New Phytol. (1999), **144**, 163–172

Characterization of two arbuscular mycorrhizal fungi in symbiosis with *Allium porrum*: colonization, plant growth and phosphate uptake

S. DICKSON[1,3]*, S. E. SMITH[1,3] AND F. A. SMITH[2,3]

[1]*Department of Soil and Water, University of Adelaide, Waite Campus, PMB 1, Glen Osmond, South Australia 5064, Australia*
[2]*Department of Environmental Biology, University of Adelaide, South Australia 5005, Australia*
[3]*Centre for Plant Root Symbioses, University of Adelaide, Waite Campus, PMB 1, Glen Osmond, South Australia 5064, Australia*

Received 16 November 1998; accepted 1 June 1999

SUMMARY

Two arbuscular mycorrhizal fungi, *Scutellospora calospora* and *Glomus* sp. 'City Beach', were grown in soil conditions suitable for colonization of *Allium porrum*. Effects on plant growth and phosphate uptake were examined. Isolates of *S. calospora* (including the one used here) have been shown by others to vary in their stimulation of plant growth, and appear to be inefficient in the transfer of P from the fungus to the host. Our hypothesis was that such isolates of *S. calospora* may be more aggressive colonizers than other fungi, thus preventing growth increases or producing strong growth depressions in the host plant. In fact, inoculation with either fungus increased growth of plants in their respective soils both low in P (P_0) and with added P (P_1) by 42 d. The effect on growth due to mycorrhizal symbiosis (i.e. mycorrhizal growth response, % MGR) at 42 d was higher in plants grown in P_0 soil. Plants colonized by *Glomus* sp. 'City Beach' had a greater % MGR than plants colonized by *S. calospora*. Both fungi colonized plants to high levels. The percentage of root length colonized was higher in P_0 soil than in P_1 soil at 21 d. The internal development of *S. calospora* appeared less affected by addition of P than *Glomus* sp. 'City Beach' at the early harvests. Formation of arbuscules followed the same trends as total colonization. Shoot P concentration was significantly higher in mycorrhizal plants than in non-mycorrhizal plants, by 21 d in P_1 soil and by 28 d in P_0 soil. Different percentage responses to added P based on total plant dry weight (% PGR) were observed at 28 and 42 d between the plants colonized by the two fungi. The increased P content due to mycorrhizal colonization (% MPR) differed with soil P. For both fungi grown in their respective soils, the response was greater in plants grown in P_0 soil. Although the isolate of *S. calospora* used is an aggressive arbuscular mycorrhizal fungus with some hosts, it promotes a strong positive plant growth response in *A. porrum* after a mild initial growth depression.

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

INTRODUCTION

The benefits of arbuscular mycorrhizal (AM) colonization on plant growth and increased nutrient uptake are widely known. In soils low in available phosphate (P), there is usually a positive growth response in the mycorrhizal plant (Smith & Read, 1997). However, mycorrhizal colonization sometimes induces growth depressions (Smith, 1980). The situation may arise that a fungal endophyte is utilizing plant carbohydrates but supplying nutrients such as P in limited amounts, if at all (Janos, 1987; Smith & Smith, 1996; Johnson *et al.*, 1997). Where P supply to non-mycorrhizal plants is not growth limiting, growth depressions may be due to the increased carbon cost imposed by mycorrhizal colonization. They may be promoted by low temperature or low light (Hayman, 1974) or particular fungus–plant combinations, all of which can change the cost–benefit equation (Mosse, 1973a; Graham & Eissenstat, 1994; Graham *et al.*, 1997).

Smaller growth responses have been observed in

*Author for correspondence (fax +61 8 8303 6511; e-mail sdickson@waite.adelaide.edu.au).

Trifolium subterraneum colonized with isolates of *Scutellospora calospora* in comparison with other fungi (Abbott & Robson, 1985b; Thomson *et al.*, 1986; Jakobsen *et al.*, 1992a), even though *S. calospora* produced more external hyphae in the soil than the other fungi investigated. Jakobsen *et al.*, (1992b) suggested that limited transport of P into *T. subterraneum* by *S. calospora* could be caused either by low rates of translocation in external hyphae, or by reduced transfer across the fungus–plant interface. With *Cucumis sativus*, *S. calospora* was shown to accumulate large amounts of ^{32}P in its hyphae, but very little was transported to the host (Pearson & Jakobsen, 1993b). These results suggest that in some situations this fungus can depress growth to different extents and that these effects may be modulated by the host.

Smith *et al.* (1994) suggested that as *S. calospora* was capable of taking up P from the soil, the inefficiency of the fungus may have been due either to poor development of fungal structures inside the root or to a lower rate of transfer per unit area of symbiotic interface (i.e. flux across the interface). Since arbuscules are considered to be the major site of nutrient transfer to the plant, a reduction in size or number of these structures would result in a smaller surface area available for P transfer. Previous studies using *S. calospora* have given data on the percentage (or fraction) of root length colonized, but have not provided information on the occurrence, density or activity of the various mycorrhizal structures (Abbott & Robson, 1985b; Thomson *et al.*, 1991; Jakobsen *et al.*, 1992a).

Comparisons between plants grown in soils with different levels of P can also indicate the degree of efficiency of P transfer of the fungus. However, interpretation is complicated by a reduction in colonization and arbuscule production when soil P is increased (Smith, 1982; Amijee *et al.*, 1989) and hence a reduction in the mycorrhizal growth response of the plant (Hall *et al.*, 1977). Increased soil P caused reduced growth of infection units of *Glomus intraradices* grown in symbiosis with *C. sativus*, and numbers of arbuscules and vesicles were also lower (Bruce *et al.*, 1994). *Scutellospora calospora* has been reported to be more sensitive than *Glomus fasciculatum* to increasing supplies of soil P, in that percentage colonization in the root was reduced to a greater extent (Thomson *et al.*, 1986). If this was correlated with reduced arbuscular development, reduced transfer to the plant might again be expected. A method which takes account of these complexities is needed to compare the effects of different fungi.

Two mycorrhizal fungi, *S. calospora* and *Glomus* sp. 'City Beach', were examined to determine whether they produce different effects on growth at early stages of plant colonization under soil conditions in which the host plants are heavily colonized. These are not the same for the two fungi. We examined plant growth, percentage colonization by various structures, length of external hyphae, and P uptake by the plant (*Allium porrum*) in the presence and absence of the mycorrhizal fungi in plants grown at two low soil P levels. Data for these parameters are reported here. Three formulae were used to examine similarities and differences between the two fungi with regard to mycorrhizal growth response and P addition. Area of symbiotic interface, inflow of P into the plant, and flux values were also determined, and are the subject of a separate paper (Dickson *et al.*, 1999).

MATERIALS AND METHODS

Experimental design

Soil and inoculation. Preliminary experiments indicated that the two fungi, *Scutellospora calospora* (Nicolson & Gerdemann) Walker & Sanders (WUM 12(2)) and *Glomus* sp. 'City Beach' (WUM 16), were very sensitive to soil pH. We were unsuccessful in producing a soil-based mix that gave a high amount of colonization by both fungi. As this was essential for our experiments, soils from two sites, Mallala and Kuitpo, South Australia, were used to take account of the pH preferences of the two fungi. In this way, both fungi were under the conditions in which they have been previously shown to colonize *Allium porrum* L. Soil and sand were autoclaved at 121°C for 1 h on two occasions over a 3 d period.

Soil was mixed 1 : 9 with sand to produce soil : sand mixes. Inoculum of *Glomus* sp. 'City Beach' (CB) was mixed into Mallala soil : sand mix which had pH 7.4 (1 : 5 soil measured in 0.01 M $CaCl_2$) and 0.83 mg kg^{-1} extractable P (Colwell, 1963). *Scutellospora calospora* (Sc) was inoculated into Kuitpo soil : sand mix pH 5.3, 2.29 mg kg^{-1} P. Both fungi were originally obtained from the Department of Soil Science and Plant Nutrition, University of Western Australia, and the isolate WUM 12(2) has been used in previous work by others with *T. subterraneum* and *C. sativus* (Abbott & Robson, 1985b; Jakobsen *et al.*, 1992a,b; Pearson & Jakobsen, 1993a,b).

Black plastic tube pots (diameter 6 cm, height 26 cm) were used containing 1 kg soil : sand mix with or without mycorrhizal inoculum. The inoculum was dry pot-culture material of *Trifolium subterraneum* L. cv. Mt Barker colonized by the appropriate fungus. Of these cultures, 15 % by wt of colonized root fragments and soil containing spores and external hyphae was thoroughly mixed throughout the soil : sand mixture. Non-mycorrhizal pots contained the same weight of material from an uninoculated pot culture of *T. subterraneum*. Two levels of 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.

Plant material. Seeds of *Allium porrum* L. cv. Musselburgh were surface sterilized in sodium hypochlorite (4.2% w/v) and planted into pots prepared as described. Two to three seeds were planted in each of five holes to produce a large enough quantity of root material for early harvests. Seedlings were not thinned out after germination, to avoid disruption of the developing fungal hyphal network. The surface of the soil was covered with plastic beads to prevent the newly planted seeds from being washed out of the soil upon watering and to reduce evaporation.

The experiment was carried out in a growth room with a 14 h photoperiod and temperatures of 23 and 18°C in the light/dark phases, 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 were randomly rearranged at each watering. The pots were given 10 ml of nutrient solution minus P based on the Long Ashton recipe (Smith & Smith, 1981) 2 wk after planting and weekly thereafter. There were 18 pots per treatment. Plants were harvested at 14, 21, 28 and 42 d. At the first two harvests six pots of each treatment were used: three pots to measure plant growth and P concentration, and three pots to measure external fungal hyphae, root length, mycorrhizal colonization, and determination of interface area using image analysis. For the remaining harvests, three pots of each treatment were used to obtain all measurements.

Plant harvest and determination of P concentration, total root length and mycorrhizal colonization

The plants were separated into roots and shoots and fresh weights recorded. Root material was cut into approx. 1 cm segments. Weighed root subsamples were taken for determination of dry weight and P concentration, and for measurement of percentage colonization and areas of symbiotic interface. Shoot material and root subsamples were oven-dried at 80°C and analysed for P concentration by the phosphovanado-molybdate method (Hanson, 1950).

Root subsamples of known weight were stained with Trypan blue using a modification of the method of Phillips & Hayman (1970). Percentage colonization was determined by the method of McGonigle *et al*. (1990). Briefly, root segments were mounted on slides, and the occurrence of internal hyphae, arbuscules and vesicles determined at the intersections between roots and an ocular cross-hair at ×160 magnification. A minimum of 150 intersections per sample was used. Total root length was determined by the grid intersect method (Tennant, 1975).

Calculation of responses to mycorrhizal colonization and P application

Although the soils were different for the two AM fungi and their respective non-mycorrhizal controls, the following equations were used to enable us to examine similarities and differences between the plants in response to mycorrhizal colonization and the addition of P to the soil.

The percentage response of plants to mycorrhizal colonization in terms of total plant dry weight (mycorrhizal growth response, % MGR) in P_0 and P_1 soils was calculated using the following equation:

$$\% \text{ MGR} = \frac{\text{DW (M)} - \text{mean DW (NM)}}{\text{mean DW (NM)}} \times 100 \quad \text{Eqn 1}$$

The percentage response in growth of plants to P addition (phosphate growth response % PGR) for both mycorrhizal and non-mycorrhizal plants was calculated as:

$$\% \text{ PGR} = \frac{\text{DW } (P_1) - \text{mean DW } (P_0)}{\text{mean DW } (P_0)} \times 100 \quad \text{Eqn 2}$$

The percentage response of plants to mycorrhizal colonization in terms of P accumulation (% MPR) was calculated as:

$$\% \text{ MPR} = \frac{\text{P content (M)} - \text{mean P content (NM)}}{\text{mean P content (NM)}} \times 100 \quad \text{Eqn 3}$$

(DW, plant dry weight (mg); M, mycorrhizal plant; NM, non-mycorrhizal plant grown at the same level of soil P as the mycorrhizal plant; P_0, no additional P to the soil; P_1, 12.4 mg P kg^{-1} added to soil; P content, μg P per plant).

Measuring length of external hyphae in soil

The length of external hyphae was determined at each harvest using a method modified from Abbott & Robson (1985b). Pots were watered immediately before harvest. Three cores of soil (13 mm diameter) were taken from each pot and mixed thoroughly together. A 3 g subsample was added to 225 ml double-distilled water containing sodium hexametaphosphate (35.7 g l^{-1}) and stirred vigorously on a magnetic stirrer for 10 min. Stirring speed was reduced and two separate aliquots of 5 ml were taken and filtered through an 8.0 μm Millipore® filter. Soil and hyphae retained on the filter were stained with 1 ml of a freshly prepared solution of fluorescein diacetate (FDA) (10 μg FDA ml^{-1} in 60 mM sodium phosphate buffer pH 7.4; Söderström, 1977). Samples were observed by fluorescence microscopy to distinguish metabolically active fungal hyphae against a dark soil background (Sukarno, 1994). The length of active hyphae was calculated by the grid intersect method (Tennant, 1975) with a grid eyepiece micrometer at ×160 magnification on a Zeiss Standard Lab 16 microscope. Zeiss excitation filter BP 450–490 and barrier filter LP 520 were used. This procedure was completed within 1 h to

avoid the Millipore filter drying out and any possible fading of fluorescence or loss of enzymatic activity. There was no attempt to differentiate between hyphae of mycorrhizal fungi and fungal saprophytes during this process. Mycorrhizal colonization was not seen in the roots of non-mycorrhizal plants, although hyphae of soil fungi were present in the pots. Values for non-mycorrhizal pots were not subtracted from those for mycorrhizal pots due to possible competition between saprophytic and mycorrhizal fungi (Sukarno, 1994). Results are presented for mycorrhizal pots only, and are expressed as m hyphae per m root.

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

Plant growth

Total plant dry weight. Non-mycorrhizal (NM) plants grown in the Mallala soil-based mix tended to be larger than those grown in the Kuitpo soil-based mix (Table 1), despite the lower level of available P in the former. Differences were not always significant, but were large enough to preclude direct comparison of CB and Sc plants. At the first three harvests there were no significant differences between dry weights of mycorrhizal plants (CB or Sc) and their respective NM controls in P_0 soil. However, there was a tendency for plants colonized by CB to have greater dry weight than their NM controls, while plants colonized by Sc tended to have a lower dry weight.

Adding P to the soils (P_1) increased the total dry weight of NM plants by 21 d (controls for CB) or 28 d (controls for Sc). Mycorrhizal plants (both CB and Sc) also responded to additional P, although significant differences in weight between plants in P_0 and P_1 soil were not seen until 42 d. Plants colonized by CB were larger than their NM controls at 42 d, but plants colonized by Sc did not show a significant positive response.

Mycorrhizal growth response. Although absolute values for weights in the two soils cannot be directly compared, the calculation of the percentage mycorrhizal growth response, % MGR (Eqn 1) gives a basis for comparison of the relative effects of mycorrhizal colonization on plant growth. This is shown in Table 1. In P_0 soil, a small positive response was observed at the early harvests for plants colonized by CB, while no effect or a slight depression in growth occurred with Sc. At 28 d the % MGR to Sc was significantly lower than that to CB. However, significant positive growth responses to colonization were apparent at 42 d in both sets of mycorrhizal plants. Values for % MGR in P_1 soil were generally lower than in P_0 soil (Table 1) and no increased growth due to either fungus was observed at the early harvests. Plants colonized by CB showed

Table 1. *Total dry weight, roots plus shoots of non-mycorrhizal (NM) controls of* Allium porrum *and plants colonized by* (*a*) Glomus sp. '*City Beach*' (*CB*) *or* (*b*) Scutellospora calospora (*Sc*) *and their corresponding percentage mycorrhizal growth response* (% *MGR*) *in 2 soil treatments* (P_0 *and* P_1)

	Soil treatment P_0 Dry weight (mg)			P_1		
Days	NM	Colonized	% MGR	NM	Colonized	% MGR
(a) *Glomus* sp. 'City Beach'						
14	3.6±0.1	4.0±0.4	12.4±10.3	3.8±0.5	3.7±0.1	−2.8±1.9
21	7.9±0.8[b]	9.2±0.9	16.0±10.8	11.0±0.6[b]	10.5±1.9	−5.3±17.4
28	16.9±1.3[b]	18.3±0.8	7.9±4.6[c]	24.7±2.6[b]	22.4±4.0	−9.6±16.3
42	22.8±4.0[a,b]	48.4±2.5[a,b]	112.2±10.9[b]	68.5±9.5[a,b]	97.7±4.2[a,b]	42.6±6.1[b,c]
(b) *Scutellospora calospora*						
14	3.1±0.2	2.8±0.3	−7.6±10.5	3.5±0.1	3.3±0.4	−3.9±12.7
21	7.9±0.9	7.6±0.6	−4.3±7.3	9.1±0.3	8.5±0.9	−6.2±10.2
28	13.1±0.7	10.2±1.2[b]	−22.2±9.3[c]	19.2±2.7	20.6±2.3[b]	7.4±11.9
42	18.7±2.7[a,b]	44.6±6.0[a,b]	138.1±32.2[b]	56.4±7.4[b]	64.9±4.1[b]	15.1±7.2[b,c]

CB and corresponding NM controls were grown in Mallala soil-based mix. Sc 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±SE, $n=3$. Significant difference by Tukey's test ($P<0.05$) between [a]NM and mycorrhizal plants at the same soil P level, [b]P_0 and P_1 levels of soil P, and [c]plants colonized by CB and Sc at the same soil P level at the same harvest (% MGRs only). Two-way ANOVAs were carried out for significant interactions. Where there was no interaction, one-way ANOVAs were used to determine significance.

Table 2. *Root length per plant of 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 Root length per plant (cm)		P_1	
	Days	NM	NM	Colonized
(a) *Glomus* sp. 'City Beach'				
14	7.0 ± 0.5	7.4 ± 0.3	7.3 ± 0.2	8.6 ± 1.9
21	16.4 ± 1.5	17.6 ± 4.2	20.0 ± 2.9[a]	10.2 ± 1.4[a]
28	33.9 ± 3.1[b]	38.7 ± 2.1	56.2 ± 7.0[b]	43.0 ± 5.9
42	50.1 ± 7.3[a,b]	101.3 ± 9.3[a,b]	136.4 ± 24.4[b]	144.7 ± 8.6[b]
(b) *Scutellospora calospora*				
14	8.3 ± 1.2	8.2 ± 2.4	7.8 ± 2.1	8.7 ± 1.3
21	14.4 ± 0.7[b]	19.7 ± 4.0	22.0 ± 1.2[a,b]	16.6 ± 0.4[a]
28	31.6 ± 3.4[a,b]	15.1 ± 3.5[a,b]	65.8 ± 6.0[a,b]	35.7 ± 4.7[a,b]
42	56.1 ± 12.0[b]	69.4 ± 15.4	176.8 ± 14.6[a,b]	83.9 ± 7.4[a]

Soil conditions and statistical analysis as for Table 1.

Table 3. *Percentage of root length colonized by total mycorrhizal colonization or by arbuscules only using Trypan blue staining in* Allium porrum *with 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	
Days	Total	Arbuscules	Total	Arbuscules
(a) *Glomus* sp. 'City Beach'				
14	6.3 ± 5.6	2.0 ± 1.7	13.0 ± 4.2[b]	2.6 ± 1.5[b]
21	62.3 ± 9.5	51.3 ± 7.0[a]	32.6 ± 9.6	18.4 ± 10.6[a]
28	72.4 ± 6.1	58.0 ± 5.2	70.0 ± 6.3	53.3 ± 9.5
42	81.0 ± 6.2	68.4 ± 8.1	71.3 ± 3.2[b]	48.7 ± 4.6[b]
(b) *Scutellospora calospora*				
14	49.1 ± 7.5[b]	1.2 ± 1.4[b]	51.6 ± 2.0[b]	0.8 ± 0.3[b]
21	81.5 ± 6.0[b]	48.3 ± 2.5[b]	72.0 ± 2.8[b]	49.3 ± 7.1[b]
28	82.3 ± 5.5[b]	51.7 ± 5.6[b]	85.0 ± 5.7[b]	59.8 ± 5.6[b]
42	89.6 ± 1.3[b]	57.2 ± 8.5[a,b]	70.6 ± 14.9[b]	27.7 ± 8.0[a,b]

Soil conditions as for Table 1. Values are the means ± SE, $n = 3$. Significant difference by Tukey's test ($P < 0.05$) between [a]P_0 and P_1 levels of soil P and [b]total and arbuscular colonization within the root 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.

a positive MGR at 42 d whereas for plants colonized by Sc the MGRs were not significantly different from zero.

Root length and root:shoot ratios. Mycorrhizal inoculation produced similar trends in root length per plant to those of total plant dry weight (Table 2). Root length of plants increased over time in all treatments. In P_0 soil, plants colonized by CB had significantly longer root length than NM controls by 42 d (Table 2a). Root length of NM and mycorrhizal plants (CB) grown in Mallala P_1 soil did not differ significantly except at the 21 d harvest.

Plants in P_0 soil colonized by Sc had greater root length than their NM controls except at 28 d when NM roots were much longer (Table 2b). In P_1 soil, plants colonized by Sc had shorter roots than their NM controls at 21 d and later.

There was also a trend for mycorrhizal plants (CB and Sc) to have lower root:shoot ratios (R:S) than their NM controls in P_0 soil (results not shown). R:S ratios for mycorrhizal plants were lower than for NM controls after the first harvest. Ratios were further reduced when P was added to the soil (results not shown).

Mycorrhizal colonization

Total fungal colonization. Results of total fungal colonization (colonization by all fungal structures:

Table 4. *Shoot phosphate concentration of 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	
	Shoot P concentration (μg mg^{-1})			
Days	NM	Colonized	NM	Colonized
(a) *Glomus* sp. 'City Beach'				
14	5.2 ± 0.3	5.0 ± 0.4	5.6 ± 0.6	5.6 ± 0.2
21	2.5 ± 0.2[b]	2.7 ± 0.3[b]	3.3 ± 0.2[b]	3.6 ± 0.2[b]
28	1.3 ± 0.1[a,b]	2.1 ± 0.1[a,b]	2.6 ± 0.1[a,b]	4.1 ± 0.3[a,b]
42	1.0 ± 0.1[a,b]	1.3 ± 0.1[a,b]	2.2 ± 0.1[a,b]	2.9 ± 0.1[a,b]
(b) *Scutellospora calospora*				
14	6.2 ± 0.5	6.0 ± 0.3	6.3 ± 0.2	6.0 ± 0.3
21	2.9 ± 0.4	2.7 ± 0.0[b]	3.3 ± 0.0	3.9 ± 0.4[b]
28	1.6 ± 0.1[a,b]	2.5 ± 0.1[a,b]	2.5 ± 0.0[a,b]	3.6 ± 0.1[a,b]
42	1.0 ± 0.0[a,b]	1.6 ± 0.1[a,b]	1.8 ± 0.1[a,b]	3.2 ± 0.2[a,b]

Soil conditions as for Table 1. Values are the means ± SE, $n = 3$. Significant difference by Tukey's test ($P < 0.05$) between [a]NM and mycorrhizal plants at the same level of soil P, [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 5. *Changes in plants in response to added phosphate* (% *PGR*) *of non-mycorrhizal* (*NM*) *controls of* Allium porrum *and plants colonized by* Glomus *sp.* '*City Beach*' (*CB*) *or* Scutellospora calospora (*Sc*)

	Mallala soil		Kuitpo soil	
Days	NM	CB	NM	Sc
14	5.7 ± 15.0	−8.6 ± 1.8	13.0 ± 3.3	17.5 ± 15.6
21	39.6 ± 7.8[b]	14.0 ± 21.0	15.4 ± 3.2[b]	13.1 ± 12.2
28	46.12 ± 15.3	22.5 ± 22.1[c]	46.0 ± 20.4	101.4 ± 22.4[c]
42	200.3 ± 41.6[a]	101.8 ± 8.6[a,c]	201.1 ± 39.2[a]	45.6 ± 9.1[a,c]

Soil conditions as for Table 1. Values are the means ± SE, $n = 3$. Significant difference by Tukey's test ($P < 0.05$) between [a]NM and mycorrhizal plants, [b]NM control plants for CB and Sc, and [c]plants colonized by CB and SC 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.

hyphae, arbuscules and vesicles) are shown in Table 3. Colonization of plant roots by CB was very low (6%) at 14 d, but had significantly increased by 21 d, and remained high throughout the experiment. In P_1 soil, total percentage colonization by CB at 14 d was similar to that in P_0 soil, but subsequent colonization was slower to develop.

Plants were highly colonized by Sc at 14 d, and at this time intercellular hyphae predominated within the root (Table 3b). Levels of colonization by Sc significantly increased after 14 d and remained high in the later harvests. Spread of Sc within the root was as extensive in P_1 as in P_0 soil, and at 21 d Sc appeared less sensitive than CB to P addition.

Formation of arbuscules. In P_0 soil the percentage of root containing arbuscules of CB followed the same trends as total colonization (Table 3a). The percentage of arbuscular root length was not significantly different from that of total percentage colonization. Addition of P to the soil significantly reduced the percentage of arbuscules at 21 d.

The percentage of arbuscular root length formed by Sc was significantly lower than total colonization at all harvests in both P_0 and P_1 soil (Table 3b). Arbuscules of Sc appeared less affected by P addition than those of CB at the beginning of the experiment, but the percentage was greatly reduced at 42 d.

Uptake of P

Concentration of P in plants. At 14 d in P_0 soil, there was a high concentration of P in shoots of all NM plants and mycorrhizal plants colonized by CB (Table 4a). Shoot P concentration decreased throughout the experiment in both treatments, but

Table 6. *Phosphate response to mycorrhizal colonization in terms of total phosphate content* (% *MPR*) *with* Allium porrum *plants colonized by* Glomus *sp.* '*City Beach*' (*CB*) *or* Scutellospora calospora (*Sc*) *in 2 soil treatments* (P_0 *and* P_1)

	CB		Sc	
	Soil treatment			
Days	P_0	P_1	P_0	P_1
14	7.8 ± 6.6^{b}	0.9 ± 3.0	-8.0 ± 2.1^{b}	-8.3 ± 9.8
21	37.8 ± 7.9^{b}	2.9 ± 14.2	3.0 ± 13.1^{b}	36.7 ± 7.2
28	96.8 ± 14.2	50.7 ± 33.5	52.8 ± 15.4	82.2 ± 21.4
42	247.8 ± 31.7^{a}	$88.6 \pm 11.0^{a,b}$	323.3 ± 56.8^{a}	$134.9 \pm 3.7^{a,b}$

Soil conditions as for Table 1. Values are the means $\pm$ SE, $n = 3$. Significant difference by Tukey's test ($P < 0.05$) between $^{a}P_0$ and P_1 levels of soil P and ^{b}plants colonized by CB and Sc at the same level 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.

by 28 d mycorrhizal plants had significantly higher shoot P concentrations than equivalent NM controls. Phosphate concentrations in roots (results not shown) showed similar trends to those in shoots. In P_1 soil the results were similar for both NM and CB plants at 14 d. By 21 d, all plants had significantly higher concentration of P in shoots than the P_0 plants. The decline of P concentration in shoots over time was slower in P_1 than in P_0 plants. Phosphate concentrations of roots (results not shown) showed similar trends to those of shoots. Shoot P concentration in NM plants and mycorrhizal plants colonized by Sc followed the same trends (Table 4b).

Responses to added P. Percentage responses to added P based on % PGR were calculated from Eqn 2, and give a basis for comparison between the two soils used (Table 5). Non-mycorrhizal controls for CB and Sc responded similarly to P except at 21 d, when the controls for Sc showed a smaller response. Plants colonized by CB showed smaller responses to added P than their NM controls. Plants colonized by Sc showed varying response values compared with NM controls, greater at 28 d but showing a smaller response at 42 d.

Mycorrhizal P response. The percentage mycorrhizal responses in terms of % MPR, calculated using Eqn 3, are shown in Table 6. Both sets of plants showed progressive increases in % MPR in both P_0 and P_1 soils. In P_0 soil, responses produced by Sc were significantly lower at the first two harvests than those produced by CB, but were similar at the third and fourth harvests. In other words, the % MPR response appeared to be delayed in plants colonized by Sc. Responses to CB in P_1 soil were smaller than those in P_0 soil and significant at 42 d. The % MPR responses to Sc were greater in P_1 soil than those in P_0 soil at the first three harvests, but significantly lower by 42 d. In P_1 soil, responses to Sc were significantly greater than those to CB at 21 and 42 d.

Table 7. *Length of metabolically active external hyphae from pots 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
Days	Length of hyphae (m m^{-1} total root length)	
(a) *Glomus* sp. 'City Beach'		
14	1375 ± 345	1135 ± 260
21	645 ± 110	1210 ± 555
28	195 ± 105	154 ± 20
42	105 ± 20^{a}	50 ± 25^{a}
(b) *Scutellospora calospora*		
14	755 ± 435	425 ± 170
21	290 ± 105	400 ± 100
28	470 ± 115^{a}	160 ± 20^{a}
42	100 ± 45	60 ± 5

Soil conditions as for Table 1. Values are the means $\pm$ SE, $n = 3$. Significant difference by Tukey's test ($P < 0.05$) between $^{a}P_0$ 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.

Length of external hyphae

The length of metabolically active external hyphae produced by CB (measured as m g^{-1} f. wt soil) did not change significantly over the period of the experiment, and values ranged from 0.97 ± 0.39 to 1.54 ± 0.38. The length of metabolically active external hyphae, calculated as m m^{-1} total root length, decreased throughout the experiment for CB and both soil P levels as length of roots increased (Table 7a). Significant differences in hyphal length between P levels were seen only at 42 d.

Scutellospora calospora also did not show any significant change in the length of metabolically active external hyphae over the experiment, with

hyphae ranging from 0.41 ± 0.19 to 0.75 ± 0.13 m g^{-1} f. wt soil. The length of metabolically active external hyphae (m m^{-1} root) decreased over time and with P addition (Table 7b). Differences between P levels were observed in hyphal lengths only at 28 d.

DISCUSSION

Comparisons of the effectiveness of different AM fungi in terms of colonization, growth response and mineral nutrition inevitably involve compromise. Because different fungi show preferences for soils with different properties (e.g. pH and texture), some may be ineffective in colonizing their hosts when a single soil type is used (Abbott & Robson, 1985a; Abbott *et al*., 1987). This may result in misleading conclusions about the potential effectiveness of an AM fungus if it were to be grown under more suitable soil conditions. An alternative strategy, adopted here, is to use a growth medium based on apparently optimal soil types, amended as appropriate, in this case with sand and a common nutrient supply. As can be seen from the slight differences in weights of NM plants in the two soil mixes, we did not completely succeed in obtaining properly 'matched' soil mixes and hence could not properly compare plants colonized by CB with those colonized by Sc. Nevertheless, responses of NM plants in the two soils to added P (% PGR) were similar (Table 5), suggesting that plants colonized by CB and Sc should also have had similar access to additional P, other factors being equal. The results as a whole, and the use of Eqns 1–3, give a good basis for characterization of the two AM fungi under conditions in which they were both able to establish effective symbiosis with *A. porrum*. Both of the AM fungi grown in their respective soils were, after a delay and depending on the level of soil P, able to increase growth of *A. porrum* and increase plant P uptake compared to their NM controls (Tables 1, 4, 6). These effects were associated with extensive colonization that included formation of intra-radical hyphae and arbuscules and external hyphae (Tables 3, 7). There were some quantitative differences in the development of the two fungi, as discussed below. In their respective P_0 soils, neither CB nor Sc produced a growth response until the 42-d harvest, although the mycorrhizal symbiosis, including growth of hyphae and formation of arbuscules, was established much earlier and had resulted in an increased P content by 21 d (compare Tables 1 and 6). Increases in shoot P concentration also occurred earlier, by 28 d. The use of the different soil mixes precludes direct comparison between the fungi, but these results indicate that regardless of a growth response, both fungal symbionts were still effective in terms of P transfer to *A. porrum* when grown in their respective P_1 soils. Addition of P to the soils produced different effects on the responses of the two fungi. In P_1 soil, CB showed increased concentration of P in the plant at 28 d and a growth response at 42 d. *Scutellospora calospora* produced a significant increase in P concentration from 21 d onwards (Table 4). However, this was not translated into an increased growth response, at least during the period of the experiment.

Despite this lack of a growth response, the plants grown with Sc developed extensive mycorrhizal colonization, including functional arbuscules. Thus it seems that absence of an eventual positive growth response by *A. porrum* was not due to lack or limitation of P transfer from Sc to the host. This is shown most clearly by the P responses to colonization (% MPR) which by 42 d were as large for Sc as for CB in their respective P_0 soils, and in the P_1 soils were larger for Sc (Table 6). Absence of a positive growth response at early harvests (and a tendency towards growth depression) after fungal growth had become well established may have been due to an increased C drain induced by the mycorrhizal fungi. Growth depressions caused by mycorrhizal fungi have been shown to occur in small-seeded plants at early growth stages due to limited C reserves (Jakobsen, 1995).

Most of the previous work with *S. calospora* has used *T. subterraneum* and *C. sativus* as host plants. However, there were early studies with *Allium cepa* in which *S. calospora* caused large growth increases after initial lags or depression (Furlan & Fortin, 1973, 1977). Thus the tendency to produce a growth depression in the host may relate to the species of host plant used and the P supply relative to C cost. Confirmation will require direct comparison of different plant species in the same experiment.

The characteristics of arbuscular mycorrhizal colonization are dependent upon the species of fungi as well as the host plant. Different patterns of hyphal development within and outside the root have been proposed (Abbott *et al*., 1992), and differences in colonization within the root by the two species investigated have been shown (Brundrett *et al*., 1994, 1996). In this experiment patterns of colonization by the two fungi differed in detail, with more rapid formation of intraradical fungal hyphae, but not arbuscules, by Sc (see Table 3).

Addition of P has been shown to reduce the average rate of growth of infection units, reduce arbuscule numbers within roots of *C. sativus* (Bruce *et al*., 1994), and cause arbuscules to be smaller and fewer in *A. cepa* (Mosse, 1973b). These findings are important, as the arbuscule is considered the major site of nutrient transfer to the plant. Limitations in size, number or function of arbuscules will influence the rate of nutrient transfer into the plant. Reports of failure by *S. calospora* to translocate P and Zn to host plants (Jakobsen *et al*., 1992b; Bürkert & Robson, 1994) might demonstrate a relation to arbuscule formation or function, and deserve further study.

Both fungi used in this experiment have previously been shown to produce large quantities of external hyphae which increased as the plants aged (Abbott & Robson, 1985b; Sukarno, 1994). Previous estimates using Trypan blue stain range from 80 to 1422 m m^{-1} root length for harvests up to 54 d (Sanders & Tinker, 1973; Abbott & Robson, 1985b; Jakobsen *et al*., 1992a). Using a vital stain (FDA) we have shown that lengths of metabolically active external hyphae are similar to the highest estimates at the first harvest and, importantly, that values decline with plant age. We found few significant differences between the length of external hyphae in P_0 or P_1 soils.

In summary, the results presented here demonstrate that both Sc and CB, when grown in their respective soils, form functional mycorrhizas with *A. porrum*, resulting in transport of P to the host. However, growth responses were different in detail. These responses may be influenced by soil type, P availability or fungal colonization. In P_0 (Kuitpo) soil there was a delayed but eventually large growth response to Sc. In P_1 (Kuitpo) soil there was no growth response to Sc. Some previous studies may not have been carried out over sufficient time to demonstrate positive growth responses. Thus, whether or not Sc promotes increased growth may depend on duration, growth conditions, host species, and possibly which isolate of Sc is used. However, isolate WUM 12(2) is known to be relatively effective in other hosts (see Introduction).

The data collected allow calculations of P inflow into the plant, area of arbuscular and hyphal interfaces, and P fluxes across these interfaces. Hence the efficiency of the two fungi in terms of P transfer can be studied in more detail, and this work is reported in a separate paper (Dickson *et al*., 1999).

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.

REFERENCES

Abbott LK, Robson AD. 1985a. The effect of soil pH on the formation of VA mycorrhizas by two species of *Glomus*. *Australian Journal of Soil Research* **23**: 253–261.

Abbott LK, Robson AD. 1985b. Formation of external hyphae in soil by four species of vesicular–arbuscular mycorrhizal fungi. *New Phytologist* **99**: 245–255.

Abbott LK, Robson AD, Gazey C 1987. Selection of inoculant VA mycorrhizal fungi. In: Norris JR, Read DJ, Varma A, eds. *Seventh NACOM – Mycorrhizae in the next decade, Gainesville, Florida*. Gainsville, FL, USA: US Institute of Food and Agricultural Sciences, University of Florida, A13.

Abbott LK, Robson AD, Jasper D, Gazey C. 1992. What is the role of VA mycorrhizal hyphae in soil? In: Read DJ, Lewis DH, Fitter AH, Alexander IJ, eds. *Mycorrhizas in ecosystems*. Wallingford, UK: CAB International, 37–41.

Amijee F, Tinker PB, Stribley DP. 1989. The development of endomycorrhizal root systems, VII. A detailed study of effects of soil phosphorus on colonization. *New Phytologist* **111**: 435–446.

Bruce A, Smith SE, Tester M. 1994. The development of mycorrhizal infection in cucumber: effects of P supply on root growth, formation of entry points and growth of infection units. *New Phytologist* **127**: 507–514.

Brundrett M, Melville L, Peterson RL (eds). 1994. *Practical methods in mycorrhizal research*. Waterloo, Canada: Mycologue Publications.

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N. 1996. *Working with Mycorrhizas in forestry and agriculture*. Canberra, Australia: Australian Centre for International Agricultural Research.

Bürkert B, Robson AD. 1994. ^{65}Zn uptake in subterranean clover (*Trifolium subterraneum* L.) by three vesicular–arbuscular mycorrhizal fungi in a root-free sandy soil. *Soil Biology and Biochemistry* **26**: 1117–1124.

Colwell JD. 1963. The estimation of the phosphorus fertilizer requirements of wheat in southern New South Wales. *Australian Journal of Experimental Agricultural and Animal Husbandry* **3**: 190–197.

Dickson S, Smith SE, Smith FA. 1999. Characterization of two arbuscular mycorrhizal fungi in symbiosis with *Allium porrum*: inflow and flux of phosphate across the symbiotic interface. *New Phytologist* **144**: 173–181.

Furlan V, Fortin JA. 1973. Formation of endomycorrhizae by *Endogone calospora* on *Allium cepa* under three temperature regimes. *Le Naturaliste Canadien* **100**: 467–477.

Furlan V, Fortin JA. 1977. Effects of light intensity on the formation of vesicular–arbuscular mycorrhizas on *Allium cepa* by *Gigaspora calospora*. *New Phytologist* **79**: 335–340.

Genstat 5 Committee. 1987. *Reference manual*. Oxford, UK: Clarendon Press.

Graham JH, Eissenstat DM. 1994. Host genotype and the formation and function of VA mycorrhizae. *Plant and Soil* **159**: 179–185.

Graham JH, Duncan LW, Eissenstat DM. 1997. Carbohydrate allocation patterns in citrus genotypes as affected by phosphorus nutrition, mycorrhizal colonization and mycorrhizal dependency. *New Phytologist* **135**: 335–343.

Hall IR, Scott RS, Johnstone PD. 1977. Effect of vesicular–-arbuscular mycorrhizas on response of 'Grasslands Huia' and 'Tamar' white clovers to phosphorus. *New Zealand Journal of Agricultural Research* **20**:349–355.

Hanson WC. 1950. The photometric determination of phosphorus in fertilizers using the phosphovanado–molybdate complex. *Journal of the Science of Food and Agriculture* **1**: 172–173.

Hayman DS. 1974. Plant growth responses to vesicular–arbuscular mycorrhiza. IV. Effect of light and temperature. *New Phytologist* **73**: 71–80.

Jakobsen I. 1995. Transport of phosphorus and carbon in VA mycorrhizas. In: Varma A, Hock B, eds. *Mycorrhiza, structure, function, molecular biology and biotechnology*. Berlin/Heidelberg, Germany: Springer Verlag, 297–324.

Jakobsen I, Abbott LK, Robson AD. 1992a. External hyphae of vesicular–arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. *New Phytologist* **120**: 371–380.

Jakobsen I, Abbott LK, Robson AD. 1992b. External hyphae of vesicular–arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 2. Hyphal transport of ^{32}P over defined distances. *New Phytologist* **120**:509–516.

Janos DP. 1987. VA mycorrhizas in humid tropical ecosystems. In: Safir GR, eds. *Ecophysiology of VA mycorrhizal plants*. Boca Raton, FL, USA: CRC Press, 107–134.

Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist* **135**: 575–586.

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. 1990. A new method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. *New Phytologist* **115**: 495–501.

Mosse B. 1973a. Advances in the study of vesicular–arbuscular mycorrhizas. *Annual Review of Phytopathology* **11**: 170–196.

Mosse B. 1973b. Plant growth responses to vesicular–arbuscular mycorrhiza. IV. In soil given additional phosphate. *New Phytologist* **72**: 127–136.

Pearson JN, Jakobsen I. 1993a. The relative contribution of hyphae and roots to phosphorus uptake by arbuscular mycorrhizal plants measured by dual labelling with ^{32}P and ^{33}P. *New Phytologist* **124**: 489–494.

Pearson JN, Jakobsen I. 1993b. Symbiotic exchange of carbon and phosphorus between cucumber and three arbuscular mycorrhizal fungi. *New Phytologist* **124**: 481–488.

Phillips JM, Hayman DS. 1970. Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**: 158–160.

Sanders FE, Tinker PB. 1973. Phosphate flow into mycorrhizal roots. *Pesticide Science* **4**: 385–395.

Smith FA, Smith SE. 1981. Mycorrhizal infection and growth of *Trifolium subterraneum*: comparison of natural and artificial inocula. *New Phytologist* **88**: 311–325.

Smith FA, Smith SE. 1996. Mutualism and parasitism: diversity in function and structure in the 'arbuscular' (VA) mycorrhizal symbiosis. *Advances in Botanical Research* **22**: 1–43.

Smith SE. 1980. Mycorrhizas of autotrophic higher plants. *Biological Reviews* **55**: 475–510.

Smith SE. 1982. Inflow of phosphate into mycorrhizal and non-mycorrhizal *Trifolium subterraneum* at different levels of soil phosphate. *New Phytologist* **90**: 293–303.

Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis, 2nd edn*. Cambridge, UK: Academic Press.

Smith SE, Dickson S, Morris C, Smith FA. 1994. Transport of phosphate from fungus to plant in VA mycorrhizas: calculation of the area of symbiotic interface and of fluxes from two different fungi to *Allium porrum* L. *New Phytologist* **127**: 93–97.

Söderström BE. 1977. Vital staining of fungi on pure cultures and in soil with fluorescein diacetate. *Soil Biology and Biochemistry* **9**: 55–63.

Sukarno N. 1994. *Effects of selected fungicides on vesicular–arbuscular mycorrhizal symbiosis*. PhD thesis, University of Adelaide, Adelaide, Australia.

Tennant D. 1975. A test of a modified line intersect method of estimating root length. *Journal of Ecology* **63**: 995–1001.

Thomson BD, Robson AD, Abbott LK. 1986. Effects of phosphorus on the formation of mycorrhizas by *Gigaspora calospora* and *Glomus fasciculatum* in relation to root carbohydrates. *New Phytologist* **103**: 751–765.

Thomson BD, Robson AD, Abbott LK. 1991. Soil mediated effects of phosphorus supply on the formation of mycorrhizas by *Scutellospora calospora* (Nicol. & Gerd.) Walker and Sanders on subterranean clover. *New Phytologist* **118**: 463–469.

Zar JH. 1984. *Biostatistical analysis*. Englewood Cliffs, NJ, USA: Prentice-Hall International.