The role of mycorrhizal symbiosis in plant intraspecific competition and population structure

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Abstract

The role of mycorrhizal symbiosis in plant intraspecific competition and population structure

The overall objective of this project was to investigate the effects of the symbiotic association of plants with vesicular-arbuscular mycorrhizal fungi on the intensity of intraspecific competition and its consequences on population structure.

I performed four main glasshouse experiments using a non-cultivated species, *Rhodanthe chlorocephala* ssp. *rosea*, or a cultivated species, *Trifolium subterraneum*. I grew the plants at different plant densities, under different levels of resources (phosphorus and/or light), in environments with homogeneous and/or patchy distribution of phosphorus (P).

In pots with homogeneous distribution of P, the addition of P to *R. chlorocephala* and mycorrhizal infection in *T. subterraneum* increased plant biomass of single plants. However, these beneficial effects were reduced by increasing plant density. Shading of plants of *T. subterraneum* did not generally alter these effects. Mycorrhizal symbiosis and the addition of P always increased the intensity of plant intraspecific competition.

In trays with patchy or homogeneous distribution of P, mycorrhizal infection and patchy distribution of P increased the total biomass and size inequality of populations of plants of *T. subterraneum*. Individual biomass was determined by the local soil P concentration in patchy environments and by mycorrhizal infection in low density treatments. Mycorrhizal infection, but not patchy P distribution, increased relative competition intensity.

My results emphasise that the main effects of mycorrhizas at the individual level cannot be expected to be apparent at the population level, because of the influence of density-dependent processes. However, infected individuals with a strong response to the symbiosis would have an advantage in situations of competition. This scenario can explain the maintenance of the symbiotic ability even under conditions such as dense populations, where there is no obvious advantage of the symbiosis at the population level.

Declaration

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

I consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying.

November 1998 *Signed*

Evelina Facelli

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Chapter 1

General introduction

1.1. Introduction

Plant competition is the interaction between plants with a shared requirement for an essential resource in limited supply, that results in the reduction in fitness of one or more of them. Its intensity (reduction of individual performance) increases, within certain limits, as the number of interacting individuals increases (Begon *et al.* 1990; Silvertown and Lovett Doust 1993). Competition affects individual growth (Harper 1977), the structure and dynamics of the populations (Weiner 1990; Watkinson 1997) and contributes to the structuring of communities (Grace 1995a). The relative limitation in availability of different resources (light, nutrients, water) (Wilson 1988b) and their spatial and temporal distribution determines the outcome of the competition and its role as a regulator of population and community structure (Fowler 1988; Weiner 1990; Wedin and Tilman 1993). The outcome of competition is also modified by the interactions of plants with other organisms such as herbivores, pathogens and symbionts (Silvertown and Lovett Doust 1993). Reciprocally, competition alters the outcome of those interactions. This may result in complex networks of interactions which include indirect effects and high order interactions (Strauss 1991). The study of combinations of interactions is just beginning, and is producing important advances in our understanding of ecological systems (Gange and Brown 1997). The effect of the symbiotic association of plants with vesicular-arbuscular (VA) mycorrhizal fungi on the outcome of intraspecific competition of cultivated and non-cultivated plants is the subject of the present project.

1.2. Background

1.2.a. Resources and plant competition

As the density of a population increases the utilisation of resources in limiting supply increases and plants begin to compete for those resources, which results in plastic

responses. The birth rate and the death rate of plants parts vary (Harper 1977). For instance, as light becomes limiting, shaded leaves translocate N to new fast growing leaves which have a higher rate of photosynthesis, shaded leaves become senescent, but the whole plant rate of photosynthesis is maintained or even increased (Mooney and Chiarello 1984). Another plastic response to density is the change in allocation of photoassimilates to roots or shoots depending upon the availability of light, water, and/or nutrients (Ericsson 1995). Nevertheless, as the availability of limiting resources declines the size of individual plants decreases. This reduction in the size of the individuals is of ecological significance because the size of a plant is correlated with its probability of survival, the frequency and probability of flowering and the amount and quality of the seed produced (Solbrig 1981; Sarukhan *et al.* 1984; Weiner and Thomas 1986; Klinkhamer *et al.* 1987; Weiner 1988a; Hartnett 1990; Weiner 1995; cf. Aarssen and Taylor 1992). Genetic characteristics such as susceptibility to pathogens and herbivores, which indirectly affect reproductive output, are also size dependent (Weiner 1988b; Begon *et al.* 1990; Crawley 1997b). Small plants might not overcome the losses of biomass produced by herbivory (Thomas and Weiner 1989; Crawley 1997b), whereas bigger plants are more likely to survive and are some times preferred by herbivores (Crawley and Weiner 1991).

Although the outcome of competition is a reduction of reproductive output, this does not always mean a decrease in the relative contribution to the next generation of all the competitors. In extreme cases, small plants can be suppressed by bigger, stronger competitors. The suppressed ones will not make any contribution to the next generation whereas the relative contribution of the dominant plants may remain unchanged or even increase (Begon *et al.* 1990).

Because of its direct effect on plant size, competition for limiting resources modifies the way the total biomass of a population is distributed between the individuals (Turner and Rabinowitz 1983; Weiner and Thomas 1986). Even in equal-aged populations plants vary in their size and relative growth rates. They have uneven distributions of biomass or size hierarchies, which might be due merely to genetic variation (Bonan 1991). However, the

asymmetric or symmetric distribution of resources between plants will change these size hierarchies. The distinction between these two types of distributions has lead to two different models explaining the interaction between competition and size inequality (degree to which the biomass is concentrated within a small fraction of the population (Weiner and Thomas 1986)): the resource depletion and resource pre-emption models (Weiner and Thomas 1986; Weiner 1988b). In the first model (resource depletion) competition reduces the relative growth rate of all the individuals by the same proportion, reduces variance of growth rates and reduces variation in sizes. Thus, in this model resource acquisition is proportional to plant size (Weiner 1990). This model is also called symmetric or two-sided competition and applies when competition for nutrients predominates. It predicts that at high density, plants will be smaller but the population will have less inequality than at low density (Weiner and Thomas 1986). In the second model (resource pre-emption), competition increases the variation in relative growth rates and increases variation in sizes. Large plants obtain a more than proportional share of the resources (relative to sizes) (Weiner 1990) and this increases their competitive ability which results in a positive feedback on plant size. This phenomenon is also called snowball cumulation, asymmetric or one-sided competition and it was observed only when competition for light was predominant (Wilson 1988a). This second model predicts that at high density plant populations will have more inequality than at low density (Weiner and Thomas 1986).

Although these two models are generally accepted, alternative analyses and recent experiments show that the degree of asymmetry of the interaction depends on the spatial and temporal distribution of the resource , the spatial distribution of the individuals in the population, neighbourhood competition and the mobility of the resource (Huston 1986; Miller and Weiner 1989; Weiner 1990; Bonan 1991). Weiner (1990) suggested that if nutrients are distributed homogeneously and the uptake is proportional to root size, the competitive interaction will be more symmetric, whereas if patches with more nutrients can be reached by large individuals, asymmetric competition will predominate. This hypothesis has not been tested yet. Turner and Rabinowitz (1983) found that populations with an initial random spatial distribution of individuals had an unexpected increase in size inequality

at decreasing density. Huston (1986) interpreted this as a consequence of variation in the exponential growth rates produced by the random variation in the number of neighbours despite the symmetric competition for nutrients. However, Bonan (1991) demonstrated that increased size variability at increasing density is directly related to neighbourhood competition and does not depend on the initial spatial distribution of individuals. It is obvious that there is need for further investigations in this field.

The availability of resources not only regulates plant fitness, population structure and growth but it also determines species coexistence under certain conditions of stability (Grace 1995a). Different species may coexist if the population growth rate of each of them is limited by a different resource (Silvertown and Lovett Doust 1993). However, if they are limited by the same resources, but there are changes in the relative abundance of these resources in time and space, the resource ratio hypothesis (Tilman 1982) predicts that those species might also co-occur (Grace 1995a; cf. Hubbell 1979). This hypothesis also requires that each species should be a superior competitor at a particular point along a light-nutrient gradient. The competitive dominant species will be the one that can reduce the concentration of the limiting resource to the lowest level (R* (Tilman 1982)) and still maintain its population. In accordance with this hypothesis it should be possible to predict the outcome of interspecific competition knowing the ability of the interacting species to reduce the concentration of an available soil nutrient in monoculture (Wedin and Tilman 1993). At the moment the R* hypothesis has been tested only once for vascular plants (Tilman 1997). This was done in a series of experiments with only one set of competing plants with one limiting resource (nitrogen) (Tilman and Wedin 1991b; Tilman and Wedin 1991a; Wedin and Tilman 1993). The results indicated that the individual R* could be used to predict the outcome of interspecific competition in that community.

Several indices are used to quantify the intensity of competition (Grace 1995b; Markham and Chanway 1996). Different indexes lead to conflicting conclusions, therefore a comparison between them should carefully consider the terms and operational definitions used in their calculation (Grace 1995b). The relative reduction in biomass or growth rate (relative competition intensity or RCI) is commonly used to estimate competition effects

(Markham and Chanway 1996). Freckleton and Watkinson (1997) criticised its suitability to compare effects of interspecific competition under different conditions and proposed the use of a process-based model as an alternative approach. However, the use of such models was objected to by Markham (1997), who suggests a complementary use of both approaches to establish the net effect of competition and determine the actual processes producing the effect. Nevertheless, the use of RCI to study intraspecific competition seems appropriate, particularly in experimental conditions where plant densities are arbitrarily set.

Resources are not homogeneously distributed in the environments occupied by plants. As a consequence of this heterogeneity , there is high level of variability in probability of survival, growth rates and fecundities, plant density and community composition (Fowler 1988), which in turn will increase resource heterogeneity (Reynolds *et al.* 1997). Environmental heterogeneity (temporal and spatial) might have three main effects: 1) it could hinder the detection of the action of regulating factors, eg. failure in detection of the effect of plant density on plant performance (Fowler 1984), 2) it could promote the coexistence of species because morphological and physiological differences in how species respond to variation in the environment would allow them to coexist despite competing for the same resources (Chesson and Huntly 1989), and 3) it could be a source of random noise in the system and therefore delay competitive exclusion and simultaneously weaken the action of factors that promote coexistence (Fowler 1988).

Information coming from low levels of organisation, such as physiological responses of part of a plant or a whole plant to changes in the environment, is often used to infer fitness or even the outcome of plant interactions (Mooney and Chiarello 1984). Therefore, to understand the effects of spatial heterogeneity in regulating populations, it is necessary first to know the effects of such environments on individual plants (Mooney and Chiarello 1984; Fowler 1988).

In nutrient-enriched soil patches roots proliferate more than in nutrient-poor patches, they produce more biomass through longer roots (Jackson *et al.* 1990) and/or by the formation of new roots (Drew 1975; Gross *et al.* 1993). In rich patches the capacity of roots to take up phosphorus (P) is also higher than in poor patches (Jackson *et al.* 1990). This probably

occurs because of an increase in the activity of P carriers or pumps (Jackson *et al.* 1990); S. Ayling, personal communication]. High concentration of P in patches not only increases root proliferation and uptake capacity but it also increases the relative amount of P available for the plants in the soil solution (Caldwell *et al.* 1992). When the same amount of P that is added to an area is applied in patches instead of homogeneously, the amount of P released to the soil solution in those enriched patches is more than proportional to the amount added. This results in an increase of P uptake independently of any increase in root proliferation or in uptake capacity (Kovar and Barber 1989; Caldwell *et al.* 1992). Single plants growing in pots with a patchy distribution of nutrients had more biomass than plants growing in pots with homogeneous distribution of nutrients, because the amount of P available for the plants in the patchy pots was higher (Cui and Caldwell 1996a). The effects of patchiness on the total biomass of a plant population have still to be investigated. However, it is possible to predict that, depending upon the scale of heterogeneity (Wijesinghe and Hutchings 1997), the total biomass will be higher in patchy soils, providing that the plants have the capacity to fully explore the patches.

As mentioned before, the distribution of nutrients would not only have effects on total biomass, but also on size distribution. If patches with more nutrient can be reached by large individuals, asymmetric competition will occur (higher size inequality) (Weiner 1990). In consequence, for plants growing in competition, a rapid access to nutrients which deprive competitors of these nutrients is of more ecological significance than maximum nutrient uptake or the average nutrient concentration in soil (Campbell *et al.* 1991; Hetrick 1991). Plants may effect this rapid access through changes in root morphology and/or nutrient uptake efficiency. In experiments with single plants these two characteristics were affected by mycorrhizal associations (Hetrick *et al.* 1988; Smith and Read 1997). However, there is a lack of information about the effects of the symbiosis when there is competition.

1.2.b. Resources and mycorrhizal symbiosis

1.2.b.1. Overview of mutualisms involving plants

Mutualisms involving plants range from the common examples of ant-acacia interactions (Janzen 1966) and pollination mutualisms (Begon *et al.* 1990) to the more inconspicuous associations between roots of plants and certain soil microorganisms such as mycorrhizal fungi and nitrogen-fixing associations of *Rhizobium* and legumes (Watkinson 1997).

Despite the widespread occurrence of mutualistic associations, there is little understanding of the effects of the mutualists on density and growth of the host plant populations (Addicott 1986; Law 1989; Watkinson 1997). There is no consensus about which are the proper conceptual frameworks to study these interactions. Furthermore, empirical demonstrations of the existence of an effect on the host plant populations dynamics are very difficult (Watkinson 1997). Removal of ants from acacia trees reduced both survival and growth of the plant (Janzen 1966). Clones of *Danthonia spicata* infected with endophytic fungi had higher survival and growth than uninfected clones when planted in dense swards. Other experiments showed that endophytic fungi increased plant abundance by reducing the probability of grasses being eaten by herbivores or affected by some plant diseases (Clay 1990; Latch 1994; Joost 1995). However, experiments to quantify the effect of pollinators on plant population dynamics of *Cypripedium acaule* and *Lathyrus versus* (pollinated by bumble-bees), in which pollinators were replaced by artificial pollination, gave inconsistent results (Watkinson 1997).

One of the most widespread mutualisms involving plants is that with vesicular-arbuscular mycorrhizal fungi. About 80% of higher plants are able to form vesicular-arbuscular mycorrhizas (VAM) and the association occurs in a wide range of ecosystems (Fitter 1989). Although the effect of VAM on the growth of individual host plants has been intensively studied (Smith and Read 1997), the understanding of their role in populations dynamics is still rudimentary (Law 1989; Allen 1991; Smith and Read 1997).

1.2.b.2. Brief description of the symbiosis

Vesicular-arbuscular mycorrhizas are symbiotic associations of fungi with roots. They increase the phosphorus uptake of infected plants and probably the uptake of other macro-nutrients such as K, and N (Smith and Read 1997). There is evidence that they promote the uptake of some micronutrients (e.g. Zn (Burkert and Robson 1994)) and they might improve plant water relations (Read 1992). Some recent studies have shown that roots of mycorrhizal plants are protected from infection by pathogenic fungi (Fitter and Garbaye 1994; Newsham *et al.* 1995a). As a consequence of the increased P uptake, mycorrhizal plants have more biomass and higher growth rate than non-mycorrhizal plants. Commonly this association is mutualistic but under particular conditions (e.g. low irradiance (Hayman 1974; Son and Smith 1988)), it might be parasitic (Smith and Smith 1996). The fungi, obligate symbionts, require plant assimilates to survive, and up to 4-20% of assimilates are transferred by the plant to the mycorrhizal fungi (Jakobsen 1993). The net benefit of mycorrhizal infection depends on the balance between the P uptake by the plant and the C transferred to the fungi (Fitter 1991; Koide 1991b; Graham *et al.* 1992).

1.2.b.3. Experimental difficulties

The ability of most vascular plants to form mycorrhizas and the wide distribution of this symbiosis in different ecosystems (Fitter 1989) have given rise to the hypothesis that VAM must have an important role in population and community dynamics (St. John and Coleman 1983; Reeves 1985; Allen and Allen 1990; Koide and Li 1991). Although, some recent field experiments provided evidence of a direct effect of mycorrhizal symbiosis on population dynamics (Merryweather and Fitter 1995b; Merryweather and Fitter 1995a; Merryweather and Fitter 1996), other attempts of studying the effect of mycorrhizas in the field have had, in general, inconclusive results (Fitter 1985; McGonigle 1988; Carey *et al.* 1992; Newsham *et al.* 1995a). The difficulties of establishing proper controls, among other factors, makes results from field experiments hard to interpret (Fitter 1985). The application of fungicides or biocides to eliminate mycorrhizal fungi also affects other organisms present in the soil, potentially producing confounding effects (Koide and Li

1989). Although the fungicide benomyl has been widely used to reduce the infection in the field, there are still problems with respect to the appropriate application techniques to use and doses to apply (Koide *et al.* 1988a; Cade-menun and Berch 1996). The lack of control in field experiments of arthropods (Finlay 1985; Boerner and Harris 1991; Fitter and Sanders 1992) and nematodes (Finlay 1985) that feed on spores and hyphae of mycorrhizal fungi adds more complexity to the interpretation of their results.

1.2.b.4. Effect of VAM on plant populations

Since the effect of mycorrhizas on single plants is to increase plant growth rate through an increase in phosphorus uptake per unit root length, it might be expected infected plants growing in phosphorus-deficient soils to be more productive than plants growing in absence of VAM infection (McGonigle 1988; Fitter 1989; Carey *et al.* 1992). However, under natural conditions, there is no clear evidence for increased plant growth (Fitter 1989; Carey *et al.* 1992; West *et al.* 1993; Newsham *et al.* 1995a). Nevertheless, some studies showed that mycorrhizal infection might be beneficial to plants under particular environmental conditions and at particular times on the plants life cycle as postulated by Fitter (1989). Mycorrhizas might increase plant fitness, which has a direct effect on population dynamics. Mycorrhizal symbiosis increased the accumulation of P in shoots and roots that could be used in the production of reproductive organs in a perennial alpine herb (Mullen and Schmidt 1993), increased the inflow of P at flowering time in a wild strawberry (Dunne and Fitter 1989) and produced the differential allocation of photoassimilates to production of tillers in grasses (Miller 1987). Results from several experiments performed with *Abutilon theophrasti*, *Avena fatua* and *Vulpia ciliata* ssp. *ambigua* provide direct evidence of the potential of mycorrhizas to affect fitness. Mycorrhizas enhanced vegetative growth and/or reproductive output of plants of *A. theophrasti* and *V. ciliata* (Carey *et al.* 1992; Stanley *et al.* 1993), and offspring of mycorrhizal mothers of *A. theophrasti* and *A. fatua* had faster growth rates and higher reproductive output than offspring of non-mycorrhizal plants (Koide and Lu 1992; Shumway and Koide 1994). There is also evidence

of indirect positive effects of mycorrhizas on fitness through the protection of the hosts against root pathogenic fungi (Newsham *et al.* 1995a).

Despite the important increments in P uptake and biomass observed in single mycorrhizal plants, as density increases these plant responses to the infection drastically decline (Bååth and Hayman 1984; Koide 1991a; Allsopp and Stock 1992a). In these experiments, in which P was the only limiting resource, the effect of VAM on plant competition was not investigated. The effect of VAM on intraspecific competition was investigated only in monocultures set as controls for interspecific competition studies which showed an increase on intraspecific competition due to the symbiosis (Hartnett *et al.* 1993; West 1996; Watkinson and Freckleton 1997).

The effect of VAM on population structure is not clear. There is some evidence that mycorrhizal plants at high density might have higher inequality in size and/or fecundity than non-mycorrhizal plants (Allsopp and Stock 1992a; Shumway and Koide 1995). Mycorrhizal plants of a fynbos species, *Otholobium hirtum*, with high growth response to mycorrhizal infection, had a higher coefficient of variation (CV) of shoot mass than non-mycorrhizal plants, at high plant density. On the other hand, mycorrhizal and non-mycorrhizal plants of a less responsive species (see 1.2.b.5, below), *Aspalathus linearis,* had similar CVs (Allsopp and Stock 1992a). Mycorrhizal plants of *Abutilon theophrasti*, a highly responsive species, did not develop different size inequality compared to non-mycorrhizal plants at high density, but they did have greater inequality in fecundity (Shumway and Koide 1995).

In general, there is scarcity of research on the role of mycorrhizas in population dynamics (Allen 1991; Crawley 1997a; Smith and Read 1997; Watkinson 1997). In particular, the dearth of information about the response of mycorrhizal plants to competition for different resources and the effect of the symbiosis on the intensity of plant competition indicates the need for further investigations. Furthermore, the interactive effects of these factors in environments with patchy distribution of nutrients are still unexplored.

1.2.b.5. Effect of VAM on plant communities

Although most of the terrestrial vascular plants are able to become infected by different species of mycorrhizal fungi, it is considered that there is a continuum of plant responses to mycorrhizal infection ranging from positive to neutral to negative (Johnson *et al.* 1997). It is this variation in responsiveness among plant species that might affect community productivity and structure (Janos 1980; Allen and Allen 1984; Allen and Allen 1990; Koide 1991b; Koide and Li 1991).

The responsiveness (the difference in growth between a non-mycorrhizal and a mycorrhizal plant at a particular available nutrient level (Reeves 1985)) of a species to mycorrhizal infection is related to plant P deficit (Koide and Li 1990; Koide 1991b), root morphology (Hetrick 1989; Pate 1994) and intrinsic growth rate (Koide 1991b). Plants with low P demand would have a limited potential to respond to large phosphorus inputs (Koide 1991b). Plants with roots having high root hair density and/or relatively high root fineness show less mycorrhizal responsiveness (Hetrick 1989). Species of lower growth rate will have lower P demand and higher tissue P concentration, which would result in a low response to mycorrhizal infection (Koide 1991b).

Although some researchers have highlighted the potential role of the VA hyphal network in structuring plant communities (Read *et al.* 1985; Grime *et al.* 1987; Read 1990; Allen 1991), it is possible that the amounts of nutrients (particularly P) and C transferred through mycorrhizal links are very small and might not affect plant fitness (Newman and Eason 1993; Fitter *et al.* 1998). There is evidence that mycorrhizal hyphae connect roots of same and different species (Chiarello *et al.* 1982; Newman *et al.* 1994), and that there is transport of carbon, N and P through these hyphal connections (Heap and Newman 1980a; Heap and Newman 1980b; Francis and Read 1984; Newman and Ritz 1986; Newman and Eason 1993; Fitter *et al.* 1998). However, there is no evidence supporting net gain of mineral nutrients by one living plant from another via VA mycorrhizal hyphae (Ritz and Newman 1984; Newman 1988).

The potential role of mycorrhizas in structuring plant communities through changes in species diversity or richness and population abundance was demonstrated in a few

investigations in controlled and field conditions (Grime *et al.* 1987; Gange *et al.* 1990; Gange *et al.* 1993). In a microcosm experiment where a turf community was simulated, mycorrhizas increased species diversity after one year (Grime *et al.* 1987). Mycorrhizas reduced the biomass of the dominant species (*Festuca ovina*) and increased the biomass of subordinate species (*Scabiosa columbaria*, *Hieracium pilosella*, *Plantago lanceolata*). The authors postulated that this was due to the transfer of carbon from the dominant species to the subordinates through the mycorrhizal hyphal network. However, this interpretation was criticised by Bergelson and Crawley (1988). They pointed out that mycorrhizas most likely exerted a negative effect on the dominant (which biomass was reduced in a 30%), releasing the subordinates from competition.

The application of fungicide in the field resulted in lower plant species richness in two early secondary successional plant communities (Gange *et al.* 1990; Gange *et al.* 1993). This change in community structure was attributed to the positive effect of mycorrhizas on the establishment of seedlings of perennial forbs. The reduction of mycorrhizal infection using fungicide also reduced the abundance of mycorrhizal species in a lichen-dominated community (Newsham *et al.* 1995b). These authors suggest that, in that community, mycorrhizas have a significant role in determining the competitive abilities of higher plant species.

The differential mycorrhizal responsiveness of the species present in a community might allow some of them to obtain more soil resources in competition with their neighbours (Allen 1996), and in consequence alter the outcome of competition between species. This was demonstrated in pot experiments that compared the outcome of intra- and interspecific competition for two mycorrhizal species with different degree of responsiveness (Hall 1978; Hartnett *et al.* 1993; Hetrick *et al.* 1994). Strong competitive effects of *Andropogon gerardii*, the species with high mycorrhizal responsiveness, on *Elymus canadensis* disappeared in the absence of mycorrhizas, indicating that its competitive dominance in tallgrass prairie is highly dependent upon its mycorrhizal associations (Hartnett *et al.* 1993; Hetrick *et al.* 1994). Similar results were obtained with

two pasture species, *Trifolium repens* (highly responsive) and *Lolium perenne* (less responsive) (Hall 1978).

Interestingly, a pioneer study of mycorrhizas and plant competition (Fitter 1976) did not show an increase in the competitive ability of the more responsive species (*Holcus lanatus)* due to mycorrhizal infection, but that the outcome of competition was related to the negative effect of mycorrhizas on the less responsive one (*Lolium perenne)*. Inoculation and competition increased the percentage infection of *Lolium perenne* and reduced its biomass and P uptake. This negative effect of mycorrhizas on species with less responsiveness mirrors the results of Grime *et al.* (1987) in their microcosms experiment (Bergelson and Crawley 1988). In contrast, Koide and Li (1991) found that *Abutilon theophrasti*, a highly responsive species, was not benefited in its competitive interaction with *Setaria lutescens*, a non-responsive species, because the latter was not negatively affected by the infection and maintained its high competitive ability.

When two species of similar responsiveness were used, mycorrhizal infection increased competition, probably due to the positive effect of mycorrhizas on the intraspecific component of the competitive interaction between the two species (West 1996; Watkinson and Freckleton 1997).

These seminal and contradictory results highlight the need for more research on the role of mycorrhizas in plant competitive interactions.

1.2.b.6. Cultivated vs non-cultivated species

Mycorrhizal symbiosis has been considered one of the adaptations of native plants from low nutrient environments to cope with scarce nutrient availability (Bowen 1980; Chapin 1980; Pate 1994). There is consensus in the literature that highly dependent species dominate communities in poor soils and in late successional stages (Janos 1980; Allen and Allen 1990; Francis and Read 1994). However, because of their low growth rates, wild plants from low nutrient environments are expected to have low responsiveness (Koide 1991b). Some wild plants are less responsive to increasing phosphorus availability and to mycorrhizal infection than cultivated ones (Koide *et al.* 1988b; Bryla and Koide 1990).

However, species from low-nutrient South African fynbos communities rely on mycorrhizal associations for seedling establishment and survival (Allsopp and Stock 1992b; Allsopp and Stock 1993).

Investigation of the effects of the symbiosis on plant performance and population dynamics of wild plants from low nutrient environments is fundamental for the understanding of natural systems. It is also paramount for the development of revegetation and/or rehabilitation programs of arid and semi-arid ecosystems where plant available phosphorus is often low (Reeves *et al.* 1979; Jasper *et al.* 1991). Although there is consensus that the sustenance or reintroduction of mycorrhizal associations would be a fundamental step in restoration and revegetation programs (Cuenca and Zovero 1992; Miller and Jastrow 1992; Reddell and Milnes 1992; Herrera *et al.* 1993), the mechanisms through which the symbiosis regulates changes in vegetation are yet to be studied (Smith and Read 1997).

1.3. The project

Although several researchers point out the need for more field studies to investigate the ecological role of mycorrhizal symbiosis, this review of the literature clearly indicates that there is a dearth of basic information fundamental to interpreting the conflicting results from field experiments. This basic information can only be obtained in simple systems to avoid confounding effects due to improper controls. The aim of this project, therefore, was to investigate the role of mycorrhizal symbiosis in plant competition and population structure using a reductionist approach.

There were two exclusive lines of research:

a) to test the hypothesis that mycorrhizas reduce R* (Wedin and Tilman 1993) and might increase in consequence the competitive ability of infected plants (Reeves 1985), or

b) to investigate the effect of mycorrhizal symbiosis on intraspecific competition and population structure.

Preliminary experiments demonstrated that it was not possible to proceed with the first line of research due to the lack of appropriate methodology (Chapter 2). Thus, the project focused on the effects of mycorrhizal symbiosis on the intensity of competition and population structure of a non-cultivated (Chapter 3) and a cultivated species (Chapters 4 and 5) under different levels of resources (P and/or light), in environments with homogeneous and/or patchy distribution of a limiting resource (P).

Chapter 2

Preliminary experiments

I describe in this chapter a series of preliminary experiments I performed to select the soil and the native plant species I would later use in the main experiments. Since plant response to mycorrhizal infection is more evident in soils of low P status (Smith and Read 1997) and I planned to study the effects of the infection on native and cultivated plants (*Trifolium subterraneum* L. cv. Mt. Barker), I sought a low nutrient soil from areas with undisturbed native vegetation proper for the growth of the cultivated species.

I also studied in a preliminary experiment the feasibility of testing the effect of mycorrhizal infection on Tilman's R* hypothesis (Wedin and Tilman 1993).

2.1. Selection of soil and determination of available P

2.1.1. Objectives

This preliminary experiment had two main objectives:

a) Selection of the soil for use in further experiments

b) Selection of a method to determine the reduction in soil available P effected by plants.

2.1.1.a. Selection of soil

The effectiveness of using fungicides to reduce mycorrhizal infection in pot and field experiments has been inconsistent (Fitter 1985; McGonigle 1988; Carey *et al.* 1992). Therefore I used autoclaved soil in all experiments to ensure non-mycorrhizal control treatments were indeed free of mycorrhizal fungi. In this way, I thus obtained a simple system to investigate the complex interactions among plant density, mycorrhizal infection, resource availability, plant competition and soil heterogeneity. However, sterilisation of soils at high temperature might change the availability of nutrients and even increase the concentration of some elements to levels toxic for plants (Larsen 1966; Smith and Smith

1981) Thus, one of the aims of the first experiment was to select a soil with low P concentration that did not produce toxic effects on plants when autoclaved. In the same experiment I also estimated the mycorrhizal infectivity of the soils in the event that the inoculation of native plants with cultivated fungi failed and I needed to use native soil as a source of inoculum (Moora and Zobel 1996).

2.1.1.b. Selection of a method to estimate soil P available for the plants

One of the possible lines of research of the project was to test the hypothesis that mycorrhizal infection reduces Tilman's R* (Wedin and Tilman 1993), the level to which plants reduce soil nutrients. As a pre-requisite, it was therefore necessary to be able to measure the P left in the soil by the plants after their growth period. In the same soils assessed for infectivity, I tested two methods of measuring available P for plants: a) Calcium chloride-extractable P (Rayment and Higginson 1992) and b) Sodium bicarbonate-extractable P (Colwell 1963). The P extracted with the first method is highly correlated with the P concentration of the soil solution, it measures the intensity component of labile P and it has been reported to be a good predictor of the yield of several crops in P-deficient soils (Stevenson 1986; Rayment and Higginson 1992). The second method is more generally used to assess plant-available P in soils and measures the intensity component of available P and part of the P on the soil solid phase (quantity component). Phosphorus extracted with this second method is also correlated with plant growth (Colwell 1963; Stevenson 1986).

2.1.2. Materials and methods

I compared soils from three different areas:

1) Middleback Station (Middleback), located 15 km west of Whyalla, on the Eyre Peninsula (32° 57' S, 137° 24' E). The climate of the area is characterised by hot summers (mean daily maximum = 28.9 °C), and mild winters (mean daily maximum = 7.3 °C). The average rainfall is 210 mm with a slight predominance in winter, and substantial among-year variability. Soils are predominantly brown calcareous earths with clay-loam texture, and

$CaCO_3$ is present at variable depths. The pH is slightly alkaline, and nutrient availability is low. The vegetation is a low open woodland, dominated by sparse individuals of *Acacia papyrocarpa* (western myall) with low chenopod shrubs occurring in the spaces between the trees (*Maireana sedifolia* (bluebush) and *Atriplex vesicaria* (bladder saltbush)). No definite annual plants communities can be associated to this vegetation type.

2) Ferries Mc Donald Conservation Park (Ferries Mc Donald), located 40 km South of Murray Bridge (35° 10' S, 139° 17' E). The climate of the area is characterised by hot summers (mean daily maximum = 27.6 °C), and mild winters (mean daily maximum = 8.4 °C). The average rainfall is 294 mm concentrated in winter. The soil is a siliceous fine sand with a neutral to acid surface layer. There are low levels of mineral nutrients in the soil. Organic matter has accumulated in the upper layers, particularly under perennial plants. The vegetation is an open scrub dominated by *Eucalyptus incrassata* and *Melaleuca uncinata*, open areas between the dominant species are sparsely vegetated by low growing shrubs and herbaceous annuals.

3) Waite Hills Land (Hills), located 8 km South East of Adelaide (34° 56' S, 138° 36' E). The climate of the area is characterised by hot dry summers (mean monthly temperature = 24.5 °C), and cool wet winters (mean monthly temperature = 11.2 °C). The average rainfall is 625.3 mm concentrated in winter. The soils vary considerably, mainly determined by topography. The soil was sampled on the western edge of the hills, where soils are dominated by relatively deep red-brown earths. The vegetation is an open-woodland/woodland dominated by *Eucalyptus microcarpa* and *Eucalyptus leucoxylon*. The understorey includes *Allocasuarina verticillata*, *Acacia pycnantha* and *Dodonaea viscosa*. Some patches of native grasses (*Themeda triandra*, *Danthonia* spp) form the ground cover.

I estimate mycorrhizal infectivity of these soils by a bioassay technique (Jasper *et al.* 1987; Brundrett and Abbott 1995), using plants of *Trifolium subterraneum* L. cv. Mt. Barker as "trap" plants.

The experiment had a factorial design with completely randomised blocks and included 3 factors: soils at three levels (Middleback, Ferries Mc Donald, and Hills); autoclaving at two levels (autoclaved soil, and non-autoclaved soil) and plants at two levels

(plants present, P, and plants absent, A). Each of the 12 resulting combinations was replicated 5 times. I surface sterilised seeds of *T. subterraneum* by soaking them for 10 minutes in 2% sodium hypochlorite with two drops of Tween 80 and rinsed them thoroughly in deionised water. I germinated seeds in Petri dishes with wet filter paper in the dark, at 25 °C. I planted 6 pre-germinated seedlings in pots containing 460 g of soil when the radicles were approximately 1 cm in length. I added a dense suspension of *Rhizobium trifolii* to all the pots to ensure effective nodulation and N_2 fixation (Smith and Smith 1981). I watered the plants as needed with deionised water.

I harvested the plants at 8 weeks and separated shoots and roots. I washed and weighed the roots and took one sub-sample (150 mg) from each pot for estimation of mycorrhizal infection. I dried the remaining material (shoots and roots minus the samples) at 70 °C for 48 h, and weighed it. I washed the root sub-samples, cleared them with 10% KOH at room temperature for 4 days and stained them with 0.05% trypan blue in lactoglycerol for one hour (a modification of the method of Phillips and Hayman (1970)). I determined root length and mycorrhizal root length using a line intersect method (Giovanetti and Mosse 1980), under a dissecting microscope with x 20 magnification.

I assessed soil P concentrations before planting and after the harvest by the two methods mentioned above. I determined plant P concentrations colorimetrically using the phosphovanado-molybdate method (Hanson 1950) after digestion of the dried, ground plant material in a mix of concentrated perchloric and nitric acids (1:6 v/v).

I analysed the data using SAS GLM procedure and SNK test to compare means (SAS 1985).

2.1.3. Results

The percentage infection was about 35% in Hills soil, 37% in Middleback soil and 5% in the Ferries Mc Donald soil.

None of the soils when autoclaved had toxic effects on plants of *T. subterraneum.*

The biomass of *T. subterraneum* was higher in the Hills soil than in the other two soils (Table 2.1).

Autoclaving increased plant biomass in Hills soil whereas there was no effect of autoclaving on plant biomass in the other two soils.

The presence of plants did not change the measured amount of labile or available soil P in the pots (Table 2.1).

2.1.4. Discussion and conclusions

I selected the Hills soil for future experiments because it supported better growth of plants of *T. subterraneum,* had relatively high infectivity, and when autoclaved did not have a toxic effect on plants. Autoclaving, however, increased plant biomass in this soil. This effect was observed before in *Lolium perenne*, *Rumex acetosa* and *Trifolium repens* grown in fumigated soil (Newman *et al.* 1977), and was related to the reduction in pathogenic microbial populations. However, the effect of sterilising soil by irradiation, heat or chemical treatment has different effects on plant growth depending upon the microbial population present in the soil and the plant species used (Newman *et al.* 1977). For instance, irradiating soil increased the biomass of *Lolium multiflorum* and reduced the biomass of *Trifolium repens* (Newman *et al.* 1977). Is because of these changes in microbial activity and the possible changes in chemical properties of the soil during the sterilisation process that the use of sterile soil as control in mycorrhizal studies is not valid (Smith and Smith 1981; Koide and Li 1989). An alternative approach is to sterilise all the soil and then to inoculate part of it with mycorrhizal inoculum (Smith and Smith 1981; Koide and Li 1989). This inoculum may be obtained from pot cultures (a mix of sterile soil, pieces of infected roots and fungal spores and hyphae) or from field soils (Koide and Li 1989). When using field soil as inoculum, the addition of soil sievings or filtrates to the non-mycorrhizal treatments may provide of similar microbial activity to the one in the mycorrhizal treatments (Koide and Li 1989).

Thus, for the main experiments I used autoclaved Hills soil and I inoculated part of it with spores of a mycorrhizal fungus.

Although R* from different plants has been measured before (Wedin and Tilman 1993), the calculations were based on nitrogen use by the plants and not on P. The

analytical methods I used for P determinations were those recommended to relate P concentration in the soil solution with plant uptake and growth (Stevenson 1986; Rayment and Higginson 1992). However, the relatively small amounts of P mobilised by plants were undetectable by these techniques in the very low P soils chosen (A. Alston, P. W. Moody, p. communications). It is also possible that the activity of roots and/or micro-organisms in the rhizosphere had increased the amount of soluble phosphates through dissolution of insoluble P sources by organic acids (Stevenson 1986). In consequence, the direct estimation of the R* by measuring P pools is extremely difficult, if not impossible, with the analytical methodology currently available. Therefore I could not test the hypothesis that mycorrhizas reduce R* by directly measuring changes in P pools in soil.

2.2. Selection of host species

a. Cultivated species

I chose *T. subterraneum* because it is a species with a high responsiveness to mycorrhizal infection and because there is abundant information about how individual plants respond to the symbiosis (Smith and Smith 1981; Abbott 1982; Abbott and Robson 1984). There is also information about how different soil P concentrations (Nadian *et al.* 1996) and different levels of light affect the response of individual plants to the symbiosis (Tester *et al.* 1985; Tester *et al.* 1986).

b. Non-cultivated species

To select the native species to use in the main experiments I performed a series of germination trials and an experiment to test the response of the selected species to mycorrhizal infection.

2.2.1 Germination trials

2.2.1.1. Objective

I required a native plant from low nutrient environments that formed associations with mycorrhizal fungi, with a short life cycle and even germination. The latter trait was

important because I required that the experiments commence with plants of equal size and age. Since I wanted to compare the results obtained using natives plants to those from experiments using *T. subterraneum*, a native legume would have been preferred, therefore I included *Kennedia postrata* and *Swainsona stipularis* in the trials.

2.2.1.2. Material and methods

I tested a total of 15 species from the Ferries Mc Donald Conservation Park, the Waite Hills and other species suggested by native plants growers. I employed germination systems commonly used for native plants (Glossop 1980; Sharman *et al.* 1989; Armitage 1993); John Stafford, Jacqui Merckenschlager and Phil Collins, personal communications) (Table 2.2).

2.2.1.3. Results

Most of the seeds tested had high bacterial and fungal contamination in Petri dishes, low rates of germination, and very slow and sparse germination (Table 2.2).

Germination improved in aluminium trays in the glasshouse for some of the species, but for most of them it was still very uneven (Table 2.2).

Kennedia postrata had a good germination rate (15 %) but there were more than 10 days interval between the emergence of the first and the last seedling (Table 2.2).

Bracteantha bracteata and *Rhodanthe chlorocephala* ssp. *rosea* had the highest germination rates and the germination concentrated in few days in both Petri dishes and aluminium trays (Table 2.2).

2.2.1.4. Discussion and conclusion

Although both legumes tested (*K. postrata* and *Swainsona stipularis*) showed a reasonable germination rate, they presented the problem of the inoculation with the proper strain of *Rhizobium* in autoclaved soils. Only few strains of *Rhizobium* were isolated from native soils and the response of native plants to inoculation with native strains needed to be

investigated (Lawrie 1983); A. Lawrie; A. Gibson, p. communications). Therefore, I chose *Bracteantha bracteata* and *Rhodanthe chlorocephala* ssp. *rosea* for further screening.

2.2.2. Response of *Bracteantha bracteata* and *Rhodanthe chlorocephala* ssp. *rosea* to inoculation with mycorrhizal fungi

2.2.2.1. Objectives

Bracteantha bracteata and *Rhodanthe chlorocephala* ssp. *rosea* had the highest germination rates, and there was information about the conditions they required for growth and flowering in glasshouse and growth room conditions (Sharman and Sedgley 1988; Sharman 1989; Sharman *et al.* 1989). Thus the next step was to test if they formed associations with cultivated mycorrhizal fungi.

2.2.2.2. Materials and methods

I planted 2 pre-germinated seedlings of *B. bracteata* and *R. chlorocephala* ssp *rosea* in 400 g pots containing an autoclaved mix of Hills soil and sand (50:50), with and without the addition of 1 mg kg^{-1} of P as Na_2HPO_4. I added a suspension of surface sterilised spores (Smith and Dickson 1997) of the mycorrhizal fungus *Gigaspora margarita* Becker and Hall to one third of the pots, and I inoculated the other third with 10% (w/w) inoculum of the mycorrhizal fungus *Glomus intraradices* (soil containing pieces of infected roots of *T. subterraneum* and spores). I left the rest of the pots as controls (soil mix without mycorrhizal inoculum). I also planted pre-germinated seedlings of *R. chlorocephala* in pots containing non-autoclaved WA soil from the area of its provenance to test if this species formed associations with native mycorrhizal fungi. After 4 weeks I harvested the plants and sampled the roots to screen them for mycorrhizal infection as described above. Roots were observed under a dissecting microscope with x 20 magnification.

2.2.2.3. Results

Both species were infected by *G. margarita* and *G. intraradices*.

There was no response to P addition, although there was a trend of more biomass in pots with added P.

B. bracteata had weaker radicles and many plants died after transplanting.

R. chlorocephala was infected by mycorrhizal fungi in the native non-autoclaved soil.

2.2.2.4. Conclusion

Although *R. chlorocephala* was not indigenous to the areas where the soils were sourced, I chose it for the main experiments because it had a relatively high germination rate, the emergence of the seedlings occurred in a short interval, and it grew well in the Hills soil/sand mix.

Table 2.1. Summary of the results of the comparison of three soils from different undisturbed native vegetation areas. Different letters indicate significant differences between means within each row (SNK, $p \leq 0.05$), GLM analysis, $n = 5$. P0, extractable P measured before planting. P1, extractable P measured after harvest. Labile P, extracted with calcium chloride. Available P, extracted with sodium bicarbonate.

soils	Ferries McDonald Conservation Park				Middleback Station				Adelaide Hills			
treatment	autoclaved		non-autoclaved		autoclaved		non-autoclaved		autoclaved		non-autoclaved	
pH	5.8 c		6.2 b		7.5 a		7.4 a		5.7 c		5.5 d	
labile P0 (µg P/g soil)	0.13 c				0.36 a		0.29 b		0.13 c		0.11 c	
available P0 (mg P/g soil)	1.8 d		1.5 d		12.4 a		10.2 b		9.3 cb		8.9 c	
plants present	yes	no	yes	no	yes	no	yes	no	yes	no	yes	no
labile P1 (µg P/g soil)	0.05 de	0.04 de	0.01 e	0.03 e	0.66 a	0.62 ab	0.56 bc	0.55 bc	0.09 d	0.12 d	0.10 d	0.08 d
available P1 (mg P/g soil)	3.0 cd	3.0 cd	2.2 d	2.3 d	9.2 a	10.5 a	9.8 a	8.6 a	5.9 b	5.9 b	5.0 bc	6.0 b
shoot biomass (mg)	361 b		338 b		381 b		449 b		713 a		502 b	
shoot [P] (mg P/g tissue)	0.5 c		0.3 d		0.6 b		1.0 a		0.7 b		0.7 b	

Table 2.2. Summary of the results from the germination trials (percentage of germination). Species are from Ferries Mc Donald Conservation Park (F), from Waite Hills Face (H) or suggested by growers (G). Treatments: legumes were always scarified; **S**: seeds surface sterilised with sodium hypochlorite, 15 minutes; **N**: seeds no surface sterilised; **B**: seeds immersed in boiling water; **H**: seeds heated in oven 105 °C, 15 minutes. Results: **C**: seeds contaminated; **slow**: emergence interval >6 days; **fast**: emergence interval ≤ 5 days. Nomenclature of the species follows (Black 1974) unless authority is specified in the table.

germination systems	Petri dish filter paper 25 °C - dark	Petri dish filter paper 25 °C - light	Petri dish water agar	pot soil/sand growth room	tray vermiculite growth room	tray sand/vermiculite glasshouse
Kennedia postrata (H)	**<10%-S, C 0%-B-H, C**			**<20%-S, N**	**0%-B-H, C <20%-S, fast**	
Swainsona stipularis (G)						**<5%**
Bracteantha bracteata (Vent.) A. Anderb. & Haegi (H)	**<10%-S, C <5%-N, C**				**<20%, slow**	**>30%**
Danthonia caespitosa (F)	**> 20%-S-N, slow**					
Enchilaena tomentosa (H)	**0%-C**					
Helichrysum apiculatum (H)				**<10%-S <20%-N**		
Helipterum corymbiflorum (G)						**<5%**
Hibbertia sericea (H)	**0%-C**					
Ragodia candolleana (F)	**0%-C**					
Rhodanthe chlorocephala ssp rosea Paul G. Wilson (G)	**<5%-S, slow**	**>80%-N, fast**	**>80%-N, fast**			**>40%**
Schoenia cassineana (H)						**<5%**
Senecio lautus (F)				**<10%-S <20%-N**		
Stipa elengantissima (F)	**<5%**					
Themeda australis (H)	**<5%**					

Chapter 3

Effects of P availability, mycorrhizas and plant density on the performance of *Rhodanthe chlorocephala* ssp. *rosea* (pink everlasting)

3.1. Introduction

Plants from low nutrient environments have special root characteristics and/or physiological adaptations to facilitate nutrient acquisition and/or nutrient-use efficiency. Higher proportion of fine roots and/or high production of root hairs, and the allocation of a greater proportion of assimilates to root growth are listed amongst these adaptations (Bowen 1980; Chapin 1980; Pate 1994). One important characteristic is the formation of mycorrhizal associations (Bowen 1980; Pate 1994). It is generally acknowledged, on the base of the wide spread occurrence of mycorrhizal roots in pristine systems, that plants growing in a low phosphorus environment respond to, and/or rely on, this symbiosis for nutrient acquisition (Bowen 1980). However, some wild plants are less responsive to increasing phosphorus availability and to mycorrhizal infection than cultivated ones (Koide *et al.* 1988b). Australian pristine systems are not an exception to the common presence of mycorrhizal fungi and their association with native plants is well documented (Abbott and Robson 1977b; Jehne and Thompson 1981; McGee 1986; Logan *et al.* 1989). Several species of *Eucalyptus* and *Acacia* have a positive growth response to inoculation with mycorrhizas as well as to addition of P (Malajczuk *et al.* 1975; Barrow 1977; Malajczuk *et al.* 1981; Jasper *et al.* 1988; Reddell and Milnes 1992). However, the addition of P to other Australian native plants from low nutrient environments has had inconsistent results and their response to mycorrhizal infection is not known (Specht 1963; Barrow 1977; Maier *et al.* 1994).

The positive effect of mycorrhizal symbiosis on plant growth through an increase in P uptake has been observed in single plants. How the symbiosis affects population density and growth is little known (Addicott 1984; Law 1989). However, there are some reports

that as density increases the positive effect of mycorrhizal infection on plant biomass decreases (Bååth and Hayman 1984; Koide 1991a; Hartnett *et al.* 1993). It is assumed that as density increases and plants compete for a limiting resource, an increase in the supply of this resource reduces the intensity of plant competition (Chapin and Shaver 1985; Goldberg 1990); but cf. (Gurevitch *et al.* 1990; Belcher *et al.* 1995). Thus, I expected that increases in P availability to the plant through P additions or mycorrhizal infection would reduce plant competition intensity in low P soils.

The overall objective of this experiment was to study to what extent an annual species adapted to a low nutrient environment relies on mycorrhizal symbiosis for P acquisition and if this response is altered by plant density. The specific questions were:

1) Does *Rhodanthe chlorocephala* (Hook.) Paul G. Wilson (pink everlasting) rely on mycorrhizal symbiosis for P acquisition?
2) If yes, is this response modified by increasing densities?
3) If not, does R. *chlorocephala* respond to addition of P?
4) If yes, is this response modified by increasing densities?
5) How does the presence of the symbiosis and/or the addition of P affect the intensity of intraspecific competition?

3.2. Materials and methods

The plant:

Rhodanthe chlorocephala (Hook.) Paul G. Wilson (pink everlasting) is a native species that grows in low phosphorus (P) soils in Western Australia (WA) (5.5 ppm, $NaHCO_3$ available P (Colwell 1963), and pH of 5.8). In preliminary tests, *R. chlorocephala* was infected by native mycorrhizal fungi when grown in a WA soil (probably by a 'fine endophyte" or *Glomus tenue* (Greenall) Hall (Abbott 1982)) and by cultivated fungi (*Gigaspora margarita* Becker and Hall and *Glomus intraradices* (Schenck and Smith) when it was grown in an autoclaved inoculated soil/sand mix.

The experiment:

The experiment had a factorial design with complete randomised blocks and included 3 factors: mycorrhizas at 2 levels (pots with mycorrhizal inoculum (M) and pots without mycorrhizal inoculum (NM)), phosphorus at 3 levels (no added P (P0), 5 ppm of added P (P1) and 15 ppm of added P (P2), and plant density at 3 levels (1, 6 and 18 plants per pot). Each of the 18 resulting combinations had 5 replications. I grew the plants in an autoclaved Adelaide Hills soil/sand mix (50:50) with a phosphorus (P) concentration of 5 ppm ($NaHCO_3$ extractable P (Colwell 1963); pH 5.5 in 10 mM $CaCl_2$). Both values were similar to the soil of the area of provenance of the seeds of *R. chlorocephala*. I thoroughly mixed soil with water containing 0, 5 or 15 ppm of P as $NaH_2PO_4.2H_2O$. I left the soil in plastic bags for two days prior to planting. I planted pre-germinated seedlings in 1 kg pots at the indicated densities. I inoculated half of the pots placing a suspension of surface sterilised spores (Smith and Dickson 1997) of the mycorrhizal fungus *G. margarita* (approx. 10 spores per plant) under each seedling. I watered the plants as needed every two days and applied 5 ml per week of a nutrient solution lacking P (Smith *et al.* 1985) to ensure plant growth was only limited by P. I conducted the experiment in a growth room, with a day length of 14 h and day/night temperatures of 20°C/15°C, respectively. Light intensity was 260 $\mu m\ m^{-2}\ s^{-1}$ (Sharman 1989). I registered the height, number of branches and number of inflorescens every week. I harvested the plants at 7 weeks when the first inflorescences began to form.

At harvest, I separated shoot and roots. I washed and weighed the roots and took one sub-sample (200 mg) from each pot, which was then stored in 50% ethanol for further estimation of mycorrhizal infection and root length. I dried the remaining material (shoots and roots minus the samples) at 70 °C for 48 h, and weighed it. I washed the root sub-samples, cleared them with 10% KOH for 4 days at room temperature, and stained them with 0.05% trypan blue in lactoglycerol for one hour (a modification of the method of (Phillips and Hayman 1970)). I determined root length using a line intersect (Giovanetti and

microscope slides (perpendicular to the longest side of the slide) per sub-sample, I scored the roots under 160 x magnification in a microscope provided by a cross hair eyepiece, I registered the presence/absence of infection (internal fungal structures) on about 50 intersection points per slide and then I calculated the percentage infection as number of intersection points with presence of fungal structures divided by total number of intersection points (about 150 per sub-sample) times by 100.

I determined total P concentration in dried, ground shoot tissue by inductively coupled plasma atomic emission spectroscopy (ICP-AES) after digestion of the samples in a mix of nitric and perchloric acid (Hanson 1950).

I calculated the relative competition intensity (RCI) as (adapted from Grace (1993)):

$$RCI = \frac{(S - C)}{(S)}$$

where S was the biomass of single plants and C was the individual biomass of plants growing in dense pots.

I analysed the data using ANOVA or GLM (when number of replicates differed because of missing samples) SAS procedures (SAS 1986), using the Tukey test to compare treatment means. I transformed the data as needed to improve normality. I used a Rank transformation prior to ANOVA to achieve normal distributions of the number of forming inflorescences and the number of shoots per plant (SAS 1986).

3.3. Results

Both inoculated and non-inoculated plants were infected with mycorrhizal fungi, but not by *G. margarita.* The use of the magnified intersection method (McGonigle *et al.* 1990) allowed me to determine that most of the infection was due to a "fine endophyte", probably *Glomus tenue*, very similar to the one described by (Abbott 1982) (Plate 3.1a,b). In the inoculated plants it was not possible to separate accurately how much of the infection was due to the "fine endophyte" and how much to *G.margarita.* It seemed that the 'fine endophyte" was present in all the samples and generally its infection had a high density of

arbuscules and internal hyphae (Plate 3.1a,b). *G.margarita* was seldom alone in one piece of root (Plate 3.2a). In general, it occupied the same piece of root as the "fine endophyte" and it was therefore, difficult to separate them (Plate 3.2b). Thus, the values of infection correspond to both fungi together.

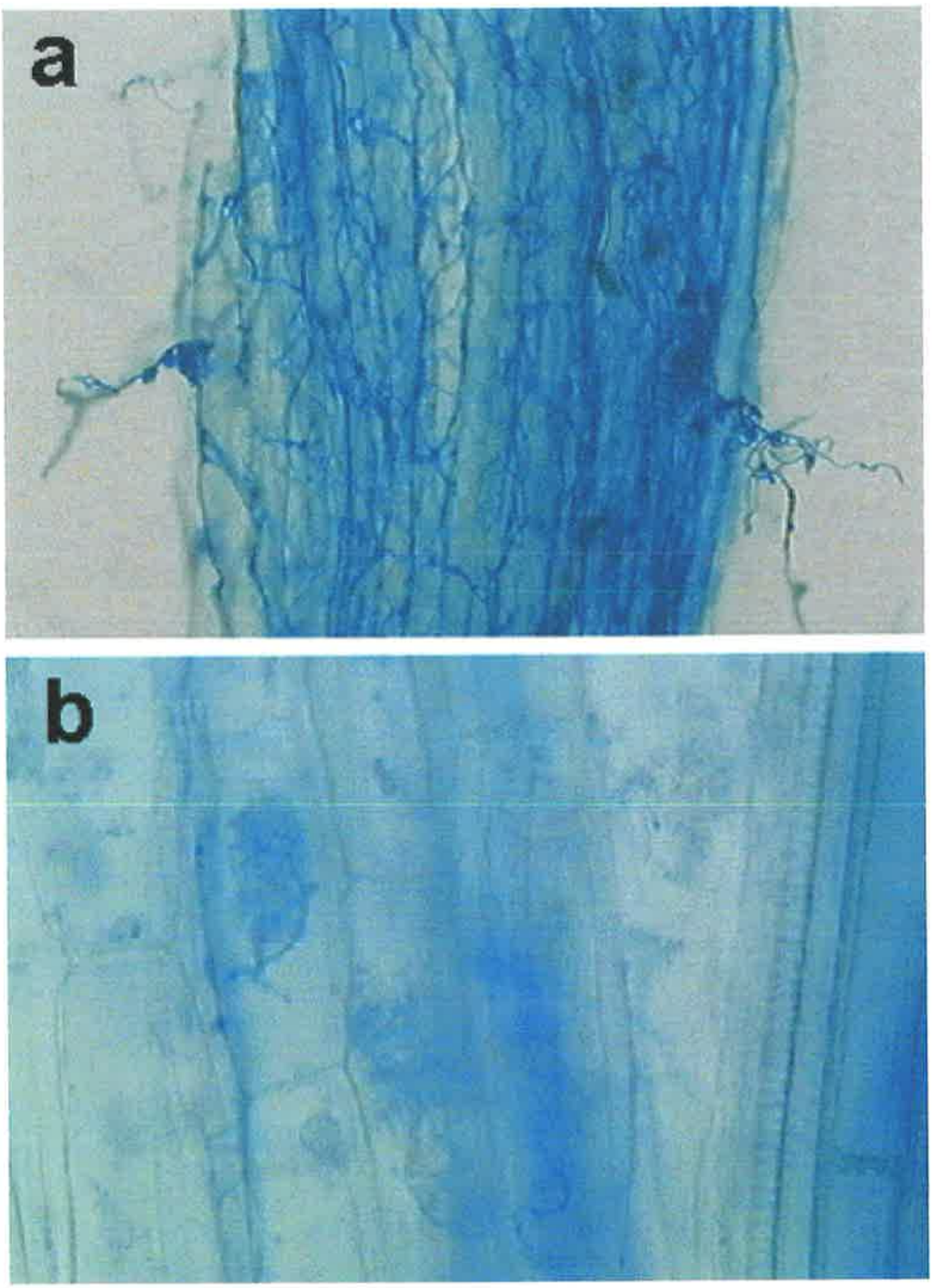


Plate 3.1. "Fine endophyte". a) External and internal hyphae and b) arbuscules, in roots of *Rhodanthe chlorocephala* ssp. *rosea* stained with trypan blue (see Material and methods for procedures). Images from compound microscope at magnifications of x 200 (a) and x 400 (b).

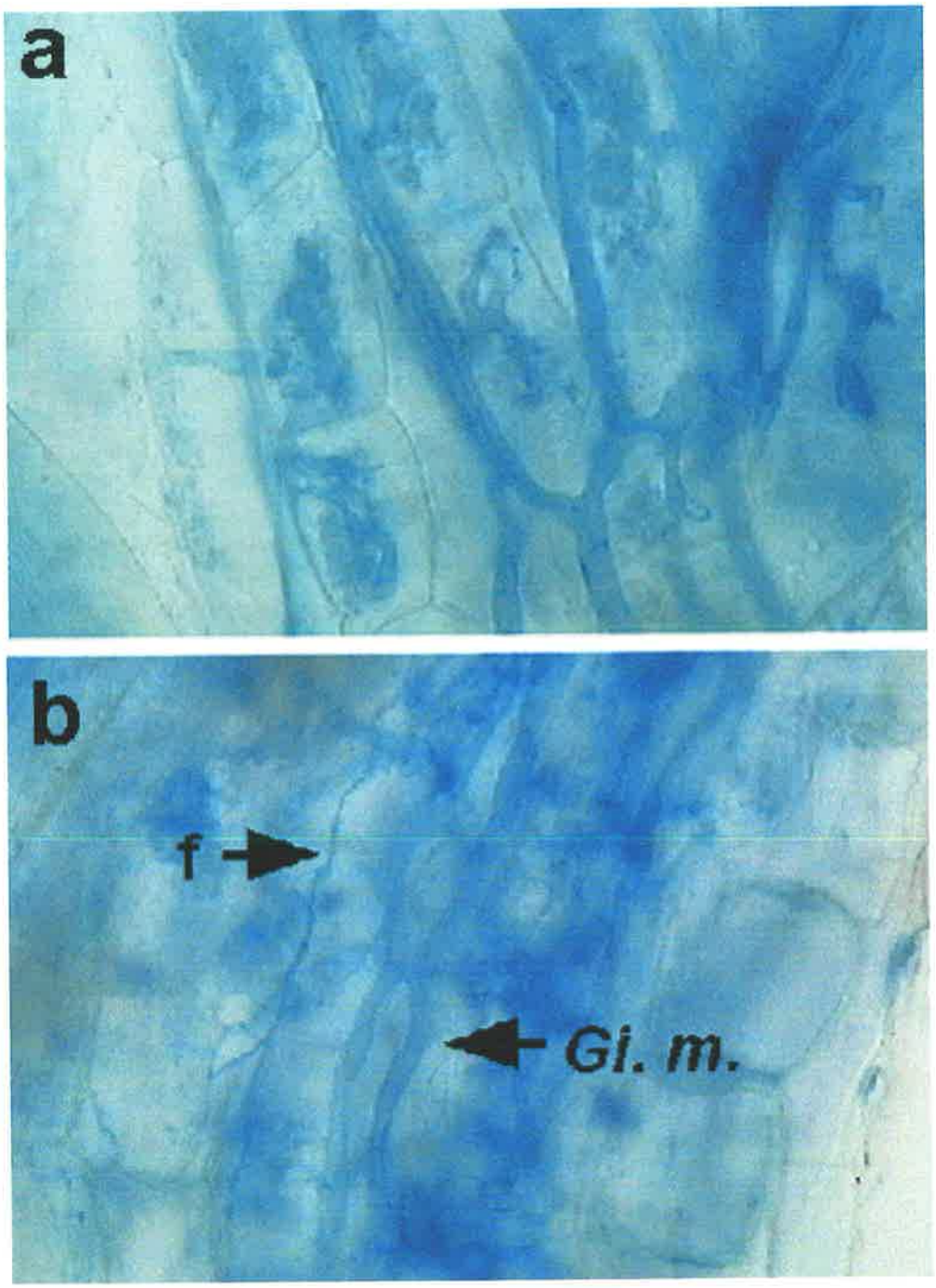


Plate 3.2. a) Internal hyphae and arbuscules of *Gigaspora margarita*. b) Internal hyphae of "fine endophyte" (f) and *G. margarita* (*Gi.m.*) in roots of *Rhodanthe chlorocephala* ssp. *Rosea* stained with trypan blue (see Materials and methods for procedures). Images from compound microscope under x 400 magnification.

The percentage of infection was only affected by the addition of P (Table 3.1). Plants grown in soils without addition of P had the highest percentage infection (about 40%) independently of the number of plants per pot, whereas plants grown with added P (P1 and P2) had infection percentages below 20% (Fig. 3.1).

Due to the contamination of the controls I could not evaluate the effects of mycorrhizal infection on plant performance, thus the following results refer to data pooled for both inoculated and non-inoculated plants.

Phosphorus and plant density treatments had interactive effects on shoot P concentration and shoot P content per plant (Table 3.1). Shoot P concentration increased as added P increased (Fig. 3.2a). At the highest level of added P, single plants had lower shoot P concentration than plants grown at the highest density (Fig. 3.2a). At the other levels of added P density did not modify the shoot P concentration. The amount of shoot P per plant increased with the addition of P at each density. Within each level of added P an increase in density drastically reduced the individual shoot P content (Fig. 3.2b).

Phosphorus and plant density had interactive effects on individual plant biomass (Table 3.2), which increased within each density with the addition of 5 ppm of P. The addition of more P did not result in a further increase in individual biomass. Within each level of P, an increase in density drastically reduced the individual biomass (Fig 3.3). Shoot and root biomass per individual had a similar pattern, but root biomass did not vary with density at the highest level of added P (data not shown).

Phosphorus and plant density had interactive effects on root:shoot ratio (Table 3.2). Addition of P reduced the root:shoot ratio only at high densities. Within each level of added P, increasing density increased root:shoot ratio (Fig. 3.4).

Phosphorus and plant density had interactive effects on number of shoots per plant (Table 3.2). Addition of P increased number of shoots per plant at low density, and within each level of P, plant density reduced the number of shoots per plant (Fig. 3.5a), whereas the addition of P increased the number of buds per plant independently of plant density (Fig. 3.5b, Table 3.2).

There was a reduction in biomass due to plant density (RCI different from zero, Student *t*, $P \leq 0.0001$) (SAS 1986) in all the treatments except in pots with 6 plants without added P. The RCI was affected independently by density and phosphorus (Table 3.3). Addition of P increased RCI and so did density (Fig. 3.6).

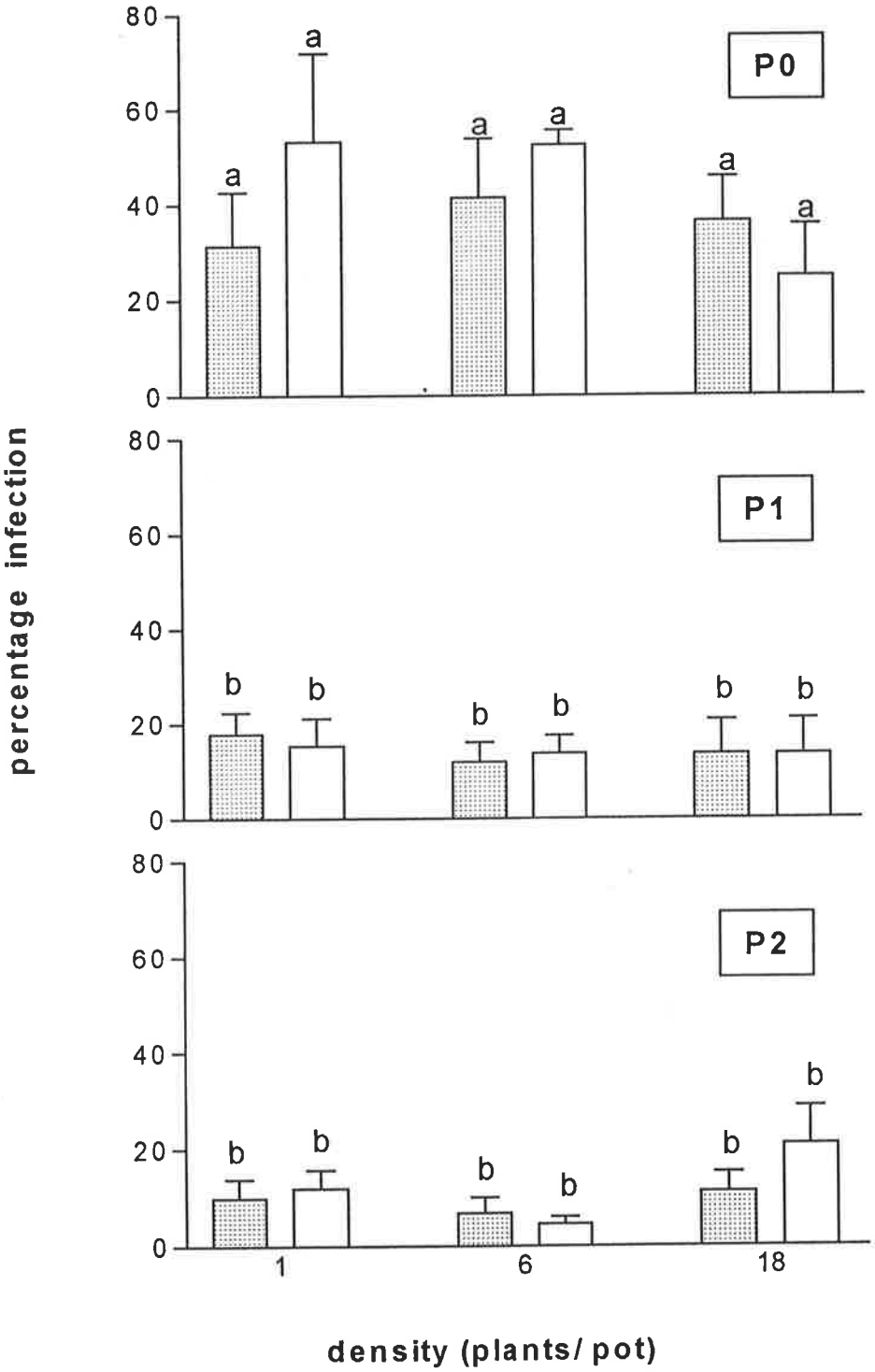


Figure 3.1. Percentage of infection of plants of *Rhodanthe chlorocephala* ssp. *rosea*, inoculated (shaded bars) or not (clear bars) with the mycorrhizal fungus *Gigaspora margarita*. Plants were grown at different densities, 1, 6 and 18 plants per pot, and at three levels of added P, P0, P1 and P2 (no added P, 5 ppm and 15 ppm of added P, respectively). Different letters indicate significant differences between means (Tukey, $p \leq 0.05$). Data are means and SE, $n = 5$.

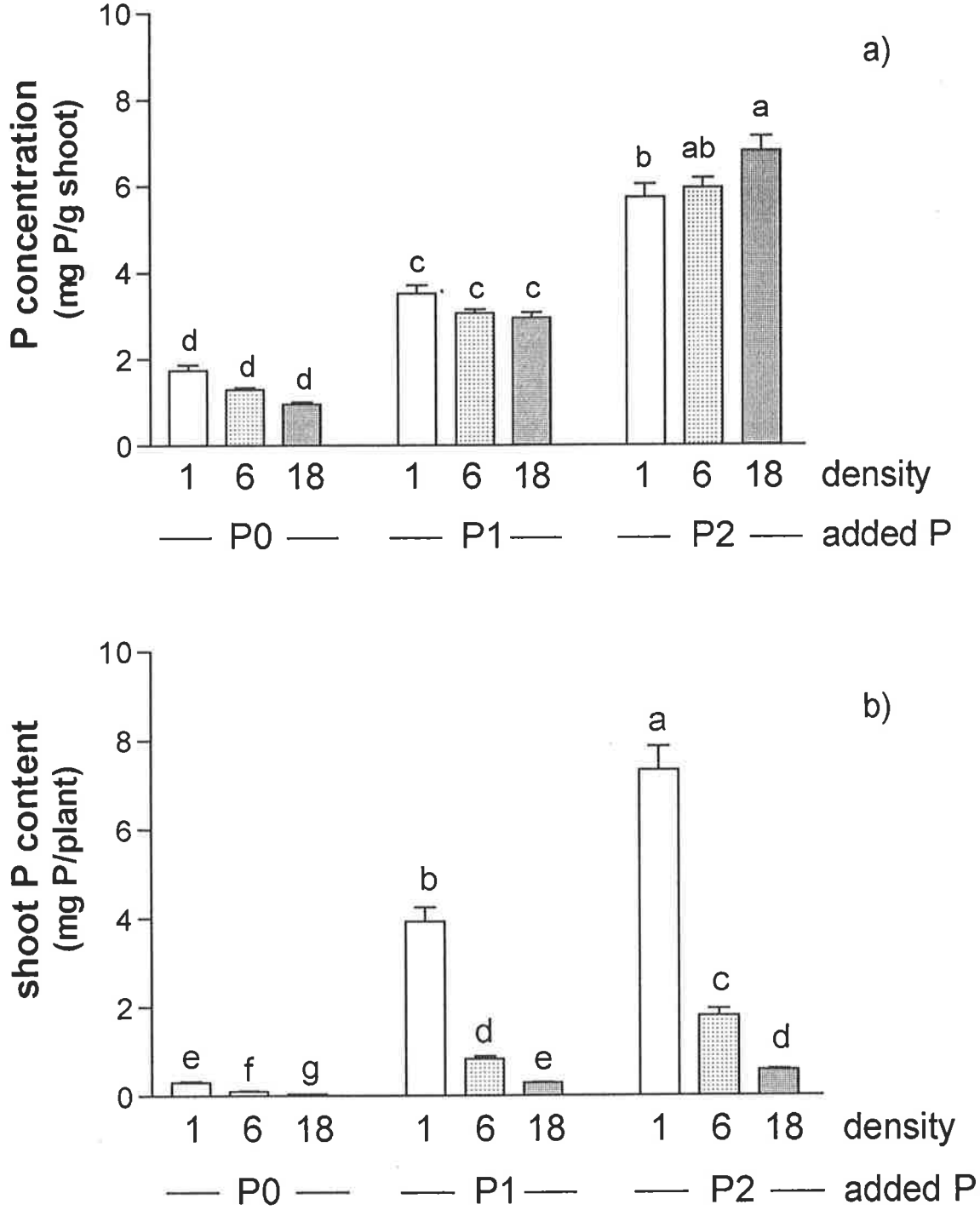


Figure 3.2. Shoot P concentration (a) and shoot P content of individual plants (b) of *Rhodanthe chlorocephala* ssp. *rosea,* grown at different densities, 1, 6 and 18 plants per pot, and at three levels of added P, P0, P1 and P2 (no added P, 5 ppm and 15 ppm of added P, respectively). Different letters indicate significant differences between means (Tukey, $p \leq 0.05$). Data are means and SE, $n = 5$.

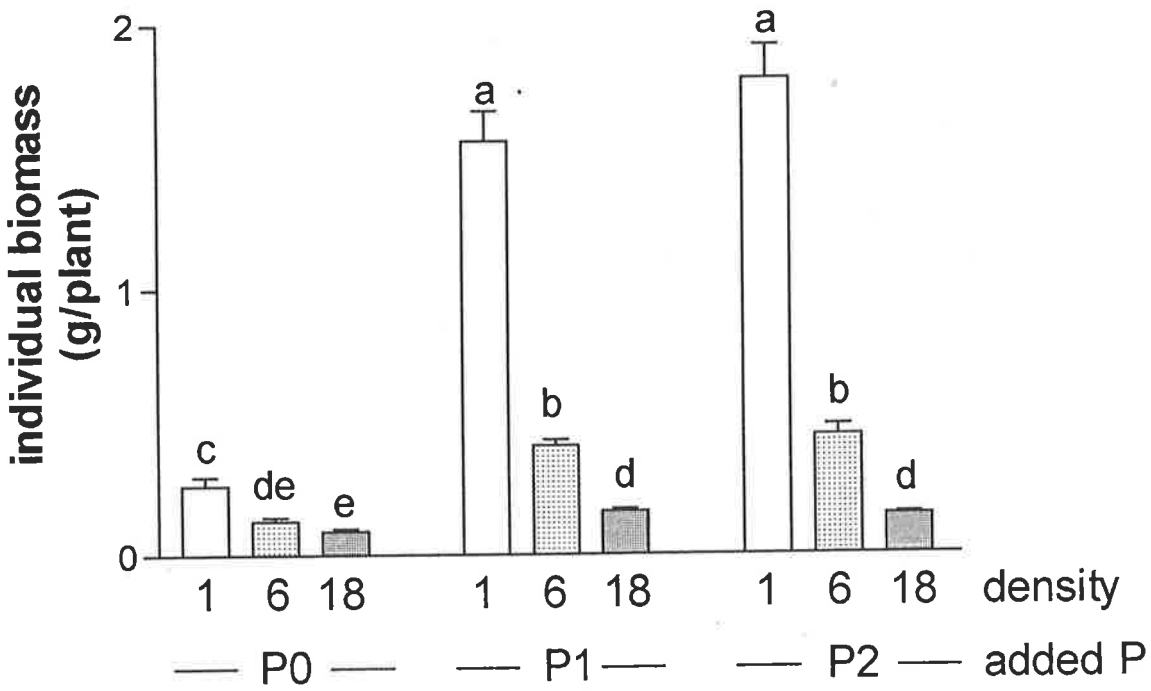


Figure 3.3. Biomass of individual plants of *Rhodanthe chlorocephala* ssp. *rosea*, grown at different densities, 1, 6 and 18 plants per pot, and at three levels of added P, P0, P1 and P2 (no added P, 5 ppm and 15 ppm of added P, respectively). Different letters indicate significant differences between means (Tukey, $p \leq 0.05$). Data are means and SE, n = 5.

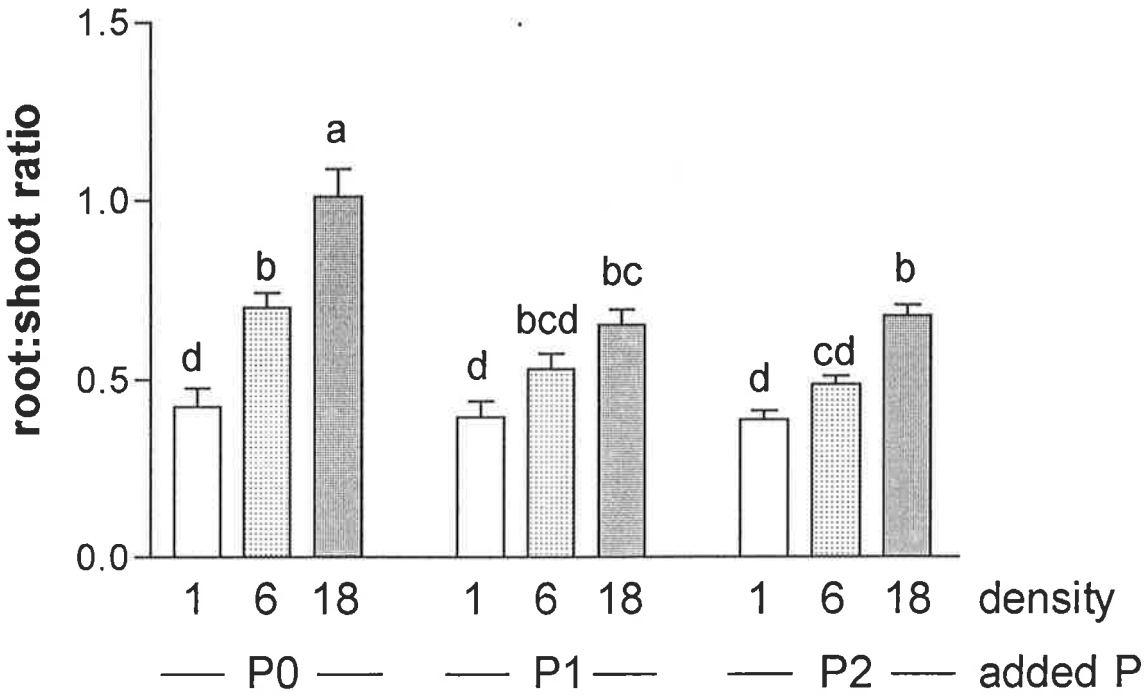


Figure 3.4. Root:shoot ratio of plants of *Rhodanthe chlorocephala* ssp. *rosea* grown at different densities, 1, 6 and 18 plants per pot, and at three levels of added P, P0, P1 and P2 (no added P, 5 ppm and 15 ppm of added P, respectively). Different letters indicate significant differences between means (Tukey, $p \leq 0.05$). Data are means and SE, $n = 5$.

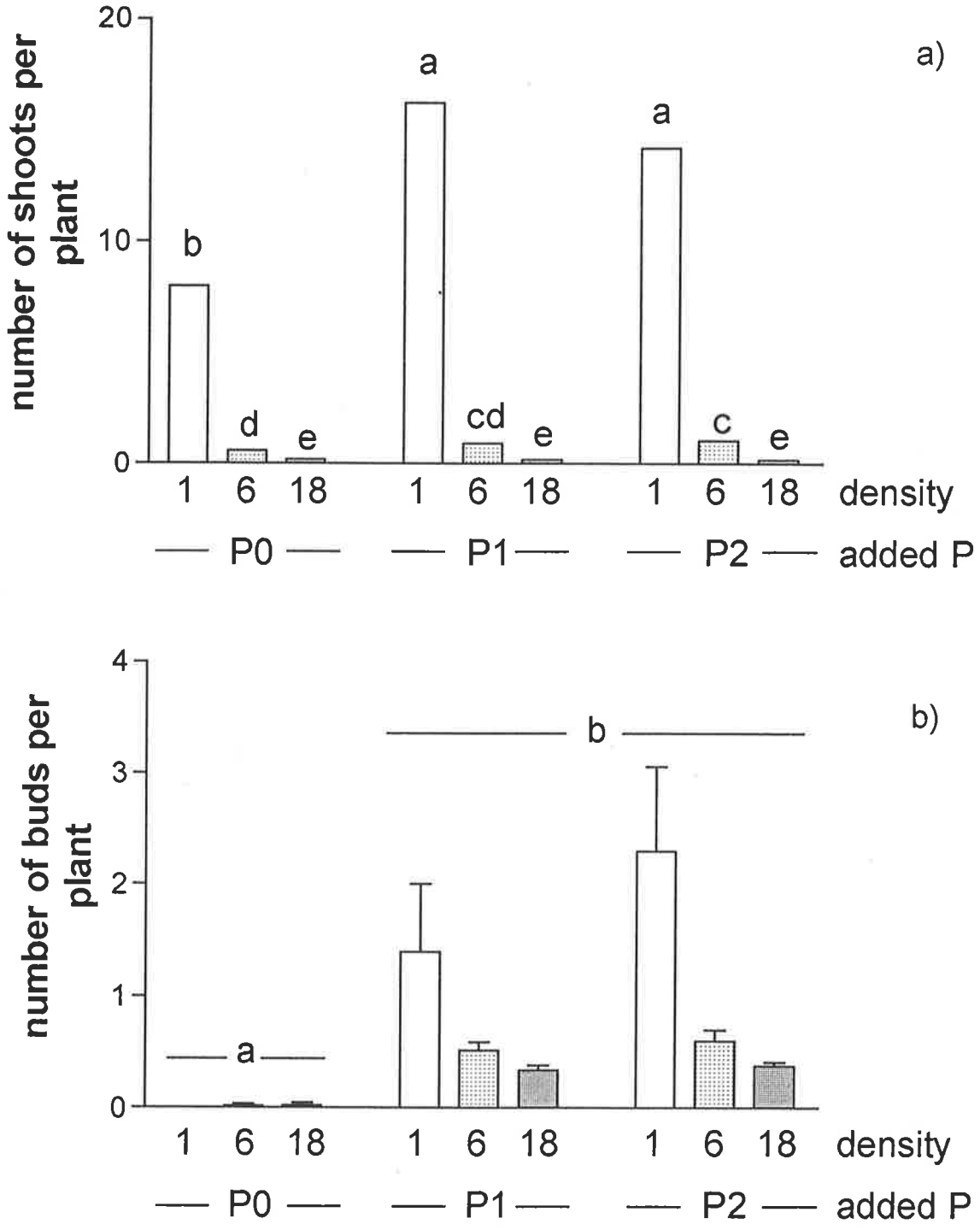


Figure 3.5. Number of shoots per plant (a) and number of buds per plant (b) of plants of *Rhodanthe chlorocephala* ssp. *rosea*, grown at different densities, 1, 6 and 18 plants per pot, at three levels of added P, P0, P1 and P2 (no added P, 5 ppm and 15 ppm of added P, respectively). Different letters indicate significant differences between means (Tukey, $p \leq 0.05$). Data are means and SE, $n = 5$.

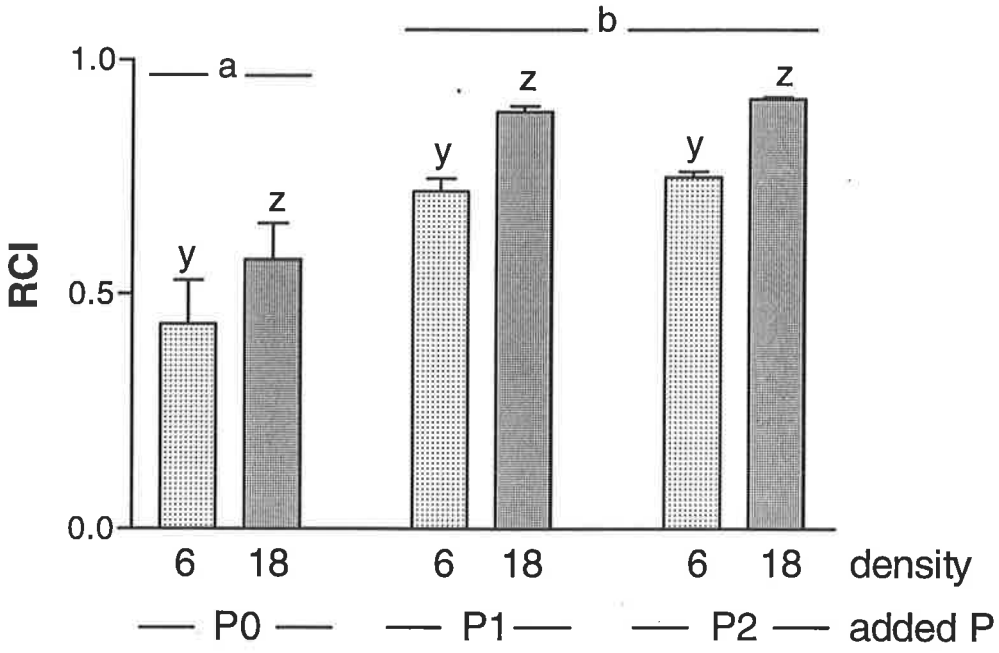


Figure 3.6. Relative competition intensity (RCI, see formula on text) of plants *Rhodanthe chlorocephala* ssp. *rosea*, grown at different densities, 1, 6 and 18 plants per pot, at three levels of added P, P0, P1 and P2 (no added P, 5 ppm and 15 ppm of added P, respectively). Different letters indicate significant differences between means (Tukey, $p \leq 0.05$). Data are means and SE, n = 5.

Table 3.1. Results of ANOVA testing the effects of mycorrhizal infection, addition of P and plant density on percentage of infection, shoot P concentration and individual shoot P content of plants of *Rhodanthe chlorocephala* ssp. *rosea*, inoculated or not with the mycorrhizal fungus *Gigaspora margarita*. Plants were grown at three different densities, and at three different levels of added P.

ANOVA/GLM		percentage infection			shoot P concentration			individual shoot P content		
transformations		arcsin SQRT(ratio)			no			log10(P content)		
factors	df	MS	F	P > F	MS	F	P > F	MS	F	P > F
phosphorus	2	0.9333	15.52	0.0001	178.32	500.72	0.0001	13.074	795.02	0.0001
mycorrhizas	1	0.0258	0.43	0.5144	0.1227	0.34	0.5592	0.0409	2.49	0.1193
P * M	2	0.0191	0.32	0.7284	0.1834	0.52	0.5998	0.0090	0.54	0.5824
density	2	0.0183	0.30	0.7385	0.3857	1.08	0.3444	7.8249	475.83	0.0001
P * D	4	0.0955	1.59	0.1880	2.6061	7.32	0.0001	0.0672	4.09	0.005
M * D	2	0.0230	0.38	0.6842	0.6225	1.75	0.1818	0.0043	0.26	0.7704
P * M * D	4	0.0724	1.20	0.3176	0.7101	1.99	0.1052	0.0118	0.72	0.5842

Table 3.2. Results of ANOVA testing the effects of mycorrhizal infection, addition of P and plant density on individual plant biomass, root:shoot ratio, number of shoots per plant and number of buds per plant of plants of *Rhodanthe chlorocephala* ssp. *rosea*, inoculated or not with the mycorrhizal fungus *Gigaspora margarita*. Plants were grown at three different densities, and at three different levels of added P.

ANOVA/GLM		individual plant biomass			root:shoot ratio			shoots per plant			buds per plant		
transformations		log10(plant biomass)			no			rank			rank		
factors	df	MS	F	P > F	MS	F	P > F	MS	F	P > F	MS	F	P > F
phosphorus	2	2.8479	252.46	0.0001	0.3606	19.10	0.0001	1.3759	28.01	0.0001	19.757	59.38	0.0001
mycorrhizas	1	0.0279	2.47	0.1205	0.0424	2.24	0.1387	0.0285	0.58	0.4492	1.9238	5.78	0.0189
P * M	2	0.0126	1.12	0.3321	0.0004	0.02	0.9781	0.1466	2.99	0.0572	0.4609	1.39	0.2573
density	2	5.3006	469.88	0.0001	1.0829	57.35	0.0001	34.923	710.81	0.0001	0.6035	1.81	0.1709
P * D	4	0.2972	26.35	0.0001	0.0843	4.46	0.0029	0.5281	10.75	0.0001	0.5875	1.77	0.1459
M * D	2	0.0042	0.37	0.6896	0.0179	0.95	0.3929	0.0799	1.63	0.2043	1.9740	5.93	0.0042
P * M * D	4	0.0197	1.74	0.1506	0.0043	0.23	0.9213	0.0481	0.98	0.4248	0.6179	1.86	0.1281

Table 3.3. Results of ANOVA testing the effects of mycorrhizal infection, addition of P and plant density on the relative competition intensity (RCI, see formula on text) of plants of *Rhodanthe chlorocephala* ssp. *rosea*, inoculated or not with the mycorrhizal fungus *Gigaspora margarita*. Plants were grown at three different densities and at three different levels of added P.

ANOVA		RCI		
transformations		no		
factors	df	MS	F	P > F
phosphorus	2	0.6576	31.08	0.0001
mycorrhizas	1	0.0188	0.89	0.3509
P * M	2	0.0831	3.93	0.0270
density	1	0.3718	17.57	0.0001
P * D	2	0.0017	0.08	0.9236
M * D	1	0.0021	0.1	0.7524
P * M * D	2	0.0133	0.63	0.5372

3.5. Discussion

I could not study the effects of mycorrhizal infection on the performance of *R. chlorocephala* because of the high contamination of inoculated and non-inoculated pots by a wild mycorrhizal fungus. Importantly, a small increase in P availability increased plant performance and probably the fitness of *R. chlorocephala*. These beneficial effects were reduced by density. Furthermore, the addition of P increased the intensity of intraspecific competition.

The anatomy of the contaminant fungus was similar to the one described as *Glomus tenue* by Hall and Abbott (1981) and Abbott (1982). I observed the same structures in roots of *T. subterraneum* and *R. chlorocephala* grown in WA soils from the site of provenance of the seeds of pink everlasting. Since I used sterilised soil from the Adelaide Hills and not WA soils to prepare the soil/sand mix for the experiment, and I added only spores as inoculum, I assumed that seeds were the most likely source of contamination. The soil mix was autoclaved twice at 120 °C for 1 hour (with one day between treatments), and I surface sterilised the spores of *G. margarita* but not the seeds, because this treatment reduced the percentage of germination (see Chapter 2). In any case, the protocols to surface sterilise seeds and spores are similar (Smith and Dickson 1997), thus it is probable that surface sterilisation of the seeds would not kill spores of mycorrhizal fungi.

G. tenue has very small spores (< 12 μm) (Hall 1977; Wilson and Trinick 1982), which could have been easily dispersed by wind to the inflorescences of *R. chlorocephala* (Warner *et al.* 1985; Walker 1988; Smith and Read 1997). Once in the inflorescences the spores could have been trapped between the hairs of the achene. The presence of spores of mycorrhizal fungi in seeds had been reported only once before (Taber 1982). This author found that seeds collected from the soil by sieving had spores inside the teguments. She suggested that hyphae exploring the soil had penetrated the seeds and/or capsules and found a suitable environment to sporulate. However, the inflorescences of *R. chlorocephala* were cut from the plants and the seeds were not in contact with the soil. I am not aware of any other report of seeds as carriers of spores of mycorrhizal fungi, but it could be a common situation in natural conditions. This finding adds one more difficulty to the already very

complex research of the ecological role of mycorrhizal symbiosis in non-cultivated plants populations.

The relatively high proportion of roots infected by "fine endophyte" suggested that it probably has higher competitive ability than *G. margarita*. However due to the lack of proper controls I could not test this assumption. Studies of competition between mycorrhizal fungi had been done by different protocols with several different fungal and host species (Abbott and Robson 1981; Wilson and Trinick 1983; Wilson 1984; Lopez-Aguillon and Mosse 1987; Hepper *et al.* 1988; Pearson *et al.* 1993). Thus results from those studies are very difficult to compare (Hepper *et al.* 1988). In general when two fungi are evenly dispersed in the soil, both infect the root system, but the relative amounts of infection can be proportional to the numbers of propagules of each fungus (Wilson 1984). However the placement of the fungi in the soil and the fungal species interacting affects the outcome of competition (Lopez-Aguillon and Mosse 1987; Hepper *et al.* 1988). It was found that fungi evenly distributed displaced the inoculated ones (Hepper *et al.* 1988). However, in the same experiment one of the fungi, reported as having high competitiveness in other experiments, was unable to infect roots in the presence of other fungi (Hepper *et al.* 1988).

There are several mechanisms proposed to explain the exclusion of one fungus by another. There may be antagonism between hyphae in the rhizosphere or between hyphae within the root (Hepper *et al.* 1988). It is also possible that the host cells invaded by one fungus become immune to invasion by another (Hepper *et al.* 1988). Wilson (1984) suggested that if exclusion is due to a change in the physiological or nutritional status of the root, the relative efficiencies with which the fungi are able to utilise nutrients will be important. This was supported by the results of (Pearson *et al.* 1993), which showed that the outcome of competition between *Scutellospora calospora* and *Glomus sp.* depended upon their relative efficiency (carbon drain of the fungus relative to P supply) and the carbohydrate supply to the fungus by the plant. Competitive effects also depend upon the rate of formation of the infection points and upon the amount of infection formed per propagule (Wilson and Trinick 1983). *G. teme* is relatively slow in forming infection points

and has more infection per unit of inoculum (Wilson and Trinick 1983). Thus if roots are occupied by another fungus, *G. tenue* may be a poor competitor. However it may form large numbers of secondary points of infection (originated from a single hypha running along the outside of a root) (Wilson and Trinick 1983; Lopez-Aguillon and Mosse 1987), thus displacing the competitor at a later stage (Wilson and Trinick 1983). In my experiment with *R. chlorocephala*, if *G. tenue* was in the fruits, it might have had an initial advantage. Since the seeds were pre-germinated in water agar, the seedlings were probably already infected when planted (cf. Powell (1980)). The fungus could both pre-empt nutrients from the roots and spread more efficiently than *G. margarita* which does not form many secondary entry points (E.F. personal observation, Lopez-Aguillon and Mosse (1987).

The reduction of mycorrhizal infection of *R. chlorocephala* (proportion of magnified intersections with mycorrhizal infection) by addition of P agreed with the knowledge that the infection is regulated by the nutritional status of the root, which depends in part of the availability of soil nutrients (Koide and Li 1990; Hetrick 1989). Although, the negative effect of addition of P on the infection is generally found at relatively higher soil P concentrations (Baon *et al.* 1993; Nadian *et al.* 1996), the sensitivity of the response differs in different hosts (Smith and Read 1997). Mycorrhizal infection might be very low both at very low and very high soil P concentrations. At very low levels of P (and what is low or high depends on the species considered) plant growth is limited and there is not a potential benefit for the plant from forming mycorrhizal associations (Koide and Li 1990). At increasing soil P availability the infection increases until a certain value where phosphorus availability starts to affect mycorrhizal development (extramatrical hyphae or arbuscule development) and/or roots grow at a higher rate than the fungus, thus the ratio root length infected/root length decreases (Jasper *et al.* 1979; Abbott *et al.* 1984; Gianinazzi-Pearson and Gianinazzi 1989).

The percentage infection was not modified by an increase in plant density. This result supports previous findings (Abbott and Robson 1984; Koide 1991a; Allsopp and Stock 1992a; West 1996). However, there have been some exceptions where increasing densities reduced the percentage of root length infected (Bååth and Hayman 1984; Chapter

4). Therefore, there is not a distinct pattern which allows a clear understanding of the effects of density on the percentage of infection.

The response of *R. chlorocephala* to addition of P reflected some of the characteristics of wild species from infertile soils pointed out by Chapin (1980). The increase in tissue P concentration at increasing amounts of added P was not expressed by the same relative increase in plant biomass. It seems that once plants reached their metabolic threshold, they accumulated P (luxury consumption) (Chapin 1980; Koide 1991b). This P could subsequently be allocated to reproductive organs. In fact, plants that received more P had more buds per plants. Although I harvested plants when buds were only incipient, I observed in additional (unharvested) pots that the differences in number of inflorescens persisted until flowering was completed. Similar results were found previously in experiments that compared the response to addition of P of Australian non-cultivated plants and cultivated plants (Specht and Groves 1966; Christie and Moorby 1975). As nutrient availability increased, species from fertile sites (e.g. *Cenchrus ciliaris* and *T. subterraneum*) responded with great increases in growth rates, whereas species from infertile sites (e.g. *Thyridolepis mitchelliana*, *Astrebla elimoides*, *Acacia suaveolns*, *Casuarina pusilla*, *Banksia ornata*) showed less growth response and an increase in tissue concentrations (Specht and Groves 1966; Christie and Moorby 1975; Chapin 1980). Plants from infertile areas have less P demand and hence their P deficit is lower and they have a smaller response to P additions. An increase in biomass as P supply increases would lead to continuous growth and bigger plants which will require more nutrients and water (Koide 1991b). This strategy is obviously not desirable in plants from infertile sites. Furthermore, the luxury accumulation of P might easily lead to enhanced P translocation to reproductive organs in plants from these infertile sites (Specht and Groves 1966) since they have a low proportion of structurally-bound P (Specht and Groves 1966; Chapin 1980).

Plants of *R. chlorocephala* maintained the same root:shoot ratio despite addition of P. When mineral nutrients are limiting a shift in carbohydrate allocation to roots is expected. Hence, an increase in nutrients available to the plant should result in lower root:shoot ratios (Ericsson 1995). However, plants from infertile sites do not have such

plastic responses to nutrient additions (Chapin 1980), as demonstrated for native Australian tree seedlings (Barrow 1977) and Australian native grasses (Christie and Moorby 1975). *R. chlorocephala* responded in this respect as a species characteristic of stressful environments, with low plasticity in allocation pattern (Grime 1977; Chapin 1980). However, there is the possibility that plant growth was limited by a nutrient (nutrients) other than P.

Most of the adaptations mentioned above to infertile soils have been studied in perennial plants. There is some information for annual plants from infertile soils to be highly responsive to addition of nutrients (Witkowski 1989; Carpenter *et al.* 1990). Nevertheless, I found that *R. chlorocephala*, an annual plant, with a short life cycle and probably a higher growth rate than the perennials from those soils, also presents characteristics associated with low-nutrient soils.

The biomass of individual plants decreased as density increased, which is expected if competition for resources occurs. However, when P was added, the RCI increased. Individual *R. chlorocephala* plants at the intermediate level of added P (5 ppm) were about 5-fold bigger than individual plants that did not receive extra P. As density increased, plants competed for P at all levels, but the difference in biomass between single and crowded plants was bigger when P was added. This counter-intuitive result, indicating greater intensity of competition at higher resource levels, supports other findings (Wilson and Newman 1987; Wilson 1988b; Gurevitch *et al.* 1990) and indirectly supports the hypothesis that competition is less important in infertile soils (Grime 1979; Grace 1990).

The fact that an increase in biomass or reproductive output of *R. chlorocephala* could be achieved only at low levels of P addition is the fundamental importance for revegetation projects and cultivation of native plants. Thus, it is important to chemically analyse soils to assess their P concentrations before fertilisation is advised (Maier *et al.* 1994). If levels of P are low, it is possible that native species will respond to mycorrhizal infection in the same way as to addition of P. It has being shown that plants that did not have a growth response to mycorrhizal infection might accumulate P and use it for the formation of reproductive organs (Mullen and Schmidt 1993) or to produce more vigorous

offspring than non-mycorrhizal plants (Koide and Lu 1995). Thus the introduction of mycorrhizal fungi in revegetation sites or the inoculation of plants in cultivated areas may produce similar effects to addition of P, or may enhance the effects of small P additions. Unfortunately I could not study the effects of the symbiosis on *R. chlorocephala* because of the lack of proper controls. Since Asteraceas are very common in Australian pristine ecosystems and most of them are infected by mycorrhizal fungi (Warcup and McGee 1983), the need for more investigations in this field is obvious. I suggest an experiment to test if spores of wild mycorrhizal fungi are present in seeds of *R. chlorocephala*. This experiment should use of *R. chlorocephala* and *T. subterraneum* (control for contamination of the growth media with spores of *G. tenue*) as indicators of the presence of spores. Seeds of both species should be germinated in sterile sand or water agar and their roots screened for mycorrhizal infection after two or three weeks.

Chapter 4

Interactive effects of mycorrhizal symbiosis, intraspecific competition and resource availability on *Trifolium subterraneum* L. cv. Mt. Barker

4.1. Introduction

It is well established that vesicular-arbuscular mycorrhizal (VAM) symbiosis increases plant phosphorus (P) uptake (Abbott and Robson 1977a; Smith and Read 1997). Studies of the effects of the symbiosis on single plants have shown that the increased P uptake leads to increased plant growth and that the availability of resources such as light and P modify this response (Smith and Read 1997). High levels of available soil P (Koide and Li 1990) and low levels of irradiance (Tester *et al.* 1985) reduce the host response. Furthermore, the plant biomass response to the symbiosis is greater when the availability of P limits plant growth. However, there is scant information about how these effects at the individual level affect processes at the population level (Addicott 1986).

It has usually been assumed that at high plant densities, an increase in the supply of a limiting resource decreases the intensity of plant competition for that resource (Chapin and Shaver 1985; Goldberg 1990). However, recent experimental work and reviews question this assumption. In pot experiments, regulating fertility and available space (Gurevitch *et al.* 1990), and in natural gradients of nutrient and light (Belcher *et al.* 1995), it was found that competition intensity was not influenced by different levels of available resources. A review of several studies on root and shoot competition showed that there was little evidence to support the assumption that adding resources reduces competition (Wilson 1988b). Furthermore, there is evidence showing that competition intensity could increase when limiting resources are increased (Wilson and Newman 1987; Wilson 1988b). How habitat productivity (mainly soil resources) affects the intensity of competition is also the subject of intense debates (Thompson 1987; Tilman 1987a; Grace 1990; Grace 1993).

The effects of competition depend upon the kind of the resource limiting growth. Competition for soil resources (nutrients or water) may lead to a reduction in mean plant size (symmetric competition) while competition for light may increase size variation by dominance and suppression (asymmetric competition) (Weiner 1990). Therefore, it could be expected that mycorrhizal symbiosis will change the competitive interactions between individuals within a population through an increase in nutrient supply to the plant and a consequent increase in growth of individuals. It has also been proposed that the hyphal connections between plants might allow a more even distribution of nutrients, leading to a reduction in size inequality (Shumway and Koide 1995) and probably a reduction in the intensity of intraspecific competition. These changes would also affect the individuals size distribution and fitness.

Although there are few data on the effect of this symbiosis on plant populations, some experiments have shown that increasing plant densities reduce the plant response to mycorrhizal infection measured as an increase in growth and/or P content (Bååth and Hayman 1984; Koide 1991a; Hartnett *et al.* 1993) and that at high densities mycorrhizal plants have higher size or reproductive inequality (Allsopp and Stock 1992a; Shumway and Koide 1995). The few studies of the effect of the symbiosis on plant competition have mainly addressed interspecific interactions of species with different responsiveness to mycorrhizal infection and/or root structure. In most of them, mycorrhizal symbiosis increased competitive ability of plants with higher response to the infection or less extensive root systems (Fitter 1977; Hall 1978; Grime *et al.* 1987; Koide *et al.* 1988a; Allsopp and Stock 1992a; Hartnett *et al.* 1993; Shumway and Koide 1995), but this advantage decreased with increasing plant densities (Crush 1995). When two species of similar responsiveness were used, VAM infection increased competition (West 1996). Although in their respective monocultures West (1996) found that there was no interaction between mycorrhizal infection and competition, a further analysis of the data showed that the intraspecific component of the competitive interaction between the two species increased when plants were mycorrhizal (Watkinson and Freckleton 1997).

I performed glasshouse experiments using *Trifolium subterraneum* L. cv. Mt. Barker as the host plant and *Gigaspora margarita* Becker and Hall as the fungal symbiont to answer three main questions:

1) How does mycorrhizal infection affect the intensity of competition at a low level of available P?

2) Is this effect modified by a reduction of light availability?

3) How does the symbiosis affect plant size distribution?

I selected *T. subterraneum* as the host because the response of single plants of this species to mycorrhizal infection under different levels of P and light has been studied in detail (Smith 1982; Tester *et al.* 1985; Tester *et al.* 1986; Nadian *et al.* 1996).

4. 2. Materials and methods

4.2.1. Effects of mycorrhizas and density (experiment 1)

This experiment had a factorial design with complete randomised blocks and included 2 factors: plant density at 6 levels (1, 3, 6, 10, 14, and 18 plants per pot) and mycorrhizas at 2 levels (pots with mycorrhizal inoculum (M) and pots without mycorrhizal inoculum (NM)). Each of the 12 resulting combinations was replicated 5 times. I grew the plants in autoclaved soil collected from a Mediterranean type Eucalyptus forest (Waite Hill Land, Adelaide) with a very low concentration of extractable P (Colwell 1963), 6 $\mu g\ g^{-1}$ of $NaHCO_3$ extractable P and a neutral pH (pH 6.4 in 10 mM $CaCl_2$). This amount of available P has been reported to limit growth of *T. subterraneum* in previous pot experiments (Nadian *et al.* 1996). Autoclaving this soil did not reduce growth of *T. subterraneum* (Chapter 2).

I planted the seedlings of *T. subterraneum* in 11 cm diameter pots containing 470 g of soil. Immediately before planting, I inoculated half of the pots with spores of *G. margarita* at an average of 10 spores/plant and added a dense suspension of *Rhizobium trifolii* to all the pots to ensure effective nodulation and N_2 fixation (Smith and Smith 1981).

I grew the plants in a growth room set at 14 h light and 10 h darkness, with a light intensity in the photoperiod of 400 $\mu m\ m^{-2}\ s^{-1}$. Day time temperatures were 22 °C, and

night time temperatures were 15 °C. I watered the pots to 12% w/w every second day with deionised water and applied 2.5 ml per week of a nutrient solution lacking N and P (Smith and Smith 1981) (5 ml were added the first week) to ensure plant growth was only limited by P.

After 8 weeks I harvested the experiment and separated shoots and roots. I washed and weighed the roots and took one subsample from each of three replications, which was then stored in 50% ethanol for estimation of mycorrhizal infection and root length. I dried the remaining material (shoots and roots minus the subsamples) at 70 °C for 48 h, and weighed it. I washed the root subsamples, cleared them with 10% KOH and stained them with 0.05% trypan blue in lactoglycerol (a modification of the method of (Phillips and Hayman 1970)). I determined root length and mycorrhizal root length using a line intersect method (Giovanetti and Mosse 1980).

I determined total P concentrations in dried, ground tissue by inductively coupled plasma atomic emission spectroscopy (ICP-AES) after digestion of the ground plant material (shoots or roots) in a mix of concentrated perchloric and nitric acids (1:6 v/v).

The response to mycorrhizal infection was calculated as the relative biomass response (RBR):

$$RBR = \frac{(M - NM)}{(NM)} \qquad (1)$$

where NM is the biomass of individual non-mycorrhizal plants and M is the biomass of individual mycorrhizal plants, from corresponding pots within each block.

The relative competition intensity (RCI) was calculated as:

$$RCI = \frac{(S - C)}{(S)} \qquad (2)$$

(adapted from (Grace 1993)) where S is the biomass of plants grown singly and C is the biomass of individual plants growing in dense pots, from corresponding pots within each block.

4.2.2. Effects of mycorrhizas, density and shading (experiment 2)

The design of this experiment was a split plot factorial which included 4 factors: plant density (at 4 levels: 1, 6, 14 and 24 plants per pot), mycorrhizas (at 2 levels: pots with mycorrhizal inoculum (M) and pots without mycorrhizal inoculum (NM)), light (at 2 levels: 400 $\mu m\ m^{-2}\ s^{-1}$ (L, full light) and 180 $\mu m\ m^{-2}\ s^{-1}$ (S, shade); and harvest (at 2 levels: first harvest at 4 weeks (H1) and second harvest at 8 weeks (H2). There were eight subplots, 4 under shade and 4 under full light (randomly assigned). Within each of them I randomised density, harvest and mycorrhizal levels. Hence, each combination of levels had 4 replications.

Soil, host plant, and mycorrhizal fungus, as well as the planting, watering and fertilising procedures, were the same as in experiment 1. I grew the plants at non-saturating irradiances of either 400 or 180 $\mu m\ m^{-2}\ s^{-1}$ ((Hesketh and Moss 1963) cited in (Tester *et al.* 1985; Tester *et al.* 1986)), provided by incandescent and fluorescent lamps. I reduced light intensity by placing a frame with a plastic mesh on top of the corresponding subplots.

After the first harvest I weighted shoots and roots individually to determine size distributions. However, at the second harvest the roots were intermingled and separation was impossible. I took three root subsamples per pot at each harvest. I determined mycorrhizal infection, P concentration, biomass plant response and intensity of competition as in experiment 1.

4.2.3. Data analysis

I analysed the data using ANOVA and the Ryan-Einot-Gabriel-Welsch Multiple Range (REGWQ) test to compare means (Day and Quinn 1989). I transformed the data when necessary to improve normality (SAS 1986).

In experiment 2, principal component analysis was used as an exploratory tool to study if the differences or similarities between size distributions were associated with density, infection or light. It was performed using relative frequencies of size classes (biomass classes) as variables. Within each treatment the distributions were centred around the

modal size class (to avoid bias for different plant sizes). Treatments with 1 plant per pot were not included in the analysis because the number of individuals was too low to yield a frequency distribution. Coefficient of variation (CV), Skewness, Kurtosis and Gini coefficient were calculated for grouped M and NM pots of densities 6 and 24 to provide a complete description of their size distributions (Hara 1988), and the Kolmogorov-Smirnov test was used to compare these distributions (Sokal and Rohlf 1981).

4.3. Results

4.3.1. Effects of mycorrhizas and density

The percentage of root length infected by mycorrhizal fungi in inoculated plants was about 40% (0% for non-inoculated plants), and independent of plant density (Fig. 4.1, ANOVA after arcsin ($\sqrt{}$ratio) transformation, $p = 0.8142$).

Mycorrhizal infection increased shoot and root P concentrations independently of density. Density did not generally modify shoot or root P concentration (Fig. 4.2a, Table 4.1).

Mycorrhizal infection and plant density increased whole plant P content per pot (data not shown, ANOVA, $p \leq 0.0001$). Whole plants and shoots reached the maximum P content with 10 plants per pot while roots had their maximum with 3 plants per pot (REGWQ, $p \leq 0.05$).

The effects of mycorrhizal infection and plant density on the P content of shoots and roots of individual plants (plant P content per pot/number of plants per pot) were not independent (Fig. 4.2b, Table 4.1). Increasing densities markedly reduced the P content of shoots and roots of individual mycorrhizal plants while only slightly reduced the P content in non-mycorrhizal plants. Phosphorus content was always higher in shoots of individual mycorrhizal plants than in non-mycorrhizal ones, whereas the P content of roots of individual mycorrhizal plants was higher only at low densities (1 and 3).

Mycorrhizal infection and plant density had interdependent effects on whole plant, root, and shoot biomass per pot (data not shown, ANOVA, significant interactions, $p \leq 0.0001$). Mycorrhizal plants had higher whole plant biomass and shoot biomass per pot

than non-mycorrhizal plants at all densities. However, their root biomass was higher only at low densities (1-6). At higher densities (10-18) there were not significant differences between root biomass of mycorrhizal and non-mycorrhizal plants (REGWQ, $p \leq 0.05$).

Mycorrhizal infection and plant density had interdependent effects on individual whole plant, shoot and root biomass (plant biomass per pot/number of plants per pot) (Fig. 4.3, Table 4.2). Increasing densities markedly reduced the biomass of individual mycorrhizal plants but only slightly affected the biomass of individual non- mycorrhizal plants. Mycorrhizal infection increased individual whole plant and shoot biomass only at densities 1,3 and 6 and individual root biomass only at densities 1 and 3.

The root:shoot (R:S) ratio of mycorrhizal plants was lower than that of non-mycorrhizal plants (Fig. 4.4, Table 4.2) and the effects of plant density were inconsistent.

The effects of mycorrhizal infection and plant density on the root length of individual plants (root length per pot /number of plants) were also interdependent (data not shown, ANOVA, $p \leq 0.0009$). Only single mycorrhizal plants had longer roots than non-mycorrhizal plants since density reduced the root length of mycorrhizal plants. Increasing densities did not affect the root length of non-mycorrhizal plants (REGWQ, $p \leq 0.05$).

The increase in biomass due to mycorrhizal infection (RBR) was negatively related to plant density (Fig. 4.5, ANOVA after $\log_{10}$ (RBR+1) transformation, $p \leq 0.0001$): single plants had an increase in biomass of about 800% but at 3 plants per pot the response was reduced to less than 300% and was nil at higher densities.

Mycorrhizal infection had a significant positive effect on relative competition intensity (RCI) (Fig. 4.6, ANOVA, $p \leq 0.0001$), whereas plant density had a marginal effect on the intensity of competition (ANOVA, $p = 0.0613$).

4.3.2. Effects of mycorrhizas, density and shading

4.3.2.a. *Harvest 1*

Plants growing in inoculated pots were infected by mycorrhizal fungi at 4 weeks, with percentage infection around 30% regardless of light intensity (only roots from densities 6 and 24 were screened because the material from density 1 was damaged, probably due to

the accidental use of KOH with a concentration higher than 10%) (Fig. 4.7, ANOVA after arcsin (√ratio) transformation, $p = 0.5753$). At this harvest there was no significant biomass response (Fig. 4.8a). However, a competitive effect (RCI different from zero, Fig. 4.8b) was apparent in almost all mycorrhizal treatments and in shaded non-mycorrhizal treatments at high densities. Mycorrhizal plants had higher RCI than non-mycorrhizal plants independently of density and light treatments (ANOVA, $p \leq 0.0009$).

4.3.2.b. *Harvest 2*

Plant density had a negative effect on percentage infection that was independent of light (Fig. 4.9, ANOVA after arcsin (√ratio) transformation, $p \leq 0.0001$). Single plants had higher percentage infection (about 60%) than plants at higher densities (about 35%). Shading did not have any effect on the percentage infection (ANOVA, $p = 0.6964$).

The effects of mycorrhizal infection, plant density, and light on root P concentration were interdependent (Fig. 4.10a, Table 4.3). Light had an independent effect on shoot P concentration, while mycorrhizal infection and plant density had interdependent effects on this variable (Table 4.3). Mycorrhizal plants at densities 1 and 6 had higher shoot and root P concentrations than non-mycorrhizal plants, but at higher densities there were no significant differences. Shading increased root P concentration of single mycorrhizal plants only, and increased shoot P concentration independently of mycorrhizal infection or plant density.

Mycorrhizal infection, plant density and light had interdependent effects on whole plant, shoot and root P content of individual plants (Fig. 4.10b, Table 4.3). Mycorrhizal infection increased individual whole plant P only at densities 1 and 6. Increasing densities reduced P content of individual plants in mycorrhizal treatments, and had no effect on the P content of individual non-mycorrhizal plants. Shading only reduced P content of single plants. Individual shoot and root P followed a similar pattern.

The effects of mycorrhizal infection, plant density and light on root biomass per pot were interdependent (data not shown, ANOVA, significant interactions M*D, $p = 0.0114$; M*L, $p = 0.0381$; L*D, $p = 0.0271$). Mycorrhizal infection and plant density also had

interdependent effects on shoot biomass per pot (ANOVA, significant interaction, $p = 0.0076$), whereas light had no effect on this variable (ANOVA, $p = 0.1327$). Shading reduced root biomass per pot of mycorrhizal plants at all densities and non-mycorrhizal plants at high densities. Mycorrhizal plants had higher shoot biomass per pot at increasing densities, independently of light (REGWQ, $p \leq 0.05$).

The effects of mycorrhizal infection, plant density and light on individual biomass of whole plants, shoots and roots were interdependent (Fig. 4.11, Table 4.4). Under both levels of light mycorrhizal infection increased individual plant biomass only at low densities (1 and 6). Increasing densities reduced the individual biomass of mycorrhizal plants but did not modify the individual biomass of non-mycorrhizal plants. Shading reduced biomass of mycorrhizal plants only at the lowest density, and had no effect at higher densities or on non-mycorrhizal plants. Individual shoot and root biomass followed similar patterns.

Mycorrhizal infection, plant density and light had interdependent effects on the root length of individual plants (data not shown, ANOVA, significant interaction M*D, $p \leq 0.0001$ and significant interaction L*D, $p \leq 0.03$). The root length of single mycorrhizal plants was higher than that of single non-mycorrhizal plants, and decreased as density increased (ANOVA, significant interaction, $p \leq 0.0001$,). In contrast, the root length of individual non-mycorrhizal plants was unaffected by increasing densities (REGWQ, $p \leq 0.05$). Single shaded mycorrhizal and non-mycorrhizal plants had shorter roots than individual plants grown at higher densities (REGWQ, $p \leq 0.05$).

The effects of mycorrhizal infection and plant density on root:shoot ratio were interdependent (Fig. 4.12, Table 4.4), as also were the effects of light and plant density (Fig. 4.12, Table 4.4). Single mycorrhizal plants had lower root:shoot ratios than mycorrhizal plants at other densities and all non-mycorrhizal treatments. Shading reduced root:shoot ratios at high densities independently of mycorrhizal status.

The relative biomass response (RBR) to mycorrhizal infection was different from zero at all densities, except densities 24 under full light and 6 under shade (Fig. 4.13). Density had a negative, independent effect on RBR (ANOVA, after $\log_{10}$ (100+RBR) transformation, $p \leq 0.0001$): single mycorrhizal plants had an average RBR of 600%, which

was reduced at 6 plants per pot to 100% and to almost zero at higher densities. Shading had no effect on biomass response to mycorrhizal infection (ANOVA, $p = 0.3126$).

Relative competition intensity (RCI) was larger than zero at all densities and both light intensities in mycorrhizal treatments and at 24 plants per pot in non-mycorrhizal, shaded pots (Fig. 4.14). Light, mycorrhizal infection and plant density had independent effects on RCI (ANOVA, $p = 0.0069$, $p \leq 0.0001$, and $p = 0.0038$, respectively). Mycorrhizal and high density treatments had higher RCI, while, shading decreased the intensity of competition.

The differences between size distributions were associated with mycorrhizal infection and plant density and seemed to be independent of light intensity (Fig. 4.15). The first axes of the principal component analysis (PC1 and PC2) explained 79% of the variation in size distribution between populations (Fig 4.14, Table 4.5). The higher eigenvectors corresponded to the size classes closest to the modal class (Table 4.5). This allowed the separation of distributions with fewer individuals in the modal class (6ML-6MS) from the more leptokurtic ones. Shading treatments did not allow differentiation of any of the distributions. Within each density group, shaded and non-shaded plants were close together. All the distributions from density 14 were very close together as well. Thus I selected only 4 distributions from treatments under full light, 6M, 6NM, 24M and 24NM, for further analysis (Fig 4.16). The size distribution of mycorrhizal plants growing at 6 plants per pot was significantly different to the rest (Kolmogorov-Smirnov Two-Sample test, $D_{0.001,\ 12} = 0.53422$, (Sokal and Rohlf 1981). The probability used was equivalent to 0.05, as for a Bonferoni correction for multiple pair comparisons (SAS 1985). The distribution of sizes of mycorrhizal plants at density 6 was more heterogeneous and characterised by a higher proportion of plants in classes larger than the modal class. Non-mycorrhizal treatments and/or high density ones had more homogeneous size distributions. This distribution was apparently characterised by a higher CV and Gini coefficient, and lower skewness and kurtosis (Table 4.6). However no statistical analysis could be conducted due to lack of true replicates.

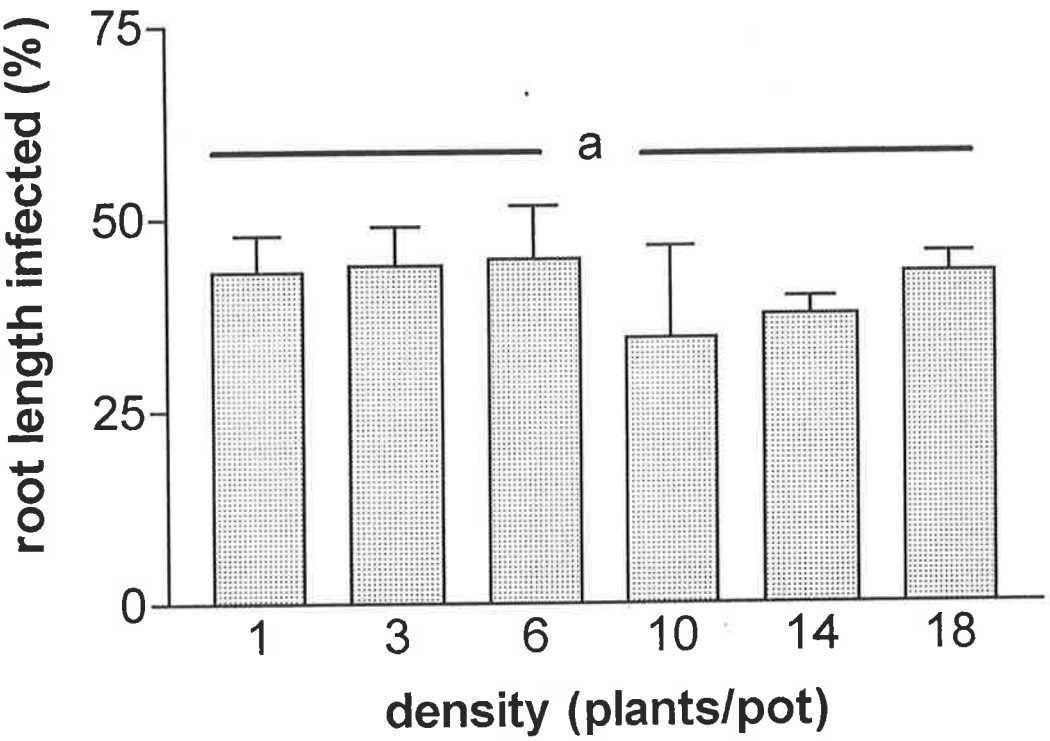


Figure 4.1. Percentage of root length infected of plants of *Trifolium subterraneum* grown at different densities. Different letters indicate significant differences between means (REGWQ, $p \leq 0.05$). Data are means and SE, $n = 5$.

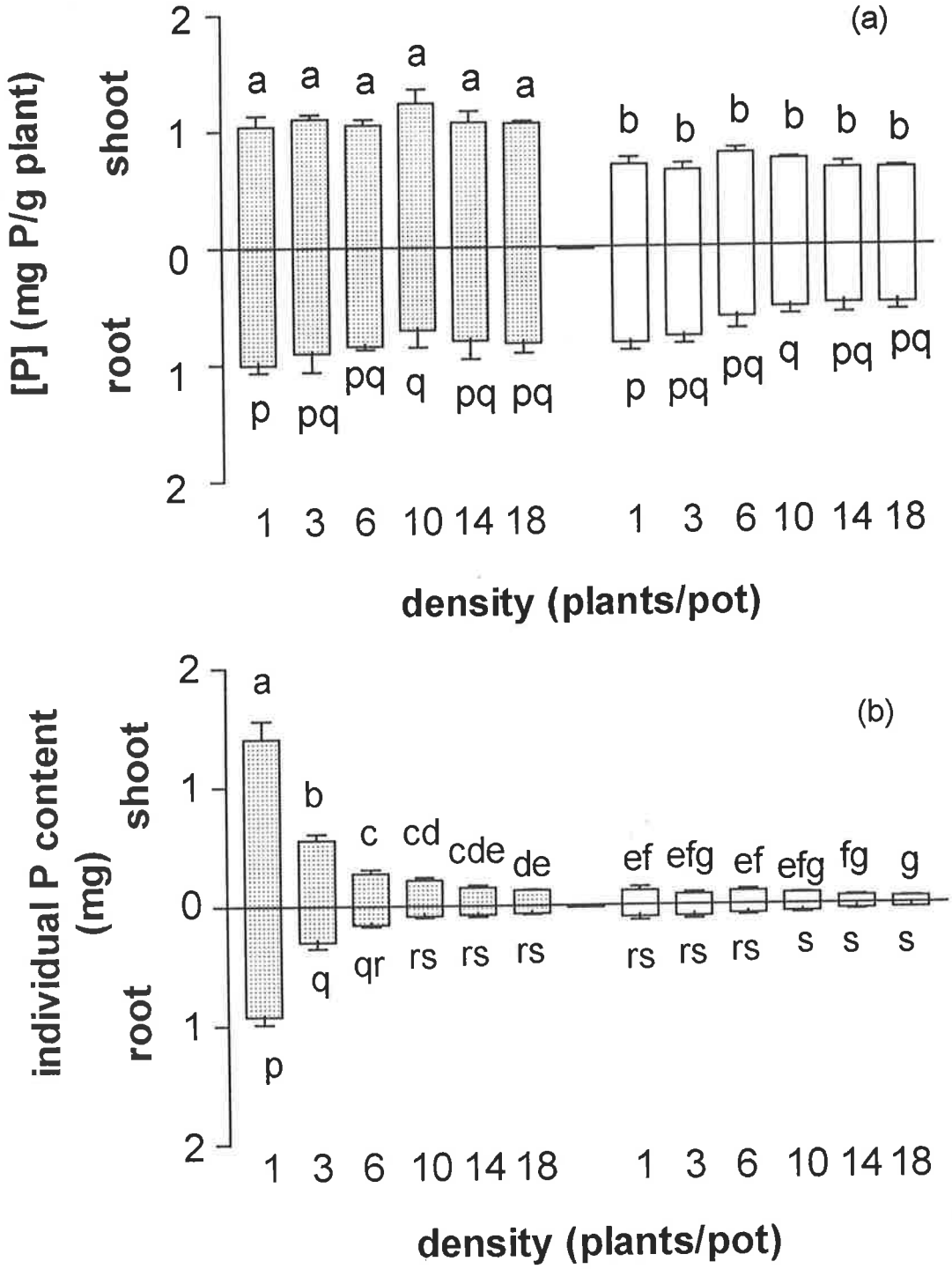


Figure 4.2. P concentration (a) and P content (plant P content per pot/number of plants per pot) (b) of shoots and roots of individual mycorrhizal (shaded bars) and non-mycorrhizal (clear bars) plants of *Trifolium subterraneum* grown at different densities. Different letters indicate significant differences between means (REGWQ, $p \leq 0.05$). Shoot and root data were analysed separately (different set of letters). Data are means and SE, $n = 5$.

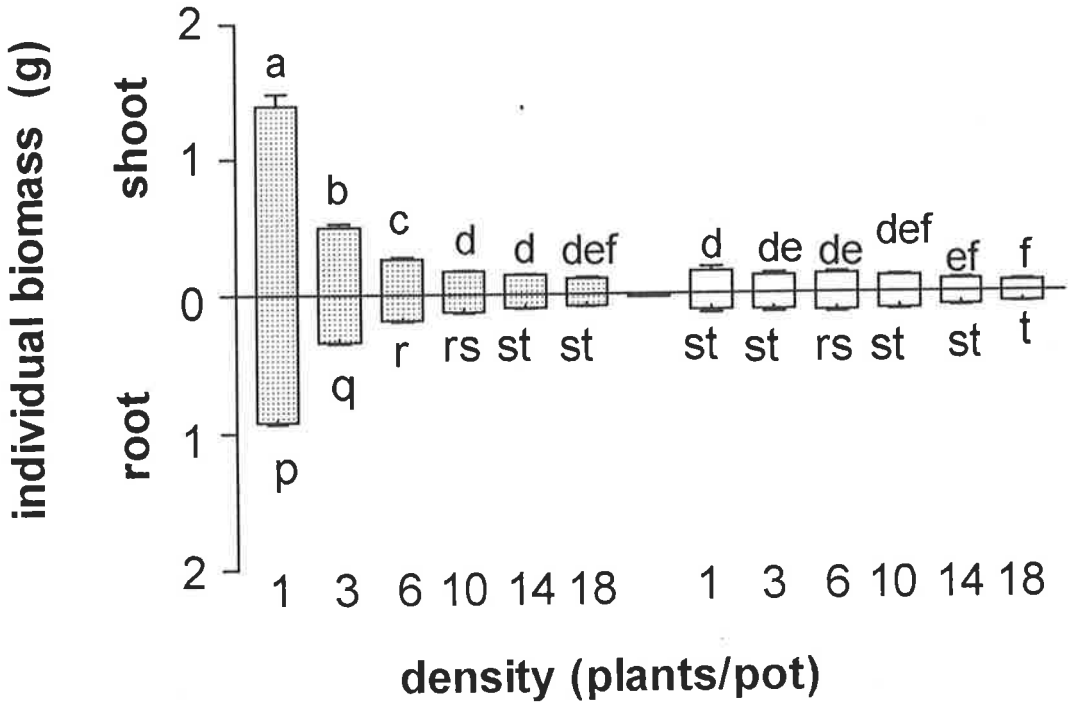


Figure 4.3. Individual shoot and root biomass (shoot or root biomass per pot/number of plants per pot) of mycorrhizal (shaded bars) and non-mycorrhizal (clear bars) plants of *Trifolium subterraneum* grown at different densities. Different letters indicate significant differences between means (REGWQ, $p \leq 0.05$). Shoot and root data were analysed separately (different set of letters). Data are means and SE, $n = 5$.

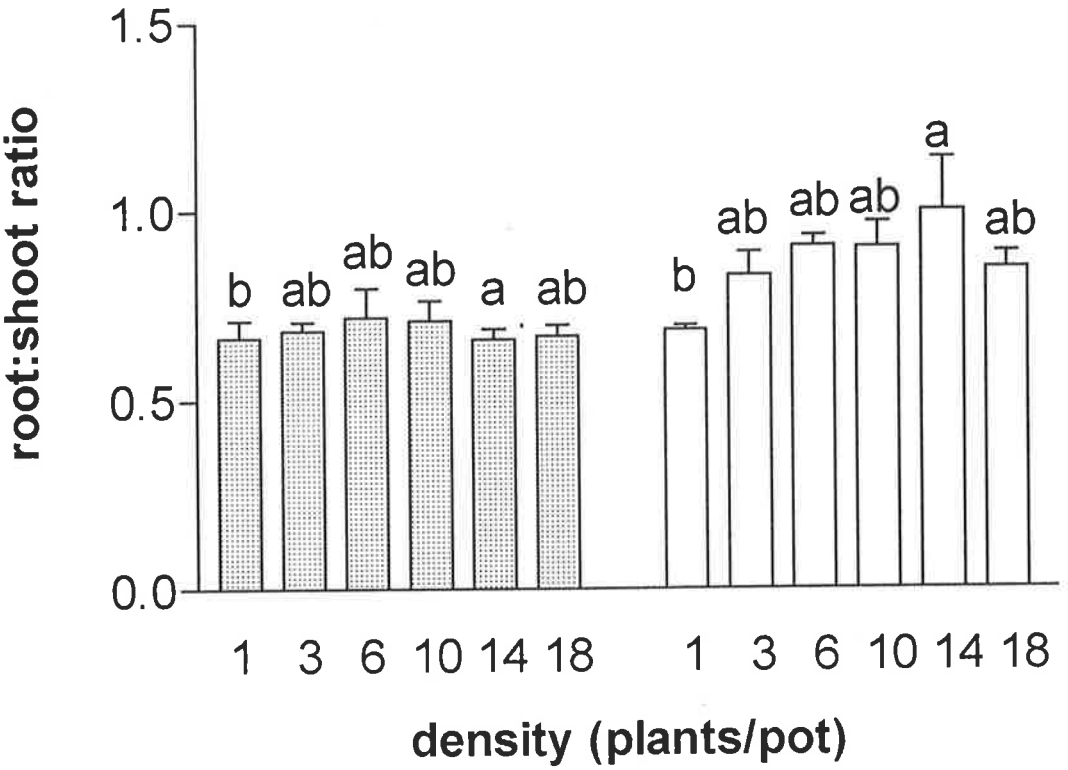


Figure 4.4. Root:shoot ratio (R:S) of mycorrhizal (shaded bars) and non-mycorrhizal (clear bars) plants of *Trifolium subterraneum* grown at different densities. Different letters indicate significant differences between means (REGWQ, $p \leq 0.05$). Data are means and SE, $n = 5$.

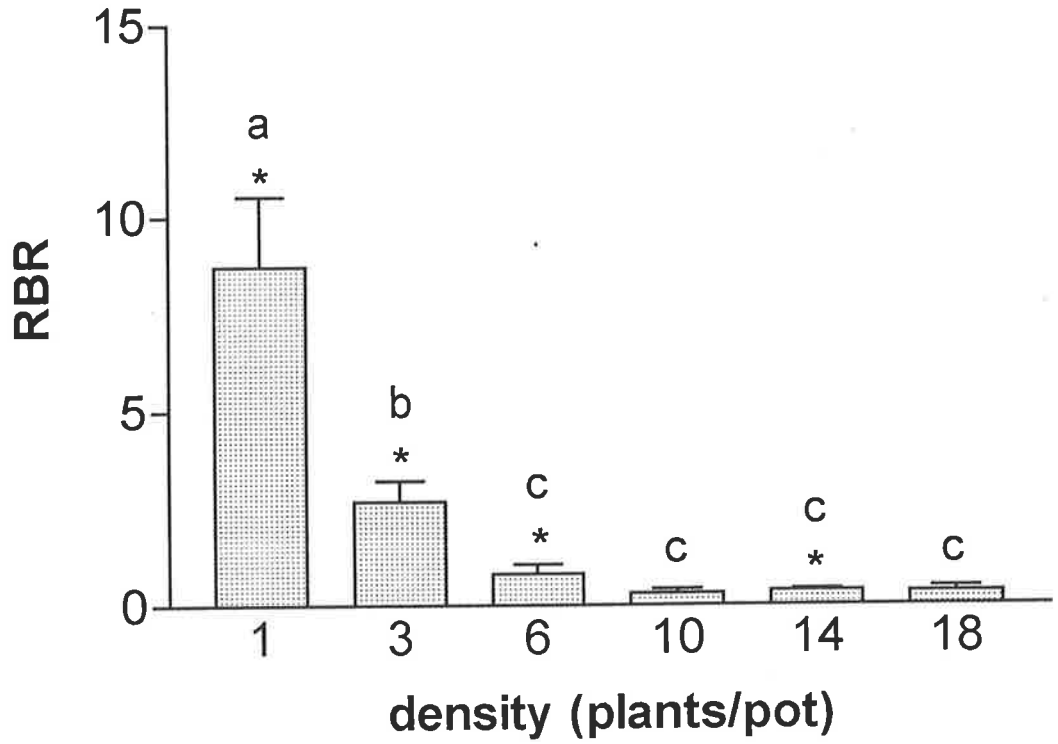


Figure 4.5. Relative biomass response (RBR) of mycorrhizal plants of *Trifolium subterraneum* grown at different densities (see text for calculations, formula (1)). Different letters indicate significant differences between means (REGWQ, $p \leq 0.05$). Stars indicate means significantly different from zero (Student's *t*, $p \leq 0.05$). Data are means and SE, n = 5.

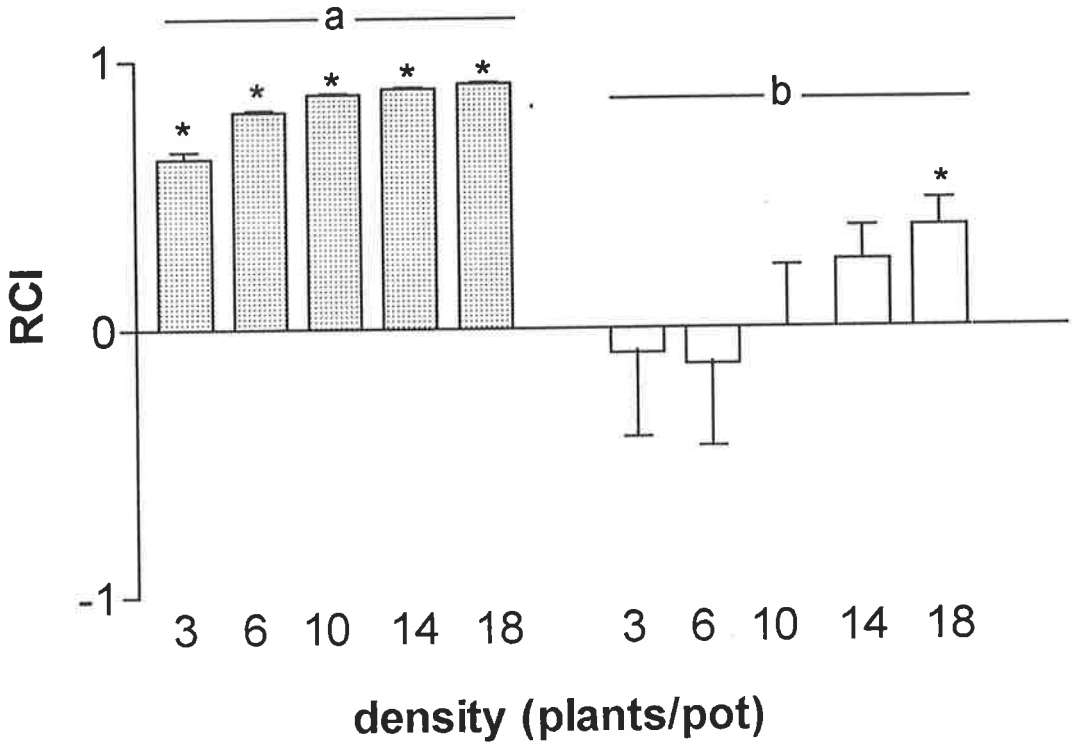


Figure 4.6 Relative competition intensity (RCI) of mycorrhizal (shaded bars) and non-mycorrhizal (clear bars) plants of *Trifolium subterraneum* grown at different densities (see text for calculations, formula (2)). Different letters indicate significant differences between means (REGWQ, $p \leq 0.05$). Stars indicate means significantly different from zero (Student's *t*, $p \leq 0.05$). Data are means and SE, n = 5.

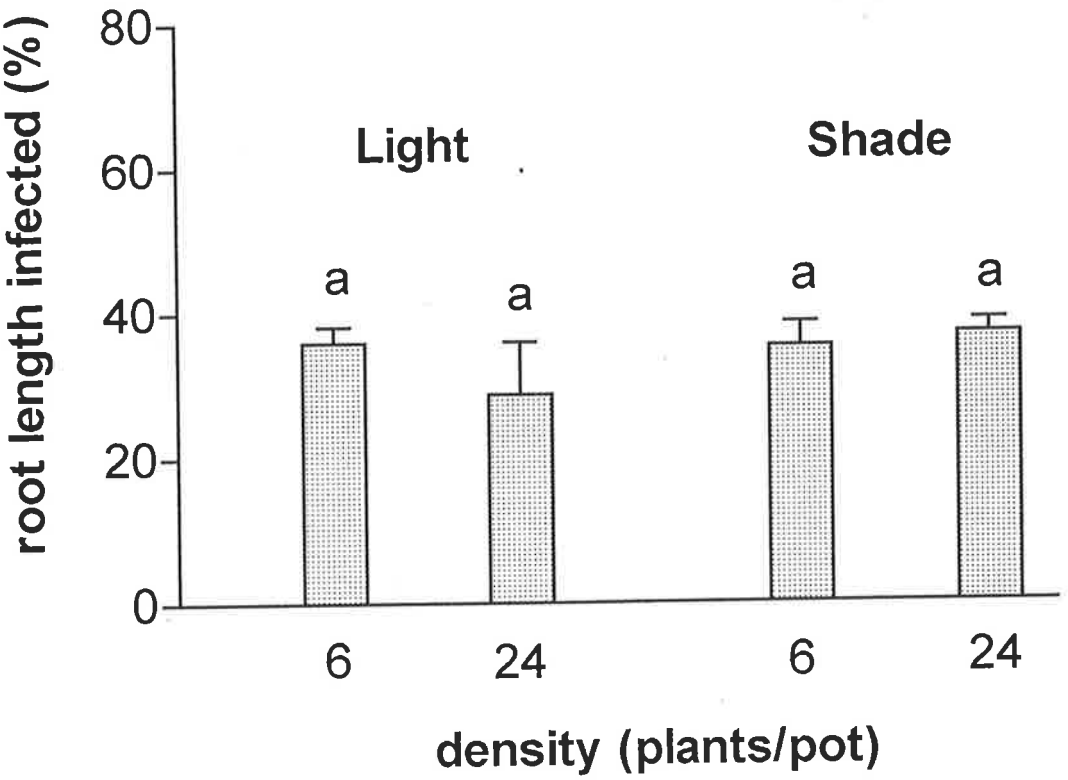


Figure 4.7. Percentage of root length infected of plants of *Trifolium subterraneum* grown at different densities and at two light intensities (Light: 400 μm m^{-2} s^{-1} and Shade: 180 μm m^{-2} s^{-1}), harvested at 4 weeks (Harvest 1). Different letters indicate significant differences between means (REGWQ, $p \leq 0.05$). Data are means and SE, n = 4.

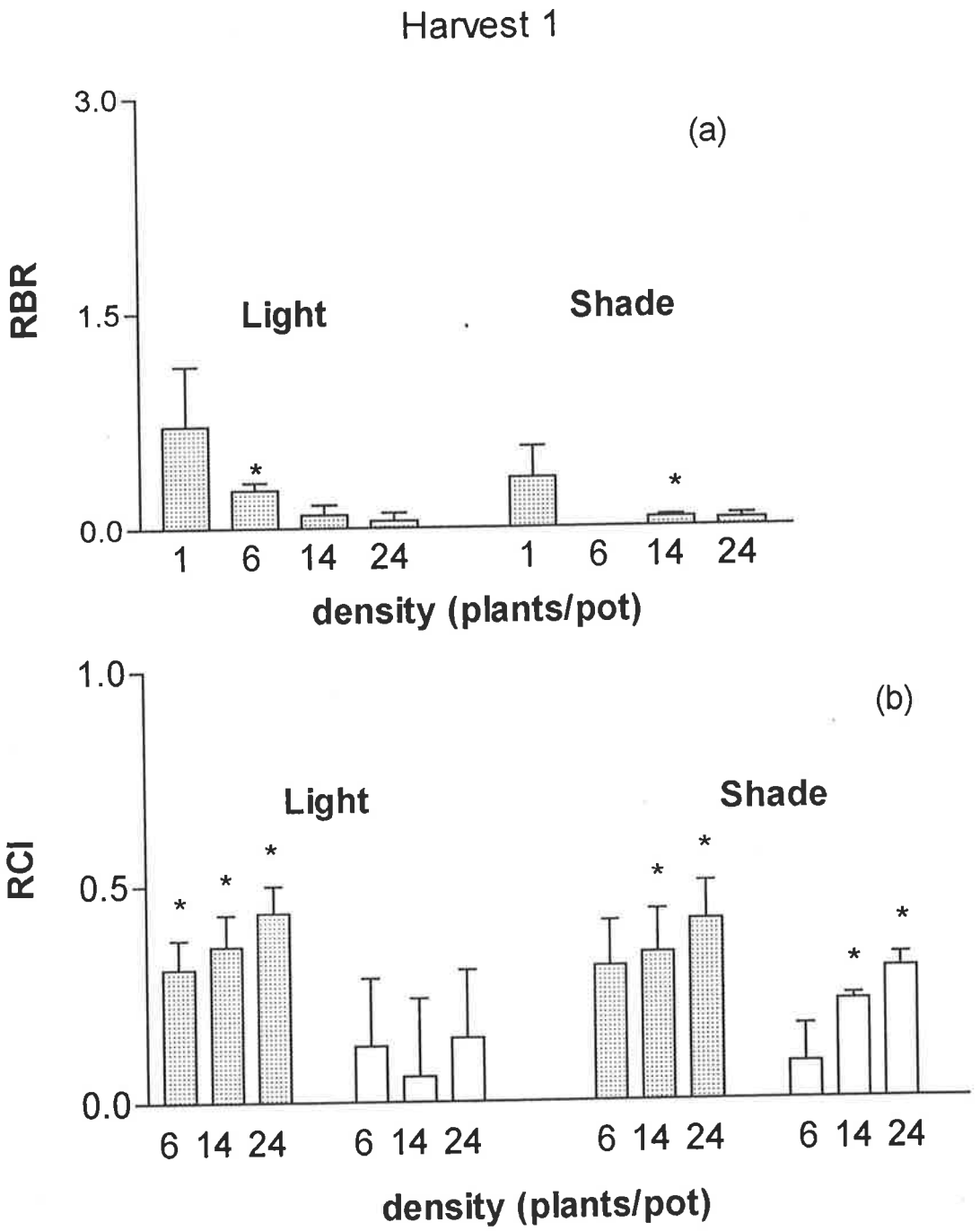


Figure 4.8. Relative biomass response to mycorrhizal infection (RBR) (a) and relative competition intensity (RCI) (b) of mycorrhizal (shaded bars) and non-mycorrhizal (clear bars) plants of *Trifolium subterraneum* grown at different densities and at two light intensities (Light: 400 μm m^{-2} s^{-1} and Shade: 180 μm m^{-2} s^{-1}) (see text for calculations, formula (1) and (2)), harvested at 4 weeks (Harvest 1). Stars indicate means significantly different from zero (Student's *t*, $p \leq 0.05$). Data are means and SE, n = 4.

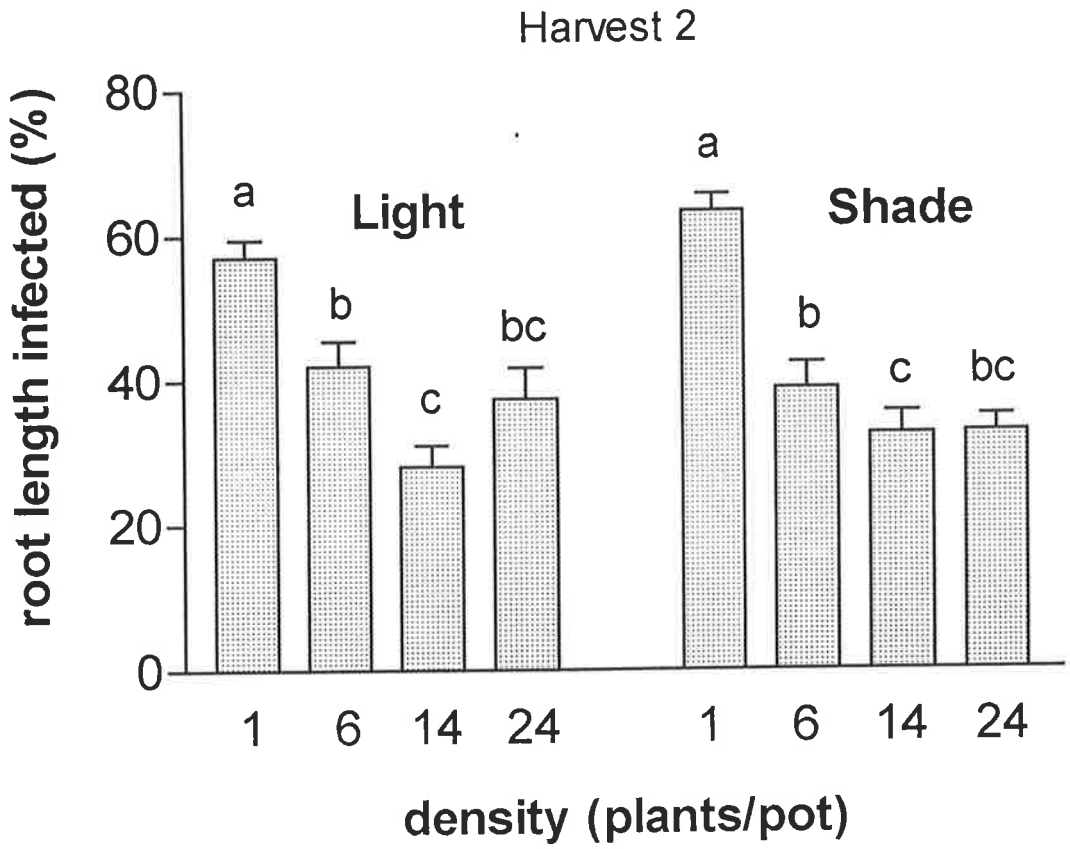
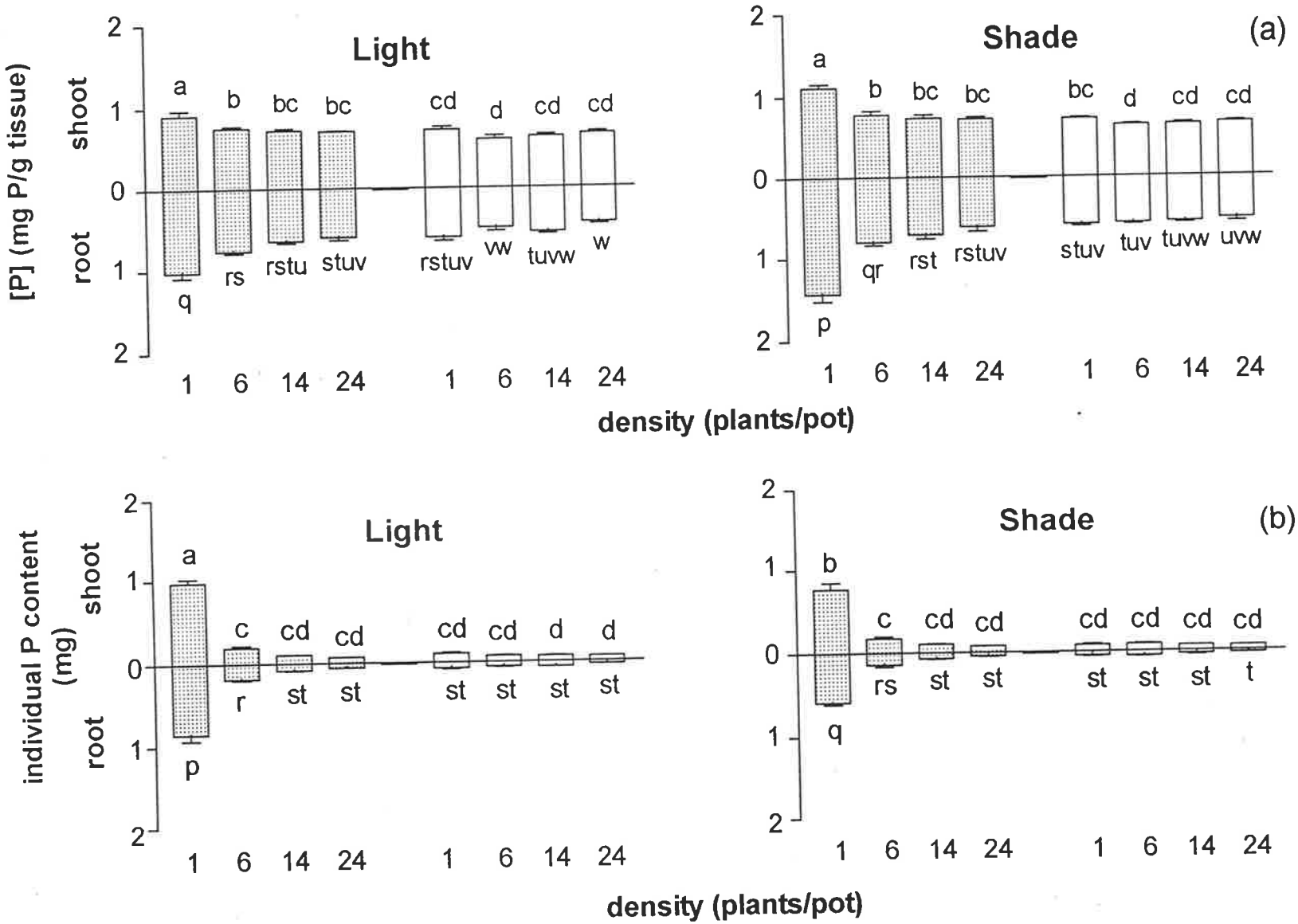


Figure 4.9. Percentage of root length infected of plants of *Trifolium subterraneum* grown at different densities and at two light intensities (Light: 400 μm m^{-2} s^{-1} and Shade: 180 μm m^{-2} s^{-1}), harvested at 8 weeks (Harvest 2). Different letters indicate significant differences between means (REGWQ, $p \leq 0.05$). Data are means and SE, n = 4.

Figure 4.10. Shoot and root P concentrations (a) and P content (plant P content per pot/number of plants per pot) (b) of shoots and roots of individual mycorrhizal (shaded bars) and non-mycorrhizal (clear bars) plants of *Trifolium subterraneum* grown at different densities and at two light intensities (Light: 400 μm m^{-2} s^{-1} and Shade: 180 μm m^{-2} s^{-1}), harvested at 8 weeks (Harvest 2). Different letters indicate significant differences between means (REGWQ, $p \leq 0.05$). Shoot and root data were analysed separately (different set of letters). Data are means and SE, $n = 4$.


Light
Shade
(a)
[P] (mg P/g tissue)
shoot
root
density (plants/pot)
Light
Shade
(b)
individual P content (mg)
shoot
root
density (plants/pot)

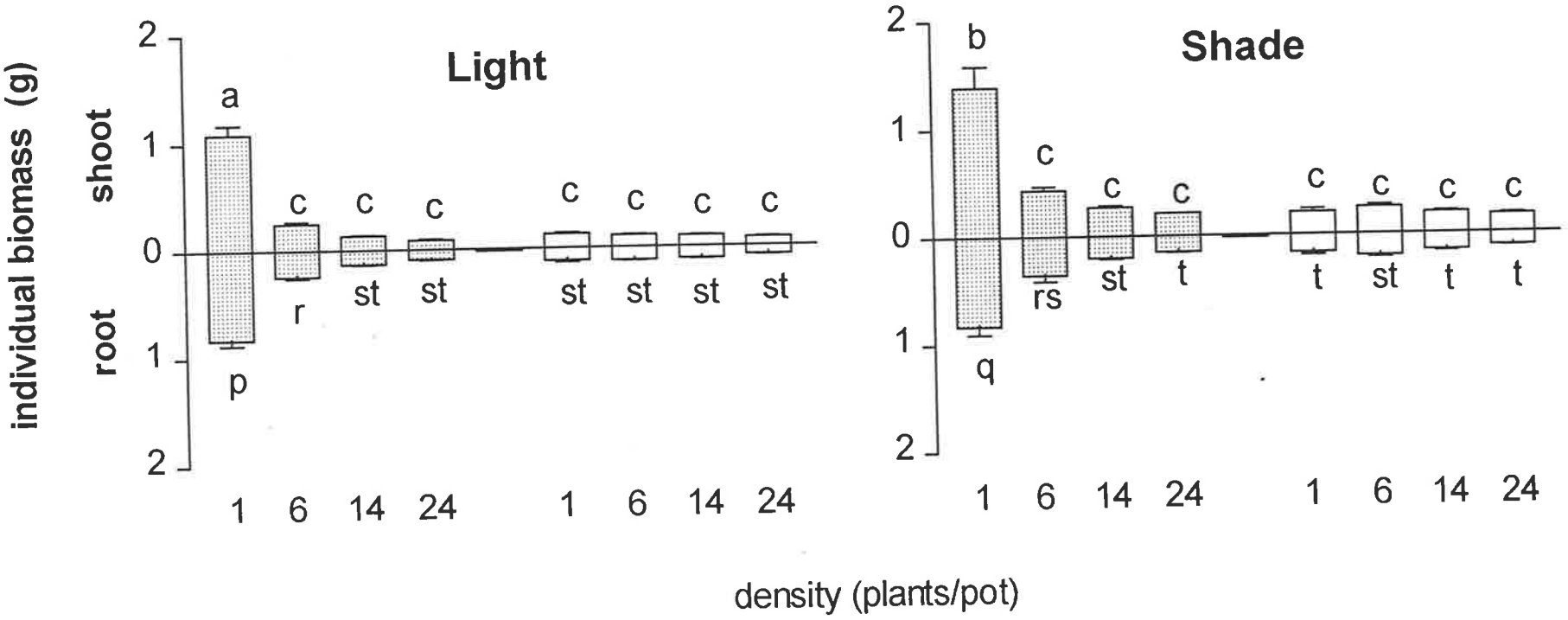


Figure 4.11. Individual shoot and root biomass (shoot or root biomass per pot/number of plants per pot) of plants per pot) (b) of shoots and roots of mycorrhizal (shaded bars) and non-mycorrhizal (clear bars) plants of *Trifolium subterraneum* grown at different densities and at two light intensities (Light: 400 μm m^{-2} s^{-1} and Shade: 180 μm m^{-2} s^{-1}), harvested at 8 weeks (Harvest 2). Different letters indicate significant differences between means (REGWQ, $p \leq 0.05$). Shoot and root data were analysed separately (different set of letters). Data are means and SE, n = 4.

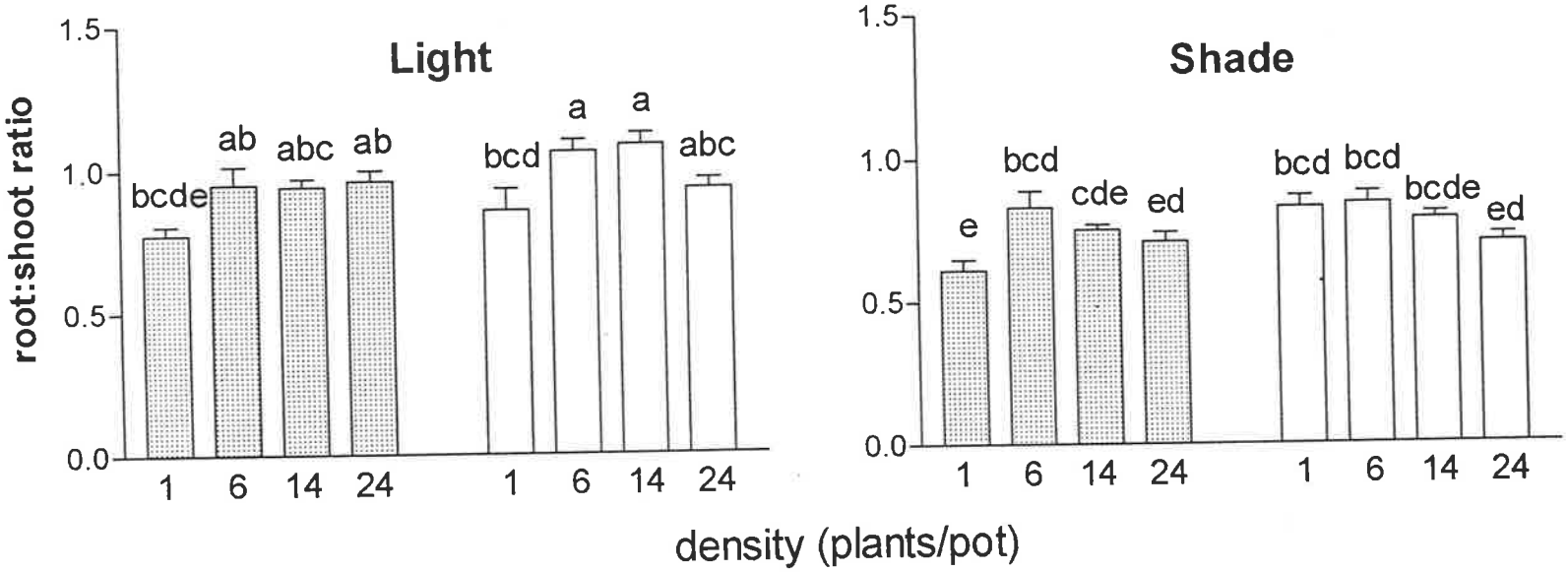


Figure 4.12. Root:shoot ratio (R:S) of mycorrhizal (shaded bars) and non-mycorrhizal (clear bars) plants of *Trifolium subterraneum* grown at different densities and at two light intensities (Light: 400 μm m^{-2} s^{-1} and Shade: 180 μm m^{-2} s^{-1}), harvested at 8 weeks (Harvest 2). Different letters indicate significant differences between means (REGWQ, $p \leq 0.05$). Data are means and SE, $n = 4$.

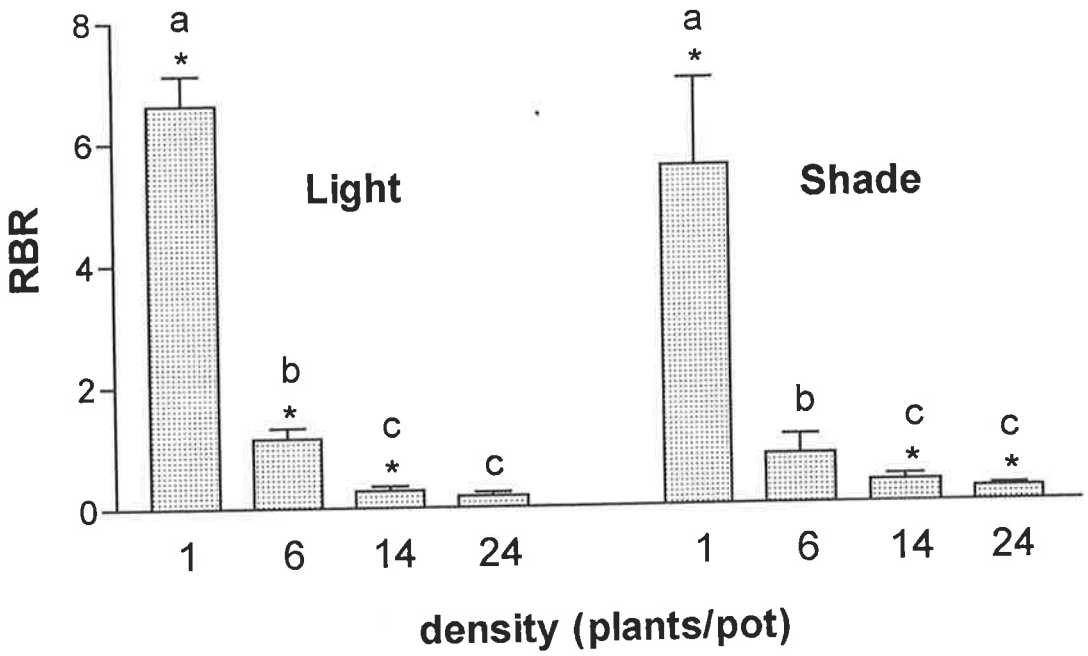


Figure 4.13. Relative biomass response to mycorrhizal infection (RBR) of plants of *Trifolium subterraneum* grown at different densities and at two light intensities (Light: 400 μm m^{-2} s^{-1} and Shade: 180 μm m^{-2} s^{-1}), harvested at 8 weeks (Harvest 2). Different letters indicate significant differences between means (REGWQ, $p \leq 0.05$). Stars indicate means significantly different from zero (Student's *t*, $p \leq 0.05$). Data are means and SE, n = 4.

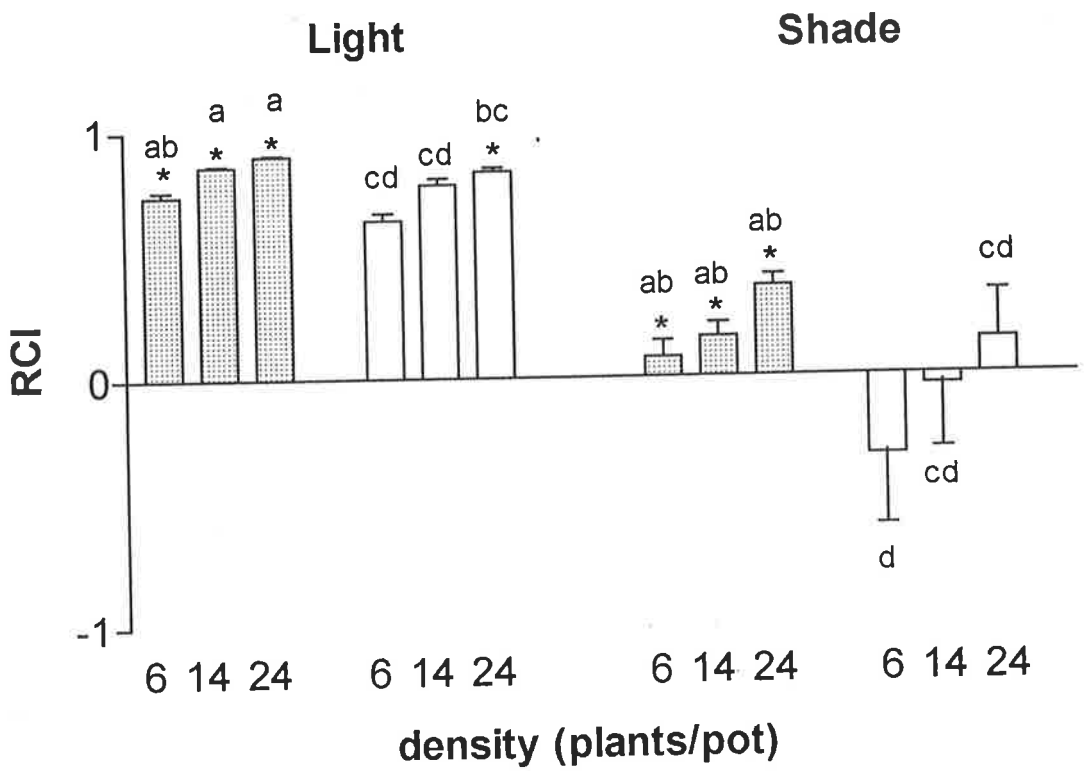


Figure 4.14. Relative competition intensity (RCI) of mycorrhizal (shaded bars) and non-mycorrhizal (clear bars) plants of *Trifolium subterraneum* grown at different densities and at two light intensities (Light: 400 μm m^{-2} s^{-1} and Shade: 180 μm m^{-2} s^{-1}) (see text for calculations, formula (2)), harvested at 8 weeks (Harvest 2). Stars indicate means significantly different from zero (Student's *t*, $p \leq 0.05$). Data are means and SE, n = 4.

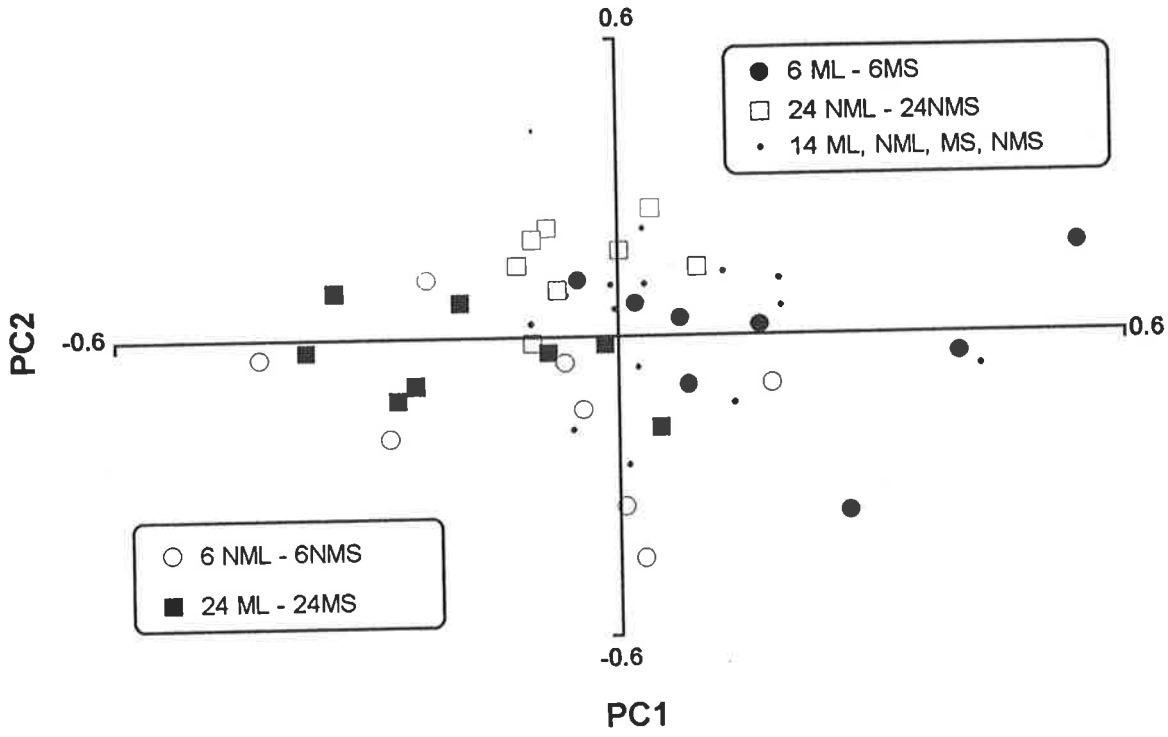


Figure 4.15. Array of the relative frequency distributions of plants of *Trifolium subterraneum* grown at different densities and at two light intensities (Light: 400 μm m^{-2} s^{-1} and Shade: 180 μm m^{-2} s^{-1}), harvested at 8 weeks (Harvest 2), in the space determined by two principal components (PC1 and PC2).

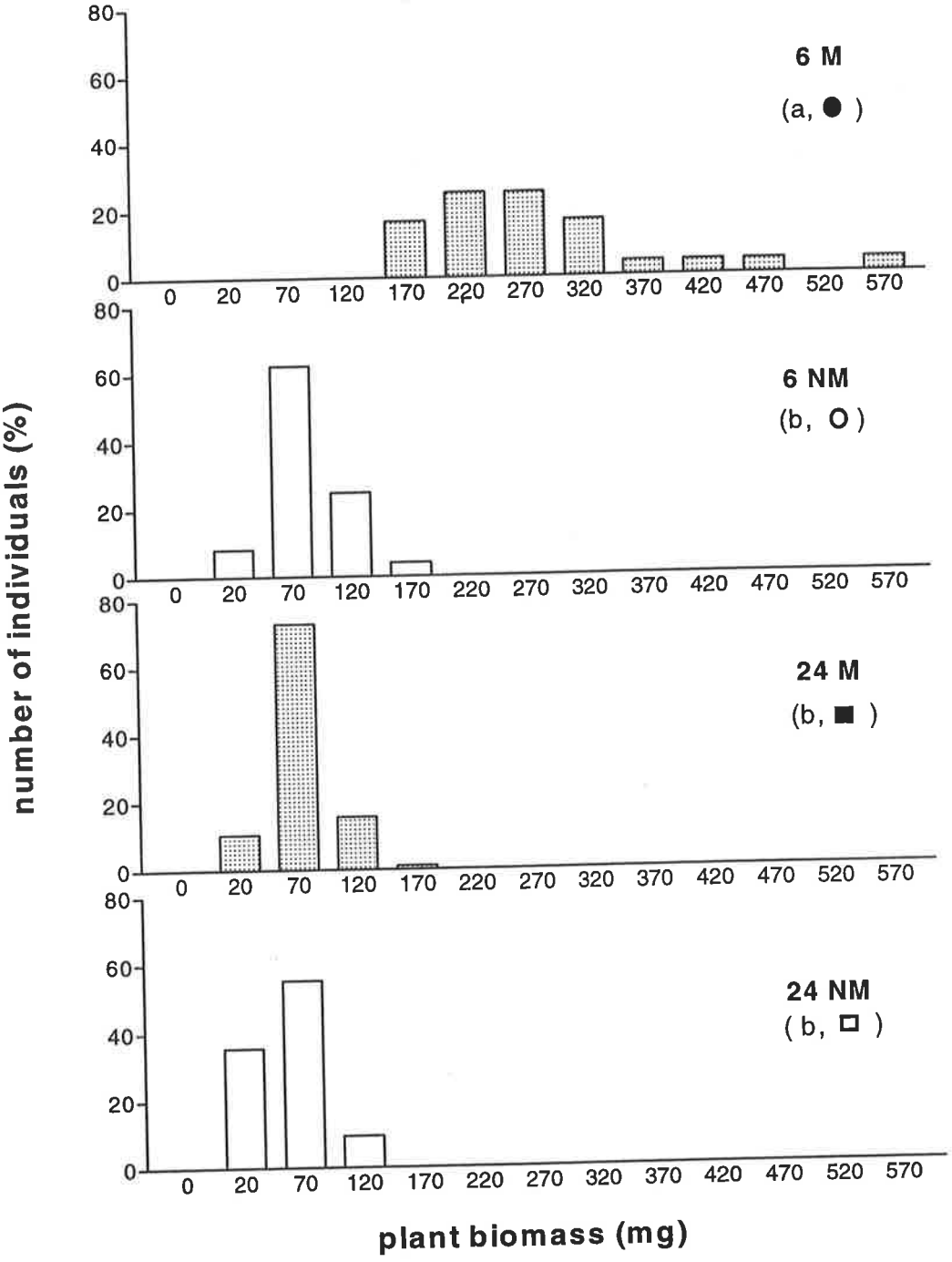


Figure 4.16. Relative frequency distributions of individual plant sizes (biomass) of mycorrhizal (M) (shaded bars) and non-mycorrhizal (NM) (clear bars) treatments under full light at densities 6 and 24. Different letters in parentheses indicate distributions are significantly different (Kolmogorov-Smirnov test, $n = 12$, $p \leq 0.05$, corrected by Bonferoni's formula for number of comparaisons = 6). Symbols in parentheses correspond to Fig. 4.15 (PC).

Table 4.1. ANOVA of shoot and root P concentrations and individual P content (plant P content per pot/number of plants per pot) of mycorrhizal and non-mycorrhizal plants of *Trifolium subterraneum* grown at different densities (1-18 plants per pot).

ANOVA		P concentration (mg P/g tissue)				individual P content (mg)			
		root		shoot		root P content		shoot P content	
transformations		no		no		log10 (P)		log10 (P)	
factors	df	F value	Pr>F	F value	Pr>F	F value	Pr>F	F value	Pr>F
mycorrhizas	1	120.64	0.0001	15.63	0.0003	233.47	0.0001	69.03	0.0001
density	5	1.33	0.2701	2.87	0.0250	39.09	0.0001	23.02	0.0001
D * M	5	0.92	0.4790	0.31	0.9025	13.80	0.0001	6.57	0.0001

Table 4.2. ANOVA of the individual whole plant, shoot and root biomass (plant biomass per pot/ number of plants per pot) and root:shoot (R:S) ratio of mycorrhizal (M) and non-mycorrhizal (NM) plants of *Trifolium subterraneum* at different densities (1-18 plants per pot). Mean squares (MS) and F values given. Degrees of freedom in parentheses. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$; ns, not significant. $n = 5$.

ANOVA	individual biomass (g)									R:S ratio		
	whole plant			shoot			root					
transformation	log10			log10			log10			no		
factors	MS	F value		MS	F value		MS	F value		MS	F value	
mycorrhizas (1)	9.66	227.08	****	12.11	281.72	****	6.92	142.80	****	0.47	40.69	****
density (5)	2.97	69.78	****	3.22	74.87	****	2.69	55.49	****	0.03	2.76	****
M * D (5)	1.53	35.99	****	1.37	31.78	****	1.74	35.84	****	0.03	2.21	*

Table 4.3. ANOVA of shoot and root P concentrations and individual P content (plant P content per pot/number of plants per pot) of mycorrhizal and non-mycorrhizal plants of *Trifolium subterraneum* at different densities (1-24 plants per pot) and at two light intensities (Light: 400 µm m^{-2} s^{-1} and Shade: 180 µm m^{-2} s^{-1}). Means and SE, n = 4. Different letters indicate significant differences between means (REGWQ, $p < 0.05$). ANOVA: mean squares (MS) and F values given. Degrees of freedom in parentheses. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$; ns, not significant.

ANOVA	P concentration (mg P/g tissue)						individual P content (mg)								
	shoot			root			whole plant			shoot			root		
transformations	log10			log10			no			no			no		
factors	MS	F		MS	F		MS	F		MS	F		MS	F	
light (1)	0.006	5.03	***	0.032	15.28	***	0.093	14.39	***	0.014	5.81	*	0.034	18.31	****
mycorrhizas (1)	0.102	82.82	****	0.418	198.1	****	3.023	465.9	****	0.874	346.3	****	0.645	347.5	****
density (3)	0.034	27.95	****	0.110	52.21	****	2.019	311.3	****	0.604	239.3	****	0.414	223.3	****
L * D (3)	0.001	1.08	ns	0.001	0.56	ns	0.061	9.46	****	0.013	5.42	**	0.017	9.20	****
L * M (1)	0.002	1.25	ns	0.001	0.46	ns	0.063	9.73	**	0.011	4.51	*	0.020	11.26	**
M * D (3)	0.01	7.81	****	0.040	19.11	****	1.800	277.5	****	0.531	210.5	****	0.375	202.2	****
L * M * D (3)	0.002	1.77	ns	0.011	5.23	***	0.042	6.55	***	0.008	3.19	*	0.013	7.30	***

Table 4.4. ANOVA of individual whole plant, shoot and root biomass (plant biomass per pot/number of plants per pot), and root:shoot (R:S) ratio of mycorrhizal and non-mycorrhizal plants of *Trifolium subterraneum* at different densities (1-24 plants per pot) and at at two light intensities (Light: 400 μm m^{-2} s^{-1} and Shade: 180 μm m^{-2} s^{-1}). Means and SE, n = 4. Different letters indicate significant differences between means (REGWQ, $p < 0.05$). ANOVA: mean squares (MS) and F values given. Degrees of freedom in parentheses. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$; ns, not significant.

ANOVA	individual biomass (g)									R:S ratio		
	whole plant			shoot			root					
transformations	no			no			no			no		
factors	MS	F		MS	F		MS	F		MS	F	
light (1)	0.287	29.69	****	0.050	11.15	**	0.096	81.18	****	0.561	98.45	****
mycorrhizas (1)	2.548	263.6	****	0.879	192.9	****	0.433	365.7	****	0.078	13.82	***
density (3)	1.634	169.1	****	0.583	128.0	****	0.265	224.4	****	0.074	13.07	****
L * D (3)	0.161	16.76	****	0.042	9.36	****	0.038	32.37	****	0.019	3.36	*
L * M (1)	0.170	17.60	****	0.040	8.88	****	0.044	37.67	****	0.001	0.13	ns
M * D (3)	1.442	149.2	****	0.514	112.8	****	0.234	197.6	****	0.02	3.44	*
L * M * D (3)	0.127	13.14	****	0.029	6.41	**	0.034	29.02	****	0.013	2.32	ns

Table 4.5. First five principal components (A), their corresponding eigenvalues and the variation and accumulated variation explained by each Principal Component, and eigenvectors (B) corresponding to the modal class (0) and to three ranges to the left (L50, L100, L150) and to the right (R50, R100, R150) of the modal class.

A. Principal components

component	eigenvalue	variation	accumulated
PRIN1	0.037067	0.476434	0.476434
PRIN2	0.024685	0.317281	0.793715
PRIN3	0.008857	0.113848	0.907563
PRIN4	0.004273	0.054926	0.962488
PRIN5	0.001718	0.022079	0.984567

B. Eigenvectors

ranges	PRIN1	PRIN2	PRIN3	PRIN4	PRIN5
L150	0.000000	0.000000	0.000000	0.000000	0.000000
L100	0.098000	0.035751	0.201480	0.811511	-0.260330
L50	0.509400	0.575978	-0.435326	-0.227471	-0.167873
0	-0.842563	0.257705	-0.295735	-0.031837	-0.066201
R50	0.113786	-0.757268	-0.528476	-0.034484	-0.038629
R100	-0.021486	-0.159110	0.545252	-0.488219	-0.551489
R150	0.033056	-0.010118	0.235571	-0.184144	0.684683

Table 4.6. Different statistical measures of size inequality: coefficient of variation (CV), kurtosis, skewness and Gini coeficient, of the distributions of sizes shown in Fig. 4.16, corresponding to mycorrhizal (M) and non-mycorrhizal (NM) plants of *Trifolium subterraneum* grown under full light (L) at 6 or 24 plants per pot.

	6 ML	6 NML	24 ML	24 NML
CV	36.6	30.4	25.2	31.8
Kurtosis	-0.79	7.58	10.18	4.12
Skewness	0.92	2.72	3.13	2.21
Gini	0.19	0.167	0.139	0.179

4.4. Discussion

Both experiments showed that mycorrhizal symbiosis increases intraspecific competition and that the positive effects of the symbiosis at the individual level are reduced as density increases, to become negligible at high densities.

Increasing densities had different effects on percentage infection in the two experiments, even though the environmental conditions were similar. While in the first experiment plant density did not modify infection, in the second experiment single plants had the highest percentage of infection (60%) and plants at higher densities had infection percentages similar to the ones in the first experiment (30%). Variable responses of infection to increasing densities have been reported (Abbott and Robson 1984; Bååth and Hayman 1984; Koide 1991a; Allsopp and Stock 1992a). Of these studies, only (Abbott and Robson 1984) discussed the relationship between plant density and infection. They found that the proportion of arbuscules in roots of *T. subterraneum* was higher at low than at high densities, although the percentage infection was similar at all densities. They postulated that increased competition for light at higher densities may have reduced the supply of carbohydrates to roots and fungus, and affected mycorrhizal development. Even though the evaluation of the development of mycorrhizal structures was beyond the scope of my experiments, the results seem not to support this interpretation. If light was the only factor limiting the formation of mycorrhizas, a reduction of more than 50% in light intensity, which is enough to have a negative effect on mycorrhizal structures in *T. subterraneum* (Tester *et al.* 1985; Tester *et al.* 1986), would be expected to result in a lower percentage infection. However, in the second experiment low light intensity did not modify infection. More detailed observations to estimate the number of entry points and the relative proportion of different fungal structures (Bruce *et al.* 1994) at different plant densities could be useful in clarifying the actual effect of density on the formation and function of mycorrhizas.

The higher P concentration in mycorrhizal plants than in non-mycorrhizal plants and its reduction by increasing plant densities agrees with previous reports (Pairunan *et al.* 1980; Koide 1991a; Allsopp and Stock 1992a). Mycorrhizal plants may have had higher P

concentrations because they were more efficient in P uptake (Smith and Read 1997). In the second experiment, where both shoot and root P concentrations were reduced by increasing plant densities, the P concentration of mycorrhizal plants at high densities was below the level indicated as limiting for plant growth (1.5 μg/mg dry tissue (Tester *et al.* 1985)), suggesting that growth of these plants was limited by P availability. It has also been proposed that mycorrhizal plants have higher P concentrations because their growth is C-limited due to the need for C to the maintenance of fungal structures (Smith and Read 1997). The increase in root P concentration of single mycorrhizal plants under shade would support this hypothesis. However, at increasing densities, mycorrhizal and non-mycorrhizal plants had similar (lower) P concentrations, regardless of light intensity. Mycorrhizal plants might have acquired P more efficiently, but at higher densities the amount of available P was as limiting as it was for non-mycorrhizal plants at all densities.

The decrease in both individual plant P content and plant biomass at increasing densities observed in both experiments agrees with results reported for *T. subterraneum* grown at different densities in sterilised soil and therefore probably in the absence of mycorrhizal fungi (Stern 1965), and also, in the presence of mycorrhizal fungi (Abbott and Robson 1984). In both examples the negative effect of density was attributed to competition for light. Abbott and Robson (1984) suggested that a reduction in light availability would reduce mycorrhizal development, as well as root growth, as a consequence of limited supply of carbohydrates. However, in my experiment, shading did not have a synergetic effect with increasing densities. I did observe that shading reduced root biomass per pot of mycorrhizal plants at all densities, and that of non-mycorrhizal plants at high densities. This increase in the relative biomass of shoots in response to reduced light intensity has been reported before in both mycorrhizal and non-mycorrhizal plants (Bethlenfalvay and Pacovsky 1983; Crawley 1986) and is consistent with modified allocation to photosynthetic tissues when carbohydrate supply decreases (Ericsson 1995). On the other hand, when mineral nutrients are limiting there is a shift of carbohydrate allocation to roots (Ericsson 1995). Hence, an increase in nutrients available to the plant, for example as a result of mycorrhizal infection, should be expected to result in lower

root:shoot ratios (Smith and Read 1997). Indeed, in the first experiment all mycorrhizal plants had lower root:shoot ratios than non-mycorrhizal plants, although the effect of density was inconsistent. In the second experiment, shading and density increased relative biomass allocation to shoots, reducing the R:S ratios of shaded mycorrhizal and non-mycorrhizal plants at higher densities.

The large decrease in the relative biomass response to mycorrhizal infection at increasing densities confirms other findings where density reduced the positive effect of mycorrhizal infection, measured either as an increase in biomass (Bååth and Hayman 1984), or an increase in P uptake (Koide 1991a). Mycorrhizal root systems are able to take up P not physically accessible to non-mycorrhizal roots, which is likely to generate a greater overlap of the P-depletion zones at higher densities (Koide 1991a). A reduction of individual biomass at increasing density was observed only in mycorrhizal plants and probably reflected the differential effect of density on the overlapping P-depletion zones (see discussion below).

Surprisingly, the reduction in the relative biomass response to mycorrhizal infection was not intensified by shading. A similar effect of reduction in light intensity (from saturating levels) on the response to mycorrhizal infection has been reported previously in soybean (Bethlenfalvay and Pacovsky 1983). However, when Bethlenfalvay and Pacovsky (1983) grew plants at non-saturating levels of irradiance and non-limiting levels of available P, a reduction of irradiance resulted in a decrease in fungal biomass, active fungal structures, percentage infection, and plant growth response (see also Tester *et al.* 1985; Son and Smith 1988; Reinhardt and Miller 1990). At increasing densities the negative effect of low light intensity might be expected to be stronger (Abbott and Robson 1984), due to a decrease in photosynthate availability for both symbionts, and hence an increase in relative C drain by the fungus (Bethlenfalvay and Pacovsky 1983; Abbott and Robson 1984; Tester *et al.* 1985; cf. Hayman 1974; Son and Smith 1988). Although shading reduced root and shoot biomass at low densities, the lack of effect of shading at high densities on plant biomass, P content, and mycorrhizal infection suggests that the availability of P had a very

important role in regulating the effects of mycorrhizal infection in the conditions of the second experiment.

It was expected that at increasing densities the individual biomass of *T. subterraneum* would decrease in non-mycorrhizal treatments as well as in mycorrhizal ones, as previously found for *Abutilon theophrasti*, *T. subterraneum*, *Holcus lanatus* and *Dactylis glomerata* (Stern 1965; Koide 1991a; West 1996). However, in my experiments the biomass and the P content of individual non-mycorrhizal plants was almost the same at all densities, and approximately 10 times less than that of a single mycorrhizal plant. It appears that each non-mycorrhizal plant was only able to access a small proportion of the nutrients available in the pots due to the small size of its root system, and that growth was therefore limited by nutrient availability. Consequently, even at higher densities there was little or no overlap of P depletion zones and no effect of reducing light. On the other hand, single mycorrhizal plants depleted most of the available P in the pots: their average P content was about 2 mg which represents approximately 75% of the total available P in the pots (pots had 470g of soil containing 5.9 μg P/g, or 2.714 mg P/pot). These plants had more shoot and root biomass and higher requirements for resources other than P. Consequently, as density increased competition for those other limiting resources increased. Competition did not occur in non-mycorrhizal pots because of the limited plant growth (Reader 1990). Non-mycorrhizal plants did not compete for P because they were unable to deplete the supply, although they were P limited (Goldberg 1990). Similarly, Reader (1990) found that the removal of neighbours did not affect the recruitment of *Hieracium floribundum* in unfertilised plots, but increased recruitment in fertilised ones, suggesting that competition was constrained by low nutrient supply. Lack of competition would allow coexistence of plants in environments where nutrients have low rates of movement in soil or high rates of supply relative to demand (Grace 1995b). Importantly, the presence of mycorrhizas alters this relationship by altering the effective rate of movement of nutrients to the roots. These counter-intuitive results, indicating greater intensity of competition at higher resource levels support other findings (Wilson 1988b; Gurevitch *et al.* 1990) and indirectly support the hypothesis that competition is less important in infertile soils (Grime 1979; Grace 1990).

However, there is also evidence supporting the hypothesis that competition is equally important in low or high nutrient environments (Tilman 1987b). In their recent review, Goldberg and Novoplansky (1997) presented a third hypothesis and pointed out the need to include the temporal dynamics of soil resources when studying the role of competition along resource gradients. They assume that soil resources are supplied in pulses and the relative importance of competition depends on the intensity and frequency of pulses and the length of interpulse periods. To test this hypothesis fully, the intensity and temporal dynamics of all the processes that increase soil resource availability need to be included in competition studies. Because mycorrhizas increase intraspecific competition intensity at least at some densities (my results; (Watkinson and Freckleton 1997)), through an increase in nutrient availability and therefore may have an important role in altering the dynamics of soil resources, they should be taken into account in such studies.

My results emphasise that the main effects of mycorrhizas at the level of individual plants cannot be expected to be apparent at a population level, because of the influence of density-dependent processes. This strong dependency of mycorrhizal effects on plant density might explain why results of field studies of the effects of mycorrhizal infection on P uptake by plants have been conflicting (Fitter 1985) and have rarely shown the expected increase in biomass due to the symbiosis (McGonigle 1988).

My results on plant size distribution showed some interesting trends. The apparently high values of CV and Gini coefficient of the size distributions of mycorrhizal populations followed a pattern previously observed in populations with asymmetric competition attributed to competition for light. However, it is possible to have asymmetric competition when there is pre-emption of nutrients by individuals with larger roots (Weiner 1986), or in this case more effective roots. This could explain the particular size distribution of mycorrhizal populations growing at 6 plants per pot. My data did not support the hypothesis of a more even distribution of nutrients (reduced inequality) between plants resulting from mycorrhizal hyphal connections between them (Grime *et al.* 1987; Law 1989). This agrees with other findings where mycorrhizal plants showed higher inequality in terms of biomass (Allsopp and Stock 1992a) or fecundity (Shumway and

Koide 1995) as density increased (cf. (Maffia and Janos 1993). In these experiments, as in mine, the sources of inoculum used were pieces of roots or spores from which each plant became infected separately. It is possible that the results would have been different if the plants were established on an intact mycorrhizal network (Newman *et al.* 1992) which linked the plants together from the beginning of the experiment.

The results presented in this chapter show that mycorrhizas increase competition and consequently increase size inequality. This effect, which is accompanied by an increase in individual plant biomass at low densities, it is not necessarily a detrimental one for the population because bigger plants have higher reproductive output (Weiner 1995).

However, it would affect the genetic structure of the population. Indeed, a mycorrhizal individual growing at low density might have relatively higher fitness than a non-mycorrhizal one. At high densities any non-infected individual (or individuals which become infected later) would make a much lower contribution to the next generation. Although there is no increase in the average biomass of the population due to mycorrhizal infection, infected individuals with a strong response to the symbiosis would have an advantage in situations of competition, thus the ability to become infected earlier would have been a character that would be selected for. This scenario can explain the maintenance of the symbiotic ability even under conditions such as dense populations where there is no obvious advantage of the symbiosis at the population level.

Chapter 5

Interactive effects of mycorrhizal symbiosis, plant density and nutrient heterogeneity on *Trifolium subterraneum*

5.1. Introduction

Competition for nutrients is one of the factors that regulates population structure (Weiner 1990) and species coexistence (Grace 1995a). The distribution of nutrients is seldom homogeneous. Patchy distribution of nutrients is common to most environments and it occurs even at a very small scale (3-4 cm) (Antonovics *et al.* 1987; Fowler 1988; Jackson and Caldwell 1993; Robinson 1994; Reynolds *et al.* 1997). Plants have different plastic and genotypic responses to this soil nutrient heterogeneity (Antonovics *et al.* 1987) and it has been postulated that their response to nutrient-enriched soil patches may be more important in determining competitive success than the average nutrient concentration in soil (Campbell *et al.* 1991). For plants growing in competition with others, a rapid access to high-nutrient patches would be more important to deprive competitors of these nutrients than maximum nutrient uptake (Hetrick 1991). It is known that individual mycorrhizal plants (in particular those infected by vesicular-arbuscular mycorrhizal, VAM, fungi) have more efficient and rapid access to phosphorus under experimental conditions (Smith and Read 1997). Although most plant species are able to form this type of association (Smith and Read 1997), its effect on the plant response to soil nutrient patchiness have been seldom investigated (cf. Cui and Caldwell (1996a)).

Roots proliferate more in nutrient-enriched soil patches, producing more biomass through longer, more branched roots (Drew 1975; Jackson *et al.* 1990) and/or generating new roots (Gross *et al.* 1993). Root P uptake capacity also increases in rich patches (Jackson *et al.* 1990).

In addition to individual plasticity there are genetic characteristics that modulate the response to nutrient heterogeneity (Campbell *et al.* 1991). These authors suggested that dominant and subordinated species differ in the scale, precision and rate with which they

capture nutrients from the environment. Dominant, fast growers, have extensive (long and thick) root systems, an adaptation to coarse-grained or high-scale foraging, whereas subdominants produce finer roots, an adaptation to undepleted local patches or high-precision foraging.

The presence of P in patches of high concentration not only increases root proliferation and uptake capacity, it also increases the relative amount of P available for the plants in the soil solution (Caldwell *et al.* 1992). When P is applied in greater concentrations to a small fraction of soil instead of in a more dilute application to a larger fraction of soil, the amount of P released to the soil solution in enriched patches results in a larger increase of P uptake even without an increase in root proliferation or in uptake capacity (Kovar and Barber 1989; Caldwell *et al.* 1992). It can be predicted therefore that a plant population growing in an environment with patchy distribution of soil P would have more biomass than a population growing in an environment with the same total amount of P but distributed homogeneously, because the amount of P available for the plants will be higher in the first one.

The distribution of sizes should be also different in homogeneous or patchy environments. It has been postulated that an initial advantage (e.g. bigger size) is of fundamental importance in determining the outcome of plant competition. Bigger plants would have higher competitive ability, grow more and displace smaller ones. In turn, this will have a positive feedback on size of the large plants and their competitive ability (snowball competition) (Wilson 1988a). In a patchy environment, then, plants that grow in a high quality patch would have an initial advantage and this would lead to a very uneven distribution of sizes. However, the snowball effect or cumulative competition has been shown only in experiments with competition for light (Wilson 1988a). Furthermore, this author suggested that there is no reason to expect it to occur with root (nutrient) competition (Wilson 1988a).

Individual plant biomass usually decreases at high densities (Mack and Harper 1977). This reduction is more intense if plants are competing for nutrients (root competition) than if plants are competing for light (Wilson 1988b; Weiner 1990). Another

characteristic of competition for nutrients is that it is symmetric. It yields individuals with similar sizes (plant biomass) or populations with low size inequality (Weiner 1990). However, it has been postulated that if limiting soil resources are distributed in patches that can be reached and exploited by larger individuals, then this would result in asymmetric competition (Weiner 1990).

Despite all the above, in an experimental set up where soils with different fertility were placed in a checkerboard design, it was found that a patchy nutrient distribution did not affect either mean plant biomass nor the size distribution of individuals in populations of *Abutilon theophrasti* (Casper and Cahill Jr. 1996). Amongst other factors, the presence of mycorrhizas was postulated as a possible cause of the small effects of the patchy distribution of nutrients on the structure of the populations (Casper and Cahill Jr. 1996).

There is no information on the effect of mycorrhizal infection on the structure of plant populations growing in heterogeneous environments. There is, however, some information about how VAM infection responds to enriched soil microsites and how single plants are affected by both the symbiosis and a patchy distribution of nutrients. It was found that, as with roots, mycorrhizal hyphae increase branching when they reach soil enriched with organic matter (St. John *et al.* 1983). In pots where a barrier to root growth, but not to hyphae was used, hyphae transported P to the host in the root-free soil equally well with either homogeneous or heterogeneous distribution of nutrients in pots (Cui and Caldwell 1996b). Duke *et al.* (1994) found that the frequency of arbuscules in roots of *Agropyron desertorum* was reduced in plants growing in enriched soil patches although in those patches the growth of mycorrhizal hyphae seemed to be enhanced and the transport of P to the plants was not affected. These authors concluded that root proliferation and uptake capacity were likely to be more important for exploitation of enriched patches than mycorrhizal activity.

The effect of mycorrhizas on plant populations in patchy environments would also probably depend upon plant density. Although there are few studies on the effect of mycorrhizal symbiosis on plant populations, some pot experiments (with homogeneous distribution of nutrients) have shown that increasing plant density reduces the plant

response to mycorrhizal infection measured as an increase in growth and/or P content (Bååth and Hayman 1984; Koide 1991a; Hartnett *et al.* 1993). Furthermore, at low densities, mycorrhizal plants have higher size or reproductive inequality (Chapter 4; Allsopp and Stock 1992a; Shumway and Koide 1995). It has also been found that the relative reduction in plant biomass due to an increase in density (relative competition intensity) was higher when plants were mycorrhizal (Chapter 4; Hartnett *et al.* 1993; Watkinson and Freckleton 1997).

It is possible that if mycorrhizas increase plant growth in rich patches (more hyphal proliferation and more root growth) they will increase snowball competition and hence size inequality (Weiner 1990) relatively more in heterogeneous than in homogeneous environments. However, it is also possible that the hyphal network connecting mycorrhizal plants that may translocate nutrients between them (Chiarello *et al.* 1982), could reduce size inequality (Shumway and Koide 1995) and the intensity of the competition (Grime *et al.* 1987; Ozinga *et al.* 1997). Furthermore, it was postulated that in homogeneous environments where the availability of P to the plants is relatively low, mycorrhizas would be more beneficial than in heterogeneous ones (Cui and Caldwell 1996a). However, these authors did not find interactive effects of mycorrhizas and pattern of nutrient distribution on plant biomass (shoot, root or root:shoot ratio), when single plants of *Agropyron desertorum* were exposed to heterogeneous or homogeneous (uniform) distribution of nutrients.

Since there is scant information on the effect of mycorrhizal infection and soil P distribution on the performance of individual plants and, to our knowledge there is no information on their interactive effects on population structure, we investigated the effects of this interaction on synthetic populations of *Trifolium subterraneum*. We addressed the following questions:

1) How does patchiness in soil P distribution affect mycorrhizal infection and the response of individual plants to that infection in low and high density populations?
2) Does patchiness in soil P distribution and mycorrhizal infection affect total and mean biomass and if so, is this effect altered by plant density?

3) How does patchiness in soil P distribution and mycorrhizal infection affect the populations size distribution and intraspecific competition intensity?

4) Is the size of the individuals determined by local P concentrations or does it depend upon the neighbourhood situation (number of adjacent patches with high P)?

5.2. Methods

To simulate patchiness in soil P distribution I used trays filled with soil with different P concentrations in randomly selected patches.

5.2.1. Experimental design

The experiment had a factorial design with complete randomised blocks and included 3 factors: plant density at 2 levels (1 and 4 plants per patch), mycorrhizas at 2 levels (plants with mycorrhizal inoculum (M) and plants without mycorrhizal inoculum (NM), and patchiness of nutrient distribution at 2 levels (homogeneous soil (Ho) and patchy soil (Pa)). Each of the 8 resulting combinations was replicated 6 times.

5.2.2. Symbionts

I used *Trifolium subterraneum* L. cv. Mt. Barker as the host plant and *Gigaspora margarita* Becker and Hall as the fungal symbiont. I selected *T. subterraneum* because its response to mycorrhizal infection at different densities (Chapter 4) and at different soil phosphorus concentrations (Nadian *et al.* 1996) in homogeneous environments (pots) is well documented.

5.2.3. Soil

I grew the plants in a 50:50 autoclaved sand and soil mix with different amounts of added P. The soil was collected from a Mediterranean type Eucalyptus forest (Waite Hills, Adelaide) and had very low available P concentration (6 $\mu g\ g^{-1}$ of $NaHCO_3$ extractable P (Colwell 1963), and neutral pH (6.4) in 10 mM $CaCl_2$). The soil-sand mix (called soil

hereafter) had a P concentration of 5 μg g^{-1} of $NaHCO_3$ extractable P (Colwell 1963). I divided the soil into three lots: low (L) P soil, with no addition of P; high (H) P soil, with the addition of 35 mg P kg^{-1}; and average (A) P soil, with the addition of 20 mg P kg^{-1}. Phosphorus was added by spraying a solution of $NaH_2PO_4.H_2O$ (only water for L soil) onto thin layers of soil, mixing and incubating for one week before planting.

5.2.4. Trays

To create soil patchiness experimentally I set up a system of trays similar to the one used by Casper and Cahill Jr. (1996). I filled 55 x 55 x 12 cm trays through a wooden grid of 49 cells of 7 x 7 x 12 cm (patches). The size of each patch provided a volume of soil similar to that used in our previous pot experiment with the same symbionts (Chapter 4). Trays with patchy distribution of P (Pa) had 25 patches filled with low (L) P soil and 24 patches filled with high (H) P soil. The patches with high or low P soils were randomly arranged, with each tray having an independent randomization. The only restriction was that of the 25 patches in the centre of the tray (the ones to be harvested), 13 had low P soil and 12 had high P soil. Trays with homogeneous P distribution (Ho) had all the patches filled with average (A) P soil. Thus, the amount of P in homogeneous and patchy trays was almost the same. Once the trays were filled with soil I carefully removed the grid avoiding any soil mixing and leaving no barriers between patches. I placed elastic threads on top of the trays to mark the relative position of the patches during planting. I planted half of the trays (low density) with one pre-germinated seedling of *T. subterraneum* per patch and the other half (high density) with 4 seedlings per patch. I inoculated half of the trays with spores of *G. margarita* at an average dose of approximately 10 spores/plant. I added a dense suspension of *Rhizobium trifolii* to all the trays to ensure effective nodulation and N_2 fixation (Smith and Smith 1981). Plants grew in a glasshouse with partially controlled temperatures (no less than 5°C or more than 35°C) and with natural light supplemented from 7 am till 7 pm by two 400 volt lamps. I watered every second day with deionised water and applied 5 ml kg^{-1} soil of a nutrient solution (lacking N and P) per week (Smith

and Smith 1981) (10 ml were added the first week) to ensure plant growth was not limited by nutrients other than P.

5.2.5. Measurements

I harvested the shoots of all the plants in the central 25 patches of each tray at 8 weeks, and weighed them after drying for 48 h at 80 °C. I harvested the roots of one randomly selected patch from each homogeneous tray. For each patchy tray I harvested the roots of randomly selected patches with high and low local P concentration and different numbers of adjacent patches with high P concentration. The aim of this was to have samples from as many different neighbourhoods as possible (different number of adjacent patches with high P). It was difficult to harvest the roots without disturbing the soil and roots of neighbouring patches. This limited the number of root systems that I could harvest. Nevertheless, I harvested at least three root systems per neighbourhood, a total of 278 samples. It was not possible to separate the roots of individual plants in the high density treatments. I washed and weighed the roots and took one sub-sample from each patch harvested. These sub-samples were then stored in 50% ethanol for further estimation of mycorrhizal infection and root length. I dried the remaining material at 70 °C for 48 h, and weighed it. I washed the root sub-samples, cleared them with 10% KOH and stained them with 0.05% trypan blue in lactoglycerol (a modification of the method of (Phillips and Hayman 1970)). I determined root length and mycorrhizal root length using a line intersect method (Giovanetti and Mosse 1980).

To assess the effect of the patchiness and density on the plant response to mycorrhizal infection I calculated the relative biomass response per tray (RBR_{tray}), as follows:

$$RBR = \frac{(M - NM)}{(NM)} * 100 \qquad (1)$$

where NM was the individual biomass of non-mycorrhizal plants per tray and M was the individual biomass of mycorrhizal plants per tray, from corresponding trays within each block. To assess the effect of local P content (the patch soil P content) on the plant

response to mycorrhizal infection I calculated the relative biomass response per patch (RBR_{patch}) using also formula (1) and in this case, NM was the average individual biomass of non-mycorrhizal plants in patches with high, low or average local soil P, and M was the average individual biomass of mycorrhizal plants in patches with high, low or average soil P, from corresponding trays within each block.

To assess the effect of patchiness and mycorrhizal infection on the reduction of biomass due to density, I calculated the relative competition intensity per tray (RCI_{tray}) as follows:

$$RCI = \frac{(S - C)}{(S)} * 100 \qquad (2)$$

(adapted from (Grace 1993)), where S was the individual plant biomass in trays with low plant density and C was the individual plant biomass in trays with high plant density, from corresponding trays within each block. To assess the effect of neighbourhood on the relative competition intensity of individual patches (RCI_{neigh}), I use formula (2) where S was the biomass of a single plant growing in a patch with low or high P with a certain neighbourhood (number of adjacent patches with high P), and C was the biomass of an individual plant growing in a patch with low or high P, at high density, with the same neighbourhood. High and low density patches with same neighbourhood were paired within each block. To assess the effect of local P on relative competition intensity (RCI_{patch}), I used the same formula (2) where S was the average individual biomass per tray of plants growing in patches with high, low or average local P, and C was the average individual biomass per tray of plants growing at high density in patches with high, low or average local P, from corresponding trays within each block.

5.2.6. Data analysis

I used ANOVA SAS procedure (SAS 1986) to investigate the effects of patchiness, mycorrhizal infection and plant density on total shoot biomass per tray, individual biomass per tray, RBR_{tray} and RCI_{tray}.

I performed regression analyses (PRISM 1994) to assess whether the variation in (per patch) percentage infection, individual shoot and root biomass, root length, and RCI_{neigh}, in the patchy trays, was related to the number of adjacent patches with high P concentration. Since the regression analyses showed that only a small proportion of this variation was related to the number of adjacent patches with high P concentration, I could investigate the effects of local P on these variables using ANOVA or GLM analysis (SAS 1986). The small effect of neighbourhood was therefore included in the unexplained error. A GLM analysis was required in the case of shoot biomass because factor combinations corresponding to different soil P concentrations had different number of replicates (12 high P patches, 13 low P patches and 25 average P patches per tray). When only some of the patches had been sampled (percentage infection, root biomass and length) I randomly selected one high P patch and one low P patch from each patchy tray to compare with corresponding patches from homogeneous trays. I used Tukey test to compare means (SAS 1986). I transformed the data when necessary to improve normality (SAS 1986).

I studied the inequality of the size distributions of the different populations (trays) by calculating the Gini coefficient (Weiner 1986).

5.3. Results

I had to discard one block from the data analysis because the plants showed little growth and nil or negative response to mycorrhizal infection. The two winter months during which the experiment was carried out were particularly overcast, and despite the supplement of light, this block, placed at the south-west side of the glasshouse, seemingly did not get enough light.

5.3.1. Infection

The percentage of root length infected by *G. margarita* was low (less than 20%) in all inoculated treatments (Figs. 5.1 and 5.2). Local phosphorus and plant density had independent effects on percentage infection (Table 5.1). Plants growing in high P patches had the lowest percentage infection, the ones growing in low P patches the highest, and the

ones in homogeneous trays had intermediate values (Fig. 5.1). The percentage infection (Fig. 5.1) and the actual root length infected (data not shown) were reduced by increased plant density (Table 5.1). Mycorrhizal infection was not affected by number of adjacent patches with high P, in patchy trays (Fig. 5.2). There was no variation in percentage infection with the increase in the number of adjacent patches with high P (slopes not significantly different from zero, Fig. 5.2). The regression analysis showed that the percentage infection was higher in patches with low P than in patches with high P (elevations significantly different, Fig. 5.2) as did the ANOVA that included patches with average soil P (Fig. 5.1).

RBR_{tray} (relative plant biomass response to mycorrhizal infection per tray) was negatively related to plant density (ANOVA, df = 1, $p \leq 0.03$), and not affected by patchiness. At low density the RBR_{tray} was around 50% and not different from zero at high density (Fig. 5.3a). RBR_{path} was also negatively related to plant density (ANOVA, df = 1, $p \leq 0.0007$), and not affected by local P concentrations (Fig. 5.3b).

5.3.2. *Effects at tray level*

Patchiness increased total shoot biomass per tray (Fig. 5.4a) and individual shoot biomass per tray (shoot biomass per tray /number of plants per tray) (Fig. 5.4b) independently of mycorrhizal infection and density (Table 5.2). Density and mycorrhizas had interdependent effects on both variables (although the effects on total shoot biomass were significant at a probability slightly higher than the conventional value of 0.05) (Table 5.2). At low density mycorrhizal plants had higher total and individual shoot biomass, but at high density, there were no differences between mycorrhizal and non-mycorrhizal plants (Fig. 5.4, a-b).

Patchiness had a positive independent effect on size inequality, measured with Gini coefficient (Fig. 5.5, Table 5.2). Mycorrhizal infection and density had interdependent effects (although notice $p \leq 0.0513$). In low density and mycorrhizal treatments individual size inequality was higher (Fig. 5.5).

5.3.3. *Neighbourhood effect*

Only in four of the 8 factor combinations (1MH, 1ML, 4ML and 4NML) (Fig. 5.6, a, b and d) was a small proportion of the variability of the individual shoot biomass explained by a positive linear relationship with the number of adjacent patches with high P. For the other treatments there was no significant relation between those two parameters (Fig. 5.6, b-d). The individual biomass of mycorrhizal plants growing at low density in patches with high (1MH) or low P (1ML) increased as the number of adjacent patches with high P increased (Fig. 5.6a). A similar effect was observed in mycorrhizal and non-mycorrhizal plants growing at high density in low P patches (4ML and 4NML, respectively) (Fig. 5.6, b and d).

In all factor combinations (except 4MH) the individual root biomass was independent of the number of adjacent patches with high P concentration (Fig. 5.7, a-d). The individual root biomass of mycorrhizal plants growing at high density in patches with high P (4MH) decreased as the number of adjacent patches with high P increased (Fig. 5.7b). A similar effect was found in individual root length (data not shown).

5.3.4. *Effects at patch level*

Phosphorus increased total shoot biomass per patch independently of mycorrhizal infection and density, whereas density and mycorrhizas had interdependent effects (Table 5.3). Plants growing in high P patches had the highest shoot biomass. Mycorrhizal plants, at low densities had higher shoot biomass than non-mycorrhizal plants. At high density there was no differences between mycorrhizal and non-mycorrhizal plants (Fig. 5.8a).

Phosphorus and mycorrhizas had independent effects on individual shoot biomass per patch, but both factors had interdependent effects with density (Table 5.3). At low density mycorrhizal plants had higher individual shoot biomass per patch, but at high density, there were no differences between mycorrhizal and non-mycorrhizal plants (Fig. 5.8b). Plants growing in high P patches at low density had higher individual shoot biomass than the rest (Fig. 5.8b).

Phosphorus, plant density and mycorrhizas had interdependent effects on root biomass (total per patch and individual per patch) (Fig. 5.9, a-b) and root length (total per patch and individual per patch) (data not shown) (Table 5.4). In patches with high P mycorrhizal and non-mycorrhizal plants had higher root biomass than in patches with low P, at both densities. At high density total root biomass was higher than at low density in all the patches, with the exception of mycorrhizal plants in patches with average P and non-mycorrhizal plants in patches with high P that showed no differences between densities (Fig. 5.9a). Root length per patch had a similar pattern (data not shown). The individual root biomass of mycorrhizal and non-mycorrhizal plants at high or low density was higher in high P patches than in the low P ones. The corresponding values for patches with average P were intermediate. In low P patches, an increase in density did not reduce individual root biomass. In high P patches, only the root biomass of non-mycorrhizal plants was reduced by density and, in average P patches, only the root biomass of mycorrhizal ones was reduced (Fig. 5.9b). Individual root length mirrored this pattern.

5.3.5. Competition

The relative competition intensity, RCI_{tray}, was higher in mycorrhizal populations independently of patchiness (Fig. 5.10a, Table 5.5a) and, when calculated for groups of patches with different local P (RCI_{patch}), was higher in mycorrhizal patches independently of local P (Fig. 5.10b, Table 5.5b). It was also independent of the number of adjacent patches with high P (RCI_{neigh}) (Fig. 5.11).

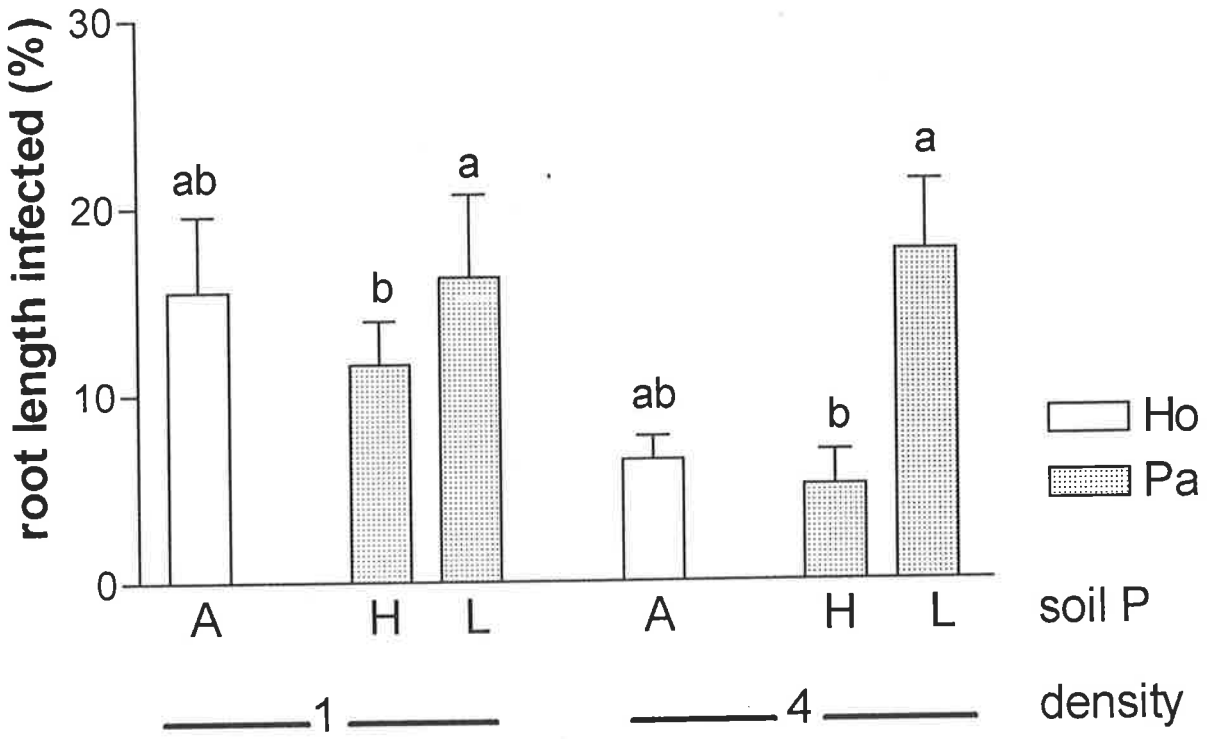


Figure 5.1. Percentage root length infected of plants of *Trifolium subterraneum* grown at low or high density (1 and 4 plants per patch, respectively) in patches with high (H) or low (L) soil P in patchy trays (Pa, shaded bars) or in average (A) soil P in homogeneous trays (Ho, clear bars). Data are means and SE, n = 5. Different letters indicate significant differences between the means of treatments with different local P (Tukey, $p \leq 0.05$). (Density had a negative and independent effect, Table 5.1).

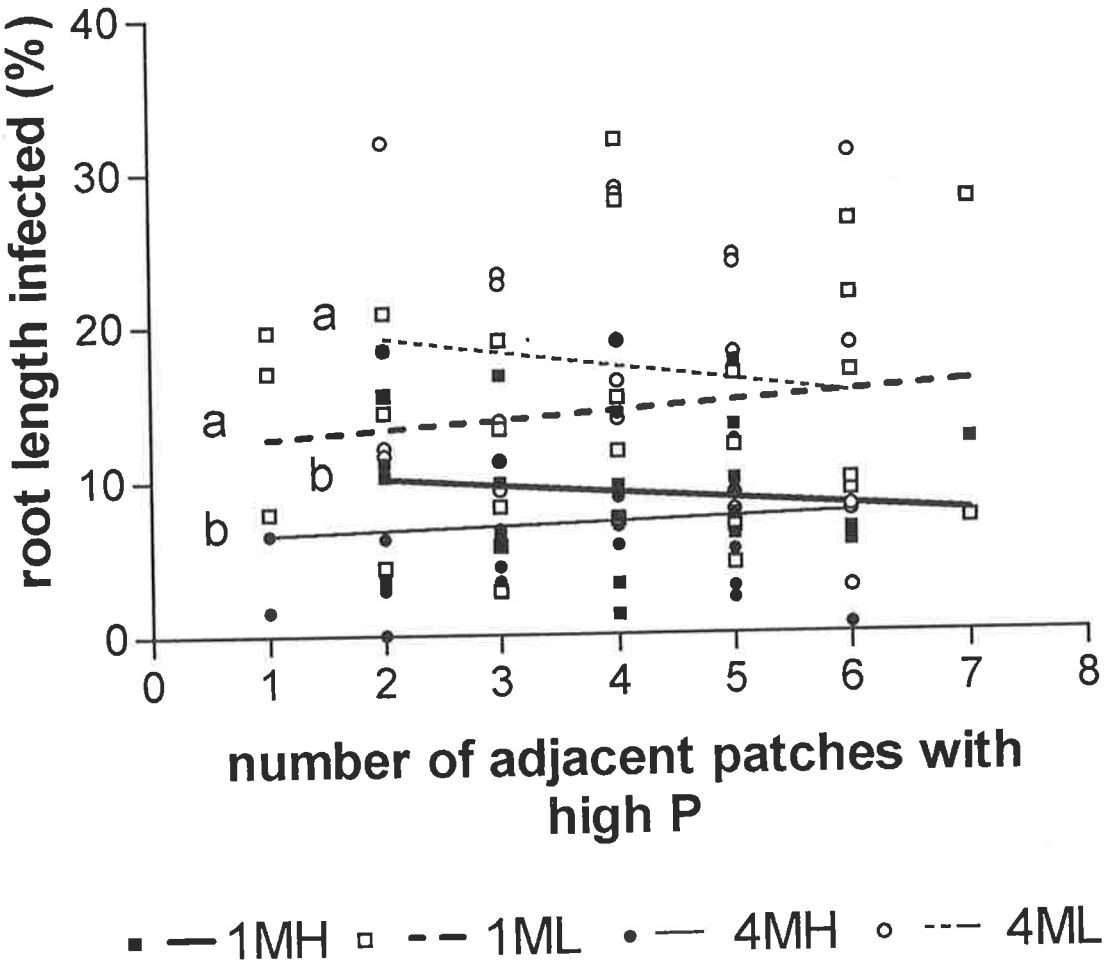


Figure 5.2. Effect of neighbourhood (the number of adjacent patches with high P) on the percentage root length infected of plants of *Trifolium subterraneum* grown at low density (1 plant per patch), in patches with high or low soil P (1MH and 1ML, respectively) or at high density (4 plants per patch), in patches with high or low soil P (4MH and 4ML, respectively).

1MH, $y = 11.02 - 0.4566x$; slope = 0; $r^2 = 0.022$ 4MH, $y = 6.268 + 0.2513x$; slope = 0; $r^2 = 0.006$

1ML, $y = 12.15 + 0.5719x$; slope = 0; $r^2 = 0.017$ 4ML, $y = 20.95 - 0.9123x$; slope = 0; $r^2 = 0.025$

Lines with elevations significantly different are labelled with different letters. = 0 indicates slope does not significantly deviate from zero ($p \leq 0.05$, (Prism 1994)).

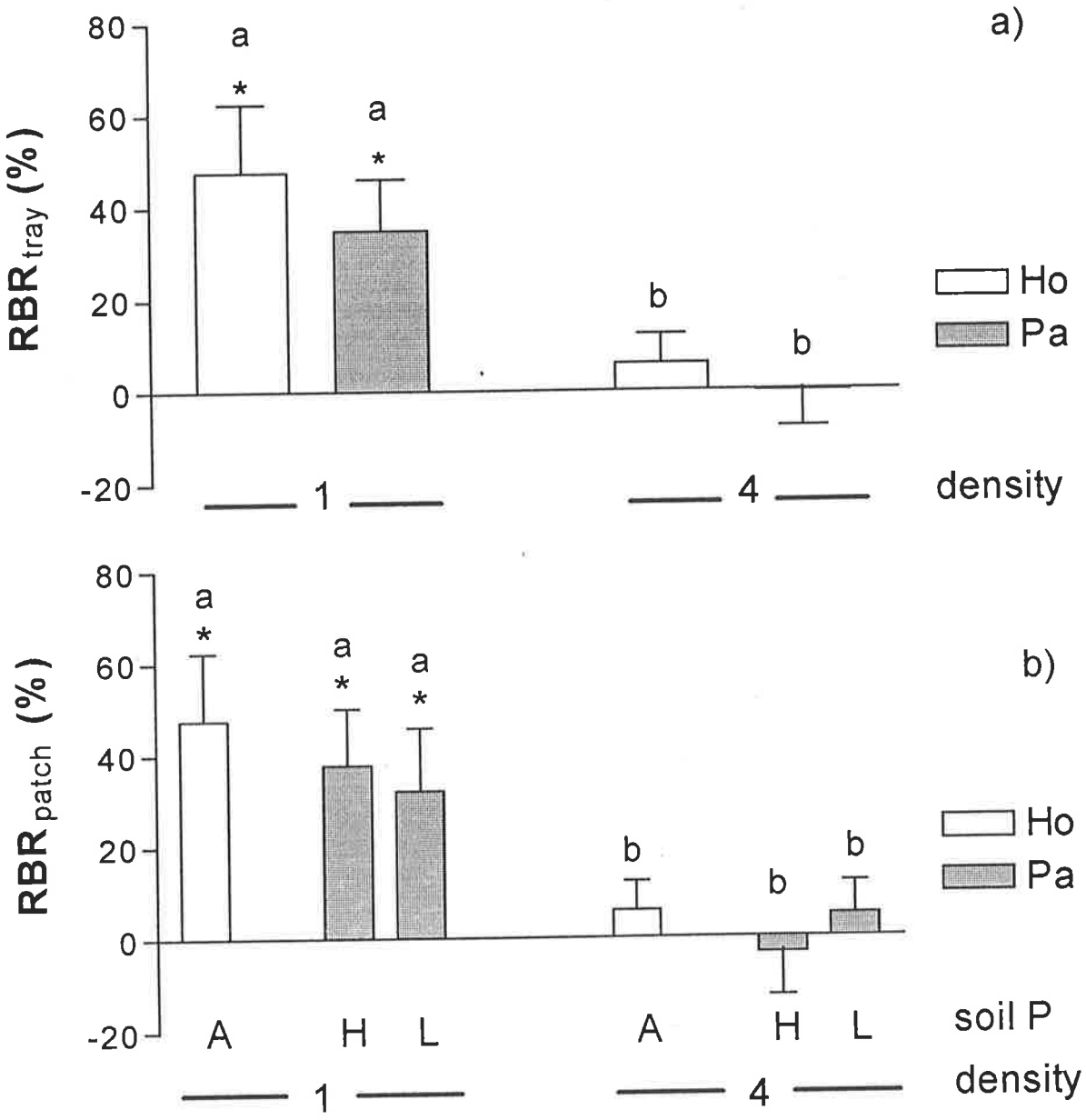


Figure 5.3. Relative biomass response to mycorrhizal infection (RBR, see text for calculations, formula (1)) of plants of *Trifolium subterraneum* grown at low or high density (1 and 4 plants per patch, respectively) in patches with high (H) or low (L) soil P in patchy trays (Pa, shaded bars) and with average (A) soil P in homogeneous trays (Ho, clear bars). Data are means and SE, n = 5. Stars indicate means significantly different from zero (Student's *t*, $p \leq 0.05$). Different letters indicate significant differences between means (Tukey, $p \leq 0.05$). a) RBR_{tray}, calculated to evaluate the effect of patchiness on RBR and b) RBR_{patch} calculated to evaluate the effect of local P on RBR.

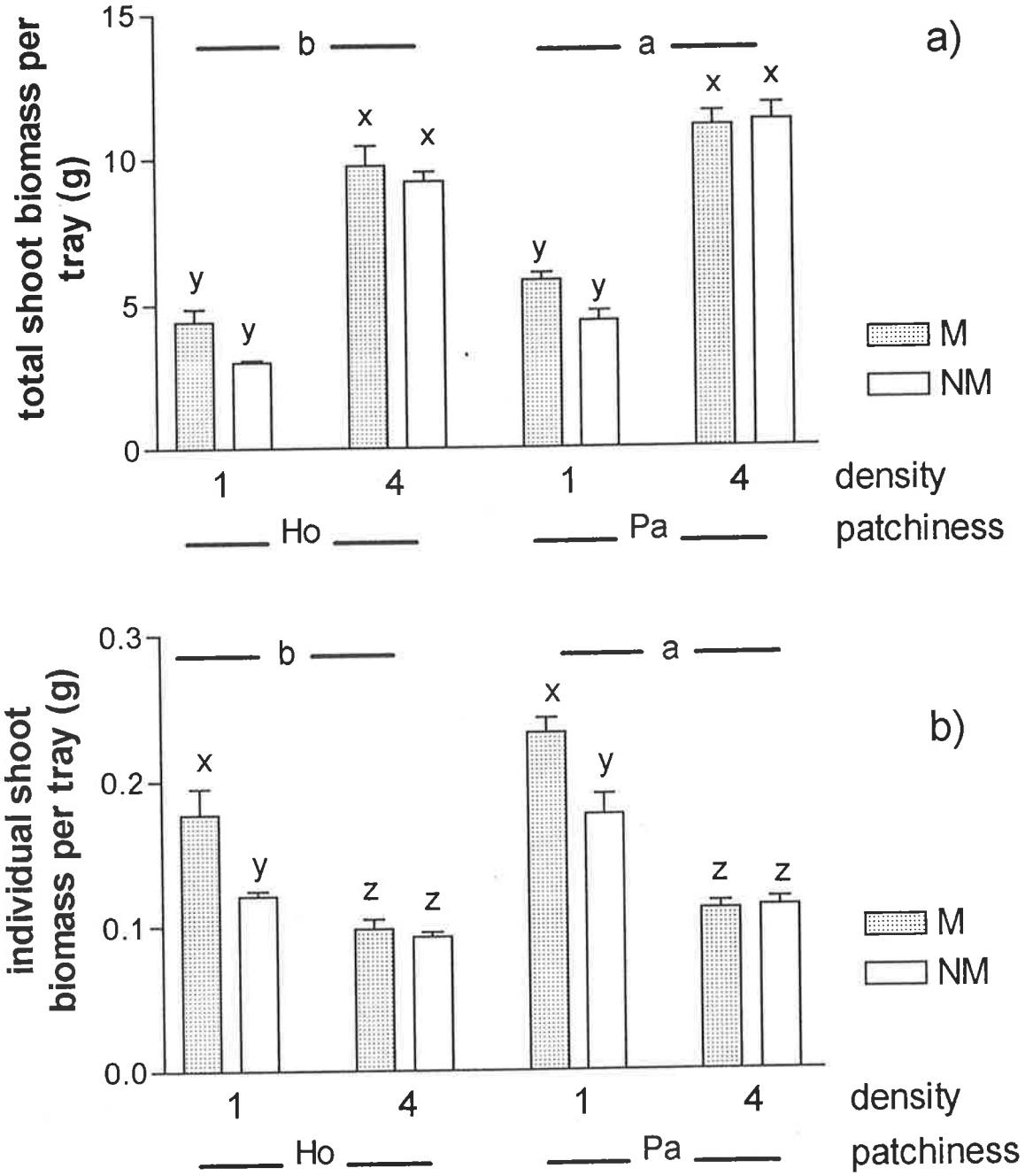


Figure 5.4. Shoot biomass per tray. a) total shoot biomass and b) individual shoot biomass (total shoot biomass per tray/number of plants per tray) of mycorrhizal (M, shaded bars) and non-mycorrhizal (NM, clear bars) plants of *Trifolium subterraneum* grown at low or high density (1 and 4 plants per patch, respectively), in homogeneous (Ho) or patchy (Pa) trays. Data are means and SE, n = 5. Different groups of letters used to indicate significant differences between means (Tukey, $p \leq 0.05$) within density (x, y, z) and patchiness (a, b) (factors with independent effects).

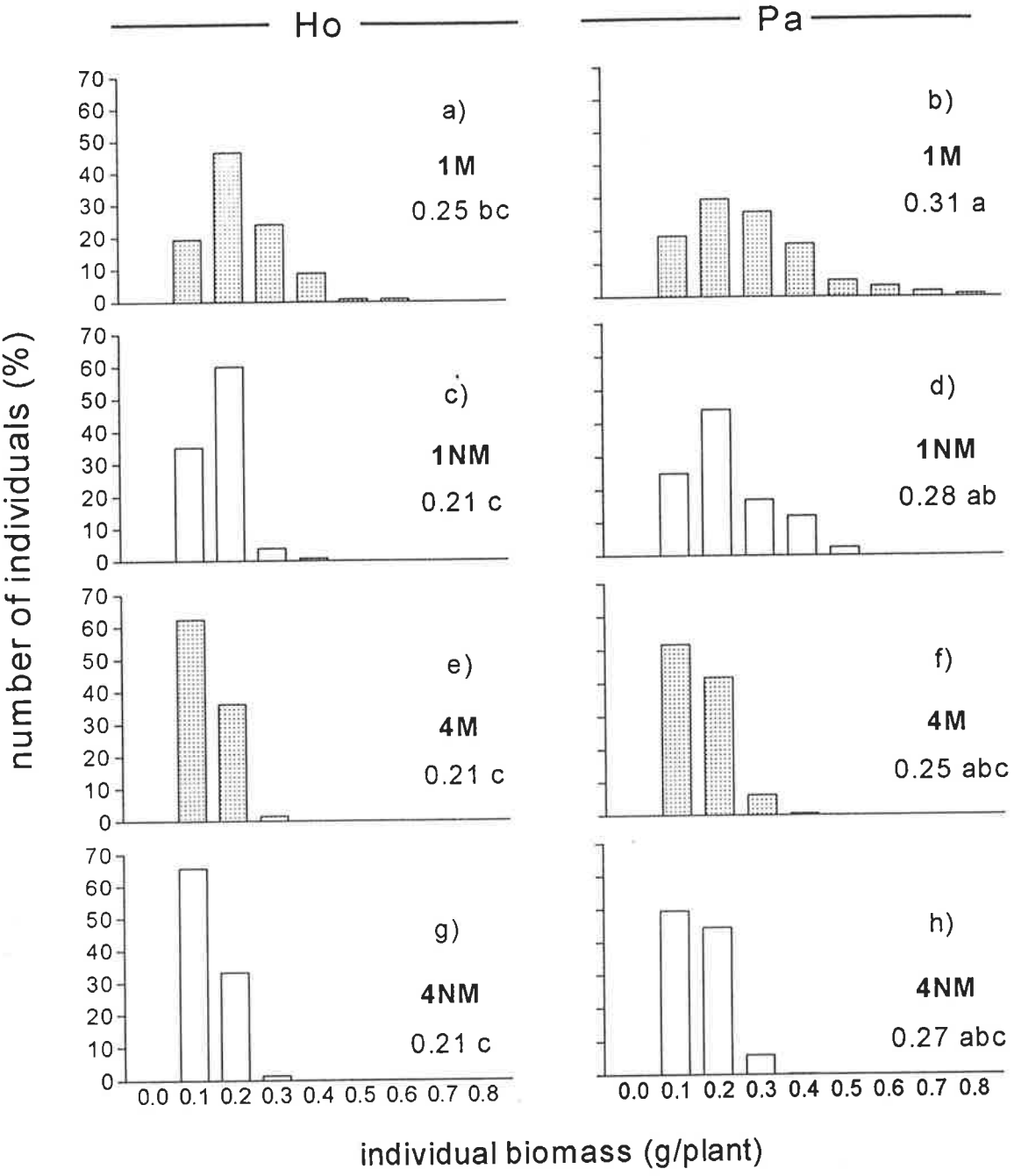


Figure 5.5. Individual size distributions of mycorrhizal (M, shaded bars) and non-mycorrhizal (NM, clear bars) plants of *Trifolium subterraneum*, grown at low (a-d) or high (e-h) density (1 and 4 plants per patch, respectively) in homogeneous (Ho) or patchy (Pa) trays, and the corresponding values of Gini coefficient. (Graphs show distribution of sizes of all the individuals from same treatments pooled together; Gini coefficient was calculated for each tray individually, and means are shown). Different letters indicate significant differences between means (Tukey, $p \leq 0.05$), $n = 5$.

Figure 5.6. Effect of neighbourhood (the number of adjacent patches with high P) on the individual shoot biomass of plants of *Trifolium subterraneum,* a) mycorrhizal plants grown at low density in patches with high (1MH) or low (1ML) soil P, b) mycorrhizal plants grown at high density, in patches with high (4MH) or low (4ML) soil P, c) non-mycorrhizal plants grown at low density, in patches with high (1NMH) or low (1NML) soil P, d) non-mycorrhizal plants grown at high density, in patches with high (4NMH) or low soil (4NML) soil P.

1MH, $y = 0.1876 + 0.0327x$; slope $\neq 0$; $r^2 = 0.07$

1ML, $y = 0.0795 + 0.0202x$, slope $\neq 0$; $r^2 = 0.08$

4MH, $y = 0.1297 + 0.0020x$, slope $= 0$, $r^2 = 0.003$

4ML, $y = 0.0523 + 0.0085x$, slope $\neq 0$; $r^2 = 0.197$

1NMH, $y = 0.1877 + 0.0118x$, slope $= 0$, $r^2 = 0.03$

1NML, $y = 0.1009 + 0.0057x$, slope $= 0$, $r^2 = 0.01$

4NMH, $y = 0.1309 + 0.003x$, slope $= 0$, $r^2 = 0.012$

4NML, $y = 0.0503 + 0.0087x$, slope $\neq 0$, $r^2 = 0.21$

Lines with elevations significantly different are labelled with different letters. $= 0$ indicates slope does not significantly deviate from zero, $\neq 0$ indicates slope significantly deviate from zero ($p \leq 0.05$, (Prism 1994)).

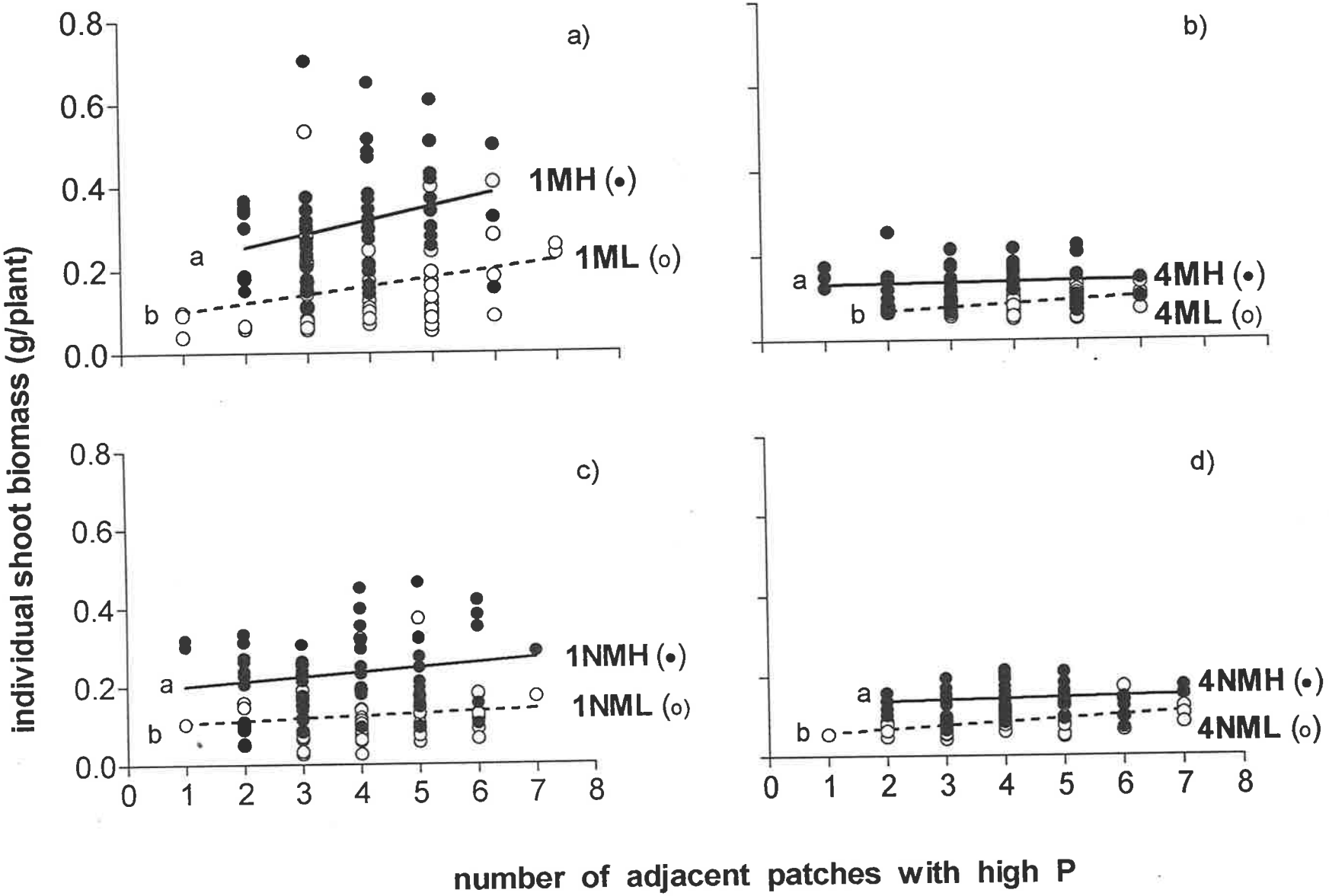

a)
b)
c)
d)
1MH (•)
1ML (o)
4MH (•)
4ML (o)
1NMH (•)
1NML (o)
4NMH (•)
4NML (o)
a
b
individual shoot biomass (g/plant)
number of adjacent patches with high P
0.0
0.2
0.4
0.6
0.8
0 1 2 3 4 5 6 7 8

Figure 5.7. Effect of neighbourhood (the number of adjacent patches with high P) on the individual root biomass of plants of *Trifolium subterraneum.* a) mycorrhizal plants grown at low density in patches with high (1MH) or low (1ML) soil P. b) mycorrhizal plants grown at high density, in patches with high (4MH) or low (4ML) soil P. c) non-mycorrhizal plants grown at low density, in patches with high (1NMH) or low (1NML) soil P. d) non-mycorrhizal plants grown at high density, in patches with high (4NMH) or low soil (4NML) soil P.

1MH, $y = 0.1389 + 0.0046x$, slope = 0; $r^2 = 0.016$

1ML, $y = 0.0536 - 0.0004x$, slope = 0; $r^2 = 0.001$

4MH, $y = 0.0963 - 0.007x$, slope $\neq 0$, $r^2 = 0.214$

4ML, $y = 0.0246 + 0.0016x$, slope = 0; $r^2 = 0.087$

1NMH, $y = 0.1416 - 0.0006x$, slope = 0, $r^2 = 0.01$

1NML, $y = 0.0385 + 0.0013x$, slope = 0, $r^2 = 0.02$

4NMH, $y = 0.0733 + 0.001x$, slope = 0, $r^2 = 0.003$

4NML, $y = 0.0244 + 0.001x$, slope = 0, $r^2 = 0.057$

?, because the slopes differ so much, it is not possible to test whether the intercepts differ significantly.

Lines with elevations significantly different are labelled with different letters. = 0 indicates slope does not significantly deviate from zero, $\neq 0$ indicates slope significantly deviate from zero ($p \leq 0.05$, (Prism 1994)).

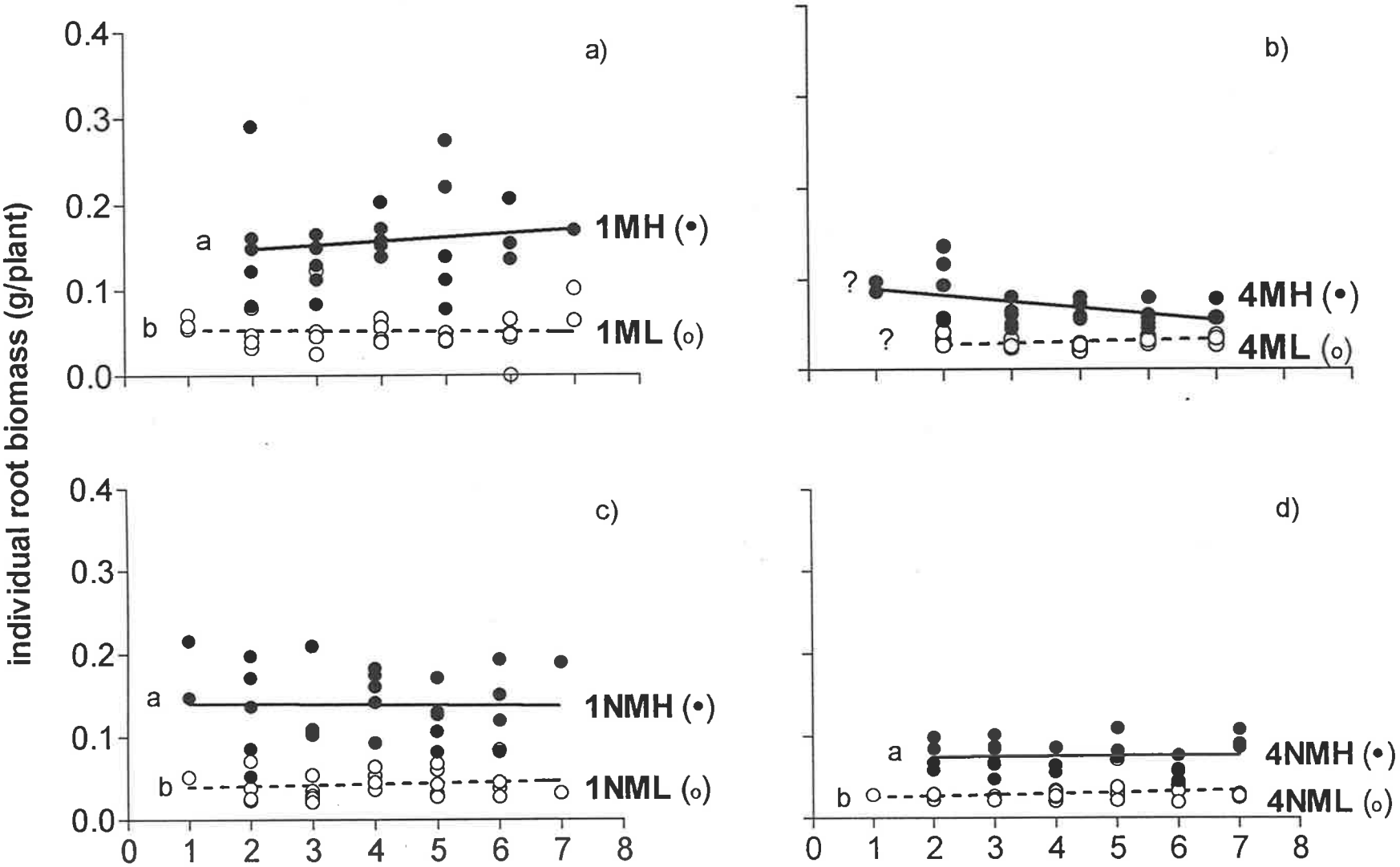

individual root biomass (g/plant)
0.4
0.3
0.2
0.1
0.0
a)
a
b
1MH (•)
1ML (o)
b)
?
?
4MH (•)
4ML (o)
c)
a
b
1NMH (•)
1NML (o)
d)
a
b
4NMH (•)
4NML (o)
0 1 2 3 4 5 6 7 8
number of adjacent patches with high P

Figure 5.8. Shoot biomass per patch. a) total shoot biomass and b) individual shoot biomass (total shoot biomass per patch/number plants per patch) of mycorrhizal (M, shaded bars) and non-mycorrhizal (NM, clear bars) plants of *Trifolium subterraneum* grown at low or high density (1 and 4 plants per patch, respectively) in patches with high (H) and low (L) soil P in patchy trays (Pa), and in patches with average (A) soil P in homogeneous (Ho) trays. Data are means and SE, n = 5. Different letters indicate significant differences between means (Tukey, $p \leq 0.05$).

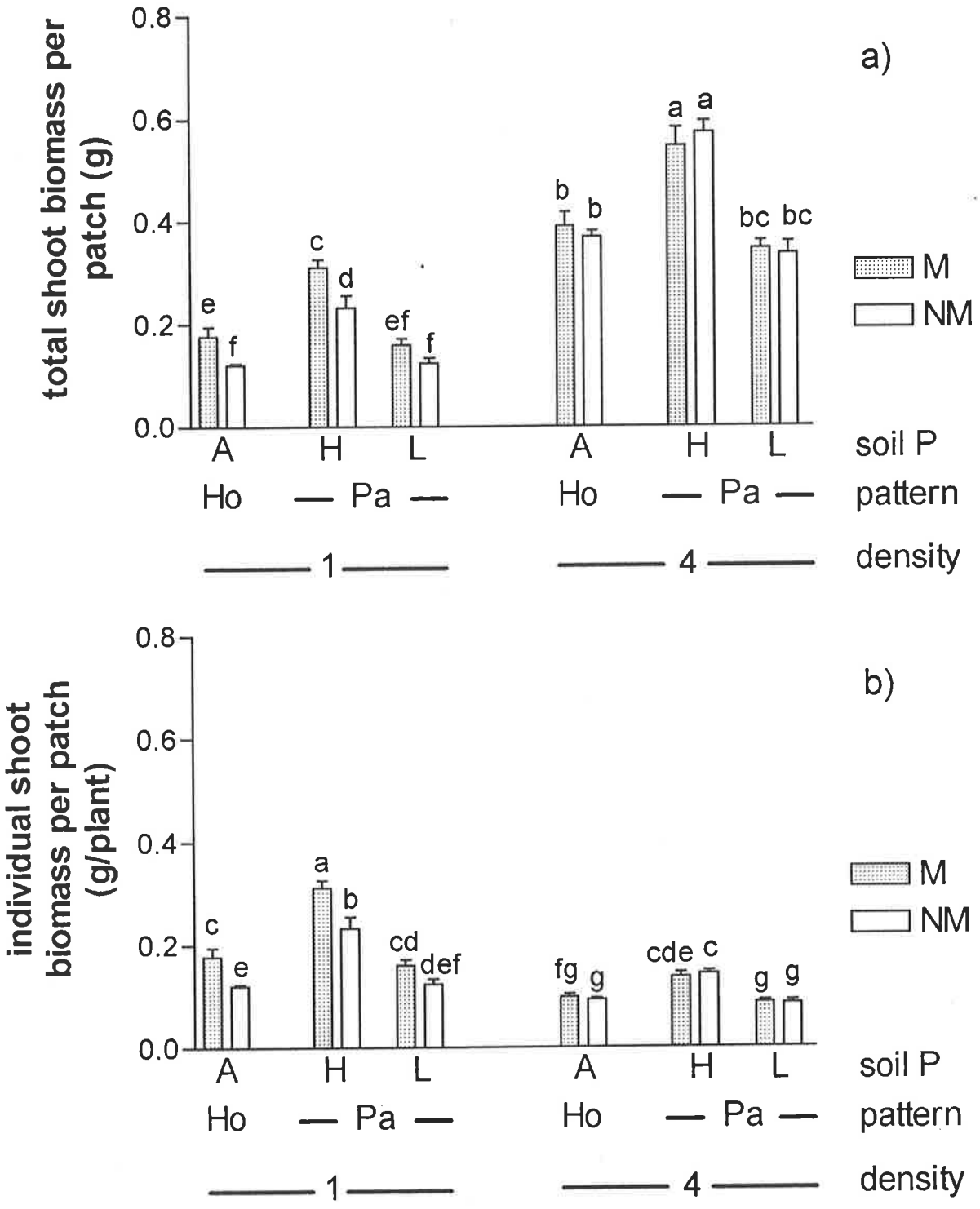
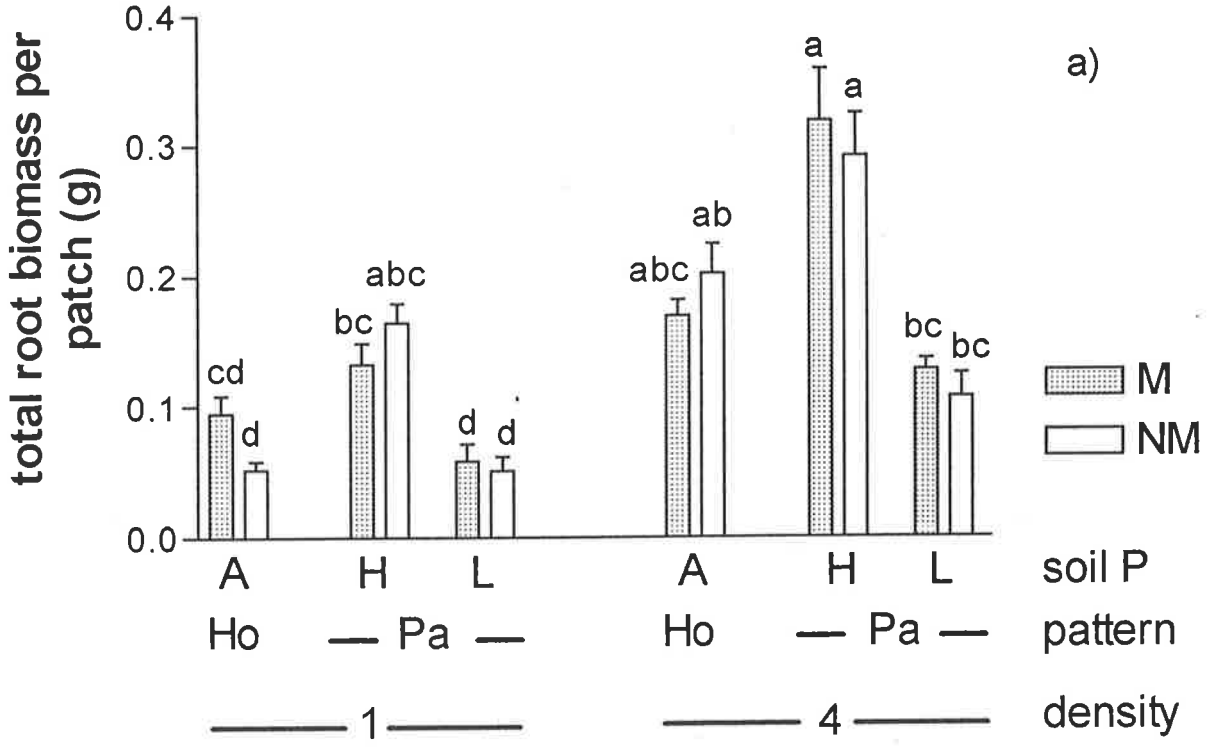
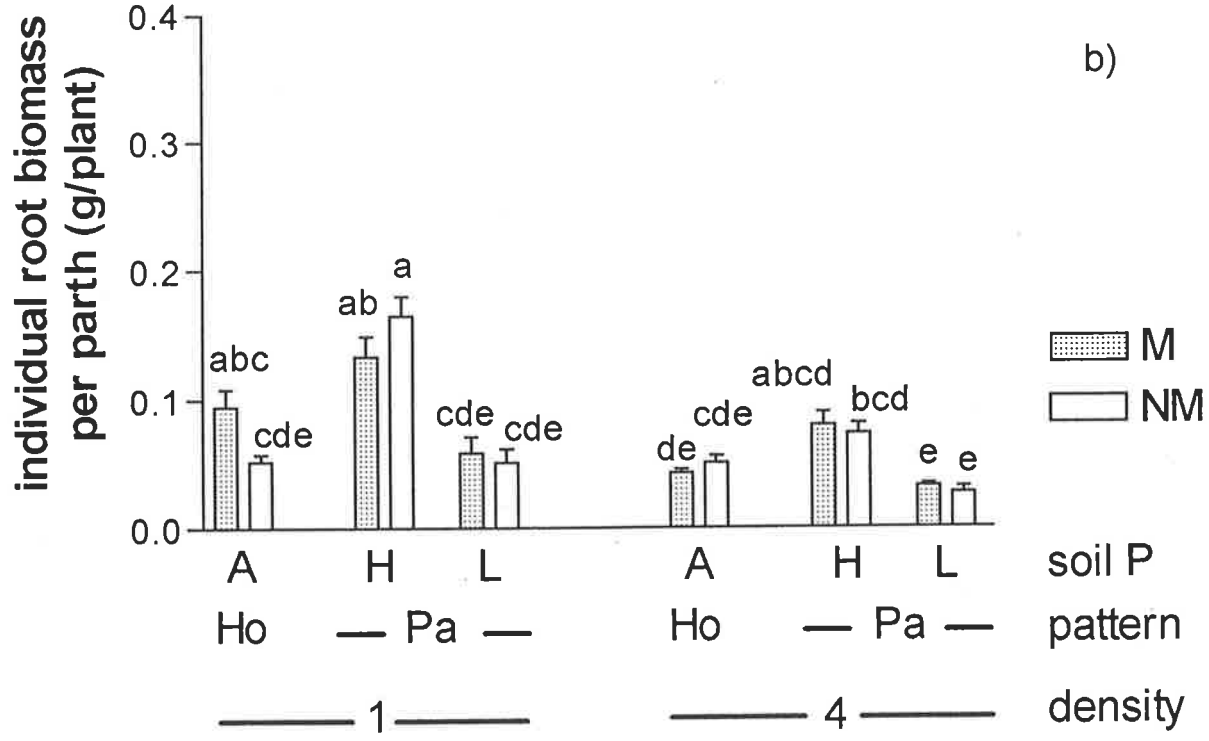

a)
total shoot biomass per patch (g)
0.8
0.6
0.4
0.2
0.0
e
f
c
d
ef
f
b
b
a
a
bc
bc
M
NM
A
H
L
A
H
L
soil P
Ho
Pa
Ho
Pa
pattern
1
4
density
b)
individual shoot biomass per patch (g/plant)
0.8
0.6
0.4
0.2
0.0
c
e
a
b
cd
def
fg
g
cde
c
g
g
M
NM
A
H
L
A
H
L
soil P
Ho
Pa
Ho
Pa
pattern
1
4
density

Figure 5.9. Root biomass per patch. a) total root biomass and b) individual root biomass (total shoot biomass per patch/number plants per patch) of mycorrhizal (M, shaded bars) and non-mycorrhizal (NM, clear bars) plants of *Trifolium subterraneum* grown at low or high density (1 and 4 plants per patch, respectively) in patches with high (H) and low (L) soil P in patchy trays (Pa), and in patches with average (A) soil P in homogeneous (Ho) trays. Data are means and SE, n = 5. Different letters indicate significant differences between means (Tukey, $p \leq 0.05$).


a)
total root biomass per patch (g)
0.4
0.3
0.2
0.1
0.0
cd
d
bc
abc
d
d
abc
ab
a
a
bc
bc
M
NM
A
H
L
A
H
L
soil P
Ho
Pa
Ho
Pa
pattern
1
4
density


b)
individual root biomass per parth (g/plant)
0.4
0.3
0.2
0.1
0.0
abc
cde
ab
a
cde
cde
de
cde
abcd
bcd
e
e
M
NM
A
H
L
A
H
L
soil P
Ho
Pa
Ho
Pa
pattern
1
4
density

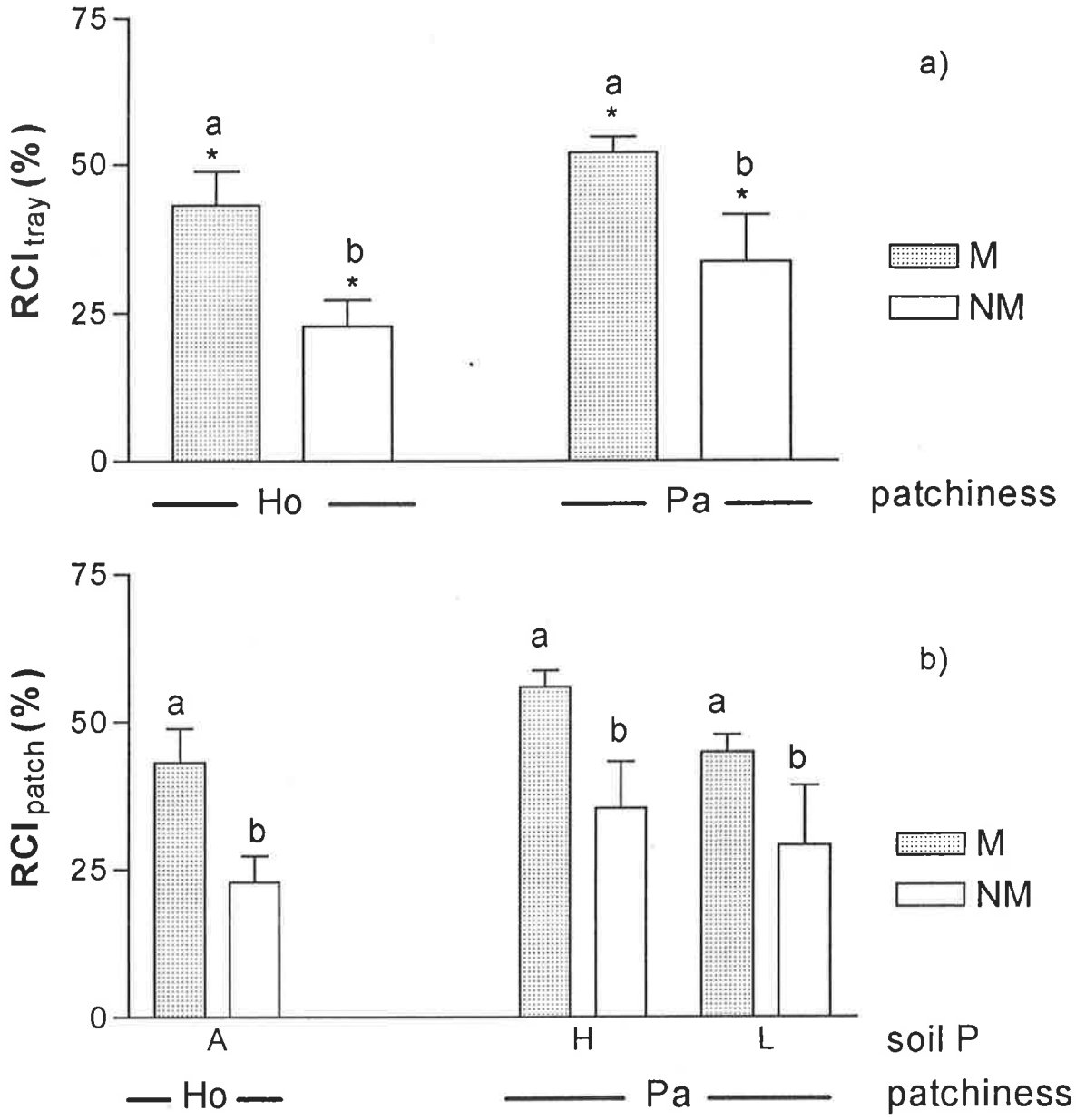


Figure 5.10. Relative competition intensity (RCI) of mycorrhizal (M, shaded bars) and non-mycorrhizal (NM, clear bars) plants of *Trifolium subterraneum*, grown at low or low density (1 and 4 plants per patch, respectively) in patches with high (H) or low (L) soil P in patchy trays (Pa), and in patches with average (A) soil P in homogeneous (Ho) trays. Data are means and SE, n = 5. Stars indicate means significantly different from zero (Student's *t*, $p \leq 0.05$). Different letters indicate significant differences between means (Tukey, $p \leq 0.05$). a) RCI_{tray}, calculated to evaluate the effect of patchiness on RCI and b) RCI_{patch} calculated to evaluate the effect of local P on RCI.

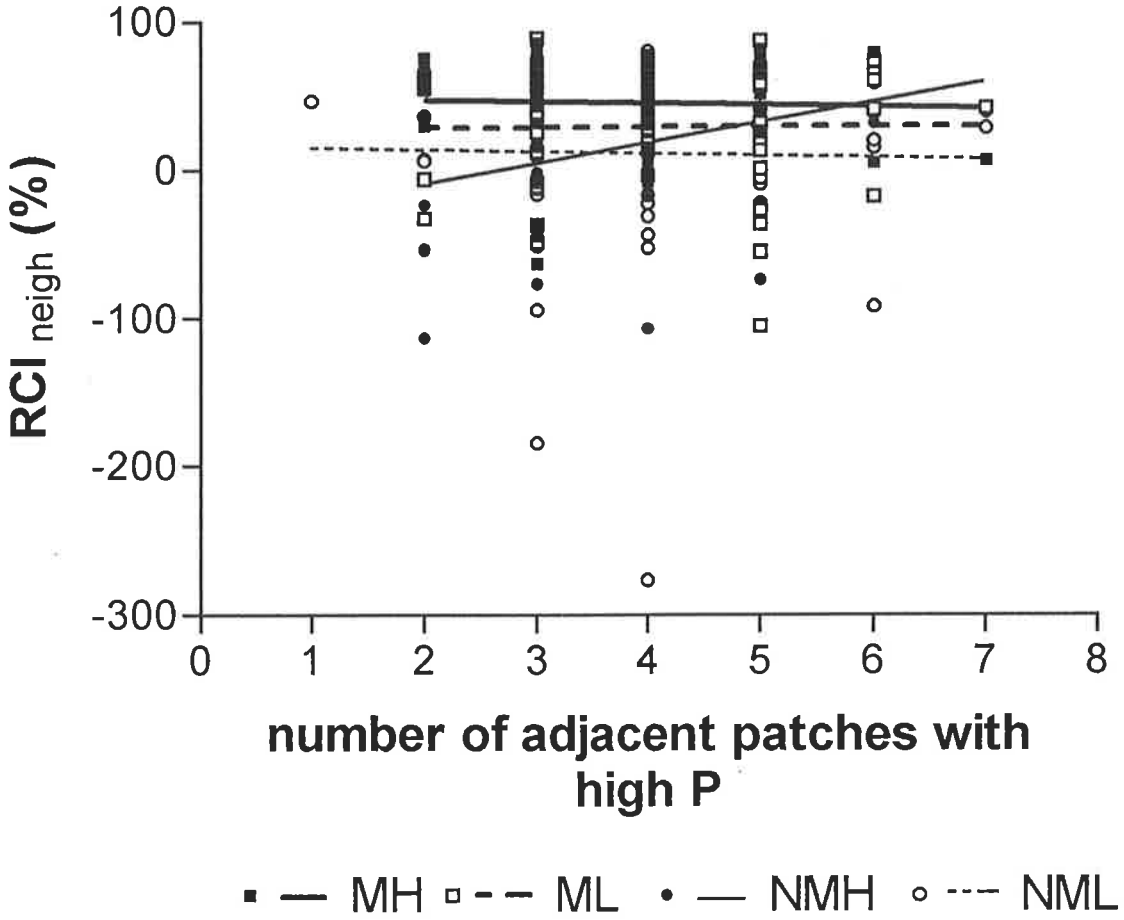


Figure 5.11. Effect of neighbourhood (the number of adjacent patches with high P) on relative competition intensity, RCI_{neigh} (see text for calculations, formula (2)) of mycorrhizal (M) and non-mycorrhizal (NM) plants of *Trifolium subterraneum*, grown in patches with high (H) or low (L) soil P.

Table 5.1. Results of ANOVA testing the effect of plant density and local soil P on the percentage infection and the root length infected of mycorrhizal plants of *Trifolium subterraneum*, grown in patches with high, low or average soil P, at high or low densities, n = 5.

ANOVA		percentage infection			root length infected		
transformations		arcsin (SQRT ratio)			log10 (length)		
factors	df	MS	F value	Pr > F	MS	F value	Pr > F
phosphorus	2	0.0501	5.01	0.0172	0.0657	0.59	0.5655
density	1	0.0507	5.08	0.0356	2.3328	20.82	0.0001
M * Pat.	2	0.0198	1.98	0.1644	0.3226	2.88	0.0797

Table 5.2. Results of ANOVA testing the effects of mycorrhizal infection, plant density and patchiness, on shoot biomass (total and individual) and Gini coefficient of the size distributions of mycorrhizal and non-mycorrhizal plants of *Trifolium subterraneum*, grown in trays with homogeneous or patchy soil P distribution, at high or low densities, n = 5.

ANOVA		total shoot biomass per tray			individual shoot biomass per tray			Gini coefficient		
transformations		no			log10 (biomass)			no		
factors	df	MS	F value	Pr > F	MS	F value	Pr > F	MS	F value	Pr > F
patchiness	1	23.4258	23.82	0.0001	0.1145	33.52	0.0001	0.0333	34.19	0.0001
mycorrhizas	1	6.3226	6.43	0.0171	0.0562	16.45	0.0004	0.0019	1.93	0.1754
M * Pat.	1	0.3365	0.34	0.5633	0.0027	0.78	0.3837	0.0002	0.20	0.6560
density	1	350.008	355.86	0.0001	0.4853	142.00	0.0001	0.0086	8.83	0.0060
D * Pat.	1	0.2043	0.21	0.6520	0.1305	3.82	0.0607	0.0009	0.93	0.3425
M * D	1	3.7927	3.86	0.0596	0.0449	13.14	0.0011	0.0040	4.15	0.0513
M * D * Pat.	1	0.3085	0.31	0.5799	0.0000	0.01	0.9200	0.0000	0.03	0.8533

Table 5.3. Results of GLM analysis testing the effects of mycorrhizal infection, plant density and local soil P on shoot biomass (total and individual) of mycorrhizal and non-mycorrhizal plants of *Trifolium subterraneum*, grown in patches with high, low or average soil P, at high or low densities, n = 5.

GLM		total shoot biomass per patch			individual shoot biomass per patch		
transformations		SQRT (biomass)			log10 (biomass)		
factors	df	MS	F value	Pr > F	MS	F value	Pr > F
phosphorus	1	3.0627	374.78	0.0001	8.6342	308.39	0.0001
mycorrhizas	1	0.2613	31.98	0.0001	1.0467	37.39	0.0001
M * P	2	0.0055	0.67	0.5111	0.0207	0.74	0.4773
density	1	11.6510	1425.71	0.0001	8.6612	309.36	0.0001
D * P	2	0.0076	0.93	0.3949	0.2337	8.35	0.0003
M * D	1	0.1742	21.31	0.0001	0.7160	25.57	0.0001
M * D * P	2	0.0118	1.45	0.2357	0.0202	0.72	0.4855

Table 5.4. Results of ANOVA testing the effects of mycorrhizal infection, plant density and local soil P on root biomass (total and individual) and root length (total and individual) of mycorrhizal and non-mycorrhizal plants of *Trifolium subterraneum*, grown in patches with high, low or average soil P, at high or low densities, n = 5.

ANOVA		total root biomass per patch			individual root biomass			total root length per patch			individual root length		
transformations		log10 (biomass)			log10 (biomass)			log10 (length)			log10 (length)		
factors	df	MS	F value	Pr > F	MS	F value	Pr > F	MS	F value	Pr > F	MS	F value	Pr > F
phosphorus	1	1.3213	81.26	0.0001	1.3222	80.08	0.0001	2.2567	108.62	0.0001	2.2567	108.62	0.0001
mycorrhizas	1	0.0004	0.03	0.8719	0.0004	0.03	0.8709	0.0122	0.59	0.4479	0.0122	0.59	0.4479
M * P	2	0.0357	2.19	0.1255	0.0350	2.12	0.1342	0.0643	3.09	0.0569	0.0643	3.09	0.0569
density	1	1.7941	110.33	0.0001	0.6277	38.02	0.0001	0.7144	34.38	0.0001	1.6537	79.60	0.0001
D * P	2	0.0098	0.60	0.5523	0.0010	0.59	0.5598	0.0117	0.56	0.5747	0.0117	0.56	0.5747
M * D	1	0.0101	0.62	0.4345	0.0106	0.64	0.4277	0.0455	2.19	0.1471	0.0455	2.19	0.1471
M * D * P	2	0.0714	4.39	0.0192	0.0717	4.34	0.0200	0.0867	4.17	0.0230	0.0867	4.17	0.0230

Table 5.1. Results of ANOVA testing the effect of plant density and local soil P on the percentage infection and the root length infected of mycorrhizal plants of *Trifolium subterraneum*, grown in patches with high, low or average soil P, at high or low densities, n = 5.

ANOVA		percentage infection			root length infected		
transformations		arcsin (SQRT ratio)			log10 (length)		
factors	df	MS	F value	Pr > F	MS	F value	Pr > F
phosphorus	2	0.0501	5.01	0.0172	0.0657	0.59	0.5655
density	1	0.0507	5.08	0.0356	2.3328	20.82	0.0001
M * Pat.	2	0.0198	1.98	0.1644	0.3226	2.88	0.0797

5.4. Discussion

Our results showed that as predicted, populations of *T. subterraneum* growing in patchy environments had higher biomass. These populations had more size inequality and the size of the individuals was determined by local P concentrations and little affected by neighbourhood. Mycorrhizas increased plant biomass and size inequality in low density populations. Importantly, these effects were independent of nutrient distribution. I failed to detect any effect of heterogeneous nutrient distribution on relative competition intensity, which was in turn, increased by the presence of mycorrhizas.

5.4.1. Infection and biomass plant response

The percentage infection was relatively low compared with values from a previous growth room experiment with the same symbiotic partners, where single shaded plants of *T. subterraneum* had around 60% of root length infected (Chapter 4). The values of irradiance measured at noon in the glasshouse were similar to the irradiance received by those shaded plants (around 180μm m^{-2} s^{-1}). However, the total irradiance received per day was probably much less in the glasshouse than in the growth room, despite light supplementation, because it was a very overcast winter. This reduction in irradiance was probably enough to reduce the number of fungal entry points (Tester *et al.* 1986), decreasing, in consequence, the percentage infection. However, this reduction did not prevent internal fungal development and effectiveness since I observed a high density of arbuscules (data not shown) and plants growing at low density had an increase in plant biomass due to the infection of about 50%.

The differences in percentage infection between low and high P patches mirrored previous works (Nadian *et al.* 1996) and support what it is known about the regulation of mycorrhizal infection by root nutritional status (Jasper *et al.* 1979; Gianinazzi-Pearson and Gianinazzi 1989; Koide and Li 1990). Since mycorrhizal plants growing in high P patches showed higher biomass than non-mycorrhizal plants, I discounted the possibility of a negative effect of soil P on the development of arbuscules as observed by Duke *et al.* (1994). Although plants in the high P patches had the lowest percentage infection they also had the longest roots, and it is possible that roots grew at a higher rate than the fungal

infection, generating a low root length infected/root length ratio (Gianinazzi-Pearson and Gianinazzi 1989). The assessment of proliferation of external hyphae (Jakobsen 1992; Olsson *et al.* 1995) and a more detailed study of fungal development (McGonigle *et al.* 1990; Bruce *et al.* 1994) were beyond the scope of the present work. Nevertheless, I suggest the use of the same experimental set up to further investigate the effect of soil P distribution on mycorrhizal fungal development.

The putative increase in plant biomass due to mycorrhizal infection was observed only in low density populations. This agrees with results from studies done with the same symbionts (Chapter 4) and with other plant and fungal species (Bååth and Hayman 1984; Koide 1991a; Hartnett *et al.* 1993). Importantly, the positive effect of the symbiosis on the biomass of low density populations occurred in both homogeneous and patchy trays. This result did not support the hypothesis that mycorrhizas are more beneficial in homogeneous environments where less soil P is available (Cui and Caldwell 1996a). Plants growing in either average, high or low P patches had similar relative increase in biomass due to mycorrhizal infection. Although the amount of P available in the low P patches limited plant growth, mycorrhizal plants could forage for more P than non-mycorrhizal plants and show a relative increase in biomass similar to mycorrhizal plants from patches with more available P (average or high soil P). The higher amount of P available for plants in the soil solution in high P patches, which is reached sooner and more efficiently by mycorrhizal roots (Smith and Read 1997), probably compensated for the low percentage infection observed. Thus, mycorrhizal plants in high P patches also had about 50% more biomass than non-mycorrhizal plants. These results showed that there is no single mechanism that explains how patchy distribution of P affects the plant response to mycorrhizal infection, and that the actual amount of P available in the patches regulates the magnitude of this response.

5.4.3. Effects at tray (population) level

Although the total amount of P was similar in patchy and homogeneous trays, plants in the patchy trays had higher shoot biomass. The P concentration in the high P patches

yielded an increase in biomass that overcompensated for the low growth of the plants in the low P patches, in the patchy trays. This effect was expected because the amount of the total P release to the soil solution in high P patches is more than proportional to the total soil P content (Caldwell *et al.* 1992; Robinson 1994). It is also probable that plants growing in those high P patches had a better nutritional status, this should permit high rates of uptake of all nutrients (S. Ayling, personal communication). Roots reaching the patches with high P concentration would respond with more proliferation of lateral roots (Drew 1975; Jackson *et al.* 1990) and the initiation of new roots (Drew 1975; Gross *et al.* 1993) which have higher rates of P uptake than older roots due to their relatively higher metabolic activity. In addition, roots reaching high P concentrations would have higher P uptake capacity, probably due to an increase in the activity of P carriers or pumps (Jackson *et al.* 1990). The increase of P uptake capacity of roots in response to high local P concentration has been studied in species from low nutrient environments and it was pointed out that these effects would probably be more evident in cultivated than in native species (Caldwell *et al.* 1992). In fact, in our work using a cultivated species, the positive effect of a patchy distribution of P was evident even at high density. However, the independence of the effect of patchiness from density is in conflict with other findings (Cahill Jr. 1995; Casper and Cahill Jr. 1996). Casper and Cahill Jr. (1996) expected stronger effects of patchiness on plant biomass of *Abutilon theophrasti* at low density, where competition was thought to be less important, but in fact patchiness increased individual biomass only at an intermediate density, making their results hard to interpret. Alternatively, Cahill Jr. (1995) found that the plant response to soil heterogeneity could be both density and species dependent. The two species tested in their experiment, *Ambrosia artemisiifolia* and *Phytolacca americana*, had different responses to soil heterogeneity. *P. americana* behave similarly to *T. subterraneum* and had higher biomass in heterogeneous soils. However, the plant biomass of *A. artemisiifolia* was not affected by density or patchiness.

These contrasting results are not completely surprising since physiological plasticity and evolutionary traits interact to determine the response of plants to soil heterogeneity (Campbell *et al.* 1991). *T. subterraneum*, a cultivated, "fast grower" forb, with high

responsiveness to addition of nutrients, showed "high precision foraging", similar to Campbell *et al.*'s (1991) findings in subordinate ("slow growers") species from fertile and unfertile environments. Although our results do not agree completely with their findings, it is important to highlight the fact that all the plants in their study were from non-cultivated species. The dominant "fast growers" were shrubs and the subordinate species were forbs.

The effect of patchiness on population biomass will also depend upon the scale of heterogeneity (Addicott *et al.* 1987; Stuefer *et al.* 1996; Wijesinghe and Hutchings 1997). A clonal plant (*Glechoma hederacea*) grown in heterogeneous environments with patches of different size but similar total amount of nutrients, had more biomass in the environments with bigger patches and responded to environments with small patches as if they were homogeneously poor (Wijesinghe and Hutchings 1997).

An increase in density produced a reduction in individual biomass in all treatments, independent of the patchiness. As in our previous experiment in homogeneous pots (Chapter 4), the relative competition intensity was increased only by mycorrhizal infection. Homogeneous and patchy trays had similar RCI because the reduction in biomass due to density was similar in average, low and high P patches. Plants in the high P patches in low density trays were bigger than plants in average and low P patches. Since the same occurred in high density trays, the reduction due to density was similar for all type of patches. In contrast, the reduction in biomass due to density was bigger in mycorrhizal plants because the infection benefited plants only at low density. At high density, mycorrhizal plants were of similar size to non-mycorrhizal plants.

5.4.4. Neighbourhood effect

As I mentioned before, plants growing in high P patches were bigger than the rest. Hence, I expected that, due to competition (Mack and Harper 1977), the biomass of individuals in each patch would be reduced as the number of adjacent patches with high P increased. However, I did not detect any negative effect of immediate neighbourhood on individual biomass (Mack and Harper 1977), probably because I harvested the plants when leaves started to overlap and compete for light. I used an indirect measurement of

neighbourhood interference, however direct and more complicated analyses by other workers have given similar results (Waller 1981; Fowler 1984). Its has been pointed out that patchiness could hide apparent effects of competition (Fowler 1984; Fowler 1988) and that sorting out the different mechanisms involved in this interference is very difficult (Waller 1981; Fowler 1984). In fact, I found that mycorrhizal plants at low density had more biomass as the number of adjacent patches with high P increased. The proportional increase was similar for plants growing in high or low P patches. Thus, mycorrhizal roots had foraged for P more efficiently, thus providing plants with more P as they reached more enriched patches. This high foraging capacity, independent of local P, suggests that hyphae did not necessarily proliferate more in rich patches (St. John *et al.* 1983) but they probably explored areas with high or low P equally (Cui and Caldwell 1996b). At high density, the effect of mycorrhizas diminished (Chapter 4) and only plants with low local P benefited from high P patches in the neighbourhood, probably because they were more P limited than plants in high P patches.

The experimental set up used here had important differences from the one used by (Casper and Cahill Jr. 1996). The use of one type of soil with differences only in the P concentration of the patches provided a very simple system to study the effect of soil heterogeneity. The random distribution of patches with different P concentration allowed us to study the effect of both local soil P and different neighbourhoods on individual plant biomass. Since I harvested before leaves overlapped considerably, I could study the effects of nutrient distribution and mycorrhizas without their interaction with competition for light. A second step would be to allow this competition to happen and also to conduct experiments with an established hyphal network.

5.4.5. Size inequality

As expected, a patchy soil nutrient distribution led to higher inequality in plant size distribution (cf. Casper and Cahill Jr. (1996)). Since local P concentrations regulated the size of the plants, patchy trays had higher size inequality than homogeneous ones (Fig. 5.5). The proportion of individuals with small size (within the 0.1 g class) was similar in

homogeneous and patchy trays. In the homogeneous trays there were almost no individuals in the bigger classes (more than 0.2 g), whereas in the patchy trays about more than 30% of the individuals were in bigger classes. It is known that even in even-aged populations, individuals differ in their growth rate (Firbank and Watkinson 1987; Weiner 1990). When competition for nutrients regulates plant size distribution (resource depletion model), the relative growth rate of all the individuals is expected to be reduced by the same proportion since resource acquisition is proportional to plant size (Weiner 1990). However, if the distribution of nutrients is patchy and large individuals can reach enriched patches, asymmetric competition will regulate size distribution (resource pre-emption model, snowball cumulation effect) (Weiner 1990). Large plants will obtain more than a proportional share of the resources relative to size and the variation in relative growth rates will increase and give rise to the variation in sizes (Weiner 1990). Our results, which show that snowball cumulation is possible when nutrient competition occurs, support Weiner's (1990) hypothesis of asymmetric competition in patchy environments and contradict the suggestion of Wilson (1988a) that snowball cumulation occurs only when competition for light is predominant. The experiments that supported this idea (Newbery and Newman 1978) used plants with different sizes (different initial advantage) which were planted in homogenous soils.

The presence of mycorrhizas in low density trays also increased size inequality, as observed before (Chapter 4). Some plants may have became infected earlier and in consequence could absorb the available P faster or respond more efficiently to the infection. These plants had an initial advantage that was reflected in their bigger sizes. Since mycorrhizal plants can reach more P than non-mycorrhizal ones, the effect on size distribution is similar to a patchy distribution of P, a small initial advantage is translated into bigger size and probably enhanced plant fitness (Chapter 4). The high inequality of mycorrhizal populations at low density did not support the idea that the hyphal network connections between plants redistribute nutrients and reduce variation in size of individuals (Grime *et al.* 1987; Law 1989). Our findings agree with other studies done in homogeneous environments (Allsopp and Stock 1992a; Shumway and Koide 1995; cf.

Maffia and Janos (1993)). In those experiments, as in ours, the sources of inoculum used were pieces of roots or spores from which each plant became infected separately. It is possible that the results would have been different if the plants were established on an intact mycorrhizal network (Newman *et al.* 1992) which linked the plants together from the beginning of the experiment.

I have now shown that plants of equal size and age growing in a favourable, nutrient rich patch will have an initial advantage over those of the same population growing in unfavourable patches, and that this initial advantage could lead to asymmetric competition. A similar situation will develop when plants become infected by mycorrhizal fungi earlier than others. If size differences are maintained after strong competition for light occurs (that stage was not reached in our experiment), those larger individuals would have more chance to competitively exclude others, and hence develop a greater chance of reproductive success (Jackson and Caldwell 1989; Campbell *et al.* 1991; Hetrick 1991).

Chapter 6

General discussion and future research

6.1. General discussion

The main effects of mycorrhizal symbiosis on single plants, nominally, increase in plants biomass, growth rate and/or fitness due to an increased P uptake, are seldom found in populations of host plants growing in natural conditions (Fitter 1985; McGonigle 1988; Carey *et al.* 1992; Newsham *et al.* 1995a). The results of this project showed that these main effects at the individual level cannot be expected to be apparent at the population level because of the influence of density-dependent processes.

In pots with homogeneous distribution of P, the addition of P to *Rhodanthe chlorocephala* and mycorrhizal infection in *Trifolium subterraneum,* increased plant biomass of single plants. However, the beneficial effect of P addition and mycorrhizal infection was reduced by plant density. Surprisingly, a reduction in the light intensity received by plants of *T. subterraneum* did not generally alter the plant response to density or to mycorrhizal infection. Although single shaded plants had less biomass, the lack of effect of shading at high densities on biomass, P content, and mycorrhizal infection of plants of *T. subterraneum* suggests that the availability of P has a very important role in regulating the effects of mycorrhizal infection on plant populations.

Since the biomass and the P content of individual non-mycorrhizal plants was almost the same at all densities, it appears that each non-mycorrhizal plant is only able to access a small proportion of the available nutrients due to the small size of its root system, and that growth is therefore limited by nutrient availability. Consequently, even at higher densities there is little or no overlap of P depletion zones and no effect of reducing light. Thus, non-mycorrhizal plants might not compete for P because of their limited growth (Reader 1990). On the other hand, single mycorrhizal plants depleted most of the available P and had more shoot and root biomass and higher requirements for resources other than P. Consequently,

as density increased mycorrhizal plants compete for P and other resources because of their enhanced growth.

Similar to the effect produced by mycorrhizas on the relative competition intensity (RCI) of plants of *T. subterraneum*, the addition of P to plants of *R. chlorocephala* also increased their RCI. As density increased there was always a reduction in the biomass of individual plants, but the RCI was bigger when P was added. These counter-intuitive results, indicating greater intensity of competition at higher resource levels support other findings (Wilson and Newman 1987; Wilson 1988b; Gurevitch *et al.* 1990) and indirectly support the hypothesis that competition is less important in infertile soils (Grime 1979; Grace 1990).

Absence of competition may allow coexistence of plants in environments where nutrients have low rates of movement in soil or high rates of supply relative to demand (Grace 1995b). Hence, it is of fundamental importance for the interpretation of results from investigations on the effects of plant competition, to establish which is the mycorrhizal responsiveness of the plants involved.

My results show that mycorrhizal infection and patchy distribution of P increase the total biomass and size inequality of populations of plants of *T. subterraneum*. This is partially in conflict with previous studies (Cahill Jr. 1995; Casper and Cahill Jr. 1996) where little effect of patchiness was found on plant populations. However, those results may be questioned since patchiness was obtained with different soils, which probably have different effects on root growth (S. Ayling, personal communication). In contrast, in my experiments I used only one type of soil, and patchiness was obtained by addition of different levels of P.

The results of my last experiment also show that in a patchy environment the biomass of individual plants is determined by the local soil P concentration. Similarly, mycorrhizal infection in low density treatments determines the size of individual plants independently of patchiness in soil P distribution. However, mycorrhizal infection, but not patchy P distribution, increases RCI.

In the patchy environments the biomass of individual mycorrhizal plants at low density increased as the number of adjacent patches with high P increased. The

proportional increase in biomass was similar for plants growing in high or low P patches. Thus, mycorrhizal roots may forage for P more efficiently, providing plants with more P as they reach more enriched patches. This high foraging capacity, independent of local P, suggests that hyphae do not necessarily proliferate more in rich patches (St. John *et al.* 1983) but they probably explore areas with high or low P equally (Cui and Caldwell 1996b).

The high size inequality of mycorrhizal populations at low density in homogeneous and patchy trays did not support the idea that the hyphal network connections between plants redistribute nutrients and reduce variation in size of individuals (Grime *et al.* 1987; Law 1989). This findings agree with other studies done in homogeneous environments (Allsopp and Stock 1992a; Shumway and Koide 1995).

Plants growing in patchy environments also have higher size inequality, independently of their mycorrhizal status. These results supported Weiner's (1990) hypothesis that if patches with more nutrients can be reached by large individuals, asymmetric competition occurs and this leads to populations with higher size inequality. Consequently, plants of equal initial size and age growing in a favourable, nutrient rich patch will have an initial advantage over those of the same population growing in unfavourable patches. A similar situation will develop when plants become infected by mycorrhizal fungi earlier than others. If size differences are maintained after strong competition for light occurs, those larger individuals would have more chance to competitively exclude others, and hence develop a greater chance of reproductive success (Jackson and Caldwell 1989; Campbell *et al.* 1991; Hetrick 1991). It is evident then, that for plants growing in competition, a rapid access to nutrients which deprive competitors of these nutrients is of more ecological significance than maximum nutrient uptake or the average nutrient concentration in soil (Campbell *et al.* 1991; Hetrick 1991).

The strong dependency of mycorrhizal effects on plant density might explain why results of field studies of the effects of mycorrhizal infection on P uptake by plants have been conflicting (Fitter 1985) and have rarely shown the expected increase in biomass due to the symbiosis (Mc Gonigle 1988).

Importantly, in dense populations, although the benefit from the symbiosis is not apparent, the initial advantage of plants that become infected early or respond more efficiently to the symbiosis, has an effect on plant size distribution due to asymmetric competition for nutrients. This asymmetric competition may contribute to the high fitness of those phenotypes. This scenario can explain the maintenance of the symbiotic ability even under conditions such as dense populations.

6.2. Future research

The high level of contamination of native plants with a wild mycorrhizal fungus in the first main experiment brings attention to another possible difficulty in experimental field studies on the ecological role of mycorrhizal symbiosis. Some wild mycorrhizal fungi are easily dispersed by wind and could be carried by seeds or fruits with hairy ornamentations (Warner *et al.* 1985; Walker 1988; Smith and Read 1997). This type of plant propagule are common in the Asteraceae family, and members of this family are important constituents of the Australian native flora. I suggest to investigate the relative importance of these seeds and fruits as dispersal agents of spores of mycorrhizal fungi. I also propose to explore the possibilities of growing native plants from cuttings. The use of cuttings would allow to exclude infection from seed-derived spores in studies of the response to mycorrhizal infection of many species from low nutrient environments.

Plant density had different effects on the percentage infection in the experiments performed using *T. subterraneum*. In the first one plant density did not affect the percentage infection, whereas in the second and the third it had a negative effect on it. The only investigation of the effect of plant density on percentage infection that I am aware of is the study of Abbott and Robson (1984), also in *T. subterraneum*. They postulate that a negative effect of plant density on the percentage of infection is due to a reduction in the formation of arbuscules as a consequence of a reduction in carbon transfer to the roots as competition for light increases. I suggest a more detailed study of the development of fungal structures (arbuscules, vesicles, and internal and external hyphae) as plant density increases, using vital staining (Smith and Dickson 1991; Smith and Dickson 1997) and the

magnified interceptions technique (McGonigle *et al.* 1990), is needed to clarify the actual effect of plant density on the formation and function of mycorrhizas

Another aspect of the symbiosis that needs further research is the activity of fungal hyphae in environments with patchy distribution of nutrients. The scarce information available on this aspect of the symbiosis is contradictory (St. John *et al.* 1983; Cui and Caldwell 1996b). The study of proliferation of hyphae in patchy and homogeneous trays was beyond the scope of my research. However the results of Chapter 5 suggest that fungal hyphae may explore areas with high or low P equally (Cui and Caldwell 1996b). This result is in conflict with the observation of more proliferation of hyphae in richer patches (St. John *et al.* 1983).

The experimental system described in Chapter 5 could be useful to study hyphal and root proliferation and function at a different scale of heterogeneity that the one used before (Jackson and Caldwell 1989; Jackson *et al.* 1990; Cui and Caldwell 1996b). These authors exposed the root system of a single plant to patches of different soil nutrient concentrations. In the experimental setting I propose, each plant grows in a patch of a certain nutrient concentration.

Finally, another area that needs further investigation is the actual ecological role of the hyphal network. Several studies (including mine) of the effect of mycorrhizas on the structure of populations of the host plants showed that mycorrhizal populations have higher size inequality. This is in conflict with the idea of a hyphal network transferring nutrients and/or photoassimilates from plant to plant (Grime *et al.* 1987; Law 1989). In most of those experiments, as in mine, the sources of inoculum used were pieces of roots or spores from which each plant became infected separately. I propose to investigate the effect of density and mycorrhizal infection on host plant populations established on an intact mycorrhizal network (Newman *et al.* 1992) which might link the plants together from the beginning of the experiment.

The understanding of the complex of interactions between plants and micro-organisms is, at present, rudimentary (Gange and Brown 1997). Although it is clear that

micro-organisms alter plant inter and intraspecific interactions, the importance of these indirect interactions is yet to be fully assessed. Therefore, studies such as the one presented here and those which combine field research with glasshouse experiments are promising. A reductionist approach of the research is required as a first step to produce basic information for the understanding of complex interactions in the framework of ecological theory.

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