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Extraradical development and contribution to plant performance of an arbuscular mycorrhizal symbiosis exposed to complete or partial rootzone drying

Elke Neumann · Barbara Schmid · Volker Römheld · Eckhard George

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Abstract Sweet potato plants were grown with or without *Glomus intraradices* in split-root pots with adjacent root compartments containing a soil with a low availability of phosphate. One fungal tube, from which root growth was excluded, was inserted into each root compartment. During 4 weeks before harvest, the soil moisture level in either both or only one of the two root-compartments of each pot was decreased. Controls remained well watered. Low soil moisture generally had a negative effect on the amount of extraradical mycelium of *G. intraradices* extracted from the fungal tubes. Sporulation in the fungal tubes was much higher compared with the soil in the root compartment, but remained unaffected by the soil moisture regime. Concentrations of P in extraradical mycelium were much lower than usually found in plants and fungi, while P concentrations in associated mycorrhizal host plant tissues were in an optimum range. This suggests efficient transfer of P from the extraradical mycelium to the host plant. Despite the negative effect of a low soil moisture regime on extraradical *G. intraradices* development, the symbiosis indeed contributed significantly to P uptake of plants exposed to partial rootzone drying. The possibility that extraradical arbuscular mycorrhizal fungal development was limited by P availability under dry soil conditions is discussed.

E. Neumann (✉) · E. George
Institute of Vegetable and Ornamental Crops (IGZ),
14979 Grossbeeren, Germany
e-mail: Neumann@igzev.de

B. Schmid · V. Römheld
Institute of Plant Nutrition (330), Hohenheim University,
70593 Stuttgart, Germany

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Introduction

Arbuscular mycorrhizal (AM) plants have been shown to withstand periods of drought better compared with non-AM controls and to maintain higher biomass production under dry soil conditions (Augé 2001). Frequently these effects could be attributed to an improved mineral nutritional status of AM plants (Al-Karaki and Clark 1998; Kwapata and Hall 1985). A higher internal water use efficiency (WUE) of AM compared with non-AM plants, irrespective of the plant nutritional status, has also been reported (Pinior et al. 2005; Porcel and Ruiz-Lozano 2004), but the precise physiological reasons behind these observations are not yet completely understood.

Recent studies indicate that the extension of the AM fungal mycelium in the soil is an important determinant for the contribution of the AM symbiosis to plant performance under drought stress (Augé et al. 2003, 2007). However, not much is known about how a low soil moisture regime affects the extraradical development of AM fungi. Soil dryness has been shown to increase (Bethlenfalvay et al. 1988; Khalvati et al. 2005) or decrease (Staddon et al. 2003) the length density of extraradical AM fungal hyphae in previous studies, but reasons for these observations are not known to date.

The AM fungi are obligate biotrophs and rely on their plant host for energy supply (Bago and Bécard 2002). Particularly when the whole plant rooting zone falls dry, a decreased plant photosynthetic capacity due to water deficiency and stomata closure might lead to a decreased

supply of the fungal symbiont with carbohydrates. Furthermore, soil dryness usually leads to a decrease in the mobility of P and other mineral elements in the soil solution (Gahoonia et al. 1994; Marschner 1995). Results of previous studies indicate that not only plant growth but also AM fungal development may be limited by a low availability of mineral nutrients in the soil (Treseder and Allen 2002). However, with a diameter of between 1 and 20 μm, AM fungal hyphae have access to fine soil pores which remain filled with soil solution even under low soil moisture regimes (Faber et al. 1991). This may render the AM fungal colony less sensitive toward decreasing soil water potentials compared with plant roots.

In several microorganisms such as yeasts or bacteria, deficiency of water, carbohydrates, or mineral nutrients stimulates the formation of resting propagules like spores (Grelet 1957; Schaeffer et al. 1965). In AM fungal inoculum production, it is a common practice to expose AM-colonized plants to severe drought stress prior to the harvest of the inoculum, to promote AM fungal sporulation (e.g., Moreira et al. 2007). Whether sporulation in AM fungi is indeed triggered by decreasing soil moisture and how drought-induced alterations in extraradical development would affect AM contribution to plant growth and nutrient uptake has not yet been investigated in detail. Particularly when drought stress is temporarily or spatially limited, the maintenance of a functional AM fungal mycelium in dry soil might be important for the outcome of the AM symbiosis.

In a vertical split-root experiment, Neumann and George (2004) could show that AM fungal hyphae were able to take up P from dry topsoil overlaying moist subsoil. This indicated that in plants exposed to partial rootzone drying, extraradical hyphae remained active in the dry soil part. However, effects of soil dryness on extraradical development of the AM fungus were not investigated in this study. It could also not be excluded that the AM fungal mycelium in the dry topsoil was supplied with water from the moist subsoil, hydraulically lifted and exuded into the rhizosphere by the host plant roots.

The first aim of the present experiment was to study the effect of a low soil moisture regime on the amount, structure, and mineral element content of the extraradical mycelium of an AM fungus. To assess whether the exposure of the host plant shoot to water deficiency would have an influence on AM fungal mycelium development in dry soil, split root-systems of the host plants were exposed either to complete or partial rootzone drying. The hydraulic transfer of water between the two root-parts of each plant was prevented. The second aim of this experiment was to compare the effect of partial and complete rootzone drying on growth, mineral element uptake, and WUE between +M and −M sweet potato plants.

Materials and methods

Stem cuttings of the Chinese sweet potato cultivar 'Hongxin' were rooted in aerated 3 mM $CaSO_4$ solution. The length of the stem of each cutting was approximately 12 cm. After the first root tips had emerged, the lower 4 cm of each stem were split vertically into two parts of equal size. The plants were cultivated in aerated nutrient solution (concentration of the element/applied form: N, 5 mM/Ca $(NO_3)_2 \cdot 4H_2O$; P, 0.5 mM/KH_2PO_4; K, 1.5 mM/KH_2PO_4 and K_2SO_4; Ca, 3.5 mM/Ca $(NO_3)_2$ and $CaSO_4 \cdot 2H_2O$; Mg, 0.6 mM/$MgCl_2 \cdot 6H_2O$; S, 1.5 mM/$CaSO_4$ and K_2SO_4; Fe, 40 μM/Fe-EDTA; B, 56 μM/H_3BO_3; Mn, 3.6 μM/$MnSO_4$; Zn, 1.5 μM/$ZnSO_4$; Cu, 1.6 μM/$CuSO_4$; and Mo, 0.07 μM/$(NH_4)_6Mo_7O_{24}$) for two more weeks, until roots established on each side of the split stem had a length of approximately 10 cm. Then, one plant was transferred into each split-root pot. These were constructed from two black 1 L plastic planting containers (Pöppelmann Teku Tainer 1.0, Teku, Germany) fastened together, side by side, with adhesive tape. Each container (= root compartment) was filled with 1,100 g dry soil at a bulk density of 1.3 g cm^{-3}. The soil had been dry heated two times for 24 h at 85°C with an interval of 48 h at room temperature to eliminate AM fungal propagules. Prior to heating, the sieved (2 mm) soil contained (mg kg^{-1}) 5.2 and 3.4 $CaCl_2$ (0.0125 M)-extractable NH_4^+ and NO_3^-, respectively, 4.4 acetate lactate-extractable (CAL; Schüller 1969) P, 58 CAL-extractable K, and 1.93 (Fe), 1.75 (Mn), 0.10 (Zn), and 0.16 (Cu) diethylenetriamine pentaacetic acid-extractable micronutrients. The soil had a pH (0.01 M $CaCl_2$) of 7.3 and 0.2% organic matter. It was classified as loamy sand (45.2% sand, 42.0% silt, 12.8% clay). The soil in all treatments was fertilized with 200 mg K (K_2SO_4), 200 mg N (NH_4NO_3), 100 mg Mg ($MgSO_4$), 50 mg P ($Ca(H_2PO_4)2 \cdot H_2O$), 10 mg Zn ($ZnSO_4 \cdot H_2O$), 10 mg Cu ($CuSO_4$), and 8 mg Fe ($FeNH_4$-citrate) per kg dry soil.

Inoculum of the AM fungus *Glomus intraradices* (BEG 110) was propagated on chickpea plants in open pot culture in the greenhouse for about 8 weeks, using the same soil as described above. The inoculum was air-dried and stored in a cold room (4°C) for 2 years before it was used for inoculation of sweet potato plants in this experiment. It consisted of a mixture of colonized chickpea root pieces and adhering soil containing extraradical mycelium and spores. Inoculum, representing 7.4% *w/w* of the growth substrate, was homogeneously mixed with the soil before it was filled into the split-root pots. The inoculum for the nonmycorrhizae (−M) treatments was filtered with deionized water (70 ml per 50 g dry inoculum through Blue Ribbon filter paper, Schleicher and Schüll, Germany) before being autoclaved. The filtrate was added to the soil of the −M treatments to encourage a microflora similar as in the mycorrhizae (+M) treatments.

One fungal tube constructed from a 50-ml polyethylene bottle (55 ml including the bottle neck; inner diameter= 2.5 cm) was buried vertically in the center of each root compartment. Each fungal tube had two windows (3.0 cm width; 3.5 cm height) covered by a nylon membrane (Sefar Nitex 03-30/18, Sefar, USA; mesh width=30 μm). The small mesh width of this membrane allowed only hyphae but not roots to penetrate. Stability of the tubes was provided by an additional cover of stiff polyamide net (Sefar Nitex 08-1000/44, Sefar, USA; mesh width=1 mm). The tubes were filled with 55 ml of a 1:1 (weight) mixture of 40 μm wet sieved soil and glassbeads (diameter=1.5–2 mm). This substrate allows for the complete extraction of AM extraradical mycelium from fungal tubes after harvest (Neumann and George 2005). The wet sieved soil was produced from the same substrate as used for the root compartments. Before it was used in the experiment, the wet sieved soil was dried at 65°C and heated for 12 h at 90°C to eliminate fungal propagules. The wet sieved soil was fertilized with the same amounts of nutrients as the soil in the root compartments.

The experiment was conducted in a glasshouse at Hohenheim University in Stuttgart, Germany (48°25′ N, 9°11′ E), from August to October. In October, the experiment received 16 h supplemental lighting of 400 μmol photons m^{-2} s^{-1} at bench height during the day provided by Osram HQL-R 400 W lamps. The split-root pots were set up completely randomized. Each of the six AM inoculation × irrigation treatments was replicated four times.

Daily evapotranspiration from the split-root pots was estimated gravimetrically. The soil water content in individual root compartments was estimated using time domain reflectometry (TEKTRONIX, 1502 C/Tektronix, Beaverton, OR, USA) according to Roth et al. (1990). A 12 cm-head with three pins was installed in each root compartment. The soil water content was measured daily before plants were watered, and the soil water content after watering was calculated from the amounts of water added to the compartments. The mean soil water content was estimated by averaging these two values, assuming that the depletion of water from the root compartments between the irrigation intervals was approximately linear.

During the first week after transplanting, the plants were covered with a plastic bag to reduce evapotranspiration. After the bags were removed, water lost from the root compartments was replaced with deionized water to maintain an average soil water content of approximately 24% *w/w* in all pots until 8 weeks after transplanting. This soil moisture level reflected 69% of the soil water holding capacity (WHC; pF=2.2). Thereafter, water supply was reduced to both ($^{-}$W/$^{-}$W) or only one ($^{+}$W/$^{-}$W) of the two-root compartments of each split-root pot. In all $^{-}$W root compartments, soil water content was gradually decreased to approximately 12 % *w/w* (34.5% of WHC; pF=3.5) in the course of 1 week and maintained at this level for another 3 weeks before harvest. The average soil water contents throughout the drought stress period were slightly lower for $^{+}$W root compartments when the adjacent root compartment was $^{-}$W instead of $^{+}$W (Table 1). This was due to a stronger depletion of soil water from $^{+}$W compartments when the other root part was exposed to dry soil. The +M plants also depleted soil water between the irrigation intervals faster compared with −M treatments, leading to lower average water contents in +M root compartments. Throughout the drought stress period, plants of the $^{-}$W/$^{-}$W treatment were temporarily wilted but not irreversibly damaged.

Four weeks after initiating the drought treatments, plants were harvested and roots were washed from the soil. Representative root samples (0.5 g fresh weight) were taken from each root compartment. The AM fungal colonized root length and the root length with intraradical AM fungal spores was assessed by a modified intersection method (Kormanik and McGraw 1982; Tennant 1975) after staining of the root samples with trypan blue in lactic acid (Koske and Gemma 1989). Between 250 and 300 intersections were counted per sample.

Two soil cores (diameter=2 cm/length=8–9 cm) were taken from each +M root compartment and dried at 50°C in a drying oven. Representative samples of mycelium from these soil samples were obtained by bringing 50 g of dried soil into suspension by stirring with approximately 500 ml water. The suspension was allowed to settle for 10 s before the supernatant was poured over a 40-μm sieve. This procedure was repeated four times before the mycelium was collected from the sieve using forceps and a Pasteur pipette and transferred to 2.5 ml Eppendorf tubes.

The extraradical mycelium from fungal tubes was extracted as described by Neumann and George (2005) and freeze dried at −30°C for 4 days (freeze dryer P15K, Piatkowski GmbH, Munich, Germany). After the DW of the mycelium had been determined, subsamples of approximately 5 mg were transferred to 2.5 ml Eppendorf tubes. These subsamples as well as the mycelium samples obtained from the root compartments were stained overnight at room temperature with a few drops of 0.05% trypan blue in lactic acid. Stained samples were transferred to a laboratory blender (Waring Blendor 8010, Waring, USA) with 200 ml tap water and blended at low speed for 45 s. Aliquots (20 ml) of the suspension were used to assess the length of hyphae and the number of AM spores by the agar film method (Bååth and Söderström 1980). By the method used for extraction of extraradical mycelium from the soil in the root compartments, it could not be completely excluded that parts of mycelium entangled with heavier soil particles remained within the soil fraction and

were not transferred into the 40-μm sieve. Thus, data obtained for these mycelium samples were not evaluated in relation with the amount of soil extracted. Over all soil samples extracted by this method, the minimum and maximum numbers of AM spores obtained were 77 and 355 per cm^3 dry soil, respectively. Between 0.73 and 4.36 m of hyphae were extracted per cubic centimeter dry soil.

The remaining freeze-dried mycelium from fungal tubes was digested in a microwave after 3 ml HNO_3 and 1 ml H_2O_2 was added to each sample. The samples were taken up into 10 ml H_2O before concentrations of P, K, Ca, Mg, S, Mn, Cu, and Zn were measured by inductively coupled plasma optical emission spectrometry (Varian Vista Pro Radial, Varian Inc., USA).

The plant material was dried at 65°C in a drying oven. After their DW was estimated, shoots, roots, tubers, and leaves lost over the growth period were analyzed for their P, Cu, and Zn concentration separately. Samples of 200 mg of ground plant material were dry ashed at 500°C, oxidized with 5 ml of 1:3 diluted HNO_3, and taken up into 25 ml of 1:30 diluted HCl. Phosphorus concentrations in the samples were analyzed colorimetrically with a spectrophotometer at 436 nm wavelength after staining with ammonium molybdate–vanadate solution (Gericke and Kurmies 1952). Concentrations of Cu and Zn were measured by atomic absorption spectrometry (AAS; ATI Unicam 939/Solaar, Thermo Electron, USA).

Statistics were calculated using the SigmaStat 2.03 Programme. Values obtained for the two root-parts of $^{+}W/^{+}W$ and $^{-}W/^{-}W$ plants were averaged for each replicate before statistical analysis was performed (n=4).

Results

Intraradical and extraradical AM fungal development

Roots of −M plants did not show AM fungal colonization. No mycelium could be obtained from root compartments or fungal tubes of −M treatments, indicating that contributions of other soil fungi to the mycelium obtained from +M soil were negligible.

In +M treatments, the extent of AM fungal root colonization was in a high range, but decreased slightly when roots grew in ^{-}W soil, irrespective of the irrigation treatment in the adjacent root compartment (Table 2). Intraradical AM fungal sporulation was unaffected by the irrigation treatment. The DW of the extraradical mycelium obtained from the fungal tubes decreased by approximately 50% when roots grew in ^{-}W soil. Similar to the intraradical root colonization, there was no effect of the treatment of the other root part on the DW of the mycelium in the fungal tubes. In the fungal tubes, the hyphal length and the spore

Table 1 Mean soil water content in the root compartments throughout the drought stress period

	Nonmycorrhizal plants				Mycorrhizal plants				Significant interactions (P values; three-way ANOVA)
Irrigation treatment	$^{+}W/^{+}W$	$^{+}W/^{-}W$		$^{-}W/^{-}W$	$^{+}W/^{+}W$	$^{+}W/^{-}W$		$^{-}W/^{-}W$	
Root compartment	$^{+}W^{a}$	^{+}W	^{-}W	$^{-}W^{a}$	$^{+}W^{a}$	^{+}W	^{-}W	$^{-}W^{a}$	
Mean soil water content (% w/w)	24.50±0.17	24.38±0.32	12.07±0.14	11.92±0.09	22.94±0.12	21.43±0.32	11.91±0.16	11.46±0.49	$M\times S$ (<0.001) $M\times/S$ (<0.001) $S\times/S$ (0.010) $M\times S\times/S$ (0.007)

Shown are the mean values ± standard deviations. The right part of the table shows the P values for significant interactions between mycorrhizal inoculation (M), the soil moisture in the root compartment (S), and the soil moisture in the combination compartment ($/S$), revealed by the three-way ANOVA. Statistical interpretation of the main effects of the individual factors was not performed, since all factor's effects depended upon the levels of the other factors

[a] The values obtained for the two root-compartments of each split-root pot were averaged and the ANOVA was performed on a balanced data set (n=4)

Table 2 Intra- and extraradical development of the AM fungus

Irrigation treatment	$^{+}$W/$^{+}$W	$^{+}$W/$^{-}$W		$^{-}$W/$^{-}$W	*P* values (two-way ANOVA)		
Root compartment	$^{+}$W[a]	$^{+}$W	$^{-}$W	$^{-}$W[a]	*S*	/*S*	*S*×/S
AM fungal colonized root length (percent of the total root length)	86.3±1.9	88.1±2.7	80.6±4.8	77.5±4.1	**<0.001**	0.185	0.718
Root length showing intraradical sporulation (percent of the AM fungal colonized root length)	4.4±1.7	8.1±3.9	7.8±1.1	8.8±3.5	0.164	0.353	0.127
DW of mycelium obtained from fungal tubes (mg per compartment of 55 ml)	40.7±11.0	47.4±20.3	21.6±14.8	19.2±13.0	**0.009**	0.559	0.780
Length of hyphae in the fungal tubes (m per cm^3 substrate)	4.93±0.74	8.39±3.72	3.82±1.38	3.59±1.11	**0.015**	0.104	0.149
Extraradical sporulation (number of AM fungal spores per m hyphae length)							
Fungal tube	623±204	500±50	499±142	386±38	0.089	0.929	0.090
Root compartment	71±21	78±29	139±22	67±13	–	–	**0.010**

Shown are the mean values ± standard deviation. The right part of the table shows the results of the two-way ANOVA. *P* values indicative of a significant ($P<0.05$) influence of the soil moisture in the root compartment (*S*), the soil moisture in the combination compartment (/*S*), or an interaction between both factors (*S*×/*S*) are printed in bold letters. Statistical interpretation of the main effects of *S* or /*S* was not performed in case the size of one factor's effect depended upon the level of the other factor

[a] The values obtained for the two root-compartments of each split-root pot were averaged and the ANOVA was performed on a balanced data set ($n=4$)

numbers per unit substrate were significantly decreased in response to the $^{-}$W treatment. However, the hyphal length per unit mycelium DW and the number of spores per meter hyphal length were unaffected by the irrigation treatment.

Spore numbers per unit hyphal length were generally much higher in the AM fungal tubes compared with the root compartments. The mean values (±standard deviation) for numbers of spores obtained per cubic centimeter substrate averaged over all $^{+}$W and $^{-}$W tubes were 1,698±912 and 3,619±1,588, respectively. In the $^{-}$W root compartment of the $^{+}$W/$^{-}$W treatment, the number of spores per meter hyphae length was approximately two times higher compared with all other treatments.

Element concentrations in the extraradical mycelium obtained from the fungal tubes did not differ depending on the irrigation treatment (results not shown). Thus, values for all +M treatments were averaged (Table 3). The P, K, Ca, Zn, Mn, and Cu contents of the extraradical mycelium were severely decreased in response to the $^{-}$W treatment, irrespective of the irrigation treatment in the adjacent root compartment (Table 4).

Evapotranspiration during the growth period and plant WUE

Daily evapotranspiration averaged over 2 days increased during the experiment with increasing plant size, but was not different between +M and −M treatments during the first 39 days after planting (data not shown). Thereafter, the mean values for +M plants were significantly higher compared with the corresponding −M controls in the $^{+}$W/$^{+}$W and $^{+}$W/$^{-}$W treatments until harvest. In the $^{-}$W/$^{-}$W treatments, daily evapotranspiration averaged over 2 days was higher for +M plants only until 6 days after the beginning of the drought stress period. Thereafter, no differences in evapotranspiration between +M and −M plants could be measured.

From 6 days after the beginning of the drought stress period, daily evapotranspiration decreased in the $^{-}$W/$^{-}$W as well as the $^{+}$W/$^{-}$W treatment compared with the respective well-watered controls (Fig. 1). The decrease in daily evapotranspiration in response to partial rootzone drying compared with well-watered controls was much larger for −M compared with +M treatments.

In the +M plants, the WUE was increased in response to the $^{+}$W/$^{-}$W or $^{-}$W/$^{-}$W treatment compared with the well-watered control (Table 5). No such effect could be observed in the −M treatments.

Plant biomass and uptake of P, Zn, and Cu

The +M plants could be visually distinguished from the −M controls by their larger shoot size, starting 5 weeks after planting. At the time of harvest, +M plants had a higher total DW compared with the −M controls within the same irrigation treatment (Table 6). In the −M plants, partial rootzone drying decreased plant growth and uptake of P, Zn, and Cu to the same extent as did complete rootzone drying. In +M plants, complete but not partial rootzone drying had a negative effect on plant growth and element uptake. The different irrigation treatments had no effect on the distribution of the root or tuber DW between the two-

Table 3 Element concentrations in mycelium obtained from fungal tubes

	P mg g DW^{-1}	K	Ca	Mg	S	Zn µg g DW^{-1}	Mn	Cu
Element concentration in the mycelium	0.78±0.36	1.28±0.31	13.54±3.27	6.10±2.02	0.46±0.28	373.45±170.49	137.30±25.13	178.74±9.14

Since values were not different depending on the irrigation treatment (data not shown), the table shows the mean values ± standard deviations for pooled data

root compartments (data not shown). Only shoots and tubers were included into the calculation of the element contents, since it could not be excluded that in roots, Zn and Cu were at least partly bound to plant or AM fungal cell walls without being taken up into the plant cytoplasm (Strassner et al. 1999).

In all irrigation treatments, shoot concentrations of P, Zn, and Cu were much higher for +M compared with −M plants. The irrigation treatment had no effect on P and Zn concentrations in the shoot. In +M plants, partial rootzone drying decreased Cu concentrations in the shoot compared with the other irrigation treatments.

Colonization by AM fungi increased P, Zn, and Cu concentrations in the roots (Table 7). The irrigation treatment did not affect root concentrations of P and Zn, but +M roots had a lower Cu concentration when grown in $^{-}$W compared with $^{+}$W soil. The P concentrations in the roots were in a similar range as values obtained for shoots of the plants in the same treatment. Mycorrhizal roots had much higher concentrations of P compared with the associated extraradical mycelium obtained from the fungal tubes. Concentrations of Zn and Cu were lower in roots compared with the AM fungal mycelium, but much higher than in the corresponding shoots.

Discussion

A low soil moisture regime negatively affected intra- and extraradical development of *G. intraradices* in the present experiment. To which extent this observation can be attributed to a decreased supply of photosynthates to roots in dry soil, or direct negative effects of soil dryness on AM fungal proliferation, remains speculative. However, the DW of the extraradical mycelium obtained from fungal tubes was decreased by approximately half in response to the $^{-}$W treatment, whereas growth of +M roots and intraradical AM fungal colonization were not or only slightly affected by soil dryness. These results may suggest that a low soil moisture regime had a direct negative effect on extraradical growth of the AM fungus.

To our knowledge, our study provides the first report on concentrations and contents of major nutritional elements in soil grown extraradical mycelium of an AM fungus. Although we cannot exclude that the mycelium we analyzed contained dead or physiologically inactive hyphae, the extremely low concentrations of P and S in the extraradical mycelium of *G. intraradices* are surprising. Phosphorus concentrations in extraradical mycelium measured in a previous experiment (Neumann and George

Table 4 Total element content of the AM fungal mycelium obtained from fungal tubes (in microgram per tube of 55 ml)

Irrigation treatment	$^{+}$W/$^{+}$W	$^{+}$W/$^{-}$W		$^{-}$W/$^{-}$W	*P* values (two-way ANOVA)		
Root compartment	$^{+}$W[a]	$^{+}$W	$^{-}$W	$^{-}$W[a]	*S*	*/S*	*S*×*/S*
P	30.45±4.75	34.34±10.87	12.07±7.70	13.42±4.73	**<0.001**	0.496	0.739
K	53.43±15.48	57.34±10.16	25.42±20.68	29.80±21.90	**0.031**	0.721	0.983
Ca	638.79±287.36	631.38±344.76	319.37±246.45	287.19±270.87	**0.041**	0.894	0.933
Mg	302.86±137.83	292.39±169.60	152.89±136.09	135.65±134.83	0.056	0.852	0.964
S	15.17±6.96	17.13±13.08	8.93±6.42	15.73±18.89	0.550	0.494	0.703
Zn	10.72±2.46	15.53±7.41	6.42±2.55	7.26±2.42	**0.012**	0.211	0.373
Mn	6.00±2.09	6.11±3.15	3.25±2.50	2.95±2.51	**0.042**	0.943	0.873
Cu	5.14±0.53	7.05±2.21	3.14±1.02	3.31±1.15	**0.001**	0.156	0.227

For statistics, see Table 2

[a] The values obtained for the two-root compartments of each split-root pot were averaged and the ANOVA was performed on a balanced data set (n=4)

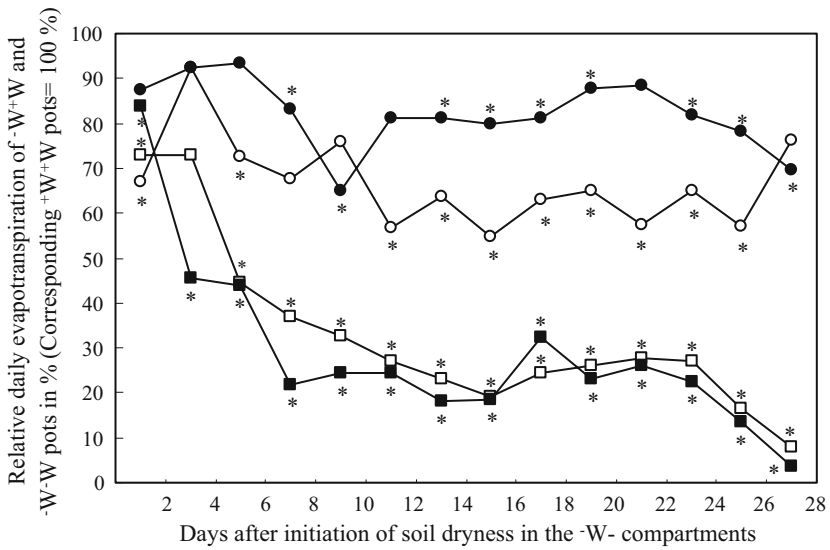

Fig. 1 Daily evapotranspiration per split-root pot averaged over 2 days during the drought stress period. Shown are the mean values for mycorrhizal (*closed symbols*) and nonmycorrhizal (*open symbols*) plants exposed to partial ($^{+}$W/$^{-}$W; *circles*) or complete ($^{-}$W/$^{-}$W; *squares*) rootzone drying, in percent of the values obtained for the corresponding well-watered ($^{+}$W/$^{+}$W) controls. *P<0.05 values are significantly (t test) different from the corresponding $^{+}$W/$^{+}$W treatment

2005) were considerably higher (between 3.62 and 3.35 mg g DW^{-1}). Reported values for sporocarps of some edible ectomycorrhizal mushrooms are in a range of between 2.1–8.1 mg P and 1.1–6.1 mg S g DW^{-1} (Colpaert et al. 2005; Sanmee et al. 2003). Our results thus suggest that P availability may have been a limiting factor for extraradical development of the AM fungus, particularly under conditions of soil dryness.

Concentrations of heavy metals in the AM fungal mycelium were much higher compared with values usually found in plants. They were, however, in a similar range compared with values found in fruiting bodies of some saprophytes and ectomycorrhizal fungi grown in non-contaminated soils (Sanmee et al. 2003; Yamaç et al. 2007). With respect to the low S concentrations in the AM fungal tissue, it seems rather unlikely that metals were bound to metallothioneins in *G. intraradices* in our study, as suggested for Cd and Cu in *Gigaspora margarita* (Lanfranco et al. 2002). Instead, they might have been compartmented in vacuoles or bound to the fungal cell walls (Joner et al. 2000).

The length of hyphae obtained from the fungal tubes was in a similar range compared with results of previous studies where the length of hyphae of *G. intraradices* per unit growth medium was measured in fungal tubes filled with a sand substrate (Drew et al. 2006; Smith et al. 2004). However, the numbers of spores obtained per cubic centimeter substrate from the fungal tubes in our study far exceeded the results of most previous counts of *Glomus* spores in experimental systems and in the field. Pot cultures of *Glomus* spp. most often revealed values of between 20 and 300 spores per gram growth substrate (e.g., Gryndler et al. 2003; Silva et al. 2005; Struble and Skipper 1988). Reports on spore numbers in field samples are usually in a range of between <1 and 100 AM spores per grams soil (e.g., Dandan and Zhiwei 2007; Mathimaran et al. 2005; Oehl et al. 2004).

In our study, numbers of spores per unit hyphal length were between 3.6 and 8.7 times higher in fungal tubes compared with the soil in the root compartment. This is in agreement with results by St-Arnaud et al. (1996), who found up to six times higher hyphal length densities and up to 17 times higher numbers of spores in hyphal compartments compared with corresponding root compartments in a dual in vitro culture of *G. intraradices* and Ri T-DNA transformed carrot roots. The precise reason for such increased sporulation at a distance from host plant roots remains speculative. It has been shown that root exudates play an important role in AM extraradical mycelium differentiation in early stages of symbiosis formation

Table 5 The WUE expressed as the amount of plant DW formed per liter of water lost by evapotranspiration throughout the whole growth period

	Nonmycorrhizal plants			Mycorrhizal plants		
Irrigation treatment	$^{+}$W/$^{+}$W	$^{+}$W/$^{-}$W	$^{-}$W/$^{-}$W	$^{+}$W/$^{+}$W	$^{+}$W/$^{-}$W	$^{-}$W/$^{-}$W
WUE (g per L)	3.00±0.36	2.53±0.53	2.80±0.29	2.75±0.13	3.45±0.19	3.28±0.46

Shown are the mean values ± standard deviation. There was a significant interaction between mycorrhizal inoculation and the irrigation treatment (two-way ANOVA; P=0.013). Statistical interpretation of the main effects of the individual factors was not performed due to interdependence of the factor's effects

Table 6 Total plant (including root) and root DW, element content of shoot, and tubers and tissue element concentrations at the time of harvest

	Nonmycorrhizal plants			Mycorrhizal plants			*P* values (two-way ANOVA)		
Irrigation treatment	$^{+}W/^{+}W$	$^{+}W/^{-}W$	$^{-}W/^{-}W$	$^{+}W/^{+}W$	$^{+}W/^{-}W$	$^{-}W/^{-}W$	*M*	*W*	*W*×*M*
Total plant DW[a] (g per plant)	15.24±2.59	10.63±2.82	10.87±1.70	19.68±1.48	22.82±1.86	15.48±3.83	**<0.001**	**0.007**	**0.008**
Total root DW[b] (g per plant)	3.04±0.36	2.45±0.87	2.71±0.19	2.75±0.56	4.02±0.64	2.87±0.83	–	–	**0.021**
Total P content of shoot and tubers[a] (mg per plant)	8.92±1.81	5.87±1.63	5.81±0.95	33.17±2.82	30.58±2.12	22.77±3.12	–	–	**0.004**
Shoot P concentration (mg g DW^{-1})	0.72±0.15	0.71±0.08	0.75±0.05	1.93±0.14	1.63±0.16	2.03±0.33	**<0.001**	0.059	0.115
Total Zn content of shoot and tubers[a] (mg per plant)	0.25±0.07	0.16±0.05	0.18±0.03	0.68±0.12	0.67±0.02	0.49±0.06	–	–	**0.027**
Shoot Zn concentration (μg g DW^{-1})	19.40±3.22	18.58±1.32	19.68±1.61	51.18±15.95	40.93±3.55	45.43±5.30	**<0.001**	0.328	0.430
Total Cu content of shoot and tubers[a] (mg per plant)	0.11±0.02	0.07±0.02	0.07±0.01	0.26±0.02	0.25±0.01	0.17±0.02	–	–	**<0.001**
Shoot Cu concentration (μg g DW^{-1})	8.65±0.49	8.24±1.02	8.87±0.88	16.65±1.06	13.73±0.96	15.40±1.60	**<0.001**	**0.016**	0.083

Shown are the mean values ± standard deviation. The right part of the table shows the results of the two-way ANOVA. *P* values indicative of a significant ($P<0.05$) influence of mycorrhizal inoculation (*M*), the irrigation treatment (*W*), or an interaction between both factors (*W*×*M*) are printed in bold letters. Statistical interpretation of the main effects of *M* or *W* was not performed in case the size of one factor's effect depended upon the level of the other factor

[a] Leaves lost over the growth period were included into the estimation of these values

[b] Tubers were not included into these values

(Akiyama et al. 2005), and it might be possible that such signal substances influence mycelium architecture also in later stages of the plant/AM fungal association. In the fungal tubes, the number of spores formed per unit hyphal length was largely unaffected by the drought stress treatment, perhaps because sporulation in this part of the extraradical mycelium was already at its maximum in all treatments. It is, however, also possible that the soil moisture content in the fungal tubes decreased more slowly compared with the soil in the root compartment and that differences in soil moisture between the ^{-}W and ^{+}W treatments were smaller in the fungal tubes.

In the root compartments, an increase in the number of spores per unit hyphal length was observed in response to soil dryness when the other root part remained well watered. Whether this was mainly due to increased spore formation or decreased hyphal elongation compared with the AM mycelium in all other root compartments cannot be resolved by the data obtained within this study. It could be speculated that the $^{-}W/^{+}W$ plants, which probably maintained a higher rate of photosynthesis compared with the $^{-}W/^{-}W$ treatments, were better able to supply their fungal symbiont with carbohydrates required for filling of spores with energy-rich lipids.

Compared with standard values cited by Bergmann (1992), tissue concentrations of P in shoots of −M plants were indicative of severe deficiency. Concentrations of Zn and Cu were also in a low but not growth-limiting range for −M plants. The concentrations of P, Zn, and Cu in the shoots of +M plants were in an optimum range, indicating that AM colonization had contributed significantly to their uptake. In −M plants, partial and complete rootzone drying decreased plant growth and nutrient uptake to the same extent. Probably water as well as P deficiency were limiting factors for growth of −M $^{-}W/^{+}W$ and $^{-}W/^{-}W$ plants. Despite a decrease in the amount of AM fungal mycelium obtained from fungal tubes and increased sporulation in the ^{-}W root compartment, +M $^{-}W/^{+}W$ plants did not differ in their DW or P uptake compared with corresponding $^{+}W/^{+}W$ plants. In +M plants exposed to complete rootzone drying, P uptake was decreased by more than one third compared with the well-watered controls. Whether a decreased supply of the AM fungus

Table 7 Concentrations of phosphorus, zinc, and copper in the roots at the time of harvest

	Nonmycorrhizal plants				Mycorrhizal plants				*P* values (three-way ANOVA)			
Irrigation treatment Root compartment	$^{+}W/^{+}W$ $^{+}W^{a}$	$^{+}W/^{-}W$ ^{+}W	^{-}W	$^{-}W/^{-}W$ $^{-}W^{a}$	$^{+}W/^{+}W$ $^{+}W^{a}$	$^{+}W/^{-}W$ ^{+}W	^{-}W	$^{-}W/^{-}W$ $^{-}W^{a}$	*M*	*S*	*/S*	Significant interactions (*P* value)
Root P concentration (mg g DW^{-1})	0.79±0.06	0.88±0.03	0.82±0.09	0.87±0.06	2.18±0.20	1.99±0.17	1.86±0.08	1.97±0.12	–	–	–	*M*×*S* (0.037) *M*×/*S* (0.049)
Root Zn concentration (μg g DW^{-1})	88.2±29.3	71.3±17.2	83.7±8.5	79.2±6.0	128.2±23.1	138.5±46.7	117.9±36.0	115.6±9.6	**<0.001**	0.440	0.994	
Root Cu concentration (μg g DW^{-1})	60.9±7.3	50.0±9.4	60.2±7.7	55.5±4.9	86.5±16.2	85.8±21.8	57.4±10.8	71.8±0.7	–	–	0.219	*M*×*S* (0.010)

[a] The values obtained for the two root-compartments of each split-root pot were averaged and the ANOVA was performed on a balanced data set ($n=4$)

Shown are the mean values ± standard deviation. The right part of the table shows the results of the three-way ANOVA. *P* values indicative of a significant ($P<0.05$) influence of mycorrhizal inoculation (*M*), the soil moisture in the root compartment (*S*), or the soil moisture in the combination compartment (/*S*) are printed in bold letters. Significant interactions between the three factors are given in the last column on the right side of the table. Statistical interpretation of the main effect of a factor was not performed in case the size of the factor's effect depended upon the level of another factor

with plant photosynthates under water deficiency contributed to a decreased P uptake of $^{-}W/^{-}W$ but not of $^{-}W/^{+}W$ plants compared with the well-watered control remains speculative.

Differences in shoot size and leaf area were probably a major reason for a higher daily evapotranspiration of +M compared with −M plants exposed to $^{+}W/^{+}W$ or $^{-}W/^{+}W$ conditions in the second half of the growth period. Surprisingly, ^{+}M and ^{-}M plants grown in $^{-}W/^{-}W$ soil did not differ in their daily evapotranspiration during the last 3 weeks before harvest, despite considerable differences in shoot size. Apparently, ^{+}M and ^{-}M plants were not different in their ability to deplete dry soil for water. This result may also point to more efficient water saving mechanisms in ^{+}M compared with ^{-}M plants, which might be either the result of an improved P nutritional status or direct effects of AM root colonization on the physiology of plant water saving mechanisms (Augé 2001).

An increased plant WUE in response to partial or complete rootzone drying has been observed frequently and can probably be attributed to water saving mechanisms induced in the shoot in response to hormonal signals produced by roots in drying soil (Dodd 2007; Dry and Loveys 1999). Partial rootzone drying is sometimes applied to crop plants under irrigation, as a strategy to increase their WUE and decrease the demand for irrigation water (Davies et al. 2002; Saeed et al. 2008). In our study, only +M plants showed an increased WUE in response to partial rootzone drying and maintained the same growth and P uptake as well-watered controls. This indicates that AM fungal root colonization may be an important determinant for the success of partial rootzone irrigation techniques in plant production. Whether in $^{-}W/^{+}W$ plants the mycelium in the ^{-}W soil remained active, or increased activity of AM fungal hyphae in the ^{+}W root compartment compensated for a decreasing contribution to P uptake of the symbiosis in the neighboring ^{-}W soil, is not known. The P concentrations in +M roots were more than two times higher compared with the associated extraradical mycelium. This suggests that the latter one efficiently transferred acquired P to its host plant and that AM fungi are able to profusely sporulate despite low tissue P concentrations.

In conclusion, our results indicate that a low availability of P, as well as a decreased supply with plant photoassimilates, may be responsible for the negative effect of a low soil moisture regime on extraradical AM fungal development. When AM root systems are exposed to spatially limited drought, extraradical AM fungal mycelium may form spores in favor of hyphal length in the dry soil parts. However, the severe decrease in the total amount of extraradical mycelium obtained from fungal tubes in ^{-}W soil suggests that neither partial nor complete rootzone drying are suitable means to increase the total number of spores per unit substrate in AM fungal inoculum production. Irrespective of the soil moisture

regime, AM fungal sporulation appeared to be greatly enhanced in the fungal tubes in absence of host plant roots. Possible differences between extraradical mycelium development in vicinity and distance of plant roots need to be considered in experiments where fungal tubes are employed. The largest relative contribution of the AM symbiosis to plant growth and P uptake could be observed when plants were exposed to partial rootzone drying. This suggests that root colonization by AM fungi may be of particular importance for performance of mycotrophic plants under conditions of spatially limited soil dryness.

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