THE OCCURRENCE AND ECOPHYSIOLOGY OF VESICULAR-ARBUSCULAR MYCORRHIZAL PLANTS IN THE CAPE FLORISTIC REGION

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ABSTRACT

The mycorrhizal status of plants of three lowland evergreen shrublands of the Cape Floristic Region, South Africa, was investigated, and levels of vesicular-arbuscular mycorrhizal (VAM) infection among VAM plants determined. Most plant species are VAM, and no indigenous ectomycorrhizal species were found. In lowland fynbos, 23 % of the species form no mycorrhizas, while mycorrhizas are absent from 18 % and 27 % of the species in renosterveld and strandveld respectively. In renosterveld and strandveld, non-mycorrhizal plants are ruderal species with low cover. In contrast, non-mycorrhizal species in fynbos include members of the woody Proteaceae and reed-like Restionaceae, which make up more than half the above ground biomass in older vegetation. Average VAM infection levels among VAM plant species are lowest in strandveld, intermediate in fynbos, and highest in renosterveld among perennials and geophytes. It is concluded that edaphic features, in particular, soil phosphorus levels, control infection levels and the mycorrhizal profile of the vegetation. Despite structural similarities among the lowland vegetation types, functional attributes, as they relate to mycorrhizas and nutrient acquisition, are very different and it is expected that the factors controlling community development differ among these three vegetation types. A high proportion of potentially VAM plant species in fynbos were non-mycorrhizal in the field, especially in the first two growing seasons following fire, suggesting that inoculum was patchily distributed, possibly as a result of dominance by non-mycorrhizal species.

Representatives of evergreen, sclerophyllous fynbos shrubs were grown in pot culture, in low nutrient soil with or without VAM inoculum, for several months in order to investigate aspects of their growth and development in response to mycorrhizas. Growth of these plants when non-mycorrhizal was controlled by seed phosphorus reserves and most of these plants were unable to extract phosphorus under low nutrient conditions in the absence of mycorrhizas. Addition of soluble phosphorus increased growth of non-mycorrhizal plants and phosphorus concentrations, but not growth, of VAM plants. Vesicular-arbuscular mycorrhizas are essential for successful establishment of slow growing, evergreen shrubs.

Those species which do not rely on VAM infection for phosphorus uptake exhibit root morphological adaptations for nutrient uptake.

Mycorrhizal responses in terms of mass and phosphorus content of VAM plants were negatively related to the log of seed phosphorus reserves for a group of 15 evergreen indigenous VAM species. Seed size among these plants is seen as a compromise between establishment and dispersal in an environment where VAM inoculum may be patchily distributed. In response to mycorrhizas, biomass and phosphorus allocation was shifted towards the shoots for these sclerophyllous species under well watered conditions and growth rates were correlated with dry mass root:shoot ratios. Growth responses to mycorrhizas may be a result of changing allocation patterns favouring the production of photosynthetic tissue. However, under less well watered conditions of cyclical drying, seedlings of one of these species, *Phylica cephalantha*, increased root:shoot ratios when VAM. Among these slow growing evergreen species, changes in allocation patterns in response to environmental conditions may be facilitated when the seedlings are VAM. Host plant physiology is influenced by mycorrhizas as was shown by an increase in leaf conductance accompanying VAM infection of *Phylica cephalantha* plants.

The effect of VAM infection on growth of seedlings of two evergreen indigenous species, growing at different densities in pot culture, was investigated. The species represented the extremes of mycorrhizal dependence among VAM plants. Growth depressions brought about by resource depletion at higher densities are greater for VAM than for non-mycorrhizal plants and there was no indication that mycorrhizas ameliorated the competitive effects of larger individuals on smaller ones. It is concluded that the cost-benefit equilibrium of being VAM changes with density, and that this has profound effects on population development.

By integrating the results of these studies, the significance of mycorrhizas in plant population and community processes, and ecosystem functioning in the Cape Floristic Region, is discussed.

CONTENTS

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CHAPTER 1

Introduction

Vesicular-arbuscular mycorrhizas

The roots of representatives of most plant taxa form vesicular-arbuscular (VA) mycorrhizas with fungi from the family Endogonaceae (Trappe 1987). VA mycorrhizas are a mutualistic relationship formed by a fungal partner, an obligate biotroph, which produces many branched haustoria, the arbuscules, in the inner cortical cells of plant roots (Harley & Smith 1983). Arbuscules develop from intercellular hyphae, while extraradical hyphae grow out into the surrounding soil environment and in some genera, lipid-rich vesicles are formed intercellularly. Large, asexual spores are produced in the soil or within the root, and have been the basis for delineating species among vesicular-arbuscular mycorrhizal (VAM) fungi, as sexual reproduction appears to be absent (Morton 1988). Spore morphological features are conservative and may not reflect physiological diversity among clones (Morton 1990). Fungal structures typical of VA mycorrhizas have been found among fossils of the earliest land plants leading to the hypothesis that the colonization of a land environment by plants was facilitated by mycorrhizas (Pirozynski & Malloch 1975). However arbuscules, which are regarded as indicative of the biotrophic nature of the symbiosis, have only been seen in fossils from the Triassic (Stubblefield, Taylor & Trappe 1987). An important consequence of the great age of the vesicular-arbuscular mycorrhizal relationship is that it is probably ancestral to other mycorrhizal associations and to the non-mycorrhizal roots typical of some taxa (Trappe 1987).

VAM fungi have limited saprotrophic abilities and rely on host plants for photosynthate to satisfy their carbohydrate needs. A dual transport of phosphorus into the host plant accompanies the efflux of carbohydrate at the interface between the arbuscule and the plant plasma membrane (Schwab, Menge & Tinker 1991) and increased phosphorus content of VAM plants is the most common effect of the mutualism on plants (Harley & Smith 1983).

Phosphorus levels in many soils are low and phosphorus depletion zones develop around plant roots which are not rapidly replenished due to the slow rate of diffusion through the soil (Bieleski 1973). Under low soil nutrient conditions, and in competitive situations, the

hyphae of VAM fungi are responsible for phosphorus uptake for most plants. Although both VAM fungi and plant roots probably acquire phosphorus from the same soil fraction (Smith & Gianinazzi-Pearson 1988, Bolan 1991), the plants benefit as the cost of supporting the fungal hyphae is less than that of producing roots to grow beyond the depletion zone (Harley 1989). Unlike ectomycorrhizas and ericoid mycorrhizas there is no evidence that extracellular enzymes are produced in quantities that affect the availability of soil nutrients (Smith & Gianinazzi-Pearson 1988).

The usual response to the enhanced phosphorus nutrition of VAM plants is enhanced growth, but VA mycorrhizas influence other aspects of mycorrhizal plant physiology, including drought resistance and water relations (Nelsen 1987), disease resistance (Graham 1988), uptake of several other soil nutrients (Saif 1987), resistance to toxic soil conditions (Gildon & Tinker 1983) and plant hormone levels (Allen, Moore & Christensen 1980). In most cases these effects can be ascribed to the improved phosphorus nutrition of the mycorrhizal plant rather than to direct intervention by the mycorrhizas in the physiology of the plant (Read 1986, Smith & Gianinazzi-Pearson 1988). However, the maintenance of mycorrhizal fungi requires an energy input from the plant, and thus physiological effects of the mycorrhizal state will not be identical to those induced by an identical influx of phosphorus from a non-mycorrhizal source.

While the relationship is obligate for the fungal partner of the mutualism, plant dependency is more variable and depends upon interactions between plant root morphological and architectural features and soil characteristics, which determine the availability of phosphorus (St John 1980, Hetrick 1991, Koide 1991a). Specificity among susceptible plant and vesicular-arbuscular mycorrhizal fungal species is absent, although the outcome of interactions between the fungi and plants may differ according to which species or clones are interacting. VAM fungal species or clones have differing capacities to infect plant roots (Dhillion 1992) and the amount of extracellular hyphae produced (Abbott & Robson 1985) and the quantity of phosphorus transported along the hyphae (Jakobsen, Abbott & Robson 1992) varies between species. Numerous studies indicate that the magnitude of plant

responses to VAM infection are highly variable (Harley & Smith 1983, Abbott & Robson 1884, McGonigle 1988).

The significance of VA mycorrhizas extends beyond their effects on individual plant nutrition. Non-nutritional effects of VA mycorrhizas include the contribution of the extraradical mycorrhizal hyphae to soil structure by increasing the size of soil aggregates (Miller & Jastrow 1991). Enhanced aggregation improves soil stability and water percolation, thus decreasing runoff volumes, which may be important to plant survival in arid environments where precipitation events are rare. Water transport along the extraradical hyphae of densely infected roots may not be as insignificant as previously thought and hyphae, by virtue of their small diameter and closer contact with soil particles, may have access to soil water that is unavailable to plant roots under dry soil conditions (Fitter 1985, Read 1991).

The mycorrhizal hyphal network in the soil is an important source of infectivity for plants establishing themselves (Read, Koucheki & Hodgson 1976). In communities with wide age structures, young plants may rapidly become mycorrhizal without having to bear the full carbon cost if the infecting hyphae are already attached to the root systems of other plants. VAM spores, which can be reintroduced by wind or animal vectors, may be important for re-establishing mycorrhizal infection in highly disturbed ecosystems (Allen 1988a). Experimental evidence indicates that resources such as carbon and phosphorus pass along the VAM hyphal network from one plant to another (Chiariello, Hickman & Mooney 1982, Francis & Read 1984). This leads to the possibility of larger plants acting as nurse plants to smaller ones, thus facilitating their establishment (Grime *et al.* 1987); however it is not clear whether ecologically significant quantities of resources pass from large source plants to smaller sink plants (Newman 1988). Whether transfer is mediated by a common hyphal network or by less direct means is not unequivocal (Newman & Ritz 1986, Haystead, Malajczuk & Grove 1988). As the hyphal network established by VAM fungi is relatively stable compared to those of other fungal populations in the soil, VAM mycelium may be

largely responsible for maintaining tight nutrient cycles (Newman 1988, Pankow, Boller & Wiemken 1991).

Natural ecosystems can be characterized by the relative importance of the suite of mycorrhizal types present (Read 1991). Edaphic attributes, particularly those associated with nutrient cycling, which in turn are influenced by climatic and disturbance regimes, are important in determining the types of mycorrhizas that will be favoured in particular ecosystems. In nothern temperate and boreal systems the association between mycorrhizas and various systems processes is reasonably well understood when compared to the factors selecting for various mycorrhizal types in tropical and mediterranean ecosystems.

Cape Floristic Region

The Cape Floristic Region lies mostly in the mediterranean climate zone of the S. W. and S. Cape Province, South Africa. The evergreen, sclerophyllous shrublands of this region are phylogenetically, structurally and functionally distinct from the vegetation to the north (Linder, Meadows & Cowling 1992, Stock & Allsopp 1992). Fynbos, the dominant vegetation of this region, has evolved in response to the particularly nutrient poor, leached soils and frequent, stochastic fires. Tissue turnover is very low in the vegetation, and above ground litter is very slow to decompose (Mitchell *et al.* 1986). Fire is the main mechanism for releasing nutrients from above ground plant matter (van Wilgen & le Maitre 1981, Brown & Mitchell 1986, Stock & Lewis 1986) and plays a major role in determining reproductive strategies among plants in fynbos (le Maitre & Midgley 1992). Structural diversity in the shrublands of the Cape Floristic Region is quite low (Campbell 1985), but plant species diversity is remarkably high, especially at the beta and gamma levels of scale in the landscape (Cowling, Holmes & Rebelo 1992). Over 8500 plant species occur in the Cape Floristic Region, of which 68 % are endemic (Bond & Goldblatt 1984).

Despite the seemingly xerophytic characteristics of the dominant growth forms, sclerophylly is an adaptation for improving nutrient use efficiency of plants growing on very low nutrient

soils (Loveless 1962, Beadle 1966, Specht & Rundel 1990), and water relations and drought resistance of sclerophyllous plants vary (Salleo & Lo Gullo 1990). Photosynthetic capacity of sclerophyllous leaves may be relatively low (van der Heyden & Lewis 1989), but their longevity ensures that carbon fixation over their lifespan may be high. Much of this carbon is not used for vegetative growth, as growth rates are low and inflexible, but may be used to construct fire resistant or evading structures, and may be available to support the carbon requirements of mycorrhizas. Even when soil nutrient levels are artificially raised, plant growth responses are limited, and most of the added nutrients may be stored, raising tissue concentrations but not contributing to vegetative growth (Witkowski, Mitchell & Stock 1990). Indications are that reproductive output is stimulated by enhanced nutritional status of the plants (Stock *et al.* 1989).

Mycorrhizas in the Cape Floristic Region

Previous studies on plant root systems in the Cape Floristic Region have shown that vesicular-arbuscular mycorrhizas (Low 1980, Hoffman & Mitchell 1986, Mitchell & Read 1987, Berliner, Mitchell & Allsopp 1989) are common, and ericoid mycorrhizas are formed by members of the Ericaceae (Robinson 1973, Straker & Mitchell 1985). In the Cape Floristic Region and Australia, members of a number of important plant families are non-mycorrhizal, in particular the Proteaceae and Restionaceae, . These non-mycorrhizal taxa produce morphologically distinct root structures which facilitate nutrient uptake (Lamont 1982). Species in the Proteaceae, Restionaceae or Ericaceae are dominant in fynbos communities (Campbell 1985).

The aims of this thesis are twofold. Firstly, to establish the types of mycorrhizas present in plant communities in the Cape Floristic Region of South Africa (Chapter 2). To do this, the occurrence, distribution and intensity of VAM infection is examined in three lowland sclerophyllous vegetation types of the western coastal foreland, and factors influencing their mycorrhizal characteristics and ecological significance are discussed (Chapter 3). These three lowland shrublands, *viz.* fynbos, renosterveld and strandveld, differ edaphically and

floristically, but are geographically close to each other and subject to the same climatic regime. The second aim is to investigate, in pot culture, the potential influences that VA mycorrhizas may have on the biology of seedlings of the dominant, sclerophyllous, woody growth form of the region.

Most studies of the effects of vesicular-arbuscular mycorrhiza on plants have concentrated on changes in growth of crop plants, or herbaceous wild plants. Typically these are fast growing and show variable growth responses to nutrients. The slow growth rates and low growth plasticity of woody plants from low nutrient environments has led to the expectation that they will not be much affected by mycorrhizas because their growth responses to nutrients are low (St John & Coleman 1983, Koide 1991a). However, in the low nutrient environment of fynbos soils, seedling establishment among most species may be precarious if nutrient acquisition is limited by a failure to develop mycorrhizas. The influence of mycorrhizas and phosphorus fertilization on the growth of three sclerophyllous species is examined in Chapter 4. Seed size may be critical in determining seedling establishment and dependence on mycorrhizas. The precise relationship between these factors is uncertain as conflicting evidence concerning the role of seed size and mycorrhizal dependence exists in the literature. Among grassland species, small seeded species are the first to show mycorrhizal growth responses (Read 1991), and seed size of obligately ericoid and orchid mycorrhizal plants is very small to enhance dispersability (Fenner 1985). Small seed size was associated with facultatively VAM tropical forest trees while large seeds were typical of later successional species with an absolute need for mycorrhizal infection for establishment (Janos 1980a). Among fynbos species a wide range of seed sizes is displayed (le Maitre & Midgley 1992) and it is predicted that, among long lived, woody shrub species, those with smaller seeds should have a greater reliance on vesicular-arbuscular mycorrhizas for establishment (Chapter 5).

Although improved phosphorus nutrition is the main result of mycorrhizal infection, effects other than increased growth may be experienced by mycorrhizal plants. Allocation patterns may change in response to increased nutrient uptake which may in turn influence plant

growth. Little is known of the flexibility of sclerophyllous plant growth and allocation in response to altered nutritional status or water availability. This study examines allocation patterns in response to mycorrhizal infection of 15 fynbos shrub species (Chapter 6). The effect of the interaction of restricted water availability and mycorrhizal infection on growth and water relations of one species is also examined (Chapter 7).

As plants in natural habitats seldom grow without interacting with neighbouring plants, the effect of mycorrhizas on individuals may be modified in the presence of other plants, in particular if resource sharing occurs along a hyphal network. However, in numerous plant population studies on the effect of density on population dynamics, the influence of symbionts such as mycorrhizas are seldom considered (Addicott 1986). The effect of mycorrhizas on growth and population size structure of seedlings growing at different densities is investigated in this study (Chapter 8, Allsopp & Stock in press a).

In the Cape Floristic Region with its bewildering numbers of plant species, a functional classification of the vegetation is essential if ecosystem processes are to be understood. Such an approach has proved valuable in establishing how factors such as alien plant invasion, wild flower harvesting and nutrient enrichment may influence vegetation dynamics in the Cape Floristic Region (Stock & Allsopp 1992). However, as in other ecosystems, little is known of below ground processes involving interactions between plants and micro-organisms and their effect on community development. In the characteristically nutrient poor soils of the Cape Floristic Region, such interactions may be keystone processes. In order to answer questions about the ecological significance of below ground processes, and mycorrhizas in particular, in the Cape Floristic Region, mycorrhizas are examined at a heirarchy of levels, ranging from their occurrence in the field to specific effects on the ecophysiology of individual plants. These studies are integrated to produce a process functional approach to enhance the current understanding of the factors significant in influencing plant community dynamics. An understanding of the role of mycorrhizas in plant communities thus facilitates the conservation and reconstruction of natural vegetation in the Cape Floristic Region.

CHAPTER 2

Mycorrhizal Status of Plants Growing in the Cape Floristic Region, South Africa

Introduction

It is generally accepted that most terrestrial plants probably form mycorrhizal associations between their roots and certain fungi, although the vast majority of plants growing in natural ecosystems have not had their mycorrhizal status confirmed (Trappe 1987, Newman & Reddell 1987). The mycorrhizal status of plants reflect both their taxonomic affinities and their ecology. Investigations on the mycorrhizal status of plants in various parts of the world indicate that the major terrestrial biomes can be characterized by specific mycorrhizal types (Read 1991). Surveys of mycorrhizas show that trees of forests and woodlands are either ectomycorrhizal or VAM; herbaceous plants and shrubs in grasslands and shrublands usually form VA mycorrhizas; boreal and temperate heathlands are dominated by ericoid mycorrhizal species; and disturbed ecosystems by non-mycorrhizal weed species (see Brundrett 1991 for references). While the mycorrhizal status of some floras is well documented (e.g. British Isles by Harley & Harley 1987), little is known about both the mycorrhizal associations of plants in the Cape Floristic Region and their functional role in this low nutrient ecosystem.

The vegetation of the Cape Floristic Region contrasts sharply, in terms of taxonomic composition and vegetation structure, with the surrounding southern African vegetation. The Cape flora has a high species diversity ($\pm$8500 species), around 68 % species endemism (Bond & Goldblatt 1984), and high beta and gamma species turnover (Kruger & Taylor 1979, Cowling 1990). Agriculture, urbanization and alien plant invasion are a severe threat to this flora as a result of the limited range of many plant species, and have led to the destruction of much of the lowland vegetation (Hall 1983). Mycorrhizas act as soil nutrient absorbing organs for the plants. As such they will influence the physiology of individuals and their interactions with other plants growing in the same community (Harley 1989, Read 1991). Recognizing the patterns of distribution and understanding the ecological role of mycorrhizal types in a community may be crucial to understanding the dynamics which shape plant communities.

This study collates published records of the mycorrhizal status of plants occurring in the Cape Floristic Region as defined by Bond & Goldblatt (1984). In addition, the mycorrhizal status of plants growing in three lowland vegetation types, west coast strandveld, west coast renosterveld and sand plain fynbos, is reported for the first time. The aim of this chapter is to provide information on the mycorrhizal status of plant species which may be of significance in explaining vegetation patterns and plant functioning in the Cape Floristic Region.

Study Areas

Three study sites representing west coast strandveld, at Melkbosstrand (33° 45'S 18° 27'E), west coast renosterveld on the farm Hercules Pillar (33° 46'S 18° 46'E), and sand-plain lowland fynbos at the fynbos biome intensive study site (33° 31'S 18° 32'E) at Pella, in the western coastal forelands were chosen to investigate the mycorrhizal status of a broad range of plants growing in threatened habitats in the Cape Floristic Region. The classification of the vegetation categories follows that of Moll *et al.* (1984). The strandveld vegetation, growing on coarse/medium sandy soil (organic matter 2.2 %, pH of 7.5, total phosphorus 422 $\mu g\ g^{-1}$ (Witkowski & Mitchell 1987)) is a broad leaved, sclerophyllous 1 - 2.5 m high shrubland with a large succulent component (Boucher 1983). The renosterveld vegetation, growing on a fine sand/clayey soil (organic matter 4.9 %, pH 4.1, total phosphorus 127 $\mu g\ g^{-1}$ (N. Allsopp unpublished)) is an evergreen, cupressoid or microphyllous shrubland, 1 - 2 m high, dominated by *Elytropappus rhinocerotis* with strong lowland fynbos affinities (Tansley 1982, Boucher 1983). The sand-plain lowland fynbos growing on medium textured sandy soil (organic matter 1.4-3.4 %, pH 4.6 - 4.8, total phosphorus 23 - 34 $\mu g\ g^{-1}$ (Mitchell, Brown & Jongens-Roberts 1984)) is an ericoid leaved, sclerophyllous vegetation, 0.75 - 1.5 m high with some taller shrubs, characterized by the presence of *Phylica cephalantha* (Boucher 1983). Vegetation surveys at the three sites have recorded 56, 63 and 215 perennial species at the strandveld (Siegfried 1981), renosterveld (Tansley 1982) and lowland fynbos (Boucher & Shepherd 1988) sites respectively. In addition

annuals and bulbous species are numerically important components of all three vegetation types (Boucher 1983, Bond & Goldblatt 1984).

Materials and Methods

Root collection

Roots were collected between June and early October while the soil was moist. Two collections were made at both the strandveld (during August 1987 and September 1989) and renosterveld sites (during October in 1988 and September 1989) . The lowland fynbos site was sampled six times over 4 years (June and August 1986, August and September 1987, September 1988, August 1989). Two 25 m x 25 m plots were set up at a site on each collection day. The plots at the strandveld site were situated 60 - 300 m inland of the high water mark. The renosterveld plots were on the N.W. - S.W. facing lower slopes of Joostenberg. At the lowland fynbos site, plots were randomly scattered throughout the 269 ha study site.

Roots of one or two representatives of each species occurring in the plots were sampled. In addition, plant species not in the plots, but encountered in the vicinity, were sampled. Smaller plants, including annuals, perennial seedlings and bulbous plants, were excavated with entire root systems. Roots of larger shrubs were collected by carefully tracing the root system from the main stem until young, unthickened roots were encountered. However for some species, including members of the Anacardiaceae and Ebenaceae, few young roots could be found despite extensive excavation along roots down to 1 m. At the renosterveld site some species were not sampled because they grew only in narrow cracks among rocks (e.g. *Olea* sp.). At the strandveld site the large size of dominant shrubs and density of the vegetation at ground level, as well as spininess of some species, precluded collection of these species' roots.

Young roots were removed from surrounding soil in the field and immediately placed in vials containing 10 % KOH for clearing. Wherever possible, 50 cm of root per plant was collected. Roots were cleared for 1 week at 20 ^{o}C, and then rinsed under running tap water (Smith & Bowen 1979). Where necessary, pigmented roots were decolourized with H_2O_2 or NaClO. This was followed by acidification in 1 M HCl and staining in 0.05 % Trypan blue in a lactic acid solution (Kormanik & McGraw 1982). Root segments were permanently mounted in a polyvinyl acid solution and inspected at 100 and 400 times magnification with a light microscope for mycorrhizal structures.

Plants were classified according to Cronquist (1988) and species names follow Gibbs Russell *et al.* (1985, 1987).

Literature survey

All known records of the mycorrhizal status of plants in the Cape Floristic Region were consulted. Only those records which reported the mycorrhizas of plants actually growing in the Cape Floristic Region are listed here. Confirmation of infection status of some species was undertaken by examining roots of plants growing in soil from their natural habitats in pot culture.

Results

VA mycorrhizas were characterized strictly by the presence of arbuscules in the inner cortical cells, with or without vesicles, (*sensu* Harley & Smith 1983) and were the most common type of mycorrhiza (61 % of species examined) (Table 2.1). Infections regarded as VAM, but morphologically distinct from the above types, were found in *Aristea dichotoma*, which formed intracellular coils similar to those described by Brundrett & Kendrick (1990b) in *Trillium grandiflorum*, while VAM fungi in *Orphium frutescens* and *Sebeae exacoides* formed structures typical of those seen in other members of the Gentianaceae (Jacquelinet-Jeanmougin & Gianinazzi-Pearson 1983). Infection formed by the "fine endophyte"

(Greenall 1963) was occasionally seen, but was never exclusively found on one species. Ericoid (ERIC) mycorrhizas were found in the hair roots of all members of the Ericaceae examined (Table 2.1). They are characterized by the formation of coiled and branched, fine hyphae in the cortical cells (Read 1984). Orchid (ORCH) mycorrhizas were seen in the two *Disa* spp. examined (Table 2.1) and consist of characteristic coarse, coiled intracellular hyphae (Harley & Smith 1983). No ectomycorrhizal infection was seen in the indigenous species examined. Introduced ectomycorrhizal species such as pines, oaks, poplar and eucalypts form ectomycorrhizas in the Cape Floristic Region but the ectomycorrhizal fungi were in all likelihood introduced with imported saplings (van der Westhuizen & Eicker 1987).

Ninety one of the 332 species reported formed no mycorrhizas (Table 2.1). These were concentrated in the Caryophyllidae and the families Brassicaceae, Crassulaceae, Proteaceae, Santalaceae, Zygophyllaceae, Restionaceae and Cyperaceae. Plant roots which contained occasional vesicles, but no arbuscules, were regarded as functionally non-mycorrhizal (Hirrel, Mehravaran & Gerdemann 1978).

Some earlier studies (Laughton 1964, Low 1980) have reported endophytic mycorrhizas (ENDO) as being present but descriptions or illustrations do not indicate structures which are typical of mycorrhizas as they are presently delimited (Harley & Smith 1983). Non-mycorrhizal fungi were found frequently in both mycorrhizal and non-mycorrhizal roots examined for this study. Thus reports of fungal infection as "endophytic mycorrhizas" should be viewed with caution. The most common non-mycorrhizal root inhabiting fungus was *Olpidium* sp., which forms cysts and zoosporangia which may be mistaken for VAM vesicles if care is not taken. Unidentified hyphal fungi including dark, septate hyphal fungi forming microsclerotia (DSH) similar to those described by Haselwandter & Read (1980) in alpine vegetation, were also present. The non-mycorrhizal roots of members of the Proteaceae have been shown to support a fungal flora that is distinctly different from that found in the non-rhizosphere soil (Allsopp, Olivier & Mitchell 1987). Infection by *Olpidium* sp. and other fungi was particularly heavy in the root systems of members of the Poaceae and Scrophulariaceae where they could obscure infection by VAM fungi (Table 2.1).

TABLE 2.1. A preliminary list of the mycorrhizal status of plants occurring in the Cape Floristic Region. VAM = vesicular-arbuscular mycorrhizal, ERIC = ericoid mycorrhizas, ENDO = endophytic mycorrhizas of an undesignated type (see text), ORCH = orchid mycorrhizas, ABS. = no mycorrhizas seen in specimens examined, DSH = dark septate hyphae.

	No. Of Specimens Examined	Location[1]	Source of information[2]	Mycorrhizal Type	Notes
MAGNOLIOPSIDA					
MAGNOLIIDAE					
LAURACEAE					
Ocotea bullata (Burch.) Baill.			EML	ENDO	
FUMARIACEAE					
Cysticapnos vesicarius (L.) Fedde	13	N	B,M&A	ABS.	
CARYOPHYLLIDAE					
AIZOACEAE					
Aizoon sarmentosum L.f.	1	H	NA	ABS.	
Galenia africana L.	5	N	B,M&A	ABS.	
Limeum aethiopicum Burm.	1	P	NA	ABS.	
Pharnaceum sp. cf. *P. croceum* E. Met. ex Fenzl	2	P	NA	ABS.	
Pharnaceum incanum L.	4	P	NA	ABS.	vesicles, other fungi may be present
Pharnaceum scleranthoides Sond.	1	P	NA	ABS.	

Pharnaceum sp. L.	3	N	B,M&A	ABS.	
Polpoda capensis Presl	3	P	NA	ABS.	vesicles may be present
Tetragonia fruticosa L.	7	N	B,M&A	ABS.	
Tetragonia portulacoides Fenzl	2	P	NA	ABS.	
MESEMBRYANTHEMACEAE					
Carpanthea pomeridiana (L.) N. E. Br.	2	P H	NA	ABS.	other fungi present
Carpobrotis acinaciformis (L.) L. Bol.	1	M	NA	ABS.	*Olpidium*, DSH, other fungi present
Carpobrotus edulis (L.) L. Bol.	3	P	NA	ABS.	arbuscules in one specimen, vesicles and other fungi present
Dorotheanthus bellidiformis (Burm.f.) N.E. Br.	3	P	NA	ABS.	DSH, other fungi present
Drosanthemum floribundum (Haw.) Schwant.	5	N	B,M&A	ABS.	
Drosanthemum sp. Schwant.	4	N	B,M&A	ABS.	
Jordaaniella dubia (Maw.) H. E. K. Martm.	1	M	NA	ABS.	
Lampranthus aurantiacus (DC.) Schwant.	2	P	NA	ABS.	other fungi present
Lampranthus sp. N. E. Br.	1	H	NA	ABS.	
Mesembryanthemum sp. L.	16	N	B,M&A	ABS.	
Ruschia macowanii (L. Bol.) Schwant.	3	M	NA	ABS.	vesicles, DSH, other fungi present
Ruschia sp. Schwant.	2	N	B,M&A	ABS.	
CHENOPODIACEAE					
Atriplex halimus L.*	2	N	B,M&A	ABS.	
Atriplex nummularia Lindl. *	2	N	B,M&A	ABS.	
Atriplex semibaccata R. Br. *	5	N	B,M&A	VAM	
Atriplex lindleyi Moq. *	23	N	B,M&A	ABS.	
Chenopodium murale L. *	11	N	B,M&A	ABS.	
Exomis sp. Fenzl	27	N	B,M&A	ABS.	
Manochlamys albicans (Ait.) Aell.	1	N	B,M&A	ABS.	
ILLECEBRACEAE					
Silene clandestina Jacq.	2	P	NA	ABS.	other fungi present

Silene undulata Ait.	2	M	NA	ABS.	vesicles, DSH, other fungi present
Silene sp. 1 L.	2	H	NA	ABS.	arbuscules in one specimen, other fungi present
Silene sp. 2 L.	1	N	B,M&A	ABS.	
POLYGONACEAE					
Emex australis Steinh. *	12	N	B,M&A	ABS.	
Rumex cordatus Poiret	4	P	NA	ABS.	vesicles, DSH, other fungi present
PLUMBAGINACEAE					
Limonium perigrinum (Berg.) R. A. Dyer	2	M	NA	ABS.	needs confirmation, small root samples
DILLENIIDAE					
STERCULIACEAE					
Hermannia alnifolia L.	1	P	NA	VAM	
Hermannia multiflora Jacq.	4	P	NA	VAM	
MALVACEAE					
Lavatera trimestris L. *	1	N	B,M&A	ABS.	
BRASSICACEAE					
Brassica sp. L.*	2	N	B,M&A	ABS.	
Heliophila africana (L.) Marais	1	M	NA	ABS.	
Heliophila arenaria Sond.	1	P	NA	ABS.	
Heliophila L. sp. 1	1	P	NA	ABS.	
Heliophila L. sp. 2	1	M	NA	ABS.	
ERICACEAE					
Erica bauera Andr.			RKR	ERIC	

Erica blenna Salisb.			RKR	ERIC	
Erica campanularis Salisb.			RKR	ERIC	
Erica cerinthoides L.			RKR	ERIC	
Erica clavisepala Guth. & Bol.		O	ABL	ERIC	
Erica daphniflora Salisb.			RKR	ERIC	
Erica glauca Andr.		pot	NA	ERIC	
Erica grandiflora L.f.		pot	NA	ERIC	
Erica gracilis Wendl.		pot	NA	ERIC	
Erica hispidula L.		K	FC S&M	ERIC	
Erica inflata Thunb.			RKR	ERIC	
Erica lateralis Willd.			RKR	ERIC	
Erica mammosa L.			RKR	ERIC	
Erica mauritanica L.			S&M	ERIC	
Erica perspicua Wendl.			EML	?	Hair roots not examined
Erica regia Bartling			RKR NA	ERIC	
Erica sessiliflora L.			RKR	ERIC	
Erica ventricosa Thunb.			RKR	ERIC	
Grisebachia plumosa Klotsch	4	P	NA	ERIC	
Simocheilus depressus (Licht.) Benth.		O	ABL	ERIC	
EBENACEAE					
Diospyros glabra (L.) de Winter	1	P	NA	VAM	
PRIMULACEAE					
Anagallis arvensis L. *	3	M H	NA	VAM	
ROSIDAE					
CUNONIACEAE					
Cunonia capensis L.			EML	ENDO	
Platylophus trifoliatus (L.f.) D. Don.			EML	ENDO	

BRUNIACEAE				
Staavia dodii H. Bol.	O	AG	VAM	
Staavia radiata (L.) Dahl	4 P	NA	VAM	
CRASSULACEAE				
Cotyledon orbiculata L.	2 M	NA	ABS.	vesicles, *Olpidium* present
Crassula capensis (L.) Baill.	1 H	NA	VAM	needs confirmation
Crassula dichotoma L.	5 P M	NA	ABS.	vesicles, *Olpidium* present
Crassula expansa Dryand.	2 N	B,M&A	ABS.	
Crassula filiformis (Eckl. & Zeyh.) Dietr.	1 P	NA	ABS.	DSH present
Crassula glomerata Berg.	2 M	NA	ABS.	other fungi present
Crassula oblanceolata Schonl. & Bak.f.	5 N	B,M&A	ABS.	
Crassula tomentosa Thunb.	2 M	NA	ABS.	
MONTINIACEAE				
Montinia caryophyllacea Thunb.	1 P	NA	VAM	
ROSACEAE				
Cliffortia ruscifolia Weim.	1+ H B	NA ABL	VAM	ABL no VAM
Cliffortia polygonifolia L.	3 P	NA	VAM	
Grielum humifusum Thunb.	2 N	B,M&A	VAM	
MIMOSACEAE				
Acacia cyclops A. Cunn ex G. Don *	25 P	NA	VAM	other fungi present
Acacia karroo Hayne	pot	NA	VAM	
Acacia saligna (Labill.) Wendl. *	12+P	NA H&M	VAM	VAM sometimes ABS., *Olpidium*, DSH, other fungi often present
FABACEAE				
Amphithalia ericifolia Eckl. & Zeyh.	1 P	NA	VAM	
Aspalathus albens L.	2+P	NA H&M	VAM	infection slight (NA) cluster roots present.

Aspalathus divaricata Thunb.	1	P	NA	ABS.	needs confirmation, other fungi present
Aspalathus flexuosa Thunb.	10	P	NA H&M	VAM	other fungi present
Aspalathus linearis (Burm.f.) Dahlg.		pot	NA	VAM	cluster roots present
Aspalathus spinescens Thunb.	23	P	NA	VAM	cluster roots present, *Olpidium*, DSH, other fungi present
Aspalathus ternata (Thunb.) Druce	1	P	NA	ABS.	needs confirmation
Aspalathus sp. 1 L.	2	M	NA	VAM	
Aspalathus sp. 2 L.	1	H	NA	VAM	
Indigofera sp. L.	3	M	NA	VAM	
Lotononis involucrata Benth.	2	P	NA	ABS.	needs confirmation
Medicago polymorpha L. *	2	M	NA	VAM	
Medicago sp. L. *	6	N	B,M&A	VAM	
Otholobium fruticans (L.) C. H. Stirton		pot	NA	VAM	
Otholobium hirtum (L.) C. H. Stirton	8	P	NA	VAM	
Otholobium sp. C. H. Stirton	1	H	NA	VAM	
Podalyria calyptrata Willd.		pot	NA	VAM	
Podalyria cuneifolia Vent.		pot	NA	VAM	
Podalyria sericea R. Br.	2	P	NA	VAM	
Priestleya glauca Salter	2	H	NA	VAM	
Priestleya sericea (L.) E. Mey.	1	P	NA	VAM	
Psoralea pinnata L.		pot	NA	VAM	
Rafnia angulata Thunb.	2+	P	NA H&M	VAM	ABS. in (NA)
Virgilia oroboides (Berg.) Salter		pot	NA EML	VAM	EML reports absence of fungi
PROTEACEAE					
Faurea macnaughtonii Phill.	3	S	NA EML	ABS.	EML reports ENDO, NA no fungi present in young roots, fungi with vesicles in dead roots
Hakea sericea Schrad. *		pot	NA	ABS.	
Leucadendron laureolum (Lam.) Fourc.		pot	NA	ABS.	
Leucospermum parile (Salisb. ex Knight) Sweet	3	P	NA	ABS.	other fungi present
Protea burchellii Stapf	1	P	NA	ABS.	other fungi present

Protea scolymocephala (L.) Reich.	2	P	NA	ABS.	other fungi present
Serruria fasciflora Salisb. ex Knight	2	P	NA	ABS.	
PENAEACEAE					
Stylapteris fruticulosus (L.f.) Juss.	3	P	NA	VAM	
THYMELAEACEAE					
Cryptadenia grandiflora (L.f.) Meisn.	1+	P	NA M&R	VAM	
Passerina paleacea Wikstr.		pot	NA	VAM	
Passerina vulgaris Thoday	3	P	NA	VAM	
Struthiola sp. 1 L.	2	P	NA	VAM	
Struthiola sp. 2 L.	2	P	NA	VAM	
CORNACEAE					
Curtisia dentata (Burm. f.) C. A. Sm.			EML	ENDO	
SANTALACEAE					
Thesium densiflorum A. DC.	2	P	NA	ABS.	other fungi present
Thesium sp. cf. *T. strictum* Berg.	1	P	NA	ABS.	
Thesium sp. 1 L.	1	P	NA	ABS.	other fungi present
Thesium sp. 2 L.	1	P	NA	ABS.	DSH, other fungi present
CELASTRACEAE					
Putterlickia pyracantha (L.) Szyszyl.	1	M	NA	VAM	
ICACINACEAE					
Apodytes dimidiata E. Mey. ex Arn.			EML	ENDO	
EUPHORBIACEAE					
Clutia alaternoides L.	2	P	NA	VAM	
Clutia daphnoides Lam.	1	M	NA	VAM	
Clutia sp. 1 L.	1	P	NA	VAM	
Clutia sp. 2 L.	1	H	NA	VAM	
Euphorbia burmannii E. Mey. ex Boiss.	2	M	NA	VAM	

Euphorbia peplus L. *	2	M H	NA	VAM	
RHAMNACEAE					
Phylica cephalantha Sond.	1	P	NA M&R	VAM	confirmed in pot experiments
Phylica ericoides L.	5	P	NA	VAM	
Phylica plumosa L.	1	P	NA	VAM	
Phylica stipularis L.	5	P	NA M&R	VAM	
Phylica sp. cf. *P. rubra* Willd.	1	M	NA	VAM	
POLYGALACEAE					
Muraltia decipiens Schltr.	1	H	NA	VAM	
Muraltia dumosa (Poir.) DC.	5	P	NA	VAM	
Muraltia thunbergii Eckl. & Zeyh.	1	P	NA	VAM	
Polygala affinis DC.	3	H	NA	VAM	fine endophyte in one
Polygala garcini DC.	3	P	NA	VAM	
Polygala virgata Thunb.		pot	NA	VAM	
ANARCADIACEAE					
Rhus rosmarinifolia Vahl	1	P	NA	VAM	DSH present
RUTACEAE					
Agathosma capensis (L.) Duemmer	2	H	NA	VAM	
Agathosma collina Eckl. & Zeyh.		pot	NA	VAM	
Agathosma gonaquensis Eckl. & Zeyh.		pot	NA	VAM	
Agathosma imbricata (L.) Willd.	6	P	NA	VAM	other fungi present
Agathosma ovata (Thunb.) Pillans		pot	NA	VAM	
ZYGOPHYLLACEAE					
Zygophyllum flexuosum Eckl. & Zeyh.	2	M	NA	ABS.	
Zygophyllum morgsana L.	14	N	B,M&A	ABS.	
Zygophyllum sessilifolium L.	1	P	NA	ABS.	
Zygophyllum spinosum L.	2	P	NA	ABS.	DSH, vesicles present

OXALIDACEAE							
Oxalis capillacea E. Mey. ex Sond.	1	P			NA	VAM	
Oxalis luteola Jacq.	3	P			NA	VAM	
Oxalis sp. cf. *O. tenuifolia* Jacq.	1	H			NA	VAM	
Oxalis obtusa Jacq.	6	P	M	N	NA B,M&A	VAM	fine endophyte, DSH present
Oxalis pes-caprae L.	11	P	M	N	NA B,M&A	VAM	
Oxalis polyphylla Jacq.	1	P			NA	VAM	
Oxalis purpurea L.	1	P			NA	VAM	
Oxalis tomentosa L.f.	1	H			NA	VAM	
Oxalis sp. 1 L.	1	B			ABL	ABS.	
Oxalis sp. 2 L.	2	N			B,M&A	VAM	
GERANIACEAE							
Erodium incarnatum (L.) L'Herit.	1	H			NA	VAM	other fungi present
Monsonia speciosa L.f.	1	H			NA	VAM	
Pelargonium elongatum (Cav.) Salisb.	1	H			NA	VAM	
Pelargonium ovale (Burm.f) L'Herit.	3	P			NA	VAM	
Pelargonium senecioides L'Herit.	2	M			NA	VAM	
Pelargonium triste (L.) L'Herit.	3	P			NA	VAM	DSH, other fungi present
Pelargonium sp. 1 L'Herit.	1	P			NA	VAM	
Pelargonium sp. 2 L'Herit. in section Myrrhium	2	M			NA	VAM	
APIACEAE							
Annesorrhiza sp. cf *A. capensis* Cham. & Schlechtd.	1	P			NA	VAM	
Annesorrhiza sp. Cham. & Schlechtd.	1	H			NA	VAM	
Chamarea capensis (Thunb.) Eckl. & Zeyh.	2	H			NA	VAM	
Torilis arvensis (Huds.) Link	2	M			NA	VAM	

LILIOPSIDA

ARECIDAE

ARACEAE				
Zandtedeschia aethiopica (L.) Spreng	2 M H	NA	VAM	
COMMELINIDAE				
RESTIONACEAE				
Cannamois parviflora (Thunb.) Pillans	1 P	NA	ABS.	
Hypodiscus willdenowia (Nees) Mast.	1 P	NA	ABS.	
Ischyrolepis monanthos (Mast.) Linder	7 P	NA	ABS.	other fungi present
Staberoha distachya (Rottb.) Kunth	1 P	NA	ABS.	
Thamnochortus punctatus Pillans	2 P	NA	ABS.	
Willdenowia arescens Kunth	3 P	NA	ABS.	
Willdenowia incurvata (Thunb.) Linder	3 P	NA	ABS.	other fungi present
CYPERACEAE				
Ficinia sp. Schrad.	4 P M	NA	ABS.	vesicles may be present
Isolepis antarctica Nees	1 P	NA	ABS.	vesicles present
POACEAE				
Aristida sp. 1 L.	2 P	NA	ABS.	*Olpidium*, other fungi present
Aristida sp. 2 L.	B	ABL	ENDO	
Bromus pectinatus Thunb.	1 M	NA	VAM	*Olpidium*, DSH, other fungi present
Bromus sp. L.	13 N	B,M&A	VAM	
Ehrharta calycina J. E. Sm.	10 P M N	NA B,M&A	VAM	VAM low at P and M, *Olpidium*, other fungi present

Ehrharta villosa Schult. f.	2	P	NA	ABS.	*Olpidium*, other fungi present
Ehrharta sp. Thunb.	3	P	NA	VAM	other fungi present
Enneapogon sp. Desv. ex Beauv.	5	N	B,M&A	ABS.	
Festuca scabra Vahl	1	P	NA	VAM	
Lolium sp. L.	32	N	B,M&A	VAM	
Pentaschistis angulata (Nees) Adamson	3	M	NA	VAM	VAM low, *Olpidium*, other fungi present
Stipagrostis zeyheri (Nees) De Winter	2	P	NA	VAM	VAM ABS. in one, other fungi present
Themeda triandra Forssk.		B	ABL	ENDO	
Tribolium uniolae (L.f.) Renvoize	1	P	NA	VAM	VAM low, *Olpidium*, DSH, other fungi present

LILIIDAE

HAEMADORACEAE

Wachendorfia parviflora W. F. Barker	5	P	NA	VAM	VAM ABS. in some, DSH, other fungi present

AMARYLLIDACEAE

Haemanthus pubescens L.f.	6	P	NA	VAM	
Haemanthus sanguineus Jacq.	1	H	NA	VAM	

ASPARAGACEAE

Myrsiphyllum asparagoides (L.) Willd.	1	P	NA	VAM	
Protasparagus capensis (L.) Oberm.	3	P	NA	VAM	
Protasparagus exuvialis (Burch.) Oberm.	2	P	NA	VAM	

ASPHODELACEAE

Anthericum rangei Engl. & Krause	1	H	NA	VAM	
Trachyandra chlamydophylla (Bak.) Oberm.	1	P	NA	ABS.	needs confirmation
Trachyandra ciliata (L.f.) Kunth	2	M	NA	VAM	DSH, other fungi present
Trachyandra hispida (L.) Kunth	2	P	NA	VAM	

Trachyandra muricata (L.f.) Kunth	2 H	NA	VAM	
Trachyandra tabularis (Bak.) Oberm.	4 P	NA	VAM	
ERIOSPERMACEAE				
Eriospermum sp. 1 Jacq. ex Willd.	2 P	NA	VAM	
Eriospermum sp. 2 Jacq. ex Willd.	1 N	B,M&A	VAM	
HYACINTHACEAE				
Albuca canadensis (L.) Leighton	5 P M N	NA B,M&A	VAM	
Albuca sp. cf. *A. spiralis* L.f.	2 P	NA	VAM	other fungi present
Albuca sp. cf. *A. tenuifolia* Bak.	1 P	NA	VAM	
Lachenalia mutabilis Sweet	2 P	NA	VAM	
Lachenalia sp. cf. *L. rubida* Jacq.	1 P	NA	VAM	
Ornithogalum thyrsoides Jacq.	2 H	NA	VAM	
Ornithogalum suaveolens Jacq.	2 H	NA	VAM	
HYPOXIDACEAE				
Spiloxene schlechteri (H. Bol.) Garside	1 H	NA	VAM	
TECOPHYLAEACEAE				
Cyanella hyacinthoides L.	1 H	NA	VAM	
IRIDACEAE				
Antholyza ringens L.	1 P	NA	VAM	DSH, other fungi present
Aristea dichotoma (Thunb.) Ker-Gawl.	1 P H	NA	VAM	intracellular hyphal coils
Babiane ambigua (Roem. & Schult.) G.J. Lewis	2 P	NA	ABS.	needs confirmation, other fungi present
Babiane sp. cf. *B. nana* (Andr.) Spreng.	1 M	NA	ABS.	needs confirmation, DSH, other fungi present
Babiane tubulosa (Burm. f.) Ker-Gawl.	1 M	NA	VAM	
Geissorhiza sp. cf. *G. aspersa* Goldbl.	1 P	NA	VAM	
Gladiolus gracilis Jacq.	1 P	NA	ABS.	needs confirmation, other fungi present
Homeria longistyla Goldbl.	3 P	NA	VAM	

Lapeirousia anceps (L.f.) Ker-Gawl.	4	P	NA	VAM	
Melasphaerula ramosa (L.) N. E. Br.	1	H	NA	VAM	
Moraea angusta (Thunb.) Ker-Gawl.	3	P	NA	VAM	other fungi present
Moreae gawleri Spreng.	1	H	NA	VAM	
Romulea schlechteri Beg.	1	P	NA	VAM	DSH, other fungi present
Watsonia meriana (L.) Mill.	2	P	NA	VAM	
ORCHIDACEAE					
Disa cornuta (L.) Sw.	1	P	NA	ORCH	
Disa uniflora Berg.		pot	NA	ORCH	

MISCELLANEOUS RECORDS

CUPRESSACEAE					
Widdringtonia nodiflora (L.) Powrie		OK	ABL	ENDO	unusual type
PODOCARPACEAE					
Podocarpus falcatus (Thunb.) R. Br. ex Mirbel	2	S	NA	VAM	
ADIANTACEAE					
Pellaea sp.	1	H	NA	ABS.	

* introduced species

LOCATION AND DOMINANT VEGETATION

B=Bainskloof, mesic mountain fynbos
H=Hercules Pillar, west coast renosterveld
J=Jonkershoek, mesic mountain fynbos
K=Kirstenbosch Botanical Gardens, in cultivation
M=Melkboschstrand, west coast strandveld
N=Nortier Experimental Farm, Lamberts Bay, west coast
O=Olifantsbos, Cape of Good Hope Nature Reserve, mesic mountain fynbos
OK=Orange Kloof, afromontane forest
P=Pella, sand plain lowland fynbos
S=Saasveld, afromontane forest
pot=plant growing in pot culture

AUTHORITY

ABL =A B Low (1980)
AG =A Gubb cited by ABL
B,M&A=R Berliner, D T Mitchell & N Allsopp (1989)
EML =E M Laughton (1964)
FC =F Coley cited by ABL
H&M =M T Hoffman & D T Mitchell (1986)
M&R =D T Mitchell & D J Read (1987)
NA =N Allsopp (this study)
RKR =R K Robinson (1973)
S&M =C J Straker & D T Mitchell (1985)

TABLE 2.2. A summary of the mycorrhizal status of the vegetation growing in sand plain lowland fynbos, renosterveld and strandveld communities, and the Cape Floristic Region (CFR). VAM=vesicular-arbuscular mycorrhizal, NM=non-mycorrhizal, ERIC=ericoid mycorrhizal, ORCH=orchid mycorrhizal.

	VAM (%)	NM (%)	ERIC (%)	ORCH (%)	UNKNOWN (%)
Lowland Fynbos	72	23	<1	1-2	3
Renosterveld	77	18	0	?	5
Strandveld	64	27	0	?	9
CFR	62	23	8	2	4

Discussion

The mycorrhizal status of many of the taxa recorded here has not previously been reported, as can be expected given the high levels of endemism and species radiation in the Cape Floristic Region and the paucity of mycorrhizal studies in this region. All the important families, as well as the twenty largest genera in the Cape Floristic Region (Bond & Goldblatt 1984), have now had some of their members examined for mycorrhizas. The endemic Penaeaceae and near endemic Bruniaceae have VAM species. Families which need further investigation are the Anacardiaceae, Ebenaceae, Juncaceae, and Celastraceae, as well as the endemic families Stilbaceae, Grubbiaceae, Roridulaceae, Retziaceae, Lanariaceae and Geissolomataceae. The lowland vegetation types have been well covered and generalizations regarding their mycorrhizas can now be made. However the mycorrhizal status of the vegetation of habitats such as forests, seasonally waterlogged soils, limestone and mountain ecosystems are less well catalogued.

The absence of ectomycorrhizas is a notable feature of this flora. Ectomycorrhizal structures are reported in many plants growing in arid regions of Australia which belong to families and genera also present in the Cape Floristic Region (Warcup 1980, Warcup & McGee 1983, McGee 1986, Bellgard 1991). Ectomycorrhizas are known to occur in the low nutrient soils of the Australian mediterranean heathlands (Chilvers & Pryor 1965, Brundrett & Abbott 1991). In addition, ectomycorrhizas have been found on trees growing in other African ecosystems (Redhead 1968, Högberg & Piearce 1986). However shrub vegetation growing on Kalahari sands adjacent to ectomycorrhizal woodlands was exclusively VAM (Högberg & Piearce 1986). The reasons for the exclusion of ectomycorrhizas from the Cape Floristic Region are not clear, although this can possibly be ascribed to the absence of an organic surface horizon which is usually associated with the presence of ectomycorrhizas (Read 1991), and to frequent disturbance by fire. For instance, in Italian mediterranean ecosystems on calcareous soils, canopy cover values for ectomycorrhizal plant species 9 years after fire was a quarter of that in unburnt forest (Puppi & Tartaglini 1991). However these explanations do not satisfactorily account for

their absence in the Cape Floristic Region as ectomycorrhizas occur in fire prone communities in Australia, with low soil organic matter (Brundrett & Abbott 1991).

The explosive speciation that the genus *Erica* has undergone in the Cape Floristic Region (±530 spp.) implies that ericoid mycorrhizas are unusually common in this area. Cowling, Straker & Deignan (1990) have suggested that edaphic specialization of the endophyte has powered this speciation, but, as yet, supporting evidence is lacking. Distinctions between *Erica* spp. are based on floral features and it seems more realistic to assume that pollinator interactions were responsible for this remarkable speciation, although differences between endophytes from calcifuge and calcicole habitats (C. J. Straker pers. comm.) may account for some edaphic specialization. An interesting feature of ericoid mycorrhizal plants in the mediterranean-climate regions of the world is their co-existence with other species, while in more temperate regions they usually form almost pure stands in areas where soil degradation has produced soil conditions which plant roots and other mycorrhizas cannot tolerate (Leake, Shaw & Read 1989).

All the non-mycorrhizal plant families in this study have been reported as such before, although some have had very few species examined for mycorrhizal colonization (Trappe 1987). Many of the non-mycorrhizal species in this study fall into the Caryophyllidae which is roughly equivalent to the Centrospermae (Cronquist 1988), a subclass regarded as non-mycorrhizal (Gerdemann 1968). Subsequent studies have shown that many species in this group are capable of forming mycorrhizas (Tester, Smith & Smith 1987), and that some families are typically mycorrhizal, eg. Cactaceae (Miller 1979). However despite these exceptions, 80 % of the species in the Caryophyllidae which have been examined are either non-mycorrhizal or facultatively mycorrhizal (Trappe 1987). Mechanisms which enable some plant species to remain non-mycorrhizal, when exposed to viable inoculum, are unclear (Tester *et al.* 1987, Koide & Schreiner 1992), although successful mycorrhizal development depends on a sequence of recognition processes between the symbionts (Gianinazzi-Pearson & Gianinazzi 1989).

In dicotyledonous species, weedy, herbaceous plants often lack mycorrhizas or are weakly mycorrhizal (Malloch, Pirozynski & Raven 1980, Trappe 1987) and it has been noted that some species are less likely to form mycorrhizas when colonizing disturbed sites than

adjacent undisturbed areas (Miller 1979, Reeves *et al.* 1979). In this study, the annuals in the Scrophulariaceae were usually non-mycorrhizal although a few individuals form typical VA mycorrhizas.

Anaerobic conditions in waterlogged soils have been invoked to explain the absence of mycorrhizas in some plants (Anderson, Liberta & Dickman 1984), and Tester *et al.* (1987) advance this as an explanation of the absence of mycorrhizas in most of the Cyperaceae. In this study the members of the Cyperaceae and the Restionaceae examined were non-mycorrhizal while growing in well drained soil with other mycorrhizal plants, although both families are often associated with waterlogged conditions, and so this does not seem to be the only reason for the exclusion of mycorrhizas from these taxa. Although Powell (1975) reports mycorrhizal structures in some roots of members of the Cyperaceae, he concludes that they are functionally non-mycorrhizal due to the possession of a fine root system. This complements Baylis' (1975) proposal that the magnolioid root form with poorly developed root hairs would be more strongly mycorrhizal than finer root systems. Two important perennial families in the Cape Floristic Region, which do not form mycorrhizas (viz. Proteaceae and Restionaceae), are characterized by the formation of cluster roots, the rootlets of which are densely covered in long root hairs (Purnell 1960, Lamont 1972a, 1982). In addition, cluster roots have been observed on members of the Cyperaceae (Lamont 1974), the genus *Aspalathus* (Fabaceae) (N. Allsopp and M. Cocks unpublished data), and Australian members of the Fabaceae (Lamont 1972b, Brundrett & Abbott 1991) which typically have low VAM infection levels. The absence or low infection levels of mycorrhizas in the taxa forming cluster roots supports the proposition that mycorrhizas will be less important when root systems are finer or root hair production dense (Baylis 1975). The loss of the ability to form mycorrhizas is regarded as an evolutionarily advanced feature (Trappe 1987).

The mycorrhizal status of the species in the Cape Floristic Region seems to be a reflection of their taxonomic position, although Newman & Reddell (1987) warn that very few families form exclusively one type of mycorrhiza or are consistently without mycorrhizas. This can be expected when world-wide the higher taxa of Angiosperms are poorly

correlated with their ecological niches (Cronquist 1988). Life form or environmental factors do not satisfactorily explain the absence of mycorrhizas in longer lived plants such as members of the Proteaceae, Restionaceae and Zygophyllaceae in the Cape Floristic Region, and this must be regarded as a taxon-related characteristic for many groups. Reports of VAM species among the Proteaceae in New South Wales, Australia (Bellgard 1991) and ectomycorrhizal *Faurea saligna* (Proteaceae) in Zambia (Högberg & Piearce 1986), indicate that the mycorrhizal status of members of this family should be investigated with respect to soil fertility, as mycorrhizas are absent in members of this family growing in the low nutrient soils of the Cape Floristic Region and Western Australia (Brundrett & Abbott 1991). Members of families such as the Aizoaceae and Mesembryanthemaceae, which are commonly found associated with disturbed areas in the Cape Floristic Region, are non-mycorrhizal when growing in undisturbed ecosystems. This supports the report that, at the ecosystem level, patterns of some mycorrhizal and non-mycorrhizal weedy species followed taxonomic divisions irrespective of growth form (Pendleton & Smith 1983). As most of the data here are obtained from plants growing in the field and mycorrhizal status was usually consistent at the family level, generalizations can be made regarding the mycorrhizal status of the Cape Flora provided cognizance is taken that exceptions may arise. The mycorrhizal status of the three lowland vegetation types is summarized in Table 2.2. If the mycorrhizal status of species listed in Bond & Goldblatt (1984) is inferred from that of taxonomically related species which have been examined, it is concluded that 62 % of the flora of the Cape Floristic Region form VA mycorrhizas, plants without mycorrhizas are the next largest group, ericoid and orchid mycorrhizas are found in less than 10 % of the flora and the mycorrhizal status of 4 % of the flora is unknown (Table 2.2).

The proportion of non-mycorrhizal species in the Cape Floristic Region is high when compared to many other vegetation types world-wide (Brundrett 1991). As non-mycorrhizal plants are normally associated with high levels of disturbance, or edaphically and climatically extreme conditions, the non-mycorrhizal flora in the Cape Floristic Region is atypical in that representatives of two of the families that dominate the vegetation of the Cape Floristic Region, the Proteaceae and Restionaceae, are non-mycorrhizal. The evolutionary and ecological significance of this needs further exploration. The diversity of

mycorrhizal types is possibly an indication that no one type of mycorrhiza nor other nutrient acquiring adaptation is pre-eminently suited to the environmental conditions in the Cape Floristic Region and that the diversity of nutrient acquisition mechanisms in this region has probably promoted plant species co-existence.

CHAPTER 3

Patterns of Vesicular-arbuscular Mycorrhizal Infection in Relation to Vegetation Type in Three Lowland Plant Communities in the South Western Cape

Introduction

The occurrence of VA mycorrhizas is widespread in natural vegetation (Brundrett 1991), but the intensity of infection can be very variable amongst individuals and species (Fitter 1989). Low temperatures and short growing season appear to exclude VAM infection at high latitudes (Bledsoe, Klein & Bliss 1990) and at very high altitudes (Read & Haselwandter 1981). The presence of ectomycorrhizal species may reduce VAM infection (Tobiessen & Werner 1980, Kovacic, St John & Dyer 1984) and the highly acid, humic soils of temperate and boreal heathlands exclude all plants except those forming ericoid mycorrhizas (Read 1991). Waterlogged conditions are also unfavourable for VAM infection (Bagyaraj, Manjunath & Patil 1979, Anderson *et al.* 1984) and soil disturbance often favours non-mycorrhizal or facultatively VAM species with low infection (Miller 1979, Reeves *et al.* 1979).

In ecosystems subject to less extreme environmental conditions, however, the factors controlling the intensity of infection are less obvious. Differences between species may be due to differing reliance on mycorrhizas for phosphorus uptake (Baylis 1975, Janos 1980b), or differences in seasonality of VAM formation (Fitter 1989). The amount of infection in roots of individual plants may be a function of their chance of encountering VAM inoculum in the soil, as well as the developmental stage when inoculum is encountered as this determines susceptibility to infection (Brundrett & Kendrick 1990b). VAM infection levels in a plant community and VAM fungal populations may be influenced by factors such as soil organic matter, soil phosphorus concentrations, percentage cover of VAM plants, and age of vegetation since disturbance (Miller, Moorman & Schmidt 1983, Anderson *et al.* 1984, Cook, Jastrow & Miller 1988, Johnson *et al.* 1991). Although simulated fires may reduce VAM infectivity of soil (Klopatek, DeBano & Klopatek 1988), it is often not clear in natural vegetation whether infection is reduced by direct effects of fire on VAM fungal populations, or if the effect is an indirect response due to changes in plant cover (Gibson & Hetrick 1988, Dhillion, Anderson & Liberta 1988).

Janos (1980b) proposed a model to describe changes in the mycorrhizal composition of plants in different seral stages of community development based on lowland tropical forest succession. Early seral stages dominated by non-mycorrhizal species are replaced by facultatively mycorrhizal species in mid-succession and then by obligately mycorrhizal species in climax vegetation. However, the authors of a study on variously aged vegetation in an alpine community concluded that the model did not fit the system (Allen *et al.* 1987). Variations in patterns of community development in response to disturbance should result in the model being modified for different vegetation types.

In this chapter the VAM infection of potentially VAM plants from different lowland shrublands of the mediterranean climate region of the Cape Floristic Region is quantified. Levels of VAM infection amongst different growth habits in sand-plain lowland fynbos, west coast renosterveld and west coast strandveld are compared and related to edaphic and vegetation characteristics. The effect of fire on infection levels among plants in fynbos vegetation is also examined. Information on the intensity of VAM infection among plants is needed to improve our understanding of the functioning of mycorrhizas in natural habitats.

Study Areas

VAM infection levels of plants growing in sand-plain lowland fynbos, in west coast strandveld and in west coast renosterveld were investigated. Location of the vegetation types and edaphic characteristics are described in Chapter 2 and Table 3.1. The climate is mediterranean and minimum daily temperatures seldom fall below 3 ^{o}C (Jarman & Mustart 1988). Annual average precipitation is approximately 520 mm (range 300 to 800 mm) and falls mainly in the winter months from May to September (Jarman & Mustart 1988).

Fire is a major disturbance feature at the 269 ha fynbos site (Brownlie & Mustart 1988) and prior to 1987, the vegetation was a mosaic of different aged vegetation, ranging from 5 to greater than 20 years old. A wild fire in November 1986 destroyed most of the vegetation except for a small area of 5 year old fynbos. The strandveld vegetation showed no signs of

fire disturbance but disturbance from burrowing mole-rats and human trampling seems to have created a mosaic of older and younger vegetation. The renosterveld site was burnt in approximately 1978 and is occasionally grazed by domestic stock which has reduced the grass cover substantially (Tansley 1982). This renosterveld is possibly a mid-successional community which may develop into Western Thicket (Campbell 1985) as species characteristic of this vegetation type such as *Olea*, *Euclea*, *Rhus* spp. are found protected among rocks and may become dominant if the site is not disturbed.

Vegetation characteristics of these three shrubland types are described in Chapter 2. Vegetation cover was estimated to be greater than 70 % at the strandveld and renosterveld sites at the time of sampling and was usually above 80 % for 4 year or older vegetation at the fynbos site (Boucher & Shepherd 1988). Cover increased rapidly in the burnt area at the fynbos site to approximately 70 % in the third year post-fire. No single species dominated strandveld and cover of individual species was less than 5 %, while 10 - 20 % of the cover is made up of non-mycorrhizal species, mostly members of the Mesembryanthemaceae and Crassulaceae. Cover of the asteraceous dominant, *Elytropappus rhinocerotis*, in renosterveld was estimated at 10 - 20 % on the plots sampled and non-mycorrhizal species contributed less than 5 % of cover. Cover by individual species seldom exceeds 5 % in 50 m^2 plots in 4 - 20 year old sand-plain lowland fynbos at Pella although in about half of these plots either *Phylica cephalantha* (Rhamnaceae) or *Thamnochortus punctatus* (Restionaceae), or both, had higher cover values (Boucher & Shepherd 1988). The Proteaceae and Restionaceae are the most important plants in the non-mycorrhizal group in the older vegetation at the fynbos site and account for a considerable proportion of the phytomass and cover (Table 3.2).

TABLE 3.1. Edaphic characteristics and vesicular-mycorrhizal spore numbers for west coast strandveld, west coast renosterveld and sand plain lowland fynbos.

	Fynbos[1]	Renosterveld[2]	Strandveld[3]
Soil texture			
Sand (%)	98	86	99
Silt (%)	1	6	1
Clay (%)	<1	8	0
pH ($CaCl_2$ extract)	4.6	4.1	7.5
Organic Matter (%)	1.4-3.4	4.9	2.2
Phosphorus ($\mu g\ g^{-1}$)			
Total	23-34	127	422
Resin extract	1.4	0.8	40
VAM spores ($(100\ g)^{-1}$)	38	90	94

[1] soil data from Mitchell, Brown & Jongens-Roberts (1984), spore numbers Berliner *et al.* (1989)
[2] N. Allsopp, unpublished
[3] soil data from Witkowski & Mitchell (1987), spore numbers from Berliner *et al.* (1989)

TABLE 3.2. Composition of above ground vegetation at the fynbos site. Biomass[1] made up by mycorrhizal (VAM) shrubs and non-mycorrhizal Proteaceae (Proteoid) and Restionaceae (Restioid) and percentage cover[2] of Proteaceae and Restionaceae in 5 x 10 m plots of various post-fire ages. A - indicates that data are not available.

	Percent of Biomass		Percent of Cover					
Vegetation Age (years)	4	9	1	3	5	12	15	19
VAM spp.	35	41	-	-	-	-	-	-
Proteoid	25	38	2	3	17	4	16	10
Restioid	37	20	3	6	17	32	32	46

[1] Data from Mitchell *et al.* (1986). Biomass was recorded for the ericoid, proteoid and restioid physiognomic groups. However the plants in the ericoid leafed group were predominantly vesicular-arbuscular mycorrhizal species, rather than members of the ericoid mycorrhizal Ericaceae. The non-mycorrhizal Proteaceae and Restionaceae were the major components of the proteoid and restioid groups respectively.

[2] Data from Hoffman *et al.* (1987)

Materials and Methods

Roots were collected during winter from 25 m x 25 m plots at the three vegetation sites as described in Chapter 2. Four plots were sampled in renosterveld and strandveld and 12 in fynbos. In 1986, plants in 5 year old fynbos were sampled, but, following a wild fire at the end of that year, root collection in 1987-1989 was confined to the burnt area at the fynbos site. Sampling at the strandveld site was biased towards younger vegetation as the climax species typically formed dense thickets which were impossible to penetrate non-destructively in order to trace root systems from the base of individual plants. Phenological data on root growth are lacking for the flora of the S. W. Cape, but field observations at the three sites sampled in this study indicate that root growth is only to be expected when the soil is moist for prolonged periods as in winter and, therefore, roots were only collected during the wet winter months.

The roots of one or two plants of every VAM species occurring in the plots were sampled. However, among some shrubs and tussocking grasses it was extremely difficult to find suitable material. Roots were cleared and stained as described in Chapter 2, cut into approximately 1 cm lengths and 50 - 200 root segments per plant were permanently mounted. Slides were scanned at 100 times magnification with a light microscope and the presence or absence of VA mycorrhizas in root sections crossing the field of view were scored. Confirmation of VAM structures (arbuscules, and associated vesicles and hyphae) was made at 400 times magnification whenever necessary. Percentage VAM infection of roots was calculated as that proportion of the total number of sections seen in the microscope field of view with either arbuscules or vesicles, provided the latter appeared to be typically mycorrhizal.

For the purpose of this study, data of VAM infection (usually only vesicles and hyphae) among individuals in typically non-mycorrhizal families (Chapter 2) were excluded. On average slightly fewer than two plants per species were examined per site. Plants were divided into three growth habit categories: annuals, perennials, which are mostly woody shrubs but include some herbaceous plants, and geophytes (both monocotyledons and dicotyledons, including hemicryptophytes).

The wet-sieving and decanting method followed by centrifugation in a 50 % sucrose was used to isolate VAM spores from the soil (Tommerup & Kidby 1979). The VAM infectivity of soil from adjacent burnt and unburnt vegetation at the fynbos site was determined 1 and 2 months after the November 1986 wild fire using the Most Probable Numbers (MPN) technique (Porter 1979). Soil was collected from the top 5 cm at points every 50 m along a line running parallel and 30 m from the burn boundary. Three soil samples were collected from the burnt and unburnt vegetation on each collection date.

Percentage infection values were arcsine transformed (Zar 1984). The number of plants with transformed percentage infection levels falling into each of five even-sized infection intensity categories were counted for the different vegetation types, growth habits and four plant families from the fynbos site. As the data was not normally distributed, non-parametric statistical analyses of the data were performed. Kruskal-Wallis one-way analysis of variance by ranks was used to compare the average VAM infection between plots, vegetation types, growth habit and, in the fynbos vegetation, between families with more than six plants sampled.

Results

Post-fire age at the fynbos site

There was a significant difference ($p < 0.0001$) in the average infection for the plots sampled at the fynbos site. Those plots with a post-fire age of less than 2 years had a significantly ($p < 0.0001$) lower average rank of VAM infection compared to those of 3 and 5 years post-fire but there were no significant differences between plots within these age groups as tested by Kruskal-Wallis one-way analysis of variance by ranks. Therefore all the samples from the 1 and 2 year post-fire vegetation were combined, as were those from the 3 and 5 year old vegetation. Few plants in plots less than 2 years post-fire had high VAM infection levels (Fig 3.1). Among the plants on the older plots there was a more even spread of plants with infection at all levels, although most plants were moderately to heavily infected

(Fig 3.1). Geophytes and perennials on the older plots had significantly higher infection levels ($p<0.01$, $p<0.001$ respectively) than those from the younger plots (Fig. 3.1).

Growth habit at the fynbos site

Most of the annuals sampled (n=40) were from the younger plots and all the annual plants (n=44) are plotted together (Fig. 3.1). Annuals at the fynbos site had very low levels of infection with decreasing numbers in the higher infection classes (Fig 3.1) and infection levels among annuals were significantly lower ($p<0.001$) than among perennials and geophytes from the younger plots. Although very few geophytes had low infection levels, in the older plots quite a few perennial plants had very low levels of VAM infection while the rest had moderate to high levels of infection (Fig. 3.1). However, the average infection rank for geophytes and perennials were the same within the same age group.

Family at the fynbos site

Of those families with six or more plants sampled there was a significant difference in average infection rank. In order of increasing infection levels were Scrophulariaceae, Poaceae, Fabaceae, Geraniaceae, Asteraceae, Oxalidaceae, Iridaceae, Asphodelaceae and Hyacinthaceae from the younger plots ($p<0.01$) and Fabaceae, Asteraceae, Iridaceae, Polygalaceae, Rhamnaceae and Thymelaeaceae from the older plots ($p<0.05$). Levels of infection of plants in the Scrophulariaceae, Iridaceae, Fabaceae and Asteraceae can be seen in Fig. 3.2. Average infection rank in these families was not significantly different for plants from the older and younger plots and the data in Fig. 3.2 were pooled. Infection levels among the plants in the Scrophulariaceae (annuals) are particularly low, but among the Asteraceae (annuals and perennials) and Iridaceae (geophytes) infection levels are fairly evenly spread among all classes except that few plants had very high infection (Fig. 3.2). Members of the Fabaceae (all perennials) have infection levels ranging from very low to very high (Fig. 3.2).

Vegetation type

Only five of the species sampled were common to all three sites, and 19 were common to two sites. There were no significant differences in infection between sampling plots at either the strandveld or the renosterveld sites. Comparing data from the 3 and 5 year post-fire fynbos samples or from all the fynbos plots with the strandveld and renosterveld data followed the same patterns, so age of the vegetation was not important in comparisons between the vegetation types. The average infection of all plants was significantly ($p < 0.0001$) different between vegetation types with strandveld having the lowest average rank and renosterveld the highest (Fig. 3.1). This pattern was repeated for geophytes ($p < 0.001$) and perennials ($p < 0.0001$) but infection was lowest among annuals at the fynbos site, followed by strandveld, with renosterveld again having the highest infection ($p < 0.0001$) (Fig. 3.1).

Although the levels of infection among annuals, perennials and geophytes are not identical at either the strandveld or renosterveld sites (Fig. 3.1), average infection was not significantly affected by growth habit at either site. Strandveld vegetation generally had decreasing numbers of plants in the higher infection classes while most of the plants at the renosterveld site had moderate to high levels of infection (Fig. 3.1). VAM spore numbers were similar in strandveld and renosterveld and lowest at the fynbos site (Table 3.2).

VAM infectivity of fynbos soils

VAM infective propagules, as determined by the MPN method, were 8, 11, and 79 per 100 g soil from unburnt vegetation and 79, 170 and 540 propagules per 100 g soil from burnt vegetation 1 month after the fire. In the second month, infectivity was 22, 22 and 79 propagules per 100 g soil from unburnt vegetation and 49, 70 and 140 for burnt vegetation. Despite the variation in infectivity of the samples, these values are not significantly different using calculated confidence limits (Alexander 1965). Further samples taken in March and June became contaminated in the greenhouse.

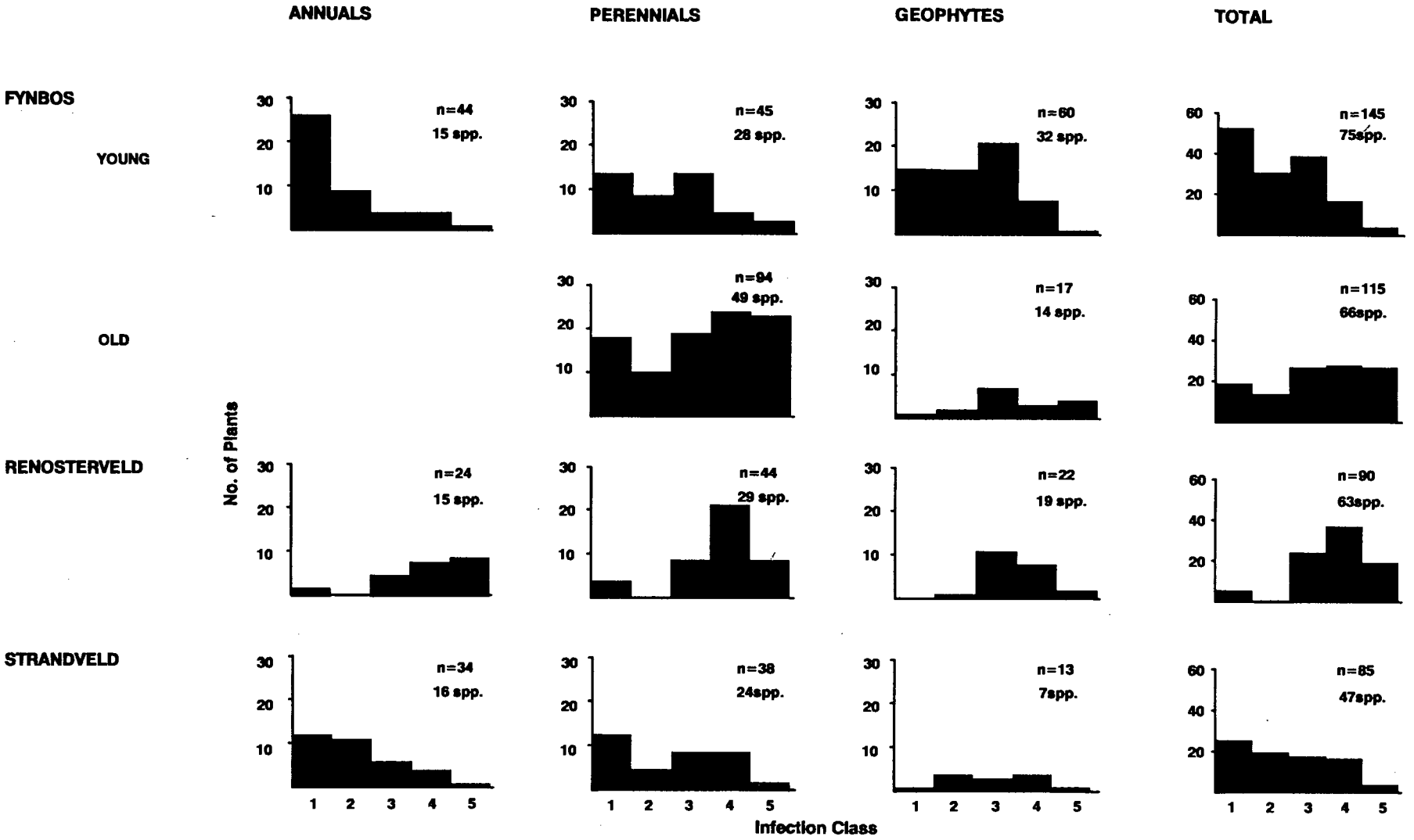

FIGURE 3.1. Infection levels among vesicular-arbuscular mycorrhizal plants growing in fynbos, renosterveld and strandveld. Number of plants with infection in infection classes 1 to 5. 1 = 0 - 17.9 %, 2 = 18.0 - 35.9 %, 3 = 36.0 - 53.9 %, 4 = 54.0 - 71.9 %, 5 = 72.0 - 90 % of root length infected, percentage data arcsine transformed.

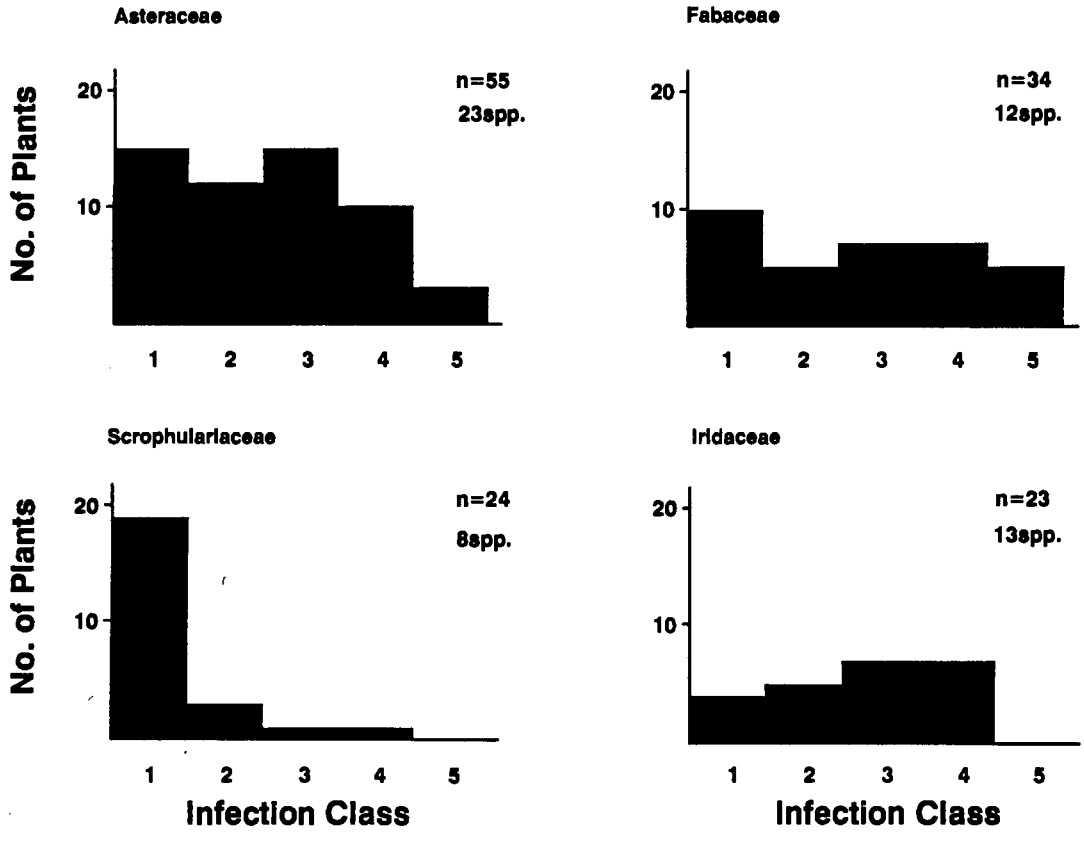

FIGURE 3.2. Numbers of plants in four families from fynbos, with vesicular-arbuscular mycorrhizal infection levels falling in five intensity classes. See Fig. 1 for explanation of class size.

Discussion

The age of the vegetation after fire seems to be an important factor influencing levels of infection among potentially VAM plants in fynbos despite variation in date of sampling which may be expected to obscure pattern (Gay, Grubb & Hudson 1982, Sanders & Fitter 1992a). The low post-fire infection levels are largely a result of the low levels of infectivity associated with annuals. Most annual plants had completed their vegetative growth and were flowering when collected, and therefore the lack of VAM infection could not be ascribed to short exposure to field inoculum. Factors that contribute to low levels of infectivity in annuals are that VAM colonization of roots is restricted to a short period of their life history (Brundrett & Kendrick 1990a) and, as annuals typically have high growth rates, roots may grow faster than the capacity of VAM fungi to infect them (Dodd & Jeffries 1986). In addition, the ruderal lifestyle of annuals is often associated with lowered mycorrhizal dependence and infection (Trappe 1987). Annuals dominate very early post-fire fynbos environments probably by exploiting the temporarily enhanced nutrient status of the soil (Brown & Mitchell 1986, Stock & Lewis 1986), and by reducing the carbon drain of maintaining mycorrhizal fungi.

The lower average VAM infection among perennials and geophytes sampled in the first and second growing season after fire suggests that fire may reduce soil infectivity, although results of the MPN measure of soil infectivity suggest otherwise. This is not a particularly reliable technique for measuring soil infectivity (Wilson & Trinick 1982), as disruption of the VAM hyphal network during soil dilution reduces infection from this source (Jasper, Robson & Abbott 1989, Evans & Miller 1990). The MPN method possibly only measures potential infectivity contributed by VAM fungal spores in the soil. However networks of VAM fungal hyphae, supported by established root systems, are thought to be the most important source of rapid VAM infection of new roots (Read *et al.* 1976, Newman 1988, Read & Birch 1988, Jasper *et al.* 1989). Destruction of above ground phytomass in the fire results in death of rootstocks from which the VAM hyphae would proliferate and many new roots may, therefore, remain non-mycorrhizal after a fire through a failure to encounter

hyphal inoculum. In addition, VAM plant species, regenerating in patches dominated by non-mycorrhizal species in the pre-fire vegetation, may remain uninfected unless VAM inoculum is reintroduced.

The intensity of infection among perennials from the three vegetation types, with fairly large numbers of plants with very low infection levels, very few with moderately low, and most with moderate to high levels of infection supports St John & Hunt's (1983) proposal that infection is a random process, but that the spread of infection along roots is exponential. This pattern also suggests that as in other ecosystems, VAM inoculum is patchily distributed in these vegetation types (Koske 1981, Walker, Mize & McNabb 1982, Allen & Allen 1990). More information on the spatial and temporal distribution of VAM fungi in the soil is needed as this will affect the establishment of individuals and, therefore the pattern of succession. The high percentage cover of non-mycorrhizal species in fynbos may increase the patchiness of VAM inoculum which in turn may affect vegetation dynamics. At the renosterveld site the higher levels of infection are probably related to the high cover of VAM plants (Miller *et al.* 1983, Benjamin, Anderson & Liberta 1989).

Edaphic characteristics, which largely determine the vegetation types in the lowland shrublands of the Cape Floristic Region, may be responsible for the different intensities of infection among fynbos, strandveld and renosterveld vegetation. Expectations that infection levels should decrease as soil fertility increases (Read *et al.* 1986) are supported by the results for the strandveld site which has the highest total phosphorus content in its soil of the three vegetation types, but the lowest average infection among VAM plants. However, VAM infection levels are not invariably correlated with soil phosphorus levels (Abbott & Robson 1991), and the renosterveld site has higher infection levels than the fynbos site despite higher levels of total phosphorus in its soil. Possibly the higher soil organic matter content, which stimulates VAM hyphal growth (St John, Coleman & Reid 1983), may contribute towards higher infection levels in renosterveld.

High levels of plant available phosphorus, as measured by resin extractable phosphorus, at the strandveld site indicate that the phosphorus cycle is not very tight, and that phosphorus

is not limiting plant growth here. Under such circumstances, VAM may be redundant; hence the low levels of infection among many of the VAM plants. However, some plants may require mycorrhizas to acquire other soil mineral nutrients which may be in short supply. The Mesembryanthemaceae and Crassulaceae and other non-mycorrhizal plants (Berliner *et al.* 1989) may be successful in strandveld due to the reduced advantage of being mycorrhizal for phosphorus acquisition in this vegetation. Strong competition among plants to acquire phosphorus in renosterveld and fynbos probably accounts for low levels of plant available phosphorus at these sites.

Data from the fynbos site, where members of some families were sampled several times suggest that plant characteristics associated with taxonomic position may influence infection. Consistent differences in levels of infection of co-occurring species is frequently reported (Anderson & Liberta 1987, Brundrett & Kendrick 1988, Sanders & Fitter 1992a), but little is known about the mechanisms which enable VAM plants to control levels of infection (Smith & Gianinazzi-Pearson 1988, Koide & Schreiner 1992). Root development, suberization and arrangement of cells influence VAM formation (Brundrett & Kendrick 1990b) and, in so far as these characteristics may be influenced by edaphic conditions, may account for some of the variation in infection among taxa and vegetation type. Although amount of root infection is not directly related to amount of extraradical hyphae (Abbott & Robson 1985), to the effectiveness of nutrient uptake of the particular fungal strains infecting the root (Sanders *et al.* 1977) or to the nutritional status of the individual plant (McGonigle 1988, Sanders & Fitter 1992b), patterns of infectivity reveal broad patterns of mycorrhizal activity in natural vegetation. Absence of mycorrhizal infection among some members of a species growing in the field may be interpreted as meaning that the species is facultatively mycotrophic (Allen & Allen 1990). While this is probably true for annuals, among slow growing perennial plants, from low nutrient environments, such a conclusion should be viewed with caution. Most perennial VAM shrubs from fynbos would appear to be reliant on mycorrhizas for phosphorus uptake in the low phosphorus soils of fynbos (Chapters 4 & 5) and lack of mycorrhizal infection in the field may indicate that the roots are growing through a nutrient poor soil patch with low biological activity.

Patterns of plant succession in the lowland shrublands of the Cape Floristic Region are insufficiently known and much of the vegetation has been transformed by human activities, so that comparative studies among different aged communities are difficult. However, it seems that renosterveld probably has strong mycotrophic tendencies. In the early post-fire stage, non-mycorrhizal or facultatively mycorrhizal annuals are temporarily abundant in fynbos, but, contrary to the model of mycorrhizal succession (Janos 1980b), biomass contributed by non-mycorrhizal species, made up by members of the Proteaceae and Restionaceae, dominate older vegetation in fynbos. Despite this dominance, numbers of potentially VAM perennial species in 4 to 20 year old fynbos vegetation at Pella average 30 (SD$\pm$5) species per 50 m^2 plot and do not diminish with vegetation age (calculated from Boucher & Shepherd 1988). VA mycorrhizas may be important in maintaining species diversity in fynbos, as has been postulated for other low-nutrient environments (Grime *et al.* 1987). The importance of mycorrhizas in the phosphorus rich strandveld soils is equivocal, as observation suggests that non-mycorrhizal species increase in importance following disturbance, as well as along a gradient of increasing aridity up the west coast (cf. the study of Berliner *et al.* (1989) in more arid strandveld). Unfortunately, the present study was unable to establish general levels of VAM infection among the thicket forming species of climax vegetation.

Levels of VAM infection are not uniform within or between vegetation types. Factors contributing to this are patchy distribution of VAM infectivity in soil, taxonomic position, growth form, disturbances such as fire, and stage in community development. Interactions between VA mycorrhizas, edaphic features and plant species contribute to the distinct characteristics of lowland vegetation types in the Cape Floristic Region.

CHAPTER 4

Mycorrhizas Stimulate Growth of Seedlings of Three Slow Growing, Sclerophyllous Fynbos Species

Introduction

The fynbos vegetation of the S. W. Cape is dominated by sclerophyllous shrubs associated with soils of amongst the lowest nutrient status worldwide (Rundel 1988). Characteristically plants from nutrient poor areas have low maximum growth and nutrient turnover rates, and low growth responses to nutrient additions, but potential for storage of such nutrients (Barrow 1977, Chapin 1980, Witkowski *et al.* 1990). Physiological demands for nutrients among these plants are not different to other plants, and nutrient requirements to maintain metabolic function are very similar to those of plants adapted to higher nutrient environments (Clarkson 1967, Field & Mooney 1986). Typically, plants have not evolved many specialized mechanisms for enhanced extraction of nutrients from low nutrient soils (Chapin 1980, Marschner 1991). An exception is the cluster roots, produced by members of the Proteaceae, a dominant fynbos family, which are particularly efficient at acquiring soil nutrients (Lamont 1982, Marschner 1991). The most common response of plants to low nutrient supply is to constrain growth within the limits of resource availability. Although this results in the production of less metabolically active tissue, nutrient deficiencies are avoided (Chapin 1988).

The perception has arisen, in a few quarters, that mycorrhizas may be of less benefit to slow growing wild plants from low nutrient environments than to faster growing, cultivated plants (St John & Coleman 1983, Koide *et al.* 1988a, Koide 1991a). The assumption underlying this is that plants with low, inflexible growth rates, high nutrient reallocation, and low tissue turnover will make low demands on their environment for nutrients such as phosphorus, whose uptake is usually enhanced by mycorrhizas. However, as the availability of soil phosphorus is often transitory, a slow uptake mechanism, to complement the low requirement over time, would be disadvantageous because wild plants rely on acquiring nutrients rapidly, in excess of immediate needs, during periods of availability (luxury consumption) (Chapin 1980). These nutrients are stored until required for growth or reproduction (Chapin 1980, Chapin, Schulze & Mooney 1990). Seedling establishment following wild fires in the fynbos is a period where efficient uptake of nutrients is particularly critical and acquisition of nutrients from the soil in competition with other plants and microorganisms would best be mediated by mycorrhizas for most plant species. In this

chapter the prediction that VA mycorrhizas are essential for the establishment of seedlings of woody, sclerophyllous plants is tested by investigating the effects of VA mycorrhizas and phosphorus fertilization on the growth and phosphorus nutrition of three species in pot culture.

Materials and Methods

Three sclerophyllous, woody plant species, indigenous to fynbos in the Cape Floristic Region (Bond & Goldblatt 1984), were grown in a low nutrient soil in a factorial experiment with mycorrhizal and phosphorus fertilizer treatments. The species, *Phylica ericoides* L. (Rhamnaceae), *Agathosma ovata* (Thunb.) Pill. (Rutaceae) and *Staavia radiata* (L.) Dahl (Bruniaceae), form shrubs up to a metre high. Seeds of *Phylica* and *Staavia* were collected from wild populations at Hagelkraal ($34^{o}40'S$ $19^{o}30'E$) and the fynbos biome intensive study site at Pella ($33^{o}31'S$ $18^{o}32'E$), respectively. Seeds of *Agathosma* were obtained from the seed collection of Kirstenbosch Botanical Gardens, Claremont, South Africa.

Phylica seeds were scarified in concentrated H_2SO_4 for half an hour. *Agathosma* seeds required no treatment other than surface sterilization with dilute NaClO. Seeds were placed in plastic petri dishes on damp sterile filter paper and incubated ($10^{o}C/20^{o}C$ temperature cycle) until sufficient of any one species had germinated to establish 30 pots with one plant per pot. *Staavia* seeds were sown in pots of sterile soil in the winter following seed collection in the previous spring. The pots were covered and left unwatered at ambient temperatures in the greenhouse until the following autumn, when watering commenced. *Staavia* seedlings emerged and were transplanted to the experimental pots at the same time as the other species were being established.

Seedlings were planted in a sterile field soil:acid washed sand mix (1:1) in 13 cm diameter plastic plant pots. The field soil was a sandy soil of low phosphorus and nitrogen status collected from the top 20 cm at the fynbos biome intensive study site (Mitchell *et al.* 1984, Stock & Lewis 1986) and sieved through a 2 mm mesh. VAM plants were established in half the pots by placing a layer of 35 g VAM inoculum soil containing approximately 1000

spores of *Acaulospora morrowae* Spain & Schenk and infected root fragments of *Trifolium subterraneum* L., 5 cm below the soil surface. The inoculum was provided by INVAM (West Virginia University, Morgantown, WV 26506-6057, USA). Local inoculum was not used because of difficulties in maintaining indigenous cultures to a consistent quality. Non-mycorrhizal (NM) controls received a layer of sterilized inoculum soil and a filtrate of non-sterile inoculum soil containing no VAM fungal spores. Both mycorrhizal treatments also received 50 ml of a filtrate of non-sterile freshly collected field soil (500 g soil per litre water) at planting and at 8 weeks.

Five pots of each mycorrhizal treatment received 25 ml of a 11.2 mM solution of KH_2PO_4 at 12 and 20 weeks. This is equivalent in total to about four times the amount of phosphorus (0.5 g P m^{-2}) being returned to the soil as ash following fire at the site from which the soil was collected (Brown & Mitchell 1986) and represented a total phosphorus input of 17.3 mg per pot. The experimental addition of phosphorus is greater than the highest amount of available phosphorus that these species are likely to encounter in their natural range (Witkowski & Mitchell 1987), but below the amount likely to be toxic to plant growth (Witkowski 1989). The unfertilized plants received two 25 ml aliquots of a solution containing an equivalent amount of K in the form of KCl.

Pots were randomly arranged on benches in a well-ventilated, unheated greenhouse in the Botany Department, University of Cape Town, and were randomized every two weeks. Plants were watered with deionized water three times a week. The temperature in winter was 8 ^{o}C - 25 ^{o}C and in summer 18 ^{o}C - 42 ^{o}C. Humidity fluctuated between 40 - 100 % RH. The greenhouse received between 4 h day^{-1} direct sunlight on clear days in mid-winter and 8 h day^{-1} in summer. Photosynthetically active radiation, while the sun was shining, ranged from 800 - 3500 μmol m^{-2} s^{-1}.

Plants were harvested at 44 weeks for *Agathosma* and 40 weeks for the other species. Plants were divided into shoots and roots (separated from the soil using a gentle spray of tap water) and dried at 80 ^{o}C for 72 h, weighed and stored in brown envelopes. Five plants of each mycorrhizal treatment were harvested at 8 weeks for the calculation of relative growth rates (RGRs) (Hunt 1978) for the period from 8 weeks until harvesting. Root:shoot ratios were

calculated from dry mass. Height of the plant above the cotyledons was measured at 4-weekly intervals from the twelfth week and the number of living and dead or abscised leaves at the final harvest were counted.

Before drying, a subsample of root material was collected for assessing VAM infection (approximately 50 randomly selected 0.5 - 1 cm pieces of root). Root material was cleared in 10 % KOH at 20 ^{o}C for seven days, then rinsed with tap water and acidified with 1 M HCl. Roots were stained in 0.05 % Trypan blue in lactic acid/glycerol/water (14:1:1) (Kormanik & McGraw 1982) and destained in an acidified 50 % glycerol solution. Percentage infection was calculated by scoring mounted root segments viewed at 100 x magnification as VAM or not in randomly chosen fields of view of a compound microscope.

Phosphorus content of the roots and shoots of all plants from the final harvest was measured colorimetrically (Murphy & Riley 1962), following acid digestion (Jackson 1958).

Comparison of the mycorrhizal and phosphorus treatments was by means of two-way analysis of variance and 95 % confidence intervals. When the effect of a treatment is compared for more than one species, the probability value of the species which has the lowest significant difference for that treatment, is given in the text. Student's *t* tests were used to compare the VAM infection data, which were arcsine transformed before statistical analysis (Zar 1984).

Results

Mycorrhizas ($p < 0.01$) and fertilization ($p < 0.01$) significantly affected the mass of all the species with unfertilized NM plants being the smallest (Fig. 4.1). Significant interactions between the treatments influenced *Phylica* ($p < 0.05$) and *Agathosma* ($p < 0.001$) mass. Mass of phosphorus fertilized and unfertilized VAM plants of a species were equal in all

instances (Fig. 4.1). In the case of *Staavia* and *Agathosma* the phosphorus fertilized NM plants achieved the same mass as VAM plants but fertilized NM plants of *Phylica* were smaller than the VAM plants (Fig. 4.1).

Mycorrhizas ($p < 0.05$), fertilization ($p < 0.001$) and interactions ($p < 0.01$) between the two, significantly influenced RGRs of all the species although the effects differ between species (Table 4.1). RGRs and final mass were related in *Staavia* and *Phylica* but similar sized *Agathosma* plants did not have the same RGRs (Table 4.1, Fig. 4.1).

Root:shoot ratios of dry mass tended to decrease in response to the presence of mycorrhizas and the addition of phosphorus (Table 4.1). Root:shoot ratios of *Agathosma* were highly variable and were not significantly affected by the treatments (Table 4.1). *Phylica* and *Staavia* root:shoot ratios were significantly affected ($p < 0.05$) by mycorrhizas and phosphorus fertilization.

Height at the final harvest was significantly increased by mycorrhizas ($p < 0.01$) and fertilization ($p < 0.05$) (Fig. 4.2). *Phylica* and *Agathosma* height followed the same trend as mass, but *Staavia* height differed more than mass among treatment combinations (Fig. 4.1, Fig. 4.2). Phosphorus fertilization and mycorrhizal effects on plant height became apparent at about the same age (16 - 20 weeks) (Fig. 4.2). Unfertilized, NM plants were always shorter and changed little after the twentieth week.

No NM plants became infected. VAM infection at 8 weeks was less than 10 % of the root length of *Staavia* and *Phylica* but was 26 % for *Agathosma*. By the final harvest infection levels were much higher but phosphorus fertilization had no significant effect (Table 4.1).

All unfertilized, NM plants of *Phylica*, *Agathosma* and *Staavia* produced fewer leaves than the other treatment combinations (Fig. 4.3). *Phylica* produced most leaves when VAM and fertilized, while *Agathosma* and *Staavia* produced equal numbers if fertilized or VAM (Fig. 4.3). Leaf sizes of all the species was noticeably smaller for NM, unfertilized plants,

although this was not measured. The proportion of dead leaves were significantly higher ($p < 0.05$) for the unfertilized, NM plants of *Phylica* and *Staavia* (Fig. 4.3).

Mycorrhizas ($p < 0.01$) and fertilization ($p < 0.001$) significantly influenced the total phosphorus content and phosphorus concentration of all the species. Unfertilized, NM plants had very low phosphorus contents and phosphorus concentrations while fertilized, VAM plants had the highest total phosphorus contents and phosphorus concentrations (Fig. 4.4, Table 4.1). VAM and NM *Staavia* acquired most phosphorus when fertilized, while fertilized NM *Phylica* and *Agathosma* plants acquired as much as unfertilized VAM plants (Fig. 4.4). Phosphorus concentrations in fertilized, NM plants were higher than in unfertilized, VAM plants (Table 4.1).

Distribution patterns of phosphorus between roots and shoots of *Agathosma* were very variable but not significantly different across treatments (Table 4.1). Mycorrhizas significantly decreased ($p < 0.05$) the proportion of phosphorus allocated to roots in *Phylica* (Table 4.1). Significant mycorrhizal and fertilizer treatment effects ($p < 0.01$) ensured that fertilized, NM *Staavia* plants had higher proportions of phosphorus in their roots (Table 4.1).

TABLE 4.1. The effect of vesicular-arbuscular mycorrhizas (M) and phosphorus fertilization (P) on the relative growth rates, root:shoot (R:S) ratios, P concentrations, P allocation and mycorrhizal (VAM) infection of seedlings of *Phylica ericoides*, *Agathosma ovata* and *Staavia radiata* (mean ± 1 standard error). Values followed by different letters imply that a treatment combination was significantly different ($p < 0.05$) for that species.

	Treatment						
	M	P	RGR ($g\ g^{-1}\ wk^{-1}$)	R:S mass ratio	P concentration ($\mu g\ g^{-1}$)	R:S P ratio	VAM infection (%)
Phylica							
	-	-	0.061a ±0.007	0.82a ±0.10	188a ±7	0.91a ±0.10	0
	-	+	0.130b ±0.003	0.56a ±0.03	1734b ±98	0.95a ±0.20	0
	+	-	0.142bc ±0.003	0.55a ±0.06	770c ±92	0.55b ±0.02	74.2a ±4.8
	+	+	0.145c ±0.001	0.39b ±0.04	2600d ±108	0.63b ±0.06	78.4a ±8.2
Agathosma							
	-	-	0.055a ±0.004	1.42a ±0.36	215a ±11	2.05a ±0.82	0
	-	+	0.107b ±0.003	0.90a ±0.11	1183bc ±365	1.91a ±0.51	0
	+	-	0.087c ±0.003	0.71a ±0.07	626c ±54	1.06a ±0.16	77.0a ±5.5
	+	+	0.090bc ±0.002	0.81a ±0.07	2472b ±378	0.76a ±0.05	70.2a ±5.0
Staavia							
	-	-	0.070a ±0.005	0.51a ±0.04	180a ±15	0.66a ±0.04	0
	-	+	0.120b ±0.003	0.48b ±0.08	2128b ±139	2.25b ±0.38	0
	+	-	0.117b ±0.008	0.36b ±0.04	921c ±6	0.45a ±0.07	63.5a ±2.8
	+	+	0.126b ±0.006	0.33b ±0.02	2083b ±144	0.78a ±0.10	57.8a ±3.9

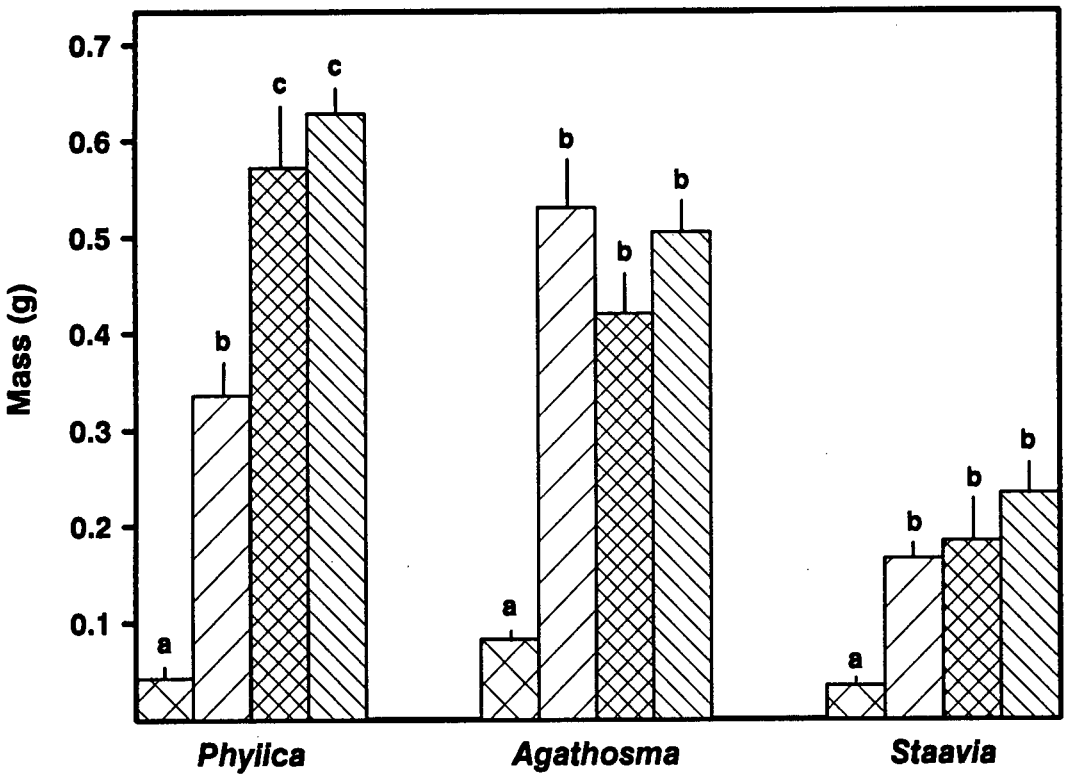

FIGURE 4.1. Mass of seedlings of sclerophyllous shrubs, *Phylica ericoides*, *Agathosma ovata* and *Staavia radiata*, grown with vesicular-arbuscular mycorrhizal and phosphorus fertilization treatments. Mass of plants which are significantly different ($p < 0.05$) from those of other treatment combinations for a species are indicated by a different letter, vertical line represents +1 standard error. Treatment combinations: ▨ non-mycorrhizal without P fertilization, ▨ non-mycorrhizal plus P fertilization, ▨ mycorrhizal without P fertilization, ▨ mycorrhizal plus P fertilization.

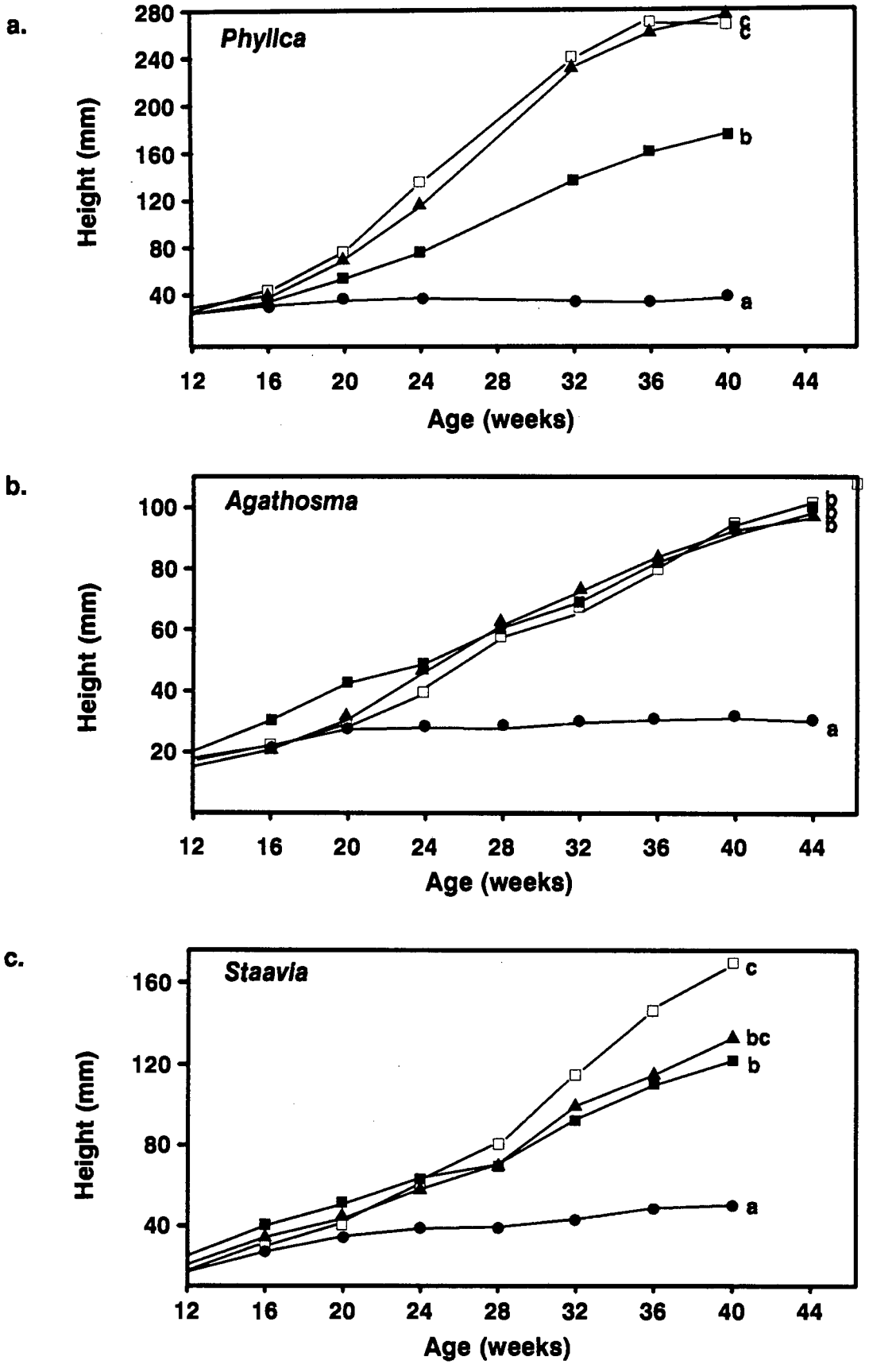

FIGURE 4.2. Height of (a.) *Phylica ericoides*, (b.) *Agathosma ovata*, and (c.) *Staavia radiata* with vesicular-arbuscular mycorrhizal and phosphorus fertilization treatments. Height of plants which are significantly different ($p < 0.05$) from those of other treatment combinations for a species are indicated by a different letter. Treatment combinations: ● non-mycorrhizal without P fertilization, ■ non-mycorrhizal plus P fertilization, ▲ mycorrhizal without P fertilization, □ mycorrhizal plus P fertilization.

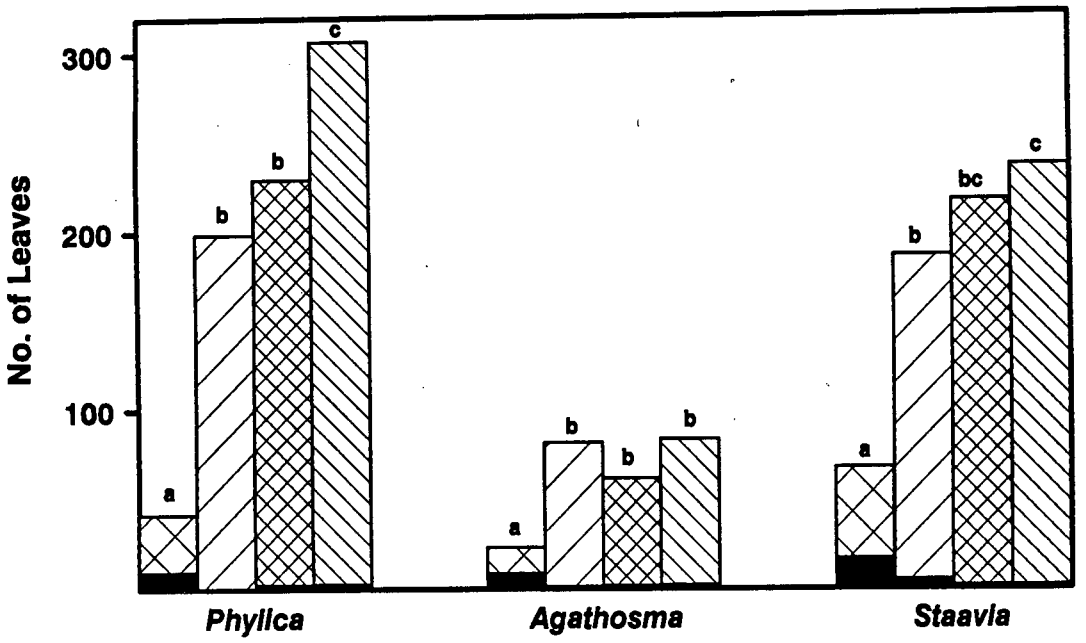

FIGURE 4.3. Total leaves, including dead leaves indicated by the black shaded region, produced by seedlings of sclerophyllous shrubs, *Phylica ericoides*, *Agathosma ovata* and *Staavia radiata*, grown with vesicular-arbuscular mycorrhizal and phosphorus fertilization treatments. Total number of leaves which are significantly different ($p < 0.05$) from those of other treatment combinations for a species are indicated by a different letter. Treatment combinations: non-mycorrhizal without P fertilization, non-mycorrhizal plus P fertilization, mycorrhizal without P fertilization, mycorrhizal plus P fertilization.

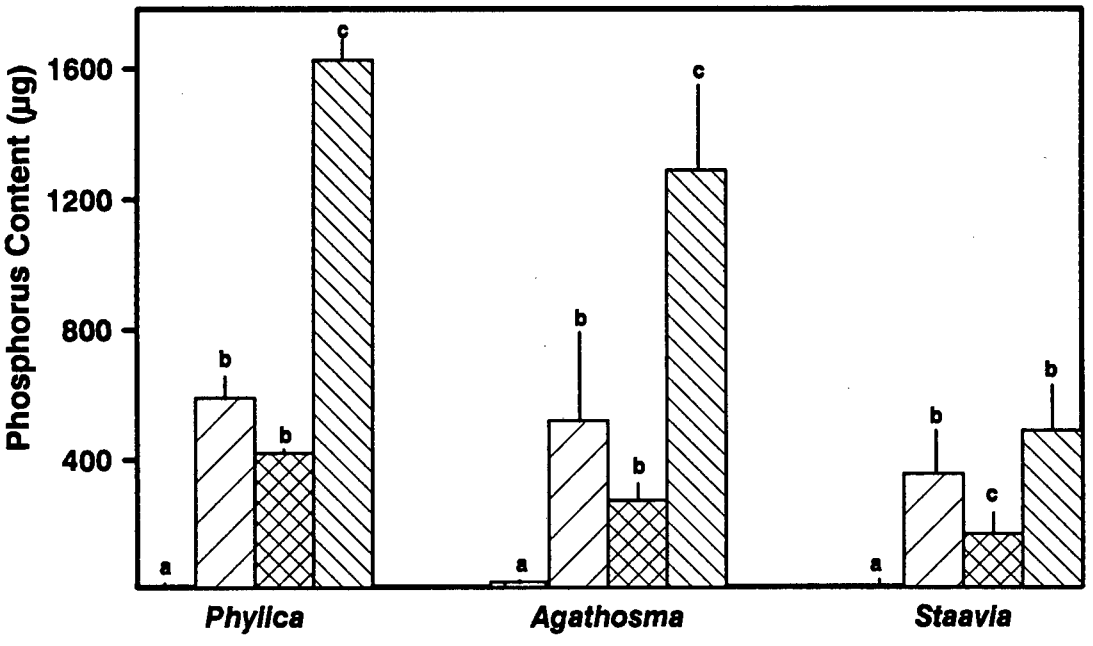

FIGURE 4.4. Total phosphorus content of seedlings of sclerophyllous shrubs, *Phylica ericoides*, *Agathosma ovata* and *Staavia radiata*, grown with vesicular-arbuscular mycorrhizal and phosphorus fertilization treatments. P contents of plants which are significantly different ($p < 0.05$) from those of other treatment combinations for a species are indicated by a different letter, vertical line represents +1 standard error. Treatment combinations: non-mycorrhizal without P fertilization, non-mycorrhizal plus P fertilization, mycorrhizal without P fertilization, mycorrhizal plus P fertilization.

Discussion

The expectation that slow growing, perennial plants from a low nutrient environment are less likely to respond to mycorrhizas than faster growing plants with more plastic nutrient responses is not supported by this study. Mass of the three representatives of sclerophyllous, woody, fynbos species showed increases over the NM controls of 5 - 14 times when VAM. Growth responses to mycorrhizas of this magnitude are among the higher values recorded for a range of both wild and cultivated annual and perennial herbaceous species (Mosse, Hayman & Arnold 1973, Crush 1974, Azcón & Ocampo 1981, Plenchette, Fortin & Furlan 1983, Saif 1987, Hetrick, Kitt & Wilson 1988, Koide & Li 1991) and woody species (Baylis 1967, Hall 1975, Menge, Johnson & Platt 1978, Janos 1980a, Pope *et al.* 1983, Borges & Chaney 1988, Michelsen & Rosendahl 1990) from many different habitats.

While NM plants in the unfertilized treatment acquired little phosphorus other than that supplied by the seeds (Chapter 5), phosphorus content of VAM plants was very much higher. Higher phosphorus content of fertilized, NM plants was paralleled by an increase in growth of these plants. It seems probable, therefore, that the main effect of mycorrhizas in increasing growth of VAM plants of *Phylica*, *Agathosma* and *Staavia* was by means of improved phosphorus nutrition. Enhanced phosphorus nutrition is the most common reason for improved growth of VAM plants (e.g. Baylis 1967, Mosse *et al.* 1973, Crush 1974, Hall 1975, Koucheki & Read 1976). Growth of VAM and fertilized NM plants was equal except for *Phylica* which did not grow to the same extent when NM despite similar total phosphorus contents. *Phylica* may be dependent on mycorrhizas for the uptake of other nutrients as well as phosphorus. For example, zinc and copper uptake of apple cuttings is enhanced by mycorrhizas when phosphorus nutrition is adequate (Gnekow & Marschner 1989). The accumulation of phosphorus in the roots of *Phylica* when NM may also have reduced the growth response (Chapter 6).

As phosphorus depletion zones are likely to develop around absorbing roots and the rate of phosphorus diffusion in soil is low, mycorrhizas are important for ensuring an adequate

phosphorus supply to most plants, in competition with other plants, under most soil conditions (Plenchette *et al.* 1983, Gianinazzi-Pearson & Gianinazzi 1983, Krikun *et al.* 1990, Bolan 1991). However, they will be particularly important under conditions, such as the experimental ones, where unfertilized soil phosphorus levels are below a threshold that NM roots can exploit.

Generally the NM seedlings showed a remarkable ability to survive despite the lack of phosphorus acquisition. The ability of slow growing species to maintain metabolic activity under conditions of nutrient stress is regarded as a key adaptive feature of plants from low nutrient environments (Clarkson 1967, Chapin 1980) and may explain the persistence of the NM controls. Nutrient translocation from older leaves, to support the continued growth of meristems and young leaves when nutrient supplies from the soil are inadequate (Chapin 1980), may account for the higher proportion of leaves lost by unfertilized NM plants. Koucheki & Read (1976) also reported a higher proportion of leaves lost by NM plants under low nutrient conditions. The lack of mortality or toxicity symptoms among the phosphorus fertilized plants indicates that phosphorus levels were not reaching toxic levels due to imbalances with other nutrients in the experimental plants (Groves & Keraitis 1976).

Allocation patterns of carbon and phosphorus were affected by mycorrhizas and phosphorus additions. While there is an expectation that root:shoot ratios should decrease in response to nutrient sufficiency (Wilson 1988, Levin, Mooney & Field 1989) and this has been seen in herbaceous plants (Atkinson 1973), as well as for slow growing heathland plants (Aerts, Boot & van der Aart 1989) and VAM woody species (Hall 1975, Michelsen & Rosendahl 1990), this is not an invariable response to mycorrhizas (Saif 1987, Miller, Jarstfer & Pillai 1987, Hetrick 1991). Among the experimental plants there was a trend towards reducing carbon allocation to roots in response to the enhanced phosphorus nutrition of VAM or fertilized plants. In addition, phosphorus translocation to shoots was enhanced by mycorrhizas while phosphorus accumulated in the roots of some NM treatments. Thus, these species are showing some plasticity in growth response, and the effect of mycorrhizas on growth may work indirectly through altering allocation patterns (Chapter 6).

While changes in mycorrhizal infection might have been expected, as the proportion of root length infected with VAM fungi often drops with increasing phosphorus fertilization, no such changes were recorded. Usually, only applications of phosphorus higher than that applied in the present study will result in a drop in VAM infection (Menge *et al.* 1978b, Thomson, Robson & Abbott 1986). The lack of change in VAM infection supports the prediction that plants will tend to retain a constant level of infection over a wide range of phosphorus availability, as increases in phosphorus supply are likely to be transitory in natural environments (Fitter 1991). The translocation of phosphorus to shoots will also maintain root phosphorus concentrations below those that may prove inhibitory to VAM infection (Menge *et al.* 1978b).

The growth rates of the unfertilized, VAM plants are typical of those for other slow growing, woody species (Jarvis & Jarvis 1964, Grime & Hunt 1975). The lack of growth response to additional phosphorus is consistent with the behaviour of such plants, but there is no indication that slow uptake of nutrients is responsible for the slow growth rate of the VAM or phosphorus fertilized seedlings. Low growth rates are associated with phosphorus storage in low nutrient environments because this allows the plant to take advantage of pulsed or unpredictable nutrient flushes, thus ensuring an adequate supply of phosphorus to support growth during periods when nutrients are unavailable (Chapin 1988, Chapin *et al.* 1990). The acquisition of phosphorus by seedlings in excess of requirements for growth in this study can be seen as taking advantage of such nutrient availability for storage.

It has been suggested that there are periods during the life of plants adapted to low nutrient environments when the mycorrhizal symbiosis is unnecessary and potentially expensive (St John & Coleman 1983, Fitter 1991). The importance of mycorrhizas may be reduced when plants rely on stored reserves for growth, but luxury consumption is probably impossible without mycorrhizas. While the requirement for nutrients for seedling growth are obvious, the requirement may be very low for mature plants due to internal cycling and slow tissue turnover. However reproduction is likely to have a high nutrient requirement and therefore dependency on mycorrhizas extends beyond the seedling stage. Studies of the costs of maintaining mycorrhizas for more mature plants are required if we are to interpret the

effects of mycorrhizas on community dynamics. Biomass production is not the only indication of the ecological significance of mycorrhizas to wild plants (Allen & Allen 1986, Miller *et al.* 1987), and other plant attributes which are affected by VAM, such as height, leaf mortality, phosphorus allocation and luxury consumption among the experimental species, may influence their ecology.

Despite low growth rates and, therefore, low phosphorus requirement, the dependency of these sclerophyllous species on mycorrhizas for acquiring phosphorus from a low nutrient soil in pot culture indicates that these species are obligately VAM during seedling establishment in their natural environments. In the low nutrient soils of the fynbos with plant available phosphorus at levels of 0.2 - 2 $\mu g\ g^{-1}$ (Mitchell *et al.* 1984, Witkowski & Mitchell 1987), these species are unlikely to encounter phosphorus concentrations of the magnitude of the phosphorus amendment in this experiment and will rely on mycorrhizas for acquiring phosphorus for growth and storage. In assessing the responses of slow growing species to mycorrhizas, it is essential to consider the biology of such plants in the context of their natural environment, and particularly to recognize the low nutrient conditions under which they grow. The results of this study refute the suggestions that plants from low nutrient environments are less reliant on mycorrhizas for nutrient acquisition than plants from more nutrient rich regions.

CHAPTER 5

Seed Reserves Influence Seedling Growth and Mycorrhizal Responses among Evergreen Shrubs from a Low Nutrient Environment

Introduction

Seed size is seen as an important ecological attribute of plants, because it reflects interactions between past environmental pressures and the evolutionary history of taxonomic groups (Hodgson & Mackey 1986). The allocation of resources to seeds is subject to the conflicting demands of effective dispersal and provisioning of the seedling for successful establishment (Fenner 1985, Howe & Westley 1986). Seed phosphorus reserves are very important for determining early seedling growth (Atkinson 1973, Fenner & Lee 1989) as the low mobility of phosphorus in the soil makes it one of the most difficult nutrients for a seedling to acquire. It might be expected, therefore, that among a group of ecologically similar species, the seedlings of poorly provisioned seeds will be highly dependent on mycorrhizas for establishment, while those of larger seeds would be more independent.

Large seeds are associated with large plants (Thompson & Rabinowitz 1989), dry habitats (Baker 1972) and later successional stages (Salisbury 1942). Hall (1975) and Janos (1980a) suggest that selection for large seeds among obligate mycorrhizal forest species is a means of ensuring establishment when the prospect of mycorrhizal infection is uncertain. Alternatively, seedlings from large seeds in shaded environments may have an advantage in supporting the carbon demands of a mycorrhizal symbiont until such a stage that the plant can photosynthesize. Seedling establishment in the fynbos is in a high light environment during mild, rainy winters following disturbance by fire, and seedling recruitment in established vegetation is not a major factor in determining community composition. Therefore, it is expected that interactions between the environment and mycorrhizas which may influence seed size and mycorrhizal response will be different to those in more shaded environments.

Members of the dominant, evergreen, shrubby vegetation of the Cape fynbos produce a wide range of seed sizes (le Maitre & Midgley 1992, Allsopp & Stock in press b) and most of these species form VA mycorrhizas (Chapter 2). As phosphorus is particularly low in these soils, it is expected that the smallest-seeded species will show the greatest mycorrhizal response while large seededness may be linked to independence from mycorrhizas for

establishment. The significance of seed size to dispersal and establishment in this low nutrient environment will be discussed in the context of the mycorrhizal responses of seedlings of a range of fynbos shrubs.

Materials and Methods

Germinated seeds of 15 species of evergreen, perennial woody plants, indigenous to the Cape Floristic Region (Bond & Goldblatt 1984) were planted in an autoclaved, infertile soil:acid washed sand mix (1:1) in 13 cm diameter plastic plant pots. Seeds were treated as set out in Table 5.1, and incubated (12 h dark/12 h light and 10^{o} C/20^{o} C temperature cycle) in plastic petri dishes on sterile damp filter paper until sufficient of any one species had germinated to establish 20 replicate pots of each treatment with one plant per pot. Dates of planting varied as germination rates were very variable but all plants were established over a two month period during the winter. Species, source of seeds, and weeks grown to final harvest are detailed in Table 5.1. Ten VAM and 10 NM plants were established for each species. Details of the soil used, the establishment of VAM and NM treatments and the growing conditions are described in Chapter 4. As it was not possible to obtain seed for a range of plant species growing in the same community, on the same soil, the use of an indigenous VAM inoculum cocktail was unlikely to reflect the fungal species composition of the soils from the natural habitats of all the species. As species specific interactions and competition between the mycorrhizal fungi (Wilson 1984) may influence the results, a VAM fungus, *Acaulospora morrowae* Spain & Schenck from INVAM (University of West Virginia, Morgantown, WV 26506-6057, USA), which has not had the opportunity to interact with any of the experimental species was chosen as a neutral compromise.

Five plants per treatment were harvested after 8 weeks of growth. Age of plants at final harvest ranged from 40 - 50 weeks (Table 5.1). Plant parts were dried at 80^{o} C for 72 h, weighed and stored in brown paper envelopes. Seed mass and phosphorus content were determined for a minimum of 20 seeds per species with their seed coats removed, except for

three of the smaller seeded species, *viz. Staavia radiata*, *Passerina paleaceae* and *Petalacte coronata*. Thus, seed weight is overestimated for these species, but Fenner (1983) points out that the seed coat usually accounts for proportionately less of the total mass when seeds are small, presumably because the seed coats are thinner.

Before drying a subsample of root material was collected for VAM infection assessment as described in Chapter 4. At the 8 week harvest roots of a species for a particular treatment were bulked to measure VAM infection. Diameters of 15 of the last order roots of NM plants of all species from the final harvest, except *Polygala virgata*, were determined at the same time as VAM infection was measured.

Nodule numbers on the root systems of the legumes and numbers of cluster roots formed by *Aspalathus linearis* and *Aspalathus spinescens* were counted. Nitrogen content of legumes was determined by Kjeldahl digestion followed by colorimetric analysis (Smith 1980). Insufficient material of NM plants precluded nitrogen analysis of the non-legume plants. Phosphorus contents of seeds and all plants from the final harvest was measured colorimetrically (Murphy & Riley 1962) following acid digestion (Jackson 1958).

RGRs for the period 8 weeks to harvest were calculated according to Hunt (1978). Responses to mycorrhizas were calculated from the equation: VAM response = $(\overline{V}_{VAM} - \overline{V}_{NM})/\overline{V}_{NM}$ where V is a variable such as plant mass, phosphorus content or RGR, and $\overline{V}$ is the mean of those variables for a particular treatment (Bryla & Koide 1990).

Student's *t* tests were used for comparisons between treatments for individual species. Differences in VAM responses, VAM infection and root diameter between species were made with one-way analysis of variance (ANOVA). Percentage data were arcsine transformed before statistical analysis (Zar 1984). Seed mass and phosphorus content were log transformed before testing the linear relationship between these variables and growth and mycorrhizal response.

TABLE 5.1. Species, germination treatments, age at final harvest and source of seeds of plants grown with and without vesicular-arbuscular mycorrhizal fungi and codes used to designate the species in the figures. KGB = Kirstenbosch Botanical Gardens, Cape Town; Pella = Fynbos Biome Study Site (33°31'S, 18°32'E); Hagelkraal (34°40'S 19°30'E); Agulhas (33°35'S 20°30'E).

Species	Code	Family	Pre-germination treatment	Age at harvest	Source of seeds
Agathosma collina Eckl.& Zeyh.	Acol	Rutaceae	fresh seed	44 wks	Agulhas
Agathosma gonaquensis Eckl.& Zeyh.	Agon	Rutaceae	fresh seed	44 wks	KBG
Agathosma ovata (Thunb.) Pill.	Aova	Rutaceae	fresh seed	44 wks	KBG
Aspalathus linearis (Burm.f.) Dahlgren	Alin	Fabaceae	conc. H_2SO_4 1 h.	40 wks	Rooibos Tea Board[1]
Aspalathus spinescens Thunb.	Aspi	Fabaceae	conc. H_2SO_4 ½ h.	40 wks	Pella
Otholobium fruticans (L.)Stirton	Ofru	Fabaceae	Boiling water	40 wks	KBG
Otholobium hirtum (L.) Stirton	Ohir	Fabaceae	Boiling water	40 wks	Pella
Passerina paleacea Wikstrom	Ppal	Thymelaeaceae	1 h. at 70 °C	40 wks	Hagelkraal
Petalacte coronata (L.) D. Don.	Pcor	Asteraceae	none	50 wks	Pella
Phylica cephalantha Sonder	Ceph	Rhamnaceae	conc. H_2SO_4 1 h.	50 wks	Pella
Phylica ericoides L.	Eric	Rhamnaceae	conc. H_2SO_4 ½ h.	40 wks	Hagelkraal
Podalyria sericea R. Br.	Pser	Fabaceae	Testa chipped	40 wks	KBG
Polygala virgata Thunb.	Pvir	Polygalaceae	none	50 wks	Parsley's seeds[2]
Psoralea pinnata L.	Ppin	Fabaceae	Boiling water	40 wks	KBG
Staavia radiata (L.) Dahl	Srad	Bruniaceae	Fresh seed 1 year in soil	40 wks	Pella

[1]Rooibos Tea Board, PO Box 64, Clanwilliam 8135, SA. [2]Parsley's seeds, PO Box 1375, Somerset West 7130, SA.

Results

Differences in mass of VAM and NM plants were not significant ($p > 0.05$) at the 8 week harvest except for *Agathosma ovata* and *Passerina paleacae* which produced larger plants when VAM (Table 5.2). By the final harvest VAM plants were heavier for all species except *Aspalathus linearis* and *Aspalathus spinescens*.

VAM infection at 8 weeks was extremely variable among the species. Infection of the bulked roots of VAM *Aspalathus spinescens, Aspalathus linearis, Phylica cephalantha* and *Staavia radiata* was less than 10 %. Ten to 50 % of root length of *Agathosma* spp., *Phylica ericoides, Polygala virgata* and *Petalacte coronata* was infected, while over 50 % of *Otholobium* spp. and *Podalyria sericea* roots were infected. VAM infection of the roots at the final harvest was less variable but was significantly ($p < 0.0001$) different between species (Table 5.2). None of the NM controls became infected. Root diameter was significantly ($p < 0.0001$) different between species (Table 5.2).

Nodule numbers on the VAM and NM *Aspalathus* spp. were equal while the *Otholobium* spp. and *Podalyria sericea* had significantly more ($p < 0.05$) nodules on VAM plants because the NM plants failed to nodulate (Table 5.3). Although VAM *Psoralea pinnata* plants had more nodules, numbers were very variable and there was no significant difference between treatments (Table 5.3). The nitrogen content of NM and VAM plants exceeded that in the seeds and was highest in VAM plants (Table 5.3). The number of cluster roots formed by the *Aspalathus* spp. were not significantly different between treatments (Table 5.3).

Total phosphorus content of all species, including those unresponsive to mycorrhizas, was significantly ($p < 0.05$) higher in VAM plants (Fig. 5.1). NM plants acquired little net phosphorus over that supplied by the seeds and only the *Aspalathus* spp., *Psoralea pinnata* and *Petalacte coronata* more than doubled their seed phosphorus when NM (Fig. 5.1).

Seed mass (excluding the seed coat) and seed phosphorus content (Table 5.2) were positively related ($r=0.78$, $p<0.0005$). There was a strong positive correlation for both log seed mass and log seed phosphorus content with mass of NM and VAM plants at the 8 week harvest (Table 5.4). By the final harvest only NM mass was positively correlated with log seed mass and log seed phosphorus (Table 5.4).

VAM mass response was highly correlated with VAM phosphorus response ($r=0.81$, $p<0.0005$), but VAM RGR response for the period 8 weeks to harvest was not correlated with either (vs. mass response $r=0.44$, $p>0.05$; vs. phosphorus response $r=0.23$, $p>0.10$). All three VAM responses were significantly different ($p<0.0001$) among species as tested by one-way ANOVA. Log seed mass and log seed phosphorus were negatively correlated with VAM mass response (Fig. 5.2, Table 5.4). Only log seed phosphorus was significantly correlated with phosphorus response (Fig. 5.2, Table 5.4) and neither seed mass or phosphorus was correlated with the RGR response (Table 5.4). Seed phosphorus concentration was not significantly correlated with any VAM response (Table 5.4). Root diameter of the last order roots of NM plants and percentage root length infected with VAM fungi were not significantly correlated with any VAM responses (Table 5.4).

TABLE 5.2. Seed mass and phosphorus content without seed coat, seedling mass at 8 weeks and final harvest (40 - 50 wks) of mycorrhizal (VAM) and non-mycorrhizal (NM) plants, % root length infected with mycorrhizas and diameter of last order roots of evergreen fynbos species. Mean ±1 standard error.

Species	Seed mass (mg)	Seed P (µg)	Mass 8 weeks (mg)		Mass final (mg)		VAM infection (%)	Root diameter (µm)
			NM	VAM	NM	VAM		
Agathosma collina	1.8±0.1	17±1	11±3	13±2	50±5	381±19	49±3	250±14
A. gonaquensis	1.9±0.1	16±1	28±4	28±3	73±5	507±67	71±3	263±9
A. ovata	2.8±0.2	14±1	11±2	17±1	84±12	422±43	77±5	266±7
Aspalathus linearis	2.6±0.1	55±2	39±3	42±5	203±36	340±68	68±4	166±7
Asp. spinescens	2.1±0.2	39±4	16±1	32±2	320±31	365±57	61±12	222±16
Otholobium fruticans	2.3±0.1	18±1	28±3	28±1	72±8	487±44	74±3	287±17
O. hirtum	4.4±0.1	16±1	57±9	59±3	101±8	583±54	77±7	266±11
Passerina paleacea	0.7±0.0	2±0	1±0	3±1	11±1	181±16	64±9	56±6
Petalacte coronata	0.3±0.0	3±0	2±0	2±1	152±17	756±45	64±4	76±3
Phylica cephalantha	5.8±0.3	36±3	23±5	26±5	262±39	721±76	76±15	283±16
Phy. ericoides	1.0±0.1	9±0	6±1	6±1	41±10	573±55	74±4	202±10
Podalyria sericea	11.0±0.4	73±7	109±6	108±9	395±39	642±58	77±5	115±5
Polygala virgata	1.6±0.1	10±0	33±4	32±4	47±2	556±24	54±3	-
Psoralea pinnata	5.4±0.3	26±2	91±10	70±8	223±14	478±30	78±7	207±8
Staavia radiata	1.1±0.0	3±0	4±0	4±0	36±5	185±33	64±3	197±6

TABLE 5.3. Number of nodules, number of cluster roots and total nitrogen content of seeds, mycorrhizal (VAM) and non-mycorrhizal (NM) seedlings of evergreen members of the Fabaceae from fynbos vegetation. Mean ±1 standard error.

Species	Nodules		Cluster roots		Total N (mg N)		
					seeds	plants	
	NM	VAM	NM	VAM		NM	VAM
Aspalathus linearis	20 ±3	27 ±7	9 ±2	5 ±2	0.24 ±0.01	3.73 ±0.55	4.72 ±0.92
Aspalathus spinescens	30 ±11	22 ±7	4 ±1	4 ±1	0.17 ±0.01	3.04 ±0.42	3.68 ±0.45
Otholobium fruticans	0 ±0	19 ±5	-	-	0.27 ±0.01	2.20 ±0.60	9.41 ±0.71
Otholobium hirtum	0 ±0	64 ±11	-	-	0.37 ±0.02	2.53 ±0.17	11.65 ±0.75
Podalyria sericea	0 ±0	34 ±9	-	-	0.81 ±0.07	4.76 ±0.71	9.35 ±0.44
Psoralea pinnata	2 ±1	13 ±6	-	-	0.53 ±0.04	1.56 ±0.17	8.40 ±1.08

TABLE 5.4. Correlation coefficients (r) and probability of significance (*p*) of relationships between various seed and plant characteristics as the independent variables and plant mass and mycorrhizal responses of seedlings of fifteen evergreen, fynbos shrub species. A - indicates that the relationship was not determined. NM=non-mycorrhizal, VAM=mycorrhizal.

		Mass (8 weeks)		Mass (final)		VAM responses		
		NM	VAM	NM	VAM	MASS	P	RGR
Log seed mass	r	+0.78	+0.82	+0.60	+0.19	-0.55	-0.38	-0.03
	p	<0.0005	<0.0005	<0.01	>0.10	<0.025	>0.05	>0.50
Log seed P	r	+0.64	+0.71	+0.74	+0.27	-0.66	-0.46	-0.14
	p	<0.005	<0.0025	<0.001	>0.10	<0.005	<0.05	>0.25
Seed P conc.	r	-	-	-	-	-0.36	-0.27	-0.25
	p	-	-	-	-	>0.05	>0.10	>0.10
VAM infection	r	-	-	-	-	-0.33	-0.20	-0.32
	p	-	-	-	-	>0.10	>0.10	>0.10
Root diameter	r	-	-	-	-	-0.25	-0.04	+0.41
	p	-	-	-	-	>0.10	>0.50	>0.05

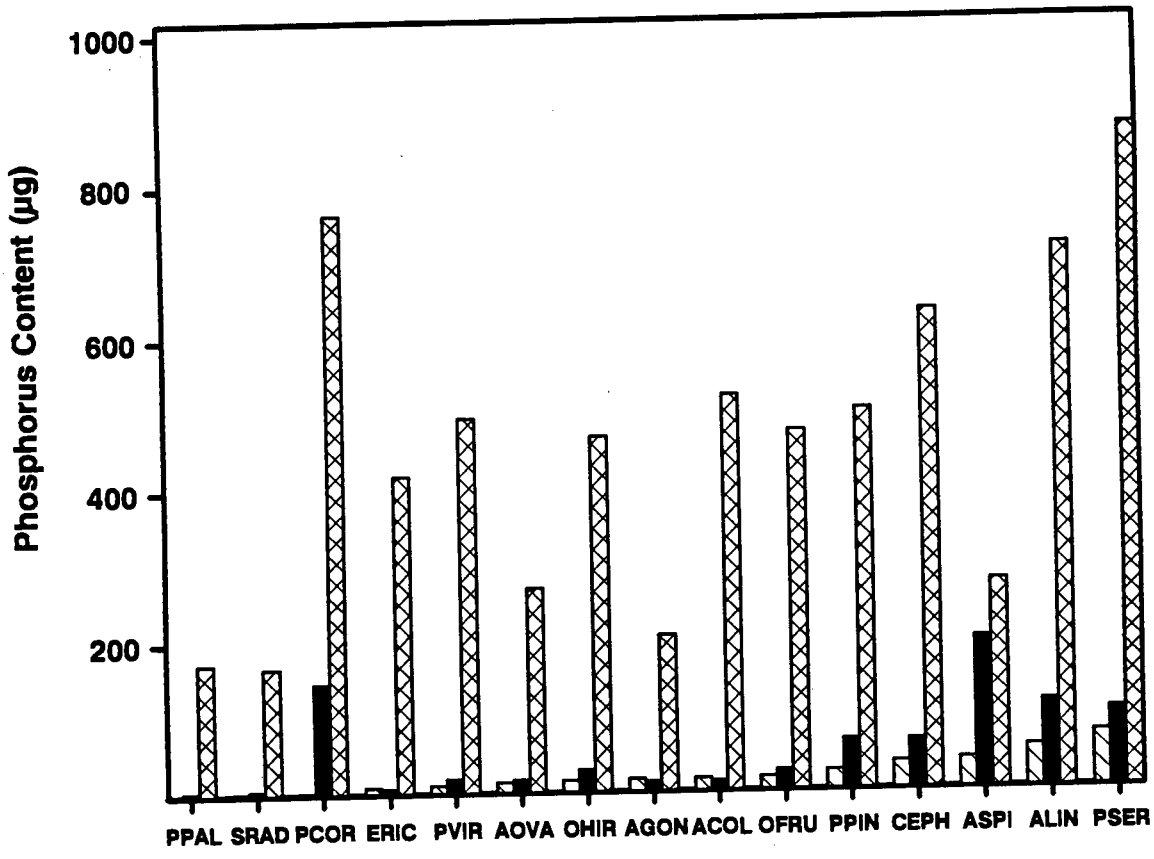

FIGURE 5.1. Phosphorus content (μg P) in seeds ▨ , non-mycorrhizal ■ and mycorrhizal ▩ seedlings of fifteen evergreen, shrubby fynbos species. See Table 5.1 for key to species codes.

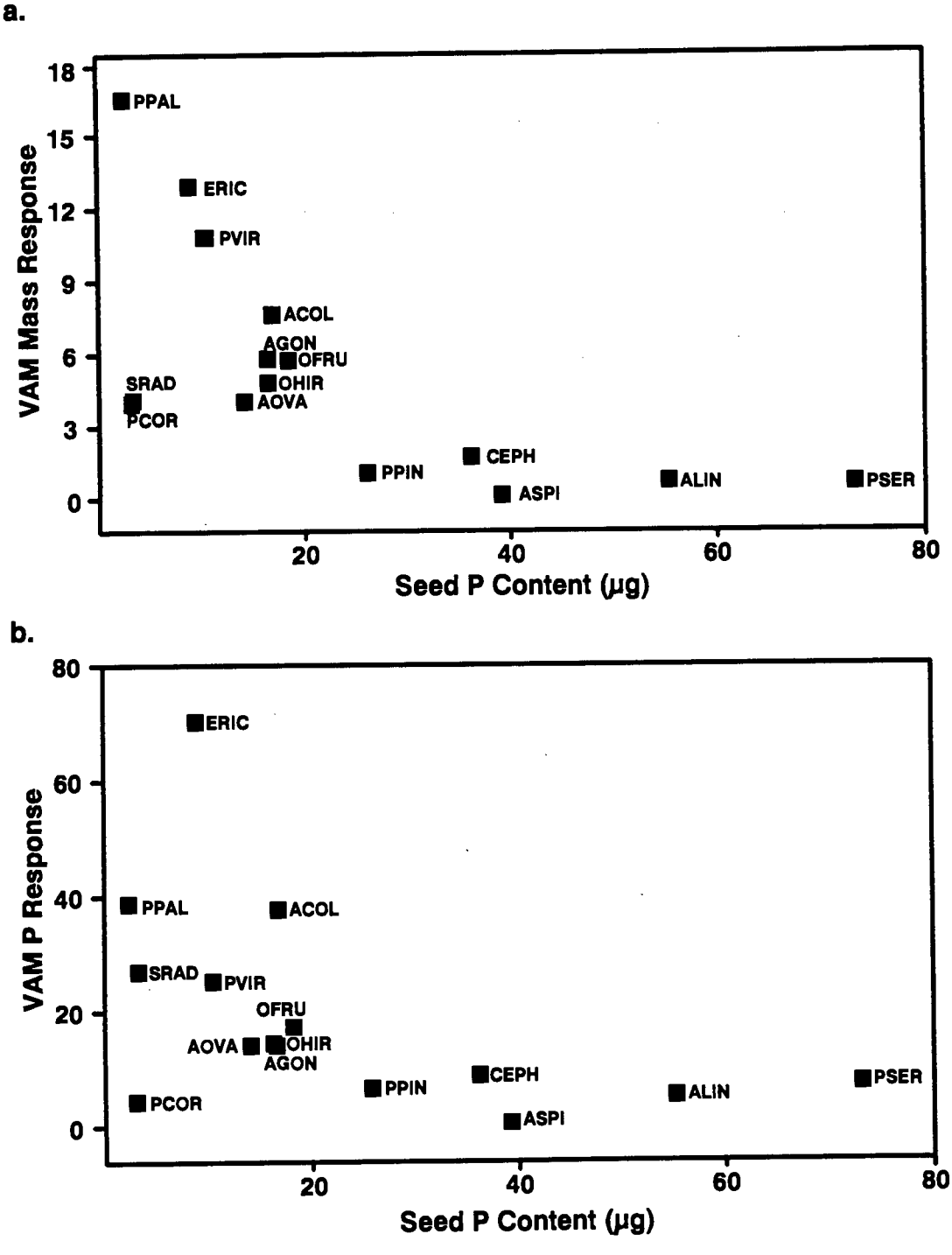

FIGURE 5.2. Mycorrhizal mass and phosphorus responses[1] of seedlings of fifteen evergreen, shrubby fynbos species in relation to their seed phosphorus content. (a.) Mass response, (b.) Phosphorus response. See Table 5.1 for key to species codes.

[1]Mycorrhizal (VAM)response=V_{VAM}-$\overline{V}_{NM}/\overline{V}_{NM}$ where V is a variable such as mass or phosphorus content (Bryla & Koide 1990).

Discussion

Seedling size at 8 weeks among these woody perennials was highly dependent on seed reserves, but as seed mass and seed phosphorus are highly correlated it is difficult to determine which of these resources is primarily responsible for growth. Similar correlations between seed reserves and seedling size have been reported when plants do not have access to other nutrients (Fenner 1983, Stock, Pate & Delfs 1990), or are deprived of phosphorus (Atkinson 1973), or when seedlings are growing in highly competitive situations (Fenner 1978, Gross 1984). Mycorrhizal effects were generally not important in determining growth at 8 weeks. By the final harvest (40 - 50 weeks), NM plant growth was still largely determined by seed resources. A similar relationship between seed size and height of NM tropical woody species was found by Janos (1980a), and persistence of woody NM seedlings on seed reserves alone can exceed one year (Baylis 1967). Seed resources are therefore important in determining the size attained by older seedlings unable to obtain mineral nutrients from their environment through a failure to become mycorrhizal.

Under the low nutrient conditions of the current experiment, VAM responses in terms of biomass gain and phosphorus acquisition are closely linked to seed size and particularly seed phosphorus reserves. Neither root diameter or length of root infected have a direct influence on VAM responses. However, with increasing seed resources, VAM mass and phosphorus responses decrease. Larger seeds will produce larger seedlings which can explore greater volumes of soil and are therefore less dependent on mycorrhizas for monopolizing space. The smaller seeded plants are more reliant on becoming VAM rapidly under low nutrient conditions in order to be competitive and thus show greater response to mycorrhizas. However, the larger seeded species must become mycorrhizal at an early stage in life, because they are unable to acquire phosphorus from this low nutrient soil any more efficiently than smaller seeded species.

Among the seeds in this experiment, dispersal of the mainly smooth and rounded seeds is by explosive mechanisms, ants or passive. There are no confounding features, such as wings, which may enhance the dispersal of large seeds over smaller, smooth seeds. The

phylogenetic constraints on seed size and number of seeds (Hodgson & Mackey 1986) are fairly similar; all the species, except *Petalacte coronata*, are from the Rosidae, and produce one or two viable seeds per ovary. Allometric considerations that plant size influences seed size (Thompson & Rabinowitz 1989) are probably not important as potential plant height (Bond & Goldblatt 1984) was not significantly correlated with seed size ($r=0.15$, $p>0.25$). The species have similar functional and structural attributes and regenerate after fire in the low nutrient soils of fynbos. Therefore this group of plants is fairly homogeneous concerning factors which may influence seed size.

The large seeds will disperse very locally, while the smaller seeds have a higher chance of more distant distribution. Species with small seeds may be less likely to face extinction because some at least will be dispersed to microsites favourable for establishment and thus escape localized stresses. Although the small-seeded species must become mycorrhizal to succeed, the probability of some seedlings encountering mycorrhizal inoculum early in life will also be higher. Large seeds have an establishment advantage in the absence of mycorrhizas, but their low dispersability without specialized aerial distribution structures, and their attractiveness to predators (Thompson 1987a), are disadvantages. The latter is overcome by the fact that larger seeds are more commonly myrmecochorous (ant-dispersed) than smaller seeds (Cowling *et al.* in press). However low dispersal seems to make populations derived from large seeds more vulnerable to local stresses, and extinction of such poorly dispersed species may contribute to speciation in the Cape flora (Cowling *et al.* in press). Many of the smaller seeded species in this study possess elaiosomes and are therefore likely to be myrmecochorous, so ant dispersal *per se* is not associated with low mycorrhizal responses. Evidence suggests that ants do not disperse seeds to nutrient rich microsites and, if anything, the reverse prevails (Bond & Stock 1989). Therefore, lower VAM dependency among large-seeded, myrmecochorous species is not overcome by dispersal to nutrient rich microsites.

Shade and drought are not important factors in early seedling establishment in fynbos heathlands and the main selective pressures for the production of larger seeds are the extremely nutrient poor soils and uncertainty in encountering mycorrhizal inoculum. VAM

infectivity of lowland fynbos soils is low (Berliner *et al.* 1989) and appears to be unevenly distributed (Chapter 3), possibly because mycorrhizal infectivity may be reduced in patches dominated for several years before fire by the non-mycorrhizal Restionaceae or Proteaceae. Growth of non-mycorrhizal crops has been shown to reduce mycorrhizal infectivity or VAM spore numbers (Kruckelmann 1975, Black & Tinker 1979). Large seeds allows establishment in such patches, independently of mycorrhizas, and in the bare soil situation following fire, movement of soil by wind and moles can facilitate recolonisation by mycorrhizal propagules (Warner, Allen & MacMahon 1987). Therefore large seeds may provide a temporal advantage in encountering mycorrhizal inoculum while the greater dispersability of smaller seeds provides a spatial advantage.

The lack of mycorrhizal response among larger seeded shrubby plants in the Rosidae in this study is significant in view of the non-mycorrhizal state of the Proteaceae (Rosidae) which is considered to be derived from the ancestral VAM state (Pirozynski & Malloch 1975, Trappe 1987). Proteaceae have acquired independence from mycorrhizas by forming cluster roots (Lamont 1982) and by the production of the largest seeds among shrub species in the fynbos (le Maitre & Midgley 1992, Allsopp & Stock in press b) which enable them to establish without external nutrients (Stock *et al.* 1990). Some large seeded species of the Fabaceae may have the potential for becoming non-mycorrhizal in the fynbos as members of the Fabaceae have developed cluster roots in both the low nutrient soils of South Africa (Chapter 2) and Australia (Lamont 1972b, Brundrett & Abbott 1991). The concurrent development of cluster roots and large seeds may be an alternate mechanism allowing for the evolution of non-mycorrhizal species among certain taxa in low nutrient environments. The non-mycorrhizal state is usually associated with herbaceous, weedy species with widely dispersed small seeds that colonize highly disturbed areas (Miller 1979, Reeves *et al.* 1979, Trappe 1987).

Although seed reserves exert a strong influence on VAM mass and phosphorus responses, RGR responses are uncorrelated with seed reserves. Thompson (1987a) proposes that large seed size and low growth rates have evolved independently to provide solutions to the

problem of seedling establishment in hostile environments. VAM RGR responses may be an indication of the growth plasticity of species in response to nutrient additions.

While most of the species were dependent on mycorrhizas for acquiring soil phosphorus from low nutrient soils, increasing seed size was associated with reduced VAM mass and phosphorus responses. Seed size is seen as the outcome of the conflict between dispersability and provisioning of seedlings among woody species. Mycorrhizal dependency of the seedlings is a function of the probability that seeds produced by a plant will encounter mycorrhizal inoculum either spatially or temporally during establishment.

CHAPTER 6

Growth and Allocation Patterns of Seedlings of Evergreen Shrubs from a Low Nutrient Environment in Response to Vesicular-arbuscular Mycorrhizas

Introduction

Growth rates of the dominant evergreen, woody vegetation typical of low nutrient environments are slow and inflexible, and increased nutrient availability usually results in storage of the additional nutrients rather than in increased growth (Chapin 1980, Witkowski *et al.* 1990). It has, however, been shown that seedlings of slow growing, sclerophyllous fynbos shrubs respond to mycorrhizas and phosphorus fertilization with enhanced growth, as well as luxury consumption, when grown in low nutrient soils (Chapter 4). Biomass production does not reflect the only effect of mycorrhizas on plant growth, as allocation patterns may also change in response infection (Miller *et al.* 1987) and mycorrhizal infection usually, but not invariably, lowers the root:shoot ratio of plants (Hetrick 1991).

Hyphae of mycorrhizal fungi increase the surface area available for nutrient absorption as they explore the soil beyond rhizosphere depletion zones for nutrients such as phosphorus (Bolan 1991). Increased phosphorus uptake by mycorrhizas results in enhanced growth rates which have been attributed to three main mechanisms. Firstly, growth may be increased through a stimulation of the photosynthetic rate per unit leaf area due to increased leaf phosphorus concentrations (Foyer & Spencer 1986, Sivak & Walker 1986); or secondly, by increased carbon allocation to photosynthetically active tissues of the shoots relative to roots due to the alleviation of nutrient stress (Bloom, Chapin & Mooney 1985, Wilson 1988, Körner 1991); and thirdly, because of increases in tissue production which result from the greater availability of a growth limiting substrate such as phosphorus. From a study on *Plantago*, with or without mycorrhizas, at different phosphorus levels, it was concluded that the increase in carbon assimilation rate due to improved nutrition was offset by the carbon demands of mycorrhizal roots; and that the increased growth of the mycorrhizal plants was due to the shift in carbon allocation from root to shoot growth (Baas, van der Werf & Lambers 1989).

If the alleviation of phosphorus limitations on growth shifts carbon allocation to shoots (Bloom *et al.* 1985, Hunt & Lloyd 1987, Wilson 1988, Ingestad & Ågren 1991), it may be expected that plants which respond to mycorrhizas with the highest proportional allocation of biomass to photosynthetically active tissue will have the highest relative growth rates (RGRs) (Tilman

1988, Hilbert 1990). Similarly phosphorus translocation to the shoots may stimulate growth, although luxury consumption may be expected among slow growing, woody species (Chapin 1980).

The effect of mycorrhizas on growth and phosphorus nutrition of seedlings of a range of evergreen and sclerophyllous, slow growing plants is examined in this chapter to determine whether differences in growth rates among these species can be attributed to changes in biomass and phosphorus allocation in response to mycorrhizas.

Materials and Methods

Germinated seeds of 15 species of evergreen, perennial woody plants (Table 5.1), indigenous to the Cape Floristic Region (Bond & Goldblatt 1984) were planted in a sterilized low nutrient soil:acid washed sand mix (1:1) in 13 cm diameter plastic plant pots with mycorrhizal (VAM) and non-mycorrhizal (NM) treatments as described in Chapter 4.

Five plants per treatment were harvested after 8 weeks of growth and at the final harvest where the age of plants ranged from 40 - 50 weeks as described in Chapter 5. Plants were divided into shoots and roots which were separated from the soil using a gentle spray of tap water. RGRs were calculated (Hunt 1978) for the period from germination to 8 weeks (0 - 8 wk) of growth using seed dry mass for time zero, and for the period from 8 weeks to final harvest (8 wk - harvest). Root:shoot ratios were calculated from plant dry mass at both the 8 week and final harvest. Root:shoot ratios were not calculated for 8 week old plants of *Petalacte coronata*, *Agathosma gonaquensis* and *Agathosma collina* as only total mass was recorded for these species. The RGR response to mycorrhizas was calculated from $(RGR_{VAM} - \overline{RGR}_{NM})/\overline{RGR}_{NM}$ for the 8 wk - harvest RGRs (Bryla & Koide 1990)

Phosphorus contents of roots and shoots from the final harvest were measured as described in Chapter 4. Phosphorus allocation to roots and shoots was measured using a root:shoot ratio of phosphorus content.

Student's *t* tests were used for comparisons between treatments for each species. Differences between the root:shoot ratios and phosphorus allocation patterns for the VAM and NM treatments were tested by two-way analysis of variance (ANOVA) for all the species. Dry mass root:shoot ratios were used as a covariate for the ANOVA on phosphorus allocation. Linear relationships between allocation and growth were tested using correlation analysis.

Results

At 8 weeks RGRs of VAM plants were only significantly ($p<0.05$) higher for *Agathosma ovata* and *Passerina paleacea*, and dry mass root:shoot ratios were not significantly different between treatments for any species (Table 6.1).

Mass of VAM plants were significantly ($p<0.05$) higher for all species except *Aspalathus linearis* and *A. spinescens* at the final harvest, while phosphorus concentrations were significantly ($p<0.05$) greater when plants were VAM except for *Aspalathus spinescens* and *Petalacte coronata* (Fig. 6.1). Dry mass root:shoot ratios were significantly ($p<0.0001$) smaller for VAM plants, and phosphorus allocation to shoots increased significantly ($p<0.05$) with root:shoot ratios as a covariate when plants were VAM (Fig. 6.2). RGRs for the period from 8 weeks to harvest were significantly ($p<0.01$) higher for all VAM plants except for *Aspalathus linearis* and *A. spinescens* (Table 6.1).

Root:shoot ratios at 8 weeks was positively correlated with 0 - 8 wk RGRs for VAM and NM plants (Fig. 6.3) while a negative relationship between root:shoot ratios at the final harvest and 8 wk - harvest RGRs was found for both VAM and NM plants (Fig. 6.4). A similar relationship between phosphorus distribution between roots and shoots and 8 wk - harvest RGRs was found for the NM plants; however, the correlation was weak for VAM plants (Fig. 6.5). Neither shoot or whole plant phosphorus concentrations were correlated with RGRs at the final harvest ($r=0.36$ - 0.38, $p>0.05$). Allocation of phosphorus in roots relative to shoots was positively correlated with root:shoot ratios for NM plants ($y=0.809x+0.097$, $r=0.84$,

$p<0.0005$) but not for VAM plants ($r=0.23$, $p>0.10$). VAM RGR response (Table 6.1) was positively correlated with the NM root:shoot ratio and the ratio of NM root phosphorus:shoot phosphorus at the final harvest ($y=2.06x-0.79$, $r=0.64$, $p<0.005$ and $y=1.88x-0.82$, $r=0.60$, $p<0.01$, respectively). A weak positive relationship was found between RGR response and VAM root:shoot ($y=5.23x-1.87$, $r=0.49$, $p<0.05$), but no relationship to VAM root phosphorus:shoot phosphorus ($r=0.04$). There were no relationships between root:shoot ratios of plants at 8 weeks and VAM RGR response.

TABLE 6.1. Relative growth rates (RGR) for the periods 0 - 8 weeks and 8 weeks to harvest and root:shoot (R:S) ratios at eight weeks for vesicular-arbuscular mycorrhizal (VAM) and non-mycorrhizal (NM) seedlings of evergreen, perennial shrubs from low nutrient habitats in the fynbos ecosystem and their VAM RGR responses[1] ±1 standard error. A - indicates no data.

Species	RGR (0-8wk) ($g\ g^{-1}\ wk^{-1}$)		RGR (8wk-harvest) ($g\ g^{-1}\ wk^{-1}$)		R:S ratio (8 week)		VAM RGR response
	NM	VAM	NM	VAM	NM	VAM	
Agathosma collina (Rutaceae)	0.23 ±0.02	0.25 ±0.01	0.039 ±0.003	0.096 ±0.001	-	-	1.40 ±0.03
Agathosma gonaquensis (Rutaceae)	0.25 ±0.03	0.26 ±0.02	0.044 ±0.002	0.095 ±0.005	-	-	1.17 ±0.08
Agathosma ovata (Rutaceae)	0.16 ±0.02	0.23 ±0.01	0.056 ±0.005	0.088 ±0.003	0.54 ±0.05	0.58 ±0.05	0.56 ±0.05
Aspalathus linearis (Fabaceae)	0.34 ±0.01	0.34 ±0.02	0.047 ±0.007	0.063 ±0.009	0.74 ±0.09	0.67 ±0.09	0.32 ±0.14
Aspalathus spinescens (Fabaceae)	0.25 ±0.01	0.27 ±0.05	0.093 ±0.004	0.091 ±0.005	0.61 ±0.06	0.38 ±0.08	-0.02 ±0.04
Otholobium fruticans (Fabaceae)	0.31 ±0.01	0.31 ±0.01	0.029 ±0.004	0.089 ±0.003	0.49 ±0.05	0.53 ±0.02	2.02 ±0.09
Otholobium hirtum (Fabaceae)	0.31 ±0.02	0.32 ±0.01	0.019 ±0.003	0.071 ±0.003	1.14 ±0.25	0.96 ±0.08	2.71 ±0.14
Passerina paleacea (Thymelaeaceae)	0.08 ±0.03	0.19 ±0.01	0.065 ±0.005	0.123 ±0.003	0.33 ±0.05	0.23 ±0.01	0.89 ±0.05
Petalacte coronata (Asteraceae)	0.22 ±0.06	0.24 ±0.03	0.100 ±0.003	0.139 ±0.002	-	-	0.39 ±0.02
Phylica cephalantha (Rhamnaceae)	0.15 ±0.03	0.17 ±0.03	0.100 ±0.005	0.139 ±0.003	0.23 ±0.03	0.23 ±0.02	0.36 ±0.05
Phylica ericoides (Rhamnaceae)	0.11 ±0.03	0.13 ±0.01	0.061 ±0.003	0.142 ±0.008	0.26 ±0.05	0.33 ±0.04	1.33 ±0.05
Podalyria sericea (Fabaceae)	0.29 ±0.01	0.28 ±0.01	0.039 ±0.004	0.055 ±0.003	0.32 ±0.02	0.45 ±0.05	0.41 ±0.07
Polygala virgata (Polygalaceae)	0.37 ±0.06	0.37 ±0.03	0.008 ±0.001	0.067 ±0.001	0.69 ±0.09	0.88 ±0.11	6.59 ±0.12
Psoralea pinnata (Fabaceae)	0.35 ±0.02	0.32 ±0.01	0.029 ±0.002	0.060 ±0.002	2.18 ±0.17	1.26 ±0.43	1.09 ±0.07
Staavia radiata (Bruniaceae)	0.14 ±0.01	0.15 ±0.02	0.074 ±0.005	0.117 ±0.008	0.17 ±0.04	0.19 ±0.02	0.58 ±0.08

[1] VAM RGR response = $(RGR_{VAM}-RGR_{NM})/RGR_{NM}$ (Bryla & Koide 1990).

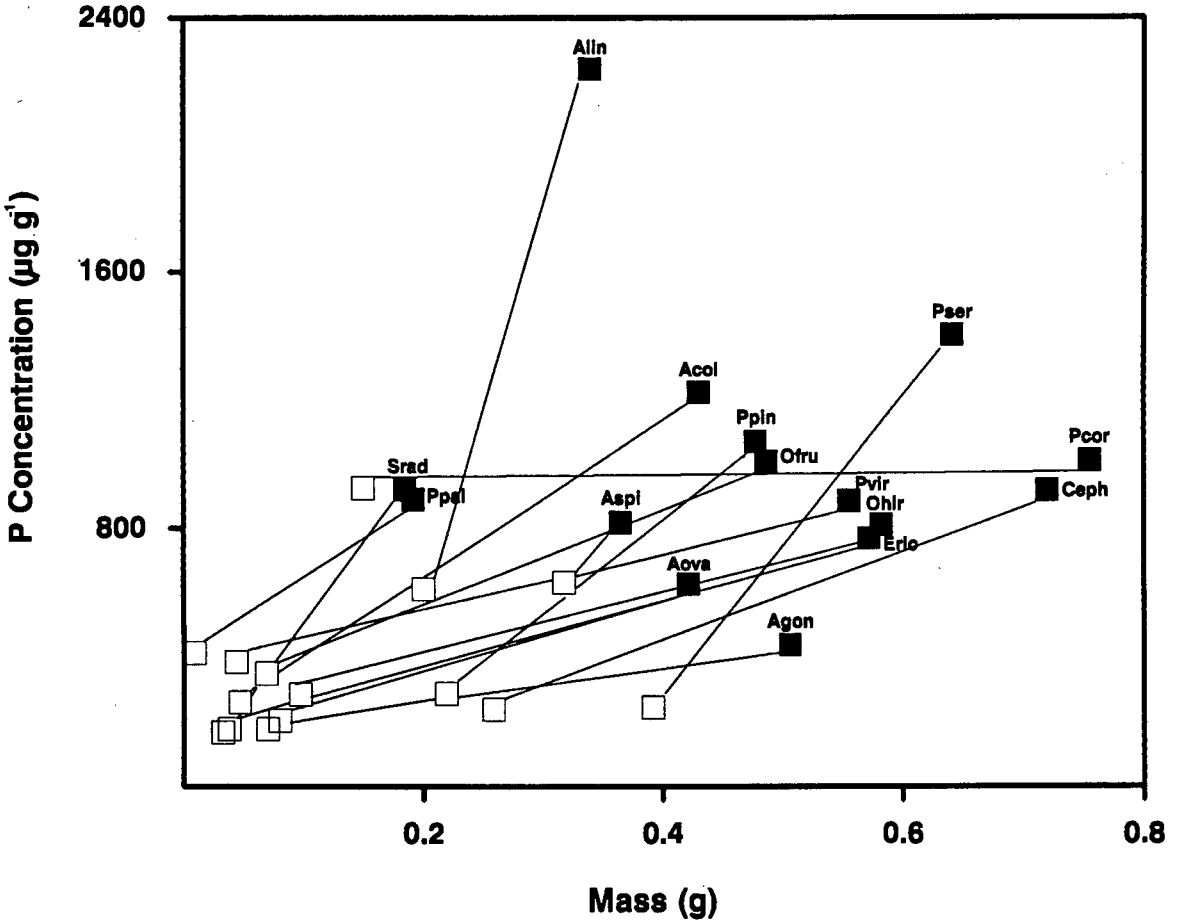

FIGURE 6.1. Mass and phosphorus concentration of non-mycorrhizal (□) and vesicular-arbuscular mycorrhizal(■) seedlings of evergreen, slow growing fynbos shrubs. Lines connect non-mycorrhizal and mycorrhizal plants of the same species. Acol = *Agathosma collina*, Agon = *Agathosma gonaquensis*, Aova = *Agathosma ovata*, Alin = *Aspalathus linearis*, Aspi = *Aspalathus spinescens*, Ofru = *Otholobium fruticans*, Ohir = *Otholobium hirtum*, Ppal = *Passerina paleacea*, Pcor = *Petalacte coronata*, Ceph = *Phylica cephalantha*, Eric = *Phylica ericoides*, Pser = *Podalyria sericea*, Pvir = *Polygala virgata*, Ppin = *Psoralea pinnata*, Srad = *Staavia radiata*.

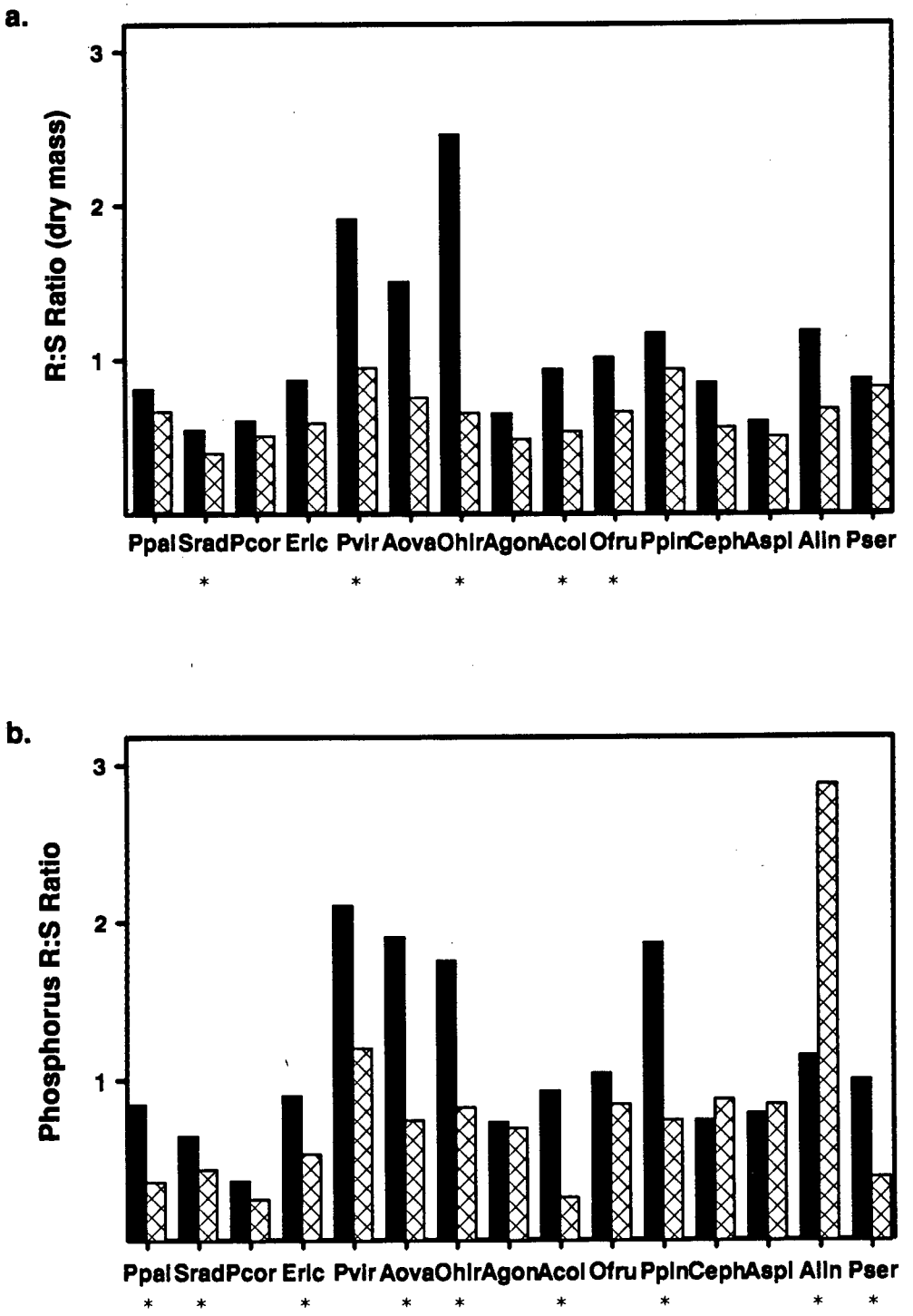

FIGURE 6.2. (a.) Biomass root:shoot (R:S) ratios and (b.) Phosphorus allocation as measured by the ratio of P in roots to that in shoots for non-mycorrhizal (■) and vesicular-arbuscular mycorrhizal (⊠) seedlings of evergreen, slow growing fynbos shrubs. Asterisks (*) mark those species for which the treatments were significantly different as tested by *t* tests. Species as in Fig. 6.1.

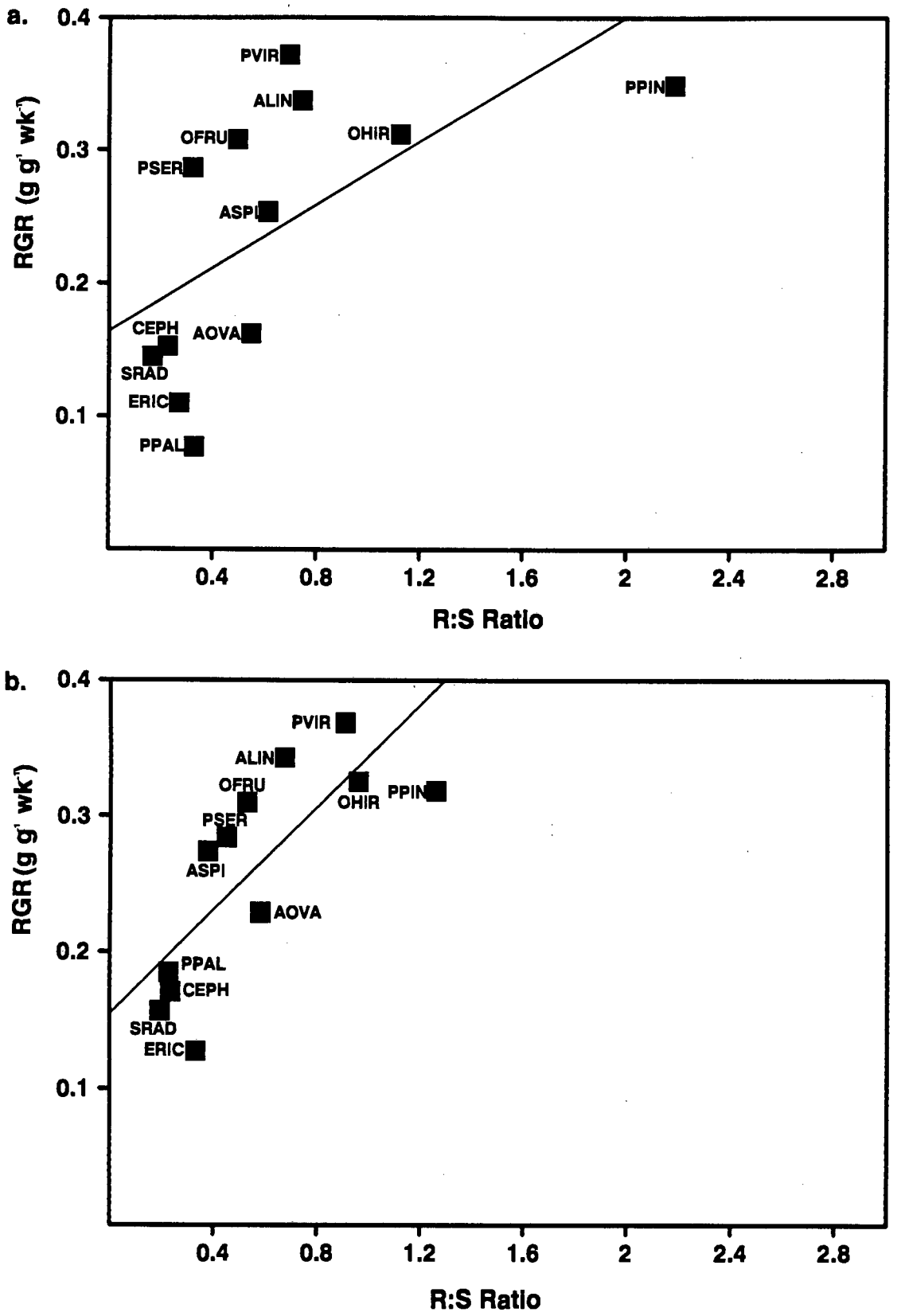

FIGURE 6.3. Relationship between root:shoot ratios at 8 weeks and the relative growth rates for the 0 - 8 wk period for (a.) non-mycorrhizal ($y=0.11x+0.166$, $r=0.60$, $p<0.025$) and (b.) vesicular-arbuscular mycorrhizal ($y=0.18x+0.154$, $r=0.76$, $p<0.0025$) seedlings of evergreen, slow growing fynbos shrubs. Species as in Fig. 6.1.

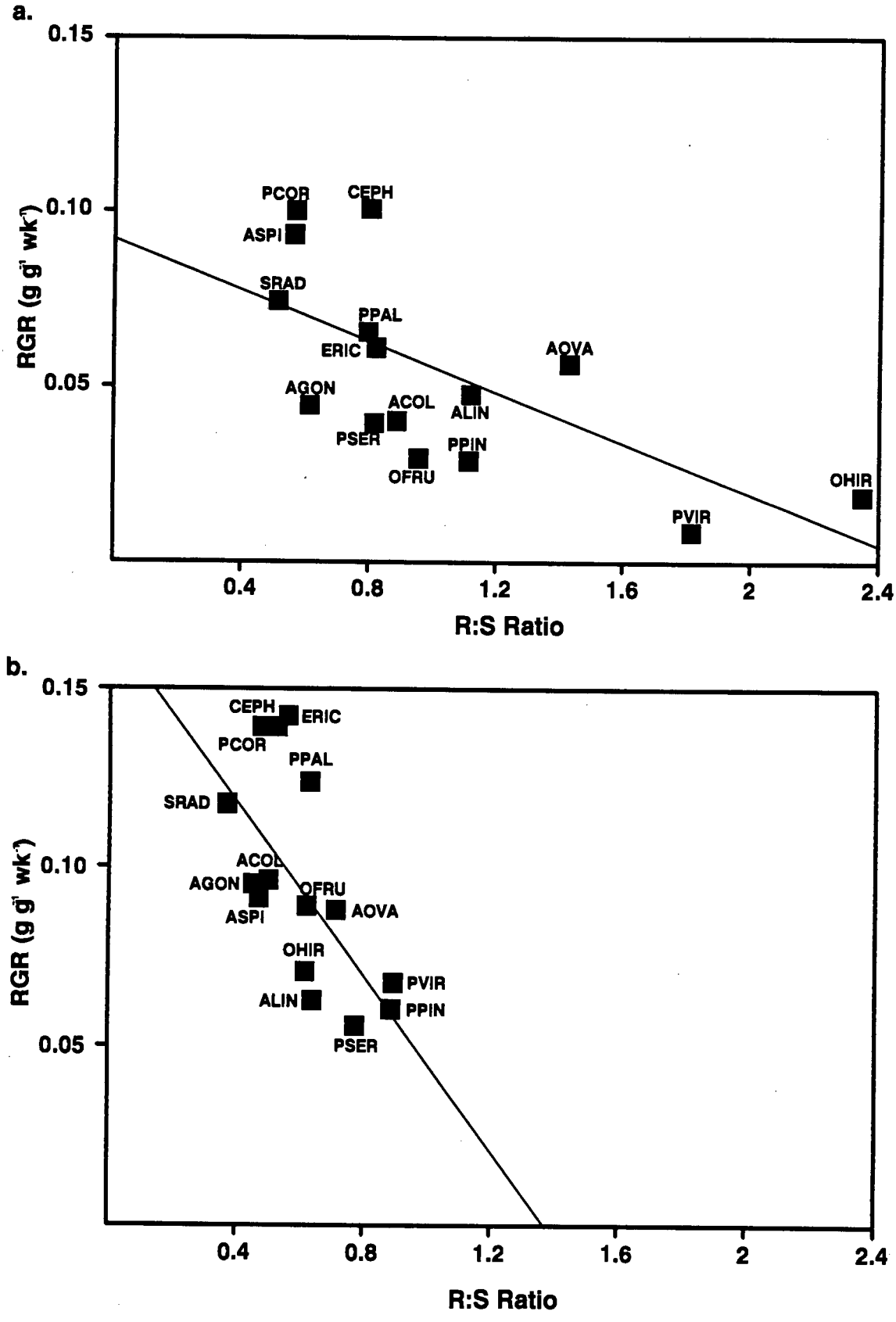

FIGURE 6.4. The relationship between root:shoot ratios and relative growth rates for the final harvest for (a.) non-mycorrhizal ($y=-0.038x+0.092$, $r=0.67$, $p<0.005$) and (b.) vesicular-arbuscular mycorrhizal ($y=-0.125x+0.172$, $r=0.64$, $p<0.01$) seedlings of evergreen, slow growing fynbos shrubs. Species as in Fig. 6.1.

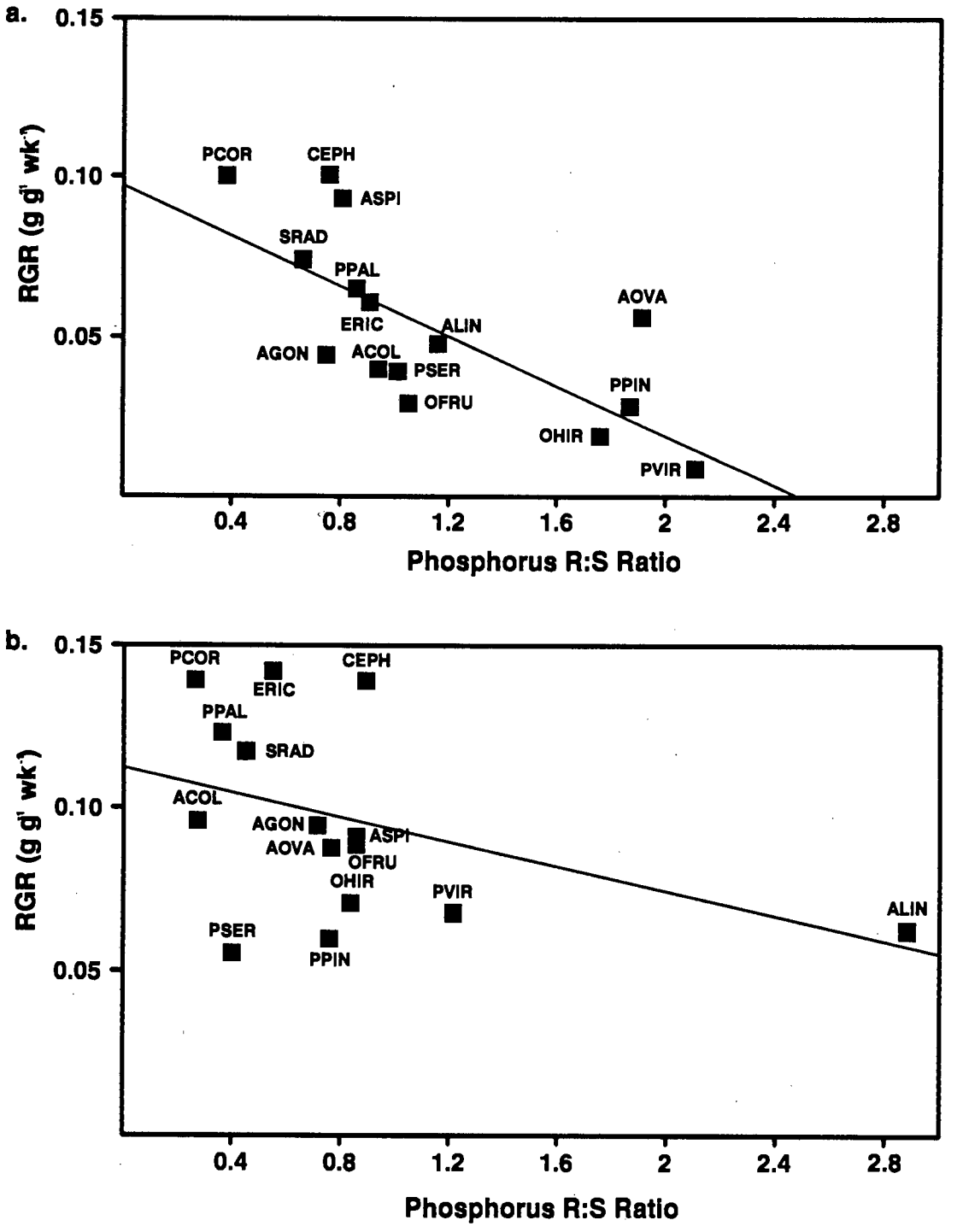

FIGURE 6.5. The relationship between phosphorus allocation as measured by the ratio of P in roots to that in shoots and relative growth rates for (a.) non-mycorrhizal ($y=-0.039x+0.098$, $r=0.72$, $p<0.0025$) and (b.) mycorrhizal ($y=-0.020x+0.112$, $r=0.43$, $0.05<p<0.10$) seedlings of evergreen, slow growing fynbos shrubs. Species as in Fig. 6.1.

Discussion

Although improved phosphorus nutrition of mycorrhizal plants appears to be the main reason for improved growth of these plants, phosphorus concentration and plant mass increases were not consistent among species. Differences in productivity or nutrient use efficiency of the VAM and NM plants and the different species may be ascribed to differences in carbon and phosphorus allocation between roots and shoots. Despite the expectation of low plasticity in growth responses of slow growing plants, among the experimental plants, the trend is for carbon allocation to shift from the roots to shoots when the phosphorus deficiency is alleviated by mycorrhizas. Similarly, phosphorus allocation to shoots is increased by VAM infection. This supports predictions that plants will adjust allocation so that their growth is equally limited by all resources (Bloom *et al.* 1985, Wilson 1988, Levin *et al.* 1989, Hilbert 1990, Ingestad & Ågren 1991).

The 0 - 8 wk RGRs of the seedlings in this study are very similar to those obtained by Grime & Hunt (1975) for maximum RGRs of woody species, including shrubs with microphyllous leaves, and may be regarded as close to their maximum potential RGRs. Contrary to predictions (e.g. Tilman 1988, Hilbert 1990) that root:shoot ratios should be negatively correlated with maximum RGRs, both VAM and NM root:shoot ratios at the 8 week harvest are positively correlated with 0 - 8 wk RGRs for both VAM and NM plants, with the slope being steeper for VAM species. A similar trend was found among a group of 68 herbaceous wetland species, where a weak positive relationship was found for the period from day 10 to day 30 after germination (Shipley & Peters 1990). Moreover, a positive relationship exists between the ratio of the RGRs of roots and shoots and RGRs of the 132 species studied by Grime & Hunt (1975) (Hunt & Lloyd 1987). There is a tendency for faster growing species in Grime & Hunt's (1975) study to allocate more mass to roots during the juvenile stage although the reverse was not true for the slow growing species (Hunt & Lloyd 1987). However, a pattern of decreased biomass allocation to roots does emerge among the slow growing plants of the present study when they are VAM. It would be interesting to test how mycorrhizas affect patterns among potentially VAM species in the studies of Shipley & Peters (1990) and Hunt & Lloyd (1987), as the plants were apparently grown without mycorrhizas. Seed reserves still had a strong influence on 8

week old seedling size (Chapter 5) and ontogenetic effects on root:shoot allocation and RGRs must be considered in predicting growth of VAM plants on the basis of allocation models among young seedlings.

However, at the final harvest, root:shoot ratios were negatively correlated with 8 wk - harvest RGRs of the plants. The slope for the VAM plants was steeper because the species with the highest root:shoot ratios when NM showed the greatest reduction in root:shoot ratios when these plants were mycorrhizal. Increased RGRs among these slow growing plants is related to changes in biomass allocation in response to mycorrhizas and RGR seems to be a function of root:shoot ratio which is controlled by resource availability and plant plasticity.

The strong correlation between NM 8 wk - harvest RGRs and the ratio of phosphorus in roots and shoots indicates that, when phosphorus nutrition is very poor, differences in phosphorus allocation to shoots may be influencing photosynthetic rates, which in turn are limiting growth. However the relationship becomes much weaker when the plants are VAM and, furthermore, there is no correlation between plant or shoot phosphorus concentrations and RGRs among either VAM or NM plants or between root:shoot allocation of carbon and phosphorus allocation of VAM plants. Lack of correlation between carbon and mineral nutrient allocation patterns have been reported among other plants (Abrahamson & Caswell 1982, Körner & Renhardt 1987). Once phosphorus stress has been alleviated by mycorrhizas, growth responses to additional phosphorus in terms of biomass increase may not be obvious among wild plants (Miller *et al.* 1987). Among slow growing plants from low nutrient habitats, RGRs are usually rather low and inflexible and phosphorus will accumulate in tissue (luxury consumption) rather than stimulate more growth (Chapin 1980). However, mycorrhizas are probably essential for building up the nutrient stores required to maintain growth during unfavourable conditions and for provisioning seeds during reproduction. Phosphorus requirements for processes other than photosynthesis and tissue growth may also explain the lack of correlation between phosphorus allocation and growth. For example, legumes sometimes show higher phosphorus allocation to roots than other plants (Pate *et al.* 1990), presumably because of the phosphorus requirements of nitrogen fixation.

Although caution must be exercised in extrapolating these results to the field situation where plasticity in root:shoot ratios in response to changes in one resource may disadvantage the plant in the acquisition of other resources and the survival of stresses such as drought (Chapin 1988), the root:shoot ratios of the plants in this study are similar to those of field-grown seedlings of sclerophyllous plants from a nutrient-poor environment in Western Australia (Pate *et al.* 1990). However, when *Phylica cephalantha* was grown with high and low watering treatments with cyclical drying, root:shoot ratios increased in response to decreased water availability as well as to mycorrhizas (Chapter 7).

Reduced root:shoot ratios may not reflect a reduction of carbon allocation to roots in real terms, because carbon requirements by mycorrhizal fungi will not be insignificant (Baas *et al.* 1989, Fitter 1991) and, among VAM and NM plants of similar size, the higher phosphorus concentrations of VAM plants are usually ascribed to the carbon demands of the mycorrhizal fungi (Stribley, Tinker & Rayner 1980). However the effects of alleviation of phosphorus deficiency in *Plantago* on growth, root:shoot ratios, and phosphorus concentrations in roots and shoots by either mycorrhizas or phosphorus fertilization, were fairly similar (Baas & Kuiper 1989). Among slow growing plants the costs of maintaining VAM fungi are possibly much lower than those of root growth, because these plants are in a position to fix more carbon than can be used for tissue production (Baas 1989). This may account for the strong relationship between root:shoot ratios and RGRs irrespective of the mycorrhizal status of the plants in this study.

Mycorrhizal RGR response could not be predicted from root:shoot ratios of 8 week old seedlings, possibly because ontogenetic considerations, unrelated to mycorrhizas, were controlling allocation at this stage. The NM root:shoot allocation patterns for phosphorus and mass at the final harvest were positively correlated with VAM RGR responses. The species with the highest root:shoot ratios and highest allocation of phosphorus to roots when NM had the largest relative changes in these parameters when they were VAM and hence the greatest increases in RGRs. However, contradictory reports of relationships between root:shoot ratios and mycorrhizal responses are reported in the literature (e.g. Menge *et al.* 1978a, Azcón & Ocampo 1981, Saif 1987, Hetrick *et al.* 1988, Koide *et al.* 1988a). Generally root fineness,

root hair number and length, and root architecture may reflect a species' potential to respond to mycorrhizas better than root biomass (Hetrick 1991). The ability of *Aspalathus linearis* and *A. spinescens* to obtain soil phosphorus when NM, and hence the reduced VAM responses of these species, is probably due to their possession of cluster roots (Chapter 2 & 5) rather than root:shoot biomass allocation.

Despite species specific characteristics such as shoot and root morphology and architecture which may influence plant productivity, the relationship between the allocation of biomass to roots and shoots, and growth rates of seedlings of a group of evergreen, slow growing shrubs from a low nutrient environment, is strong. This study suggests that the growth and allocation responses of these plants, to the alleviation of nutrient stress by mycorrhizas, are qualitatively similar to growth responses of faster growing species. Changes in biomass allocation influence growth rates, while phosphorus allocation only stimulates growth within the limits imposed by slow growing, relatively nutrient-unresponsive woody species, for which phosphorus storage is an important adaptation.

CHAPTER 7

Vesicular-arbuscular Mycorrhizas Influence Phosphorus Nutrition, Growth and Water Relations of a Sclerophyllous Shrub

Introduction

Investigations on the effects of VA mycorrhizas on plants show that, in addition to improving their phosphorus nutrition, their water relations are altered (Nelsen 1987). The water relations of different species are not uniformly affected by mycorrhizas but mycorrhizal plants often show increased leaf conductance or transpiration (Allen *et al.* 1981, Huang, Smith & Yost 1985, Fitter 1988) or drought stress tolerance (Allen & Boosalis 1983, Nelsen & Safir 1982, Busse & Ellis 1985). Most studies have related the differences in the water relations of mycorrhizal and non-mycorrhizal plants to the improved phosphorus nutrition of VAM plants (Nelsen 1987, Fitter 1988, Graham, Syvertsen & Smith 1987, Syvertsen & Graham 1990), but some reports indicate that mycorrhizas may affect leaf conductance and drought tolerance independently of plant nutritional status (Allen & Allen 1986, Augé, Schekel & Wampel 1987). Mechanisms whereby phosphorus nutrition may influence plant water relations are not clear. Although phosphorus concentrations are not directly involved in controlling stomata (Terry & Ulrich 1973, Wong, Cowan & Farquhar 1985a, Jacob & Lawlor 1991), the net rate of photosynthesis may be reduced under phosphorus deficient conditions (Machler, Schnyder & Nösberger 1984, Foyer & Spencer 1986, Sivak & Walker 1986, Hart & Greer 1988, Kirschbaum & Tompkins 1990) leading to conditions in the mesophyll which may affect stomatal conductance (Wong, Cowan & Farquhar 1985b).

Investigations on the effects of VA mycorrhizas on plant water relations have been carried out predominantly on agricultural crops, although studies on the effects of VA mycorrhizas on the growth and water relations of wild plants such as grasses and forbs (Allen *et al.* 1981, Allen & Allen 1986, Hetrick, Kitt & Wilson 1986) and woody perennials (Osonubi *et al.* 1991) have been conducted. Crop and wild plants studied so far usually come from relatively fertile soils and have rapid growth rates with short life cycles. Perennial plants from low nutrient environments such as the mediterranean heathlands of South Africa and Australia are evergreen, sclerophyllous shrubs with intrinsically slow growth rates and low phosphorus availability is reflected in very low leaf phosphorus concentrations (Rundel 1988). Although these plants are subjected to seasonal drought, sclerophylly is regarded as

an adaptation to low nutrients (Loveless 1962) and is not a modification for drought tolerance (Salleo & Lo Gullo 1990). When nutrients are supplied to these plants, growth responses are small or absent (Specht 1963, Witkowski *et al.* 1990). The responses of these plants to VAM infection may therefore differ from those of faster growing plants. In this study the effects of VA mycorrhizas on the phosphorus nutrition, growth and water relations of seedlings of *Phylica cephalantha* Sonder were investigated. Treatments included mycorrhizal infection, high and low watering regimes and fertilizer additions. *P. cephalantha*, an ericoid leaved sclerophyllous shrub, is a common representative of the sub-canopy shrub layer of lowland fynbos, and regenerates after fire from seed or by resprouting (Boucher 1983).

Materials and Methods

Plant culture

Seeds of *Phylica cephalantha* (Rhamnaceae), collected from plants growing at the fynbos biome intensive study site (Jarman & Mustart 1988) at Pella (33° 31' S, 18° 32' E), South Africa, were acid scarified for one hour, rinsed and then germinated on damp filter paper. Seedlings were transferred to polystyrene pots, with holes punched in the base, containing a sterile soil:acid washed sand mixture (1:1). The soil, collected from the top 20 cm at the study site, is a fine sandy soil of low phosphorus and nitrogen status (Mitchell *et al.* 1984, Stock & Lewis 1986). To establish VAM plants in the sterile sand:soil mixture, the pots were inoculated with 15 ml soil containing *Acaulospora morrowae* Spain & Schenk spores and root fragments of *Cynodon dactylon* (L.) Pers. on which the inoculum had been increased. The inoculum was obtained from INVAM (West Virginia University, Morgantown, WV 26506-6057, USA). Non-mycorrhizal (NM) controls were established by adding sterilized inoculum soil with a spore-free wash of the inoculum soil. All plants

were initially grown for 3 months in a greenhouse before transfer to controlled environment growth chambers. Plants were rotated weekly to avoid positional effects.

The effects of VA mycorrhizas on *P. cephalantha* seedlings were tested in two experiments. In the first, plants were grown under low nutrient conditions with or without mycorrhizas, and in the second, a factorial design with mycorrhizal, watering and phosphorus fertilizer treatments was implemented.

Experiment 1: Effect of VAM under low nutrient conditions

VAM and NM plants in 175 ml pots containing a sand/soil mixture were grown for a further five months in a controlled environment growth chamber (light 16 h, PAR 335 μmol m^{-2} s^{-1}, 25 ^{o}C/10 ^{o}C day/night temperatures, daytime relative humidity 50 %). Plants were watered every three days with distilled water, the amount determined gravimetrically so that gravimetric soil moisture was approximately 2 % on the third day of the drying cycle. When the plants were 8 months old, pre-dawn xylem pressure potentials of seven plants from each treatment, watered to saturation the previous evening, were determined. Transpiration rates of a further 10 plants from each treatment were measured over a 3 day drying cycle after which plants were harvested and xylem pressure potentials determined. The root systems of the plants used to determine pre-dawn xylem pressure potentials were used to confirm the presence of mycorrhizas in inoculated plants.

Experiment 2: Factorial experiment with VAM, phosphorus and watering treatments

Mycorrhizal, phosphorus and watering treatments were applied in a 2x2x2 factorial design to 3 month old plants growing in a larger soil volume (250 ml pots containing the sterile sand/soil mixture) in a growth chamber (light 16 h, PAR 390 μmol m^{-2} s^{-1}, 25^{o} C/15^{o} C, day/night temperatures, daytime relative humidity 50 %). There were six replicates per

treatment combination. A 20 ml volume of a balanced 1/10th strength Hoaglands nutrient solution (Hewitt 1966) containing micronutrients was administered weekly after transfer to the growth chamber. Phosphorus was supplied as KH_2PO_4 (3.1 μg P ml^{-1}). Either 30 ml or 50 ml of distilled water was provided on the first day of a 4 day drying cycle to simulate a low or high watering regime. Transpiration rates were measured daily over the drying cycle during the third and eighth week after transfer to the growth chamber. The plants were harvested at approximately 5 months old, immediately after the last transpiration readings.

Determination of plant parameters

Transpiration rates were measured with a Li-Cor steady state porometer (Model LI-1600, Li-Cor Inc., Box 4425, Lincoln, Nebraska 68504, USA) by placing the whole shoot in a cylindrical chamber. Plants were watered as normal before 09h00 on day one of a drying cycle and transpiration rates measured each day between 11h00 and 14h00 throughout the drying cycle. The order in which the plants were measured changed every day, as did their position in the growth chamber. Leaf area was used to calculate transpiration rates per unit area. Leaf conductance was calculated from transpiration rate and leaf temperature (Von Caemmerer & Farquhar 1981). An average leaf temperature for the whole plant was obtained by looking down on the top of the plant with the view finder of the infra-red thermometer (Instatherm model 14-220D-4, supplied by Protea Physical and Nuclear Instrumentation (Pty) Ltd., Rondebosch 7700, South Africa). The arrangement of the leaves around the stem is such that a high proportion of leaves can be seen through the view finder in this manner. Xylem pressure potentials were measured with a Scholander pressure bomb (PMS Instruments Co., 2750 N. W. Royal Oaks Drive, Corvallis, Oregon 97330, USA).

Leaf area was measured with a Skye leaf area meter, (Skye Instruments Ltd., Unit 5, Ddole Industrial Estate, Llandrindod Wells, Powys LD1 6DF, UK), immediately after the last set

of transpiration readings. Roots were washed free of soil and all plant parts were dried at 80 ^{o}C and weighed. Leaf specific mass was calculated from dry leaf mass and leaf area. The phosphorus content of the roots and shoots (leaves plus stem) was determined by the method of Murphy & Riley (1962) following acid digestion (Jackson 1958). In the second experiment nitrogen content of the roots and shoots were determined colorimetrically following Kjeldahl digestion (Smith 1980).

Roots were cleared and stained according to the method of Phillips & Hayman (1970), omitting phenol from the stain. Roots in the second experiment were subsampled by randomly removing 50 segments (0.5 - 1.0 cm long) from the root system and scoring the presence or absence of VAM infectivity of segments seen in fields of view of a light microscope at 100 times magnification.

Statistical analysis

Comparison of VAM and NM plants in the first experiment was by Student's *t* tests whereas, data from the second experiment were analysed by three-way analysis of variance (ANOVA). Percentage VAM infection data were arcsine transformed (Zar 1984) before two-way ANOVA.

Results

Plant biomass and leaf area

Root mass was significantly higher ($p < 0.001$) for all VAM plants (Table 7.1, Fig. 7.1) but the effect of mycorrhizas on shoot mass varied in the two experiments. In the low nutrient experiment, shoot mass of VAM plants was less than that of the NM plants (Table 7.1).

Shoot mass was significantly higher ($p < 0.05$) for VAM plants in the factorial experiment (Fig. 7.1). Watering regime, phosphorus addition and interactions between the treatments had no significant effect on root or shoot mass in the factorial experiment. Root:shoot ratios were greater for VAM plants (Tables 7.1 & 7.2) and plants receiving less water had higher root:shoot ratios in the factorial experiment (Table 7.2).

Leaf area followed the pattern of shoot mass by being lower for VAM plants in the low nutrient experiment (Table 7.1) and higher for VAM plants in the factorial experiment (Table 7.2). Leaf specific mass was not affected by treatments in the first or second experiment (Tables 7.1 & 7.2). Differences in calibrating the leaf area meter due to changes in ambient light conditions are thought to be responsible for the differences in leaf specific mass of the plants in the first and second experiment.

Nutrient content and mycorrhizal infection

All VAM plants had significantly ($p < 0.001$) higher root and shoot phosphorus concentrations (Table 7.1, Fig. 7.2). While there was an increase in root phosphorus concentration in response to an interaction ($p < 0.05$) between VAM and phosphorus fertilization in the factorial experiment, phosphorus fertilization alone and watering regime did not affect phosphorus levels in the factorial experiment. The total phosphorus content of NM seedlings in the low nutrient and factorial experiment were 46.8 μg (SD$\pm$11.7) and 45.9 μg (SD$\pm$15.9) P plant^{-1}, respectively.

The presence of VAM, addition of phosphorus and the high watering regime all increased root nitrogen concentrations in the factorial experiment (Table 7.2), but shoot nitrogen concentrations were only increased by the presence of VAM infection.

In the low nutrient experiment, VAM infection of the VAM plants appeared to be heavy, but was not quantified as the roots did not clear sufficiently. In the factorial experiment, the infection levels of the VAM plants that received phosphorus fertilizer were lower than the other VAM plants (Table 7.2).

Water relations

VAM plants had significantly ($p < 0.01$) higher leaf conductances in both experiments (Tables 7.3 & 7.4, Fig. 7.3). Conductances tended to decrease more during the drying cycle for VAM plants but were fairly constant over the drying cycles of NM plants (Table 7.3, Fig 7.3). In the factorial experiment, soil moisture was reduced to 0.21 % and 1.36 % for the low and high watering levels respectively on day 4 of the drying cycle. VAM plants receiving the low watering treatment had the highest leaf conductances (Fig 7.3). This was a frequently significant ($p < 0.05$) interaction effect between mycorrhizal and watering treatments (Table 7.4).

Pre-dawn xylem pressure potentials of shoots in the low nutrient experiment were not significantly different among treatments immediately after watering (Table 7.1). After the 3 day drying cycle, xylem pressure potentials were significantly lower for VAM plants in this experiment (Table 7.1). Xylem pressure potentials were not determined in the factorial experiment.

TABLE 7.1. Phosphorus concentration, dry mass, root:shoot (R:S) ratios, leaf area, leaf specific mass (LSM) and xylem pressure potential (XPP) of *Phylica cephalantha* seedlings grown under low nutrient conditions ±1 standard error. Significance values from *t* tests: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, NS not significant.

	P Concentration		Mass		R:S Ratio	Leaf area	LSM	XPP	
	Root ($\mu g\ g^{-1}$)	Shoot	Root (mg)	Shoot		(cm^2)	($g\ cm^{-2}$)	Day 1 (MPa)	Day 3
NM	154 ±9	132 ±9	144 ±10	183 ±15	0.84 ±0.11	7.59 ±0.66	190 ±5	-0.29 ±0.21	-0.74 ±0.07
VAM	355 ±16	257 ±18	213 ±14	130 ±10	1.75 ±0.19	5.14 ±0.36	198 ±5	-0.38 ±0.32	-1.00 ±0.05
Significance	***	***	***	**	***	**	NS	NS	**

TABLE 7.2. Root:shoot (R:S) ratios, leaf area, leaf specific mass (LSM), percentage VAM infection of roots and nitrogen concentration in roots and shoots of *Phylica cephalantha* seedlings grown in a factorial experiment with mycorrhizal (NM=non-mycorrhizal, VAM=mycorrhizal), P fertilizer (-P=no P, +P=with P) and water treatments (L=low water treatment, H=high water treatment) ±1 standard error. Variance by two or three way analysis of variation, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, NS not significant. Values in ANOVA table are F values, d.f.=1,40, interactions between treatments produced no significant effects.

	R:S Ratio	Leaf Area (cm^2)	LSM ($g\ cm^{-2}$)	VAM Infection (%)	Nitrogen concentration Root ($mg\ g^{-1}$)	Nitrogen concentration Shoot ($mg\ g^{-1}$)
NM -P L	0.49 ±0.04	8.50 ±1.23	223 ±9	0 ±0	6.69 ±0.26	6.83 ±0.29
NM -P H	0.46 ±0.04	10.19 ±1.23	212 ±10	0 ±0	6.36 ±0.85	6.26 ±0.33
NM +P L	0.55 ±0.04	8.89 ±0.62	214 ±7	1.7 ±1.1	6.93 ±0.46	7.18 ±0.25
NM +P H	0.45 ±0.05	7.63 ±0.78	226 ±7	0 ±0	9.96 ±0.94	6.86 ±0.26
VAM -P L	0.66 ±0.04	9.69 ±0.88	215 ±8	59.8 ±3.8	8.50 ±0.21	6.38 ±0.16
VAM -P H	0.49 ±0.03	10.98 ±1.29	231 ±8	53.8 ±3.5	9.84 ±0.94	6.09 ±0.03
VAM +P L	0.62 ±0.09	10.61 ±1.24	230 ±7	38.7 ±5.5	8.93 ±0.66	6.16 ±0.22
VAM +P H	0.58 ±0.03	11.95 ±0.59	216 ±6	45.7 ±4.9	10.26 ±0.98	5.75 ±0.21
ANOVA	3-WAY	3-WAY	3-WAY	2-WAY	3-WAY	3-WAY
Main effects						
mycor	6.5 ***	6.4 *	1.3 NS	- -	11.4 **	11.6 ***
fert	0.3 NS	0 NS	0.9 NS	8.4 **	4.3 *	3.5 NS
water	4.9 *	0.9 NS	0.7 NS	0 NS	5.7 *	0.8 NS

TABLE 7.3. Leaf conductance ±1 standard error of non-mycorrhizal (NM) and mycorrhizal (VAM) *Phylica cephalantha* seedlings growing under low nutrient conditions through a 3 day drying cycle. Significance values from *t* tests: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, NS not significant.

	Leaf conductance (mmol m^{-2} s^{-1})		
	Day 1	Day 2	Day 3
NM	91 ±13	83 ±13	72 ±13
VAM	437 ±89	299 ±32	254 ±45
Significance	**	***	***

TABLE 7.4. Three way analysis of variance of leaf conductance of *Phylica cephalantha* seedlings in response to a factorial experiment with mycorrhizal, phosphorus fertilizer and water treatments, over drying cycles in weeks 3 and 8 of the water treatment. Values are F values, d.f.=1,40, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, NS not significant.

	Week 3				Week 8		
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 3	Day 4
Main effects							
A mycorrhizas	22.2 ***	18.6 ***	15.8 ***	18.9 ***	26.8 ***	22.7 ***	22.9 ***
B fertilizer	0.7 NS	0 NS	0.2 NS	0.5 NS	0 NS	0.1 NS	0 NS
C water	4.7 *	1.7 NS	0.3 NS	0.2 NS	3.5 NS	1.7 NS	1.7 NS
Interactions							
AxB	2.2 NS	1.7 NS	1.8 NS	1.8 NS	0 NS	0.1 NS	0 NS
AxC	6.7 *	4.2 *	3.1 NS	2.3 NS	4.3 *	4.4 *	2.5 NS
BxC	4.6 *	2.5 NS	3.8 NS	1.6 NS	0.9 NS	4.7 NS	2.4 NS
AxBxC	2.1 NS	0.1 NS	0.2 NS	0.2 NS	0 NS	0.1 NS	0.3 NS

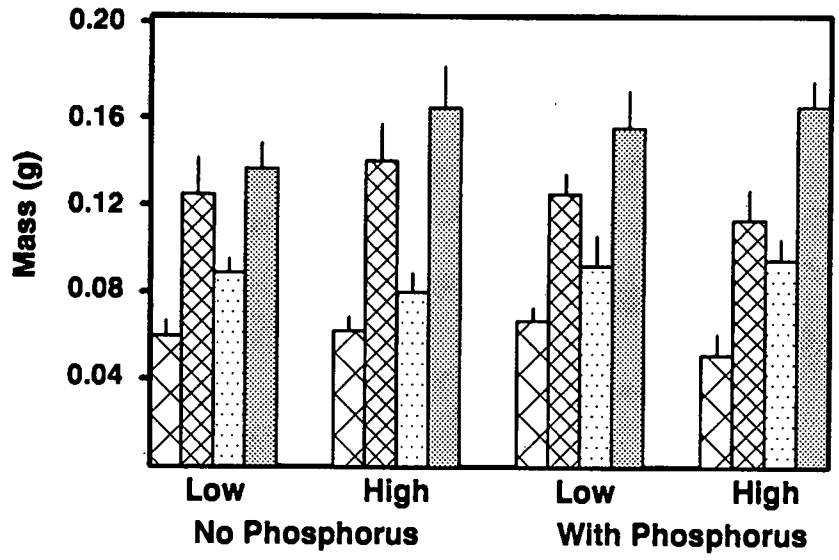

FIGURE 7.1. Mass of roots and shoots of *Phylica cephalantha* seedlings in response to mycorrhizal, phosphorus (with or without P) and water treatments (high or low water treatments) in a factorial experiment. Vertical line represents +1 standard error. ▨ = non-mycorrhizal root, ▩ = non-mycorrhizal shoot, ⬚ = mycorrhizal root, ▦ = mycorrhizal shoot.

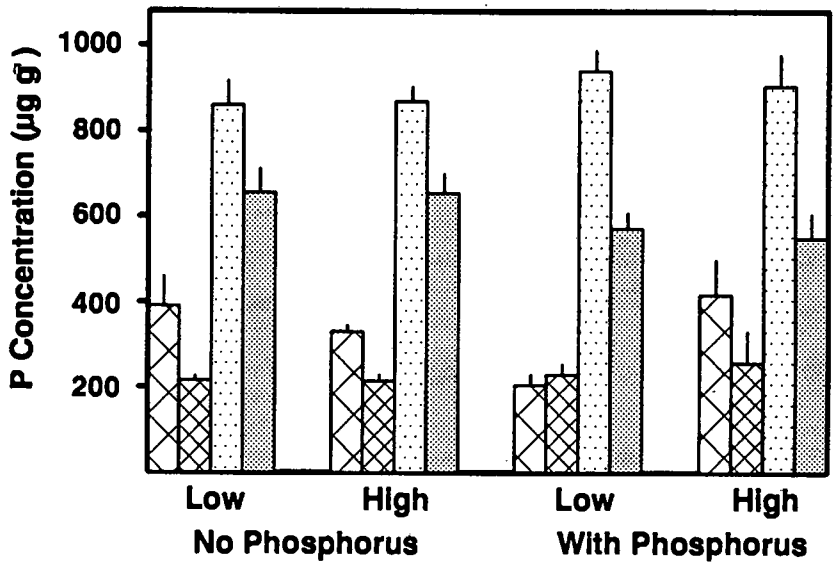

FIGURE 7.2. Phosphorus concentration in roots and shoots of *Phylica cephalantha* seedlings in response to mycorrhizal, phosphorus fertilizer (with or without P) and water treatments (high or low water treatments) in a factorial experiment. Vertical line represents +1 standard error. ▨ = non-mycorrhizal root, ▩ = non-mycorrhizal shoot, ⬚ = mycorrhizal root, ▦ = mycorrhizal shoot.

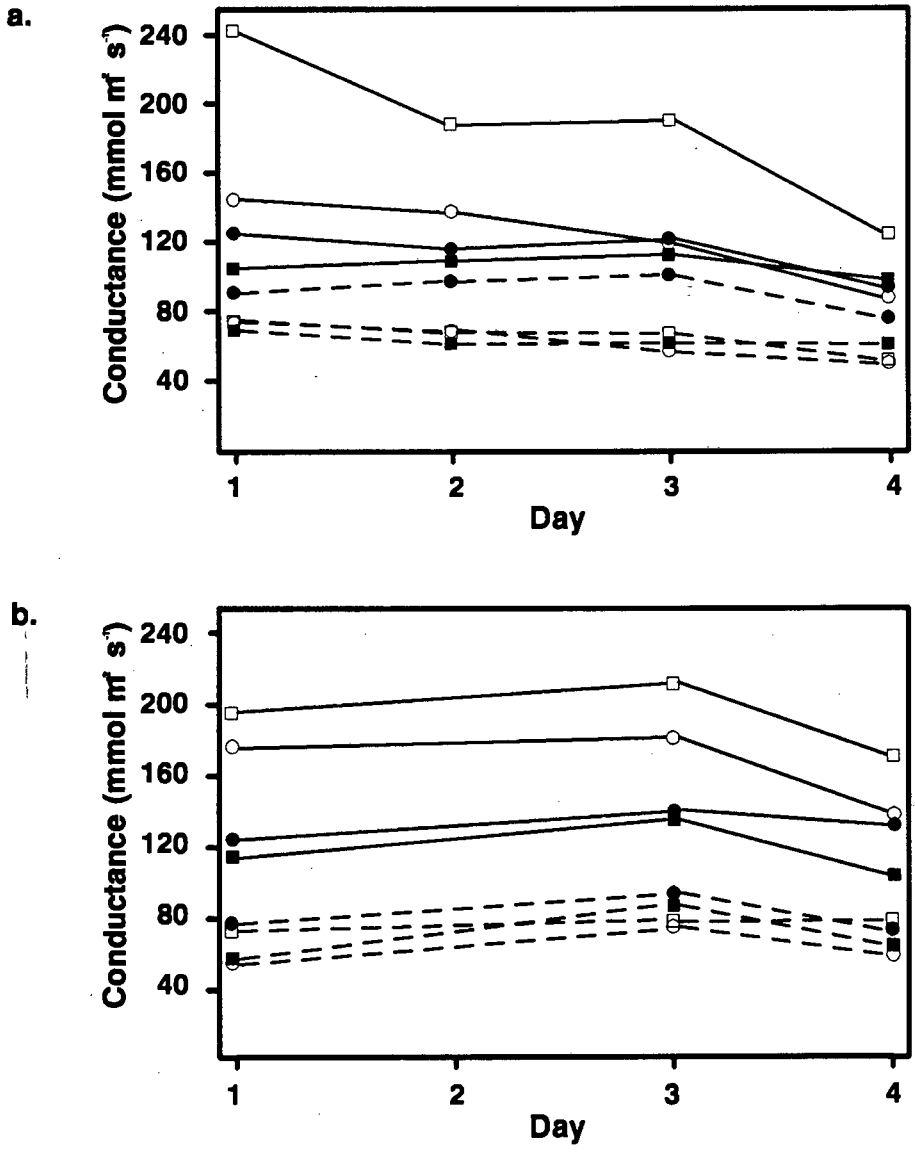

FIGURE 7.3. Leaf conductance of *Phylica cephalantha* seedlings over a four day drying cycle in response to mycorrhizal, phosphorus and watering treatments in a factorial experiment, during the third week (a), and the eighth week (b), of the application of the fertilizer and water treatments. —— = mycorrhizal plants, – – = non-mycorrhizal plants, □ = no P + low water regime, ○ = P + low water regime, ■ = no P + high water regime, ● = P + high water regime.

Discussion

The results of this study show that *P. cephalantha* seedlings react in a manner similar to many other plants, with different growth rates and life forms, by increasing their tissue phosphorus concentrations in response to mycorrhizas. Plant growth in the factorial experiment responded positively to VAM infection, but the plants in the low nutrient experiment showed reduced shoot growth in response to VAM infection. The shoot growth depression in these plants may be due to competition between the plant and the fungus for carbon under generally low nutrient conditions brought about by the small soil volume and dilution of the soil with sand. Growth depressions in non-sclerophyllous plants in response to VAM infection have been reported (Buwalda & Goh 1982, Koide 1985a, Osonubi *et al.* 1991).

Average seed phosphorus content in *P. cephalantha* is 36.1 μg P (SD$\pm$15.5), and the NM seedlings show a less than 28 % increase in total phosphorus. Phosphorus concentrations in the soil, even with phosphorus supplied in a soluble form, were below those that these roots can exploit (Föhse, Claassen & Jungk 1988) when non-mycorrhizal. Similarly Mosse *et al.* (1973) reported that roots of two of three tropical fodder species studied were incapable of taking up phosphorus when non-mycorrhizal. It appears that *P. cephalantha* is an obligate VAM species with respect to phosphorus nutrition, even under conditions of moderate phosphorus supplementation. Seed phosphorus is sufficient to support early seedling establishment and survival but subsequent development and success of *P. cephalantha*, as in other sclerophyllous shrubs (Chapter 5), is dependent on the plant becoming mycorrhizal. The role of mycorrhizas in acquiring nitrogen did not appear as critical as phosphorus uptake to the seedlings, although mycorrhizas were responsible for increasing nitrogen content.

The reduction in VAM colonization of roots of the plants receiving the phosphorus supplement may be a consequence of the higher phosphorus concentration in these roots, a factor which has been shown to control VAM infection in other plants (Menge *et al.* 1978b, Stribley *et al.* 1980) although VAM infection was not reduced among other sclerophyllous

species by phosphorus fertilization (Chapter 4). Slow growing, sclerophyllous shrubs, such as the study species, usually respond to additional nutrients by luxury consumption (Chapin 1980, Witkowski *et al.* 1990) which can however lead to nutrient toxicity and tissue death (Groves & Keraitis 1976). A feedback mechanism which controls phosphorus uptake by reducing mycorrhizal infection may be desirable under such conditions, although evidence for such a mechanism from these studies is equivocal.

Carbon allocation patterns as indicated by root:shoot ratios show that VAM plants allocated more carbon to roots than NM plants. This is the reverse of the trend observed for the same species in another study (Chapter 6) but was similar to root:shoot ratios reported for NM and VAM citrus seedlings under drought conditions (Graham *et al.* 1987). In the low nutrient experiment, in the present study, the overall higher root:shoot ratios, irrespective of mycorrhizal status, is possibly caused by the low nutrient conditions induced by the smaller pot size, but in the factorial experiment, root:shoot ratios are of similar magnitude to those in Chapter 6. All plants in the current study were subjected to repeated drying cycles, whereas in the previous study (Chapter 6), plants were well watered and the soil was never allowed to dry out. Thus, under conditions of restricted water availability, mycorrhizal plants may be able to allocate more carbon to root growth compared to non-mycorrhizal plants, which may enhance their water capturing capacity. However, all plants receiving less water had higher root:shoot ratios in the factorial experiment, so plasticity in allocation is not confined to the VAM plants.

Leaf conductance of *P. cephalantha* reacts to VAM infection in a similar manner as in many other plants (Allen & Boosalis 1983, Koide 1985b, Huang *et al.* 1985, Nelsen 1987, Fitter 1988), although VA mycorrhizas do not enhance the conductance of *Citrus* sp. compared to NM plants of similar nutritional status (Syvertsen & Graham 1990). This indicates that while morphological and life history features of *P. cephalantha* are very different to these plants, physiological processes associated with plant water relations are probably similar. The presence or absence of mycorrhizas was the main factor affecting leaf conductance which was consistently higher in VAM plants. Probably the most important reason contributing to increased leaf conductance in *P. cephalantha* was phosphorus availability, as

phosphorus concentrations were always higher in VAM plants. Although, the relative increase in root biomass among VAM plants may have increased their water uptake capacity, there was no correlation between the root:shoot ratios of NM or VAM plants and their leaf conductances in either experiment. Other plant parameters such as leaf area did not change consistently in response to VAM infection, and leaf specific mass was unaffected by mycorrhizas in the two experiments. Therefore, neither can be responsible for the differences in leaf conductance. If increased leaf conductance is related to the stimulation of photosynthesis through improved phosphorus nutrition, it can be assumed that carbon assimilation is greater for the mycorrhizal plants. This may allow mycorrhizal plants greater flexibility in carbon allocation in response to environmental conditions.

The xylem pressure potentials of the experimental plants, after a drying cycle, were higher than those associated with drought stressed sclerophyllous plants in the field (Miller, Miller & Miller 1983) and they may never have been severely water stressed. These experiments are inconclusive about the role of VAM in enhancing drought stress tolerance of *P. cephalantha*. Leaf conductance was not lowered by the low watering regime; on the contrary, interactions between VAM infection and the low water treatment tended to increase conductance. Field data from a number of studies on fynbos plants indicate that they continue transpiring and fixing carbon throughout most of the year, despite the summer drought period (Miller *et al.* 1983, van der Heyden & Lewis 1989, Richardson & Kruger 1990, Smith & Richardson 1990). This is usually ascribed to the deep taproots of most of these plants reaching water. The higher root:shoot ratios of VAM *P. cephalantha* may well be an advantage in this environment as a large root system may help it survive the summer drought period, while continuing to transpire, adopting a water spending strategy coupled with drought avoidance.

This study on *P. cephalantha* indicates that the biology of this sclerophyllous plant is intimately linked to its mycorrhizal symbiont. As a seedling it is an obligate mycorrhizal plant as, in the absence of mycorrhizas, it is unable to extract phosphorus from the soil. Leaf conductance is lowered and its capacity to fix carbon may be compromised and therefore, its ability to develop suitable morphological responses to enhance its survival in

its natural environment may be reduced. In particular carbon allocation to roots in VAM *P. cephalantha* seedlings, when subjected to cyclical drying, may be a strategy that will enhance long term survival under dry conditions.

CHAPTER 8

Density Dependent Interactions between Vesicular-arbuscular Mycorrhizal Fungi and Even-aged Seedlings of Two Perennial Fabaceae Species

Introduction

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 & 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 less 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 effect 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 & Watkinson 1990) and competition for scarce resources is usually responsible. In two experiments comparing the growth and phosphorus 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 & Hayman 1984, Koide 1991b). At higher plant densities, fewer differences were observed between mycorrhizal and non-mycorrhizal plants as the phosphorus depletion zones of their roots overlapped. 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 look only 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 & Hardwick 1986, Hara 1988). Various measures of plant size distributions exist (Hara 1988). Weiner & 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 which are closely correlated in most cases (Weiner & Thomas 1986).

In a survey of 16 experiments, inequalities in shoot size increased with increasing density in 14 cases (Weiner & 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 will be linked together by a hyphal network and that resources may pass along this network from source to sink plants (Chiariello *et al.* 1982, Francis, Finlay & Read 1986). If this is the case, 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. It is therefore expected that mean plant size will 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 chapter, 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, is investigated.

These species were chosen for testing the individual mycorrhizal, plant density and interactive effects of mycorrhizas and plant density as they show different responsiveness to mycorrhizas (Chapters 5 & 6).

Materials and Methods

Otholobium hirtum and *Aspalathus linearis* are endemic to the fynbos region of South Africa. Both species are evergreen members of the Fabaceae. *Otholobium* has hairy, trifoliate leaves while *Aspalathus* has needle-like leaves. Recruitment of seedlings in fynbos occurs during winter following wild fires. Members 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 & Allsopp 1992).

Otholobium seeds were collected from adult plants at the fynbos biome intensive study site, Pella ($33^{o}31'$ S, $18^{o}32'$ E), and seeds of *Aspalathus* were obtained from the Rooibos Tea Control Board, Clanwilliam, South Africa. *Otholobium* seeds were immersed in boiling water which was allowed to cool, drained off and the seeds were incubated for 12 h at 4^{o} C. *Aspalathus* seeds were acid scarified for 1 hour (Engelbrecht, Smit & Knox-Davies 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 phosphorus and nitrogen (Mitchell *et al.* 1984, Stock & 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 & 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 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. *Aspalathus* and *Otholobium* 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%, day length 16 h, day/night temperatures 10 ^{o}C/25 ^{o}C, PAR 320 μmol m^{-2} s^{-1}) and watered three times a week with distilled water.

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 *Otholobium* seedlings were successfully separated and weighed but those of *Aspalathus* 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 *Aspalathus*. Nodules were counted and a subsample of roots from each pot cleared and stained to determine VAM infection (Phillips & Hayman 1977). Total phosphorus and nitrogen of the pooled plant material from each pot was determined by the molybdenum-blue method for phosphorus (Murphy & Riley 1962) after digestion in a tri-acid mix (Jackson 1958) and nitrogen was determined colorimetrically (Smith 1980) following Kjeldahl digestion using a selenium catalyst.

Relative growth rates (RGR) for the period 0 to 30 days and 31 to 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 VAM and 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 coefficient of variation (CV) of plant growth in each pot was calculated using the mass of individual shoots of *Otholobium* and *Aspalathus*. 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 square root transformed before analysis (Zar 1984).

Results

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

Absence of mycorrhizas and increases in density reduced plant mass of *Otholobium* but differences in size between VAM and NM *Otholobium* plants diminished with increasing plant density (Table 8.1, Fig. 8.1a). *Aspalathus* plants were also smaller at higher densities, and VAM plants were smaller than NM plants at all densities (Table 8.2, Fig. 8.1b). Plant mass of singly grown VAM *Aspalathus* was similar to that obtained by naturally infected *Aspalathus* plants (0.209 ± 0.033 g) grown in unsterile field soil and therefore the lack of growth response to mycorrhizas cannot be ascribed to incompatibility 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 (Figs. 8.1a & b).

VAM *Otholobium* plants were taller and had more leaves than NM plants while NM and VAM *Aspalathus* plants did not differ in these parameters (Tables 8.1 & 8.2). Increasing density resulted in shorter plants with fewer leaves. Leaves of VAM *Aspalathus* plants were shorter than those of NM plants and leaf length decreased with increasing density (Table 8.2). VAM infection of the roots was not affected by plant density for either species (Tables 8.1 & 8.2). Nodulation was highest for singly grown VAM *Otholobium* plants, absent in the single NM *Otholobium* plants, with differences between NM and VAM plants decreasing with increasing density (Table 8.1). NM *Aspalathus* plants had more nodules than the VAM plants, and nodulation decreased with increasing density (Table 8.2). Number of root clusters were the

same for VAM and NM *Aspalathus* plants but decreased with increasing density (Table 8.2). Root:shoot ratios were lower for VAM *Otholobium* plants but were unaffected by density (Table 8.1). Neither mycorrhizal or density treatments affected *Aspalathus* root:shoot ratios (Table 8.2).

The shoot mass coefficient of variations of VAM *Otholobium* plants were higher than NM plants and VAM *Otholobium* shoot mass coefficient of variations increased from density one to density four but were unaffected by higher densities (Table 8.1). NM *Otholobium* coefficient of variations increased with increasing density. Coefficient of variations of VAM and NM *Aspalathus* shoots were similar and increased with increasing density (Table 8.2).

By day 30 mycorrhizas, but not density, were affecting RGRs of *Otholobium* (Table 8.1). However increasing density was depressing *Aspalathus* RGRs at this stage although mycorrhizas had no influence (Table 8.2). RGRs for the period 31 to 120 days decreased for both species with increasing density but mycorrhizas generally increased RGRs of *Otholobium* and decreased those of *Aspalathus* (Table 8.1 & 8.2).

The total phosphorus accumulated by all the plants in a pot increased with increasing density but most of this was accounted for by the phosphorus available in the seeds (Table 8.3). Similar amounts of soil phosphorus were accumulated at all densities except by NM *Otholobium* plants which acquired very low amounts of soil phosphorus (Table 8.3).

Phosphorus concentrations were higher in VAM plants. A significant interaction ($p < 0.001$) of mycorrhizal and density treatments is seen in the decrease of phosphorus concentrations of VAM *Otholobium* plants with increasing density, with the opposite trend in NM *Otholobium* (Table 8.1). Phosphorus concentration of VAM *Aspalathus* plants were higher than NM plants and this difference increased at higher densities (Table 8.2). Phosphorus content of all *Aspalathus* (Table 8.2) and VAM *Otholobium* plants decreased with increasing density but phosphorus content of NM *Otholobium* plants remained constant at all densities (Table 8.1). VAM *Otholobium* plants had accumulated almost 14 times more phosphorus than NM plants at the lowest density but this dropped to 1.6 times at the highest density (Table 8.1). Differences

between VAM and NM *Aspalathus* phosphorus content were slight and decreased with increasing density (Table 8.2).

Nitrogen concentration of VAM *Otholobium* was higher than NM plants but did not change much with density (Table 8.1). Nitrogen concentration in *Aspalathus* was unaffected by mycorrhizas but decreased with increasing density (Table 8.2). Both increasing density and absence of VAM lowered nitrogen content of *Otholobium* plants but increasing density alone lowered the nitrogen content of *Aspalathus* plants (Tables 8.1 & 8.2).

TABLE 8.1. Mean ±1 standard error of plant parameters of *Otholobium hirtum* plants grown at densities of 1, 4, 8 and 16 plants per pot with (VAM) or without (NM) vesicular-arbuscular mycorrhizal inoculum. Significance values as determined by two-way analysis of variance: NS=not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. CV=Coefficient of variation.

	TREATMENT								ANOVA		
	NM 1	NM 4	NM 8	NM 16	VAM 1	VAM 4	VAM 8	VAM 16	MYC	DEN	MxD
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	***	**	***
R:S 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	***	***	***

TABLE 8.2. Mean ±1 standard error of plant parameters of *Aspalathus linearis* plants grown at densities of 1, 4, 8 and 16 plants per pot with (VAM) or without (NM) vesicular-arbuscular mycorrhizal inoculum. Significance values as determined by two-way analysis of variance: NS=not significant, * $p<0.05$, ** $p<0.01$ *** $p<0.001$. CV=Coefficient of variation.

	TREATMENT								ANOVA		
	NM 1	NM 4	NM 8	NM 16	VAM 1	VAM 4	VAM 8	VAM 16	MYC	DEN	MxD
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
R:S 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 ±0.0	36.6 ±8.7	55.8 ±7.2	57.5 ±2.6	30.5 ±0.0	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 ($\mu g\ g^{-1}$)	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

TABLE 8.3. Sources of phosphorus in *Otholobium hirtum* and *Aspalathus linearis* grown at different densities (1, 4, 8 and 16 plants per pot) in a low phosphorus soil with (VAM) and without (NM) vesicular-arbuscular mycorrhizal inoculum. Mean ±1 standard error, percentage values following mean in square brackets.

	Total P accumulated in plants ($\mu g\ pot^{-1}$)		P from seeds ($\mu g\ pot^{-1}$)	P from soil ($\mu g\ pot^{-1}$)	
	NM	VAM		NM	VAM
O. hirtum					
1	19 ±1	267 ±13	16 ±1	3 [15%]	251 [94%]
4	75 ±4	299 ±11	65 ±5	10 [14%]	234 [78%]
8	141 ±5	357 ±14	130 ±10	11 [8%]	227 [63%]
16	302 ±12	500 ±13	260 ±21	41 [14%]	239 [48%]
A. linearis					
1	239 ±23	269 ±22	55 ±5	184 [77%]	214 [79%]
4	413 ±18	448 ±26	221 ±22	191 [46%]	227 [51%]
8	656 ±38	667 ±31	442 ±44	213 [33%]	225 [34%]
16	1164 ±56	1141 ±34	884 ±88	279 [24%]	256 [22%]

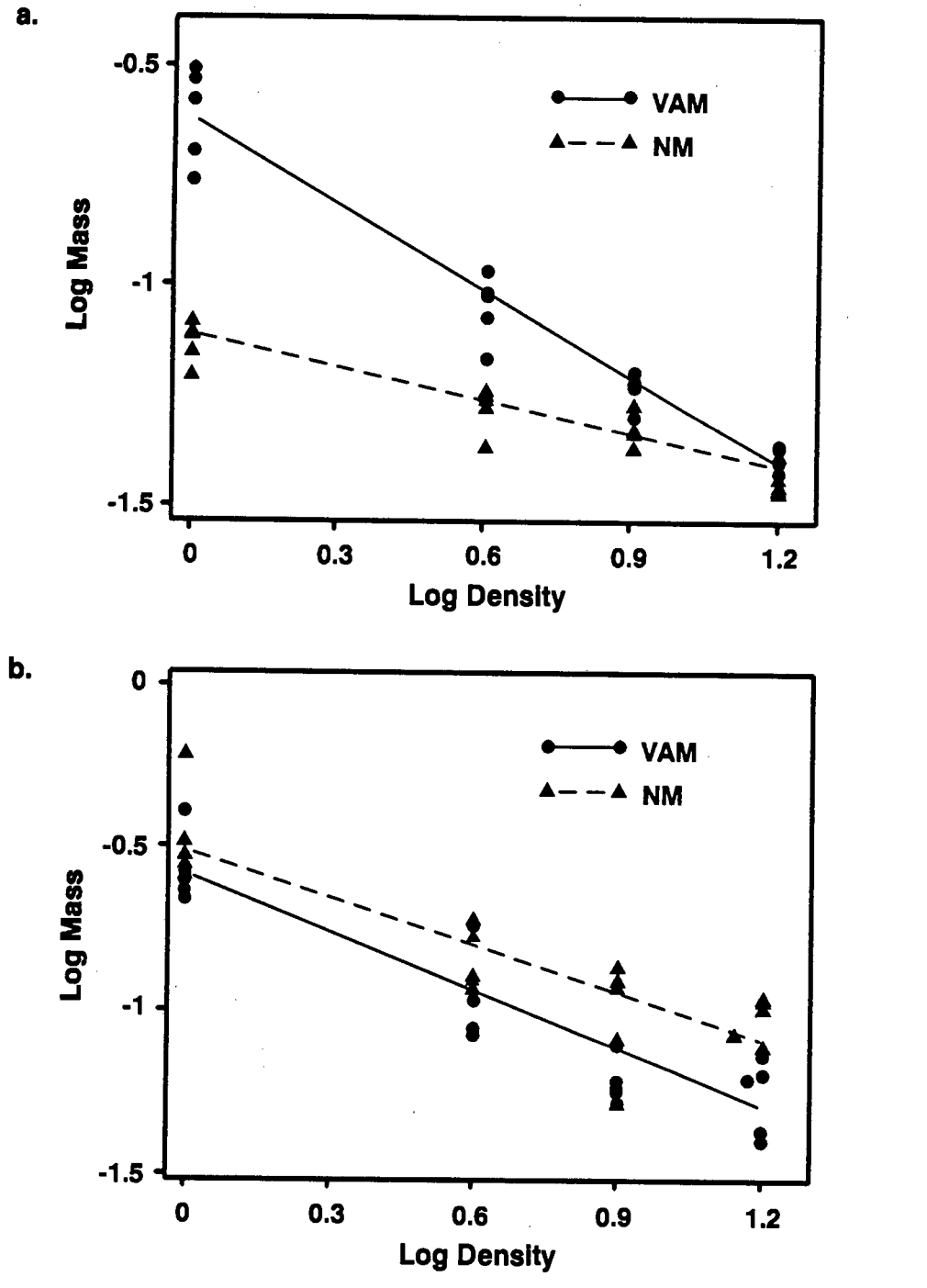

FIGURE 8.1. Effect of final plant density on mean plant mass when (a.) *Otholobium hirtum* and, (b.) *Aspalathus linearis* are grown with (VAM) or without (NM) vesicular-arbuscular mycorrhizal inoculum. Slopes of VAM and NM plants are significantly different ($p < 0.05$).

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 phosphorus, are possibly the most important limiting resource in these experiments. Apparently *Aspalathus* and VAM *Otholobium* plants at the lowest density had taken up almost all available phosphorus and phosphorus depletion occurred at a lower density of plants than in another experiment of similar design (Koide 1991b). Nitrogen acquired per pot from non-seed sources was greater at higher densities and nitrogen fixation may be contributing to the plants' nitrogen nutrition.

The effect of mycorrhizas on plant growth is often attributed to the improved phosphorus nutrition of VAM plants (Harley & Smith 1983). Increased phosphorus uptake will usually result in elevated growth when other factors affecting growth are not limiting. The greater growth of VAM *Otholobium* is accompanied by much greater phosphorus influx into VAM plants and VAM plants were able to acquire soil phosphorus even at high densities of plants. On the other hand the total phosphorus content of the NM *Otholobium* plants is almost entirely derived from the seed (cf. Chapter 5). It appears therefore that the concentration of soil phosphorus must be below the threshold (Föhse *et al.* 1988) that NM roots of *Otholobium* can exploit. In this soil, phosphorus uptake by *Otholobium* is mediated by the mycorrhizal fungus and *Otholobium* 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 *Otholobium*, the effect of being NM is detrimental at all densities in this low nutrient soil. The mycorrhizal influence on phosphorus uptake in *Otholobium* decreases in proportion to the decrease in available phosphorus per plant as density increases and is not related to changes in intensity of VAM infection with increasing density. The decrease in mycorrhizal mediated phosphorus 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 *Aspalathus* plants acquired very similar quantities of P as NM plants but had higher tissue phosphorus concentrations due to smaller plant mass. Both VAM and NM

Aspalathus 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 (Lamont 1982). The cluster roots of *Aspalathus* and the VAM fungus have similar efficiencies in acquiring soil phosphorus. *Aspalathus* can be regarded as a species which has little or no mycorrhizal dependency, at least under the experimental conditions.

As the VAM fungi were not able to confer any nutrient uptake advantage to the *Aspalathus* plants, under these growing conditions, there is 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 1985a) or inadequate light (Smith & Gianinazzi-Pearson 1990).

The 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 *versus* log density which are steeper for the VAM plants (Figs. 8.1a & 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 has risen. At higher densities, carbon input is often lower because shading effects and the lower nutrient status of the plants decreases their photosynthetic efficiency (Field & 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 a major occurrence 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 & Antonovics 1986). Shaw & 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 coefficient of variations are more targeted for extinction than ones with higher coefficient of variations despite similar average growth. 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 coefficient of variations of the denser VAM *Otholobium* plants and all *Aspalathus* 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 & 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 highlight areas in population development where mycorrhizas may be of significance, and 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

CHAPTER 9

General Discussion

In this chapter the ecological significance of mycorrhizas in the Cape Floristic Region is evaluated on the basis of evidence provided by field studies on VAM occurrence, and laboratory based experiments to determine the effects of VA mycorrhizas on various aspects of growth of sclerophyllous and evergreen shrubs. Attention is focussed on three lowland vegetation types which are threatened by human activities and alien plant invasion.

Members of all the important families and the twenty largest genera in the Cape Floristic Region have now been examined for mycorrhizas (Chapter 2). VAM plant species dominate, and non-mycorrhizal species make up a substantial proportion of the flora. Unlike other mediterranean ecosystems (Puppi & Tartaglini 1991), including those on very nutrient poor soils in Australia (Brundrett & Abbott 1991), there is no ectomycorrhizal component to the vegetation. The reasons for this are not clear, but extremely low nutrient conditions and frequent fires may be partial explanations. This divergence in the occurrence of ectomycorrhizal species needs to be examined, not only to determine why they are absent in the S. W. Cape, but also to elucidate what allows them to be prevalent in a climatically and edaphically similar environment in western Australia. Ericoid mycorrhizal species are also common, and in certain mountain fynbos communities members of the Ericaceae dominate the vegetation (Campbell 1985).

Superficially, the mycorrhizal composition of the three lowland vegetation types are similar (Chapter 2). However, the taxonomic and life form composition of the non-mycorrhizal portion differs between the vegetation types, as do the levels of mycorrhizal infection among VAM plants (Chapter 3). Non-mycorrhizal species are insignificant in renosterveld and VAM infection levels are high among most plants, including annual life forms. In the high phosphorus strandveld soils, the non-mycorrhizal species are typically members of the Mesembryanthemaceae, Crassulaceae and Zygophyllaceae, and levels of infection among VAM species are low. Infection levels are slightly higher among VAM species in low phosphorus fynbos soils, however, non-mycorrhizal species from the Proteaceae and Restionaceae dominate the vegetation. These non-mycorrhizal species are atypical in that they are slow growing and long-lived. Members of the Mesembryanthemaceae, Crassulaceae and Zygophyllaceae, and similar non-mycorrhizal taxa, are present in fynbos,

but are typical of disturbed or extreme (e.g. cracks in rocks) habitats. Evolutionary pressures that led to the loss of the mycotrophic state are probably very different for these two groups of non-mycorrhizal plants.

Low levels of plant available phosphorus in the renosterveld soils of intermediate total phosphorus status indicate that competition among plants and micro-organisms for this nutrient is probably high in this community. In this ecosystem, VAM plants are favoured and VA mycorrhizas may ensure a tight phosphorus cycle (Newman 1988, Pankow *et al.* 1991). Renosterveld community dynamics are poorly understood and most of the possible renosterveld areas have been under cultivation or severely disturbed for several centuries (Rebelo 1992). Potential reconstruction and conservation efforts in this ecosystem should recognize the highly mycotrophic nature of the natural vegetation, as mycorrhizal infectivity may be reduced in transformed landscapes by cultivation and fertilization practices (Kruckelmann 1975, Black & Tinker 1979, Thompson 1987b).

VAM species are not favoured by the high phosphorus soils of strandveld, where nutrient cycling, particularly of phosphorus, is not tight (Witkowski 1991), and ruderal species without mycorrhizas are successful. VA mycorrhizas are found among strandveld thicket forming species, even though their reliance on them for nutrient acquisition may be slight as indicated by low levels of infection.

In fynbos, strong competition among plants to acquire phosphorus may be expected. High carbon:nutrient ratios in the litter produced by fynbos result in very slow rates of decomposition, and nutrient release from above-ground phytomass is facilitated by fire (Mitchell *et al.* 1986). Ruderals with very low mycorrhizal dependence take advantage of the post-fire nutrient flush in this community (Brown & Mitchell 1986, Stock & Lewis 1986) although geophytes and grasses may dominate in other young post-fire fynbos communities (Kruger 1983). The ephemerals are soon replaced by shrubby or reed-like species. The former possess VA mycorrhizas, ericoid mycorrhizas or have non-mycorrhizal roots while the latter, mainly members of the Restionaceae, are non-mycorrhizal (Chapter 2).

Root modifications associated with the non-mycorrhizal Proteaceae and Restionaceae seem particularly effective at acquiring phosphorus. Proteoid roots of members of the Proteaceae acidify their rhizosphere soil with organic acids, resulting in the release of plant available phosphorus from aluminium and iron phosphates (Marschner 1991). Proteoid roots are usually found growing in association with organic matter, where their greatly increased surface area facilitates nutrient uptake (Lamont, Brown & Mitchell 1984). Similar cluster roots have been developed by members of the Fabaceae in South Africa (Chapters 2 & 5) and Australia (Brundrett & Abbott 1991). In the South African genus *Aspalathus*, the results of pot experiments indicate that their cluster roots are as effective as mycorrhizas for phosphorus uptake (Chapters 5 & 8). Whilst VAM hyphae may also proliferate in patches of high organic matter (St John *et al.* 1983), their ability to acquire phosphorus, other than in a soluble form, is limited (Smith & Gianinazzi-Pearson 1988, Bolan 1991). In contrast, ericoid mycorrhizas take up nitrogen in the form of amino acids and produce extracellular phosphatases and proteases, enabling them to utilise phosphorus and nitrogen respectively from organic matter (Stribley & Read 1980, Straker & Mitchell 1986). In the very low nutrient environments of fynbos, niche differentiation in terms of utilizing different soil nutrient resources may promote co-existence between VA mycorrhizal, non-mycorrhizal and ericoid mycorrhizal species. However, the VAM symbiosis may be at a competitive disadvantage to other nutrient acquiring strategies, which may short-circuit the decomposition process; and hence members of the non-mycorrhizal Proteaceae and Restionaceae and ericoid mycorrhizal Ericaceae or Epacridaceae dominate the low nutrient environments of South African fynbos (Campbell 1985) and west Australian kwongan (George, Hopkins & Marchant 1979).

Differences in the types of mycorrhizal and non-mycorrhizal plants making up three lowland vegetation types, and in the levels of mycorrhizal infection in plants, indicate that fluxes of nutrients and energy through the systems will differ. The nutrient requirements of different plants will be satisfied by uptake from different nutrient compartments in the soil depending on their root modifications for nutrient capture, and their carbon budgets will be influenced by the costs of maintaining mutualisms or root morphological modifications. Thus the

potential for mycorrhizas to regulate community processes in these three structurally similar shrublands will differ.

Despite the dominance in terms of biomass or cover of members of the Proteaceae, Restionaceae or Ericaceae in both lowland and mountain fynbos, the majority of species growing in these communities are potentially VAM (Chapter 3). From the results of greenhouse experiments (Chapters 4 & 5), most of these species appear to be obligately dependent on mycorrhizas for phosphorus acquisition and establishment under low soil phosphorus conditions. Among VAM shrubs in fynbos, the possibility that VA mycorrhizas may promote or maintain species diversity should be explored further. High species diversity has long been associated with low nutrient soils, but the mechanics promoting this are not well understood. It has been suggested that hyphal links between VA mycorrhizal plants increase species diversity because carbon and nutrients are shared among plants, with larger plants acting as source and smaller plants as sinks (Grime *et al.* 1987). Evidence against this as a mechanism promoting co-existence is that variation of plant size of seedlings grown at high densities in pots did not decrease when they were mycorrhizal (Chapter 8), so resource sharing along the hyphal network seems unlikely. Other pot experiments with VA mycorrhizas concur with this result as larger plants usually suppress smaller ones of the same species (Ocampo 1986, Eissenstat & Newman 1990), and particular species are favoured when plants are grown in species mixes (Fitter 1977, Hall 1978, Allen & Allen 1984). A strategy of sharing resources would result in a more even sized population, which may be more prone to extinction than one in which individuals competed with each other for resources (Shaw & Antonovics 1986). Hence, individuals in mycorrhizal plant populations may have a higher chance of survival if reallocation of resources from source to sink plants is not an important feature. Both the effects of interactions between mycorrhizas and density on population dynamics, and the transfer of resources along mycelial networks, must be investigated in the field.

Patchiness in plant species distribution is a recognized feature of species-rich communities (Grubb 1986), and mycorrhizal inoculum distribution may explain some of these patterns. Levels of infection among plants in fynbos were highly variable, indicating that the hyphal

network is by no means densely established in fynbos soils, and plant roots may fail to encounter inoculum while susceptible to infection (Chapter 3). This occurs after fire, when recruitment of plants from seeds and resprouting are important in determining subsequent community development in fynbos. VAM infection is heterogeneously distributed (Chapter 3), possibly as a result of a mosaic of VAM and non-mycorrhizal species dominating localized areas for several years. Irregularity of mycorrhizal inoculum distribution in the landscape will have a number of consequences in terms of plant establishment, vegetation composition, species diversity and plant interactions. In fynbos, community composition and development may be influenced by which species are able to establish themselves from seeds in particular VAM rich or poor patches following fire.

Patchiness of VAM inoculum in the soil may influence seed size in fynbos. Low nutrient soils favour the production of large seeds in order to ensure establishment (Fenner 1985, Stock *et al.* 1990) but large-seeded species may be poorly disseminated, and therefore face extinction from localized pressures. Seedlings of many VAM species have an obligate requirement for mycorrhizas in order to obtain phosphorus from low nutrient soils in pot culture (Chapter 5) and will fail to establish if they germinate in areas with low inoculum potential. Widespread dispersal should increase the chances of encountering inoculum and hence may favour the production of smaller seeds among VAM species in the fynbos environment.

The size attained by non-mycorrhizal seedlings of VAM plant species is closely correlated with seed reserves, and mycorrhizal responses are highest among smaller seeded fynbos shrub species grown from seed in pot culture (Chapter 5). As seed size diminishes, the ability of seedlings to monopolize space may be increasingly dependent on mycorrhizal infection. Large-seeded species may be able to establish a competitive presence in patches without becoming mycorrhizal, whereas smaller seeded species would have to become mycorrhizal rapidly, in order to be competitive. Eventually VAM infection of the seedlings from large seeds is necessary for their continued growth. Seed size among woody species in a low nutrient environment represents a trade-off between dispersability and provisioning of seedlings, with no single seed size conferring an absolute advantage to VAM plant

species. It is postulated that the adoption of the non-mycorrhizal condition in the Proteaceae was dependent on the parallel development of cluster roots and large seeds in this taxon.

Pot culture has proved a valuable method for establishing the effects of mycorrhizas on plant growth and physiology, but results should be interpreted with care concerning their ecological significance to species in their natural environment. Mycorrhizal growth responses of seedlings of slow growing, woody, sclerophyllous species are of the same order of magnitude as those of faster growing plants (Chapters 4 & 5), refuting suggestions that low nutrient requiring species may not respond to mycorrhizas (St John & Coleman 1983, Koide 1991a). As with faster growing plants, the main effect of VA mycorrhizas is enhanced phosphorus nutrition of the seedlings and mycorrhizas appear essential for their establishment in the low nutrient soils of fynbos. However, as growth rates are much slower among these wild plants, the effects of mycorrhizas on growth are much slower to manifest themselves (Chapters 4 & 6). Unlike the growth of more plastic plants, mycorrhizal plants do not respond to additional phosphorus amendments with increased growth rates; rather tissue phosphorus concentrations increase (Chapter 4). Low and inflexible maximum relative growth rates, and luxury consumption are characteristic of woody, sclerophyllous plants (Grime & Hunt 1975, Chapin 1980). Uptake of nutrients when transiently available in the soil is a key feature contributing to these plants' survival in natural environments (Chapin *et al.* 1990) and is facilitated by VA mycorrhizas.

The absence of infection among potentially mycorrhizal geophytes and perennials in the field (Chapter 3) may give the impression that these plants are facultatively mycorrhizal in fynbos (Allen *et al.* 1987). This interpretation fails to appreciate that in many natural habitats high vegetative productivity is only encountered in the early stages of vegetation re-establishment following disturbance (Pankow *et al.* 1991). Among older plants the ability to store and recycle nutrients is high, while growth rates are very low (Pate & Dixon 1982, Chapin 1988). Nevertheless reproduction may claim a high proportion of a plant's annual phosphate uptake (Kuo *et al.* 1982, Witkowski 1990). In the seasonal environment of most of the Cape Floristic Region, plants often have to re-establish mycorrhizal roots every winter. Failure to become mycorrhizal at any one time may not severely compromise their

survival, but may account for fluctuations in reproductive output. Thus mycorrhizas can influence post-fire community composition through their effect on the nutrition of the previous generation.

To a limited extent, field levels of infection reflect the performance of plant species in pot culture (Chapters 3 & 5). For example, members of the Thymelaeaceae, Polygalaceae and Rhamnaceae had high levels of infection in the field and high mycorrhizal responses in pot culture. Members of the Fabaceae had very variable levels of mycorrhizal response, which mirror field levels of infection; *Aspalathus* spp. often had low infection in fynbos, and were unresponsive to mycorrhizal infection in pot culture, while the reverse was found for *Otholobium hirtum*. Great variability in response to VA mycorrhizas is a feature of the Fabaceae (Crush 1974, Lioi & Giovanetti 1987, Saif 1987), and some members of this family are ectomycorrhizal or non-mycorrhizal (Högberg & Piearce 1986, Brundrett & Abbott 1991).

The influence of mycorrhizas on biomass partitioning is seldom addressed explicitly although changes in allocation patterns may affect plant growth and ecology (Miller *et al.* 1987, Körner 1991). Allocation patterns among the seedlings responded to the mycorrhizal assisted alleviation of phosphorus deficiency by increasing biomass and phosphorus partitioning to shoots (Chapter 6), supporting predictions based on the concept of balanced growth (Wilson 1988, Hilbert 1990). As mycorrhizal fungi impose their own carbon costs on plants (Harley 1989, Fitter 1991), this may be expected to confound patterns of carbon allocation, and, under extremely low nutrient conditions, the shift in growth to root production, plus the carbon demand of the mycorrhizas resulted in reduced shoot growth of mycorrhizal *Phylica cephalantha* seedlings (Chapter 7). However, plants with better nutrition are in a position to fix carbon in excess of growth requirements and this is then available for the VAM fungus (Baas 1989). Under well watered conditions, shoot growth is enhanced in mycorrhizal plants, and differences in biomass allocation to roots and shoots among the species can be used to predict the relative growth rates of older seedlings (Chapter 6). It is concluded that increased growth in response to mycorrhizas is a function of increased biomass allocation to photosynthetic tissue. However, the results of a later

experiment on the effects of mycorrhizas on the growth of one of these species when subjected to cyclical drying appears to contradict this conclusion (Chapter 7). Under cyclical drying growing conditions, increased biomass was aportioned to the roots of mycorrhizal plants as water availability decreased. Nonetheless, leaf conductances were higher among mycorrhizal plants, indicating that their potential for carbon fixation was increased. Growth of these species in response to mycorrhizas is therefore enhanced by a combination of increased allocation to photosynthetic tissue (Baas *et al.* 1989) and an increased carbon fixing capacity of the photosynthetic tissue (Allen *et al.* 1981, Smith & Gianinazzi 1988).

Allocation patterns among slow growing species are assumed to be genetically fixed in order to avoid responses to temporary changes in environmental conditions which may be inappropriate for normal functioning (Bloom *et al.* 1985). However, mycorrhizal enhancement of phosphorus nutrition of slow growing species may permit them to alter their allocation patterns during the seedling stage. Plasticity in allocation at the seedling stage may permit adjustments in growth that enhance survival in their immediate neighbourhood. Among mycorrhizal dependent species such adjustments are facilitated by mycorrhizas and delays, in becoming mycorrhizal in the field, may reduce their ability to adapt to their environment.

In pot culture, the cost of maintaining the symbiosis increases relative to the benefits, irrespective of the mycorrhizal dependency of the plant species, when grown at higher densities (Chapter 8, Allsopp & Stock in press a). Among obligate mycorrhizal species, mycorrhizal benefit is reduced in dense populations. The effect of mycorrhizas on population dynamics depends upon which part of the host's life cycle is affected and what effect this has on interactions with other plants (Addicott 1986). Mycorrhizal influences on competition and co-existence may only be solved by examining the effect of mycorrhizas on interactions between plants in the field although practical considerations of manipulating mycorrhizas in plant communities are formidable. Even in annual or herbaceous

communities where effects may appear quite rapidly, the results of removing VAM fungi have been equivocal (Fitter 1986, Koide *et al.* 1988, Gange, Brown & Farmer 1991), partly because a VAM specific fungicide has not been discovered. In shrublands the microcosm approach of Grime *et al.* (1987) would only be suitable for examining interactions among very young plants. An alternative approach has been to manipulate mycorrhizas while reconstructing highly disturbed ecosystems (Miller 1987, Allen 1988b). Opportunities for such studies should increase in South Africa if mining companies and other developers are required to reestablish natural vegetation. In the Cape Floristic Region, marginal farmlands, dam construction sites, coastal housing developments and areas cleared of alien vegetation may all require the reconstruction of the below ground biota to ensure successful revegetation of the indigenous biota.

The integration of the various levels of the study of the influences of mycorrhizas on growth of sclerophyllous seedlings and their occurrence in the field has proved valuable in explaining aspects of reproductive biology, vegetation patterns and plant community functioning in different natural shrublands in the Cape Floristic Region. This information facilitates a greater functional understanding of community processes in these shrublands. In order to improve management and conservation of this highly distinctive floral region, further attention concerning the impacts of various disturbances on community processes is required, in order to develop predictive models. Future mycorrhizal studies should concentrate on developing a greater understanding of the ecophysiology of mycorrhizal plants and of mycorrhizal mediated processes in the field. Obtaining a clearer picture of the dynamics and infectivity of mycorrhizal mycelium in time and space in the soil is a priority, as is a quantification of the severity and frequency of ecosystem disturbances on below ground processes. Population level studies on the effects of mycorrhizas on individual plant species should be combined with process functional studies in order to develop a greater understanding of competition and co-existence in communities. Autecological mycorrhizal studies may contribute to the preservation of endangered plants and promote the successful cultivation of wild flowers. Currently the wild flower industry is heavily reliant on exploiting wild populations, although the sustainability of this resource under present

management techniques is doubtful. Progress towards a holistic understanding of ecosystems requires a careful evaluation of the significance of mycorrhizas at many levels of scientific endeavour in biological sciences.

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