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Influence of colonisation by an arbuscular mycorrhizal fungus on the growth of seedlings of *Banksia ericifolia* (Proteaceae)

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Abstract Tap and primary lateral roots of seedlings of the putatively non-mycorrhizal *Banksia ericifolia* became marginally colonised when grown in an established mycelium of an arbuscular mycorrhizal (AM) fungus in the laboratory. A similar degree of colonisation was found in seedlings from an open woodland. All colonies lacked arbuscules. Two factors influencing colonisation and associated growth of host plants were examined experimentally: concentration of P in the soil and organic energy associated with the fungus. While some inoculated seedlings were slightly smaller when colonised by AM fungi, the results were inconsistent and never statistically significant. Seedlings take up insignificant quantities of soil P during early growth, even in the presence of abundant added P. Though colonisation was minor in all cases, an existing mycelium, whether or not connected to a companion plant, slightly increased the amount of root of *B. ericifolia* colonised by an AM fungus. All seedlings grew slowly. Shoots were significantly larger than roots, until the initiation of proteoid roots which commenced at about 40 days after germination, with both relatively high and low P supply.

Keywords Arbuscular mycorrhizal fungi · *Glomus pellucidum* · *Banksia ericifolia* · Proteaceae · Impoverished soil

Introduction

The soils derived from sandstone in the Sydney region are severely nutrient impoverished. Plants found on these soils have evolved mechanisms to enhance uptake and/or maximise the use of minerals, including sclerophylly (Beadle 1962), parasitism, carnivory, and formation of proteoid roots or mycorrhizas (Handreck 1997). These mechanisms enable plants to grow in soil with extremely low concentrations of P and/or increase the uptake of P from soil. However, while the low levels of P in Australian soils (Beadle 1962) may account for the ubiquity of mycorrhizas in Australian plant communities (Bellgard 1991; Brockoff and Allaway 1989; Brundrett and Abbott 1991; McGee 1986), members of some families commonly lack colonisation.

Members of the family Proteaceae are a common and major structural component of the vegetation found on sandstone soils in the Sydney region. Early studies of Proteaceae indicated that plants lacked mycorrhizas in root systems (Khan 1978; Malajczuk and Lamont 1981; Malajczuk et al. 1981). A more recent field survey found "typical" arbuscular mycorrhiza (AM) in roots of *Conospermum longifolium* Sm., *C. taxifolium* Sm. and *Telopea speciosissima* R.Br. in eastern NSW (Bellgard 1991). Vesicles and internal hyphae, but not arbuscules, were reported in roots of *Persoonia levis* (Cav.) Domin, *P. pinifolia* R.Br., *Hakea dactyloides* (Gaertn.) Cav., *Grevillea buxifolia* (Sm.) R.Br., *G. oleoides* Sieber ex Schult., *G. sphacelata* R.Br. (Bellgard 1991) and *G. ilicifolia* R.Br. (McGee 1986). Extensive hyphal colonisation was observed in fine but not proteoid roots of *Stenocarpus salignus* R. Br. used in parks and gardens of Sydney, Australia (M. Fokkes, unpublished data). In a preliminary experiment, internal hyphae but no arbuscules were observed in the roots of inoculated seedlings of *Banksia ericifolia* L.f., *Petrophile sessilis* Sieber ex Schult. & F. and Mustard, *Brassica* sp. L. (G.S. Pattinson, unpublished results) growing in soil with extremely low available P. The functional and ecological importance of the presence of hyphae of AM fungi in roots of Proteaceae is unknown.

Mycorrhizas are commonly associated with increased plant uptake of P from soil, though the response is determined, in part, by the P status of the soil relative to the physiological requirements of the plant. However, AM fungi in soil may reduce growth of seedlings. Growth and survival of a range of plant species, which were found

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to be free of mycorrhizas in pastures, were reduced in the presence of AM fungi in experimental conditions (Allen et al. 1989; Francis and Read 1995). The authors speculated that the reduced growth and survival were due to either competition for minerals between plant and mycorrhizal fungus, or the fungus imposing a carbon drain or having an allelopathic affect on the host. Whatever the mechanism, the same need not apply to woody species, such as the Proteaceae.

Many woody species of Proteaceae growing in soils overlying sandstone in the Sydney region have serotinous seed stores (Bradstock 1991). Fire kills the adult plant of *B. ericifolia* and seeds are released altogether after fire (Bradstock 1991; Bradstock and Myerscough 1981). The seedbed following fire has increased light, available water and minerals (Keith 1996), favouring seedling establishment. Fire reduces density of propagules of AM fungi in underlying soil (Pattinson et al. 1999), which in turn may influence the plant species composition during establishment (Gange et al. 1990, 1993; Grime et al. 1987). Seedlings of non-mycorrhizal plants may grow and establish more readily in soil following fire because densities of propagules of AM fungi have been reduced and seedlings are able to access more of the mineral resources than mycorrhizal seedlings.

Alternatively, the association between AM fungi and the non-host seedling may be transient. Colonisation may enhance initial uptake of P, enabling increased survival of seedlings. Colonisation may decline as the seedlings mature and the plant uses other mechanisms, such as proteoid roots, for mineral uptake. The aims of this experiment were to determine the potential of seedlings of *B. ericifolia* to be colonised by AM fungi and to quantify the effect of colonisation by AM fungi on seedlings of *B. ericifolia*.

Materials and methods

The influence of P on colonisation of B. ericifolia

The experiment had two stages, establishment of the hyphal network and the transplantation of seedlings into the hyphal network. The experiment comprised a 2×3x3 factorial of mycorrhizal status (with or without the addition of mycorrhizal inoculum), three phosphorus (P) treatments (0, 0.3 and 1 mg P per week), and three harvests (19, 33 and 47 days).

Pots with two compartments separated by a mesh were constructed from rectangular plastic tubing (100×50 mm) cut into 50- and 30-mm lengths. A stainless steel screen mesh, with a pore size of 32 μm, was glued between the lengths of the plastic tubing enabling hyphal but limiting root, penetration. The tubing was capped at each end by a plastic sheet 2 mm thick and one of the narrow, long sides was sawn off to form an elongated pot (50×80×95 mm: W × L × H). The smaller section of the pot is referred to as the hyphal compartment and the larger side as the companion plant compartment. The pots were filled with autoclaved (70 min at 121°C) soil mix consisting of 3 parts top soil (pH 5.3, EC 25 μS cm^{-1}, 1:5 H_2O) collected from a mixed eucalypt community at Lucas Heights and 7 parts river sand. Inoculum was derived from pot cultures of *Glomus pellucidum* McGee & Pattinson (isolate NH 1.5) grown on leeks, *Allium porrum* L. cv. Jumbo. The soil was rinsed from the leek roots and the roots cut into approximately 10-mm lengths and thoroughly mixed.

A single seedling of *Dodonaea triquetra* Wendl. was placed in the companion plant compartment of each pot and half the pots were inoculated by placing infected fresh root material (1 g) adjacent to the roots at transplantation. The pots were watered via a wick immersed in a reservoir of water. The pots were placed in a growth room with a day length of 13 h at a PAR at plant height of 700 μmol m^{-2} s^{-1} at 25°C. The night temperature was set at 20°C. All seedlings were grown for 9 weeks to enable fungal colonisation of the hyphal compartment. Seeds of *B. ericifolia* were germinated on a moist sand bed in the growth room and transplanted into the hyphal compartment when the root radical was approximately 10 mm long. *B. ericifolia* was chosen because it was consistently colonised by AM fungi in a preliminary experiment.

A base nutrient solution containing 3.3 mM KNO_3, 1 mM $Ca(NO_3)_2.4H_2O$, 0.6 mM $MgSO_4.7H_2O$ and 1 ml l^{-1} of micronutrient solution and FeEDTA was prepared, to which was added either 0, 44 or 145 μM KH_2PO_4. Each week, 10 ml of solution was added to each pot as appropriate. This is equivalent to 0 (P 0), 0.3 (P 0.3) and 1 (P 1) mg of P per week, respectively, thought to be inadequate, adequate or in excess of the requirements of *B. ericifolia* (Handreck 1997).

Five replicates for each treatment were destructively harvested at 19 and 33 days. Due to an error in the labelling of the treatments, only 3, 4 and 4 replicates were harvested for the mycorrhizal P 0, the non-mycorrhizal P 0.3 and P 1 treatment, respectively, at 47 days. The plants were removed from the soil and washed and the root and shoot fresh weight measured. All the roots were cleared and stained (Phillips and Hayman 1970) and the total length of roots and mycorrhizas determined by the grid intersect method (Giovannetti and Mosse 1980). The shoots were dried at 70°C for 24 h and weighed. P content of the total shoots of plants harvested at 19, 33 and 47 days was determined using the ammonium molybdate method (Allen et al. 1974).

Data were analysed using the Systat 5.04 programme (SPSS Inc.). Two-way ANOVA was used to determine differences between means for shoot dry weight, shoot P concentration and percent colonisation of *B. ericifolia* grown in the presence or absence of the hyphae of *G. pellucidum*. Data on percent colonisation were arcsine transformed (Zar 1996) prior to analysis.

Contribution of light intensity to the interaction between B. ericifolia seedlings and G. pellucidum

Compartmental pots were filled with autoclaved 30% Lucas Heights soil mix and *D. triquetra* seedlings transplanted into the companion plant compartment. Where appropriate, the seedlings were inoculated with *G. pellucidum*. The compartmental pots were placed in the growth room and plants allowed to grow for 9 weeks. *B. ericifolia* seeds weighing 22.5–32.5 mg were germinated on moist sand and transplanted into the hyphal compartment of the pots when seedlings had a root radical approximately 10 mm long.

Seedlings were either inoculated or not. Seedlings were grown under intense light (700 μmol m^{-2} s^{-1}) except for one treatment that was shaded from day 19. Hyphae either remained intact or were severed along the mesh and a plastic sheet inserted to prevent reconnection between the chambers (Table 1). Five replicate seedlings of *B. ericifolia* were harvested at 19, 33 and 47 days (Table 1). At harvest, the soil was rinsed from the roots and the shoots and roots weighed. All the roots were cleared and stained and the total length of roots and mycorrhizas determined by the grid intersect method. The shoots were dried at 70°C for 24 h and weighed.

An additional five replicates were harvested at 33 and 47 days in the ± mycorrhizal, full light treatment. The soil was washed from the roots and the plants were weighed and dried for 24 h. P content of a further 20 seeds of *B. ericifolia* weighing 22.5–32.5 mg, the total shoots of plants harvested after 19, 33 and 47 days, and the roots and shoots of the additional replicates were determined (Allen et al. 1974).

Two-way ANOVA was used to determine differences between means for the shoot dry weight, shoot P concentration and percent colonisation of *B. ericifolia* grown in the presence or absence of the

Table 1 Summary of treatments imposed on the*Dodonaea triquetra* host plants and *Glomus pellucidum* (isolate NH 1.5) growing in split pots. The host plants were inoculated with NH 1.5 and grown in high light conditions or shaded. Hyphae severed at the mesh and the imposition of a shaded treatment (50%) are indicated, together with the time when the *Banksia ericifolia* seedlings were harvested (days after planting)

Inoculation	Light (%)	Hyphae severed	Harvest
Nil	100	–	19, 33, 47
NH 1.5	100	Hyphae not severed	19, 33, 47
NH 1.5	100	Day zero	33, 47
NH 1.5	100	Day 19	33, 47
NH 1.5	50	Hyphae not severed	33, 47

hyphae of *G. pellucidum*. The shoot dry weights of seedlings of *B. ericifolia* and the host plants grown in the presence and absence of mycorrhizal fungi, in the full light treatment and the additional treatments harvested for shoot and root P analysis were pooled in the ANOVA.

Contributions of the companion plant and the presence of mycelium to the growth of B. ericifolia

Inoculated and uninoculated seedlings of *D. triquetra* were grown in the companion plant chamber as above. At the point of transplanting pregerminated seed of *B. ericifolia*, two further treatments were added to determine the effect of the companion on removal of nutrients from the hyphae in the hyphal chamber, and the presence of hyphae on removal of minerals from the hyphal chamber. In one treatment, the soil in the hyphal chamber was removed and replaced with fresh soil mix, and a shield was placed against the screen to slow the return of hyphae from the companion plant chamber. In the other treatment, the inoculated companion was replaced with fresh soil mix, leaving the hyphal network entire in the hyphal chamber. Seedlings of *B. ericifolia* were harvested from hyphal chambers of the inoculated, uninoculated and 'hyphal chamber replaced' treatments after 3 weeks, and for all four treatments after 6 and 13 weeks. Total fresh weight, root fresh weight, shoot dry weight, root length and colonisation were determined as above.

Naturally colonised seedlings of B. ericifolia and B. serrata

Five seedlings each of *B. ericifolia* and *B. serrata* L.f. were collected in September 1998 from an area of open woodland adjacent to Lucas Heights Waste Disposal Centre, NSW, Australia that was burnt in December 1997. Root material of each seedling was collected, although recovery of entire root systems was impossible. The soil was rinsed from the roots and the roots cleared and stained and examined for AM.

Results

Influence of P on the colonisation of *B. ericifolia*

Colonisation developed in all seedlings of *B. ericifolia* at each harvest in all P treatments, except at the first harvest in the treatment with the highest added P (Table 2). Only the taproot and laterals adjacent to the taproot were

Table 2 Mean (± SE) percent colonisation of roots, shoot dry weight (mg), % P concentration in shoots, total P in shoots, root length (cm), and ratio of shoot dry weight to root length (SDW/RL) (mg cm^{-1}) of *B. ericifolia* grown in pots inoculated (+AM) or not (−AM) with *G. pellucidum* and harvested at 19, 33 or 47 days. Means with different letters are significantly different ($P<0.05$) within mycorrhizal treatments. Means marked * are significantly different ($P<0.05$), within P treatments

Parameter	Day 19		Day 33		Day 47	
	+AM	−AM	+AM	−AM	+AM	−AM
Colonisation						
P 0	14 ± 6a	–	9 ± 4a	–	6 ± 1a	–
P 0.3	6 ± 2ab	–	9 ± 2a	–	8 ± 1a	–
P 1	0 ± 0b	–	9 ± 1a	–	7 ± 1a	–
Shoot dry wt.						
P 0	26 ± 3a	25 ± 1a	34 ± 3a	50 ± 2a	78 ± 2a	125 ± 9a
P 0.3	19 ± 2a	21 ± 3a	37 ± 4a	38 ± 2a	67 ± 9a*	125 ± 20a
P 1	25 ± 1a	26 ± 2a	49 ± 5a	44 ± 1a	68 ± 14a	105 ± 2a
% Shoot P						
P 0	0.98 ± 0.09a	1.24 ± 0.04a	0.87 ± 0.11a	0.61 ± 0.02a	0.26 ± 0.01a	0.20 ± 0.02a
P 0.3	1.13 ± 0.04a	1.21 ± 0.06a	0.83 ± 0.05a	0.77 ± 0.06a	0.42 ± 0.06a	0.26 ± 0.03a
P 1	1.19 ± 0.08a	1.00 ± 0.08a	0.70 ± 0.08a	0.74 ± 0.04a	0.41 ± 0.05a	0.29 ± 0.02a
Total shoot P						
P 0	249 ± 20a	306 ± 7a	286 ± 27a	304 ± 8a	206 ± 6a	239 ± 11a
P 0.3	211 ± 17a	249 ± 33a	300 ± 17a	294 ± 20a	262 ± 14a	312 ± 39a
P 1	292 ± 11a	260 ± 32a	331 ± 20a	321 ± 10a	257 ± 25a	305 ± 23a
Root length						
P 0	13 ± 2a	16 ± 3a	34 ± 6a*	58 ± 5a	119 ± 26a	182 ± 19a
P 0.3	16 ± 6a	14 ± 2a	30 ± 10a	49 ± 9a	92 ± 14a*	232 ± 27a
P 1	11 ± 2a	23 ± 2a	39 ± 6a*	64 ± 4a	72 ± 6a*	225 ± 14a
SDW/RL						
P 0	–	–	1.1 ± 0.2	0.9 ± 0.1	0.8 ± 0.2	0.7 ± 0.1
P 0.3	–	–	1.7 ± 0.4	0.9 ± 0.2	0.7 ± 0.1	0.6 ± 0.1
P 1	–	–	1.4 ± 0.3	0.7 ± 0.0	0.8 ± 0.0	0.5 ± 0.0

Table 3 Mean (± SE) percent colonisation, shoot dry weight (mg), and root length (cm) of *B. ericifolia* seedlings grown in the presence or absence of arbuscular mycorrhizal (AM) fungal hyphae and the shoot dry weight of the*Dodonaea triquetra* host plant.*(n)* indicates the number of replicates included in the statistical analysis

Parameter	19 Days		33 Days		47 Days	
% Colonisation						
Full light, +AM, hyphae intact	5 ± 4	(5)	2 ± 1	(5)	2 ± 1	(5)
Full light, hyphae severed day zero	–	–	0 ± 0	(3)	1 ± 1	(4)
Full light, hyphae severed day 19	–	–	1 ± 1	(5)	2 ± 1	(5)
Half light, +AM, hyphae intact	–	–	1 ± 1	(5)	1 ± 1	(5)
Shoot dry wt.						
Full Light, –AM	27 ± 5	(5)	56 ± 7	(9)	135 ± 27	(10)
Full light, +AM, hyphae intact	23 ± 2	(5)	39 ± 3	(10)	85 ± 16	(9)
Full light, hyphae severed day zero	–	–	35 ± 2	(3)	136 ± 17	(4)
Full light, hyphae severed day 19	–	–	43 ± 12	(5)	111 ± 14	(5)
Half light, +AM, hyphae intact	–	–	38 ± 5	(5)	86 ± 20	(5)
Root length						
Full Light, –AM	17 ± 5	(5)	64 ± 20	(5)	179 ± 77	(5)
Full light, +AM, hyphae intact	19 ± 4	(5)	52 ± 17	(5)	104 ± 24	(5)
Full light, hyphae severed day zero	–	–	37 ± 6	(3)	135 ± 19	(4)
Full light, hyphae severed day 19	–	–	49 ± 18	(5)	142 ± 15	(5)
Half light, +AM, hyphae intact	–	–	50 ± 12	(5)	106 ± 29	(5)
Shoot dry wt. of host plant						
Full Light, –AM	36 ± 8	(5)	58 ± 21	(10)	31 ± 3	(10)
Full light, +AM, hyphae intact	515 ± 133	(5)	361 ± 12	(10)	400 ± 21	(10)
Full light, hyphae severed day zero	–	–	292 ± 27	(5)	519 ± 108	(5)
Full light, hyphae severed day 19	–	–	350 ± 18	(5)	521 ± 119	(5)
Half light, +AM, hyphae intact	–	–	310 ± 13	(5)	400 ± 64	(5)

colonised. Hyphae and vesicles were present but no arbuscules were observed. The application of P at a rate of 1 mg per week appeared to delay and reduce colonisation at the first and second harvest; however, the differences were not statistically significant ($P>0.05$) at the first harvest (Table 2). Two seedlings had started to form proteoid roots by 6 weeks. The proteoid roots were not colonised by *G. pellucidum*, but were surrounded by wefts of hyphae.

The relationship between the shoot dry weight and treatments varied over the experimental period. The shoot dry weight of the seedlings of *B. ericifolia* grown in the presence and absence of the hyphae of the mycorrhizal fungus was similar at 19 and 33 days, except for the plants in the P 0 treatment and harvested at 33 days (Table 2). In the ANOVA, the factors Myc and P interacted in the analysis of the shoot dry weight at 33 days. At low P concentrations, plant growth in the presence of AM fungi tended to be slower than in the absence of AM fungi. At higher P concentrations, plants in the presence of AM fungi grew at similar or faster rates than uninoculated plants (Table 2). At 47 days, the shoot dry weight of the *B. ericifolia* seedlings grown in the inoculated soil was consistently lower in all P treatments (Table 2).

Though low overall, shoot P was variable within and between treatments. The percent concentration of P in the shoots of *B. ericifolia* seedlings was similar in the P 0.3 and P 1 treatments (Table 2). The mean concentration of P in the seedlings of *B. ericifolia* grown in the P 0 treatments, harvested at 33 and 47 days and in the presence of *G. pellucidum* appeared to be higher than in the seedlings grown in the absence of the fungus (Table 2). However, the differences were not statistically significant ($P>0.05$). The total shoot P was similar ($P>0.05$) in plants grown in the presence and absence of the AM fungus, in all P treatments and over time (Table 2).

Root length of seedlings of *B. ericifolia* was reduced in the presence of *G. pellucidum* (Table 2), which resulted in a higher ratio of shoot dry weight to root length (ANOVA, $P<0.05$) for plants harvested at 33 and 47 days (Table 2, Table 3). However, no differences in means were detected when tested by Tukey's HSD.

Contribution of light intensity to the interaction between *B. ericifolia* and *G. pellucidum*

Seedlings of *B. ericifolia* were colonised at each harvest, except those grown in the pots with hyphae severed prior to transplanting and harvested at 33 days (Table 3). Colonisation developed predominantly in the taproot. Hyphae and vesicles were present at 19 days, but only hyphae at 33 and 47 days. Arbuscules were not observed at any harvest. Six seedlings had started to form proteoid roots by 6 weeks. External hyphae surrounded the lateral and proteoid roots, from which fungi penetrated the root surface, though further colonisation did not develop. Epidermal colonisation could not be quantified readily and is not included.

Uninoculated seedlings of *B. ericifolia* appeared to be consistently larger than seedlings inoculated with *G. pellucidum*, but the differences were not statistically significant ($P>0.05$: Table 3). Inoculated seedlings of *B. ericifolia* harvested at 47 days tended to be smaller in full light and shaded treatments than in the treatments in which the hyphae were severed before planting or at 19 days ($P>0.05$).

Table 4 Mean (± SE) proportion P in shoots and roots, total P in shoots and roots of *B. ericifolia* seedlings grown in the presence or absence of AM fungal hyphae. *(n)* indicates the number of replicates included in the statistical analysis

Parameter	Day 19		Day 33		Day 47	
% Shoot P						
Full Light, –AM	1.06 ± 0.09	(5)	0.51 ± 0.06	(9)	0.22 ± 0.06	(10)
Full light, +AM, hyphae intact	1.00 ± 0.09	(5)	0.80 ± 0.08	(10)	0.27 ± 0.03	(9)
Full light, hyphae severed day zero	–	–	0.73 ± 0.04	(3)	0.14 ± 0.03	(4)
Full light, hyphae severed day 19	–	–	0.85 ± 0.21	(5)	0.19 ± 0.04	(5)
Half light, +AM, hyphae intact	–	–	0.78 ± 0.12	(5)	0.27 ± 0.06	(5)
% Root P						
Full Light, –AM	–	–	0.09 ± 0.00	(4)	0.07 ± 0.00	(5)
Full light, +AM, hyphae intact	–	–	0.07 ± 0.01	(5)	0.07 ± 0.00	(4)
Total shoot P						
Full Light, –AM	238 ± 16	(5)	257 ± 6	(9)	177 ± 9	(10)
Full light, +AM, hyphae intact	252 ± 6	(5)	292 ± 12	(10)	195 ± 2	(9)
Full light, hyphae severed day zero	–	–	249 ± 8	(3)	174 ± 7	(4)
Full light, hyphae severed day 19	–	–	277 ± 8	(5)	190 ± 26	(5)
Half light, +AM, hyphae intact	–	–	277 ± 16	(5)	192 ± 11	(5)
Total root P						
Full Light, –AM	–	–	11 ± 2	(4)	19 ± 6	(5)
Full light, +AM, hyphae intact	–	–	6 ± 1	(5)	14 ± 5	(4)

The total P in the shoots was variable and after 19 days was higher than that in seeds. Total P of shoots appeared to decline after 33 days (Table 4), though the differences were not statistically significant ($P>0.05$). The total P in the roots, though considerably less than shoots, increased from the harvest at 33 to 47 days. At 33 days, the total P in the roots tended to be higher in uninoculated than inoculated plants, though the differences were not statistically significant ($P>0.05$: Table 4).

The percent P in the shoots of seedlings of *B. ericifolia* was similar in the plants grown in the presence and absence of *G. pellucidum* at 19 days (Table 4). At 33 days, inoculated plants had a higher concentration of P in the shoots than uninoculated seedlings. At 47 days, inoculated seedlings grown in the full light and shaded treatment appeared to have higher concentrations of P in the shoots than those grown in the treatments in which the hyphae were severed before transplantation of seedlings or at 19 days, or those grown in the absence of the fungus (Table 4). However, none of the differences were statistically significant ($P>0.05$).

The root growth of inoculated and uninoculated seedlings of *B. ericifolia* was similar at 19 days. At 37 and 47 days, the uninoculated plants had longer roots (Table 3) but the differences were not statistically significant ($P>0.05$).

Contribution of the companion plant and the presence of mycelium to growth of *B. ericifolia*

Shoot dry weight of *B. ericifolia* was not significantly affected by any treatment at any harvest ($P>0.05$). Root length at 3 and 6 weeks was unaffected by treatment. The formation of extensive proteoid roots in all treatments precluded measurement of root length at 13 weeks, and root weight was an unreliable indicator of actual mass because of the presence of considerable quantities of soil within the proteoid root mass. Only tap roots of two seedlings in the chamber in which hyphae remained attached to the companion were colonised at 3 weeks. These both had less than 10% colonisation. No other seedlings were colonised at any harvest. All inoculated companion plants had more than 30% of the fine roots colonised (data not shown).

Naturally colonised seedlings of *B. ericifolia* and *B. serrata*

Hyphae, typical of AM fungi, were observed in the roots of three and four out of the five seedlings of *B. ericifolia* and *B. serrata*, respectively. Colonisation was generally slight and similar to colonisation of experimental plants. Hyphae typical of AM fungi were common on the surface of the root, occasionally penetrating the root. In roots of *B. serrata*, invaginations were observed in the epidermal cells. Septate fungi lacking clamps, resembling a *Rhizoctonia*, were also observed in the roots of *B. ericifolia* and *B. serrata*, especially in the proteoid roots.

Discussion

Seedlings of *B. ericifolia* and *B. serrata* from an open woodland were colonised by AM fungi and the former were colonised in the laboratory when inoculated with *G. pellucidum*. Colonisation was limited to taproots and to a lower extent to primary laterals of seedlings. Colonisation

consisted of hyphae and vesicles. No arbuscules of AM fungi were present in the roots of *B. ericifolia*.

The effect of colonisation on growth of *B. ericifolia* was insignificant. While some experiments resulted in minor differences in rates of growth or development between inoculated and uninoculated seedlings for short periods, other experiments did not. While selection of seed for experiments reduced variation, it is likely that the differences in growth between seedlings and experiments have no biological significance. These results are in stark contrast to the effect of AM fungi on other non-mycorrhizal plants (Allen et al. 1989; Francis and Read 1995; G.S. Pattinson, unpublished data).

The reasons for the differences between *B. ericifolia* and other plant species that normally lack mycorrhizas are unclear. The experimental soils and the soils in which *B. ericifolia* grows normally are extremely impoverished. The mechanisms enabling continued growth of seedlings might include temporary increase in uptake of P from soil, efficient use of P within seedlings, or storage of P in seed until uptake from soil is adequate for plant growth. The presence of hyphae in soil around or in root systems apparently did not alter mineral uptake, and the seedlings did not respond to additional P in the soil. Indeed, seedlings had extremely reduced root systems until the development of proteoid roots. Thus, reserves of P in seeds appears to be overwhelmingly important for growth of seedlings. The constant total shoot P over 47 days has been observed in other species of Proteaceae, including *B. laricina* C. Gardner (Stock et al. 1990), *B. grandis* Willd. (Barrow 1977) and *Hakea sericea* Schrader (Mitchell and Allsopp 1984; Stock et al. 1990). It is clear that AM fungi do not contribute to the uptake of P in this family. At least during growth of seedlings and prior to development of the proteoid roots, P from seed stores is recycled within the plant and especially retained in the shoot system. Retention of P in the shoots enables efficient use of P in establishing the shoot system.

The mechanism for control of initiation and spread of colonisation is unclear. *B. ericifolia* is normally found in open vegetation and plants are thus light saturated. An established mycelium of AM fungi appears to be essential for initiation of colonisation of roots. However, the mycelium need not be attached to a companion plant and the companion may even be shaded. These responses indicate control of fungal ingress to roots by *B. ericifolia*.

Seedlings of *B. ericifolia* control the spread of colonisation. Only primary and a few lateral roots were colonised in these investigations. Supply of P to the soil or organic carbon did not appear to modify the host control. Addition of P to the soil at levels in excess of host requirements appeared to inhibit the AM fungus directly (Amijee et al. 1993a, 1993b), rather than the interaction. Continued colonisation of fine roots is only rarely observed in Proteaceae (e.g. *Stenocarpus*, M. Fokkes, unpublished). The mechanisms regulating colonisation by AM fungi require more careful examination, though they do not appear to include response to edaphic conditions.

The ecological significance of the interaction between members of the Proteaceae and AM fungi remains to be explored. The reduced growth rates and increased mortality of non-mycorrhizal herbaceous species result in these species being competitively excluded from stable plant systems (Francis and Read 1995; Ocampo 1986). In the field, survival of seedlings of *B. ericifolia* and other *Banksia* spp. is greatest in areas that have been burnt recently (Bradstock 1991; Bradstock and Mycerscough 1981; Cowling and Lamont 1987; Zammit and Westoby 1988) and, indeed, plants disperse most of their seed onto adjacent soil, thus maintaining "thickets" of single species in some cases (Hammill et al. 1998). Increased survival of seedlings has been attributed to higher mortality in unburnt areas due to predation and water deficit. AM fungi are unlikely to reduce survival of seedlings of *Banksia* at unburnt sites.

The spatial density of AM fungi in soils underlying natural vegetation is heterogeneous (Boerner et al. 1995; Brundrett and Abbott 1994; Brundrett and Juniper 1995; McGee 1989). The heterogeneity is associated with the mycorrhizal status of the plants present and the distribution and density of the roots within the soil profile (Allen 1991; Allen and Allen 1984; Allen et al. 1989; Brundrett and Abbott 1994; Miller et al. 1983). The presence of non-mycorrhizal host plants probably reduces the density of AM fungal propagules in the surrounding soil (Read 1994; Reeves et al. 1979), especially as *Banksia* spp. form dense mats of proteoid roots at the surface of the mineral soil. The reduced densities of AM fungi may provide *B. ericifolia* seedlings a competitive advantage over potentially mycorrhizal seedlings germinating in these areas, ultimately leading to the support of thickets of non-mycorrhizal plants where AM fungi are severely depleted, surrounded by zones where mycorrhizal plants sustain the fungal symbionts. Thickets of *B. ericifolia* and similar species probably sustain the heterogeneous patterns of AM fungi under native vegetation systems.

These experiments demonstrate that seedlings of presumed non-mycorrhizal woody plants may be colonised to a very limited extent by AM fungi, especially when challenged in a hyphal network connected to a host plant. In the case of *B. ericifolia*, seedlings rapidly inhibited colonisation and were not uniformly disadvantaged by the fungi. *B. ericifolia* is likely to alter the structure of plant communities because seeds are distributed locally, inducing and sustaining clumps of the host. Dense clumps of *B. ericifolia* are likely to reduce the survival of AM fungi in the soil, reducing the chances of mycorrhizal plants, including most N-fixing plant species, of establishing themselves in competition. Because seedlings of *B. ericifolia,* and probably other species of Proteaceae, are unaffected by the presence of AM fungi, they may assume a considerable proportion of a landscape. This will have consequences for establishment of mycorrhizal species and possibly the productivity of the landscape.

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