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Long-distance transport of P and Zn through the hyphae of an arbuscular mycorrhizal fungus in symbiosis with maize

Jan JANSÁ*, Ahmad MOZAFAR, Emmanuel FROSSARD

Group of Plant Nutrition, Institute of Plant Sciences, Swiss Federal Institute of Technology (ETH) Zürich, Eschikon 33, 8315 Lindau, Switzerland

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Abstract – We studied the effect of an arbuscular mycorrhizal fungus (AMF), *Glomus intraradices*, on the uptake of phosphorus (P) and zinc (Zn) by maize (*Zea mays*) in two types of experimental containers, the Cuvettes and the Starpots. The containers consisted of a compartment colonised by both roots and AMF and two compartments only explored by AMF mycelium. Plants inoculated with AMF took up more P and Zn than non-mycorrhizal plants. The AMF transported significant amounts of ^{33}P and ^{65}Zn when the labelled nutrients were located less than 15 cm away from the roots. The uptake of both ^{33}P and ^{65}Zn was significantly correlated with the mycelium length density measured near the labelled compartment in the Cuvettes and ^{33}P and ^{65}Zn uptake rates were correlated to each other in both the Cuvettes and the Starpots. Development of the AMF mycelium also varied with the size of the buffer compartment.

arbuscular mycorrhiza / mycelium / phosphorus / zinc / maize

Résumé – Transport de phosphore et de zinc par les hyphes d'un champignon endomycorrhizien en symbiose avec le maïs. Nous avons étudié l'influence d'un champignon mycorrhizien à arbuscule (AMF), *Glomus intraradices*, sur le prélèvement du phosphore (P) et du zinc (Zn) par le maïs à l'aide de deux dispositifs expérimentaux (les containers "cuvette" et "starpot"). Chacun des dispositifs était divisé en trois compartiments : un compartiment colonisé à la fois par les racines et la mycorrhize et deux compartiments explorés uniquement par les hyphes mycorrhiziens. Les plantes mycorrhizées ont prélevé significativement plus de P et de Zn que les plantes non mycorrhizées. La mycorrhize a transporté des quantités significatives de ^{33}P et de ^{65}Zn vers la plante lorsque la taille du compartiment intermédiaire était inférieure à 15 cm. Le prélèvement de ^{33}P et de ^{65}Zn était significativement corrélé à la longueur de mycelium exprimée par gramme du substrat et mesurée dans le dispositif en "cuvette". Les prélèvements de ^{33}P et de ^{65}Zn étaient fortement corrélés entre eux dans les dispositifs "cuvette" et "starpot". Enfin nous montrons que la longueur du mycelium mesuré à différents endroits dans le compartiment intermédiaire varie avec la taille de ce compartiment et donc avec l'accessibilité des éléments nutritifs par le champignon.

mycorrhize à arbuscule / mycélium / phosphore / zinc / maïs

1. INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) colonise the roots of the majority of the terrestrial plant species [41, 43]. The AMF improve the uptake by the host plant of nutrients such as P that are strongly adsorbed onto soil particles. This improved nutrition has been explained by the extension of the AMF hyphae in zones, which are remote from the root system, allowing for the exploration of spatially unavailable nutrients [24, 25, 34, 39]. In exchange the AMF receives carbohydrates from its host plant [17, 43]. Mycorrhizal hyphae of *Glomus* sp. and *Acaulospora* sp. can transport P from distances of up to

several centimeters from host plant roots [15, 18, 19, 24, 27]. That is much further than the range of several millimetres, which root hairs can exploit [13]. The efficiency of P uptake by AMF has been related to both the spatial distribution of the AMF extraradical hyphae in the soil and to the capacity of P uptake by unit length of the hyphae [17, 19, 36, 39].

Fewer studies have been conducted to elucidate the role of the AMF in micronutrient uptake by the plants compared with those dedicated to understanding of the role of AMF in P nutrition. Beneficial effects of mycorrhiza formation on the uptake of zinc by different plant species from the soil have been suggested from experiments conducted in systems where

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* Correspondence and reprints
jan.jansa@ipw.agrl.ethz.ch

the AMF and the roots grew in the same compartment [3, 5, 6, 30]. Bürkert and Robson [4] showed that different AMF species differed in their efficiency of taking up Zn from a root-free compartment located up to 40 mm from clover roots. However, little information is available on the importance of the extraradical mycelium on the uptake of Zn and its transport to the host plant.

The objective of this study was to estimate the contribution of *Glomus intraradices* to the P and Zn nutrition of maize (*Zea mays* L.) cv. Corso. As the experimental set up and the growing conditions can strongly affect the results, two types of experimental containers were tested to achieve this objective. Both of them were made of three compartments: a plant compartment, in which both the roots and the AMF could grow, and two compartments that could only be explored by the AMF mycelium: a buffer compartment of variable length and a labelled compartment containing both ^{33}P and ^{65}Zn . Such experimental systems allowed dissecting the role of roots and the extraradical mycelium of the AMF on P and Zn uptake as proposed by Schüepp et al. [35]. The double labelling of soil allowed a direct comparison of P and Zn uptake through the same mycorrhizal network as proposed by Suzuki et al. [46].

2. MATERIALS AND METHODS

2.1. Plant and fungi

A monospore isolate of *G. intraradices* (INVAM SW205, BEG 157) isolated from a Swiss agricultural soil [22] was used for this study. The AMF inoculum was produced on *Plantago lanceolata* L. and contained about 100 spores per gram. Crude inoculum including root pieces was mixed with expanded Montmorillonite (Oil Dri Chem-Sorb WR24/18) in a ratio of 1:1 (v:v) and filled into 50 mL containers, which were subsequently planted with pre-germinated maize seedlings. Non-mycorrhizal substrate was prepared by autoclaving the soil: sand: expanded Montmorillonite mixture (1:4:5, v:v:v) and adding to it a water extract (20 mL·kg⁻¹) from the AMF inoculum (100 g inoculum in 1 L water) that has been filtered three times through a Whatman No 1 filter paper.

The seeds of maize cv. Corso (each containing a mean of 0.99 ± 0.11 mg P and 8.7 ± 0.35 µg Zn) had been surface-sterilised with 5% calcium hypochlorite for 15 minutes and germinated on moist sand at 25 °C in darkness for 2 days. Germinated seeds were grown for 2 weeks in a growth chamber (300 µmol photon·m⁻²·s⁻¹, 16 h photoperiod, 25/20 °C day/night temperature and 50/80% RH). Rooted plantlets were then transferred with the adhering substrate into the experimental containers (described below) and grown for an additional 3 weeks in the greenhouse under the same conditions as above except RH being 40–50%. Minimum light intensity was 400 µmol photon·m⁻²·s⁻¹. Plants were watered with de-ionised water and each plant was fertilised weekly with 10 mL of 7-times concentrated P-free Hoagland nutrient solution [48].

After 3 weeks of growth in the experimental containers, the radioactively labelled soil was added, and the plants were

grown in the greenhouse for an additional 25 days before being harvested and analysed.

2.2. Substrate

The soil used for this experiment was collected from the 0–20 cm horizon of an agricultural field (Eutrochrept, 22.3% clay, pH 7.4) in Eschikon-Lindau, Switzerland. The soil was autoclaved at 121 °C for 1 h, was then re-inoculated with soil bacteria by adding 20 mL·kg⁻¹ soil of soil washing (100 g unsterile soil suspended in 1 L water) that has been filtered three times through a Whatman No 1 filter paper. The soil was finally incubated at ambient temperature for 6 weeks. The availability of P and Zn in the sterilised soil was assessed by the method of isotopic exchange kinetic as described by Fardeau [10] for P and by Sinaj et al. [38] for Zn. The fraction of radioactivity remaining in the solution after one minute of exchange ($r_{1\text{min}}/R$) reached 0.543 and 0.038 for ^{33}P and ^{65}Zn , respectively. The n value (i.e. the exponent of the power function, which describes the decrease of radioactivity in the solution after one minute of exchange) was 0.227 and 0.188 for ^{33}P and ^{65}Zn , respectively. The amount of element (P or Zn) isotopically exchangeable within one minute ($E_{1\text{min}}$) that is totally and immediately plant available reached 15.4 mg P·kg⁻¹ soil and 2.4 mg Zn·kg⁻¹ soil. This soil presented an $E_{1\text{min}}$ value higher than the critical level of 5 mg P·kg⁻¹ under which P deficiency limits crop yield [14]. Such a critical level has not yet been identified for Zn.

2.3. Experimental containers and labelling

Two types of containers (the Cuvette and the Starpot) each with three compartments were used for this study (Fig. 1). In both types of containers, the spatial separation between the different compartments was ensured by a 20 µm nylon mesh. The buffer (B) and the labelled (C) compartments were only accessible for the AMF hyphae while the plant compartment (A) contained both AMF and the roots.

The Cuvette container presented a contact area between the compartments of 182 cm² (14 cm × 13 cm). The plant and the buffer compartments were filled with a substrate (substrate 1) consisting of autoclaved soil: expanded Montmorillonite: quartz sand (0.7–1.2 mm) mixed in a ratio of 1:2:2 (v:v:v). The plant compartment (14 cm × 13 cm × 4 cm, volume 728 mL) was planted with 3 maize plants. The buffer compartment had a variable width (5, 10, 15 or 20 cm). The availability of P was lower in the buffer compartment ($E_{1\text{min}} = 2.9$ mg P·kg⁻¹) than in the labelled compartment composed only of sterile soil ($E_{1\text{min}} = 15.4$ mg P·kg⁻¹). The radioactive compartment (14 cm × 13 cm × 4 cm) was filled with 400 mL of autoclaved soil (330 g dry weight) labelled both with 5.55 MBq carrier-free $^{33}\text{PO}_4$ (Amersham) and with 835 kBq ^{65}Zn (NEN- Perkin-Elmer) added as ZnCl₂ with a specific activity 86 MBq mg·Zn⁻¹. The radioisotopes were dissolved in 0.25 M HCl, and the soil was labelled by mixing 1 mL of the radioactive solution to the soil that was then incubated in the darkness at ambient temperature for 48 hours. The soil layer was under- and over-layered with 1 cm of the substrate 1. Three replicate Cuvette containers were included for each buffer compartment size in the mycorrhizal treatment, while

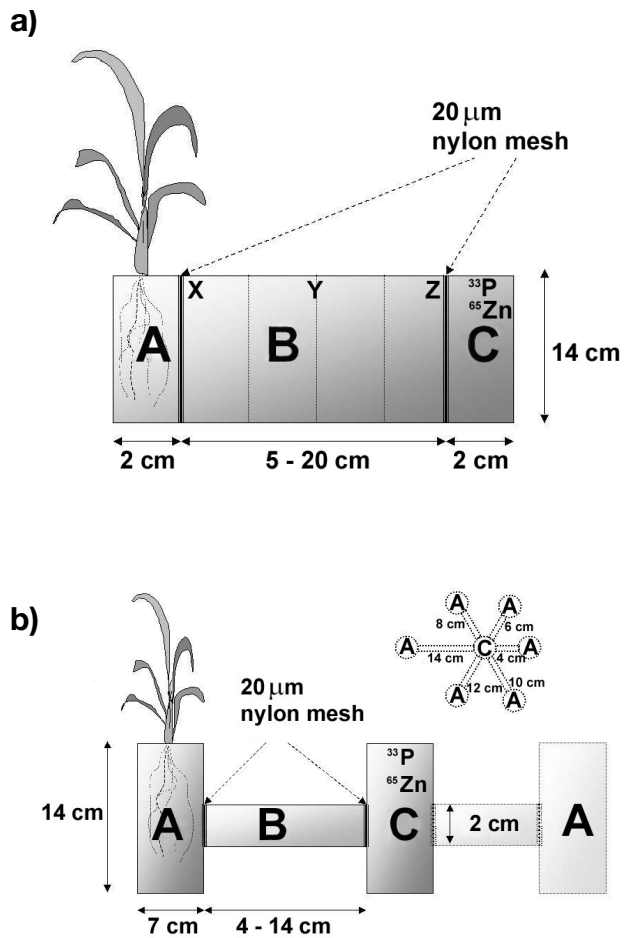


Figure 1. Cuvette container (a) and Starpot container (b) design. Both container types consist of 3 compartments: a root compartment, where the plant is grown (A), a hyphal buffer compartment (B) and a hyphal labelled compartment where the radioactive tracer is added (C). The location of sampling points for mycelium length density observation is indicated: X: 0–1 cm from the root compartment; Y: in the middle of the buffer compartment; Z: 0–1 cm from the labelled compartment.

only two replicates were included for the non-mycorrhizal controls. Each of the three maize plants was considered as a replicate. Roots from each Cuvette container (containing 3 plants) were pooled and analysed together and an average per plant was calculated.

Each Starpot container consisted of six 750 mL PVC pots where individual maize plants were grown. These pots were connected with tubes of different lengths (4, 6, 8, 10, 12 or 14 cm) of 2 cm diameter, resulting in a vertical contact area of 3.14 cm² between the different compartments. The buffer compartment tubes were filled with a sterile substrate (substrate 2), consisting of soil: sand: expanded Montmorillonite (5:2:3, v:v:v). Plant pots were filled with a substrate 1. The central compartment was filled with autoclaved soil, and a

100 mL centrifugation tube was inserted into the middle, which was later replaced with a block of 70 mL (58 g dry weight) of labelled soil. The soil was labelled by adding 1 mL of 0.25 M HCl containing 9.17 MBq ³³P₄ and 835 kBq ⁶⁵Zn as described above. Three replicates were included for both mycorrhizal and non-mycorrhizal treatments.

Watering of the plant compartment with de-ionised water was done in both types of containers by time-controlled automatic watering facility, maintaining the substrate humidity at 60–80% of its water holding capacity (WHC). Watering of the buffer compartment (in the Cuvette containers only) and of the labelled compartment (in both containers) was done using a tensiometer-controlled watering facility (Blumat, Telfs, Austria), maintaining the substrate humidity at 50–60% WHC.

2.4. Plant and substrate analysis

Shoot and root biomass was measured at harvest after drying the plant material at 105 °C for 48 hours. The aliquots (approximately 0.5 g each) of the dry biomass of the plants (shoot and roots analysed separately) were cut to pieces <1 cm, dry ashed (6 h at 550 °C), and the ashes solubilised in 2 mL of 5.6 M HCl. The concentrations of P and Zn in the extracts were measured colorimetrically using the malachite green method for P [29] and ion chromatography (Dionex DX500) for Zn [38].

The concentration of ³³P and ⁶⁵Zn in the solution was measured by β-scintillation counting after neutralisation of the extracts with NaOH, considering different energy windows (0–600 and 600–1500 keV) to distinguish between the two radioisotopes. The high energy window readings were attributed to radioactivity of ⁶⁵Zn only while the low energy window reading provided measurement of emissions from both ³³P and ⁶⁵Zn isotopes. Counting efficiency for different isotopes at different energy windows was established by extensive calibration with single and dual isotope standards. Scintillation counting was performed using a Packard 2500 TR counter and a Packard Ultima Gold scintillation cocktail. Measurements of the ³³P and ⁶⁵Zn concentration in plant samples were always performed concomitantly with the measurements of the ³³P and ⁶⁵Zn contained in the solution used to label the soil to correct for decay.

Arbuscular mycorrhizal structures (hyphae, arbuscules and vesicles) in the roots were stained after clearing the roots in 1.8 M KOH (90 °C, 1h) in a mixture of Trypan- and Methylene Blue (each 0.05% in lactic acid:glycerol:water, 1:1:1, v:v:v), following a procedure modified from Phillips and Hayman [32]. The percentage of root length colonised by AMF structures was estimated by the grid-line intersect method [16]. Mycelium length density in the substrate was estimated following the filtration-gridline method [47]. The mycelium length density was assessed in the Cuvettes by taking soil cores (depth 10 cm, diameter 1 cm) in three zones of the buffer compartment: at 0–1 cm from the root compartment (region X), in the middle (region Y), and at 0–1 cm distance from the labelled compartment (region Z) (Fig. 1). In the Starpots, mycelium length density was assessed in bulk samples taken from the connecting tubes.

Table I. Biomass production and P and Zn uptake by maize growing in Cuvette containers as affected by *Glomus intraradices*. Results of analysis of covariance (ANCOVA) are given. Distance of labelled compartment from the plants is used as a covariate (DIST), and the contrast between mycorrhizal and control treatments (TREATMENT) is shown.

	F ratio (1, 1, 57)			
	TREATMENT	DIST	Non-mycorrhizal control	Mycorrhizal treatment
Total plant DW (g)	1.94 ns	0.27 ns	8.69	8.04
P (mg per plant)	40.13 ***	5.86 *	4.81 b	10.57 a
Zn (μg per plant)	97.82 ***	5.70 **	79.9 b	139.6 a
% introduced ^{33}P transported to plant	34.22 ***	44.37 ***	0.01 b	12.10 a
% introduced ^{65}Zn transported to plant	31.86 ***	37.70 ***	0.01 b	4.33 a

F ratios (df, dfcov, residual df) are given, with a measure of the significance: ns not significant; (*) $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Different letters denote significant differences between treatment means as assessed by the LSD multiple range test ($P < 0.05$). Nine and six replicate plants were included into the mycorrhizal and non-mycorrhizal control treatments at each distance, respectively.

2.5. Statistics

Analysis of variance (ANOVA), analysis of covariance (ANCOVA), regression analysis, and comparison of correlation coefficients were done using Statgraphics® (Manugistics, Rockville MD). The significance values for correlation originate from the lack-of-fit ANOVA. Different letters denote significant differences between the treatments on a 5% probability level based on multiple range LSD F-test.

3. RESULTS

3.1. Experiment with the Cuvette containers

The total maize biomass (top and roots combined) slightly decreased, although not significantly, in the presence of *Glomus intraradices* (Tab. I). The root: shoot biomass ratio was, however, significantly increased in the mycorrhizal plants, and this effect was more pronounced for the shortest length of the buffer compartment (data not shown). P and Zn uptake was significantly higher in the mycorrhizal plants than in their non-mycorrhizal counterparts (Tab. I, Fig. 2). The concentration of P in maize tops was $1.34 \pm 0.09 \text{ mg} \cdot \text{g}^{-1}$ and $0.57 \pm 0.03 \text{ mg} \cdot \text{g}^{-1}$ for the mycorrhizal and the non-mycorrhizal treatments, respectively. The Zn concentration was $16.4 \pm 1.03 \mu\text{g} \cdot \text{g}^{-1}$ and $4.66 \pm 0.40 \mu\text{g} \cdot \text{g}^{-1}$ for the mycorrhizal and the non-mycorrhizal treatments, respectively. Significant transport of ^{33}P and ^{65}Zn to the plant occurred only in the presence of the AMF (Tab. I) but was strongly limited, when the buffer compartment length exceeded 15 cm (Tab. II).

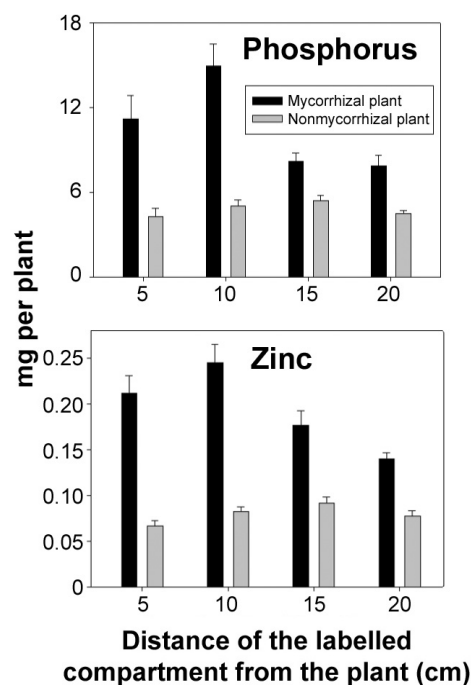


Figure 2. Effect of *Glomus intraradices* on the total P and Zn content of maize (roots plus tops) grown in the Cuvette containers. Means + SE of means are given (six replicates in the non-mycorrhizal control and nine replicates in the mycorrhizal treatment).

Maize roots were strongly colonised by the AMF in all inoculated containers. No influence of the length of the buffer compartment on either hyphal (Tab. II) or arbuscular or vesicular colonisation (data not shown) of the roots was observed. No colonisation was found in the non-mycorrhizal treatment. The percentage of ^{33}P and ^{65}Zn introduced into the labelled compartment and transported to the plant through AMF was positively correlated with the length density of the mycelium measured in the region Z close to the labelled compartment ($R^2 = 0.73$ and $R^2 = 0.71$ for ^{33}P and ^{65}Zn , respectively, $P < 0.001$ in both cases) (Tab. II). Finally the mycelium length density in the region Z (close to the labelled compartment) became significantly smaller as the size of the buffer compartment increased, while in the region X the length density of the mycelium significantly increased (Tab. II). The length density of mycelium in the region Y remained constant over the range of buffer compartment sizes explored.

3.2. Experiment with the Starpot containers

AMF reduced the total maize biomass production in the Starpots (Tab. III) and caused an increase in root: shoot biomass production compared with non-mycorrhizal plants (data not shown). This effect was more pronounced when the distance between the root and the labelled compartments was short (data not shown). Inoculation with AMF significantly increased both the P and Zn uptake by maize (Tab. III, Fig. 3). The concentration of P in maize tops was $1.27 \pm 0.06 \text{ mg} \cdot \text{g}^{-1}$ and $0.85 \pm 0.06 \text{ mg} \cdot \text{g}^{-1}$ for the mycorrhizal and the non-mycorrhizal treatments, respectively. The Zn concentration was

Table II. Colonisation of maize roots by *Glomus intraradices*, transport of ³³P and ⁶⁵Zn placed at different distances from the roots by the AMF extraradical hyphae, and length densities of AMF mycelium in three regions of the buffer compartment of the Cuvette containers. Results of ANOVA and mean values are given.

	F ratio (3, 8)	Length of the buffer compartment			
		5 cm	10 cm	15 cm	20 cm
Hyphal colonisation (% root length)	0.62 ns	98.0	93.3	94.0	95.3
Mycelium length density in region X (m·g ⁻¹ substrate)	6.14 *	2.9 c	4.1 bc	5.7 ab	7.2 a
Mycelium length density in region Y (m·g ⁻¹ substrate)	0.78 ns	6.4	6.4	7.0	8.5
Mycelium length density in region Z (m·g ⁻¹ substrate)	27.01 ***	4.7 a	6.4 a	1.1 b	0.6 b
Percentage of introduced ³³ P transported to plants	174.11 ***	26.8 a	20.8 b	0.8 c	0.0 c
Percentage of introduced ⁶⁵ Zn transported to plants	37.70 ***	8.8 a	8.1 a	0.3 b	0.0 b

F ratios (df, residual df) are given, with a measure of the significance: ns not significant; (*) $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Different letters denote significant differences between treatment means as assessed by the LSD multiple range test ($P < 0.05$). Only data from the mycorrhizal treatments were used.

Table III. Biomass production and P and Zn uptake by maize growing in Starpot containers as affected by *Glomus intraradices*. Results of analysis of covariance (ANCOVA) are given. The distance of labelled compartment from the plants is used as covariate (DIST), the contrast between mycorrhizal and control treatments (TREATMENT) is shown.

	F ratio (1, 1, 33)		
	TREATMENT	DIST	Non-mycorrhizal control / Mycorrhizal treatment
Total plant DW (g)	10.81 **	1.18 ns	9.08 a / 8.31 b
P (mg per plant)	21.44 ***	7.78 **	7.62 b / 10.48 a
Zn (µg per plant)	19.07 ***	0.80 ns	114 b / 163 a
% introduced ³³ P transported to plant	12.13 **	15.16 ***	0.0 b / 2.8 a
% introduced ⁶⁵ Zn transported to plant	13.10 **	14.06 ***	0.0 b / 1.0 a

F ratios (df, dfcov, residual df) are given, with a measure of the significance: ns not significant; (*) $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Different letters denote significant differences between treatment means as assessed by the LSD multiple range test ($P < 0.05$). Three replicate plants were included into the mycorrhizal and non-mycorrhizal control treatments at each distance.

11.6 ± 0.65 µg·g⁻¹ and 6.03 ± 0.60 µg·g⁻¹ for the mycorrhizal and the non-mycorrhizal treatments, respectively. ³³P and ⁶⁵Zn were transferred to the plants only in the presence of

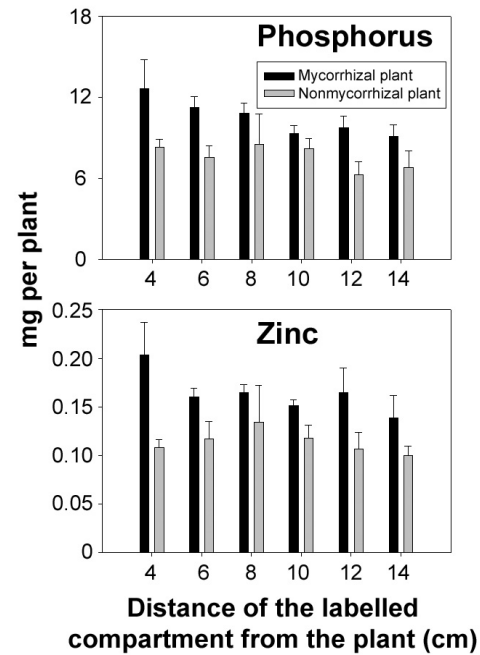


Figure 3. Effect of *Glomus intraradices* on the total P and Zn content of maize (roots plus tops) grown in the Starpot containers. Means + SE of means are given of data from three replicate plants.

AMF (Tab. III). The uptake of ³³P and ⁶⁵Zn by the mycorrhizal plants (Tab. IV) decreased as the distance between the plant and the labelled compartments increased. P uptake in the mycorrhizal plants significantly decreased with increasing distance from the labelled compartment ($R^2 = 0.32$, $P = 0.014$), while such a trend was not found for Zn uptake.

Maize root colonisation by the AMF hyphae was very high in all inoculated Starpots (Tab. IV). The length of the connecting tube between the plant and the soil compartments did not affect either AMF hyphal (Tab. IV) or arbuscular or vesicular colonisation rate in maize roots (data not shown). No colonisation was found in the non-mycorrhizal treatment. Mycelium length density measured in the buffer compartment of non-mycorrhizal control pots was (probably due to presence of mycelium of saprophytic fungi) about 1% of the mycelium length density observed in the mycorrhizal treatment (data not shown). The size of the buffer compartment (connecting tube) did not affect the mycelium density in that compartment (Tab. IV).

3.3. Comparison of P and Zn uptake in both experimental systems

³³P and ⁶⁵Zn uptake were strongly correlated to each other both in the Cuvette and the Starpot containers (Fig. 4). Furthermore the correlation coefficients were not statistically different from each other ($P = 0.76$) suggesting that a common set of mechanisms could explain the uptake of both elements.

Table IV. Colonisation of maize roots by *Glomus intraradices* and the transport of ^{33}P and ^{65}Zn placed at different distances from the roots by the AMF extraradical mycelium in the Starpot containers. Results of ANOVA and mean values are given.

	F ratio (5, 12)	Length of the buffer compartment					
		4 cm	6 cm	8 cm	10 cm	12 cm	14 cm
Hyphal colonisation (% root length)	1.60 ns	96.0	99.3	98.0	96.0	93.3	94.0
Mycelium length density ($\text{m} \cdot \text{g}^{-1}$) (in the buffer compartment)	0.63 ns	18.2	16.7	19.1	17.9	18.5	13.8
Percentage of introduced ^{33}P transported to plants	11.52 ***	9.96 a	4.31 b	1.69 bc	0.31 c	0.26 c	0.01 c
Percentage of introduced ^{65}Zn transported to plants	7.80 **	3.48 a	1.61 b	0.77 bc	0.15 bc	0.12 c	0.09 c

F ratios (df, residual df) are given, with a measure of the significance: ns not significant; (*) $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Different letters denote significant differences between treatments (LSD multiple range test, $P < 0.05$). Only data from the mycorrhizal treatments (three replicated units per each distance) are used.

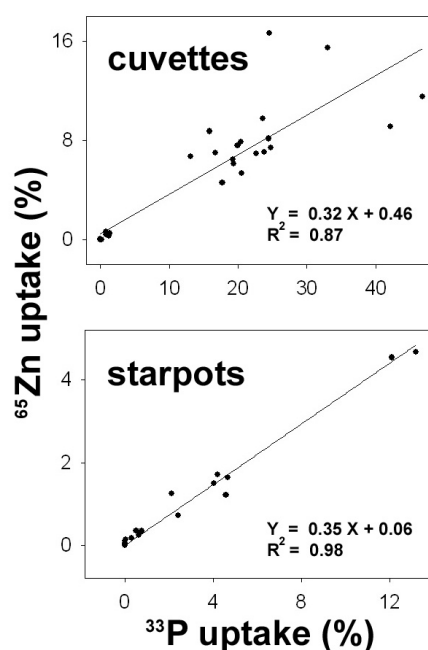


Figure 4. Correlation between the percentage of ^{33}P added to the system and taken up by maize via the AMF mycelium and the percentage of ^{65}Zn added to the system and taken up by maize via the AMF mycelium in both the Cuvette and Starpot containers. Both correlations are significant at the $P < 0.001$ level.

4. DISCUSSION

4.1. Shoot and root biomass production of maize

The decrease in biomass production observed in the presence of *G. intraradices* in the Starpot and in the Cuvette containers confirm earlier observations showing that the cost of building the mycorrhizal structures can counterbalance or outweigh the effect of an improved P and Zn nutrition on plant biomass production through mycorrhiza establishment [11,

40, 42]. Inoculation of plants with AMF has been shown to decrease the root: shoot biomass ratio previously [8, 43] that is in contrast with results obtained both in the Cuvette and in the Starpot containers. The difference between our results and those described in the literature might be explained by the different experimental containers and/or by the different growing conditions as the light intensity or the substrate moisture [9, 23, 45]. Our results are, however, only of a limited relevance for field grown maize because the root development was limited by the small substrate volume.

4.2. Uptake and transport of P and Zn by *Glomus intraradices*

Glomus intraradices significantly improved the uptake of both P and Zn by the maize plants. In both systems, the uptake of ^{33}P observed at a given length of the buffer compartment was significantly correlated to that of ^{65}Zn measured for the same size of the buffer compartment. However the absolute transfer rates of ^{33}P and ^{65}Zn were higher in the Cuvettes than in the Starpots for a given length of the buffer compartment. These differences were probably due to the different size of the contact zone between the compartments and to the different geometry of the buffer compartment (soil block versus a narrow tube for the Cuvette and the Starpot containers, respectively). In the Starpot container, the uptake of P and Zn by the AMF might also have been influenced by the competition among individual plants growing in the same experimental unit, which contained 6 plants in the 6 external tubes.

Almost 27% of the added ^{33}P were transported to the maize plants by *Glomus intraradices* in the Cuvette containers from the distance of 5 cm from the roots. Such a high transport was probably due to the high available P content of the soil used for this study, which reduced the transfer of the introduced radioisotope into less rapidly exchangeable pools, and to the extensive mycorrhizal hyphal development in the experimental containers. Furthermore in the Cuvette containers, a significant portion of the P taken up by maize was derived from the buffer compartment, which contained a substrate with a low P availability. This can be deduced from comparison of the results of this Cuvette experiment with those of a pot experiment where maize was grown in single compartment containers of the same

geometry as the plant compartments in the Cuvettes [21]. In the single compartment containers (performed under identical conditions to those described here), the colonisation of the same maize cultivar by the same isolate of *G. intraradices* as used in this study resulted in a surplus in P uptake of 19% as compared with the non-mycorrhizal plants. This gives an estimate of how much can the establishment of mycorrhizal symbiosis increase the P uptake from the root compartment [21]. In contrast, mycorrhizal maize growing in the Cuvettes with a 20 cm long buffer compartment, having no access to the P in the labelled compartment, contained about 76% more P than the non-mycorrhizal maize. These results show that *G. intraradices* took up a large quantity of P from the buffer compartment as it reached a 20 cm length.

Glomus intraradices was also efficient in taking up and transporting Zn. Almost 9% of the added ^{65}Zn was transported to the plants from the distance of 5 cm within 25 days in the Cuvette containers. The transport of ^{65}Zn by mycorrhizal hyphae observed in previous studies [4, 6, 28] was about two orders of magnitude lower than that observed in this study. This could be explained by the use of different host plants (clover versus maize), by the differences in efficiency and/or development of the AMF symbionts used, or by the differences in Zn availability in the soils used for the experiments. For example, Liu et al. [26] have shown that another isolate of *G. intraradices* played an important role in Zn uptake of maize under low Zn conditions, but not under Zn sufficient conditions. Zhu et al. [49] have shown that AMF can diminish the Zn uptake by white clover from Zn-contaminated soils compared with non-mycorrhizal plants. These results point out to the necessity of fully characterising Zn availability, e.g. by using the isotopic exchange kinetics approach [12, 38], in soils used to study the effect of AMF on Zn transport to the plants.

4.3. AMF mycelium and mycorrhiza-mediated nutrient uptake by maize

Mycelium length density in the labelled compartment explained the transport of both ^{33}P and ^{65}Zn to the plant, confirming the results of earlier works [18–20, 37, 40]. In our work, this is shown by the significant correlation observed for both radioisotopes in the Cuvette container between the radioisotope uptake and the mycelium length density measured next to the labelled compartment (region Z) and with both types of containers by the constant ratio observed between ^{33}P and ^{65}Zn uptake (Fig. 4). As the optimum concentration of P and Zn in maize are estimated to be 3000 and 25 mg · kg⁻¹ DM, respectively [2], we conclude that mycorrhizal plants were slightly deficient in these elements whereas non-mycorrhizal plants suffered from severe P and Zn deficiencies.

The mycelium of the *G. intraradices* isolate used in this study reached a distance of about 15 cm from the plant roots in the Cuvette containers. This is the largest distance from the roots at which the AMF mediated uptake of nutrients such as P and Zn has been observed [4, 18, 24, 43]. It has, however, to be established, whether this contributes significantly to mineral nutrition of field grown maize, where the mean inter-root distance is 0.3–2 cm only [1, 31]. These reported values were, however, obtained by theoretical calculation from the root length density data, and did not consider a possible heterogeneity of root distribution in the soil determined, for

example, by soil macropores [33, 44]. It is also necessary to study whether the AMF exert important nutritional effects on young plants with not fully developed root system.

In the Cuvette containers, differences in development of AMF mycelium were observed depending on the length of the buffer compartment. When the labelled soil compartment (nutrient-rich patch) was too far from the roots to be accessed by AMF hyphae, an increase in mycelium length density was observed in the proximity of the roots (Tab. II). This is the first direct evidence showing the ability of a given AMF isolate to modify its mycelium growth pattern according to the localisation of resources [7].

5. CONCLUSION

We showed with both the Cuvettes and the Starpot containers that the studied *Glomus intraradices* isolate largely increased the total P and Zn content of plants but had little effect on the plant biomass production. This isolate was efficient in taking up and transporting ^{65}Zn and ^{33}P from soil zones located less than 15 cm away from the maize root under greenhouse conditions. This isolate was also able to take up and transport significant amounts of P and probably also Zn from the buffer compartment that presented a lower nutrient availability. We also showed that the architecture of the AMF mycelium depended on the actual spatial distribution of the nutrients in the substrate. As both the Cuvette and the Starpot experiments yielded similar results, we deduce that the exploration of soil zones not accessible to the roots by a large mycelium network that can adapt to the spatial localisation of nutrients explains the high efficiency of this *G. intraradices* isolate to supply maize plants with P and Zn.

The following points remain to be clarified in further studies: (1) Do the AMF really play an important role in P and/or Zn acquisition of field grown plants where roots are not limited in their growth by mesh barrier? (2) Does the variation in nutrient uptake efficiency among different AMF isolates/species/genera relate only to the length density of mycelium produced by the AMF in the soil, or does it depend also on the isolate/species-specific density/affinity of nutrient transporters on the surface of the hyphae?

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