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The relative contribution of hyphae and roots to phosphorus uptake by arbuscular mycorrhizal plants, measured by dual labelling with ^{32}P and ^{33}P

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SUMMARY

The aim of this investigation was to measure the relative contribution of hyphae and roots to the total uptake of phosphorus by mycorrhizal plants. Cucumber plants were grown in three-compartment systems where ^{32}P was applied to a lateral root-free compartment (HC) and ^{33}P applied to an identical lateral compartment with both roots and hyphae (RHC). The cucumber seeds were sown into the main root compartment (RC) which was inoculated with one of the following three arbuscular mycorrhizal fungi: *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders [isolate WUM 12(2)]; *Glomus* sp. [isolate WUM 10(1)]; *Glomus caledonium* (Nicol. & Gerd.) Trappe & Gerdemann (isolate RIS 42) or left uninoculated to represent a control treatment. The plants were harvested at 17 and 27 d. The hyphal uptake of ^{32}P from the HC increased as follows – *S. calospora* < *Glomus* sp. < *G. caledonium*. The uptake of ^{32}P from the HC was equivalent to 7, 21 and 109 % of the uptake of ^{33}P from the RHC in plants colonized by *S. calospora*, *Glomus* sp. and *G. caledonium*, respectively. This indicates that the relative contribution of the roots in total P uptake varied greatly between the three mycorrhizal treatments. Although ^{33}P uptake was variable within treatments, the P uptake systems of roots colonized by *G. caledonium* appeared to be inactive when compared to hyphal ^{32}P uptake. This may have been due to feedback mechanisms being activated because of the high hyphal P uptake. The roots colonized by *S. calospora* had higher rates of root-P uptake compared with the control roots, suggesting that the root-P uptake systems have been stimulated by the presence of the fungus. This would ensure that the P supply was sufficient to maintain photosynthesis in this symbiosis where the fungus gives only little P in return for its use of plant carbon substrate from the host.

Key words: Arbuscular mycorrhizas, P uptake, radioisotopes, hyphal inflow.

INTRODUCTION

Hyphae of arbuscular mycorrhizal (AM) fungi increase the absorptive surface area of the root system and consequently the uptake of phosphorus into the host plant (Harley and Smith, 1983). However, AM fungi can differ markedly in their ability to increase P uptake into the plant. This may be caused by differences in rate and distance of hyphal spread away from the root (Kothari, Marschner & Romheld, 1991; Jakobsen, Abbott & Robson; 1992*a*), and by differences in the P transport capacity per unit length of hyphae (Jakobsen, Abbott & Robson, 1992*b*; Pearson & Jakobsen, 1993). The consequences of these interfungal differences for P absorption by the root are unknown at present. Quantification of the relative contributions of the fungus and the root in total P uptake will help us to identify the most important target for management of a given symbiosis towards maximal P uptake.

The contribution of the mycosymbiont to the P content of a mycorrhizal plant cannot be estimated by comparing it with a non-mycorrhizal plant, because of differences in the architecture of mycorrhizal and non-mycorrhizal root systems (Hetrick, 1991; Schellenbaum *et al.*, 1991). The hyphal contribution to P inflow has been estimated as the inflow into mycorrhizal roots minus the inflow into non-mycorrhizal control roots (Sanders *et al.*, 1977), on the assumption that the activities of the P uptake systems of mycorrhizal and non-mycorrhizal roots are similar. However, the physiology of the root is influenced by the nutrition of the mycosymbiont

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(reviewed by Clarkson, 1985) and this influence might well extend to the activity of the P uptake systems.

The potential contribution of hyphae to P uptake has been measured as the difference in P content of non-mycorrhizal plants and mycorrhizal plants grown with a root-free hyphal compartment (Li, George & Marschner, 1991*a*, *b*; Jakobsen *et al.*, 1992*a*). However, this requires that root densities in the root compartment are unusually high to prevent any differences in P uptake from the root compartment.

The objective of the present work was to distinguish between P uptake by hyphae and roots by means of dual labelling with radiotracers. Hyphae grew into a compartment with ^{32}P-labelled soil whilst both roots and hyphae grew into an identical compartment with ^{33}P-labelled soil. The relative content of the two isotopes in the host would reflect the P uptake from each of the labelling compartments.

MATERIALS AND METHODS

Plants and mycorrhizal inoculum

Surface-sterilized seeds of cucumber (*Cucumis sativus* L.) cv. Aminex (F1 hybrid) were germinated between moist tissues for 48 h before sowing. The soil received dry soil inoculum of *Glomus* sp. [isolate WUM 10(1)], *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders [isolate WUM 12(2)] or *Glomus caledonium* (Nicol. & Gerd.) Trappe & Gerdemann (isolate RIS 42). Uninoculated soil served as a control treatment.

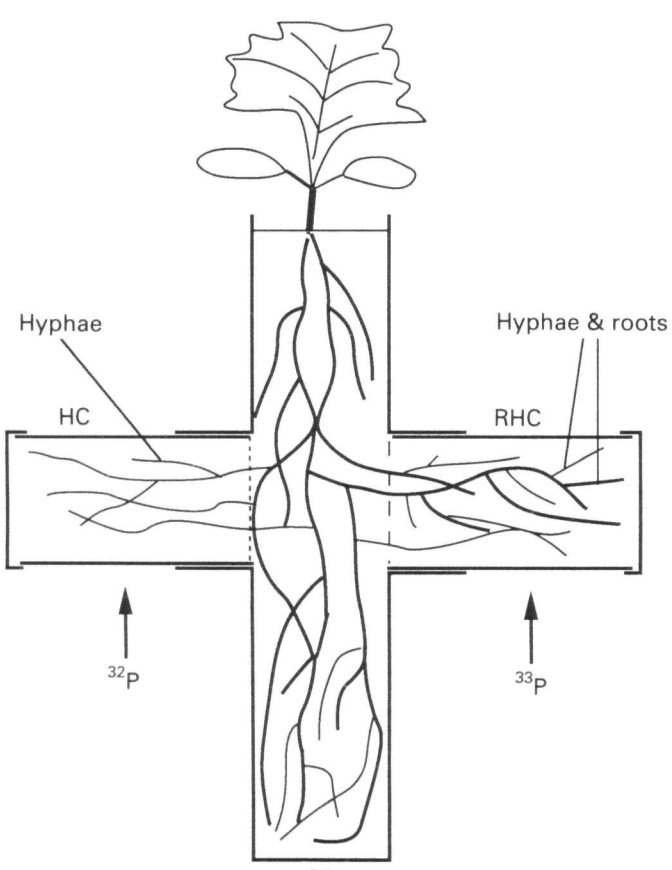

Figure 1. A schematic diagram of the PVC growth systems, showing the central root compartment (CC) and the two lateral compartments – the hyphal compartment (HC) and the root and hyphae compartment (RHC).

Growth system

A 1:1 mixture (w/w) of a sandy loam and quartz sand was partially sterilized by irradiation (10 kGy, 10 MeV electron beam) to eliminate the indigenous arbuscular mycorrhizal fungi. The soil mixture had a pH (H_2O) of 6·1 and contained 8 mg kg^{-1} soil of 0·5 M $NaHCO_3$-extractable P. A total of 390 g (with inoculum) of the soil mix was placed into open PVC tubes (270 mm length, 36 mm inner diameter) with two lateral compartments (each containing a further 110 g of soil) placed opposite each other at 100 mm from the top (Fig. 1). Each lateral compartment (70 mm length, 36 mm diameter) fitted tightly into the central root compartment (CC). Hyphae grew into the hyphal compartment (HC) through a fine mesh (37 μm) which restricted the growth of the roots. The roots + hyphae compartment (RHC) had a course mesh (700 μm) that allowed roots and hyphae to pass freely. The following nutrients were mixed thoroughly into the soil (mg kg^{-1} dry soil): KH_2PO_4 (35), K_2SO_4 (70), $CaCl_2$ (70), $MgSO_4.7H_2O$ (20), $CuSO_4.5H_2O$ (2·2), $ZnSO_4.7H_2O$ (5), $MnSO_4.7H_2O$ (10), $CoSO_4.7H_2O$ (0·33), $NaMoO_4.2H_2O$ (0·2).

Six non-inoculated plants and eight plants of each mycorrhizal treatment were prepared. Fifty grams (for *S. calospora* and *G. caledonium*) or 25 g (for *Glomus* sp.) of inoculum was mixed throughout the CC and another 50 or 25 g was banded in the CC between the two lateral compartments. The *Glomus* sp. treatment received less inoculum to attempt to equalize the inoculum potentials. Each CC received 20 ml of leachings (< 38 μm) of the three combined inocula to attempt to produce an initially similar population of soil microbes in all treatments. The lateral compartments were left empty but capped. The CC soil was watered to 70 % of field capacity and incubated at room temperature for 1 week prior to sowing. Two germinated seeds of cucumber were sown into each tube and thinned to one after emergence. Each tube received 10 mg of N as NH_4NO_3 at regular intervals amounting to 70 mg N in total. The plants were maintained in a growth chamber with a 16/8 h light/dark cycle at 21/16 °C, 60–70 % relative humidity. Osram daylight lamps provided photon flux density of 500–550 μmol m^{-2} s^{-1} (400–700 nm).

Labelling with ^{32}P and ^{33}P

Isotope-labelled soil was added to HC and RHC 5 d after sowing. $H_3{}^{32}PO_4$ and $H_3{}^{33}PO_4$ were pre-mixed into two 100 g lots of dry soil. Each of them was then mixed thoroughly with the soil to be placed in the HC

and RHC, respectively, and the final activity of each isotope was 3·7 kBq g^{-1}. The cap of each compartment was temporarily removed whilst 10 g of unlabelled soil was placed against the mesh to act as a buffer zone (8–10 mm) between labelled P and the main root compartment. Each PVC tube received 100 g of ^{33}P-labelled soil and 100 g of ^{33}P-labelled soil in the HC and RHC, respectively.

Harvests

Three non-mycorrhizal plants and four plants of each mycorrhizal treatment were selected randomly and harvested 17 d after sowing. The remainder were harvested 10 d later. The shoots were cut at the soil surface, dried at 80 °C for 24 h, weighed and ground in liquid nitrogen.

The soil was removed from the two labelling compartments and the 10 g unlabelled buffer zone soil was separated from the labelled soil. The labelled soil was weighed and thoroughly mixed. Duplicate 2 g subsamples were taken from all labelling compartments. The roots in the RHC were washed free of soil and then cleared and stained. The roots in the CC were washed and weighed. They were then subsampled for drying and staining. The dried samples were ground in liquid nitrogen.

Analyses

Ground plant material was digested in a solution of nitric/perchloric acid (4:1 v/v), mixed with scintillation fluid (3 ml sample/10 ml scintillation fluid), and analysed on a Packard TR1900 liquid scintillation counter for ^{32}P and ^{33}P activity by Dual Spectrum Analysis. Counts were automatically corrected for counting efficiency and isotope decay. The P content of these digests was measured by the molybdate-blue method (Murphy & Riley, 1962) in a Technicon AutoAnalyzer II. The duplicate 2 g of soil were blended with 250 cm^3 water and 3 cm^3 aliquots of this were filtered and then stained with Trypan Blue on 25 mm membrane filters (1·2 μm). Hyphal length was recorded in 25 random fields of view per filter (Jakobsen *et al.*, 1992*a*). The length of stained hyphae on the filters was determined by the grid-line intercept method (Tennant, 1975) at × 200 magnification. The root subsamples for staining were cleared with KOH and stained with Trypan Blue. A line-intercept method (Newman, 1966) was used to measure the root length of the stained samples and the presence or absence of mycorrhizal colonization at each intercept was used to determine the percentage colonization and mycorrhizal root length.

Statistics

The data were analyzed by one-way analyses of variance using a General Linear Model that is suitable for unbalanced data. Means were compared by an estimated value for the Least Significant Difference ($P = 0{\cdot}05$) calculated from the results of the anova. Differences between the hyphal length produced in the two lateral compartments were tested by a paired t test.

RESULTS

Plant growth and mycorrhizal colonization

Mycorrhizas were well established in the CC in all the inoculated treatments at 17 d and became more abundant by 27 d. Percent root colonization in CC was highest in the association with *Glomus* sp. at 27 d (Table 1), but the mycorrhizal root length in CC was similar with all three fungi at this stage.

The formation of mycorrhizas occurred more slowly in the inoculum-free RHC and remained lower than in the CC (Table 1). The percentage of colonization of RHC roots by *S. calospora* in particular, remained low and was similar at both harvests.

Shoots of plants colonized by *S. calospora* and by *Glomus* sp. were smaller than the controls at harvest 1, but by harvest 2 the non-mycorrhizal treatment shoots were the smallest (Table 1). Dry mass of the roots in the CC was unaffected by treatment except for the first harvest where plants colonized by *Glomus* sp. had the highest dry weight of roots of the four treatments. These differences were reflected in the root length in the CC at 17 d, whilst at 27 d the root length was lower in plants with *Glomus* sp. than in plants with *G. caledonium* and in non-mycorrhizal plants. Growth of roots into the RHC was successful in all but one replicate of the *Glomus* sp. treatment at harvest 1. Root lengths in the RHC were similar in the four treatments, except for harvest 1, where values were smaller in plants colonized by *Glomus* sp. than in the control plants.

The lengths of hyphae in the RHC and HC of the mycorrhizal treatments were corrected for background values (the four means used varied between 299–380 m) measured in the control treatment. The RHC and HC of plants contained less hyphal length when colonized by *S. calospora* than by either *Glomus* species (Table 1). There were no differences between the RHC and the HC for any of the treatments.

At 17 d shoot-P concentration was lowest in plants colonized by *S. calospora* and highest in the plants colonized by *Glomus* sp. (Table 2), while root-P concentration was highest in the *G. caledonium* treatment and lowest in *Glomus* sp. treatment. Plants colonized by *G. caledonium* or *S. calospora* had lower shoot-P concentrations at 27 d than controls or plants colonized by *Glomus* sp., whilst the only difference in the corresponding root-P concentrations were between the controls and plants colonized by *Glomus* sp. (Table 2).

At 17 d shoot-P content was highest in the control

Table 1. *Plant growth and mycorrhizal colonization of* Cucumis sativus *harvested at 17 and 27 d from sowing*

Harvest	Shoot dry mass (g)	Root dry mass (g)	Root length (m)		Colonization (%)		Hyphal length† (m)	
			RC+	RHC+	RC	RHC	RHC	HC+
17 d								
Control	0·54	0·07	22·3	6·9	0	0	—	—
Scutellospora calospora	0·49	0·09	21·1	4·9	37	9	70	113 #
Glomus sp.*	0·48	0·13	24·2	3·9	73	30	386	365
G. caledonium	0·53	0·09	20·0	6·3	45	23	475	394
LSD	0·05	0·03	4·2	2·7	13	11	121	98
27 d								
Control	1·72	0·32	55·2	13·1	0	0	—	—
S. calospora	1·95	0·30	49·5	15·0	60	9	309	287
Glomus sp.	2·04	0·31	42·1	13·5	81	36	719	843
G. caledonium	1·96	0·26	52·9	15·5	65	43	965	791
LSD	0·16	NS	9·4	NS	14	19	223	187

* One replicate failed to spread into the labelling compartment.
LSD significant at the $P = 0{\cdot}05$ level.
\# No differences were observed between the HC and RHC compartments at the $P = 0{\cdot}05$ level at any time.
+RC, main root compartment; RHC, lateral compartment with roots and hyphae; HC, lateral compartment with hyphae only.
† Hyphal lengths corrected for background.

Table 2. *Phosphorus (P) content and phosphorus concentration of* Cucumis sativus *colonized by three arbuscular mycorrhizal fungi harvested at 17 and 27 d old*

Harvest	P concentration (% d.wt)		P content (mg)	
	Shoot	Root	Shoot	Root
17 d				
Control	0·18	0·25	1·00	0·17
Scutellospora calospora	0·15	0·23	0·72	0·21
G. caledonium	0·16	0·29	0·83	0·25
Glomus sp.	0·19	0·21	0·93	0·27
LSD	0·02	0·05	0·16	0·03
27 d				
Control	0·24	0·21	4·20	0·67
S. calospora	0·21	0·24	4·10	0·74
G. caledonium	0·20	0·26	3·92	0·67
Glomus sp.	0·23	0·29	4·78	0·92
LSD	0·02	0·08	0·64	NS

Values represent the mean of three replicates for controls and four replicates for mycorrhizal treatments.
LSD significant at the $P = 0{\cdot}05$ level.

plants and lowest in the plants associated with *S. calospora* (Table 2). The corresponding root-P contents increased in the following order of inoculation treatments: control < *C. calospora* < *G. caledonium* < *Glomus* sp. (Table 2). The plants colonized by *Glomus* sp. had the highest shoot-P content at 27 d, whilst root-P contents were similar in the plants of the four treatments (Table 2).

Uptake of phosphorus isotopes

Amounts of ^{32}P in both shoots and roots at harvest 1 were unaffected by treatment. Ten days later the *G. caledonium* hyphae had transported more ^{32}P to the plant than the fungi of the other inoculation treatments (Table 3). The hyphal uptake from the HC by the *G. caledonium* mycorrhiza was greater than the total P uptake by roots and hyphae together from the RHC, whilst the uptake by hyphae of *S. calospora* and *Glomus* sp. in the HC represented only 7 and 21 %, respectively, of the total P uptake from the RHC (Table 3).

The uptake of ^{33}P from the RHC into roots and shoots at 17 d varied greatly within treatments but not between treatments (Table 3). However, by 27 d the shoot ^{33}P content in the plants inoculated with *S.*

Table 3. *^{32}P and ^{33}P content (Bq) of the shoots and roots of* Cucumis sativus *harvested at 17 and 27 d from sowing*

Harvest	^{32}P content (HC)			^{33}P content (RHC)			Hyphal uptake as % of total
	Shoots	Roots	Total	Shoots	Roots	Total	
17 d							
Control	529	70	599	8666	677	9343	—
Scutellospora calospora	347	92	439	6681	665	7346	ND
Glomus sp.*	315	92	407	5145	454	5599	ND
G. caledonium	296	193	489	4650	695	5345	ND
LSD	NS	NS		NS	NS		
27 d							
Control	923	137	1060	20429	1170	21599	—
Scutellospora calospora	2620	427	3047	40624	4479	45103	6·8
Glomus sp.	6473	1219	7692	34736	2510	37246	20·7
G. caledonium	25319	5071	30390	26112	1694	27806	109·3
LSD	3672	1402		19318	2721		

* One replicate failed to spread into the labelling compartment.
LSD significant at the $P = 0{\cdot}05$ level.

calospora was higher than that of the control treatments but similar to those of plants inoculated by the two other fungi. The ^{33}P content in the roots at 27 d was higher in plants colonized by *S. calospora* than in control plant or those colonized by *G. caledonium* (Table 3).

DISCUSSION

Dual labelling of arbuscular mycorrhizal plants with radioactive isotopes of phosphorus is a novel approach for measuring the relative contribution by hyphae and by roots to the total uptake of P by the mycorrhizas. The use of three-compartment systems allowed us to compare directly the transport into the plant of ^{32}P from a root-free hyphal compartment and of ^{33}P from a similar sized compartment containing both roots and hyphae.

The marked inter-fungal differences in capacity for transport (uptake, translocation and transfer) of P by hyphae measured in this study confirm previous observations by Jakobsen *et al.* (1992*a*, *b*) and Pearson & Jakobsen (1993). Hyphal P transport from the HC increased in the order *S. calospora*, *Glomus* sp., *G. caledonium*, but these differences were not reflected in the uptake of ^{33}P from the RHC, which was more variable within treatments. Only plants colonized by *S. calospora* had higher ^{33}P content than the controls, and the uptake of ^{33}P by plants colonized by *G. caledonium* was similar to the uptake of ^{32}P by the fungal hyphae alone.

The interpretation of these data is complicated as hyphae present in the RHC would have originated from two sources: colonized roots in the CC and colonized roots in the RHC. However, we assume that similar amounts of hyphae would have grown from CC into the HC and RHC, and that hyphae from the more slowly-colonized roots in the RHC would have contributed only marginally to the total hyphal length in the compartment. These assumptions are in agreement with the absence of marked differences in the lengths of hyphae measured in the HC and RHC of each treatment. A tendency toward higher hyphal lengths in the RHC than in the HC was observed with *G. caledonium*, which has previously been observed to spread rapidly (I. Jakobsen, unpublished results). This would also be expected to occur in the other mycorrhizal treatments as the colonization in the RHC became fully established.

We assume that hyphae directly associated with CC roots transported similar amounts of ^{32}P and ^{33}P to the plant, and that hyphae associated with RHC roots contributed only little to the transport of ^{33}P. This leads to the conclusion that all transport of ^{33}P from the RHC of plants colonized by *G. caledonium* would have occurred via the hyphae. P uptake by roots is regulated by feed-back mechanisms which may operate at the whole plant level or the cellular level (Clarkson, 1985; Marschner, 1986). We propose that the P concentration of the roots influences further uptake: the maintenance of high rates of hyphal P transport into cells of colonized parts of the root may inactivate the P uptake systems of the root.

The proposed fungal inactivation of root P uptake seemed only to occur in plants colonized by *G. caledonium* which was very effective in P uptake. Coincident increases in concentration of P in shoots and roots were not observed and further studies should pay attention to more detailed investigations of P status of colonized root cells or root tissues.

The root length densities in the RHC of about 20 cm cm^{-3} soil would have resulted in some overlapping of P-depletion zones around roots. This might have resulted in competition for ^{33}P between

roots and hyphae. However, a recent field study using similar dual-labelling techniques with peas colonized by the same isolate of *G. caledonium* suggests that competition was not an important factor since the density of pea roots in the RHC was much lower than in the present study, but the ^{32}P uptake from the HC was still higher than the ^{33}P uptake from the RHC (Jakobsen, unpublished results).

The higher uptake of ^{33}P by roots colonized by *S. calospora* than by the control roots could not be explained by the hyphal uptake and suggests that the P uptake systems were more active in roots colonized by *S. calospora* than in control roots, although increased P concentration or content was not observed in these plants. The reasons for the observed increase in ^{33}P uptake by the roots can not be explained from the results presented here, and further investigations are required.

Although the regulatory mechanisms have not yet been identified, this experiment clearly demonstrates that calculation of hyphal inflow to mycorrhizal roots may be considerably underestimated when based on comparisons between inflow to roots of mycorrhizal plants and roots of non-mycorrhizal plants (Sanders *et al.*, 1977).

A number of factors in this experiment need consideration. There is a need to control the diffusion-mediated transport of isotopes, which was not fully achieved here in spite of the use of a buffer layer of unlabelled soil at the proximal end of each labelling compartment. However, diffusion of ^{33}P from the RHC was probably similar to the diffusion of ^{32}P from the HC. It is also desirable to monitor the soil moisture in the labelling compartments, as the water uptake will be higher from the RHC than the HC. In the present work, moisture levels were maintained by frequent watering, and we checked regularly that the soil at the distal end of the RHC remained moist. The absence of roots in the HC results in a low activity of the general soil microflora in the HC compared to the RHC (Olsson, Jakobsen & Söderström, unpublished results). The importance of this for the relative availability of the radioisotope is unknown as yet. Finally it is important to know whether roots and/or hyphae descriminate between ^{31}P, ^{32}P and ^{33}P. This would involve the use of a non-mycorrhizal control with two root compartments and a mycorrhizal plant with two hyphal compartments.

This work strongly indicates that the P uptake by root cells is influenced by the presence of mycorrhizal fungi and that this effect varies between fungal species. An isolate of *G. caledonium* appears to suppress completely the direct uptake of P by roots of cucumber. The consequences of such marked changes in function of root systems should be further studied in terms of plant productivity. Measurements should be performed over a complete growth cycle and with different host plants and soils.

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