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A comparison of the development and metabolic activity of mycorrhizas formed by arbuscular mycorrhizal fungi from different genera on two tropical forage legumes

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Abstract Two glasshouse experiments were done to assess the development and metabolic activity of mycorrhizas formed by isolates of arbuscular mycorrhizal fungi (AMF) from three different genera, *Acaulospora*, *Gigaspora* and *Glomus* on either *Pueraria phaseoloides* L. or *Desmodium ovalifolium* L. plants. The second of the two experiments included three levels of a localised phosphate source in the pots. Alkaline phosphatase (ALP), stained histochemically in the intra-radical mycelium (IRM) of AMF over sequential harvests, did not provide a direct marker for the efficiency of AMF in mobilising phosphorus (P) for plant growth and development. The ability of the extra-radical mycelium (ERM) to scavenge a localised phosphate source, determined by its extraction from buried 35-μm mesh pouches, was dependent on the species of AMF tested. This work indicates that AMF from different genera have unique patterns of mycelial development when forming mycorrhizas with tropical hosts in the presence of a localised phosphate source. AMF also appear to have different mechanisms for the control of P transfer, within the mycelium, to the host. The significance of the architecture of the ERM is discussed as well as the localisation of ALP in the IRM in determining the efficiency of AMF in terms of P accumulation *in planta* and subsequent growth of plants.

Key words Alkaline phosphatase · Mycelium · *Acaulospora* · *Gigaspora* · *Glomus*

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Introduction

Arbuscular mycorrhizal fungi (AMF) are a unique and ancient group of micro-organisms (Remy et al. 1994; Simon et al. 1993) which have co-evolved with land plants. Differences in the timing of divergence of families within AMF (Simon et al. 1993), coupled with the fact that all AMF occupy the same soil/plant niche, indicate that they may have evolved to serve more than one function in plant roots. Despite this, the majority of experiments on AMF use only species of *Glomus*. This is largely because, unlike species of *Acaulospora*, *Gigaspora* and *Scutellospora*, *Glomus* are easy to produce and maintain in pure culture (Dodd and Thomson 1994) and can colonise the plant root rapidly. This may, however, mask considerable diversity in the function of AMF, knowledge of which is vital for understanding their ecological roles.

It is well documented, from studies using species of *Glomus*, that the development of the intra-radical mycelium (IRM) and extra-radical mycelium (ERM) may be increased or decreased by the concentration of phosphorus (P) in the soil (Abbott et al. 1984; Amijee et al. 1989). In addition, alkaline phosphatase (ALP), localised in the vacuole of the IRM (Gianinazzi et al. 1979), has been reported as a potential marker for the efficiency of the mycorrhizal symbiosis and it was suggested that it might be involved in P transport (Tisserant et al. 1993). There is a tendency, however, for research groups to extrapolate results from studies on species of *Glomus* to all Glomalean fungi. A few interspecific studies on AMF have highlighted that species may differ in their formation of the ERM (Abbott and Robson 1985; Jakobsen et al. 1992a,b), the localisation of ALP activity in the IRM (Ezawa et al. 1995) and their ability to supply P to the host plant (Jakobsen et al. 1992b; Pearson and Jakobsen 1993). This has been attributed to differences in the efficiency of P uptake, translocation and/or transfer to the plant between genera of AMF (Jakobsen et al. 1992a,b; Pearson and Jakobsen 1993). A criticism that can be levelled at some

of these studies is the lack of detailed harvests over the early growth of the plants when being colonised by different species of AMF.

In this study, we have measured the development of the IRM and the ERM of three species of AMF from different genera colonising either of two tropical forage legumes (*Desmodium ovalifolium* L. and *Pueraria phaseoloides* L.). These parameters were monitored by sequential harvesting of plants over an early growth period for each plant. The presence or absence of a localised phosphate source was also investigated and the rate of application. The phosphate source was enclosed in buried mesh pouches, this allowed penetration of fungal hyphae but not roots and enabled the densities of ERM near the localised nutrient source to be assessed over time. The localisation and activity of the vacuolar ALP in the mycelium of different AMF, in relation to P accumulation in plant tissues, was also studied.

Materials and methods

Plant material and growth substrate

Tropical kudzu, *Pueraria phaseoloides* L. (CIAT 9900), and *Desmodium ovalifolium* L. (IPB 13089) were used as host plants in experiments 1 and 2, respectively. The seeds of both species were surface sterilised for 15 min in 5% sodium hypochlorite (10% active chlorine), rinsed in several changes of sterile de-ionised water (dH_2O) and immersed for 5 min in dH_2O at 60 °C. The seeds were then soaked overnight in the dark in sterile dH_2O at 30 °C, rinsed in several changes of sterile dH_2O and pre-germinated at 30 °C on moist, sterile, Terragreen (Agsorb, grade 8/16) prior to planting. Terragreen, an inert attapulgite clay (Oil-Dri, Wisbech, Cambs, UK) pH 5.5–7.0, was used as a growth substrate in both experiments. This was prepared by soaking overnight in a 50:50 solution containing 5 mM [2-(N-morpholine)-ethane sulphonic acid] buffer (MES), a source of sulphur to promote growth of the ERM (Vilariño et al. 1997), and 1.4 g l^{-1} Vitafeed 102 (Vitax Ltd., Leicester, UK) containing 18% N, 0% P, 36% K with trace elements.

AM fungi

Three species of AMF from three different genera were used in experiments, *Acaulospora tuberculata* (BEG41), *Gigaspora rosea* (BEG111) and *Glomus manihotis* (BEG112). AMF were maintained in open pot cultures (2-l pots, 15 × 15 × 12 cm), with Terragreen as the growth substrate and *D. ovalifolium* as the host, under simulated tropical conditions in a glasshouse (min 18 °C/max 40 °C, relative humidity 60–80%, photoperiod 18/6 h light/dark). Supplementary 400 W metal halide lighting was used, giving a photon flux density of 400–600 μmol m^{-2} s^{-1} measured at the leaf surface (between and directly under the lights, respectively). The inoculum of each fungus, for both experiments, contained colonised root fragments, hyphae and spores in Terragreen. The amount of inoculum added in each experiment corresponded to a "saturation dose", i.e. an amount which resulted in the fastest colonisation possible for each AMF on the two hosts used under the prevailing environmental conditions. This approach allows for differences in the infectivity of propagules in different species of AMF.

Experiment 1

The aim of this experiment was to compare the development of the ERM (in a localised mesh pouch, LMP), the IRM and plant growth for each AM fungus colonising *P. phaseoloides* over three harvests, 10–20 weeks after sowing. Two-litre pots (15 × 15 × 12 cm) were half-filled with sterile Terragreen. One 5-day-old seedling of *P. phaseoloides* was planted, at the same position in each pot, 5 cm above a band of inoculum (50 g) of either *A. tuberculata* (BEG41), *Gi. rosea* (BEG111), *G. manihotis* (BEG112) or a mixture of all three species of AMF (including non-mycorrhizal plants inoculated with uncolonised root fragments in Terragreen). A 10-cm^3, 35-μm mesh pouch (Precision Textiles Ltd., Bury, UK), containing 10 g of sterile Terragreen, but no phosphate, was inserted at the same position in each pot (Fig. 1). This allowed hyphal penetration, but excluded roots. Twelve replicates of each treatment were placed in the glasshouse described above (January-May 1996). The experiment was laid out in a 3 × 4 × 5 factorial randomised block design. Four replicate plants of each treatment were harvested after 10, 13 and 20 weeks growth.

Experiment 2

The aim of this experiment was to compare the effect of localised phosphate addition on the development of the ERM (in a LMP), the IRM, the ALP activity in the mycelium and plant P content for each AM fungus colonising *D. ovalifolium* over three harvests, between 3–9 weeks after sowing. *D. ovalifolium* was used as a host instead of *P. phaseoloides* because of problems with seed viability in the latter. Both are tropical forage legumes with similar rates of growth and development. Two-litre pots (15 × 15 × 12 cm) were half-filled with sterile Terragreen. One 5-day-old seedling of *D. ovalifolium* was planted, at the same position in each pot, 5 cm above a band inoculum (50 g) of either *A. tuberculata* (BEG41), *Gi. rosea* (BEG111) or *G. manihotis* (BEG112) (including non-mycorrhizal plants inoculated with uncolonised root fragments in Terragreen). A 10-cm^3, 35-μm mesh nutrient pouch, containing 10 g of sterile Terragreen and either 0, 40 or 100 mg P kg^{-1} (Osmocote, slow release fertiliser [N:P:K 14:13:13], Zeneca, UK.) in tablet form (0, 60 or 150, 3 mm diameter pellets, respectively), was inserted at the same position in each pot (Fig. 1). Twelve replicates of each treatment were placed in the glasshouse described above (March-May 1997). The experi-

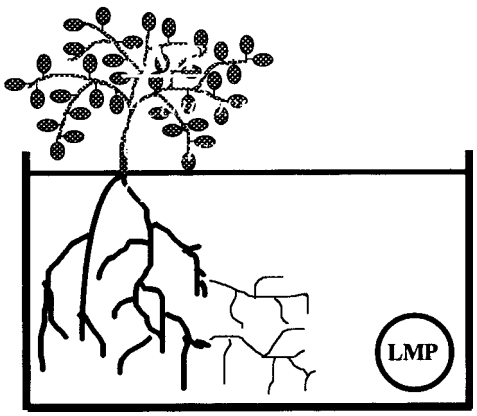

Fig. 1 A diagrammatic representation of a pot used in experiments 1 and 2 designed to compare the development of the intra- and extra-radical mycelia (IRM, ERM) and the alkaline phosphatase (ALP) activity in the mycelium of different isolates of AMF colonising one of two tropical forage legumes, with or without localised phosphate addition (0, 40 or 10 mg P kg^{-1}) in a localised mesh pouch (LMP, 10 cm^3, 35 μm mesh)

ment was laid out in a $3 \times 3 \times 4 \times 4$ factorial randomised block design. Four replicate plants of each treatment were harvested after 3, 6, and 9 weeks growth.

The surface of the pots in both experiments was covered with a 0.5 cm depth of polypropylene homopolymer black beads (Cookson, Congleton, UK) to reduce evaporation and prevent algal growth. Plants were watered regularly, by weight, to 80% water holding capacity with dH_2O and given nutrients weekly (1.4 g l^{-1} Vitafeed). The concentration of nutrients corresponded to an amount optimal for plant growth in each experiment. The concentrations of N and K in the phosphate fertiliser had no effect on plant growth in Experiment 2.

Assessment of total colonised root length, root length containing arbuscules and alkaline phosphatase activity in the intra-radical mycelium

The plant roots were washed free of soil debris using tap water and cut into 1–2 cm pieces. The roots were stained for ALP using the technique of Tisserant et al. (1993). The roots were counterstained overnight in 0.1% acid fuchsin in lacto-glycerol (lactic acid:glycerol:dH_2O, 5:1:1) at room temperature and then destained overnight in 50% glycerol. The root pieces, stained for ALP, were sampled randomly and 10×1-cm lengths mounted in 50% glycerol on a microscope slide. Three replicate slides per sample were viewed under a compound microscope at $\times 100$–400 (Axioscope, Zeiss, Germany) and assessed for total colonised root length, the root length containing arbuscules and the ALP activity in the IRM of AMF. This was done using a simple grading system. Each piece of root was graded according to the total area of the root cortex colonised by AMF and that containing only arbuscules i.e. 0% = 1, 25% = , 50% = 3, 75% = 4 and 100% = 5. The ALP activity in the IRM was graded according to the intensity of the black ALP precipitate, i.e. none = 0, up to black = 5. A mean value for the total number of fields of view assigned to each grade was calculated and determined on a root length basis. Quantification of the ALP activity in the entry point hyphae (formerly appressorium, Fig. 2) was also made using this grading system.

Quantification of lengths of extra-radical mycelium

The ERM was extracted from a 5-g air-dried sample of Terragreen from each LMP using the aqueous membrane filtration technique of Jakobsen et al. (1992a). The nitrocellulose membrane filters (Whatman 25 mm diameter, 0.45-μm pore size), containing the extracted hyphae, were transferred to microscope slides, allowed to air-dry and stained with two drops of 0.1% acid fuchsin in lacto-glycerol. The lengths of ERM were estimated over 25 fields of view along two transects forming a double-cross on the membrane using a visual estimation technique (Vosatka personal communication). A 10-mm eyepiece graticule (100×0.1 mm Graticule Ltd., Tonbridge, Kent, UK) was incorporated into the lens ($\times 10$) of a compound microscope (Axioscope, Zeiss, Germany). The micrometer scale on the graticule was orientated until it was parallel to the hyphae on the membrane filter in each field of view allowing it to be used as a ruler to estimate hyphal lengths. The lengths of ERM were estimated from four replicate membrane filters at $\times 100$ for each sample. The lengths of ERM for the mycorrhizal treatments were obtained by subtracting values of ERM lengths from a corresponding non-mycorrhizal treatment.

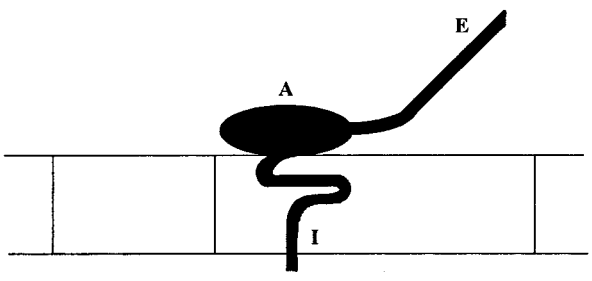

Fig. 2 A diagrammatic representation of an entry point hypha (formerly appressorium) *A* on the surface of a plant root and associated IRM (*I*) and ERM (*E*). Measurements of the ALP activity extended 50 μm from the entry point

Plant growth

The fresh weight of plant shoots was measured directly after removal from the host plant. The roots were first rinsed in tap water to remove soil particles, then blotted on tissues to remove excess water, before their fresh weights were measured. The leaves were separated from the stem, arranged on a light box and covered with a glass plate to assess the leaf area. The image of the leaves from each plant was captured with a JVC black and white video camera (UVP CCD 4722-2200/0000) using a macrolens attached to a stand 42 cm above a light box. The captured image was processed, as a field of 512×512 pixels, through a series of steps including image sharpening, contrasting and editing. The total leaf area was then estimated. The software package "PC image" (Foster and Findlay Associated Ltd., Newcastle-upon-Tyne, UK) and a synapse framestore (Synoptics Ltd., Cambridge, UK) formed the image analysis system. The root length was assessed similarly using the same image analysis system. The roots were arranged in a Petri-dish containing tap water, individual roots were separated from each other using forceps. Where root systems were too dense, a representative 1-g sub-sample was taken for measurement and spread out in a Petri-dish. The plants were oven-dried for 48 h at 70 °C before their dry weights were measured. The dried shoot and root material was then prepared for P analysis by dry-ashing and acid digestion. The P content of plant tissues was assessed using the molybdate method (Anderson and Ingram 1993) at 880 nm (UV/VIS Perkin-Elmer, Lamda 1, UK).

Statistical analysis

Data were tested for normality and analysed using two-way analysis of variance (ANOVA) on Minitab version 10 for Windows. Percentage data were arcsine transformed prior to analysis. When a significant *F*–value was obtained ($P > 0.05$), treatment means were separated by the Least Significant Difference (LSD).

Results

Experiment 1

Total colonised root length and root length containing arbuscules

The total colonised root length and the root length containing arbuscules were low and unaffected by the species of mycorrhizal fungus used to inoculate *P. phaseoloides* plants ($P < 0.05$) at first harvest (Fig. 3a, b). Values for both parameters were significantly higher in *P. phaseoloides* plants forming mycorrhizas with *G. manihotis* ($P > 0.01$) at second harvest and significantly higher with *Gi. rosea* ($P > 0.01$) at final harvest than all other mycorrhizal treatments (Fig. 3a, b). There were no significant differences in either the total colonised root length or the root length containing arbuscules in *P.*

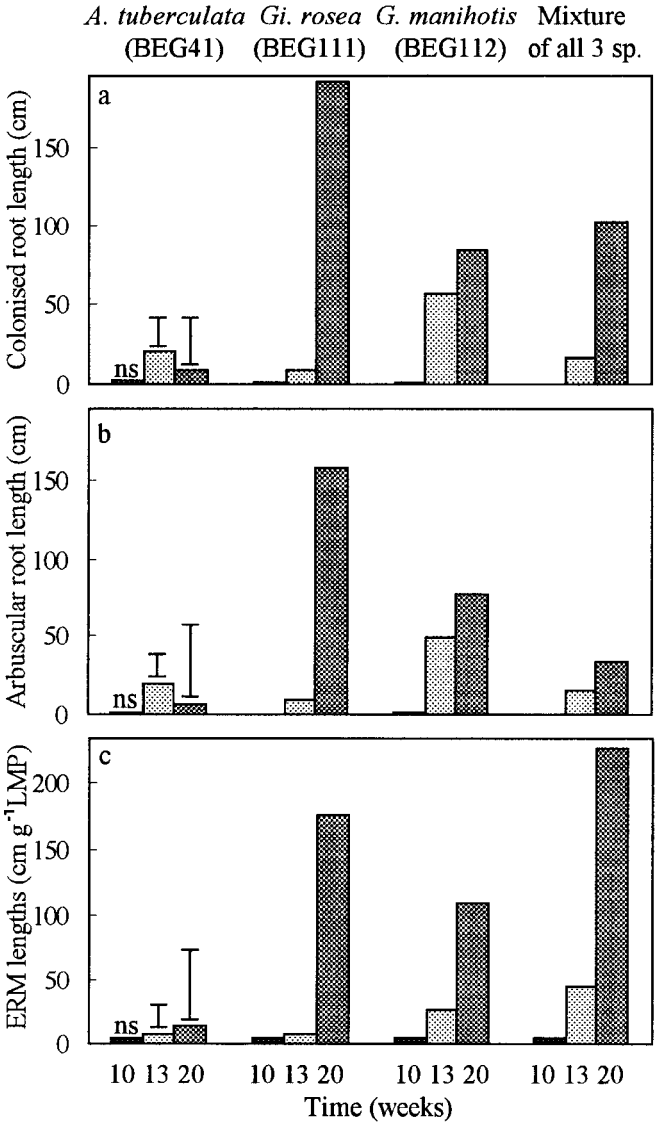

Fig. 3 Effect of AMF on (**a**) the total colonised root length, (**b**) the root length containing arbuscules (arbuscular root length) and (**c**) the lengths of ERM (in the LMPs) in *Pueraria phaseoloides* plants at each harvest (10 ■, 13 □ and 20 ■ weeks). The *bars* indicate the Least Significant Difference (LSD, $P>0.05$) between treatment means within each harvest. *ns* indicates no significant difference between treatment means

phaseoloides plants forming mycorrhizas with either *G. manihotis* or the mixture of species of AMF ($P<0.05$) at final harvest (Fig. 3a, b), and values remained low in *P. phaseoloides* plants forming mycorrhizas with *A. tuberculata* at each harvest (Fig. 3a, b).

Lengths of extra-radical mycelium extracted from the localised mesh pouch

The lengths of ERM extracted from the LMP were low and unaffected by the species of mycorrhizal fungus or the mixture of species of AMF ($P0.05$) at first harvest (Fig. 3c), but at second harvest values were significantly lower for *P. phaseoloides* plants forming mycorrhizas with either *A. tuberculata* or *Gi. rosea* ($P>0.001$) (Fig. 3c). There were no significant differences in the lengths of ERM extracted from the LMP in *P. phaseoloides* plants forming mycorrhizas with either *Gi. rosea* or the mixture of species of AMF ($P0.05$), but values for the latter were significantly higher than in plants forming mycorrhizas with *G. manihotis* ($P>0.01$) at final harvest (Fig. 3c). The lengths of ERM extracted from the LMP remained low in *P. phaseoloides* plants forming mycorrhizas with *A. tuberculata* at each harvest (Fig. 3c).

Plant growth

The leaf area was significantly higher in non-mycorrhizal *P. phaseoloides* plants and those forming mycorrhizas with either *A. tuberculata* or *G. manihotis* ($P>0.05$) than all other mycorrhizal treatments at first harvest (Table 1a). The shoot and root fresh weights and leaf area were significantly higher in *P. phaseoloides* plants forming mycorrhizas with either *A. tuberculata* or *G. manihotis* ($P>0.01$) than all other mycorrhizal treatments at second harvest (Table 1b). The root length was significantly greater in *P. phaseoloides* plants forming mycorrhizas with *Gi. rosea* ($P>0.001$) than all other mycorrhizal treatments at second harvest (Table 1b). The shoot and root fresh weights were significantly higher in *P. phaseoloides* plants forming mycorrhizas with either *A. tuberculata*, *G. manihotis* or the mixture of species of AMF ($P>0.001$) than all other mycorrhizal treatments at final harvest (Table 1c). The root dry weight was significantly higher in *P. phaseoloides* plants forming mycorrhizas with *G. manihotis* ($P>0.01$) than all other mycorrhizal treatments, except *A. tuberculata*, at final harvest (Table 1c).

Experiment 2

A. tuberculata failed to colonise the roots of *D. ovalifolium* plants during the timescale of this experiment. Results for this species of AMF are, therefore, not presented.

Total colonised root length and root length containing arbuscules

The total colonised root length and the root length containing arbuscules were low and unaffected by mycorrhizal or phosphate treatment in *D. ovalifolium* plants ($P<0.05$) at first harvest (Fig. 4a, b). The addition of localised phosphate (40 or 100 mg P kg^{-1}) significantly decreased values for both parameters in *D. ovalifolium* plants forming mycorrhizas with either *Gi. rosea* or *G. manihotis* ($P>0.05$) at second and final harvests

Table 1 Growth of non-mycorrhizal *Pneravia phaseoloides* plants or those forming mycorrhizas with either *Acaulospora tuberculata* (BEG41), *Gigaspora rosea* (BEG111), *Glomus manihotis* (BEG112) or a mixture of all three species of AMF. Plants were harvested after (a) 10, (b) 13 or (c) 20 weeks. Values are the means of four replicates (*ns* the F-value was not significant between treatment means, *nd* no data available due to the deterioration of samples)

	Mycorrhizal treatment	Shoot fresh wt. (g)	Leaf area (cm^2)	Root fresh wt. (g)	Root dry wt. (g)	Root length (cm)
a	*A. tuberculata* (BEG41)	1.0	48	0.5	0.0	43
	Gi. rosea (BEG111)	0.5	23	0.2	0.0	24
	G. manihotis (BEG112)	0.9	38	0.4	0.0	39
	Mixture of all 3 species	0.3	10	0.1	0.0	12
	Non-mycorrhizal	0.9	45	0.5	0.0	43
	LSD $P<0.05$	ns	12	ns	ns	ns
b	*A. tuberculata* (BEG41)	10.0	462	5.3	0.5	488
	Gi. rosea (BEG111)	3.4	157	2.0	0.2	747
	G. manihotis (BEG112)	10.2	413	5.1	0.5	519
	Mixture of all 3 species	3.0	128	1.7	0.2	188
	Non-mycorrhizal	4.1	180	2.5	0.2	540
	LSD $P<0.05$	2.3	93	1.0	ns	92
c	*A. tuberculata* (BEG41)	21.7	nd	14.8	1.0	2933
	Gi. rosea (BEG111)	14.0	nd	7.7	0.7	2906
	G. manihotis (BEG112)	29.8	nd	15.8	1.7	5610
	Mixture of all 3 species	25.1	nd	16.1	0.5	2614
	Non-mycorrhizal	17.2	nd	12.6	0.6	3188
	LSD $P<0.05$	2.5	nd	1.5	0.8	ns

(Fig. 4a, b). With no localised phosphate addition, the total colonised root length and the root length containing arbuscules were significantly higher in *D. ovalifolium* plants forming mycorrhizas with *G. manihotis* ($P>0.05$) than with *Gi. rosea* at second harvest (Fig. 4). This trend was reversed at final harvest (Fig. 4a, b).

Alkaline phosphatase activity in the intra-radical mycelium

With no localised phosphate addition, the ALP activity in the IRM was significantly higher in *D. ovalifolium* plants forming mycorrhizas with *Gi. rosea* ($P>0.05$) than with *G. manihotis* (BEG112) at first harvest (Fig. 4c). The addition of localised phosphate (40 or 100 mg P kg^{-1}) had no effect on the ALP activity in the IRM in *D. ovalifolium* plants forming mycorrhizas with *Gi. rosea* ($P<0.05$) at any harvest (Fig. 4c). The addition of localised phosphate (40 or 100 mg P kg^{-1}) significantly decreased the ALP activity in the IRM in *D. ovalifolium* plants forming mycorrhizas with *G. manihotis* ($P>0.05$) at second and final harvests (Fig. 4c). The ALP activity in the entry point hyphae (formerly appressorium) and associated 50-μm lengths of IRM in *D. ovalifolium* plants forming mycorrhizas with *Gi. rosea* was negligible, whilst the ALP activity in the associated 50-μm lengths of ERM was very high at each harvest, irrespective of phosphate treatment (Fig. 5a). The entry point hyphae and associated 50-μm lengths of IRM and ERM contained similar amounts of ALP activity in *D. ovalifolium* plants forming mycorrhizas with *G. manihotis* at each harvest, irrespective of phosphate treatment (Fig. 5b).

Lengths of extra-radical mycelium extracted from the localised mesh pouch

The addition of localised phosphate (40 or 100 mg P kg^{-1}) significantly decreased the lengths of ERM extracted from the LMP in *D. ovalifolium* plants forming mycorrhizas with *Gi. rosea* ($P>0.05$) at each harvest (Fig. 4d). No ERM was present in the LMP in *D. ovalifolium* plants forming mycorrhizas with *G. manihotis* at first harvest (Fig. 4d). No ERM was present in the LMP in *D. ovalifolium* plants forming mycorrhizas with either *Gi. rosea* or *G. manihotis* after the localised addition of 100 mg P kg^{-1}, at any harvest (Fig. 4d). With no addition of localised phosphate, the lengths of ERM extracted from the LMP were significantly higher in *D. ovalifolium* plants forming mycorrhizas with *Gi. rosea* ($P>0.05$) than with *G. manihotis* at second and final harvests (Fig. 4d). The localised addition of 40 mg P kg^{-1} significantly increased the lengths of ERM extracted from the LMP in *D. ovalifolium* plants forming mycorrhizas with *G. manihotis* ($P>0.001$) compared with *Gi. rosea* at final harvest (Fig. 4d).

Plant growth

There were no significant differences in either the growth or P content of *D. ovalifolium* plants between mycorrhizal or phosphate treatments (*P*0.05) at first harvest (Table 2a). The shoot fresh and dry weights and leaf area were significantly higher in *D. ovalifolium* plants forming mycorrhizas with either *Gi. rosea* or *G. manihotis* ($P>0.05$) than non-mycorrhizal plants at second harvest, irrespective of phosphate treatment (Ta-

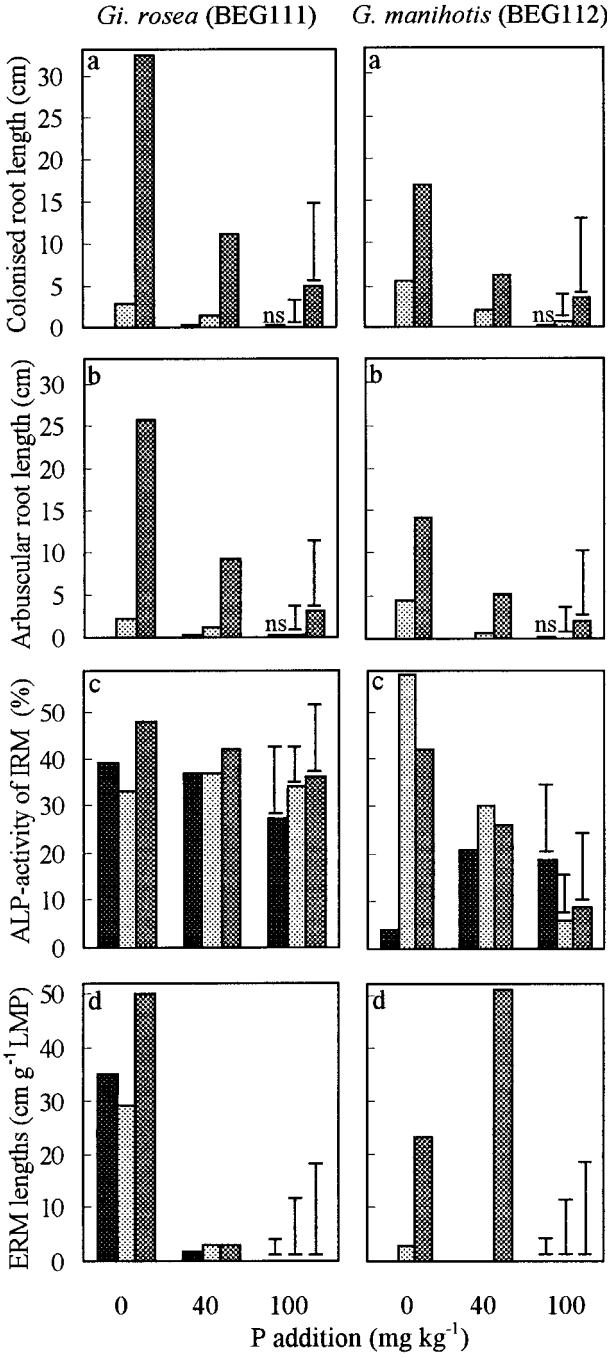

Fig. 4 Effect of AM fungus and localised phosphate addition on (**a**) the total colonised root length, (**b**) the root length containing arbuscules (arbuscular root length), (**c**) the ALP activity in the IRM and (**d**) the lengths of ERM (in the LMPs) in *Desmodium ovalifolium* plants, at three levels of a localised phosphate source (0, 40 or 100 mg kg^{-1}) in the LMPs at each harvest (3 ■, 6 □ and 9 ■ weeks). The *bars* indicate the Least Significant Difference (LSD, $P>0.05$) between treatment means within each harvest. *ns* indicates no significant difference between treatment means

ble 2b). The localised addition of either 40 or 100 mg P kg^{-1} significantly increased the shoot fresh weight, leaf area and shoot P content of *D. ovalifolium* plants ($P>0.05$), at second harvest, irrespective of mycorrhizal treatment (Table 2b). The shoot and root P contents were significantly higher in *D. ovalifolium* plants forming mycorrhizas with either *Gi. rosea* or *G. manihotis* ($P>0.05$) than non-mycorrhizal plants at final harvest, irrespective of phosphate treatment (Table 2c). The localised addition of either 40 or 100 mg P kg^{-1} significantly increased the shoot P content of *D. ovalifolium* plants ($P>0.05$) at final harvest, irrespective of mycorrhizal treatment (Table 2c). The root dry weight and root length were significantly higher in *D. ovalifolium* plants with no addition of localised phosphate ($P>0.05$) at final harvest, irrespective of mycorrhizal treatment (Table 2c).

Discussion

Our study indicates that neither internal biomass parameters (acid fuchsin stained roots) nor localised ERM densities (in the LMPs in our case) reflected the ability of AMF, from different genera, to stimulate plant growth and development. Interestingly, *P. phaseoloides* plants inoculated with *A. tuberculata* (BEG41), over a 20-week period in experiment 1, had very low levels of both the IRM and ERM (in the LMPs), but led to a significantly greater stimulation of plant growth than those colonised by *Gi. rosea* (BEG111), which had extensive development of the IRM and ERM at later harvests. Indeed, *Gi. rosea* decreased plant growth compared with non-mycorrhizal plants at 20 weeks in experiment 1. Comparisons with similar studies (Abbott and Robson 1985; Zhao et al. 1997) are not easy as different approaches, pot sizes and fungal/plant combinations were used, though Abbott and Robson (1985) and Jakobsen et al. (1992b) observed this lack of growth stimulation in studies on *Trifolium subterraneum* L. plants colonised by *Scutellospora calospora* (WUM12), which is also from the Gigasporaceae. It has been suggested for this isolate of *S. calospora* that the ERM is able to retain P within the IRM/ERM prior to translocation to the shoots of *T. subterraneum* plants (Jakobsen et al. 1992b). Interestingly, in another experiment on *D. ovalifolium*, polyphosphate (stained using 4′,6-diamidino-2-phenylindole) was shown to accumulate in the ERM of *Gi. rosea* (BEG111) but not of *G. manihotis* (BEG112) (Boddington and Dodd unpublished work). This provides further evidence that AMF in the Gigasporaceae control the transfer of P to the plant for growth and development as observed by others (Pearson and Jakobsen 1993). This does not mean that there are not other benefits to the plant as the mycorrhiza continues to develop.

The trends for development of the ERM (in the LMPs) by *A. tuberculata* (BEG41) were similar to that seen in other microcosm studies for *A. laevis* (WUM11) forming mycorrhizas with *T. subterraneum* plants (Jakobsen et al. 1992b). Jakobsen et al. (1992b) showed that the rate of P inflow to the roots of *T. subterraneum*, forming mycorrhizas with *A. laevis* (WUM11),

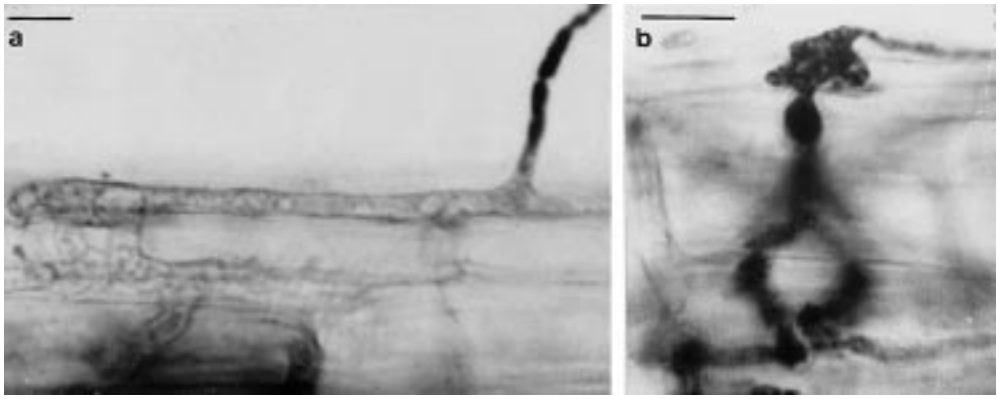

Fig. 5 Typical examples of the ALP activity in the entry point hyphae (formerly appressorium) and associated 50-μm lengths of IRM and ERM of either (**a**) *Gi. rosea* (BEG111) or (**b**) *G. manihotis* (BEG112), *bars* 50 μm

Table 2 Growth and P content of non-mycorrhizal *Desmodium ovalifolium* plants or those forming mycorrhizas with either *Gi. rosea* (BEG111) or *G. manihotis* (BEG112). Plants were harvested after (a) 3, (b) 6 or (c) 9 weeks. Values are the means of four replicates (*ns* the F-value was not significant between phosphate or mycorrhizal treatment)

	Factor	Shoot fresh wt. (g)	Shoot dry wt. (g)	Leaf area (cm^2)	Shoot P (mg)	Root fresh wt. (g)	Root dry wt. (g)	Root length (cm)	Root P (mg)
a	Mycorrhizal treatment								
	Gi. rosea (BEG111)	1.1	0.2	31.0	1.6	0.6	0.0	62	0.1
	G. manihotis (BEG112)	1.0	0.2	27.3	1.5	0.6	0.0	53	0.1
	Non-mycorrhizal	1.1	0.2	30.4	1.6	0.6	0.0	67	0.1
	LSD $P<0.005$	ns	ns	ns	ns	ns	ns	ns	ns
	Phosphate treatment								
	0 mg P kg^{-1}	0.9	0.1	26.1	1.5	0.5	0.0	50	0.1
	40 mg P kg^{-1}	1.0	0.2	25.7	1.5	0.6	0.0	61	0.1
	100 mg P kg^{-1}	1.4	0.2	37.0	1.7	0.7	0.0	70	0.1
	LSD $P<0.05$	ns	ns	ns	ns	ns	ns	ns	ns
b	Mycorrhizal treatment								
	Gi. rosea (BEG111)	18.2	2.4	795	12.2	6.3	0.4	129	0.7
	G. manihotis (BEG112)	15.3	2.6	874	14.5	6.8	0.4	135	1.1
	Non-mycorrhizal	9.2	1.4	563	12.7	3.9	0.2	89	0.7
	LSD $P<0.05$	4.5	0.8	240	ns	2.1	ns	ns	ns
	Phosphate treatment								
	0 mg P kg^{-1}	8.4	1.4	498	10.3	4.9	0.3	111	0.7
	40 mg P kg^{-1}	15.0	2.2	882	14.8	6.0	0.4	121	0.8
	100 mg P kg^{-1}	17.1	2.8	862	15.6	6.1	0.4	124	1.0
	LSD $P<0.05$	4.5	0.8	240	3.4	ns	ns	ns	ns
c	Mycorrhizal treatment								
	Gi. rosea (BEG111)	36.7	7.3	1711	21.1	14.8	1.4	337	1.5
	G. manihotis (BEG112)	35.7	6.9	1607	22.3	12.7	1.2	276	1.3
	Non-mycorrhizal	33.9	6.3	1535	14.3	14.3	1.2	350	1.1
	LSD $P<0.05$	ns	ns	ns	6.0	ns	ns	ns	0.3
	Phosphate treatment								
	0 mg P kg^{-1}	32.0	6.6	1441	14.1	15.8	1.5	412	1.3
	40 mg P kg^{-1}	37.8	7.3	1742	21.3	13.4	1.2	305	1.3
	100 mg P kg^{-1}	36.6	6.7	1670	22.3	12.7	1.1	246	1.3
	LSD $P<0.05$	ns	ns	ns	6.0	ns	0.3	65	ns

was approximately three times higher than that by either a *Glomus* spp. (WUM10) or *S. calospora* (WUM12) over a 47-day period. This led to significantly higher shoot and root growth in plants colonised by the *A. laevis* isolate. They concluded that it was the increased spread of the ERM of this fungus that led to this effect. In our study, we do not know if the low lengths of ERM (in the LMPs) measured for plants colonised by *A. tuberculata* (BEG41) reflected the presence of the ERM outside the LMP, nearer the main zone of root growth. Dodd (1994) showed, however, that the architecture of the ERM for *A. morrowiae* (PHIL11 A) and *G. manihotis* (BEG112) were also very different. This may influence P uptake, transport and/or transfer efficiency to the plant rather than total lengths or densities of ERM in soil cores. It was also apparent that the growth of plants inoculated with the mixture of species of AMF was not significantly differ-

ent from that observed when forming mycorrhizas with either *A. tuberculata* (BEG41) or *G. manihotis* (BEG112) alone. Obviously, we do not know which AMF species in the mixture colonised the plant and when, but future studies using molecular approaches for detection of AMF *in planta* may help elucidate this (Clapp et al. 1995; Tisserant et al. 1998).

The effect of a localised phosphate source, applied at different rates, had unique effects on the spread of the ERM (in the LMPs) in *D. ovalifolium* plants colonised by either *Gi. rosea* (BEG111) or *G. manihotis* (BEG112). The ERM (in the LMPs) formed by *Gi. rosea*, with no addition of localised phosphate, reached approximately 40 cm g^{-1} of substrate within 3 weeks after inoculation. In contrast, the ERM (in the LMPs) of *G. manihotis* increased gradually to approximately 20 cm g^{-1} at 9 weeks. In addition, localised phosphate (40 mg P kg^{-1}) stimulated proliferation of the ERM (in the LMPs) for *G. manihotis* but restricted the ERM of *Gi. rosea* to low levels. The reason behind the differences in hyphal proliferation (in the LMPs) between the two AMF is uncertain, but may indicate that *Glomus* spp. are better adapted to exploit or scavenge for sources of phosphate at higher concentrations. Interestingly, the level of P in the roots of plants colonised by *Gi rosea* (BEG111) may have been higher than in those colonised by *G. manihotis* (BEG112) (not quite statistically significant, *P*0.06) at final harvest. There is evidence, therefore, that the mechanisms for P uptake and transfer are different between the two AMF and this may reflect differences between these genera in terms of their natural ecological roles or life cycle requirements from the symbiosis.

The levels of IRM declined with localised phosphate addition as observed by other authors for isolates of *Glomus* spp. (Abbott et al. 1985; Amijee et al. 1989). However, the amount of ALP active IRM differed between both AMF in the present experiment. *Gi. rosea* (BEG111) had a constant amount of ALP activity in the IRM across the range of localised phosphate added whilst that of *G. manihotis* (BEG112) declined with increasing rates of phosphate addition. Ezawa et al. (1995), using isolates of *Gi. rosea* and *G. etunicatum* forming mycorrhizas with either marigold or leek plants over a 6-week period, observed that the ALP activity in the IRM of *Gi. rosea* was retained in the intercellular hyphae after the decline of arbuscules. In the same study, the ALP activity in the IRM of *G. etunicatum* was confined to the arbuscules and declined along with the arbuscular root length. They suggested, therefore, that this could indicate differences in the sites of P transfer between species of AMF from the two genera. In our study, we did not note a decline in arbuscule presence in plants colonised by *Gi. rosea* (BEG111) over time so cannot comment on the observations of Ezawa et al. (1995) about localisation of ALP activity in the hyphae rather than the arbuscules. Their study did not, however, correlate the ALP activity in the IRM with the P content of the plant. In our study, the P content of *D. ovalifolium* plants was similar, irrespective of the ALP activity in the IRM of *Gi. rosea* (BEG111) or *G. manihotis* (BEG112). This suggests differences in the efficiency of transfer of P from the fungus to the plant, which may be independent of histochemically stained ALP activity in the IRM. Further evidence for this was provided by Larsen et al. (1996), who observed an inhibitory effect of the fungicide benlate on hyphal P transport to *Cucumis sativus* L. plants by *Glomus caledonium* (BEG15), over a 47-day period, with no concurrent decrease in the ALP activity in the IRM.

Alkaline phosphatase activity was not detected in the zone of the entry point hyphae in mycorrhizas formed by *Gi. rosea* (BEG111), but was in those formed by *G. manihotis* (BEG112) and in *Glomus coronatum* (BEG49) in another experiment (Boddington and Dodd unpublished work). The reason for this is unclear, but may relate to the differential expression of phosphate transporters in the IRM and ERM, as observed by Harrison and van Buuren (1996) for an isolate of *G. versiforme* or to symbiosis-specific phosphatases in the IRM and ERM of AMF as indicated by Tisserant et al. (1998) in *G. microaggregatum* (BEG56). Further work is needed to clarify this.

The use of a range of AMF from different genera is a necessity if more knowledge on their ecological function is to be acquired. There are unique problems in this though, as observed with *A. tuberculata* (BEG41) in this study, where inoculum of the same age, colonised *P. phaseoloides* plants but not *D. ovalifolium* plants. Whilst this could be explained by host specificity, a more likely explanation is that it relates to the importance of the infective propagules in the inoculum. It has been shown, for example, that *A. laevis* (WUM11) (Gazey et al. 1993; Tommerup 1983), *A. trappei* (WUM19) and *Acaulospora* spp. (WUM18) (Gazey et al. 1993) produce spores which pass through a dormancy phase before germinating. This is a well-recognised characteristic of *Acaulospora* spp. which can remain quiescent for up to 8 months (Tommerup 1983). Dormancy is not often seen for species of *Glomus* or *Gigaspora*, but is found for many species of *Scutellospora* (Dodd personal observation). In such cases, it is further evidence for the unique life cycle strategies of AMF from different genera.

In conclusion, this study has shown clear intergeneric differences in the development of both the IRM and ERM of different isolates of *Acaulospora*, *Gigaspora* and *Glomus* in association with two seedlings of tropical forage legumes. Multiple harvests allowed comparison of the dynamics of AMF development from the three genera to be made. Although two different tropical legumes were grown (under similar growth and environmental conditions) the same rates of spread of *G. manihotis* (BEG112) and *Gi. rosea* (BEG111) were observed on each host. AMF from the three genera had unique mycelium development in plant roots and soil and appear to have different mechanisms for the con-

trol of P transfer to the host. Our study indicates that the ALP activity in the IRM of AMF, as revealed by histochemical staining, could be a functional marker useful for monitoring the metabolic activity of arbuscular mycorrhizas, but not for comparing the efficiency of different AMF species for P uptake or transfer in the two tropical legumes tested. There is, therefore, urgent need for more in depth analysis of the formation of mycorrhizas and their metabolic activity, not merely root colonisation alone. The differences in the localisation and efficiency of the ALP in the IRM and ERM of AMF from different genera may be of significance, though further work on this is required. The functional roles of AMF from different genera need further clarification so that their ecological strategies can be predicted, e.g. for use in revegetation of natural ecosystems such as sand dunes, where species from the Gigasporaceae appear to proliferate (Gemma and Koske 1988).

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