likely derives from our application of on-line measurements, the repeated wetting-drying cycles and the high repetition rate of the water content measurements.

Our results indicate a relative contribution of the mycorrhizal fungus to the direct and indirect total water uptake of the plants of about 20%. Indirect water uptake may result from water being effluxed from hyphae to soil with following uptake by roots. This value accords with some other estimates in the literature (22%, Faber et al. 1991; 24%, Cui and Nobel 1992) and the general notion that the contribution is important (Ruiz-Lozano and Azcon 1995) and contradicts studies showing the contribution to be negligible (Sanders and Tinker 1973; Cooper and Tinker 1981; Graham and Syvertsen 1984; Fitter 1985; George et al. 1992; Koide 1993). One possible explanation for the differences in results regarding the overall contribution of mycorrhiza to plant growth might derive from the growth conditions of the plants possibly playing an important role for the existence and the activity of the mycorrhiza. For example, it has been reported that an optimum water supply during plant development (George et al. 1992) may lead to a decreased formation of mycorrhiza and their reduced activity. By contrast, repeated drying periods presumably induce an increased capability to obtain water from the soil by mycorrhizal plants (Khalvati et al. 2005). An equivalent effect was reported for phosphorus, which is obviously important for plant-AMF interaction: increasing soil phosphate levels resulted in a reduction in the percent mycorrhizal infection of roots (Sanders and Tinker 1973).

Finally, it has been reported that hyphae tend to increase in length with the progress of drying (Querejeta et al. 2003).

It is important to note that our value of 20% may be regarded as a lower limit of the total contribution of the AMF because their hyphae also exist and contribute to water uptake in the plant compartment as well. Indeed, because of the higher volume of the plant compartment, their contribution may be even higher than that of the hyphae ending in the hyphal compartment. However, over the course of the experiment, the hyphal compartment had higher water content than did the plant compartment, possibly resulting in an increased capability of hyphae to transport water into the plant compartment compared to natural conditions. Water may also be effluxed from the hyphae into the drier soil in the plant compartment and it may become indirectly available to the plant. Therefore the value of 20% may overestimate the direct hyphal contribution to the total water uptake with part of it possibly representing indirect transfer to the plant.

A previous investigation (Khalvati et al. 2005) that also investigated the decline in water content during drying periods obtained values of 0.146 g g^{-1} and 0.121 g g^{-1} for the AMF and control treatments, respectively, yielding also a relative hyphal contribution of about 20%. At the same time, AMF also induced a 100%-increase in the shoot dry weight and a 19%-increase in the root weight of the plants. Thus, although AMF result in a clear difference in drying rates, it is unclear whether this effect originates from the increased dry weights (indirect effect) and/or from the increased water flow in the hyphae (direct effect). Indeed, the ratio of the total water uptake by the plant between the AMF and non-AMF treatments obtained in our investigations (68.7 g day^{-1}/47.1 g day^{-1}= 1.46) is often held to be related to the corresponding ratios in the dry weights of the shoots and roots (Koide 1993; Augé et al. 2001). We obtained the dry weight ratio 1.49, which is nearly identical to the ratio above. Therefore, our measurements agree with the well-known water use efficiency for the relationship between water consumption and dry weight of shoots and roots.

Further quantitative comparisons with previous results reveal a large degree of variation. For instance, our estimate of 64.4±12.3 cm^{-2} for hyphal density is clearly higher than the two values reported in literature obtained at different times after sowing by Faber et al. (1991) of 0.67 cm^{-2} and 18.6 cm^{-2}. Further, our measured water flow in single hyphae of 0.35 μl day^{-1} is much lower than reported values of 2.4 μl day^{-1} measured for a single hyphal entry point (Allen 1982). This discrepancy may be explained by the branching of hyphae which makes it probable that single hyphae exhibit smaller flows than the flows in entry points. The higher values of 18.2 and 9.0 μl day^{-1} as obtained for two different experiments with plant-hyphae-chambers (Faber et al. 1991) may originate from the clearly different number of hyphae crossing the air gap combined with a possible flexibility in the rate of water flow in individual hyphae such that a comparatively small number of hyphae can compensate for a given water deficit by increasing the water flow in any single hypha.

Similarly, the value that we obtained for bulk velocity in hyphae (0.31 cm min^{-1}) provides an indication of the capability of the hyphae, especially when compared to values of 0.037 cm min^{-1} obtained from the phosphorus transport and of 5 cm min^{-1} calculated under the assumption that all the additional water transpired by plants with mycorrhiza is transferred by hyphae (Sanders and Tinker 1973). Additional velocity values from the literature are 2.1 cm min^{-1} (Allen 1982) and 16.1 cm min^{-1} and 7.9 cm min^{-1} (Faber et al. 1991). Again, explanations for the large range of values derive from any or all of differences in the experimental setup (including the duration of drought), the state of plant and hyphae development (Faber et al. 1991), and/or the species of fungi applied for the experiment (Ruiz-Lozano and Azcon 1995).

We note that watering events necessarily resulted in sharp increases in the water content values in the plant compartments. Although the total weight was restored, the measured values of the peak water content after watering were lower than the initial one. This discrepancy can in part be explained by the non-accounted increasing weight of shoots and roots. The final dry weight of shoots and roots yield 23.2 g (AMF) and 15.6 g (non-AMF, see above), equivalent to fresh weights of 103 g and 69 g, respectively. These weights were not considered during re-watering. At the end of the experiment, they correspond to water content values being lower by 0.030 g g^{-1} and 0.020 g g^{-1} than the initial value of 0.23 g g^{-1}. In this way, a considerable part of the decline in

the peak values during re-watering can be explained, the exact reasons for the total decline and its variation, however, remain unclear.

Although the watering interval was 1 day only, the measured values may continue to increase over a longer time span. One must consider that the water content sensors integrate over the whole depth interval from 7.5 cm to 22.5 cm and that water content is not uniform within the sensitive volume as indicated in Fig. 1. Especially at the start of the re-watering, the water content is much lower than the saturated water content of 0.37 g g^{-1}, and the corresponding hydraulic conductivity is rather low. Therefore, the associated water flow movements often lasted more than 1 day and could be responsible for maximum values of water content occurring even about 2 days after watering. Thus, our analyses of the drying period began only with a constant decline in water content values.

Although watering was restricted to the plant compartment only, clear increases in water content were also obvious in the hyphal compartment with AMF (and to a lesser degree in the non-AMF treatment), especially prior to the drying periods starting on day 35, 42, and 50. This demonstrates that water was transferred from the plant compartment into the hyphal compartment during the watering events. The existence of hyphae even in the compartments of the non-AMF treatment (9±5 cm^{-2}) may provide an explanation because the possibility of bidirectional water flow in hyphae has recently been reported (Allen 2007; Querejeta et al. 2003); our observed increases in water content in the hyphal compartment, and especially the differential increases between the AMF and non-AMF treatments, may be a confirmation for the hypothesis of a bidirectional flow. However, it cannot be excluded that this increase was due to preferential flow along the compartment walls followed by a partial filling of the holes in the barrier and a transfer into the hyphal compartment. Probably, this effect was facilitated by the dry soil which tends to be hydrophobic. On the other side, the clear difference between the effects in the AMF and non-AMF treatments points to an effect which depends on the existence of hyphae crossing the barrier. Furthermore, a channel for the water transfer from the plant to the hyphal compartment without influence of hyphae would be independent on time which is not suggested by the small increases observed during the first two re-wetting events.

However, the small increase in water content during re-watering in the non-AMF treatment is obviously not compensated for by water loss in the subsequent drying period, presumably caused by the restricted time period to accomplish this effect. This leads to the slightly increasing water content in the hyphal compartment.

In conclusion, our application of a plant-hyphal chamber with an air-gap in combination with the on-line, non-destructive, and sensitive water content measurement in each of the two compartments provides unambiguous support for the presence of water flow in hyphae and a significant 20% contribution of the hyphae to the direct and indirect total water uptake of the plant. In addition, we were able to provide the first estimate of the flux density for water in the hyphae (22.8 μl cm^{-2} day^{-1}). Although discrepancies still exist regarding the exact contribution of AMF to the water balance of the plant, we are hopeful that our experimental protocol will provide a standard to yield comparable measures across studies.

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References

Allen MF (1982) Influence of vesicular-arbuscular mycorrizae on water movement through Bouteloua gracilis (H.B.K.) Lag ex Steud. New Phytol 91:191–196

Allen MF (2007) Mycorrhizal fungi: highways for water and nutrients in arid soils. Vadose Zone J 6:291–297

Ames RN, Reid CPP, Porter LK, Cambardella C (1983) Hyphal uptake and transport of nitrogen from two ^{15}N-labelled sources by Glomus mosseae, a vesicular-arbuscular mycorrhizal fungus. New Phytol 95:381–396

Augé RM, Stodola AJW, Tims JE, Saxton AM (2001) Moisture retention properties of a mycorrizal soil. Plant Soil 230:87–97

Augé RM (2004) Arbuscular mycorrhizae and soil/plant water relations. Can J Soil Sci 84:373–381

Cooper KM, Tinker PB (1981) Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. IV. Effect of environmental variables on movement of phosphors. New Phytol 88:327–339

Cui M, Nobel PS (1992) Nutrient status, water uptake and gas exchange for three desert succulents infected with mycorrhizal fungi. New Phytol 122:643–649

Dean TJ, Bell JP, Baty AJB (1987) Soil moisture measurement by improved capacitance technique. Part I. Sensor design and performance. J Hydrol 93:67–78

Egerton-Warburton LM, Querejeta JI, Allen MF (2007) Common mycorrhizal networks provide a potential pathway for the transfer of hydraulic lifted water between plants. J Exp Bot 58:1473–1483

Faber BA, Zasoski RJ, Munns DN, Shackel K (1991) A method for measuring hyphal nutrient and water uptake in mycorrhizal plants. Can J Bot 69:87–94

Fitter AH (1985) Functioning of vesicular-arbuscular mycorrhizals under field conditions. New Phytol 99:257–265

George E, Häussler GE, Vetterlein D, Grogus E, Marschner H (1992) Water and nutrient translocation by hyphae of Glomus mosseae. Can J Bot 70:2130–2137

Graham JH, Syvertsen JP (1984) Influence of vesicular-arbuscular mycorrhiza on the hydraulic conductivity of roots of two citrus rootstocks. New Phytol 97:277–284

Khalvati MA, Hu Y, Mozafar A, Schmidhalter U (2005) Quantification of water uptake by arbuscular mycorrhizal hyphae and its significance for leaf growth, water relations, and gas exchange of barley subjected to drought stress. Plant Biol 7:706–712

Koide RT (1993) Physiology of the mycorrhizal plant. Adv Plant Pathol 9:33–5

Kormanik PP, McGraw AC (1982) Quantification of vesicular-arbuscular mycorrhizas in plant roots. In: Schenck NC (ed) Methods and principles of Mycorrhizal research. The American Phytopathological Society, St. Paul, Minnesota, pp 37–45

Li XL, George E, Marschner H (1991) Extension of the phosphorus depletion zone in VA-mycorrhizal white clover in a calcareous soil. Plant Soil 136:41–48

Querejeta JI, Egerton-Warburton LM, Allen MF (2003) Direct nocturnal water transfer from oaks to their mycorrhizal symbionts during severe drying. Oecologia 134:55–64

Rhodes LH, Gerdemann JW (1978) Hyphal translocation and uptake of sulphur by vesicular-arbuscular mycorrhizae of onions. Soil Biol Biochem 10:355–360

Ruiz-Lozano JM, Azcon R (1995) Hyphal contribution to water uptake in mycorrizal plants as affected by the fungal species and water status. Physiol Plant 95:472–478

Ruth B (1999) A capacitance sensor with planar sensitivity for monitoring soil water content. Soil Sci Soc Am J 63:48–54

Ruth B, Munch JC (2005) Field measurements of water content in the top soil using a new capacitance sensor with a flat sensitive volume. J Plant Nutr Soil Sci 168:169–175

Ruth B, Grundmann S, Schroll R (2008) Simulation of exterior conditions in permanently closed soil chambers by controlling air flow, soil water content, and temperature. Water Air Soil Pollut Focus 8:243–250

Sanders FE, Tinker PB (1973) Phosphate flow into mycorrhizal roots. Pestic Sci 4:385–395

Subramanian KS, Charest C, Dwyer LM, Hamilton RI (1995) Arbuscular mycorrhizas and water relations in maize under drought stress at tasseling. New Phytol 129:643–650

Thomas AM (1966) In situ measurement of moisture in soil and similar substances by fringe capacitances. J Sci Instrum 43:21–27

Unestam T, Sun YP (1995) Extramatrical structures of hydrophobic and hydrophilic ectomycorrhizal fungi. Mycorrhiza 5:301–311

Warren JM, Brooks JR, Meinzer FC, Eberhart JL (2008) Hydraulic redistribution of water from Pinus ponderosa trees to seedlings: evidence for an ectomycorrhizal pathway. New Phytol 178:382–394