Phosphorus acquisition strategies within arbuscular mycorrhizal fungal community of a single field site

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Received 20 November 2004. Accepted in revised form 21 March 2005

Key words: compartmented containers, functional diversity, Glomus, radioisotope labeling

Abstract

Diversity in phosphorus (P) acquisition strategies was assessed among eight isolates of arbuscular mycorrhizal fungi (AMF) belonging to three *Glomus* species, all obtained from the same field site. Maize (*Zea mays* L. cv. Corso) was used as a test plant. Compartmented cultivation containers coupled with ³³P radioisotope labeling of soil P were employed to estimate (1) the distance from the roots that AMF were able to acquire soil P from, (2) the rate of soil colonization, (3) the efficiency of uptake of soil P by AMF, (4) benefits provided to maize in terms of P acquisition and growth. *Glomus mosseae* and *G. intraradices* took up P 10 cm from roots, whereas *G. claroideum* only up to 6 cm from the roots. *G. mosseae* most rapidly colonized the available soil volume and transported significant amounts of P to maize from a distance, but provided no net P uptake benefit to the plants. On the other hand, both *G. intraradices* and three out of four *G. claroideum* isolates significantly improved net P uptake by maize. These effects seem to be related to variability between and to a limited extent also within AMF species, in mycelium development, efficiency of hyphal P uptake and effects on plant P acquisition via the root pathway. In spite of absence of maize growth responses to inoculation with any of the AMF isolates, this study indicates remarkable functional diversity in the underground component of the studied field site.

Abbreviations: AMF – arbuscular mycorrhizal fungi; ANOVA – analysis of variance; G. – *Glomus*; HLD – hyphal length density; LSD – least significant difference; MPR – mycorrhizal P uptake response; NM – nonmycorrhizal; P – phosphorus; PAR – photosynthetically active radiation (400–700 nm); SPA – specific P activity (kBq ³³P/mg P)

Introduction

The association between terrestrial plants and arbuscular mycorrhizal fungi (AMF) is one of the most widespread and most ancient symbioses on Earth (Redecker et al., 2000; Simon et al., 1993; Trappe, 1987). It involves majority of plant species but only a small group of fungi consisting of about 150 described species (Clapp et al., 2002; Smith and Read, 1997). The specificity of AMF association with plants is relatively low (Bever, 2002). This means that the same AMF species can colonize different host plants and also that one host plant can be colonized by different AMF species. Several different AMF species can simultaneously colonize a single plant root system (Jansa et al., 2003a; McGonigle and Fitter, 1990; Merryweather and Fitter, 1998; van Tuinen et al., 1998).

AMF are able to take up mineral nutrients with limited mobility in the soil such as phosphorus (P) from beyond the P depletion zone formed around

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roots (Cooper and Tinker, 1978; Sanders and Tinker, 1971; Smith et al., 2001). In exchange, these fungi have direct access to reduced carbon compounds derived from plant photosynthesis (Shachar-Hill et al., 1995). Upon establishment of AMF colonization, plants can absorb P both directly at the soil-root interface through root epidermis and root hairs (root uptake pathway) and through AMF hyphae in soil (mycorrhizal uptake pathway; Smith et al., 2003). Functional differences have been recognized among AMF species. These include different rate of root colonization (Graham and Abbott, 2000; Hart and Reader, 2002a), mycelium spreading capacity in the soil (Jakobsen et al., 1992a,b; Smith et al., 2000), fungal P metabolism and P transfer to plants (Boddington and Dodd, 1999), and carbon requirements from the host plant (Hart and Reader, 2002b; Jakobsen et al., 2002; Smith and Smith, 1996). Most of this knowledge has been gathered from studies with a few AMF isolates belonging to different genera or species (,isolate' here refers to progeny of one or several spores of an identified AMF that has been propagated in sterilized cultivation medium under controlled conditions). Previous studies reported that e.g. Glomus sp. and Acaulospora laevis can take up P from greater distances (up to 11.7 cm) from clover roots than Scutellospora calospora (Jakobsen et al., 1992b; Li et al., 1991; Smith et al., 2000) and that G. intraradices is more efficient than G. mosseae in uptake of soil P from a distance greater than 3 cm from the roots (Drew et al., 2003). There is, however, very little other evidence about variability among different Glomus species in P uptake from the soil at different distances from roots. On the other hand, studies of functional differences within AMF species indicated important variation among isolates of the same AMF species with respect to plant growth response, mycelium growth pattern, and spore production per unit of hyphal length, while the P uptake per unit of hyphal length seemed more conserved on AMF species level (Koch et al., 2004; Munkvold et al., 2004; Stahl et al., 1990; Stahl and Christensen, 1991).

Although plant roots are often simultaneously colonized by several AMF species, functional consequences of this phenomenon remain largely unknown and speculative (Koide, 2000; Merryweather and Fitter, 1998). Koide (2000) has proposed that colonization of roots with functionally complementary AMF may be more beneficial to the plant than colonization with any of the fungi separately. It is, however, inherently difficult to support this theory quoting results of previous studies that all involved AMF isolates of different origins. The only exception we are aware of is the study of Koch et al. (2004), using isolates of *G. intraradices* from our laboratory, obtained from the same field as referenced in this paper.

To substantiate the theory of functional complementarity within a real AMF community, we first obtained about a hundred pure AMF isolates from a single field experiment in Switzerland (Jansa et al. 2002). This was one of the largest efforts to collect AMF from a single field site. Three Glomus species were selected for the functional experiments described here, because these species were dominating AMF spore community in the soil as well as they were concomitantly colonizing roots of field-grown maize (Jansa et al., 2002, 2003a). These AMF were previously identified by using spore morphology, isoenzyme profiling and DNA sequencing (Jansa et al., 2002). Although different isolates to be used here were originally obtained from differently tilled plots of the field experiment, we and others have previously shown that all of the AMF species examined here were present in all tillage treatments and that tillage did not affect physiology of G. intraradices (Jansa et al., 2002, 2003a, Koch et al., 2004). In this study we aimed to assess the variability in P acquisition strategies among those Glomus spp. isolates and to estimate their effects on P uptake and growth of maize. In particular, we studied how far from the roots were the AMF able to take up soil P, how fast were they able to spread in the soil, how efficient they were in soil P uptake, and what consequences the root colonization had for plant P uptake and growth. Maize was studied because this crop was commonly grown at the field site. We employed compartmented cultivation containers coupled with ³³P radioisotope tracing. The distance aspect (i.e. how far from the root surface do the AMF hyphae acquire P) has been included because this was the mechanism proposed by Koide (2000) as an example for complementarity of functions within AMF community.

Material and methods

Experimental design

Two experiments were carried out, each with five inoculation treatments. They were carried out sequentially, because of spatial limitation in the glasshouse. Experiment 1 included the following inoculation treatments: nonmycorrhizal control (NM), *Glomus intraradices* isolates Int1 and Int2 and *Glomus mosseae* isolates Mos1 and Mos2 (Table 1). Experiment 2 included: NM, and *Glomus claroideum* isolates ClA1, ClA2, ClB1, ClB2 (Table 1).

For each inoculation treatment, five different containers were used, one for each length of the intermediate compartment (2, 4, 6, 8, and 10 cm). There was thus a single container for each inoculation treatment and five distances between the plant and the labeled compartments. For some analyses, the length of intermediate compartment was disregarded, resulting in five replicate values for each inoculation treatment. For regression analysis, values for each compartment length were considered separately.

Biological materials

Eight monosporic AMF isolates (progenies of single AMF spores) were used in this study (Table 1), belonging to three species of *Glomus* (*G. intraradices*, *G. mosseae*, and *G. claroideum*). They were all isolated in 1999 from a single agricultural field site, namely the tillage experiment in Tänikon, Switzerland (Jansa et al., 2002). Inoculum for the pot experiments described here was produced in 800 mL pots filled with mixture of sterilized soil quartz sand (1:4; v:v) and planted with wheat (Triticum aestivum L. cv. Albis). Wheat was grown in a growth chamber for 4 months under 16 h photoperiod, 350 μ mol PAR m⁻² s⁻¹ and 25/20 °C (day/night). This allowed for spore production between 32 and 98 spores g^{-1} potting mixture, depending on AMF isolate. The inoculum was prepared by chopping the roots to 1 cm pieces and mixing them homogeneously with the potting mixture containing AMF spores. Nonmycorrhizal inoculum was prepared from nonmycorrhizal wheat grown under the same condition as the mycorrhizal wheat plants. Inoculum was stored at 4 °C for maximum of 8 weeks.

Maize (Zea mays L. cv. Corso) seeds were surface-sterilized in 5% calcium hypochlorite for 15 min and germinated on moist sand at 25 °C in darkness for 2 days. Germinated maize seeds were planted into 50 mL pots containing the inoculum mixed with expanded clay (Oil Dry Chem-Sorb WR24/18, Brenntag, Vitrolles, France) in a ratio 1:1 (v:v). Per plant, 25 mL (23.5 g dry weight) of fresh, moist inoculum was used. Maize seedlings were grown in the 50 mL pots for 2 weeks in a growth chamber under following conditions: 16 h photoperiod, 350 µmol PAR $m^{-2} s^{-1}$, 26/22 °C (day/night), and 50/80% relative air humidity (day/night), before being transferred into compartmented containers (see below). Plants were watered once a day during this period, maintaining humidity in the pots between 60 and 80%

Table 1. AMF isolates from a single field site in Tänikon (Switzerland), used in this study. Isolate identification numbers in the AMF collection in Eschikon are quoted

Abbreviation	Species	Eschikon collection number	BEG accession
Intl	Glomus intraradices Schenck & Smith	291	BEG158
Int2		141	BEG157
Mosl	Glomus mosseae (Nicol. & Gerd.) Gerdemann & Trappe	964	BEG161
Mos2		243	BEG160
ClA1	Glomus claroideum Schenck & Smith (morphotype A)	360	BEG155
ClA2		133	n.a.
ClB1	Glomus claroideum Schenck & Smith (morphotype B)*	610	BEG156
ClB2		132	n.a.

*Previously considered as Glomus clarum-like (Jansa et al. 2002); n.a. isolate not registered in BEG.

Accession numbers in the International Bank for the Glomeromycota (BEG) are shown for the isolates that were registered there.

of maximum water holding capacity of the inoculum-clay mixture.

Cultivation media

Two kinds of cultivation media were used in this study - either undiluted, sterilized field soil (here called 'soil'), or substrate mixture (here called 'substrate') consisting of the soil, quartz sand (grain size 0.7-1.2 mm), and expanded clay, mixed in a ratio of 1:2:2 (v:v:v). Soil, sand and expanded clay were all separately autoclaved (20 min, 121 °C), and the soil was then treated with an aqueous solution (20 mL kg⁻¹ soil) extracted from the original unsterile soil (100 g soil in 11 water) and filtered three times through Whatman No 1 paper filter. This was done in order to re-introduce soil bacteria into the soil. The soil was incubated for 6 weeks under glasshouse conditions before preparing the substrate. The soil was a loamy Eutrochrept with a P content of 745 (± 76.2) mg kg⁻¹ (modified extraction protocol of Saunders and Williams, 1955; briefly, the soil was incinerated at 550 °C for 6 h, extracted with boiling 5.6 M HCl for 10 min and filtered through Whatman No 40 filter paper). The amount of soil P isotopically exchangeable within 1 min $(E_{1\min}, E_{1\min})$ corresponding to the P pool readily available for plant uptake, Fardeau, 1996) was 15.4 (± 0.54) mg kg⁻¹ (estimated according to Frossard and Sinaj, 1997). P content of the substrate was 196 (± 11.4) mg kg⁻¹, and the readily available P pool (E_{1min}) in the substrate was 2.49 (± 0.24) mg kg⁻¹. This was below the critical level of 5 mg kg⁻¹, at which P availability becomes limiting for maize growth (Gallet et al., 2003).

Experimental setup and labeling

Compartmented containers described as cuvettes by Jansa et al. (2003b) were used in this study. A cuvette container consisted of three compartments: a plant compartment, where inoculated plants were grown, an intermediate compartment of variable length, and ³³P labeled compartment. Spatial separation of the different compartments was ensured by a nylon mesh with opening of 20 μ m. The contact area between the compartments was 14 × 13 cm. The plant compartment was four cm long, and its volume was 728 mL. The length of the intermediate compartment was 2, 4, 6, 8, or 10 cm. The length of the labeled compartment was 4 cm.

The plant and the intermediate compartments were filled with the substrate. Each plant compartment was planted with three 2-weeks-old maize seedling from growth chamber. These were grown for total of 40 days in the glasshouse under the following conditions: 16 h photoperiod, 26/ 22 °C (day/night), and 50% relative air humidity. The minimum light intensity (combined of solar and artificial light, 400W Eye Clean-Ace lamps, Iwasaki, Tokyo, Japan) was set at 400 µmol PAR m⁻² s⁻¹. Plants were watered by automatic tensiometer-controlled units (Blumat, Telfs, Austria) maintaining humidity of cultivation media at 60-70% of its maximum water holding capacity. Plants were fertilized with 25 mL plant⁻¹ week⁻ of modified Hoagland nutrient solution (Hoagland and Arnon, 1950) containing no P throughout the duration of the experiment.

A wooden block was inserted into the labeled compartment at the beginning of the experiment but was removed at 19 days before harvest. Soil portions to be inserted into the labeled compartments (400 mL, 330 g dry weight each, 50% of maximum water holding capacity) were labeled with ³³P. Homogeneous labeling was achieved by thorough mixing of 1 mL (2.47 MBq) of aqueous $^{33}PO_4^{3-}$ solution (carrier-free orthophosphate, Amersham Pharmacia Biotech, Piscataway NJ, USA) with each soil portion for 5 min. The labeled compartments were then filled in three layers. The bottom layer consisting of 160 mL of the substrate was covered with 400 mL of the labeled soil. The top layer consisted of 160 mL of the substrate. This setup was chosen to prevent surface drying of the labeled soil as well as to avoid leaching of radioactive P from the containers.

Available P contents of compartments (E_{1min} ; mg compartment⁻¹) were as follows: plant compartment, 1.76; labeled compartment, 5.90; intermediate compartments 2, 4, 6, 8, and 10 cm long, 0.88, 1.76, 2.64, 3.52, and 4.40, respectively.

Harvest and analyses

The third leaf of maize was harvested 9 days after labeling, dried at 105 °C for 48 h, and incinerated at 550 °C for 8 h. The ash was dissolved

in 2 mL boiling 5.6 *M* HCl, made to 50 mL volume with distilled water and filtered (Whatman No 40). The concentrations of P and ³³P were determined according to Ohno and Zibilske (1991) and by scintillation counting, respectively. The extracts were neutralized with 1 *M* NaOH and counted on Packard 2500 TR counter (Packard BioScience, Meriden CT, USA) using a Packard Ultima GoldTM (Perkin Elmer, Boston MA, USA) scintillation cocktail mixed with the samples in a ratio of 5:1 (v:v). At final harvest shoot and root biomass was determined after drying plant materials at 105 °C for 48 h. The concentrations of P and ³³P in both shoots and roots were measured as described above.

Mycorrhizal colonization structures in the roots were stained by modified procedure of Philips and Hayman (1970). Briefly, roots were cleared in 1.8 M KOH at 90 °C for 1 h, rinsed with water and neutralized in 0.5 M HCl for 30 min. Then the roots were transferred (with no further rinsing) into a mixture of Trypan- and Methylene-Blue (0.05% each in lactic acid: glycerol: water, 1:1:1, v:v:v), stained for 2 h and destained in water overnight. The percentage of root length colonized by AMF hyphae, arbuscules, and vesicles was estimated by magnified intersection method (McGonigle et al., 1990), scoring 100 root intersects per sample under compound microscope (400×). Soil cores (1 cm diameter) were taken from the intermediate compartment region adjacent to the labeled soil (i.e. at a distance of zero to 1 cm from the labeled soil) and hyphal length density (HLD) of the AMF mycelium was determined there by the filtration-gridline method (Sylvia, 1992) with Millipore RAWG02500 membranes (Millipore, Bedford MA, USA).

Calculations and statistical analysis

Plant materials (shoots and roots) from each container were pooled and the values were divided by three to obtain data on a single plant basis. P uptake of plant was calculated by subtracting average seed P content (0.96 mg) from P content of plants (shoot and roots combined). Mycorrhizal P uptake responses (MPR) were calculated according to Cavagnaro et al. (2003),

using individual P uptake values of inoculated plants (Mp) and mean P uptake value of nonmycorrhizal plant (NMp, a mean of five replicates):

$$MPR = \frac{Mp - NMp}{NMp} \times 100.$$
(1)

Specific P activity (SPA) was calculated by dividing the ³³P activity (corrected for isotope decay, kBq) by P content of the plant (mg). The MPR, SPA, ³³P transport via AMF as well as HLD at a distance from the roots in experiments 1 and 2 were combined for a common analysis. This was possible because both of the experiments were performed in the same type of containers, using identical cultivation media, plant cultivar, watering and fertilization regimes, as well as glasshouse settings (temperature, light intensity, photoperiod, and humidity). AMF inoculum for both of the experiments was produced under identical conditions. Both of the experiments were carried out for exactly the same period of time and both included NM treatment. This allowed correction for differences in plant P uptake between experiments 1 and 2 caused by factors beyond our control, by comparing relative P uptake with respect to NM control (MPR, Eq 1). Inherently, we could not correct for possible systematic differences in HLD and ³³P transport via AMF between experiments 1 and 2 because the NM controls only provided background readings of these variables (close to zero) and because different AMF isolates were used in experiments 1 and 2. Therefore, we only combined data for ³³P transport relative to plant P content (SPA) or ³³P transport on whole plant basis. The latter were solely used for regression analysis, examining the relationship between HLD and ³³P uptake from soil. All other analyses were performed separately for experiments 1 and 2.

Data were subjected to analysis of variance (ANOVA) and regression analysis, using Statgraphics[®] software version 3.1. Following significant ANOVA (P < 0.05), differences between treatment means were examined by multiple range LSD-based *F*-test considering 95% confidence intervals. Data for hyphal, arbuscular and vesicular colonization of roots were arcsin – square root transformed (Linder and Berchtold, 1976) so as not to violate the assumption of normality of residual distribution in ANOVA.

In order to compare the effect of inoculation with different AMF isolates on total P and ³³P uptake by maize with increasing length of the intermediate compartment, we used comparison of regression lines. Linear regression model was used with untransformed data since this model provided maximum explanatory value. With this approach, we were able to compare percentage of the variability in our data explained by the different models: 1. Considering all data pooled over all inoculation treatments, i.e. how the distance as the only explanatory factor affected the P uptake by mycorrhizal maize; 2. Considering the data split according to the AMF species identity (3 levels, G. intraradices, G. mosseae, and G. *claroideum*), i.e. estimating how the identification of the inoculum down to the species level contributed to further explanation of the variability in our data; 3. Considering the data split according to the AMF isolate identity (eight levels), i.e. estimating how much of the variability in the entire dataset was due to variation among both AMF species and isolates. A rigorous statistical testing of the differences between the different regression models with increasing number of explanatory factors was not possible simply because we did not have available at least three different isolates of each AMF species in this study. We used instead a very conservative estimate to compare the different regression models here: if the R^2 -value adjusted for the number of degrees of freedom (=measure of explained variability in

the dataset) did not increase by at least 1% with increasing number of explanatory factors, we considered that introduction of those explanatory factors did not contribute significantly to model improvement and hence had no significant influence on the studied parameter.

Results

Plant biomass

Biomass production upon final harvest was higher in experiment 1 than in experiment 2, with mean values for NM plants of 7.27 g and 5.04 g, respectively (P < 0.001). Biomass was not affected by inoculation with any of the eight *Glomus* isolates compared to appropriate NM controls (data not shown).

Root colonization

All of the AMF isolates used in this study were infective, with at least 45% of the root length colonized by AMF hyphae at harvest 2 (Table 2). No colonization was detected in roots of NM plants (data not shown). We observed a number of significant differences in the extent of root colonization by AMF hyphae, arbuscules, and vesicles between both the AMF species and AMF isolates (Table 2). In general, AMF hyphal colonization of roots was higher in experiment 1

Table 2.	Extent of roo	t colonization	by 8 AMF	(see Table	1) in e	experiments 1	and 2 at harvest 2
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Inoculation treatment	Н	А	V
Experiment 1			
Int1	80.4 b	34.8-	16.8 b
Int2	94.4 a	44.4-	30.4 a
Mosl	85.2 b	44.4-	0.0 c
Mos2	78.4 b	48.4-	0.4 c
Experiment 2			
ClA1	76.0 a	64.8 a	3.2 b
ClA2	56.4 bc	26.4 b	2.8 b
ClB1	44.8 c	22.4 b	5.6 ab
ClB2	60.4 b	32.0 b	12.8 a

NM treatments were removed from this analysis and the size of the intermediate compartment was disregarded here. Means (five replicates) of percentage of root length colonized by AMF hyphae (H), arbuscules (A) and vesicles (V) are shown. Different letters denote significant differences between treatment means (LSD, P < 0.05). Hyphen (-) indicates absence of significant differences between treatment means.

than experiment 2 (P < 0.001), whereas no difference between experiments was observed with respect to arbuscular colonization (P=0.19). Hyphal colonization of roots was higher for Int2 isolate than for Int1 and both *G. mosseae* isolates. Both of the *G. mosseae* isolates produced no or very few vesicles, much less than *G. intraradices* (Table 2). The extent of root colonization by both AMF hyphae and arbuscules was higher for the ClA1 isolate than for any other *G. claroideum* isolate. The extent of root colonization by vesicles was more variable among the *G. claroideum* isolates and no clear trends could be identified (Table 2).

Plant P uptake

Maize P uptake was significantly higher for plants inoculated with any of the two *G. intrara-dices* or with three out of four *G. claroideum* isolates compared to the NM control (Table 3).

No significant effect of the size of the intermediate compartment on MPR was detected when the data for all AMF inoculation treatments were pooled (Figure 1a). Splitting the data according to AMF species resulted in the regression model being highly significant, with slopes of the regression lines significantly different among different AMF species (Figure 1b). This means that maize P uptake was differentially affected by the AMF species depending on the size of intermediate compartment.

Further increase in explained variability (R^2) value) of the MPR was observed upon introducing AMF isolate identity into the regression model (Figure 1c). In particular, MPR of maize significantly increased with size of the intermediate compartment for both of the G. intraradices isolates (P < 0.05 in both cases), while there was no significant correlation between the MPR and the size of the intermediate compartment for any other inoculation treatment (Figure 1c, analyses not shown). Based on the net increase of explained variability between the models considering AMF species and AMF isolate identities, we can not rule out the possibility that there is, apart from the variability among the species, also a significant variability among AMF isolates belonging to the same species with respect to maize P uptake.

³³P transport, SPA

Significant amounts of ³³P were transported by *G. mosseae* and *G. intraradices* from distances up to 10 cm from the roots. *G. claroideum* only transported labeled P from distances up to 6 cm from the roots (Figure 1e, h). No ³³P activity was detected in the NM plants. Maize inoculated with either *G. mosseae* isolates had higher SPA than any other AMF isolate nine days after labeling (Figure 2a). At 19 days after labeling, maize inoculated with any of the two *G. mosseae* isolates only had higher SPA than one of the *G. intraradices* isolates (Int2) and all four of the *G. claroideum* isolates (Figure 2b).

The SPA at both harvests was significantly (P < 0.05) and negatively correlated with the size of the intermediate compartment when the data was pooled over all inoculation treatments (Figures 1d, g). Splitting the data according to the identity of AMF species caused substantial increase in explained variability of the SPA datasets for both the first and second harvests (Figures 1e, h). At the first harvest, highly significant differences were detected among the slopes of regression lines representing different AMF species (Figure 1e), but these were absent at second harvest (Figure 1h).

Table 3. Phosphorus uptake by maize colonized by 8 AMF (see Table 1) in experiments 1 and 2 at harvest 2 (P content – seed P), disregarding the size of the intermediate compartment

Inoculation treatment	P uptake (mg)	MPR
Experiment 1		
NM [*]	8.24 b	_
Int1	12.9 a	56.9 a
Int2	13.1 a	58.7 a
Mos1	7.99 b	-2.98 b
Mos2	7.71 b	-6.34 b
Experiment 2		
NM [*]	3.93 d	-
ClA1	6.31 a	60.7 a
ClA2	5.18 bc	31.8 bc
ClB1	4.78 cd	21.6 cd
ClB2	5.69 ab	44.9 ab

Nonmycorrhizal treatment.

Means (five replicates) of absolute values and MPR are shown. Different letters denote significant differences between treatment means (LSD, P < 0.05).

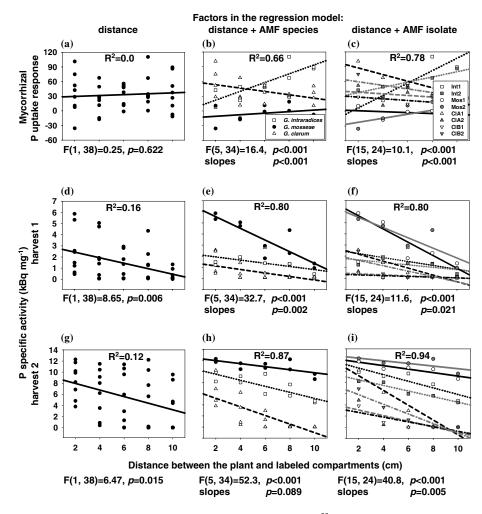


Figure 1. Regression models showing dependency on distance between plant and ³³P labeled compartments of MPR (at harvest 2) and SPA in maize (at both harvests 1 and 2). Whole plant P uptake values (P content – seed P) at harvest 2 were used for determination of P uptake response. P concentration and ³³P activity in aboveground biomass were used for determination of SPA. Three models are compared here: First, the distance between plant and labeled compartments is considered as the only explanatory factor of studied variable (a, d, g). Second, AMF species identity is considered in addition to the distance between plant and labeled compartments (b, e, h). Third, AMF isolate identity is considered as further explanatory factor in addition to the distance between plant and labeled compartments (b, e, h). Third, AMF isolate identity is considered as further explanatory factor in addition to the distance between plant and labeled compartments (c, f, i). R^2 values have been adjusted for the number of degrees of freedom. Statistical significance of the entire regression model as well as *P* value is given for test of null hypothesis that there are no differences between the slopes of the regression lines in the model. In the panels b, c, e, f, h, and i, squares indicate *Glomus intraradices*, circles *G. mosseae*, and triangles *G. claroideum*, solid regression lines in the same panels refer to *G. mosseae*, dotted lines to *G. intraradices*, and both dashed and dash-dotted lines to *G. claroideum*.

Introduction of AMF isolate identity into the regression model caused a net increase in explained variability only for SPA at the second harvest (Figure 1i). At both harvests, significant differences were detected among AMF isolates with respect to changes in SPA with increasing distance between the plant and labeled compartments (Figures 1f, i). Statistically significant differences were detected among both intercepts

(P=0.01) and slopes (P=0.04) of regression lines representing maize SPA colonized by the four *G. claroideum* isolates at second harvest. These results mean that at first harvest the identity of AMF species was sufficient to explain all observed differences in SPA among the inoculation treatments. At second harvest, the variability between the isolates of the same AMF species became important.

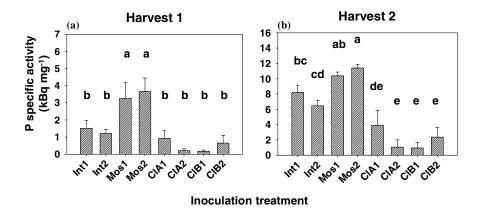


Figure 2. SPA in maize aboveground biomass (ratio of ³³P activity and P concentration) at harvests 1 and 2, disregarding the size of the intermediate compartment. Means and standard errors of means of five replicates are shown. Different letters denote significant differences between treatments (LSD, P < 0.05). NM treatment was not included in this analysis.

AMF hyphae

Highly significant correlation (P < 0.001) was found between the HLD (disregarding the AMF species identities) in the immediate vicinity of the labeled compartment, and the ³³P activity in maize plants ($R^2 = 0.85$) at the second harvest (Figure 3). At the same time, significant correlation was found between HLD and ³³P activity in maize colonized by *G. claroideum* ($R^2 = 93.5$, P < 0.001) and marginally significant correlation was also found for *G. intraradices* ($R^2 = 34.1$, P = 0.051), while there was no correlation for *G.* mosseae (P = 0.78). The slopes of regression lines for *G. claroideum* and *G. intraradices* proved to be significantly different (P < 0.001).

Discussion

Plant biomass

We did not observe any significant response of maize biomass production to AMF inoculation even if the total P uptake by the plants increased. This is consistent with previous observations by Boucher et al. (1999), who also reported no significant effects on maize shoot biomass in response to inoculation with four AMF species. Some other plant species such as tomato (*Lycopersicon esculentum* Mill.), cucumber (*Cucumis sativus* L.), or barley (*Hordeum vulgare* L.) are also notoriously known for either absence of or

negative growth responses to AMF colonization (Pearson and Jakobsen, 1993; Smith et al., 2003; Zhu et al., 2003), the reasons still not being well known. However, Cardoso et al. (2004) recently showed that maize growth did positively respond to AMF inoculation if plants were grown in tropical Oxisol. The apparent discrepancy of

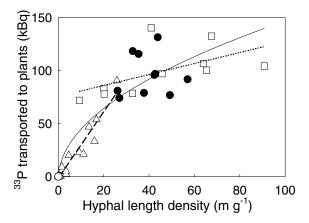


Figure 3. Relationship between HLD next to the labeled compartment and ³³P uptake by maize from labeled compartment at harvest 2 (19 days after labeling). Results of the experiments are shown using eight AMF isolates, considering five distances between plant and labeled compartments. Regression curve for data pooled over all eight AMF inoculation treatments (solid line): $y = -3.3 + 15.1x^{0.5}$, for *G. claroide-um* (dashed line): y = -1.2 + 3.1x, and for *G. intraradices* (dotted line): y = 76 + 0.52x. NM treatment was not included into the regression analysis. Open circles indicate NM, open triangles *G. claroideum*, open squares *G. intraradices*, and closed circles *G. mosseae*.

results is probably due to different properties of soil and maize cultivars used in different studies.

The difference in maize growth between experiments 1 and 2 was most likely due to variation in light intensity and temperature in the glasshouse. These two factors were impossible to fully control because they partially depended on environmental conditions outside of the glasshouse.

Root colonization

All AMF isolates used in this study well colonized maize roots, though the isolates colonized roots to different extent. This may be due to inherent differences in capacity of the AMF isolates to colonize maize or due to different infectivity of the inoculum. We did not estimate infectivity of the inoculation materials here, because we were unsure about methods for doing so: the sum of spores and vesicles (Liu and Luo, 1994) ignores infectious potential of mycelium fragments, and most probable number assay (Adelman and Morton, 1986) may be misleading when comparing inocula with different levels of dormancy or colonization rate. The latter also requires storage of the inocula during testing. which may in turn unpredictably affect AMF infectivity. We recognize that different AMF in this study could also have followed different trajectories of root colonization (Hart and Reader, 2002a; J. Jansa, unpublished observation) and that this might have contributed to the apparent functional differences reported here. Further experiments with sequential harvests will be needed to elucidate this story.

Obvious absence of vesicles in maize roots colonized by *G. mosseae* in this study was in contradiction to some previous studies (using different isolates of *G. mosseae*), reporting vesicles in the roots colonized by *G. mosseae* (Liu and Luo, 1994; McGonigle et al., 2003).

Plant P uptake

Improvement of P uptake of mycorrhizal vs. NM plants growing in containers with large root-free compartments (such as cuvettes in this study) is not surprising and has previously been reported (Jansa et al. 2003b; Li et al., 1991). The design of

such containers has been criticized for overestimation of mycorrhizal contribution to plant P uptake (Smith et al., 2004). In this context, the absence of P uptake benefits from colonization with G. mosseae is surprising (in another experiments one of our G. mosseae isolates significantly lowered P uptake in comparison to NM control; J. Jansa, unpublished observation). Glomus mosseae was transferring P from labeled soil in amounts comparable to G. intraradices, yet did not cause any increase in total P uptake of maize. This means that the contribution of G. mosseae to P uptake of plants via mycorrhizal pathway must have been offset by lower uptake through the root pathway than in the NM plants (Smith et al., 2003). This could be accomplished in two ways: either the root surface available for P uptake was lower than in NM plants, or the density and/or activity of P transporters responsible for P uptake via the root pathway was lower (=down-regulation of root uptake pathway). Previously, a different isolate of G. mosseae than used in this study suppressed the expression of P transporter MtPT2 and P-starvation inducible gene Mt4 in Medicago truncatula to the greatest extent among seven different AMF species (Burleigh et al., 2002). But, in contrast to our results, colonization of Medicago truncatula with G. mosseae significantly increased P uptake and also the growth of plants in that latter study. This indicates existence of compatible (and incompatible) pairs of AMF and plant species, as discussed elsewhere (Helgason et al., 2002; Ravnskov and Jakobsen, 1995).

P uptake via AMF

Important variation in P uptake strategies among and within three *Glomus* species is shown here. This is to our knowledge the first direct evidence showing different species of *Glomus* having access to P located at different distance from roots. Previously, we demonstrated that *G. intraradices* took up P from distances over 10 cm from maize roots (Jansa et al., 2003b). Li et al. (1991) also showed that *G. mosseae* depleted soil P at a distance of 11.7 cm from clover roots. This is consistent with our results presented here, showing isolates of both above mentioned species to take up P from distances up to 10 cm from maize roots. The information about more limited extension G. claroideum into the soil (up to 6 cm from roots) is unique as this AMF species has not been studied yet with respect to its P transport capacity over defined distances. In spite of the fact that the isolates of both G. intraradices and G mosseae transfer comparable amounts of P from remote distances to the plants, the two species seem to achieve this by different mechanisms. While the high SPA in maize colonized by G. mosseae at harvest 1 indicated high rate of hyphal extension by this AMF species into a newly available soil volume, G. intraradices appeared to extract soil P more efficiently, but its expansion into labeled soil was slower than for G. mosseae. This is supported by greater MPR of plants inoculated with G. intraradices compared to G. mosseae and by significant increase in MPR with increasing length of the intermediate compartment (indicating efficient exploitation of the intermediate compartment). Additionally, greater efficiency in P uptake by G. intraradices is also supported by results of another study where ³³P was injected into a compartment pre-colonized by the AMF, and where G. intraradices appeared to transport more ³³P than G. mosseae (J. Jansa, unpublished observation). Similarly, Drew et al. (2003) observed more efficient uptake of ${}^{33}P$ by G. intraradices than by G. mosseae from a fixed distance from roots whereas the HLD was similar for both of the AMF species.

Although we provide evidence here that (at least) some of the functional diversity can be attributed to differences between species, we also detected significant differences within AMF species (e.g. uptake of ³³P from different distances by *G. claroideum* isolates). Similarly, high levels of functional diversity with respect to hyphal growth within AMF species have recently been reported by others (Koch et al., 2004; Munkvold et al., 2004). Thus the challenge for the future will be to look more closely at the variation in P acquisition strategies among and within AMF species, using a large number of isolates (10+) for each AMF species.

Role of AMF hyphae in P uptake

The values of HLD in the intermediate compartment (especially those of *G. intraradices* and *G. mosseae*) were quite high, compared to previous glasshouse studies, where values over 25 mg⁻¹ were rarely reported (Jakobsen et al. 1992a, 2002; Smith et al. 2004). Hyphal densities in our experiments reached those encountered in field soil (Miller et al., 1995). This may be related to the fact that the labeled compartment was filled with undiluted, sterilized field soil.

Although we did not measure AMF hyphal development in the labeled compartment here (but only in the intermediate compartment very close to the labeled one), HLD was very strongly correlated with transfer of ³³P to the plants. Similar correlations between HLD in and P uptake from root-free compartments are commonly reported (Jakobsen et al., 2001; Schweiger et al., 1999) and indicate a simple and causal relationship between HLD and P uptake via mycorrhizal pathway (George et al., 1995). It appears that the curve reported in Figure 3 may reach a plateau, after which further increase in HLD would not result in any further increase in mycorrhizal ³³P uptake from the labeled compartment. This may indicate the situation when the P depletion zones of mycorrhizal hyphae overlap and virtually all available P in the labeled compartment being taken up. In this study we also provide an interesting evidence for the relationship between HLD and ³³P uptake being different for G. claroideum and G. intraradices, but being rather constant among the isolates belonging to each species. Constant P uptake per unit hyphal length on AMF species level with only little variation among isolates of the same species was recently reported for G. mosseae and G. caledonium by Munkvold et al. (2004).

Interestingly, although we observed high variation in HLD of *G. intraradices* here (higher HLD closer to plants, data not shown), amounts of ³³P transported to plants from any distance were quite similar. Possibly, the mycelium branched in the labeled compartment (where the P availability was higher than otherwise) in order to maximize P uptake from it, regardless of the distance from the plants. This indicates some plasticity of mycelium development of *G. intraradices* in response to local nutrient availability, similar to our previous observation (Jansa et al., 2003b). 174

Conclusion

We provide here an evidence for diversity in P uptake strategies among several Glomus isolates from a single field site. Both differences among and within AMF species are demonstrated. These results mean that plants colonized simultaneously by two or more of these different fungi can potentially benefit from functional complementarity in associated AMF community, as proposed by Koide (2000). This evidence is directly ecologically relevant because the isolates for this study were obtained from a single ecosystem and we have previously shown that Glomus spp. used here concomitantly colonized maize roots in the field (Jansa et al., 2003a). Research in two areas is urgently needed now to bring further understanding of the functional diversity and complementarity within AMF community. First, we have to perform experiments with AMF communities consisting of more than one AMF isolate, and the choice of the isolates should be ecologically relevant (e.g. originating from the same ecosystem if we want to gain insight into that ecosystem). Second, we have to establish tools to quantify development in roots and in soil of each AMF isolate within a mixed community.

Acknowledgements

We would like to thank to Richard Ruh for his invaluable technical assistance throughout this study. We are grateful for helpful and constructive comments on the manuscript by two anonymous reviewers. Financial support of the Swiss Federal Institute of Technology (ETH) in Zürich (project TH 41-2703.5) is gratefully acknowledged.

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Section editor: J.H. Graham