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Density dependent interactions between VA mycorrhizal fungi and even-aged seedlings of two perennial Fabaceae species

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Summary. The interaction of density and mycorrhizal effects on the growth, mineral nutrition and size distribution of seedlings of two perennial members of the Fabaceae was investigated in pot culture. Seedlings of *Otholobium hirtum* and *Aspalathus linearis* were grown at densities of 1, 4, 8 and 16 plants per 13-cm pot with or without vesicular-arbuscular (VA) mycorrhizal inoculum for 120 days. Plant mass, relative growth rates, height and leaf number all decreased with increasing plant density. This was ascribed to the decreasing availability of phosphorus per plant as density increased. *O. hirtum* was highly dependent on mycorrhizas for P uptake but both mycorrhizal and non-mycorrhizal *A. linearis* seedlings were able to extract soil P with equal ease. Plant size distribution as measured by the coefficient of variation (CV) of shoot mass was greater at higher densities. CVs of mycorrhizal *O. hirtum* plants were higher than those of non-mycorrhizal plants. CVs of the facultatively mycorrhizal *A. linearis* were similar for both mycorrhizal and non-mycorrhizal plants. Higher CVs are attributed to resource preemption by larger individuals. Individuals in populations with high CVs will probably survive stress which would result in the extinction of populations with low CVs. Mass of mycorrhizal plants of both species decreased more rapidly with increasing density than did non-mycorrhizal plant mass. It is concluded that the cost of being mycorrhizal increases as plant density increases, while the benefit decreases. The results suggest that mycorrhizas will influence density-dependent population processes of facultative and obligate mycorrhizal species.

Key words: Vesicular-arbuscular mycorrhizas – Density-dependence – Plant population dynamics – Resource depletion – Phosphorus

Many studies have examined the effect of density on plant growth, but the effect of plant mutualists such as mycorrhizas on the outcome of these experiments is seldom explicitly addressed. Most terrestrial plants form mycorrhizas and while the effect of the mutualism on individuals is fairly well established (Harley and Smith 1983), the effect on populations is less clear. Evidence that individual-level mutualism affects population dynamics is difficult to obtain (Addicott 1986). If plants grow at densities lower than those which result in rapid overlap of resource depletion zones then the influence of mycorrhizas on population growth may be the sum of the effects of mycorrhizas on the individual plants making up that community. Thus the mutualism may exist at the individual level and may influence population growth rates without affecting equilibrium population densities. However at some stage resource depletion zones are likely to overlap and then the effects of mycorrhizas on the individual are unlikely to be the same at different densities. The influence of a mutualist may depend on which part of the host's life cycle it affects and whether that part of the life cycle influences population dynamics. Addicott (1986) predicts that the more ways a mutualist benefits another species the more likely it is to affect the density of that species. Since mycorrhizas impose a carbon cost on host plants which affects the growth of very young seedlings, and because plants have differing dependencies on mycorrhizas, their effect on population dynamics may be considerable, although mycorrhizal benefit to the individual plant may not be obvious in the field.

Reduction in plant growth with increasing density is a commonly observed phenomenon (Clark 1990; Firbank and Watkinson 1990) and competition for scarce resources is usually responsible. In two experiments comparing the growth and P nutrition of mycorrhizal and non-mycorrhizal plants at different densities, response to mycorrhizal infection was greatest at the lowest density and was ascribed to the more efficient uptake of phosphorus by mycorrhizal roots (Bååth and Hayman 1984; Koide 1991). At higher plant densities, fewer differences were observed between mycorrhizal and non-mycorrhizal plants as the P depletion zones of their roots over-

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lapped. While these experiments observed the reduction in benefit of a mutualism at higher densities they did not explore the consequences on population size structure.

When investigating the effect of density on populations the range of experimental plant sizes is probably a more ecologically meaningful measure than the mean plant sizes. Studies which only look at the effect of density on mean plant size have been criticized as they fail to appreciate the effect of the range of differences in size on the dynamics of a population (Benjamin and Hardwick 1986; Hara 1988). Various measures of plant size distributions exist (Hara 1988). Weiner and Solbrig (1984) recommend that measures of plant size variability are best portrayed through measures of inequality such as the Gini coefficient or the coefficient of variation (CV) which are closely correlated in most cases (Weiner and Thomas 1986).

In a survey of 16 experiments, inequalities in shoot size increased with increasing density in 14 cases (Weiner and Thomas 1986). They concluded that in most cases competitive interference does not act in the same manner as reduction in resources caused by abiotic factors and suggest that the inequality in size is due to preemption of resources by larger plants. This will result in one-sided competition, with the larger plants suppressing the growth of smaller plants relatively more than the smaller plants can influence the larger plants, and will produce greater inequality in growth at higher densities.

One consequence of mycorrhizal plants growing at high density is that they may be linked together by a hyphal network and that resources may pass along this network from source to sink plants (Chiarello et al. 1982; Francis et al. 1986). If this is the case it may be expected that size inequality may decrease with increasing density. If resource sharing along hyphal links does not occur, increasing density will result in increasing size variability. As density increases the cost of being mycorrhizal will probably increase. We therefore expect mean plant size to decrease more rapidly with increasing density when the plants are mycorrhizal. So while the benefit of being mycorrhizal may decrease for the individual as density rises, the effect of mycorrhizas on population development may not be insignificant.

In this paper we investigate the interactive effects of mycorrhizas and density on the mineral nutrition, growth and size distribution of seedlings of two perennial species, *Aspalathus linearis* (Burm. f.) Dahlgren and *Otholobium hirtum* (L.) C.H. Stirton in a pot experiment. These species show different responsiveness to mycorrhizas and are therefore ideal for testing the individual mycorrhizal, plant density and interactive effects of mycorrhizas and plant density.

Materials and methods

Plant species

O. hirtum and *A. linearis* are endemic to the fynbos region of South Africa. Both species are evergreen members of the Fabaceae. *O. hirtum* has hairy, trifoliate leaves while *A. linearis* has needle-like leaves. Recruitment of seedlings in fynbos occurs during winter following wild fires. Species of *Otholobium, Aspalathus* and other legumes are frequently dominant in the first few years following fire, and *Aspalathus* species may form dense stands. The soils on which fynbos grows are of very low nutrient status and competition for nutrients and nutrient depletion are probably important community determinants (Stock and Allsopp 1992).

Plant growth

O. hirtum seeds were collected from adult plants at the fynbos biome intensive study site, Pella (33°31′ S, 18°32′ E), and seeds of *A. linearis* were obtained from the Rooibos Tea Control Board, Clanwilliam, South Africa. *O. hirtum* seeds were immersed in boiling water which was allowed to cool, drained off and the seeds were incubated for 12 h at 4° C. *A. linearis* seeds were acid scarified for 1 h (Engelbrecht et al. 1983) and rinsed in sterile water. Seeds were sown at double the final densities in 13 cm diameter plastic pots containing an autoclaved acid washed sand/soil mixture (1 : 1). The soil, collected from the top 20 cm at Pella, is a fine, acid sand with low total and available concentrations of P and N (Mitchell et al. 1984; Stock and Lewis 1986). Organic matter in the sand/soil mix was 0.42 % and pH was 4.54. Mycorrhizal inoculum was supplied as a mixture of soil, spores and roots from a culture of *Acaulospora morrowae* Spain and Schenck (obtained from INVAM, West Virginia University, Morgantown, WV 26506–6057, USA) growing on *Medicago sativa* L. Inoculum soil (20 g) was layered 5 cm below the final soil surface. Autoclaved inoculum soil and a spore-free wash of inoculum soil filtered through Whatman #4 filter paper was

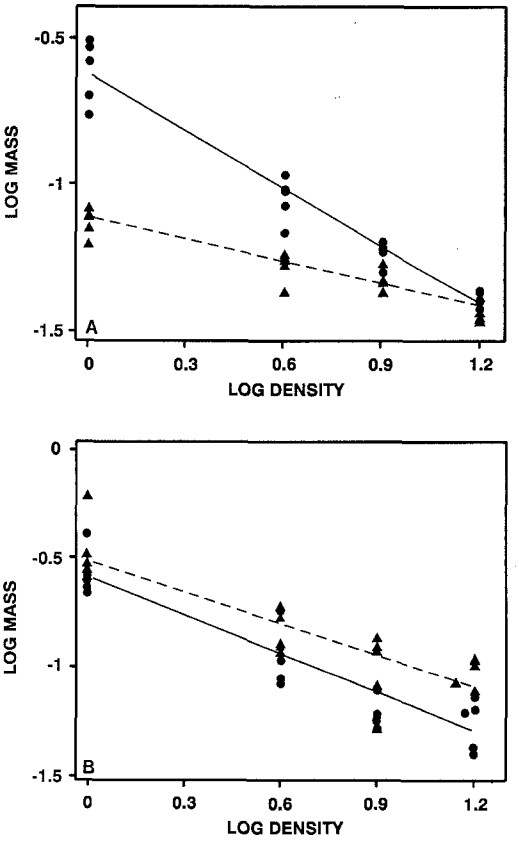

Fig. 1A, B. Effect of final plant density on mean plant mass when **A** *Otholobium hirtum* and **B** *Aspalathus linearis* are grown with *(VAM: solid lines, dots)* or without *(NM: dashed lines, triangles)* vesicular-arbuscular mycorrhizal inoculum. Slopes for VAM and NM plants are significantly different ($P<0.05$)

supplied to the non-mycorrhizal pots. All pots received a filtrate of freshly collected rhizosphere soil on the day of sowing and on day 30 to ensure a supply of rhizobia for nodulation.

Germination of both species is synchronous. *A. linearis* and *O. hirtum* pots reached the required densities 8 and 12 days respectively after sowing and these days were regarded as time zero. Plant densities of 1, 4, 8 or 16 plants per pot were maintained by pulling out additional seedlings as they emerged. Care was taken not to disturb the remaining plants and most of the root systems of weeded plants were removed. Plants were grown in a controlled environment growth chamber (RH 50%, daylength 16 h, day/night temperatures 10° C/25° C, PAR 320 μmol m^{-2} s^{-1}) and watered three times a week with distilled water.

Determination of plant parameters

Eight pots per species were established for each treatment combination (viz. presence or absence of mycorrhizas, and four densities). Three pots of each combination were harvested at day 30.

The rest of the plants (five pots per treatment combination) were harvested on day 120. Roots were freed from the soil with a gentle spray of tap water. Dry weights of individual shoots were measured. The root systems of individual *O. hirtum* seedlings were successfully separated and weighed but those of *A. linearis* seedlings were pooled as they were too brittle for accurate separation. Height of plants above the position of the cotyledons was measured and number of leaves counted. Length of youngest expanded leaf and cluster root numbers were also determined for *A. linearis*. Nodules were counted and a subsample of roots from each pot cleared and stained to determine vesicular-arbuscular mycorrhizal infection (Phillips and Hayman 1970). Total P and N of the pooled plant material from each pot was determined by the molybdenum-blue method for P (Murphy and Riley 1962) after digestion in a tri-acid mix (Jackson 1958) and N was determined colorimetrically (Smith 1980) following Kjeldahl digestion using a selenium catalyst.

Data management and statistics

Relative growth rates (RGR) for the periods 0–30 days and 31–120 days were calculated (Hunt 1978). The slopes of the log of the average mass per plant per pot plotted against the log of density (Yoda et al. 1963) was determined for the mycorrhizal (VAM) and non-mycorrhizal (NM) plants of each species and compared using the slope comparison in Zar (1984). Root : shoot ratios were calculated on a dry mass basis. The CV of plant growth in each pot was calculated using the mass of individual shoots of *O. hirtum* and *A. linearis*. The significance of the effect of mycorrhizal infection and density on plant parameters was determined by means of two-way analysis of variance (ANOVA). Percentage values were arcsine transformed before analysis (Zar 1984).

Results

Mortality was very low with deaths occurring in only two pots. One VAM and two NM *A. linearis* plants died in pots with 16 plants.

Table 1. Mean (±SE) of plant parameters of *Otholobium hirtum* plants grown at densities (DEN) of 1, 4, 8 and 16 plants per pot with (VAM) or without (NM) vesicular-arbuscular mycorrhizal (MYC) inoculum

	Treatment								ANOVA		
	NM 1	NM 4	NM 8	NM 16	VAM 1	VAM 4	VAM 8	VAM 16	MYC	DEN	MYC×DEN
Plant mass (g)	0.074 (0.003)	0.052 (0.002)	0.046 (0.002)	0.036 (0.001)	0.249 (0.023)	0.090 (0.005)	0.058 (0.002)	0.040 (0.001)	***	***	***
Height (mm)	15.8 (0.5)	15.5 (0.4)	14.8 (0.1)	16.6 (0.3)	86.6 (5.0)	29.3 (1.2)	21.1 (0.3)	18.3 (0.2)	***	***	***
Leaf number	2.8 (0.1)	3.0 (0.1)	2.9 (0.1)	2.5 (0.0)	9.2 (2.3)	5.1 (0.5)	4.2 (0.1)	3.1 (0.2)	***	*	*
VAM infection (%)	0 (0)	0 (0)	0 (0)	0 (0)	47.5 (11.2)	40.7 (5.8)	65.3 (7.2)	72.5 (4.6)	–	NS	–
Nodule number	0 (0)	2.4 (0.2)	3.7 (0.2)	3.5 (0.3)	12.6 (2.2)	4.8 (0.3)	5.2 (0.1)	2.7 (0.1)	***	**	***
Root: shoot ratio	2.66 (0.22)	2.13 (0.11)	1.92 (0.04)	1.73 (0.11)	1.27 (0.15)	1.43 (0.10)	1.51 (0.05)	1.55 (0.05)	***	NS	***
CV (%) of shoot mass	15.9 (0)	17.4 (2.0)	23.1 (2.4)	43.0 (9.9)	34.3 (0)	72.3 (11.9)	58.5 (5.1)	63.9 (8.8)	***	NS	*
RGR (0–30) ($g\ g^{-1}\ day^{-1}$)	0.067 (0.000)	0.072 (0.001)	0.063 (0.003)	0.068 (0.002)	0.075 (0.004)	0.072 (0.000)	0.075 (0.003)	0.069 (0.000)	*	NS	NS
RGR (31–120) ($g\ g^{-1}\ day^{-1}$)	0.008 (0.000)	0.003 (0.000)	0.004 (0.000)	0.000 (0.000)	0.019 (0.001)	0.009 (0.000)	0.003 (0.000)	0.001 (0.000)	***	***	***
P concentration ($\mu g\ g^{-1}$)	259 (14)	356 (10)	380 (4)	523 (17)	1118 (111)	838 (30)	767 (49)	780 (36)	***	NS	***
P content (μg)	19.1 (1.4)	18.9 (1.1)	17.6 (0.6)	18.8 (0.7)	267.4 (16.1)	74.9 (2.7)	44.7 (1.7)	31.2 (0.8)	***	***	***
N concentration ($mg\ g^{-1}$)	19.14 (0.51)	18.03 (0.61)	17.81 (0.28)	16.62 (0.38)	14.01 (0.78)	17.14 (0.26)	17.36 (0.51)	16.86 (0.23)	***	NS	***
N content (mg)	1.41 (0.07)	0.95 (0.05)	0.82 (0.03)	0.59 (0.01)	3.52 (0.45)	1.54 (0.08)	1.01 (0.02)	0.68 (0.02)	***	***	***

Significance values as determined by two-way analysis of variance: NS = not significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Table 2. Mean (±SE) of plant parameters of *Aspalathus linearis* plants grown at densities (DEN) of 1, 4, 8 and 16 plants per pot with (VAM) or without (NM) vesicular-arbuscular mycorrhizal (MYC) inoculum

	Treatment								ANOVA		
	NM 1	NM 4	NM 8	NM 16	VAM 1	VAM 4	VAM 8	VAM 16	MYC	DEN	MYC × DEN
Plant mass (g)	0.346 (0.054)	0.145 (0.012)	0.102 (0.013)	0.095 (0.005)	0.274 (0.031)	0.111 (0.016)	0.061 (0.004)	0.056 (0.005)	*	***	NS
Height (mm)	74.2 (27.0)	14.6 (5.8)	13.7 (3.5)	7.5 (0.5)	81.6 (27.8)	6.4 (1.8)	5.2 (1.1)	4.3 (1.2)	NS	***	NS
Leaf number	22.4 (5.4)	11.6 (1.3)	9.1 (0.8)	10.3 (0.3)	18.6 (2.7)	8.9 (0.9)	6.2 (0.3)	7.2 (0.8)	NS	***	NS
Leaf length (mm)	55.4 (5.4)	43.3 (2.4)	35.7 (3.9)	35.5 (0.3)	49.2 (3.7)	36.0 (2.7)	29.9 (1.3)	28.2 (2.5)	*	***	NS
VAM infection (%)	0 (0)	0 (0)	0 (0)	0 (0)	33.5 (5.6)	30.8 (6.7)	29.7 (5.3)	18.7 (4.6)	–	NS	–
Nodule number	30.6 (6.2)	16.3 (2.6)	14.5 (3.8)	11.1 (1.0)	13.2 (5.4)	6.6 (4.7)	2.9 (1.8)	6.8 (2.2)	**	***	NS
Cluster root number	8.8 (1.1)	3.0 (0.5)	1.5 (0.3)	0.9 (0.1)	7.8 (1.3)	2.2 (0.6)	0.5 (0.1)	0.4 (0.1)	NS	***	NS
Root: shoot ratio	1.03 (0.16)	0.97 (0.04)	1.08 (0.15)	0.96 (0.05)	0.83 (0.09)	1.28 (0.08)	1.34 (0.10)	1.19 (0.16)	NS	NS	NS
CV (%) of shoot mass	5.1 (0)	36.6 (8.7)	55.8 (7.2)	57.5 (2.6)	30.5 (0)	38.6 (2.7)	36.3 (4.6)	58.5 (6.0)	NS	***	NS
RGR (0–30) (g g^{-1} day^{-1})	0.053 (0.002)	0.034 (0.002)	0.033 (0.000)	0.030 (0.000)	0.044 (0.001)	0.036 (0.002)	0.036 (0.001)	0.030 (0.000)	NS	***	*
RGR (31–120) (g g^{-1} day^{-1})	0.036 (0.001)	0.032 (0.000)	0.028 (0.001)	0.029 (0.000)	0.036 (0.001)	0.028 (0.001)	0.022 (0.000)	0.023 (0.001)	***	***	NS
P concentration (μg g^{-1})	727 (72)	723 (35)	876 (123)	787 (38)	1004 (65)	1092 (128)	1366 (34)	1347 (132)	***	*	NS
P content (μg)	239.1 (22.7)	103.2 (4.5)	82.0 (4.7)	74.4 (2.1)	269.7 (22.1)	112.1 (6.4)	83.4 (3.8)	72.1 (1.3)	NS	***	NS
N concentration (mg g^{-1})	12.32 (0.53)	11.84 (0.61)	12.06 (0.58)	15.68 (0.12)	12.83 (1.78)	13.95 (1.39)	10.50 (0.51)	13.88 (1.19)	NS	*	NS
N content (mg)	4.34 (0.83)	1.74 (0.22)	1.26 (0.20)	1.50 (0.08)	3.78 (1.05)	1.49 (0.17)	0.65 (0.07)	0.81 (0.13)	NS	***	NS

Significance values as determined by two-way analysis of variance: NS = not significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Absence of mycorrhizas and increases in density reduced plant mass of *O. hirtum* but differences in size between VAM and NM *O. hirtum* plants diminished with increasing plant density (Table 1, Fig. 1A). *A. linearis* plants were also smaller at higher densities, and VAM plants were smaller than NM plants at all densities (Table 2, Fig. 1B). Plant mass of singly grown VAM *A. linearis* was similar to that obtained by naturally infected *A. linearis* plants (0.209 ± 0.033 g) grown in non-sterile field soil and therefore the lack of growth response to mycorrhizas cannot be ascribed to incompatability with the experimental VAM fungus. The slope of the log mean mass versus log density plot was significantly steeper ($P<0.05$) for VAM plants of both species (Fig. 1A, B).

VAM *O. hirtum* plants were taller and had more leaves than NM plants while NM and VAM *A. linearis* plants did not differ in these parameters (Tables 1 and 2). Increasing density resulted in shorter plants with fewer leaves. Leaves of VAM *A. linearis* plants were shorter than those of NM plants and leaf length decreased with increasing density (Table 2). VAM infection of the roots was not affected by plant density for either species (Tables 1 and 2). Nodulation was highest for singly grown VAM *O. hirtum* plants, absent in the single NM *O. hirtum* plants, with differences between NM and VAM plants decreasing with increasing density (Table 1). NM *A. linearis* plants had more nodules than the VAM plants, and nodulation decreased with increasing density (Table 2). Number of root clusters were the same for VAM and NM *A. linearis* plants but decreased with increasing density (Table 2). Root : shoot ratios were lower for VAM *O. hirtum* plants but were unaffected by density (Table 1). Neither mycorrhizal or density treatments affected *A. linearis* root : shoot ratios (Table 2).

The shoot mass CVs of VAM *O. hirtum* plants were higher than NM plants and VAM *O. hirtum* shoot mass CVs increased from density one to density four but were unaffected by higher densities (Table 1). NM *O. hirtum* CVs increased with increasing density. CVs of VAM and NM *A. linearis* shoots were similar and increased with increasing density (Table 2).

By day 30 mycorrhizas, but not density, were affecting RGRs of *O. hirtum* (Table 1). However increasing den-

Table 3. Sources of phosphorus in *Otholobium hirtum* and *Aspalathus linearis* grown at different densities in a low P soil with (VAM) and without (NM) vesicular-arbuscular mycorrhizal inoculum

	Total P accumulated in plants (μg pot^{-1})		P from seeds (μg pot^{-1})	P from soil (μg pot^{-1})	
	NM	VAM		NM	VAM
O. hirtum					
1	19 (3)	267 (30)	16 (2)	3 [15%]	251 [94%]
4	75 (10)	299 (24)	65 (11)	10 [14%]	234 [78%]
8	141 (11)	357 (31)	130 (23)	11 [8%]	227 [63%]
16	302 (26)	500 (30)	260 (46)	41 [14%]	239 [48%]
A. linearis					
1	239 (50)	269 (49)	55 (12)	184 [77%]	214 [79%]
4	413 (40)	448 (57)	221 (48)	191 [46%]	227 [51%]
8	656 (84)	667 (69)	442 (97)	213 [33%]	225 [34%]
16	1164 (123)	1141 (74)	884 (195)	279 [24%]	256 [22%]

Standard deviations in brackets below mean, percentage values following mean in square brackets

sity was depressing *A. linearis* RGRs at this stage although mycorrhizas had no influence (Table 2). RGRs for the period 31–120 days decreased for both species with increasing density but mycorrhizas generally increased RGRs of *O. hirtum* and decreased those of *A. linearis* (Table 1 and 2).

The total P accumulated by all the plants in a pot increased with increasing density but most of this was accounted for by the P available in the seeds. Similar amounts of soil P were accumulated at all densities except by NM *O. hirtum* plants which acquired very low amounts of soil P (Table 3).

P concentrations were higher in VAM plants. A significant interaction ($P<0.001$) of mycorrhizal and density treatments is seen in the decrease of P concentrations of VAM *O. hirtum* plants with increasing density with the opposite trend in NM *O. hirtum* (Table 1). P concentration of VAM *A. linearis* plants were higher than NM plants and this difference increased at higher densities (Table 2). P content of all *A. linearis* (Table 2) and VAM *O. hirtum* plants decreased with increasing density but P content of NM *O. hirtum* plants remained constant at all densities (Table 1). VAM *O. hirtum* plants had accumulated almost 14 times more P than NM plants at the lowest density but this dropped to 1.6 times at the highest density (Table 1). Differences between VAM and NM *A. linearis* P content were slight and P content decreased with increasing density (Table 2).

N concentration of VAM *O. hirtum* was higher than NM plants but did not change much with density (Table 1). N concentration in *A. linearis* was unaffected by mycorrhizas but decreased with increasing density (Table 2). Both increasing density and absence of VAM lowered N content of *O. hirtum* plants but increasing density alone lowered the N content of *A. linearis* plants (Tables 1 and 2).

Discussion

The reduction in individual plant size with increased density is a result of increasing overlap of resource depletion zones. Among the resources that become relatively less abundant at higher densities are light, water and soil nutrients. Soil nutrients, especially P, are possibly the most important limiting resource in these experiments. Apparently *A. linearis* and VAM *O. hirtum* plants at the lowest density had taken up almost all available P and P depletion occurred at a lower density of plants than in another experiment of similar design (Koide 1991). Nitrogen acquired per pot from non-seed sources was greater at higher densities and nitrogen fixation may be contributing to the plants' N nutrition.

The effect of mycorrhizas on plant growth is often attributed to the improved P nutrition of VAM plants (Harley and Smith 1983). Increased P uptake will usually result in elevated growth when other factors affecting growth are not limiting. The greater growth of VAM *O. hirtum* is accompanied by much greater P influx into VAM plants and VAM plants were able to acquire soil P even at high densities of plants. On the other hand the total P content of the NM *O. hirtum* plants is almost entirely derived from the seed, as has been found to be the case for non-mycorrhizal large seeded Proteaceae of similar environments (Stock et al. 1990). It appears therefore that the concentration of soil P must be below the threshold (Föhse et al. 1988) that NM roots of *O. hirtum* can exploit. In this soil, P uptake by *O. hirtum* is mediated by the mycorrhizal fungus and *O. hirtum* can be regarded as an obligate mycorrhizal species when growing under these experimental conditions. While the advantage of being VAM diminishes with increasing density for *O. hirtum*, the effect of being NM is detrimental at all densities in this low nutrient soil. The mycorrhizal influence on P uptake in *O. hirtum* decreases in proportion to the decrease in available P per plant as density increases and is not related to changes in intensity of VAM infection with increasing density. The decrease in mycorrhiza-mediated P uptake with increasing density is reflected in the decreasing differences in VAM and NM plant mass, height, leaf number and RGR at higher densities.

In contrast VAM *A. linearis* plants acquired very similar quantities of P as NM plants but had higher tissue P concentrations due to smaller plant mass. Both VAM and NM *A. linearis* form cluster roots and these probably play a very similar role to the proteoid roots in the Proteaceae which are thought to be responsible for nutrient uptake under low nutrient conditions (Jeffrey 1967). The cluster roots of *A. linearis* and the VAM fungus seem to have similar efficiencies at acquiring soil

P. *A. linearis* can therefore be regarded as a species which has little or no mycorrhizal dependency.

As the VAM fungi did not confer any nutrient uptake advantage on the *A. linearis* plants under these growing conditions there was a reduction in VAM plant growth which may be due to the carbon requirements of the fungus. Similar growth depressions have been observed in other VAM plants especially under conditions of adequate mineral nutrition of the NM plants (Koide 1985) or inadequate light (Smith and Gianinazzi-Pearson 1990).

Our prediction that increasing density would affect VAM plants more severely than NM plants is borne out for the two species tested, which represent extremes of VAM dependency. This is highlighted by the slopes of the log mean mass against log density, which are steeper for the VAM plants (Fig. 1A and B). The carbon requirements of the VAM fungus may be responsible for this pattern. If the costs of maintaining the symbiosis were proportional to the size of the plant (i.e. the cost-benefit model remained the same at all densities) then the slopes for the VAM and NM plants would be the same. As VAM infection did not change with density, smaller plants were supporting the same proportion of fungus as larger plants but the relative cost of supporting the fungus was higher. At higher densities carbon input is often lower because shading effects and the lower nutrient status of the plants decreases their photosynthetic efficiency (Field and Mooney 1986). Hence for mycorrhizal plants the fungal symbiont exacerbates the effects of increasing density on plant size, possibly by increasing the cost : benefit ratio as density increases. Mycorrhizal plant populations may therefore run a greater risk of extinction at high density than non-mycorrhizal populations.

This study does not provide direct evidence that mycorrhizas influence population densities as mortality was not frequent during the experiment. However, other authors have also noted that plants will persist even at very high densities which may be reducing RGRs to 0 or below (Smith 1983; Shaw and Antonovics 1986). Shaw and Antonovics (1986) warn that seedlings starting at high densities with low mortality rates are likely to suffer population extinction and populations with low plant size CVs are more likely to become extinct than ones with higher CVs despite similar average growth. Therefore mechanisms that allow variation in plant size may ensure that some plants will have suitable attributes enabling them to survive stresses due to changing environmental conditions.

The high shoot mass CVs of the denser VAM *O. hirtum* plants and all *A. linearis* plants indicates that resource sharing among plants sharing the same hyphal network may not be a feature of VAM seedling establishment in dense populations. Rather the implication is that there is preemption of resources by fitter individuals (Weiner and Thomas 1986). Those individuals who, due to factors such as larger initial seed size or to slightly earlier germination, are able to grow bigger sooner, and in the case of obligate mycorrhizal species become VAM quicker, are able to monopolize a greater amount of both the light and mineral nutrient resources. They thus depress the growth of smaller plants relatively more than their growth is affected by the smaller plants.

As these experiments were conducted on plants growing in pots in a controlled environment, the results cannot be used to predict the quantitative effects of mycorrhizas on population processes in regularly disturbed ecosystems where recruitment is most often in response to a large-scale disturbance such as fire. However simulation experiments of this nature will highlight areas in population development where mycorrhizas may be of significance, and will allow preliminary hypothesis testing before attempting to manipulate mycorrhizas in the field. This study indicates that when a species has a high mycorrhizal dependency then the mycorrhizal population may produce individuals falling in a wider size distribution and a few of these may be able to survive a new stress while the NM plants are unlikely to survive. When plants are capable of growing adequately without mycorrhizas then indications are that resource preemption increases with density irrespective of VAM status, but in this case mycorrhizal plants may show lower competitive ability. Irrespective of mycorrhizal dependency, mycorrhizas appear to impose a higher cost on the plants at higher densities. Mechanisms that reduce high seedling densities may have developed in part as a response to the deleterious effects of mycorrhizas on plant growth at high densities. This study indicates that size distributions of plants at higher densities may vary depending on the mycorrhizal dependency of the species.

While the mycorrhizal mutualism may have its greatest positive effects on individuals growing at low densities, their impact on populations at higher densities should not be ignored simply because the mycorrhizal growth responses are not obvious. Further investigations are needed to elucidate the effects of interactions of mycorrhizas and density over longer periods of the plant's history and under field conditions.

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