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Contribution by two arbuscular mycorrhizal fungi to P uptake by cucumber (*Cucumis sativus* L.) from ^{32}P-labelled organic matter during mineralization in soil

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Abstract

An experiment was set up to investigate the role of arbuscular mycorrhiza (AM) in utilization of P from organic matter during mineralization in soil. Cucumber (*Cucumis sativus* L.) inoculated with one of two AM fungi or left uninoculated were grown for 30 days in cross-shaped PVC pots. One of two horizontal compartments contained 100 g soil (quartz sand : clay loam, 1:1) with 0.5 g ground clover leaves labelled with ^{32}P. The labelled soil received microbial inoculum without AM fungi to ensure mineralization of the added organic matter. The labelling compartment was separated from a central root compartment by either 37 μm or 700 μm nylon mesh giving only hyphae or both roots and hyphae, respectively, access to the labelled soil. The recovery of ^{32}P from the hyphal compartment was 5.5 and 8.6 % for plants colonized with *Glomus* sp. and *G. caledonium*, respectively, but only 0.6 % for the non-mycorrhizal controls. Interfungal differences were not related to root colonization or hyphal length densities, which were lowest for *G. caledonium*. Both fungi depleted the labelled soil of $NaHCO_3$-extractable P and ^{32}P compared to controls. A 15–25 % recovery of ^{32}P by roots was not enhanced in the presence of mycorrhizas, probably due to high root densities in the labelled soil. The experiment confirms that AM fungi differ in P uptake characteristics, and that mycorrhizal hyphae can intercept some P immobilization by other microorganisms and P-sorbing clay minerals.

Introduction

Most studies on P uptake by plants colonized by arbuscular mycorrhizal (AM) fungi have considered uptake from inorganic P (P_i) sources, as direct uptake of organic P (P_o) by plant roots (Dalal, 1977) and presumably by AM fungal hyphae is negligible. However, a substantial part of the P taken up by field grown plants originates from P_o after mineralization (Hedley et al., 1982; McLaughlin et al., 1988a). Fresh plant residues is a type of organic matter that contains both P_i and P_o (Bromfield and Jones, 1972). Upon contact with soil P_i may be leached or converted to P_o through microbial immobilization. Microbial P together with P_o may later be released by mineralization. In this context the term "release" implies both leaching of P_i originally contained in organic matter as well as mineralization of P_o. Phosphorus released from organic matter is subject to uptake by plant roots and microorganisms as well as fixation by various organic or inorganic soil constituents. The ability of roots to utilize P released from organic matter is crucial under P deficient conditions. Nutrient uptake from mineralized organic matter through AM hyphae has been demonstrated for N (Ames et al., 1983) but not for P. Roots may accelerate mineralization of P_o (Blair and Boland, 1978), but the possible role of mycorrhiza is unknown. AM fungi could alter the ability of the host plant to utilize released P either negatively by reducing the bacterial population in the rhizosphere (Christensen and Jakobsen, 1993) and thus mineralization of organic matter, or positively by exploiting a larger volume of soil where

P is released by leaching or mineralization. AM fungi may also differ in their possible modification of host plant utilization of this P. The objective of this study was to measure the uptake of P from organic matter by hyphae and mycorrhizal roots of cucumber in association with two AM fungi.

Materials and methods

Experimental design

Plants were grown with two different AM fungi or left uninoculated and labelled organic matter was applied to either roots or hyphae. Each treatment had four replicates.

Production of ^{32}P-labelled organic matter

Subterranean clover (*Trifolium subterraneum* L., cv. Mount Barker) was grown in eight pots with 1500 g of a partially sterilized (10 kGy, 10 MeV electron beam) 1 : 1 mixture w/w of a P deficient sandy loam (Jakobsen and Nielsen, 1983) and quartz sand. Nutrients except P were added at optimum level for plant growth. Phosphorus was added at a level corresponding to 50% of maximum growth to ensure rapid and exhaustive uptake of subsequently added ^{32}P. The pots had a 12-cm^2 opening in the bottom fitted with 700 μm nylon mesh allowing roots to grow into a water-filled container below the pot (modified after Janssen, 1990). Deionized water was added to soil and water containers to maintain 60% of water holding capacity (WHC) of the soil and a water surface 0.5 cm below the nylon mesh. The water containers of each pot received 4.6 MBq ^{32}P as carrier-free H_3PO_4 after 36 days. Plants were allowed to absorb ^{32}P for 8 days, whereafter shoots were harvested, dried for 24 hours at 80 °C and ground (<0.5 mm). The resulting labelled organic matter had a P content of 1.93 mg P g^{-1} dry weight, and an activity of 163 kBq g^{-1} dry matter at the time when it was added to the labelling compartments.

Pots

Cross-shaped pots (Fig. 1) made by PVC tubes (45 mm inner diameter) and fittings were divided into three compartments: A 320 mm vertical root compartment (RC) was divided by 37 μm nylon mesh from a 70-mm horizontal hyphal compartment (HC) and by 700 μm nylon mesh from a 70-mm horizontal hyphae-root compartment (HRC). Thus, all pots contained a similar soil volume for root growth plus a hyphal compartment, irrespective of where labelled organic matter was placed.

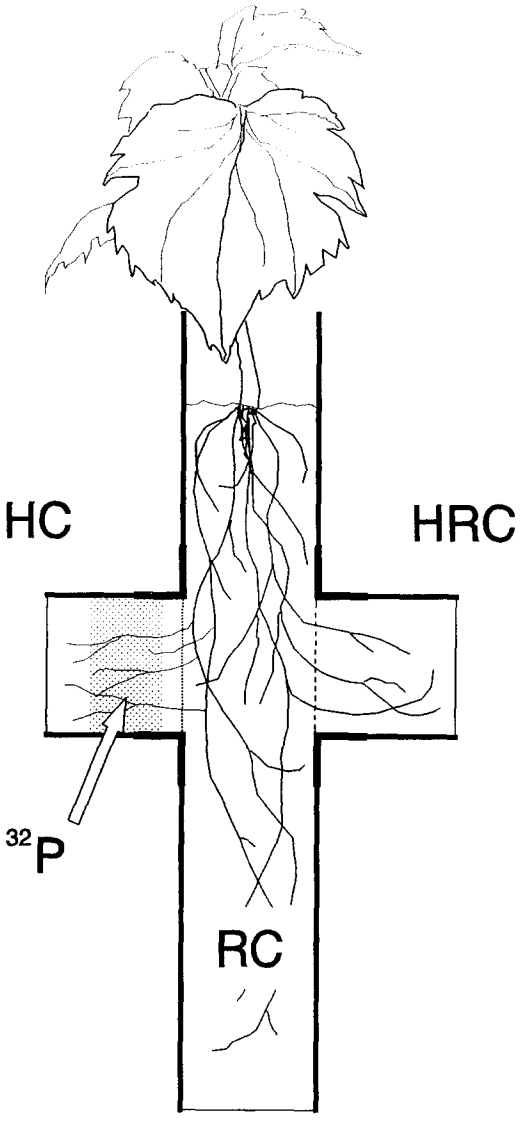

Fig. 1. Cross-shaped pots divided into three compartments: A central root compartment (RC), a hyphal compartment (HC) and a hyphae-root compartment (HRC). Organic matter labelled with ^{32}P was placed in either HC (as shown here) or in HRC.

Soil and fungal inoculum

The soil-sand mixture used was similar to that used for the production of ^{32}P- labelled organic matter. The RC of non-mycorrhizal controls received 88 mg KH_2PO_4 kg^{-1} dry soil while RC of mycorrhizal plants and all

HC and HRC received 53 mg KH_2PO_4 kg^{-1}. Basal nutrients were mixed into the soil in the following amounts (mg kg^{-1} dry soil): K_2SO_4: 72; $CaCl_2$ $2H_2O$: 72; $MgSO_4$ $7H_2O$: 20; $MnSO_4$ H_2O: 10; $ZnSO_4$ $7H_2O$: 5; $CuSO_4$ SH_2O: 2; Na_2MoO_4 $2H_2O$: 0.2; $CoSO_4$ $7H_2O$: 0.35. The dry weights of soil in RC, HC and HRC were 680 g, 170 g and 170 g, respectively. Soil in RC of the mycorrhizal treatments included 100 g mycorrhizal inoculum placed adjacent to the side compartments. Mycorrhizal inoculum consisted of the described soil from pot cultures with colonized roots of *T. subterraneum* L. and spores of either *Glomus caledonium* (Nicol. and Gerd.) Trappe and Gerdemann (isolate RIS 42) or *Glomus* sp. (WUM 10, obtained form L K Abbott, Univ. of Western Australia). Controls received 5 mL filtrate (Whatman no. 40 filter) of 10 g mixed inoculum in 250 mL water and 100 g irradiated soil. Pots with soil and inoculum were watered to 60% of WHC and incubated for 8 days for mycorrhizal spores to germinate.

Plants and growth conditions

Two pregerminated seeds of cucumber (*Cucumis sativus* L.) cv. Aminex (Fl hybrid) were sown in each pot and thinned to one per pot after seedling emergence. The plants were placed in a growth chamber with a 16/8 hours light/dark cycle (500 μmol m^{-2} s^{-1}, Osram HQI-T 250 W/D) at 20/15 °C and watered daily by weight. Nitrogen (NH_4NO_3) was added weekly to a total amount of 135 mg N per pot.

Placement of labelled organic matter

Ten days after seedling emergence one HC or HRC in each pot was filled with soil containing 81.5 kBq ^{32}P in organic matter. The labelled organic matter was homogeneously mixed into a 42-mm layer (0.5 g labelled organic matter in 100 g dry soil) separated from the RC-HC/HRC interface mesh by a 5-mm layer of unamended soil. The side compartments were then topped with unamended soil and sealed. Disks of coarse mesh (700 μm) were placed between the soil layers to facilitate separation during harvesting. The labelled soil received 2 mL 100 g^{-1} of a fresh filtrate (Whatman GF/A filters) of 10 g non-sterile soil in 250 mL water as an inoculum of indigenous microorganisms except AM fungi. For each pot the side compartment opposite to the labelled compartment was filled with soil without labelled organic matter.

Time course of ^{32}P release

An incubation treatment was included in order to monitor the change in availability of ^{32}P with time. Twenty-one pots without plants were prepared with 100 g of the same soil-sand mixture with labelled organic matter as used in HC/HRC and placed in the growth chamber. Nine pots containing 190 g labelled soil as a horizontal layer confined by coarse mesh (700μm) between 2 x 450 g unamended soil were sown with cucumber. All pots were watered to 60 % of WHC and placed in the growth chamber described above. Radioactivity was measured in 0.5 *M* $NaHCO_3$-extracts of soil samples from pots without plants after 2 hours, 2, 4, 7, 11, 18 and 30 days, and from pots with plants after 11, 18 and 30 days. The content of ^{32}P in three replicate plants was also measured at each of the last three harvests.

Analysis

Plants were harvested 40 days after seedling emergence. Dried roots and tops were ground and samples were taken for wet digestion in nitric acid : perchloric acid (4 : 1) (Johnson and Ulrich, 1959). The digests were analyzed for total P in an autoanalyzer system using ammonium molybdate/ascorbic acid (Murphy and Riley, 1962). The activity of ^{32}P was measured by Cherenkov counting using a Packard 1900 TR liquid scintillation analyzer, and counts were corrected for counting efficiency and isotopic decay. Phosphorus and ^{32}P in soil were measured similarly in 0.5 *M* $NaHCO_3$ extracts (Olsen et al., 1954). Total and mycorrhizal root lengths were measured using a line intersect technique (Tennant, 1975) after staining with trypan blue in lactoglycerol (Kormanik and McGraw, 1982). Length of mycorrhizal hyphae was measured as the difference between mycorrhizal and non-mycorrhizal samples using a membrane filter/grid-intersect method, modified after Abbott et al. (1984). Modifications included the use of 2 mL aliquots and 20 mm diameter filter area. Duplicate filters were made from two samples of each treatment, and hyphae were counted in 25 fields of vision at 200 × magnification.

Treatment effects were tested by analysis of variance and means were compared by the T'-method (Spjøtvoll and Stoline, 1973) for unplanned comparisons between samples of unequal size (one control replicate was excluded due to extremely poor plant growth, giving n=3 for controls labelled in HRC).

Table 1. Mycorrhizal colonization in root compartments (RC), total and colonized root length in hyphae-root compartments (HRC), and hyphal length in hyphal compartments (HC). Different letters in same column indicate significant ($p < 0.05$) differences between means

	Colonization in RC	Root length in HRC		Hyphal length in HC
	(%)	Total (cm cm^{-3})	Colonized (cm cm^{-3})	(m cm^{-3})
Control	0 b	28.0 a	0 a	0 b
Glomus sp.	62 a	20.8 a	6.3 b	34.6 a
G. caledonium	47 a	29.9 a	9.0 b	20.8 a

Table 2. Dry weight (DW), P conentration and ^{32}P activity of plants. Different letters in same column indicate significant ($p < 0.05$) differences between means

Inoculation	^{32}P placement	Plant DW	Plant P concentration	^{32}P in dry matter		
		(g)	(mg P g^{-1} DW)	Bq g^{-1} DW	Bq plant^{-1}	Bq cm^{-1} hyphae or root
Control	HRC (n=3)	9.07 ab	1.55 a	2297 a	20704 a	11.3
	HC	9.01 ab	1.54 a	58 c	525 d	-
Glomus sp.	HRC	6.89 c	1.36 b	1764 a	12290 b	-
	HC	8.46 ab	1.39 b	531 b	4468 c	1.9 a
G. caledonium	HRC	7.75 bc	1.35 b	2097 a	16225ab	-
	HC	9.17 a	1.20 c	764 b	6985 c	5.0 b

Results

Inoculation with AM fungi resulted in an average colonization of 62 and 47 % of the root length for *Glomus* sp. and *G. caledonium*, respectively, measured in RC adjacent to the labelling compartments (Table 1), but the colonization levels of both fungi decreased markedly with increasing distance from the site of inoculum placement (data not shown). Accordingly, roots in HRC were less colonized than those in the RC-inoculum zone (Table 1). Uninoculated controls remained uncolonized, and no roots were found in hyphal compartments. Plant dry weight was lower in mycorrhizal HRC-treatments compared to their respective HC-treatments, and in one mycorrhizal treatment compared to controls. Further, plants in all mycorrhizal treatments had lower P concentrations (Table 2).

Plants with roots accessing the labelled organic matter utilized 15–25 % of the 81.5 kBq ^{32}P initially supplied (Table 2). Non-mycorrhizal plants had a higher ^{32}P uptake than plants colonized with *Glomus* sp., whereas plants colonized by *G. caledonium* had an intermediate uptake not significantly different from the two other treatments (Table 2).

The plant uptake of ^{32}P from HC of the two mycorrhizal treatments was about 10 times higher than from HC of the non-mycorrhizal treatment (Table 2). The uptake mediated by hyphae comprised 5–9 % of the added ^{32}P corresponding to 36–43 % of the amount transported by mycorrhizal roots with direct access to the labelled soil. The two fungi did not differ significantly in hyphal length density or total amount of ^{32}P translocated to plants (Table 1), but plants inoculated with *G. caledonium* had a higher uptake of ^{32}P per unit length of hyphae compared to *Glomus* sp. (Table 2).

Table 3. Phosphorus extracted from labelled soil in HC with 0.5 *M* $NaHCO_3$ (pH 8.5) and net release of ^{32}P. Different letters in same column indicate significant $p < 0.05$) differences between means

	P and ^{32}P in $NaHCO_3$-extracts			Net release
	mg P kg^{-1}	Bq g^{-1} dry soil	Bq mg^{-1} P	(%)
Control	16.2 a	94.6 a	5851 b	12.3 b
Glomus sp.	13.3 b	87.8 ab	6606 a	16.3 a
G. caledonium	12.9 b	78.2 b	6233 ab	18.2 a

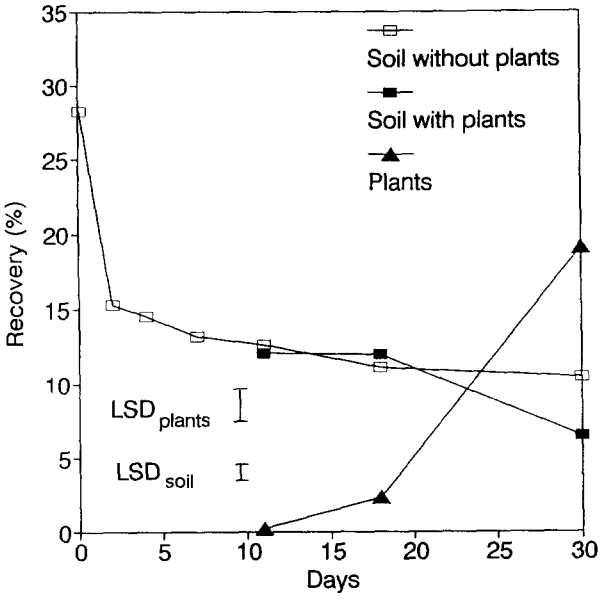

Fig. 2. Time course of ^{32}P extractable from soil (0.5 *M* $NaHCO_3$) and P content in plants expressed as percent recovery of initially added ^{32}P in organic matter. Bars indicate $LSD_{0.05}$-values (n=3).

The time course of ^{32}P release from organic matter is shown in Figure 2. During the first 2 days extractable ^{32}P decreased from 28.3% to 15.3% of the initially added ^{32}P, whereafter ^{32}P availability decreased at a slower rate. After 30 days extractable ^{32}P had been reduced to 10.4 % in soil without plants compared to 6.4% in soil with plants. At this time the plants had assimilated 19.1 % of added ^{32}P. Extraction of ^{32}P from labelled soil in HC showed that hyphae of *Glomus* sp. and *G. caledonium* had depleted 7 and 17 %, respectively, of the ^{32}P available in extracts from the control treatment (Table 3). Corresponding depletions of Pi in the same extracts were 18 and 20 %. The specific activity in these soil extracts was significantly higher for *Glomus* sp., but not for *G. caledonium*, compared to control soil (Table 3).

Release of ^{32}P from organic matter was calculated for HC (adopted from Blair and Boland, 1978):

$$\text{Net release}(\%) = 100 \cdot \frac{^{32}\text{P in plants} + \text{available } ^{32}\text{P in soil}}{\text{added } ^{32}\text{P}} \quad (1)$$

The net release was 33 and 48 % higher in the presence of hyphae of *Glomus* sp. and *G. caledonium*, respectively, than when no hyphae were present in the HC (Table 3).

Discussion

The high proportion of ^{32}P recovered in plants showed that P in the added organic matter was available for plant uptake and that the relatively high C/P ratio (*c.* 230) did not prevent a substantial net release of P upon contact with unsterile soil.

Mycorrhizal plants had lower P concentration and in one case lower dry weight compared to controls which were supplied with extra P in RC to compensate for an expected increase in P uptake by mycorrhiza.

The absence of a mycorrhizal enhancement in uptake of ^{32}P from HRC may be explained by the high root length densities measured. Phosphorus depletion zones around single roots extend 0.5–2.6 mm from the root surface (Hendriks et al., 1981; O'Keefe and Sylvia, 1992) due to uptake by root hairs and exudation of mobilizing agents. A measured average root diameter of 0.2 mm and a depletion radius between 0.93 and 1.13 mm would leave the whole soil volume in HRC efficiently exploited by roots at the present densities. The lower uptake of ^{32}P by mycorrhizal plants compared to non-mycorrhizal plants could also have resulted from lower rates of mineralization, as mycorrhizas may reduce the microbial biomass and activity in rhizosphere soil (Christensen and Jakobsen, 1993).

A maximum of 8.6 % of the ^{32}P added to HC as dried clover shoots was recovered in plants via hyphal

transport. Hyphal length densities did not differ significantly between the two fungal species, but the uptake efficiency of ^{32}P per cm hyphae for *G. caledonium* was twice that found for *Glomus* sp.. Linear regression analysis revealed a positive relationship between hyphal length density and transport of ^{32}P when plants inoculated with the same fungus were compared (r^2 = 0.87, $0.10>p>0.05$ for *Glomus* sp.; $r^2 = 0.45$, $p>0.10$ for *G. caledonium*). The viability of the hyphae was not measured, but similar soil conditions and hyphal age should minimize differences in viability and allow for a comparison between the two fungi. The difference in uptake was consistent with the results of Pearson and Jakobsen (1993).

Levels of available P_i in HC measured by 0.5 *M* $NaHCO_3$-extraction were lower in mycorrhizal treatments compared to controls, in spite of similar initial P content. A similar significant depletion of ^{32}P was caused only by *G. caledonium*. This together with an increased specific activity in soil extracts from the *Glomus* sp. treatment compared to controls indicates that the two fungi had used P from different sources.

The net release of ^{32}P in labelled soil exploited by hyphae exceeded that of controls by 33–48 %. This increased hyphal utilization of P added as organic matter is equivalent and quantitatively comparable to root-induced mineralization of P_o as found by Blair and Boland (1978) and Till and Blair (1978). An increased net release of ^{32}P mainly seems to reflect that hyphae intercepted P immobilization by other microorganisms and fixation on clay minerals at the time and place when P was released from organic matter, and that this released P was rendered less available at a later stage. This is supported by the measured decrease in extractable ^{32}P with time and consistent with the results of White and Ayoub (1983) and McLaughlin et al. (1988b). Further, mycorrhizal mobilization mechanisms could enhance utilization of $^{32}P_o$ or $^{32}P_i$ from organic matter directly by decreasing pH (Li et al., 1991), by exudating phosphatases (Dodd et al., 1987) or by increasing the uptake of simple P_o -compounds (Jayachandran et al., 1992). Only the former of these mechanisms is demonstrated for AM hyphae in the absence of roots.

The uptake of ^{32}P was only 2–6 times as high for roots as for hyphae when expressed on the basis of unit length (Table 2). These differences are small considering the large difference between surface areas per unit length of hyphae and roots as well as the highly efficient P-mobilizing mechanisms characteristic to roots (Bagshaw et al., 1972; Heial and Sauerbeck, 1984; Moghimi et al., 1978; Tarafdar and Jungk, 1987). These small differences could have two explanations: Firstly, all labile ^{32}P might have been depleted by roots in the labelled soil, whereby the mineralization of residual organic matter would become the rate limiting step for ^{32}P uptake by roots but not by hyphae. In this context the added ^{32}P could be regarded as two separate P pools: labile P (both P_i and P_o) leached from organic matter and adsorbed onto soil particles, and P held in structural fractions of organic matter liable to a slower degradation and release. A high proportion of P in clover leaves is usually found as inorganic P (Bielski, 1968; Bromfield and Jones, 1972), but much of this P_i is converted to P_o (Bromfield and Jones, 1972) and microbial P (Chauhan et al., 1981) after a short period of contact with unsterile soil. Secondly, root P depletion zones in the labelled soil were probably overlapping making the root system inefficient (Baldwin et al., 1972).

Root densities of most field-grown crops are markedly lower than those measured in the present experiment, and as much as 75 % of the soil may be unexploited by roots including root hairs (Jungk, 1984). Under such conditions mycorrhizal roots are likely to increase P uptake rates also from mineralized P_o due to the widely accepted mechanism of hyphae spreading into unexploited soil volumes (Sanders and Tinker, 1971).

The present work demonstrates that hyphae of two arbuscular fungi can transport significant amounts of P released from organic matter to their host plants. A maximum of 8.6 % of added ^{32}P in dried clover shoots was recovered in plants after 30 days by hyphal transport alone, while 25 % was taken up by uncolonized roots. The implications of these findings for field conditions where hyphal and root length densities are frequently lower are yet to be verified. Nonetheless, our results suggest that mycorrhizal hyphae can intercept biological and chemical immobilization during release of P from organic matter at sites where roots are not present. This would contribute to a closer recycling of P in organic matter and a smaller loss of P to non-labile fractions.

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