New Phytol. (1999), **144**, 173–181

Characterization of two arbuscular mycorrhizal fungi in symbiosis with *Allium porrum*: inflow and flux of phosphate across the symbiotic interface

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Received 16 November 1998; accepted 1 June 1999

SUMMARY

Individual arbuscular mycorrhizal fungi can differ markedly in their ability to improve the phosphate nutrition and growth of host plants. In particular, *Scutellospora calospora* is relatively ineffective with some hosts and a growth depression is often observed. We have examined the abilities of *S. calospora* and *Glomus* sp. 'City Beach' strain WUM 16, grown in soils which promote extensive mycorrhizal colonization, to transfer phosphate (P) to *Allium porrum*. Phosphate uptake from the low-P soils (P_0) was compared with uptake from soils amended with extra P (P_1). In order to relate P transfer to physiological characteristics of the two fungi, inflow of P via fungus to the plant was combined with the surface areas of intercellular hyphae and arbuscules (symbiotic interfaces) to calculate the amount of P transferred per unit area of interface (P fluxes). 'Hyphal inflows' and 'hyphal fluxes' were also calculated on the assumption that P uptake by the plant was the same in mycorrhizal and equivalent non-mycorrhizal (NM) plants (the validity of this assumption is discussed). With both soil P treatments, P was taken up by plants colonized by both mycorrhizal fungi to a greater extent than by the equivalent NM controls. Hyphal inflows to plants in P_0 soil that were colonized by *Glomus* sp. 'City Beach' were greatest from 14–21 d and decreased at later harvests. With P_1 soil the inflow via *Glomus* sp. 'City Beach' peaked at a very high value at 21–28 d. Hyphal inflows into plants in P_0 soil that were colonized by *S. calospora* increased throughout the experiment, and with P_1 soil they remained steady at high values. With both fungi, the surface areas of the symbiotic interfaces increased greatly after 14 d, and generally there was little effect of higher soil P. With P_0 soil, *Glomus* sp. 'City Beach' showed no significant differences in the ratio of surface areas of the two interfaces over the course of the experiment. *Scutellospora calospora* tended to produce a lower percentage of interfacial area contributed by arbuscules. With the mycorrhizal plants growing in their respective soils, there appeared no consistent differences between the two fungi with respect to fluxes of P across the interfaces. With P_0 soil, fluxes via *Glomus* sp. 'City Beach' were initially higher than those via *S. calospora*, but later they were higher with *S. calospora*. With P_1 soil the only difference was the 28–42-d period, when *Glomus* sp. 'City Beach' produced the higher flux (reflecting the low surface area at the time). The results show that relative inefficiency of *S. calospora* in its ability to transfer P, as reported by others, may result from different environmental conditions, use of different hosts, or even of different fungal isolates (strains).

Key words: AM fungi, *Scutellospora calospora*, *Glomus* sp. 'City Beach', P uptake.

INTRODUCTION

The effectiveness of arbuscular mycorrhizal (AM) fungi in increasing nutrient uptake in plants relies on the ability of fungal hyphae to take up nutrients from the soil, translocate them to the plant, and transfer them through the fungus–plant symbiotic interfaces. Mycorrhizal fungi differ in their effectiveness of

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uptake and transfer of these nutrients, and hence in their ability to enhance plant growth (Sanders *et al.*, 1977; Abbott & Robson, 1985). A reduction in plant growth will occur if the mycorrhizal fungi were utilizing plant carbohydrates during early plant development and creating a carbon drain to the plant (Janos, 1987; Smith & Smith, 1996; Johnson *et al.*, 1997).

Isolates of *Scutellospora calospora* have been shown to produce smaller plant-growth responses than other fungi (Thomson *et al.*, 1986; Jakobsen *et al.*, 1992a). Jakobsen *et al.* (1992b) used ^{32}P tracer time-course experiments to show that hyphae of *S. calospora* grown in symbiosis with *Trifolium subterraneum* grew extensively in the soil and accumulated P effectively, but very little P was transported to the plant. They suggested that P transport was limited by processes involved in hyphal translocation and/or transfer across the symbiotic interface rather than hyphal P uptake itself. *Scutellospora calospora* accumulated large amounts of ^{32}P into its hyphae but transported little to the host plant *Cucumis sativus* (Pearson & Jakobsen, 1993). By contrast, we have shown large increases in P uptake and plant growth in *Allium porrum* colonized by *S. calospora* and *Glomus* sp. 'City Beach' when grown in soil suitable for mycorrhizal colonization (Dickson *et al.*, 1999). *Scutellospora calospora* colonized roots more rapidly than *Glomus* sp. 'City Beach', but apparently did not form more arbuscules based on determination of the percentage of root length in which one or more arbuscules occurred (see method of McGonigle *et al.*, 1990). However, there are problems in comparing fungi on the basis of percentage colonization or occurrence of arbuscules because the methods do not give any information on the density of hyphae or arbuscules within the root. If density and activity of fungal interfaces can be determined, then measurements of hyphal inflow can be interpreted more meaningfully. Determination of surface areas then allows P flux across the symbiotic interfaces to be used to compare different fungi and different experimental conditions.

'*Arum*-type' mycorrhizas such as those formed by *A. porrum* have two plant–fungal interfaces: intercellular hyphae and arbuscules (Gallaud, 1905). There is a general assumption that arbuscules are the site of P transfer to the plant. The entire arbuscule is surrounded by the plasmalemma of the host cell (Cox & Tinker, 1976). Because this plasmalemma is greatly invaginated, the surface area of contact between the plant and the arbuscule is large. Image analysis has been used by Smith & Dickson (1991) to calculate the area of living fungal structures in roots of *T. subterraneum* and *A. porrum* stained with the vital stain nitroblue tetrazolium (NBT). They found that as 'infection units' age, the contribution of arbuscules to the total colonization and area of interface declines. It was also seen that differences existed between the fungi in the density of arbuscules and hyphae, even when the percentage of root length colonized was similar. Two mycorrhizal fungi from the genus *Glomus* (*Glomus mosseae* and *Glomus* sp. 'City Beach'), grown in Mallala soil–sand mix with *A. porrum*, were compared, and *Glomus* sp. 'City Beach' was found to be more effective in P uptake and in increasing plant growth (Smith *et al.*, 1994). The differences were closely correlated not with percentage colonization in the roots or with the extent of arbuscular development, but with differences in rates of transfer of P per unit area (fluxes) across the symbiotic interface.

Factors limiting the size of fluxes include the rate of P translocation to the interface through the external hyphae and the efflux from the fungus to the apoplast (Smith *et al.*, 1994). Most previous work has compared P fluxes using different AM fungi and host plants (Cox & Tinker, 1976; Smith *et al.*, 1994), but few studies have included the effects of soil P supply. Estimates of fluxes of P into *Allium cepa*, based on shoot material only, and using two soil P levels, were calculated for a single harvest period of 3–6 wk (Sukarno, 1994; Sukarno *et al.*, 1996). Results showed that the flux via *Glomus* sp. 'City Beach' was not affected by P addition to the soil.

Fluxes are important in that; they help determine the effectiveness of transfer of P to the host, and the values obtained may give an understanding of mechanisms involved in the transfer process (Smith *et al.*, 1994). Reduction in size or number of arbuscules will result in a smaller surface area available for P transfer. Alternatively, reduced flux per unit arbuscular area will also reduce transfer, even if the surface area of the interface remained the same. Either or both factors could contribute to the apparently low transfer of P from *S. calospora* to the plants. A further complication would be introduced if intercellular hyphae, as well as arbuscules, are involved in nutrient transfer. These structures make a considerable contribution to the surface area of the metabolically active interface, particularly as plants age and arbuscules become less frequent (Smith & Dickson, 1991).

Phosphate addition affects arbuscule development, as well as the percentage of root length colonized (Bolan, 1991; Smith & Read, 1997). Small increases of soil P have been shown to reduce the size of arbuscules and the numbers of both arbuscules and vesicles (Mosse, 1973; Bruce *et al.*, 1994). In this case, therefore, a smaller surface area for transfer would result. However, as additional P is added to the soil, inflow of P into the plant would be expected to increase and so fluxes may become higher.

In our previous paper (Dickson *et al.*, 1999) we showed that mycorrhizal growth responses to *S. calospora* were delayed in low P soil compared with those to *Glomus* sp. 'City Beach', and did not occur

when P was added to the soil (at least up to 42 d). This reduction of growth response could not be attributed to a lower percentage of colonization (which was not greatly reduced by P) or to failure of P uptake. Although P uptake was lower into plants colonized by *S. calospora* than into those colonized by *Glomus* sp. 'City Beach', it was nevertheless considerably higher than into the non-mycorrhizal (NM) controls. Although the use of different soils precluded direct comparison between the two fungi, we were able to examine relative effects on plant growth and P nutrition with respect to the equivalent controls, and compare effects of added soil P on growth of mycorrhizal and NM plants within limits discussed later.

In this paper we have used data from the same experiment to study the inflow of P to the plant and the transfer of P across the symbiotic interface at two soil P levels in *Glomus* sp. 'City Beach' and *S. calospora*. We examined total P content of the plant, areas of symbiotic interface, inflows of P into the plant and flux values.

MATERIALS AND METHODS

Experimental design

This experiment is described in a previous paper (Dickson *et al.*, 1999) and methods are briefly outlined here. Seeds of *Allium porrum* L. cv. Musselburgh were grown in black plastic tube pots containing 1 kg sterilized soil mix. Two soils were used to take account of the growth preferences of the two fungi, so that both fungi were under conditions in which they have been previously shown to colonize well. *Glomus* sp. 'City Beach' (WUM 16) (CB) was inoculated into 10% Mallala soil mixed with 90% sand to give 0.83 mg kg^{-1} extractable P (Colwell, 1963) and pH 7.4 (1:5 soil:0.01 M $CaCl_2$). *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders (Sc) was inoculated into a 10% Kuitpo soil mix (2.29 mg kg^{-1} P, pH 5.3). Mycorrhizal and corresponding NM control plants were grown in the same soil mix. Equations were calculated to enable us to examine similarities and differences between the plants in response to mycorrhizal colonization and the addition of P to the soil in terms of plant growth and P content (Dickson *et al.*, 1999).

Mycorrhizal pots were inoculated by mixing in 15% (w/w) of dry pot culture material of *Trifolium subterraneum* L. cv. Mt Barker. These cultures consisted of colonized root fragments with soil containing spores and external hyphae of the appropriate fungus. Non-mycorrhizal pots contained the same weight of material from an uninoculated pot culture. Two levels of phosphate (P) were used; no additional P (P_0) and 12.4 mg P kg^{-1} (P_1), added as NaH_2PO_4 and mixed through the soil before potting.

Two to three seeds were planted in each of five holes, and plants were grown in a growth room with a 14:10 h day/night cycle and temperatures of 23–18°C, respectively. Photon flux density was 500 μmol m^{-2} s^{-1}. Pots were watered to 12.5% w/w with deionized water three times a week and randomly rearranged. Nutrient solution minus P was applied 2 wk after planting and weekly thereafter (Smith & Smith, 1981). Plants were harvested at 14, 21, 28 and 42 d giving three inter-harvest periods for calculations of inflows and fluxes.

Plant material was separated into roots and shoots, and fresh weights were recorded. Root subsamples were taken either for dry weight and determination of P concentration, or for measurement of percentage colonization (Dickson *et al.*, 1999). Subsamples of known weight were used to determine total root lengths by the grid intersect method (Tennant, 1975). The areas of active symbiotic interface were determined as follows. Root samples were embedded and freeze-sectioned following a modified method of Smith & Dickson (1991). Sections were stained overnight in a solution containing NBT and sodium succinate, allowing maximum formazan production by succinate dehydrogenase activity to occur. All sections were scored for the presence of metabolically active fungal structures (arbuscules and intercellular hyphae) and a subsample used to determine numbers and perimeters of these structures using image analysis.

Calculation of area of symbiotic interface

The numbers and perimeters of fungal structures in sections (intercellular hyphae, arbuscules and vesicles) were measured using the Videopro 32 Colour Image Analysis system (Leading Edge Pty Ltd, Adelaide, S. Australia). The program used enabled the automatic discrimination of fungal structures after staining with NBT. Populations of 76 sections (both colonized and non-colonized) were measured, and the means calculated.

Mean perimeters of uninvaginated arbuscules were converted into estimates of invaginated structures using a factor of 2.07 as calculated by Cox & Tinker (1976). The surface area of arbuscules in a given length of root was calculated by multiplying the invaginated area by $4/\pi$ (Underwood, 1969; Cox & Tinker, 1976; see Smith *et al.*, 1994). This value was then multiplied by a correction factor of 1.2 to allow for variation in size between immature and mature arbuscules found within the population measured (Smith *et al.*, 1994).

The area of intercellular hyphal interface was calculated as described by Smith & Dickson (1991) and measured as one hypha per intercellular space. Measurements of hyphae of *Glomus* sp. 'City Beach' have been shown to be circular, therefore they can be considered cylindrical in length (S. Dickson & P.

Kolesik, unpublished). Results are expressed as area of interface per m root.

Calculation of P inflow, hyphal inflow and P flux across the symbiotic interface

Phosphate inflow (P uptake m^{-1} s^{-1}) was calculated by the method of Brewster & Tinker (1972) from total root length per plant and P content per plant over two consecutive harvest periods. Inflows were calculated for three inter-harvest periods: 14–21, 21–28 and 28–42 d. Inflows via the fungi (hyphal inflows) were calculated by subtracting P inflow in NM plants from that in mycorrhizal plants (Sanders & Tinker, 1973; Sanders *et al.*, 1977). This calculation assumes that inflow into non-colonized parts of mycorrhizal roots was unchanged: its validity is discussed later. In situations where inflows into NM plants were slightly negative and values for mycorrhizal plants were positive, the negative values were used in the calculation of the hyphal inflow. Means and standard errors of means of inflows were calculated using values for change in P content and root length between harvests determined by subtracting the individual values for the second harvest from the mean value at the first harvest.

Fluxes of P were calculated following the method of Smith *et al.* (1994) using the area of symbiotic interface per m root and hyphal or total inflows (disregarding the inflow subtraction of NM plants) to give maximum and minimum values. Fluxes across the arbuscular interface were calculated using the arbuscular surface areas obtained from image analysis and the percentage colonization of metabolically active arbuscules in root sections stained by NBT. Total fluxes (flux across arbuscular and hyphal interfaces) were calculated using the total fungal surface area and the percentage colonization with respect to both fungal structures. Calculations of means and standard errors of means of fluxes were determined using measurements of surface area, percentage colonization and inflow per replicate. In each of the first two harvests, three replicate pots were used to measure inflow and three replicates for mycorrhizal colonization and determination of area of interface. The means of the inflow values were used in the calculation of flux. Three individual replicates (with all data obtained from the same pot) were used for the later harvests.

Statistical analysis

Data were analysed using the programme GENSTAT 5 (Genstat 5 Committee, 1987). Multiple linear comparisons between means were made using Tukey's honestly significant difference statistic (Zar, 1984). Where no interactions were present, one-way ANOVAs were used to determine significance. In all studies $P < 0.05$ was taken to be significant.

RESULTS

P content

Total P contents of mycorrhizal and NM plants grown in the mixture based on Mallala soil are shown in Table 1a. Total P content in NM plants did not change significantly over harvest times. Plants colonized by CB had significantly greater P content than did equivalent NM plants by 21 d, and by 42 d had 348% more P than NM plants. All plants grown in Mallala soil showed increased P content in P_1 soil. NM plants grown in P_1 soil had significantly greater P content than P_0 plants at all harvest times. Mycorrhizal P_1 plants had significantly higher P content than mycorrhizal P_0 plants by 21 d. Increases in P content by 42 d were not, however, as great as in P_0 soil, with values increasing by 189%.

The total P content of NM plants in the mixture based on Kuitpo soil (P_0) also did not change significantly over time (Table 1b). Phosphate content in plants colonized by Sc was significantly higher than NM plants by 28 d, and by 42 d they contained 423% more P than their equivalent NM controls. All plants showed increased P content in P_1 soil, with NM plants having significantly greater P content than P_0 plants at all harvest times. By 21 d mycorrhizal P_1 plants had significantly higher P content than P_0 mycorrhizal plants, and by 42 d had increased by 235%. Non-mycorrhizal plants grown in P_0 Mallala and Kuitpo soils had very similar P content, as did the mycorrhizal plants (except at 28 d). Non-mycorrhizal plants in P_1 Mallala soil had higher P content after 21 d than those in Kuitpo soil, but there was no difference in mycorrhizal plants except at 42 d.

Inflows

Inflows of P into NM and mycorrhizal plants and calculated hyphal inflows are shown in Table 2.

Plant inflows. In soil with no added P, inflow of P into NM plants was significantly lower than that into plants colonized by CB for all inter-harvest periods (Table 2a). Inflow into these plants and their NM controls decreased in the 28–42 d period. Inflows of P were greater in all plants when grown in soil with added P. Inflow into NM plants declined after 14–21 d, whereas inflow into mycorrhizal plants declined after 21–28 d.

Non-mycorrhizal plants in P_0 soil grown as controls for Sc showed a high inflow from 14–21 d followed by no inflow of P in the later harvest periods (Table 2b). Inflow into plants colonized by Sc was also high for the 14–21 d period and increased through the 28–42 d period. In P_1 soil, inflows into NM plants were significantly higher than in P_0 soil at

Table 1. *Total phosphate content* (*in roots and shoots*) *of non-mycorrhizal* (*NM*) *controls of* Allium porrum *and plants colonized by* (*a*) Glomus *sp.* '*City Beach*' *or* (*b*) Scutellospora calospora *in 2 soil treatments* (P_0 *and* P_1)

	Soil treatment P_0		P_1	
	Total P content (μg per plant)			
Days	NM	Colonized	NM	Colonized
(a) *Glomus* sp. 'City Beach'				
14	17.2 ± 1.2^{b}	18.5 ± 1.1	20.6 ± 0.8^{b}	20.8 ± 0.6
21	$17.6 \pm 1.1^{a,b}$	$24.2 \pm 1.4^{a,b}$	35.2 ± 2.8^{b}	36.2 ± 5.0^{b}
28	$19.6 \pm 0.5^{a,b}$	$38.5 \pm 2.8^{a,b}$	62.1 ± 6.0^{b}	93.6 ± 20.8^{b}
42	$18.9 \pm 0.7^{a,b}$	$65.6 \pm 6.0^{a,b}$	$139.7 \pm 22.9^{a,b}$	$263.4 \pm 15.4^{a,b}$
(b) *Scutellospora calospora*				
14	16.6 ± 1.0^{b}	15.3 ± 0.4	21.2 ± 1.3^{b}	19.4 ± 2.1
21	20.2 ± 1.2^{b}	20.8 ± 2.7^{b}	$26.6 \pm 1.4^{a,b}$	$36.3 \pm 1.9^{a,b}$
28	$17.8 \pm 1.3^{a,b}$	$27.2 \pm 2.8^{a,b}$	$45.1 \pm 5.5^{a,b}$	$82.2 \pm 9.7^{a,b}$
42	$17.2 \pm 2.9^{a,b}$	$73.0 \pm 9.8^{a,b}$	$94.7 \pm 15.0^{a,b}$	$222.5 \pm 3.5^{a,b}$

'City Beach' plant and corresponding NM controls were grown in Mallala soil-based mix. S. calospora plants and corresponding NM controls were grown in Kuitpo soil-based mix P_0, no added phosphate; P_1, added phosphate (12.4 mg P kg^{-1}). Values are the means $\pm$ SE, $n = 3$. Significant difference by Tukey's test ($P < 0.05$) between [a]NM and mycorrhizal plants, [b]P_0 and P_1 levels of soil P at the same harvest. Two-way ANOVAs were carried out for significant interactions. Where there was no interaction, one-way ANOVAs were used to determine significance.

Table 2. *Inflow of phosphate to* Allium porrum *with non-mycorrhizal* (*NM*) *and mycorrhizal plants colonized by* (*a*) Glomus *sp.* '*City Beach*' *or* (*b*) Scutellospora calospora *in 2 soil treatments* (P_0 *and* P_1)

	Soil treatment P_0			P_1		
	Inflow of P (mol P m^{-1} (root) $s^{-1} \times 10^{-13}$)					
Days	NM	Colonized	HI	NM	Colonized	HI
(a) *Glomus* sp. 'City Beach'						
14–21	$2.0 \pm 5.1^{a,b}$	28.8 ± 7.1^{a}	26.8 ± 7.1	61.5 ± 11.6^{b}	79.8 ± 23.3	18.3 ± 23.3
21–28	$4.3 \pm 0.8^{a,b}$	$28.3 \pm 5.0^{a,b}$	23.9 ± 5.0	$33.7 \pm 2.5^{a,b}$	$119.1 \pm 33.2^{a,b}$	85.4 ± 33.2
28–42	$-0.5 \pm 0.5^{a,b}$	$11.4 \pm 3.1^{a,b}$	11.9 ± 3.1^{c}	$22.4 \pm 4.2^{a,b}$	$53.9 \pm 3.0^{a,b}$	31.6 ± 3.0^{c}
(b) *Scutellospora calospora*						
14–21	14.6 ± 3.3	22.5 ± 10.8^{b}	7.9 ± 10.8	8.2 ± 12.3^{a}	$74.2 \pm 8.4^{a,b}$	66.1 ± 8.4
21–28	$-5.9 \pm 3.4^{a,b}$	$19.5 \pm 6.9^{a,b}$	25.5 ± 6.9	$24.5 \pm 6.2^{a,b}$	$98.2 \pm 17.4^{a,b}$	73.7 ± 17.4
28–42	$-0.7 \pm 1.8^{a,b}$	$34.0 \pm 2.8^{a,b}$	34.7 ± 2.8^{c}	$11.7 \pm 3.1^{a,b}$	$66.6 \pm 3.7^{a,b}$	54.9 ± 3.7^{c}

HI is the calculated inflow via fungal hyphae obtained by subtraction.
Soil treatment as for Table 1. P_0, no added phosphate; P_1, added phosphate (12.4 mg P kg^{-1}). Values are the means $\pm$ SE, $n = 3$. One-way ANOVAs were used to determine significance ($P < 0.05$) between [a]mycorrhizal and NM inflows at the same soil P level, [b]P_0 and P_1 levels of soil P, and [c]HI of fungal treatments at the same soil P level at the same harvest.

the 21–28 and 28–42 d harvest periods. Inflows into plants colonized by Sc were relatively constant and substantially higher than equivalent NM controls for all harvest periods and for mycorrhizal plants in P_0 soil.

Hyphal inflows. In soil with no added P, hyphal inflows via CB declined at later harvest periods (Table 2a). They increased with P addition to the soil for the 21–28 and 28–42 d harvest periods. A large inflow occurred between 21 and 28 d, decreasing in the later period.

Hyphal inflows via Sc in P_0 soil increased over the course of the experiment (Table 2b). In P_1 soil, inflow via Sc appeared more constant for all harvest periods. Differences in hyphal inflows between the

Table 3. *Area of symbiotic interface stained by nitroblue tetrazolium* (*NBT*) *in* Allium porrum *plants colonized by* (*a*) Glomus *sp.* '*City Beach*' *or* (*b*) Scutellospora calospora *in 2 soil treatments* (P_0 *and* P_1)

	Soil treatment P_0			P_1		
	Area of symbiotic interface ($m^2\ m^{-1}$ root $\times 10^{-6}$)					
Days	Hyphae	Arbuscules	Total	Hyphae	Arbuscules	Total
(a) *Glomus* sp. 'City Beach'						
14	1.6 ± 1.9	6.6 ± 8.0	8.1 ± 10.0	3.5 ± 3.8	5.9 ± 7.2	9.4 ± 11.0
21	207.8 ± 134.1	852.5 ± 473.6	1060.3 ± 607.6	43.2 ± 35.3	190.1 ± 165.5	233.3 ± 200.8
28	420.7 ± 117.5[a]	1824.7 ± 651.2[a]	2245.5 ± 767.0[a]	59.6 ± 29.0[a]	132.7 ± 19.4[a]	192.3 ± 22.0[a]
42	275.3 ± 135.2	443.3 ± 149.6	718.6 ± 283.1	248.4 ± 85.1	314.1 ± 122.2	562.5 ± 199.6
(b) *Scutellospora calospora*						
14	13.5 ± 5.9	0.6 ± 0.4	14.1 ± 6.3	3.4 ± 4.1	0.0 ± 0.0	3.4 ± 4.1
21	280.50 ± 78.7	480.8 ± 58.6[a]	761.3 ± 60.0[a]	238.5 ± 71.5	196.3 ± 92.5[a]	434.8 ± 141.9[a]
28	360.5 ± 110.7	695.70 ± 306.1	1056.2 ± 413.0	331.3 ± 95.0	603.3 ± 190.9	934.6 ± 272.1
42	200.7 ± 45.1	786.3 ± 332.2	987.1 ± 377.4	173.7 ± 69.9	329.9 ± 89.8	503.2 ± 152.2

Soil treatment as for Table 1. P_0, no added phosphate; P_1, added (12.4 mg P kg^{-1}). Values are the means ± SE, $n = 3$. One-way ANOVAs ($P < 0.05$) were used to determine significance between [a]P_0 and P_1 levels of soil P.

Table 4. *Fluxes of phosphate calculated using plant inflow or hyphal inflow* (*HI*) *for three successive harvest periods from* Allium porrum *to either* (*a*) Glomus sp. 'City Beach' *or* (*b*) Scutellospora calospora *in 2 soil treatments* (P_0 *and* P_1)

	Soil treatment P_0				P_1			
	Flux (mol $m^{-2}\ s^{-1} \times 10^{-9}$)							
	From inflow		From HI		From inflow		From HI	
Days	Arbuscular	Total	Arbuscular	Total	Arbuscular	Total	Arbuscular	Total
(a) *Glomus* sp. 'City Beach'								
14–21	10.1 ± 5.0	8.3 ± 4.2	9.4 ± 4.7	7.7 ± 3.9	955.6 ± 1071.1	600.8 ± 657.5	218.9 ± 245.3	137.6 ± 150.6
21–28	2.3 ± 0.5[a]	1.8 ± 0.4[a]	1.9 ± 0.4[a]	1.5 ± 0.3[a]	74.1 ± 4.3[a,b,c]	56.1 ± 2.8[a,b,c]	53.1 ± 3.1[a,b,c]	40.3 ± 2.0[a,b,c]
28–42	1.0 ± 0.3[a,b]	0.8 ± 0.3[a,b]	1.1 ± 0.3[a,b]	0.8 ± 1.6[a,b]	26.6 ± 7.0[a]	15.4 ± 3.3[a]	15.3 ± 3.4[a]	8.9 ± 1.6[a]
(b) *Scutellospora calospora*								
14–21	9.6 ± 1.3[c]	5.9 ± 0.5[c]	3.4 ± 0.5[a,c]	2.1 ± 0.2[a,b]	107.6 ± 55.1	38.1 ± 9.8	95.7 ± 49.0[a]	33.9 ± 8.8[a]
21–28	3.7 ± 1.0[a]	2.3 ± 0.5[a]	4.8 ± 1.4[a]	3.0 ± 0.7[a]	15.3 ± 3.5[a,b]	40.3 ± 2.0[a,b,c]	20.1 ± 5.3[a,b]	11.45 ± 2.6[a,b,c]
28–42	4.9 ± 1.0[a,b]	3.5 ± 0.6[a,b]	5.0 ± 1.0[a,b]	3.5 ± 0.6[a,b]	9.3 ± 0.5[a,c]	8.9 ± 1.6[a]	11.8 ± 0.5[a,c]	7.7 ± 0.3[a]

Areas of arbuscular or total (arbuscular and hyphal) symbiotic interfaces were calculated at the mid point of each harvest period.
Soil treatment as for Table 1. P_0, no added phosphate; P_1, added phosphate (12.4 mg P kg^{-1}). Values are the means ± SE, $n = 3$. One-way ANOVAs ($P < 0.05$) were used to determine significance between [a]P_0 and P_1 levels of soil P, [b]fungal treatments at the same soil P level at the same harvest, and [c]fluxes calculated using inflow or HI at the same soil P level at the same harvest.

fungi were significant at the 28–42 d harvest period in both P_0 and P_1 soil with higher inflow via Sc.

Area of symbiotic interface

Data for the total perimeters of metabolically active intercellular hyphae and arbuscules per section stained with NBT were used to calculate areas of symbiotic interface formed by these structures (Table 3). In P_0 soil the areas of hyphal and arbuscular interfaces produced by CB were low at 14 d, but increased markedly by 21 d (Table 3a). In P_0 soil, arbuscules formed by CB constituted around four times more interface than hyphae, except at 42 d when the values were similar. Adding P to the soil generally reduced the mean areas of interface (hyphae and arbuscules), but differences between P_0 and P_1 soil were significant only at 28 d. At 14 d, Sc formed a relatively large number of intercellular hyphae compared with arbuscules with P_0 and P_1 soil (Table 3b). The number of arbuscules was lower with P_1 than P_0 soil at 21 d, but formation of intercellular hyphae by Sc was little affected by addition of P. The contributions of hyphae and arbuscules to the area of total interface differed

between the fungi. The ratio of arbuscular to hyphal interface with Sc was generally lower than with CB.

Fluxes across the arbuscular and hyphal interface

The data from Tables 2 and 3 are used in Table 4 to calculate the flux of P across both the arbuscular and the total (arbuscular plus hyphal) interfaces for each harvest period. Fluxes have been calculated using (i) plant inflow (i.e. ignoring uptake by root cells) and (ii) hyphal inflow. Fluxes calculated using plant inflows indicate maximum values.

Arbuscular fluxes. Fluxes for CB in P_0 soil calculated using inflows or hyphal inflows were highest between 14 and 21 d and declined thereafter (Table 4a). The addition of P resulted in much larger fluxes via CB, with differences between P_0 and P_1 soil significant for all harvests except the first time period in which there was a large variability with P_1 soil.

Fluxes for Sc in P_0 soil were relatively constant throughout the experiment (Table 4b). Differences between fungi were significant between 28 and 42 d, reflecting this different pattern. The addition of P increased fluxes for Sc significantly for all harvests. The effect of P was less marked for Sc than CB, with differences between the two fungi again significant between 21 and 28 d. As in P_0 soil, there was a general decline in fluxes over the time course of the experiment.

Fluxes across total interface. In P_0 soil, fluxes across the total (arbuscular and hyphal) interfaces were lower than those calculated from arbuscule area alone because of the larger surface area (Table 4a). This effect was particularly important for Sc, where hyphae constituted a higher proportion of the total interface especially at early harvest periods.

The effect of P addition was similar to that observed for arbuscular flux, with differences between P_0 and P_1 soil again significant for all harvest periods except with CB between 14 and 21 d.

DISCUSSION

We have shown that the growth of plants in their respective soils colonized by CB and Sc was significantly increased compared with that of NM controls by 42 d (Dickson *et al*., 1999). Total P content in plants also increased both with mycorrhizal colonization and with P addition, indicating that P was taken up into the mycorrhizal plants in greater amounts than into NM controls.

Non-mycorrhizal plants in P_0 soil were able to absorb P effectively only up to 21 d (controls for Sc) or 28 d (controls for CB), presumably reflecting the development of depletion zones around the roots. Increased soil P increased inflow of P into NM plants, as expected. Inflow was consistently higher into mycorrhizal plants than NM plants, irrespective of soil or P supply. Our estimates of hyphal inflow (Table 2) were obtained by the conventional method of subtracting values for NM plants from those for mycorrhizal plants. These estimates will of course be too low if P uptake by the roots is decreased or 'switched off' by fungal colonization, so that the fungal pathway alone is responsible for P uptake. This is suggested by measurements of P transfer in *C. sativus* obtained with radioactive tracers (Pearson & Jakobsen, 1993) and by recent evidence that expression of plant genes encoding high-affinity P transporters is reduced in mycorrhizal plants, although this is not always the case (Liu *et al*., 1998; Rosewarne, 1998). Thus the subtraction of values of NM plants from those for mycorrhizal plants gives minimal estimates of hyphal inflow, and the maximal values must be similar to those of inflows into the mycorrhizal plants without the subtraction (Table 2). Our calculated hyphal inflows were within the range $8–35 \times 10^{-13}$ mol m^{-1} (root) s^{-1} in P_0 soils and $18–85 \times 10^{-13}$ mol m^{-1} (root) s^{-1} in P_1 soils, with possible maximal values from $11–35 \times 10^{-13}$ mol m^{-1} (root) s^{-1} and $54–119 \times 10^{-13}$ mol m^{-1} (root) s^{-1} in P_0 and P_1 soils, respectively. These values lie within the range previously reported. Estimates of hyphal P inflow vary considerably, ranging from $1–19 \times 10^{-13}$ mol m^{-1} (root) s^{-1} (Jakobsen *et al*., 1992a) to 238×10^{-13} mol m^{-1} (root) s^{-1} (Nadian, 1998). Apart from uncertainty about the assumptions behind the calculations, these values are of course influenced by many factors, including levels of soil P, the fungal isolates, host plants and soil used, production of external hyphae, extent of colonization, and the time period used for the calculation.

There were differences between the fungi in the development of symbiotic interfaces, as shown in Table 3. There is a possibility that the different patterns of mycorrhizal colonization between the fungi might be confounded by the use of soil–sand mixes based on different soils. However, given that available P was the factor limiting plant growth, it is unlikely that at the two P levels the different soils (rather than the two fungi) were the cause of the differences in development of the intraradical interfaces. With plants grown in P_0 soil, Sc produced intercellular hyphae earlier than CB, and the reverse was the case with arbuscules. With P_1 soil, Sc produced more extensive intercellular hyphae than CB up to 28 d. The area of the arbuscular interface with Sc was again small at 14 d, but was high at 28 d. In summary, there was a strong tendency for Sc to produce a lower proportion of interfacial area contributed by arbuscules. Irrespective of the soil factor, the values (which are for metabolically active interfaces) are much more informative than measurements of total colonization on the basis of percentage of root length colonized, which take no account of density of colonization or activity of interfaces.

Measurements of total colonization as a percentage of root length can be greatly biased by the presence of intercellular hyphae relative to arbuscules, and give no indication of surface areas for transfer of nutrients between the symbionts. For example, the values of *c.* 50% colonization at 14 d with Sc in P_0 and P_1 soils in the same experiment (Dickson *et al.*, 1999) are now seen to represent sparse production of intercellular hyphae and very few arbuscules. In later harvests, where roots were 70–90% colonized, surface areas of active interfaces were 50–70 times larger (in P_0 soil) or 100–250 times larger (in P_1 soil) than those at 14 d, with arbuscules predominating.

In the early stages, low inflows into plants colonized by CB or Sc were associated with low areas of interface, but there were differences in fluxes of P (i.e. transfer per unit area of interface). The latter indicate differences in capacity of the fungal and plant P transporters in the two symbioses under the two soil P conditions and at different times. With P_0 soil, fluxes across the interfaces with CB were highest in the early stages and then declined, while fluxes involving Sc tended to increase with time. With P_1 soil, fluxes were much higher (even though areas of interfaces decreased), reflecting delivery of more P through the hyphae and P transporters at interfaces that could cope with increased delivery. With P_1 soil, influxes decreased with time, probably due to internal regulation of transport activity resulting from higher concentrations within the root or reduced 'plant demand'. However, as a whole there were no consistent differences between CB and Sc with respect to capacity for transfer of P to the host.

In summary, the results confirm the conclusion (Dickson *et al.*, 1999) that both fungi can take up P from soil and transfer it to *A. porrum* under conditions where extensive colonization becomes well established. There were differences both in development of the symbioses and their interfaces, and in transport capacity of the interfaces (transport per unit area of interface and hence of plasma membranes). We believe that these reflect the fungi used rather than the soil conditions, especially when extra P was added. The combination of development and activity determines the effectiveness of the mycorrhizal symbioses, provided that external hyphae can provide P to the interfaces. The relative ineffectiveness of *S. calospora* with some hosts, reported by others, may result from sub-optimal soil conditions or properties of the hosts (rather than the fungi) that may affect the development of functional interfaces for P transfer to the host, as opposed to transfer of organic C to the fungus. Definitive evidence that can determine the relative importance of arbuscular interfaces versus intercellular interfaces is still needed to help determine the physiological significance of differences in development of the two interfaces, irrespective of the cause in the experiment.

ACKNOWLEDGEMENTS

We wish to thank A. R. Humpage, S. M. Ayling and the referees for helpful comments, B. M. Wiseman for statistical advice, and the Australian Research Council for financial support.

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