COLONIZATION OF *ACACIA* SPECIES BY VA MYCORRHIZAL FUNGI IN A MEDITERRANEAN ENVIRONMENT

by

Sergio Gaiad

B.Sc. (Forestry)

This thesis is presented for the degree of
Master of Science
of the University of Western Australia

Soil Science and Plant Nutrition
School of Agriculture

October, 1989

ABSTRACT

Leguminous species are an important component of the understorey of *Eucalyptus marginata* Donn ex Sm. (jarrah) forest in south-western Australia. *Acacia pulchella* R.Br. is a common legume in this environment, playing an important role in nutrient cycling. This species is also used in revegetation programs in areas disturbed by bauxite mining in the jarrah forest. The ability to form symbiotic associations with root nodule bacteria and VA mycorrhizal fungi is one of the reasons that makes this a successful species.

The VA mycorrhizal association increases the volume of soil explored for nutrients, by growing a network of hyphae into the soil, making it possible for the plants to take up nutrients that otherwise would be out of reach. The VA mycorrhizal fungi is an obligate symbiont therefore any factor affecting the plant is likely to have an effect on the fungi. Plant activity (root and shoot growth, flowering) is strongly affected by the distribution of rainfall and changes in temperature in the highly seasonal environment of south-western Australia. The VA mycorrhizal association is a dynamic process therefore the assessment of the level of inoculum in a certain area must consider seasonal changes in fungal activity.

To test some of the factors affecting the VA mycorrhizal association of *A. pulchella*, three experiments were set up. In the first experiment, undisturbed soil cores were taken from four sites, two in natural jarrah forests, and two in areas revegetated after bauxite mining. Cores were taken at five times during the year. *Acacia pulchella* seedlings were grown for 10 weeks to test the hypothesis that VA mycorrhizal fungi would show different patterns of seasonal

variation if growing in soil from natural jarrah forest or in soil from revegetated areas.

For the second experiment, soil was collected during winter and summer, at four depth increments to 20cm. Phosphate was added to half of the soils to test the hypotheses that the infectivity of VA mycorrhizal fungi would a) change with the seasons, b) decrease with increasing depth of soil, and c) decrease with addition of phosphate.

In the last experiment, soil was collected from the depths of 0-3, 3-10, 10-20, 20-35, and 35-50cm from an undisturbed jarrah forest, and placed in wooden boxes in the same order that they were collected in the field. The boxes were placed in soil and seeds of *Acacia pulchella* were sown. Root growth and mycorrhizal development was monitored at six harvests over the following nine months.

Mycorrhizal formation in seedlings of *Acacia pulchella*, growing in undisturbed soil cores from natural jarrah forest, was positively related with rainfall. A decrease in infectivity during summer (January) coincided with the dry period when there was little root growth . With the onset of the winter rain in April/May, levels of infection rose sharply in cores collected in May, remaining at the same levels up to the last collection, in November. On the other hand there were no seasonal changes in infectivity in plants grown in cores from restored areas, with levels of infection remaining low throughout the year. The lower levels of colonization in soil cores from restored areas were expected because soil disturbance is known to decrease the capacity of VA mycorrhizal fungi to colonize roots. Nevertheless, an increase in infection was expected with increasing age of the restoration, but this was not observed in the one year of the experiment. It may be that the species of VA mycorrhizal fungi need to be adapted to the changed soil conditions after mining before they can spread through

the soil or at least survive until microclimatic conditions are more suitable.

There was no seasonal trend in formation of VA mycorrhizas by individual genera of fungi in either natural or restored areas. *Glomus* was by far the most common genus in natural jarrah forest with very few mycorrhizas formed by the other genera. More genera were present in restored areas with *Glomus*, *Gigaspora*, and fine endophyte occurring in similar proportions.

In experiment two, the percentage of root length colonized in plants grown in disturbed soil cores was higher in summer than in winter. This contrasts with the results from experiment one. The explanation for this may be that in experiment one undisturbed soil cores were used, and in experiment two the soil was dried and mixed before plants were grown. It is likely that the main type of propagule during winter was hyphae in the soil and within the roots. With drying and disturbance of the soil those propagules were probably killed, resulting in the almost complete absence of infection during the winter collection in experiment two. To reinforce that, levels of infection in experiment two were the same as in experiment one, for the summer soil collection (36% in exp.2, and 38% in exp.1).

There was no difference in the percentage of root length colonized of plants grown in disturbed cores for the various depths within the top 8cm of the soil. At the winter harvest, percentage of root length colonized deceased to almost zero in soil deeper than 8cm. In December, the level of mycorrhiza formation was constant with no difference in percentage of colonization in soils from all depths. Application of phosphorus did not affect mycorrhiza formation in the top 20cm of the soil. *Glomus* was the main genus occurring under all

circumstances, especially in the top 8cm. Few infections associated with either*Acaulospora* or *Gigaspora* were recorded in December.

In the last experiment, most of the root system of *Acacia pulchella* was located in the top 10cm, decreasing sharply with increasing depth. Root and shoot growth were slow up to the 13th week after seed germination, then increased sharply. After the 29th week root length decreased due to the dry and hot summer but shoot growth continued at the same level. Roots grew at a relative growth rate (RGR) of 0.16 (g/g/week) during the first 29 weeks after germination but the RGR for shoot was 0.13. Mycorrhizal infection followed the same pattern as that of root distribution in the soil profile, decreasing with soil depth. *Glomus* and *Gigaspora* were the dominant genera forming mycorrhizas during the first six weeks of growth of seedlings of *Acacia pulchella* but with time *Glomus* become dominant, with very few infections by *Gigaspora* .

The results from my experiments show that infectivity of VA mycorrhizal fungi in natural jarrah forest decreased during summer probably due to a decrease in moisture content in the soil, and death of the fine root system. Moreover they highlight the necessity of specifying the soil conditions under which experiments are conducted and raise the question about the validity of using disturbed soil to assess the infectivity of VA mycorrhizal fungi in natural conditions. Roots of *Acacia pulchella* seem to be mostly located in the top 10cm of the soil profile, decreasing with soil depth. Propagules of VA mycorrhizal fungi follow the same pattern. *Glomus* was by far the dominant genus of VA mycorrhizal fungi in the jarrah forest ecosystem.

Techniques to measure infectivity of VA mycorrhizal fungi in the mediterranean climate of south-western Australia should consider the minimization of disturbance of the soil to avoid loss of

potential propagules of VA mycorrhizal fungi. Moreover, the simultaneous growth of plants in glasshouse and field conditions should be used, when possible, to measure the accuracy of results from glasshouse grown plants to predict the formation of mycorrhizas in field.

ACKNOWLEDGEMENTS

I would like to thank:

- Professor Alan D. Robson and Dr. Lynette K. Abbott for their constant help, guidance and encouragement throughout the course of my work,

- the staff from the mycorrhizal lab. at the Soil Science and Plant Nutrition Depatment,

- Mr. David Jasper, Dr. Mark Brundrett, and Ms. Martine Scheltema for their comments on the manuscript,

- Ms. Natalie Hunt for her help in experiment 2 in Chapter 3,

- Alcoa of Australia Ltd. and the Department of Conservation and Land Management for allowing the collection of seedlings and soil cores in areas under their responsibility,

- Finally I thank Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA) for financial support and the Brazilian Government for providing the opportunity for me to undertake this course.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

The mediterranean environment of south-western Australia is characterized by a dry and hot summer and rainfall occurring mainly in the cooler months of the year (Beard,1983). The soils in this region are very infertile with most of the nutrients concentrated in the top 15cm in the profile (Lamont,1982a). Plant species occurring in this region have developed a number of adaptative characteristics to cope with these low levels of nutrients in the soil. *Acacia* is a well represented genus in the understorey of *Eucalyptus marginata* Donn.ex Sm.(jarrah) forests. This genus has an ability to form symbiotic associations with nodule-forming bacteria (Hansen and Pate,1987) and mycorrhizal fungi (Malajczuk *et al.*,1981; Jasper *et al.*,1987).

The VA mycorrhizal fungi are obligate symbionts (Harley and Smith,1983). Therefore, factors affecting root growth are likely to have an effect on the activity of these fungi. Root growth is highly seasonal in the jarrah forest (Dell and Wallace,1983; Shea and Dell,1981), and the capacity of VA mycorrhizal fungi to colonize roots is likely to follow the same trend. Mining activities are known to decrease the infectivity of VA mycorrhizal fungi (Jasper *et al*, 1987, 1989c). Moreover, in areas disturbed by bauxite mining, during the early stages of restoration, there are lower plant and root densities than in adjacent native forests. This makes these areas susceptible to wider variation in soil temperature and more rapid changes in soil moisture content which could lead to different patterns of seasonal variation in infectivity of VA mycorrhizal fungi compared with that in natural jarrah forests.

Many plant species occurring in the mediterranean south-western Australia maintain shoot growth well into summer (Bell and Stephens,1984). Roots growing deeper in the profile are responsible for supplying water for the plants, especially during summer (Lamont,

1982a). However, the uptake of nutrients deeper in the profile is likely to be decreased because of the lower nutrient availability and lower root density in these deeper layers. The distribution of VA mycorrhizal fungi in the profile has not been studied for this environment. Nevertheless, if mycorrhizal fungi can colonize roots deeper in the soil, and take up nutrients in summer, when plants are still growing but nutrients in the surface are not available because the soil is dry, this could be vital for the plants to achieve their nutrient requirements.

To test some of these factors affecting VA mycorrhizal infectivity I conducted three experiments using *Acacia pulchella* R.Br. as my test plant. This species is a common component of the understorey of jarrah forest and one of the species used in restoration of areas after bauxite mining in this region. In Chapter 2, I review some of the factors affecting the mycorrhizal association with special emphasis on (1) seasonal variation in both root growth and VA mycorrhizal activity, (2) response of the fungi to soil disturbance, and (3) distribution and activity of VA mycorrhizal fungi in the soil profile. In Chapter 3, I tested the hypotheses that (1) infectivity of VA mycorrhizal fungi would show different trends in soil under natural jarrah forest or in soil in restored areas after bauxite mining, (2) infectivity of VA mycorrhizal fungi would decrease with increasing depth in the top 20cm of the soil, and (3) application of phosphorus to the soil would decrease infectivity of the fungi. In Chapter 4, I followed root growth and spread of VA mycorrhizal fungi with depth, in seedlings of *Acacia pulchella* from seed germination until root activity had decreased in mid-summer.

CHAPTER 2

REVIEW OF LITERATURE

1) INTRODUCTION

Acacias have long been considered important species because of their high adaptability to different environments and their wide range of use by humans (New, 1984). Moreover, these species can play a key role in nutrient cycling in natural ecosystems (Adams and Attiwill, 1984b ; Malajczuk *et al.*, 1981) as well as in revegetation of disturbed areas (Langkamp *et al.*,1979; Cornet and Otto,1985;Jasper *et al.*,1987). The capacity of acacias to tolerate adverse conditions including extremely deficient levels of nitrogen and phosphorus has led to their success as pioneer species. Symbioses formed between acacias and both rhizobia and mycorrhizal fungi are likely to be beneficial in soils with low levels of nitrogen and phosphorus (Langkamp and Dalling,1982; Lamont,1982a).

Studies of acacias in natural ecosystems deal mainly with the assessment of nitrogen fixation (Hansen and Pate,1987; Hansen *et al.*,1987; Monk *et al.*,1981). Rates of symbiotic nitrogen fixation in native legumes vary according to plant species, soil conditions, age and density of the stand (Hansen *et al.*,1987). Values measured in Australia range from as low as 0.005 kg N $ha^{-1}year^{-1}$ for *Acacia melanoxylon* growing in a low open-forest environment (Lawrie,1981) to 12-31 kg N $ha^{-1}year^{-1}$ for *Acacia dealbata* in a wet sclerophyll forest (Adams and Attiwill,1984b). However, in adverse conditions, such as heathlands, even a minimal input of nitrogen could be vital for survival and establishment of seedlings at the very early stages of plant growth (Monk *et al.*,1981). This is emphasized by the findings of Hansen *et al.*(1987) where symbiotically fixed nitrogen was 13-61% of the total shoot nitrogen content in first year seedlings

compared with only 1.1-3.4% in the second year and well below 1% in the fourth year.

Unlike the symbiosis between species of acacia and rhizobia, the association between acacias and vesicular-arbuscular (VA) mycorrhizal fungi has received little attention until recently. Mycorrhizas are known to increase the uptake of phosphorus and other nutrients in a variety of plants (Harley and Smith,1983) and legumes seem to be particularly responsive to this association (Rose and Youngberg,1981). The improved uptake of nutrients can indirectly lead to increased nitrogen fixation (Bowen,1981; Barea and Azcón-Aguilar,1983).

Associations between acacias and mycorrhizal fungi are likely to be very important both when plants are growing in undisturbed nutrient-deficient soils and in revegetation of sites disturbed by mining. Acacias are common species in natural ecosystems of southwestern Australia (Hopper and Maslin,1978), one of the most nutrient-poor mediterranean ecosystems in the world (Specht,1979), and in rehabilitation programs (Langkamp and Dalling,1982; Jasper *et al.*,1987; Gardner and Malajczuk,1988) because of their relatively rapid establishment and growth rate. *Acacia pulchella*, a common species in south-western Australian forests, responded markedly to phosphorus application in well watered conditions (Hansen and Pate,1987). This species is known to form associations with VA mycorrhizal fungi (Malajczuk *et al.*,1981) which are known to increase nutrient uptake and may also increase tolerance of water stress (Graham *et al.*,1987; Huang *et al.*,1985; Nelsen,1987). Langkamp and Dalling (1982) working with several legumes, including four *Acacia* species, suggested that infection by mycorrhizal fungi is

essential for a successful establishment of long-term vegetation on areas rehabilitated after mining.

Mining processes decrease the infectivity of VA mycorrhizal fungi (Reeves *et al.*,1979; Rives *et al.*,1980; Warner,1983; Jasper *et al.*,1987,1989a). This decrease is probably due to a dilution of propagule density in the upper layer of the soil, to damage and loss of propagules by dessication, and to premature germination (Jasper *et al.*, 1987). The recovery of the inoculum potential in restored areas depends on time after rehabilitation (Loree and Williams,1987), plant species colonizing restored areas (Miller,1987b), and soil characteristics (Stahl *et al.*,1988).

Water, temperature, soil fertility, and availability of sites for colonization are some of the factors affecting the root length colonized by VA mycorrhizal fungi. Any factor which affects those parameters is likely to have some effect on the infectivity of those fungi. In natural ecosystems of south-western Australia, most of the nutrients are concentrated in the top 15cm of the soil profile with most of the fine roots growing in this layer of soil (Lamont,1983). With increasing depth the level of nutrient supply decreases; the roots growing deeper in the soil are essential in supplying water for the plant, especially during the dry season. If mycorrhizal fungi are important in nutrient cycling processes, how are they affected by seasonality in root growth and by soil depth? In the early stages of rehabilitation of mined areas the soil is left bare, and is exposed to a wider range of temperature variation, and is more susceptible to dessication and extremes of temperature than is soil in undisturbed sites. At the same time, the soil characteristics are generally changed by exposing the less fertile layers of the profile. In addition, loss of or damage to mycorrhizal propagules caused by soil disturbance can lead to a reduction in the extent of colonization of roots

by these symbiotic fungi. In this review I will consider some of the factors that could affect the establishment and function of VA mycorrhizas in acacias growing in natural and disturbed ecosystems.

2) FACTORS AFFECTING COLONIZATION OF PERENNIAL LEGUMES BY VA MYCORRHIZAL FUNGI IN NATURAL ECOSYSTEMS

2.1.Life Cycle of Fungi in Relation to Life Cycle of Plants

Root system and seasonality in root growth

The mediterranean environment of south-western Australia is characterized by very infertile soils, hot and dry summers, and rainfall occurring during the cooler months of the year (Fig. 2.1) (Beard,1983). Under these conditions, most of the root system is located in the top 15cm of the soil following the pattern of nutrient distribution in the soil profile (Lamont,1983). Dodd *et al.*(1984) have divided the plants occurring in the sandplain of Western Australia into five types, according to root system patterns, following the scheme proposed by Cannon(1949). For example, *Acacia pulchella* a common species in the understorey of jarrah forests, was classified as Type 1, characterized by well developed primary and lateral roots with neither type dominating. This species has a relatively shallow root system reaching a depth of just 10-42cm, with roots spreading less than one metre laterally (Dodd *et al.*,1984). The root/shoot ratio of *A. pulchella* in this study was 0.2.

Because of the low fertility of soils, plants in the jarrah forest rely heavily on nutrient cycling, and adaptative characteristics of root systems such as mycorrhizas are a common feature of plants

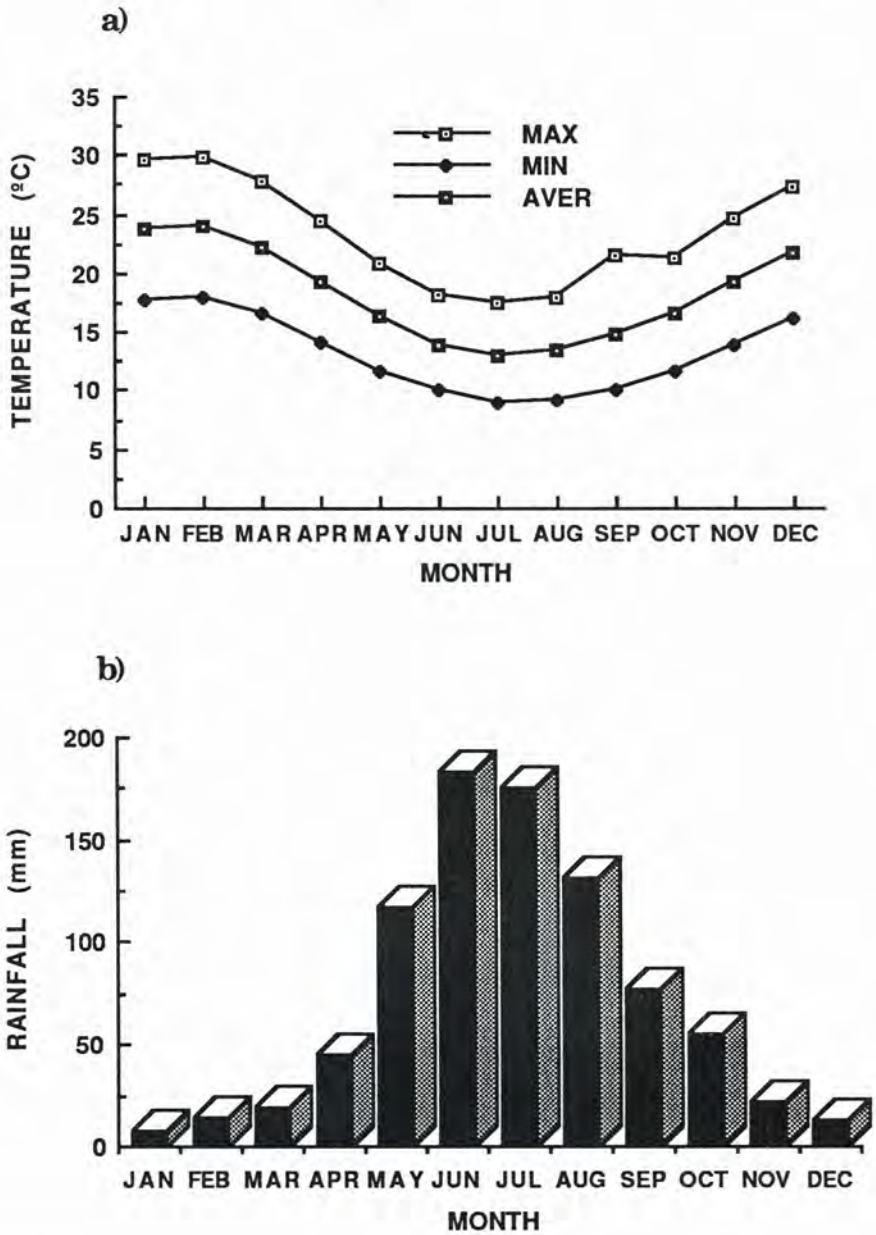

Figure 2.1: Temperature (a) and rainfall (b) average for the mediterranean region of south-western Australia where my experiments were conducted. Data from Bureau of Meteorology, Perth

growing in these conditions (see Lamont,1982a). Baylis (1975) has suggested a classification for root systems based on the diameter of the ultimate root branching, from "graminoid" (fine roots provided with root hairs) to "magnolioid" type (coarse roots with no root hairs). Studies on root morphology and root growth are scanty in forest species. In one of the few studies involving Western Australian species, Barrow (1977) showed that *A. pulchella* has a "magnolioid" type root with a mean diameter of 0.28mm and no root hairs. In contrast, jarrah (*Eucalyptus marginata*), karri (*Eucalyptus diversicolor*), and marri (*Eucalyptus calophylla*) have much thinner roots, with root hairs. Forest species generally have a lower root density than crops (Table 2.1) (Nye and Tinker, 1977) and the coarse root system in *Acacia* species means a lower ability to explore the soil.

There are two well established cycles in root growth for native plants growing in the mediterranean climate of south-western Australia. The first one, an annual cycle is due to the dry and wet season. The second cycle follows high intensity prescribed burns or wildfires. During the long dry summer period, fine roots of *E. marginata* die off during summer but new root growth starts when the soils are moistened after rain (Shea and Dell,1981). New root growth of *E. marginata* has two peaks, in spring, and in autumn (Dell and Wallace,1983) but surface root growth was observed to stop during mid-winter (B.Rochel, unpublished, cited by Lamont,1982a). Water supply rather than temperature seems to be the factor affecting root activity during summer, but it is not clear if the decrease in root activity during winter is a function of low temperature or a natural part of the physiological cycle of the plant. Although there are no similar studies for the understorey species, Hansen *et al.*(1987) found nodule activity in *A. pulchella* and *A. alata* to be confined to the wet months of the year,

Table 2.1 : Examples of root density of different plant species in the top 10cm of soil profiles.

Plant species	Forest type	Root density (cm cm^{-3})	Reference
various	dry sclerophyll	7	Carbon *et al.*(1980)
Pinus radiata	plantation	2	Nambiar(1981)
Eucalyptus regnans	wet sclerophyll	1-2	Ashton(1975)
cereals (oats,rye,wheat)	crop	5-25	Barley(1970)
Stylosanthes gracilis	crop	30	Barley(1970)
Medicago sativa	crop	20	Barley(1970)

reaching a maximum in spring (August or September). In this study, the lack of nodule activity during summer was attributed to insufficient water and low activity during winter to low temperatures. These factors also have an effect on mycorrhizal associations, as will be discussed later in this review.

After a fire, vigorous regeneration of legumes occurs within the understorey and then declines (Table 2.2). The intense new root production during the first year is likely to provide plenty of sites for colonization by VA mycorrhizal fungi and may help the spread of these fungi. After the death of a high number of seedlings between the first and second year, the young roots are easily decomposed and could play an important role in nutrient cycling. They could also be a source of inoculum of VA mycorrhizal fungi.

The stage of plant development also affects the seasonality in activity of both root growth and VA mycorrhizal fungi.

Table 2.2: Number of stems ($x10^3$) of leguminous species per ha after fire

Species	Years after fire			Reference
	1	2	3	
Acacia spp	750	200	100	Adams and Attiwill (1984a)
Acacia pulchella	80	60	20	Hansen *et al.*(1987)
Acacia alata	700	250	200	Hansen *et al.*(1987)
Acacia extensa	30	15	10	Hansen *et al.*(1987)
Bossiaea aquifolium	110	70	50	Hansen *et al.*(1987)
various	45[a]	-	-	Shea *et al.*(1979)

a - only one observation made at 11 months after fire

In annual crops there is an intense period of root growth early in the season during plant establishment and vegetative growth, then the root growth slows down during mid season and the percentage of root length colonized by VA mycorrhizal fungi remains constant during flowering and seed production (Roldan-Fajardo *et al*,1982). By the end of the season, spore production has occurred, root growth stops, and the plant dies off. In forest ecosystems where a perennial root framework is already established and different plant species have different times for flowering and setting seeds, roots stay alive throughout the year, even if new root growth stops and fine roots die during stressful periods. The interaction among rainfall distribution, low temperature, new root growth, and physiological stage of plant development with VA mycorrhizal fungi should be looked at as a whole when studying mycorrhizal associations in perennial ecosystems.

VA mycorrhizal fungi and seasonality in propagule formation.

VA mycorrhizal fungi are obligate symbionts (Harley and Smith,1983) and their activity is closely linked to activity of the host plant. Spores and hyphae in the soil and within the roots are the main source of inoculum (Bowen,1987). Spores are survival and dispersal structures while hyphae are responsible for host colonization, including the spread of the fungi between roots of the same or adjacent plants, and for nutrient uptake. Production and germination of spores is affected by the stage of plant development (Hayman,1970; Saif and Khan,1975; Giovannetti,1985), water regime, and high temperatures (Furlan and Fortin,1973;Menge,1984). Numbers of spores increased during the flowering period in wheat (Hayman,1970; Saif and Khan,1975) and in three sand dune species in Italy (Giovannetti,1985). In both cases the increase in spore number was associated with a decrease in root growth. Although there are no similar studies for forest ecosystems, the number of spores found under forest canopies is generally lower than in agricultural fields (Abbott and Robson,1977; Hayman and Stovold, 1979). Baylis (1969) suggested that this would be because in agricultural fields roots die at the end of the season, stimulating spore production, while in forests there is continuous root growth. Moreover, there is a higher density of roots in agricultural than in forest soils (Table 2.1). Increased root density was associated with increased colonization by VA mycorrhizal fungi (Warner and Mosse,1982; Abbott and Robson,1984b). This could lead to increased sporulation.

Soil moisture and temperature play an important role in spore germination. Maximum germination of spores of *Glomus*

epigaeus was attained with the soil at or above field capacity (Daniels and Trappe,1980). In contrast, there was no germination of three *Glomus* species when the soil matric potential was -0.001MPa with the maximum germination occurring at -0.01MPa (Sylvia and Schenck,1983). In the same study, 21% of spores of *Glomus etunicatum* germinated at -1.0MPa but only 5 and 6% of spores of *Glomus clarum* and *Glomus macrocarpum* respectively, germinated at that moisture potential. As highlighted by Bowen (1987), the results reported so far are quite variable, depending on the fungal species and methods used. Moreover, he emphasized that spore germination does occur at low levels of soil moisture and that moisture alone may not be the main factor affecting spore germination in field soils. However, soil moisture is likely to have an important role in spore germination in the highly seasonal environment of south-western Australia. Soils in this region can reach a matric potential of -35 MPa during summer (E.Newmam, cited by Jasper *et al.*,1989b). This matric potential is likely to inhibit most root and fungal activity. On the other hand, different fungal species have different optimum temperatures for maximum spore germination (Table 2.3). The range of temperature between 20 and 35°C seems to be the optimum for most of the fungal species while temperatures below 15°C are likely to inhibit or delay spore germination. Temperature of the soil frequently exceeds 45°C during summer in south-western Australia (Shea *et al.*,1979). However, these high temperatures occur when the levels of moisture in the soil are very low and it is unlikely that spores will germinate at that stage. Most of the spore germinaton will occur with the onset of the rainfall period when the temperatures are more favourable to spore germination.

Table 2.3: Temperature requirements for spore germination of various VA mycorrhizal fungi.

Fungi Species	Medium	Temperature (°C)		Reference
		Minimum	Optimum	
Glomus caledonium	soil	5	20-25	Tommerup,1983b
G. epigaeus	soil	10	20-25	Daniels &Trappe, 1980
G. fasciculatum	soil	15	25-35	Nadarajah &
	agar	15	25-35	Nawawi,1987
G. mosseae	agar	<15	20-25	Schenck *et al.*, 1975
Acaulospora laevis	agar	15	30-35	Nadarajah &
	soil	15	30-35	Nawawi,1987
	soil	10	20	Tommerup,1983b
Gigaspora margarita	soil		26-31	Clarke,1978
	soil	15	25-35	Nadarajah &
	agar	15	25-35	Nawawi,1987
	filter paper	20	25-35	Kobayashi,1988
G. coralloidea	agar	<15	20-34	Schenck *et al.*, 1975
G. heterogama	agar	20	25-34	Schenck *et al.*, 1975
G. calospora	soil	5	25-30	Tommerup,1983b
G. gigantea	sand	15	20-30	Koske,1981

Although spores are one of the main types of propagules, their numbers in the soil do not always have a direct correlation with the extent of root length infected (Hayman and Stovold,1979). Dormancy of spores, presence of other forms of propagules, and difficulty in recovering spores from soil for some species are some of the possible explanations for this (Abbott and Robson,1989). Tommerup(1983a) found that different species of fungi

have different periods of dormancy. *Glomus* sp had a dormancy period from one to 6 weeks depending on whether the spores had been stored in dry or moist soil. *Gigaspora calospora* had a period of dormancy from 6 to 12 weeks, but for *Acaulospora laevis* that period was 6 months regardless of the store conditions. Spores of *G. gigantea* collected from the field had a dormancy period of between 5 and 9 weeks (Gemma and Koske,1988). This period of dormancy may be of great ecological significance because it can prevent the germination of spores during stressful environmental conditions.

Germination of spores occurs when conditions of soil moisture and temperature are favourable, even if no suitable host plant root is available (Powell,1976). Some species of fungi have the ability to produce multiple germ tubes (Harley and Smith,1983). This characteristic increases the chances of contact between fungi and roots. Further, Koske (1982) has demonstrated that germ tubes emerging from spores of some species of VA mycorrhizal fungi are attracted towards the roots by volatile products.

In many ecosystems where the number of spores is low, hyphae in the soil and inside the roots are considered to be the major propagules (Jasper *et al.*,1989c; McGee,1989; Birch,1986). Hyphae may be able to colonize roots more quickly than spores (Read and Birch, 1988). Francis and Read (1984) showed that an extensive network of hyphae in the soil could form interconnections between plants. Birch (1986) demonstrated that this hyphal network was involved in initial colonization and spread of VA mycorrhizal fungi throughout the root system. The length of external hyphae produced differs among species of VA mycorrhizal fungi (Graham *et al.*,1982; Abbott and Robson,1985). For example, *Glomus fasciculatum* produced less external hyphae than *Gigaspora calospora* when

growing in soil with subterranean clover in glasshouse conditions but there was no difference in the formation of external hyphae between *A. laevis* and fine endophyte (Abbott and Robson,1985). However, the distribution of the hyphae (Abbott and Robson,1985), and the way the fungi spread (Wilson,1984) may be more important than the hyphal length in determining the extent of colonization of roots by VA mycorrhizal fungi.

The predominance of hyphae as propagules in mediterranean (Jasper *et al.*,1989c) and semi-arid (McGee,1989) environments is in some way surprising because spores are the propagules considered to be the best adapted to harsh conditions. However, Jasper *et al.*(1989b) demonstrated that hyphae of some fungal species can remain alive in soil with a matric potential of -21 MPa for a period of at least 36 days in glasshouse conditions. More important is the fact that the maintenance of the infectivity of this hyphal network was not dependent on being attached to living roots. Nevertheless, those results still need confirmation in field conditions. VA mycorrhizal hyphae can also survive in fragments of roots from the previous growing season (McGee,1987) or perennate inside long-lived live roots (Brundrett and Kendrick,1989); hyphae regrow from these propagules when the conditions become favourable. The mechanisms by which the mycorrhizal hyphae manage to survive in dry soils or inside dead and inactive roots are poorly understood but this knowledge is vital for an understanding of the ecology of VA mycorrhizal fungi in ecosystems.

Colonization of roots by VA mycorrhizal fungi.

The first step in assessing the inoculum level of VA mycorrhizal fungi in natural ecosystems is to determine what

with the extent and speed of colonization of roots in the mediterranean environment of south-western Australia (Bolan and Abbott,1983; Scheltema *et al.*,1987; Jasper *et al.*,1987). Therefore, the number of spores is unlikely to give an accurate measure of the inoculum potential of VA mycorrhizal fungi in this environment. The estimation of the number of infective propagules using the most probable number method (Porter,1979) or the bioassay technique used by Moorman and Reeves (1979) may be useful in assessing mycorrhizal potential in disturbed soil but not in natural ecosystems because it requires soil disturbance, which can damage some forms of propagules in the soil (Jasper *et al.*, 1989c). "Infectivity" of a population of VA mycorrhizal fungi has been defined as a measure of the rate and extent of mycorrhiza formation (Abbott and Robson,1981). Although this is not a precise definition, an assessment of the extent (percentage of colonization and total root length colonized) of mycorrhiza formation within a certain time under particular conditions can give an indication of the potential for colonization of new roots formed. Further information would be required to determine whether such an infectivity assessment was correlated with mycorrhiza formation in the field (Abbott and Robson,1989).

Bolan and Abbott (1983) found almost no mycorrhizal colonization in subterranean clover grown in soil disturbed at the collection time, from a jarrah forest in spring but there was heavy colonization in plants grown in soil collected from the same site in summer. The dominant VA mycorrhizal fungus present was *A. laevis*. These results indicate the differences in the type of inoculum in different seasons. The low level of colonization from soil collected in spring cannot be interpreted as a lack of fungal activity at that stage. During spring, plants are growing actively and the mycorrhizal activity

is likely to follow the same trend. Jasper *et al.*(1989a,b) suggested that mycorrhizal hyphae in the soil and within the roots are the main source of propagules in jarrah forest soil; damage to the fungal network in the soil leads to a drastic reduction in colonization by this species. Therefore methods for assessment of the infectivity of VA mycorrhizal fungi that cause the least possible damage to the hyphal network in the soil are likely to give a more accurate measurement of the potential of the fungi to colonize roots in field conditions.

The association between VA mycorrhizal fungi and roots is a dynamic process and both the root and the fungal components exhibit continuing growth and development (Harley and Smith,1983). Root growth in the forest ecosystem of south-western Australia is highly seasonal with most of the root activity occurring during the wet months of the year (Dell and Wallace,1983; Shea and Dell,1981). The spread of VA mycorrhizal colonization inside the root is strongly affected by root morphology and root density (Miller,1987a). Colonization by VA mycorrhizal fungi is likely to follow the dynamics of fine root turnover in ecosystems, therefore, the determination of mycorrhizal colonization at a single harvest may be a poor indication of the inoculum potential of the area (St John and Coleman,1983). The importance of considering the effects of seasonal changes on VA mycorrhizal activity has been demonstrated in a number of papers (Bolan and Abbott,1983; Scheltema *et al.*,1987; Brundrett and Kendrick,1988). However, the measurements of the fungal activity should be accompaned by a description of the climatic environment, plant activity (root and shoot growth, flowering), and botanical composition (Abbott and Robson,1989). These data are essential for the interpretation of results and comparisons with other studies.

Phosphorus in the soil and in the plant can regulate the mycorrhizal activity both in the soil and within the root (Cooper, 1984). Application of phosphorus fertilizer decreased the formation of VA mycorrhizas (Schwab *et al.*,1983). These authors suggested that the decrease was due to a reduction in growth of external hyphae which led to fewer secondary infections. Jasper *et al.*(1979) also found a decrease in mycorrhiza formation with soil P-amendment but they considered this was due to the decreased concentration of soluble carbohydrate inside the root. Further work by Thomson (1987), confirmed that increased phosphorus supply decreased the percentage of root length colonized by VA mycorrhizal fungi. He showed that the effect of phosphorus supply in decreasing mycorrhizal formation was mediated by an effect of P supply in decreasing the concentration of soluble carbohydrates in the roots or in the root exudates.

2.2.2. Effect of soil disturbance on VA mycorrhizal activity

Soil disturbance can decrease the infectivity of VA mycorrhizal fungi (Reeves *et al.*,1979; Rives *et al.*,1980; Warner,1983; Jasper *et al.*,1987,1989a). Clearing of the forest vegetation prior to bauxite mining in Western Australia generally occurs when rainfall starts to decrease (September or October)(Jasper *et al.*,1989c). The topsoil is usually spread immediately after stripping on an adjacent mined pit (Nichols *et al.*, 1985). At the time of clearing of the vegetation, plants in the forest are still growing actively, and the hyphae in the soil are likely to be the main type of propagule. Spores may be important also, but their low numbers may limit their contribution as propagules. Hyphae of some VA mycorrhizal fungi (eg. *A. laevis*) can survive in dry soil but lose their ability to infect roots if the hyphal network in the soil is

damaged (Jasper *et al.*, 1989b). Disturbance of the soil by removing the vegetation, stripping and respreading the soil is very severe and it is aggravated by the fact that the soil is left bare during the dry summer season. These processes have been shown to decrease the infectivity of VA mycorrhizal fungi (Jasper *et al.*,1987).

The percentage of root length colonized by VA mycorrhizal fungi in seedlings of *A. pulchella* grown in undisturbed cores for 12 weeks, was decreased from 62% in cores from natural jarrah forest to 9% in cores from restored areas (Jasper *et al.*,1987). This decrease was due to a dilution of propagule density in the upper layer of the soil, to damage of propagules or to exposure to a more susceptible condition for desiccation or premature germination. However, the biggest decrease in percentage of root length colonized by VA mycorrhizal fungi in bioassay plants of *A. pulchella* grown in undisturbed cores, collected at various stages of disturbance during bauxite mining, occurred after the clearing of the vegetation (Jasper *et al.*,1989c). The percentage of root length colonized dropped from 20% before the vegetation was cleared to 5% one month after clearing; no colonization was observed after the soil had been stripped and respread. The infectivity of the fungi appeared to depend on the hyphae remaining attached to a living plant (Jasper *et al.*,1989c). A decrease in root colonization of *Plantago lanceolata* was also attributed to the disruption of the link between the fungi and their source of carbon supply (Read and Birch,1988). The plant and the fungi were growing actively at the time of vegetation removal in the experiment of Jasper *et al.* (1989c) and it may be that the fungi were not resistant to a sudden shortage of photosynthetic products.

Recovery of the inoculum level in restored areas is dependent on plant species (Miller,1987b), time after rehabilitation

(Loree and Williams,1987), and soil characteristics (Stahl *et al.*,1988). The inoculum level of VA mycorrhizal fungi decreased during the first five years after soil disturbance but started to increase from the sixth year (Miller,1987b). The increased inoculum level was associated with the return of mycorrhizal plants to the area. The inoculum level reached the original levels in less than two years. It is unlikely the presence of non-mycorrhizal plants was a limiting factor for recovery of the inoculum levels in areas after bauxite mining in south-western Australia because *Eucalyptus spp* and leguminous species, used for rehabilitation of those areas, are potentially mycorrhizal (Malajczuk *et al.*,1981; Lamont,1982b).

In a survey comparing the levels of mycorrhizal activity in areas with different times after restoration, the number of plant species colonized by VA mycorrhizal fungi increased with increasing age of the stand but the percentage of root length colonized was usually less than 5% (Wilson,1983). Problems in clearing the roots for mycorrhizal assessment may have accounted for these low levels of colonization. Nevertheless, the number of spores in a 7 year old restored area was similar to that in an adjacent natural jarrah forest. However, the number of spores did not always correlate closely with the percentage of root colonization in the mediterranean environment of south-western Australia (Jasper *et al.*,1989c), and spore abundance alone should not be considered as an indication that the inoculum level had been restored. Levels of colonization returned to initial levels 17 to 20 months after soil disturbance in a sand and gravel mining area in England (Warner,1983). In another situation, the percentage of root length colonized increased from about 10% in the first year after restoration to more than 50% in the fourth year (Loree and Williams, 1987). There was a considerable variability in this data but the increase

to the fourth year was clear. However, only one percent of plants were mycorrhizal three to four years after the rehabilitation of a semi-arid oil-shale area (Reeves *et al.*,1979). Although the loss of infectivity of VA mycorrhizal fungi due to soil disturbance by mining activities was higher in areas of bauxite mining in Western Australia than in North American studies, this effect is thought not to be long-term (Jasper *et al.*,1989c).

The process of respreading the topsoil and mixing it with subsoil, not only damages the VA mycorrhizal propagules but may also create an unfavourable environment for growth of the fungi. Stahl *et al.*(1988) working on areas disturbed by surface coal mining, observed that VA mycorrhizal fungi promptly colonized the root system of seeded sagebrush but were not able to spread extensively in the area. They suggested that this was a response of the fungi to changes in the soil environment but they did not give an explanation of what those changes could be or how they would affect the VA mycorrhizal fungi. The level of nutrients were higher in the disturbed soil than in the native sagebrush-grassland soil in their study, which may have affected the species of fungi adapted to a previous poorer soil condition. In a range of studies, soil from restored areas generally had a similar pH to soil from undisturbed sites, whereas the restored areas had a lower concentration of organic carbon and a higher nitrogen concentration (Table 2.4). The levels of phosphorus varied according to the practice used in the restoration of the area. From these data it seems that although changes in soil chemical properties could account for some of the changes in growth of mycorrhizal fungi there is likely to be some other factor controlling the level of mycorrhizas in restored areas. While VA mycorrhizal fungi do not exibit much host specificity, they

Table 2.4. Comparison of chemical properties of soils before and after disturbance by various mining activities

Mining activity	Disturbance condition	pH	Organic Carbon (%)	Phosphorus ($\mu g\ g^{-1}$)	Nitrogen ($\mu g\ g^{-1}$)	Reference
bauxite	undisturb.	4.8	2.3	7	-	Jasper *et al.*,(1987)
	disturbed	4.7	1.8	1	-	
bauxite	undisturb	5.6	5.6	7	13	Jasper *et al.*,(1989c)
	disturbed	5.9	5.9	16	18	
coal	undisturb.	7.9	4.0	1	-	Rives *et al.*,(1980)
	disturbed	7.9	2.8	4	-	
coal	undisturb.	7.3	3.3	3	5	Gould and Liberta (1981)
	disturbed	7.8	2.0	1	26	
coal	undisturb.	7.9	n.a.[1]	4	7	Stahl *et al.*,(1988)
	disturbed	7.5	n.a.	7	21	
old road	undisturb.	8.0	1.1	3	1	Moorman and Reeves (1979)
	disturbed	8.2	0.7	5	13	

1: na, not available

may adapt to specific conditions and that soil disturbance could result in a permanent loss of some fungi adapted to particular conditions.

The diversity of species of VA mycorrhizal fungi is also affected by disturbance of the soil. The number of species of fungi decreased from six in a natural jarrah forest to three in an adjacent four year old restored area (Sas,1988). However, five out of the original six species were present in a ten year old revegetated area. In another study in a similar area, spores from five species of fungi were recovered from both one year old and seven year old restored areas after bauxite mining compared with seven species from an adjacent natural forest (Wilson, 1983). Similar results were attained by Stahl *et al.* (1988) in a surface coal mining in Wyoming. Five species were recovered in the undisturbed areas against only two in the restored area. On the other hand, there was no difference in species of fungi colonizing bioassay plants of *Acacia pulchella* whether the soil had been collected in natural jarrah forest or in restored areas after bauxite mining (Jasper *et al.*,1989c).

2.2.3. Soil depth and distribution of VA mycorrhizal fungi

There has been relatively little study of the influence of depth on the abundance of VA mycorrhizal fungi. As a general rule, the number of propagules decreases with increasing depth (Table 2.5) but this is dependent on soil characteristics and plant species covering the soil (Zajicek *et al.*,1986; White *et al.*,1989). Although most of the mycorrhizal propagules are generally in the top layers of the soil, spores and infected roots have been found up to 2.1m deep (Zajicek *et al.*, 1986). At this point, one should consider that the soil profile could be divided into two portions which have properties that could influence VA

mycorrhizal fungi in different ways. The first concerns the soil below the organic layer where soil fertility, aeration and root density decrease, where water availability increases, and where variation in soil temperature is low. The second includes the organic layer of the soil (top 20-30 cm) which holds most of the available nutrients as well as most microbial and root activity, but it is exposed to wide variations in moisture content and extremes of temperature.

Most of the studies on mycorrhizas are conducted in the top 30cm of the soil but even within this layer there may be changes with depth, especially in seasonal environments such as the mediterranean. The number of spores decreased from 14 per 100g soil, in the top 10cm of a semi-arid soil, to one at 30-40cm depth (McGee,1989). In the same study, *Scutellospora calospora* was the only species to be recovered at 30cm depth, but this species was not recovered in the top 3cm of the soil. The author suggests that this could be an adaptative characteristic of that species to survive during the summer season. In another situation, very little change was observed in inoculum potential in the top 30cm of the soil but dropped to almost zero at depth between 60 and 70cm (Schwab and Reeves,1981). Similar results were attained by Jakobsen and Nielsen (1983). The percentage of root colonized remained unchanged in the top 10cm under grass and dropped at depth between 10 and 15cm, but was higher between 5 and 10cm when in a forb vegetation type, decreasing in the top 5cm and below 10cm (Koide and Mooney,1987). These different responses seem to be related to climate and plant species characteristics, but their ecological significance remain to be determined.

Table 2.5. Changes in spore number and colonization by VA mycorrhizal fungi according to depth.

Vegetation type	Depth (cm)	Spore[1] density	Colonization[3]	Reference
Permanent pasture	0-10	56	-[2]	Smith (1978)
	10-20	22	-	
	20-30	14	-	
	30-40	4	-	
Tallgrass prairie (forb)	20	89	-	Zajicek *et al.* (1986)
	40	76	-	
	60	68	-	
	80	34	-	
	100	9	-	
Crops	0-10	49	-	Jakobsen and Nielsen (1983)
	10-20	52	-	
	20-40	23	-	
	40-60	7	-	
	60-80	4	-	
	80-100	7	-	
Grass	0-5	-	22	Koide and Mooney (1987)
	5-10	-	19	
	10-15	-	9	
Forb	0-5	-	13	Koide and Mooney (1987)
	5-10	-	30	
	10-15	-	12	
Arid land	10	1600	++	Allen (1988)
	25	3200	++	
	45	1400	+	
	75	2000	+	
Semi arid	0-10	14	-	McGee (1989)
	30-40	1	-	
Cold desert (soil type Haplargid)	10	2500	-	White *et al.*(1989)
	20	6000	-	
	30	2500	-	
	40	2000	-	
	50	1500	-	
	60	800	-	
	70	900	-	

continued over

Table 2.5 (cont)

Cold desert	10	6000	-	White *et al.*(1989)
(soil type	20	7000	-	
Torrifluvent)	30	5800	-	
	40	6000	-	
	50	5000	-	
	60	5500	-	
	70	7000	-	
Cold desert	0-14	-	70	White *et al.*(1989)
(soil type	14-29	-	84	
Haplargid)	29-78	-	62	
	78-124	-	1	
	124-165	-	0	
Cold desert	0-10	-	66	White *et al.*(1989)
(soil type	10-35	-	76	
Torrifluvent)	35-67	-	86	
	67-140	-	81	

1- number of spores/100g soil; 2- not determined;
3- % of colonization in bioassay plants

Root density (Miller, 1987a), soil fertility (Hetrick, 1984), aeration (Mosse,*et al.*, 1981; Saif, 1981), and inoculum density (Hetrick, 1984) affect activity of VA mycorrhizal fungi. Despite many *Eucalyptus* species and grasstrees (*Xanthorrhoeaceae*) occurring in the mediterranean environment of Australia having most of their root system between 20 and 70cm deep into the soil (Lamont,1981), most of the understorey species concentrate their roots in the top 15cm (Lamont,1982a) close to the organic layer. Root density follows the patterns of nutrient distribution in the profile (Lamont,1982a), therefore, the distribution of propagules of mycorrhizal fungi are likely to follow the same trend. White *et al.*(1989) recovered about 70 spores per g of soil, at 70 cm down in the soil profile and recorded 85% infection in roots occurring between 67 and 140cm, in one of four soils studied from the Red Desert in Wyoming. The number of spores in that case was extremely high and may have been caused by intermittent root

growth (Mosse and Bowen,1968). Moreover, the soil which had shown high levels of infection at depth also had an evenly distributed root system throughout the soil profile. On the other hand, Zajicek *et al.*(1986) recovered very few propagules below one metre following the decrease in root density below that point, although spores and infected roots were found at 2.1m depth. In the same way, Harris *et al.* (1987) working on stored topsoil recovered spores up to 3.5m depth in the stockpile but no infection was recorded below one metre. They suggested that in those conditions, the main source of propagules would be fresh root and that propagules below one metre would be non-viable.

Patterns of root growth and consequently root distribution in the soil profile change with vegetation types which are controlled by edapho-climatic conditions. The spread of VA mycorrhizal infection in the soil profile through linkage between plants by "arterial hyphae" (Read *et al.*,1985) was hypothesized by Zajicek *et al.* (1986). They considered that nutrients taken up by grass plants in the top layer of the soil could be transferred to forb roots at lower depths via mycorrhizal linkages between roots. On the other hand, McGee (1989) considered it unlikely that new root growth, occurring generally below 10cm in a semi-arid soil in Australia, would be infected by VA mycorrhizal propagule present in the top layer. These contradicting hypotheses highlight the lack of research in this area, and the need for an understanding of the relationship between VA mycorrhizal fungi and roots present in deeper layers in the soil. The diversity of VA mycorrhizal fungi also decreases with depth. *Glomus fasciculatum* was the only species to be recovered below 60cm in Zajicek's study. The role or the functioning of those fungi at increasing depth in the soil remains to be determined.

3. CONCLUSION

The study of the ecology of VA mycorrhizal fungi in natural ecosystems must consider that mycorrhiza formation is a dynamic process where each partner interacts with the other and with the surrounding environment. In the highly seasonal mediterranean climate of south-western Australia, water availability and variation in temperature play a major role in dictating plant, and probably fungal activity in the jarrah forest.

Soil disturbance caused by mining processes generally decreases the infectivity of VA mycorrhizal fungi in the soil but how the fungi behave after disturbance is poorly understood. Plant and root density are lower in recently restored areas than in the natural forest. This may lead to wider variation in soil temperatures and more rapid changes in soil moisture content in areas restored after mining than in natural forests. Disturbance of the soil may also change the composition of species/genera of VA mycorrhizal fungi in the area. These two factors combined may produce different patterns of seasonal variation in infectivity of VA mycorrhizal fungi between natural forest and areas restored after mining.

There is very little information on root growth of understorey species of the jarrah forest. Soils in the mediterranean environment of south-western Australia are very infertile and most of the nutrients, microbial activity and fine roots are in the top 15cm of the soil. This layer is also the most affected by the hot and dry conditions of the summer. Fine roots die and microbial activity is likely to be drastically reduced in the top layer of the soil during summer. However, many plant species maintain shoot growth well into or through the summer season. Roots deeper in the profile are thought to

be responsible for supplying water for the plant during summer but nutrient uptake is likely to be reduced because of the low level of nutrients and lower root density at depth. If mycorrhizal fungi spread at depth it could be vital for increasing the capacity of the plant to take up nutrients in a period when the nutrients in the surface zone are not available.

CHAPTER 3

SEASONAL VARIATION IN INFECTIVITY OF VA MYCORRHIZAL FUNGI IN NATURAL AND REGENERATED ECOSYSTEMS

3.1. INTRODUCTION

Leguminous species are an important component of the understorey of *Eucalyptus marginata* Donn. ex Sm. (jarrah) forests in south-western Australia. These species have the great advantage of forming symbiotic associations with root-nodule bacteria and mycorrhizal fungi (especially VA mycorrhizal fungi) that enable them to cope with the poor nutrient conditions of soils occurring in this region. Among the legumes, *Acacia* is one of the most well represented genera in this region with 336 species occurring in the South-Western Botanical Province of Australia (Hooper and Maslin,1978). Among these, the shrub *Acacia pulchella* R.Br. is a common understorey component of the jarrah forest that can germinate in dense stands after hot fires, and play an important role in nutrient cycling in the jarrah forest (Monk *et al.*,1981). *A. pulchella* is one of the leguminous species used in rehabilitation of disturbed areas after mining. Having a "magnolioid" type root, according to the classification of Baylis (Barrow,1977), this species benefits from the association with root-nodule bacteria (Monk *et al.*,1981) and VA mycorrhizal fungi (Malajczuk *et al.*,1981).

The VA mycorrhizal association basically increases the surface area of roots by growing a net of hyphae into the soil to make it possible for the plant to obtain nutrients that otherwise would be out of the reach of roots. This association becomes especially important in impoverished soils and for nutrients with low mobility in soil, such as phosphorus. The capacity of VA mycorrhizal fungi to infect and colonize roots is affected by inoculum type and concentration, and by nutrient status of the soil. Inoculum type and concentration are affected by seasonal variation in rainfall and temperature, stage of plant

development, soil disturbance, and floristic composition of the site. Nutrient status of the soil may affect fungal activity. Soils in this region are very infertile with nutrient availability decreasing with depth (Lamont,1982a). Phosphate can act to either increase or decrease mycorrhizal colonization, depending on the level applied. Concentrations of phosphate luxurious for plant growth decrease mycorrhizal infection whereas in soils with a low concentration of phosphate an additional application is likely to increase colonization of roots (Cooper,1984).

To test some of these factors I set up two experiments. In the first one, undisturbed cores were taken from four selected sites, two natural jarrah (*E. marginata*) forests, and two nearby revegetation areas after bauxite mining, where *A. pulchella* was the predominant species. Cores were taken five times during the year, to test the hypothesis that the infectivity of VA mycorrhizal fungi would show differences in seasonal variation in soil from under natural jarrah forest compared with soil from restored areas. Infectivity was assessed in terms of the percentage of root length colonized and the length of roots colonized for plants grown in glasshouse conditions, for a specific time under specific conditions. For the second experiment, soil was collected from a natural jarrah forest during winter and summer, at different depths from the top 20cm, and phosphorus was added or not to test the hypotheses that infectivity of VA mycorrhizal fungi would a) change with season; b) decrease with increasing depth of soil, and c) decrease with addition of phosphate.

3.2. MATERIAL AND METHODS

3.2.1. Experiment 1

3.2.1(a) Description of sites

All sites were in the mediterranean region of south-western Australia in the Climatic District number 9 of the Bureau of Meterorology of Australia. This region is characterized by rainfall occurring mainly between April and October with a peak in July-August when the average minimum temperature is 9-10°C, followed by a dry period from November till March with the average maximum temperature having a peak of 29-30°C in January-February (Figure 2.1). Rainfall data for the Jarrahdale region during the study period is shown in Figure 3.1.

The first site was located at a forest block called Illawarra , off Gardner Road at 32°08'S and 116°07'E and it will be referred to as N84. That area had been burnt during Spring,1984 and seeds of *A.pulchella* were germinated during Autumn/ Winter,1985. The overstorey was composed of *E.marginata* , and *Eucalyptus calophylla* R.Br.(Marri), and the understorey of *A. pulchella, Acacia urophylla* Benth., *Xanthorrhoea preisii* Endl.(black boy),*Macrozamia riedlei* (Graud.)C.A.Gardn., *Hibbertia amplexicaulis* Steud, *Hakea lissocarpha* R.Br., *Bossiaea aquifolium* Benth., and some other plants from the genera *Leucopogon, Adenanthos, Persoonia, Dryandra, Hovea*, and *Kennedia.*

The second site was located at Canning River Catchment Area, off Kinsella Road and Brookton Hwy at 32°09'S and 116°12'E and it will be referred to as N85. That area had been burnt

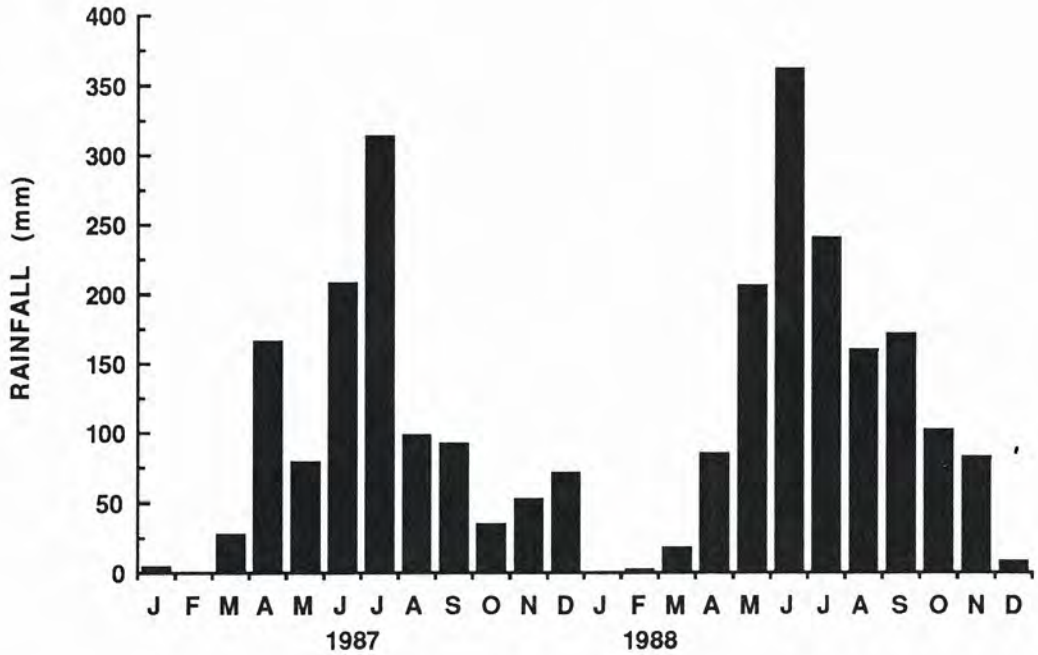

Figure 3.1. Monthly rainfall for the years 1987 and 1988 at Jarrahdale (Experiment 1). Data from ALCOA of Australia, Jarrahdale Minesite.

during Spring,1985 and *A. pulchella* seeds germinated during Autumn/Winter,1986. The overstorey species were the same as those of the previous site but the understorey had much less diversity of species being composed of mainly *A. pulchella, X. preisii, M. riedlei, Hibbertia hypericoides,* and *Hakea lissocarpha* . The previously reported mycorrhizal status of plants occurring in both sites is shown in Table 3.1. The soil in both sites was a sandy gravel with an intense concentration of fine roots and organic matter in the top 10 cm.

The two sites from restored areas were located at Alcoa of Australia - Jarrahdale Minesite, at 32°18'S and 116°06'E and they will be referred to as R1 and R2. In both sites, the former vegetation was a jarrah forest similar to the natural forests used in this study. The vegetation had been cleared, the topsoil stockpiled and the subsoil used for bauxite extraction. After mining, the topsoil was respread and a mixture of seeds of understorey species was broadcast over the area. Seedlings of *Eucalyptus* spp. were then handplanted in June at spacings of 3 m. In both sites *A. pulchella* plants were about one year old at the first harvest in October 1987. Phosphorus had been broadcast by aeroplane at rates of 450 kg/ha as superphosphate (9% P) at planting time. Chemical analysis of the soils from the four sites are shown in Table 3.2.

3.2.1 (b) Sampling

For each site, two circular plots, 10 m radius, were selected at random within an area of several hectares. The two plots were chosen to increase the sampling area within each site. Soil and root samples were taken five times throughout the year in October, 1987, and in January, May, August,and November,1988. Four

Table 3.1: Mycorrhizal status of plants occurring on the two natural jarrah forests used in experiment 1.

Plant Species	Family	Mycorrhizal Status	Reference
Eucalyptus marginata Donn. ex Sm.	Myrtaceae	ec+va	Malajczuk *et al.*,1981
E. calophylla R.Br.	Myrtaceae	ec+va	Malajczuk *et al.*,1982; Gardner and Malajczuk,1988
Acacia pulchella R.Br.	Mimosaceae	va	Malajczuk *et al.*,1981
A. urophylla Benth.	Mimosaceae	va	1
Xanthorrhoea preisii Endl.	Xanthorrhoeaceae	va	1
Macrozamia riedlei (Gaud.)C.A.Gardn.	Zamiaceae	va	Lamont,1984
Hibbertia amplexicaulis Steud.	Dilleniaceae	va	Gardner and Malajczuk,1988
Bossiaea aquifolium Benth.	Papilionaceae	va	1
Kennedia prostrata R.Br.	Papilionaceae	ec+va	Warcup,1980; Gardner and Malajczuk,1988
Hakea lissocarpha R.Br.	Proteaceae	nm	2
Adenanthos barbingera Lindley	Proteaceae	nm	2
Persoonia eliptica R.Br.	Proteaceae	nm	2
Dryandra nivea (Labill.) R.Br.	Proteaceae	nm	Lamont,1984
Conospermum sp	Proteaceae	nm	2
Leucopogon sp	Epacridaceae	er	3
Phyllanthus calycinus Labill.	Euphorbiaceae	va	Lamont,1984
Clematis pubescens Huegel ex Endl.	Ranunculaceae	va	1
Hovea trisperma Bent.	Papilionaceae	?	?

ec = ectomycorrhiza; va = vesicular arbuscular; nm = non mycorrhizal; er = ericoid mycorrhiza ;? = information not available;1 = other species in the same genus form va mycorrhizas 2 = Proteaceae are non mycorrhizal;
3 = other species in the same genus have ericoid type mycorrhiza

Table 3.2: Chemical analysis of top soil (0-10cm) from the sites used in experiment 1.

Site	Plot	pH 1:5 water	Organic Carbon (%)[1]	Mineral Nitrogen (µg/g)		Extractable Phosphorus (µg/g)[2]	Exchangeable Potassium (µg/g)[3]
				NO_3^-	NH_4^+		
N84	I	5.75	3.35	2	13	6	57
	II	6.11	2.82	3	16	3	42
N85	I	5.61	3.38	2	8	10	45
	II	5.86	3.31	2	8	8	51
R1	I	5.97	2.86	2	6	10	48
	II	6.65	1.60	3	5	11	35
R2	I	5.77	1.77	2	4	10	29
	II	5.91	2.02	2	4	7	18

[1]Walkley (1947); [2]Colwell (1963); [3]Pratt (1965)

A.pulchella plants were selected for each plot at each sampling time. Two undisturbed cores, 11cm in diameter by 14cm depth, were taken for each plant about 10cm from the base of the plant, and placed straight away in 1.5kg plastic pots. An extra soil sample was taken for each plant to count and classify spores. The cores were taken to the glasshouse area at The University of Western Australia, placed in cooling tanks, and kept at 20±2°C throughout the experiment. *A. pulchella* was sown in the undisturbed cores after the seeds had been treated in boiling water for 60 seconds to overcome hard seededness and then kept in aerated water until the start of germination. Eight seeds were sown per pot within 24 hrs of collecting the cores. Plants were thinned to two seedlings per pot two weeks after emergence. Field capacity was determined for all sites at the first soil collection in October,1987. Soil weight was recorded for every individual core taken, and an average soil moisture determined at each soil collection. Pots were watered to 80% of field capacity and maintained at that level of moisture throughout the experiment by daily watering. Seedlings were grown for 10 weeks from sowing. Fresh and dry shoot weight, fresh root weight , percentage of root length infected by VA mycorrhizal fungi and percentage of different fungal genera infecting plants were determined. Spores were also counted and classified for each soil collection.

3.2.2. Experiment 2

Soil was collected from a jarrah forest approximatly 3km from Jarrahdale townsite, 100m south of Balmoral Road. That area had been burnt two years previously, and *A. pulchella* and *A. barbinervis* were very common species in the understorey.

Soil was collected in June and December, 1987 and six plants of either *Acacia* species were selected at each time depending on the species present. For each plant, the loose litter was scraped off, and the soil within a radius of 20-30 cm from each plant was collected at depths of 0-3 cm, 4-8 cm, 9-12 cm, and 13-20 cm. The soil from the six plants, for each depth, was bulked together, mixed thoroughly, and potted into 1.5 kg plastic pots. The pots received either 33μg P per g of soil as a solution of KH_2PO_4 in 100ml of water or just 100ml of de-ionised water with no phosphorus added.

The hard seededness of *A. pulchella* seeds was overcome in the same way as in Experiment 1. Twenty five seeds were sown per pot. Seedlings were thinned to eight per pot and harvested nine weeks after sowing. Soil analysis of the original soil is shown on Table 3.3.

3.2.3 Experimental Design

The experimental design for experiment 1 was a complete factorial with four sites, five collection times, and sixteen replicates. The second experiment was also a complete factorial involving four depths, two collection times, two levels of P applied, and three replicates.

An analysis of variance was conducted for all data using the GENSTAT package (Rotham. Exp. Station) in a complete factorial.

3.2.4. VA Mycorrhizal Fungi Assessment

The general procedure for clearing the roots was according to Phillips and Hayman (1970). Roots were heated for 10-12 hours at 75°C in 10% KOH and then stained in trypan-blue (lactic-acid, 650ml;

Table 3.3: Chemical analysis of soil used in experiment 2.

Depth (cm)	pH 1:5 water	Organic Carbon (%)[1]	Mineral Nitrogen (µg/g)		Extractable Phosphorus (µg/g)[2]	Exchangeable Potassium (µg/g)[3]
			NO_3^-	NH_4^+		
0-3	5.70	2.86	1	5	2	41
4-8	5.93	1.46	1	7	2	27
9-12	6.03	1.09	1	5	2	19
13-20	6.08	0.85	1	7	2	17

[1]Walkley (1947); [2]Colwell (1963); [3]Pratt (1965)

glycerol, 600ml; DI water, 800ml; trypan-blue, 1.3g) overnight at room temperature. The KOH solution was changed after heating for 4 to 5 hours if the solution was very dark. Before replacing the KOH solution, roots were washed in water, rinsed in 10% HCl for 2 minutes, washed in water again, then the KOH was replaced and the roots heated for a further 7-8 hours. This procedure improved the clearing of the roots. The percentage of root length infected was assessed using the line intercept method (Newman,1966).

Fungal genera were identified using hyphal characteristics according to Abbott (1982). No distinction was made between *Gigaspora* and *Scutellospora* and both genera will be referred to herein as *Gigaspora* . Spores were separated by wet sieving (Giovanetti and Mosse,1980), and counted under a dissecting microscope.

3.3. RESULTS

3.3.1.Experiment 1

a) Mycorrhizal fungi in field soils

The roots of field-grown plants of *A. pulchella* were collected to assess the percentage of colonization by VA mycorrhizal fungi at the same time as each collection of soil cores. However, it was not possible to clear the roots from the field because of the high concentration of phenolic materials in the roots. Various attempts were made to clear the roots, using different methods and time of heating in KOH but none proved efficient. Therefore, I was not able to assess mycorrhizal colonization in field-grown plants. All the results

presented in this chapter refer to colonization of roots of plants grown in undisturbed soil cores, under glasshouse conditions.

The number of spores averaged between 1 and 3 per 100g of soil, for all sites at all seasons. Most of the soil samples had no spores.

b) Infectivity of VA mycorrhizal fungi

The percentage of root length of *A. pulchella* seedlings colonized by VA mycorrhizal fungi was always much higher if seedlings were grown in undisturbed soil cores from natural jarrah forest than in cores from areas restored after mining. There was no difference in the percentage of root colonization between either of the two sites from natural jarrah forest or between either of the two sites from restored areas (Fig.3.2).

Differences were observed in seasonal variation in percentage of root length colonized when plants were grown in soil cores from either site from the natural jarrah forest sites and areas restored after mining (Fig.3.2). The percentage of root length colonized decreased during the summer season (Jan) in plants grown in cores from natural forest and increased with the onset of the predominantly winter rainfall. Unlike the cores from jarrah forest, there was no clear seasonal change in colonization of roots by VA mycorrhizal fungi in cores from restored areas where the levels of colonization remained low throughout the year. Moreover, there was not an increase in infectivity of the fungi in restored areas during the study period. An increase was expected because during the year the plants had grown bigger and a gradual increase in number of propagules was expected with

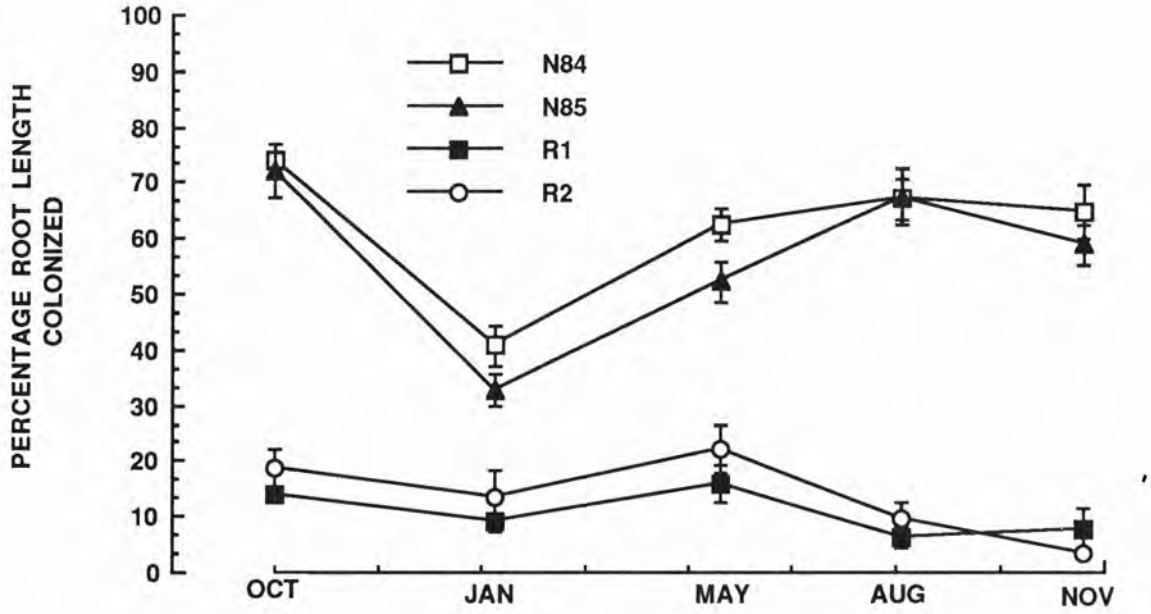

FIGURE 3.2. Seasonal variation in percentage of root length colonized by VA mycorrhizal fungi in *Acacia pulchella* seedlings grown in undisturbed cores from natural forest (N84 and N85) and restored areas (R1 and R2) in glasshouse conditions. Each point is a mean of 16 cores and bars are standard errors of means (Experiment 1).

increasing root density. There were no interactions between sites and seasons.

Seedlings of *A. pulchella* grown in cores from disturbed areas produced less root length colonized than seedlings grown in cores from natural jarrah forest (Fig.3.3). There was no difference between the two sites from restored areas after mining. On the other hand, seedlings grown in cores from site N85 generally produced more root length colonized than those grown in cores from site N84. This corresponded to higher total root length produced for seedlings grown in soil from site N85.

There were no seasonal changes in root length colonized for seedlings grown in cores from restored areas, but for seedlings grown in cores from natural jarrah forest there was a similar pattern of seasonal variation as in the percentage of root length colonized (Fig.3.3). Site N85 produced more root length colonized than site N84 at the first soil collection in October. During summer (Jan) the root length colonized decreased for both sites, and there was no difference between the two sites. Corresponding with the start of the winter rainfall, the root length colonized increased for cores collected from both sites but at different rates. Plants grown in cores from site N84 reached a plateau level of colonization in May. For seedlings grown in cores from site N85, the root length colonized increased until the August soil collection and then decreased in November to the same level as in May. There were no interactions between sites from revegetation areas and time of core collection. On the other hand, for sites from natural jarrah forest, the dry conditions of the summer season minimized the differences in root length colonization assessed in cores from the two sites but they became apparent again with the start of the rainfall period.

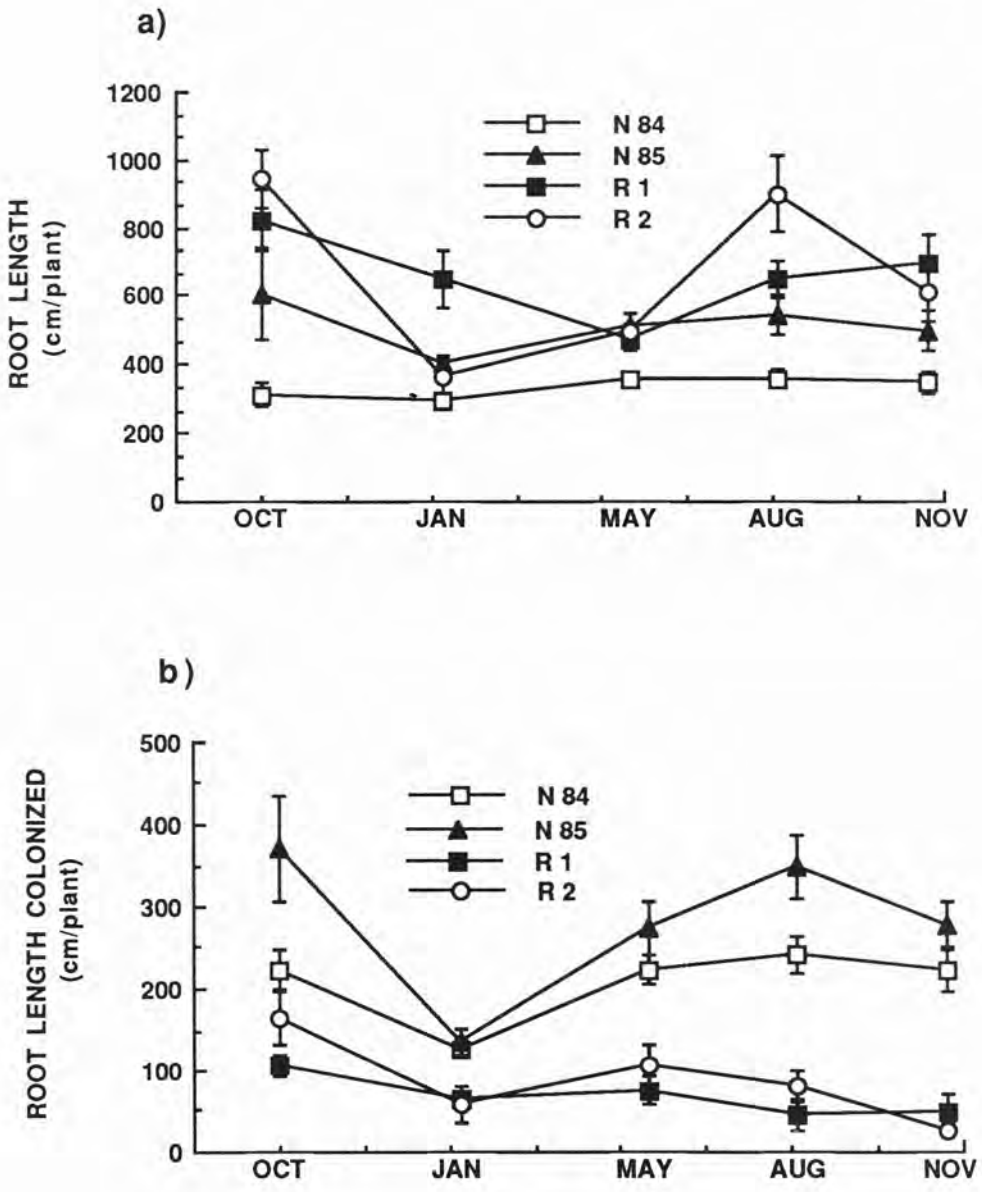

Figure 3.3. Seasonal changes in root length (a) and root length colonized by VA mycorrhizal fungi (b) of *Acacia pulchella* seedlings growing in undisturbed cores from natural forests (N84 and N85) and restored areas (R1 and R2) in glasshouse conditions (cm plant^{-1}). Each point is a mean of 16 replicates, and bars are standard errors of means (Experiment 1).

There was a distinct difference in dominance of particular genera of VA mycorrhizal fungi colonizing roots of *A. pulchella* grown in soil cores from natural jarrah forest and from restored areas (Fig.3.4 and Table 3.4). *Glomus* was by far the predominant genus in soil cores from natural jarrah forest with the other genera scoring generally less than 10% of total colonization. In soil cores from restored areas the distribution of genera was more uniform with *Glomus, Gigaspora*, and fine endophyte occurring in similar proportions. *Acaulospora* was present at very low intensity in both natural forest and restored areas. The sum of all genera at a certain time may exceed 100% because of the presence of double colonization in a piece of root. Fine endophyte was the main fungus occurring in double colonization in soils from both natural forest and restored areas. There were differences in hyphal characteristics of *Glomus* species between sites and between harvests but no attempt was made to try to classify the fungi to species level. In the same way, distinct hyphal characteristics occurred inside single cores from natural jarrah forest indicating that it was likely that there was more than one fungal species colonizing the roots, even if most of them belonged to *Glomus*. In contrast to natural jarrah forest, the hyphal characteristics of fungi occurring in roots from single cores from restored areas were uniform, suggesting that infection came from a single species of VA mycorrhizal fungus. This could reflect the low density of propagules in restored areas that allows a single species of fungus to colonize the root with no competition from other fungi.

No seasonal trend in occurrence of particular genera of VA mycorrhizal fungi could be drawn for either natural jarrah forest or restored areas (Fig.3.4 and Table 3.4). Although *Glomus* was the dominant genus observed within roots of plants grown in cores

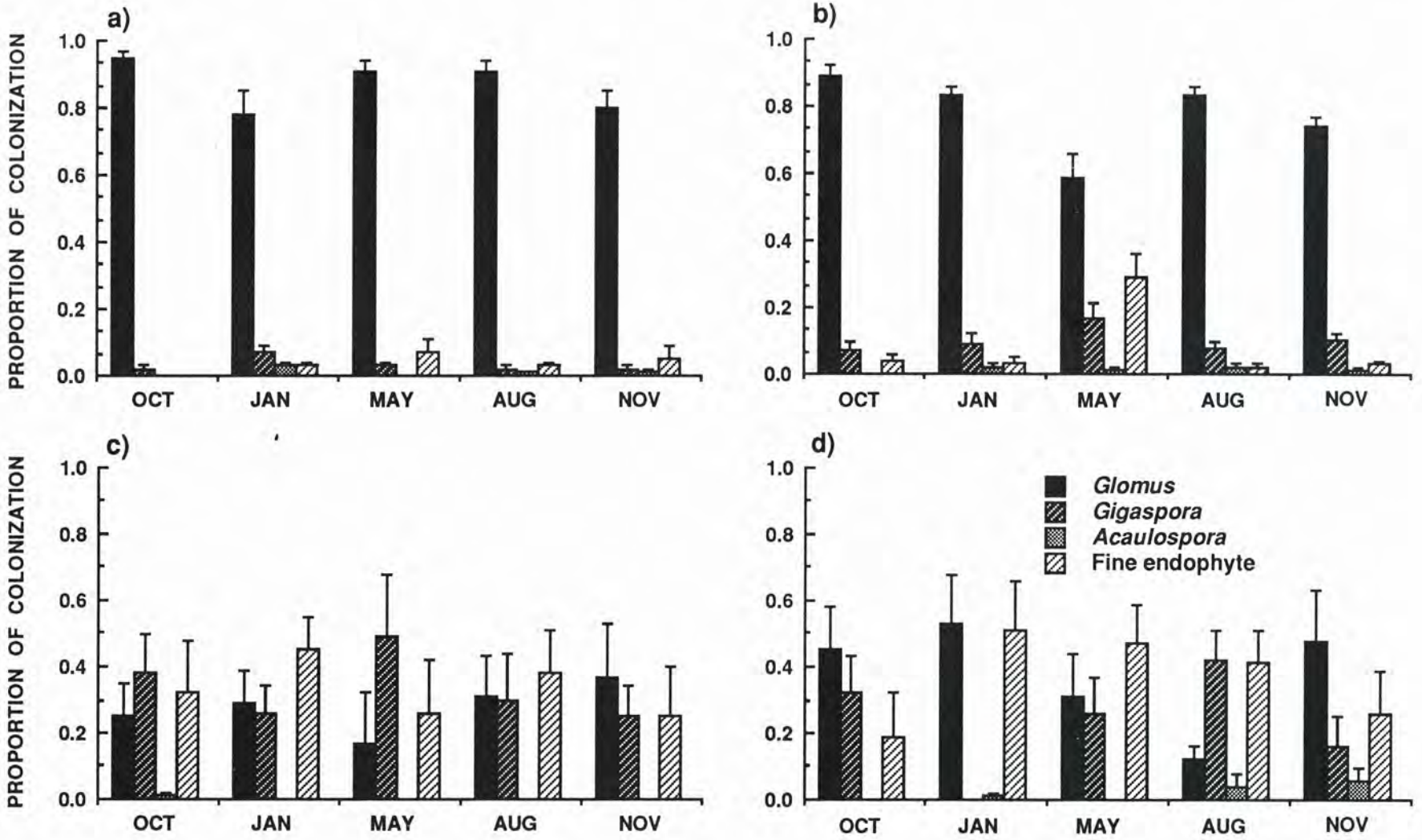
a)
b)
c)
d)
PROPORTION OF COLONIZATION
PROPORTION OF COLONIZATION
1.0
0.8
0.6
0.4
0.2
0.0
OCT
JAN
MAY
AUG
NOV
Glomus
Gigaspora
Acaulospora
Fine endophyte

FIGURE 3.4. **Proportion of total length of infected root colonized by each of four genera of VA mycorrhizal fungi in *Acacia pulchella* plants grown for 10 weeks in undisturbed cores from natural (a - site N84, and b - site N85) and restored areas (c - site R1, and d - site R2) in glasshouse conditions. Each point is a mean of 16 replicates, and bars are standard error of means (Experiment 1).**

Table 3.4. Root length (cm/pot)(± standard error) of root colonized by each of four genera of VA mycorrhizal fungi in seedlings of *Acacia pulchella* grown for 10 weeks in cores from four sites (Experiment 1).

Time	Genus	Site			
		N84	N85	R 1	R 2
October	*Glomus*	448±35	594±64	21±10	59±26
	Gigaspora	10±7	36±14	28±10	39±21
	Acaulospora	0±0	0±0	1±1	0±0
	Fine endophyte	0±0	22±13	22±12	15±10
January	*Glomus*	243±32	279±42	23±9	97±57
	Gigaspora	19±6	27±10	19±7	0±0
	Acaulospora	10±5	5±3	0±0	1±1
	Fine endophyte	10±3	9±5	38±14	37±17
May	*Glomus*	223±19	154±2	13±13	42±23
	Gigaspora	7±3	53±17	25±14	13±6
	Acaulospora	1±1	2±2	0±0	0±0
	Fine endophyte	5±7	89±27	4±2	43±17
August	*Glomus*	225±24	323±31	31±9	10±4
	Gigaspora	3±1	33±9	23±17	37±9
	Acaulospora	1±1	10±3	0±0	9±9
	Fine endophyte	6±2	6±2	10±5	37±14
November	*Glomus*	187±25	209±24	29±23	8±5
	Gigaspora	5±2	28±8	7±3	4±3
	Acaulospora	3±1	5±2	0±0	1±1
	Fine endophyte	6±4	9±5	11±7	4±2

from natural jarrah forest, colonization by *Gigaspora*, and fine endophyte reached 20 and 30% in site N85 at the third harvest (May). In cores from restored areas, at least three genera were recorded as equally abundant at each sampling time.

b) Plant Growth

In general, fresh shoot and root weight were greatest for plants grown in cores collected in late spring and summer and least for cores collected in winter (Table 3.5). There were relatively minor differences in fresh shoot and root weight for plants grown in cores.

3.3.2. Experiment 2

The percentage of root length colonized in seedlings of *A. pulchella* grown as bioassay plants in pots of mixed, and sieved soil was higher for soil collected in early summer (December) than for soil collected in winter (June)(Fig.3.5). The effect of soil depth and application of phosphorus on percentage of root length colonized was dependent on the time of soil collection. When soil was collected in winter, plants grown in soil from the top 3cm of the profile had lower levels of colonization by VA mycorrhizal fungi than did plants grown in soil from the 4-8cm layer . A very low percentage of root length colonized was observed in plants grown in soil deeper than 8cm at that time. When soil was collected in December, there was no difference in percentage of colonization in plants grown in soil from any depth. In the same way, application of phosphorus to the soil had no effect on the percentage of colonization when soil was collected in December, but the

Table 3.5: Fresh shoot and root weight (g pot^{-1}) of *Acacia pulchella* plants growing in undisturbed soil cores from natural (N84 and N85) and restored areas (R1 and R2), in glasshouse conditions (Experiment 1).

Time	SHOOT				ROOT			
	N84	N85	R1	R2	N84	N85	R1	R2
OCT	0.23	0.58	0.40	0.82	0.22	0.47	0.66	0.96
JAN	0.18	0.33	0.15	0.13	0.30	0.43	0.47	0.34
MAY	0.16	0.22	0.14	0.14	0.22	0.30	0.27	0.30
AUG	0.23	0.42	0.20	0.37	0.38	0.48	0.43	0.76
NOV	0.20	0.34	0.47	0.26	0.36	0.51	0.77	0.57
	$LSD_{SHOOT.05} = 0.17$				$LSD_{ROOT.05} = 0.18$			

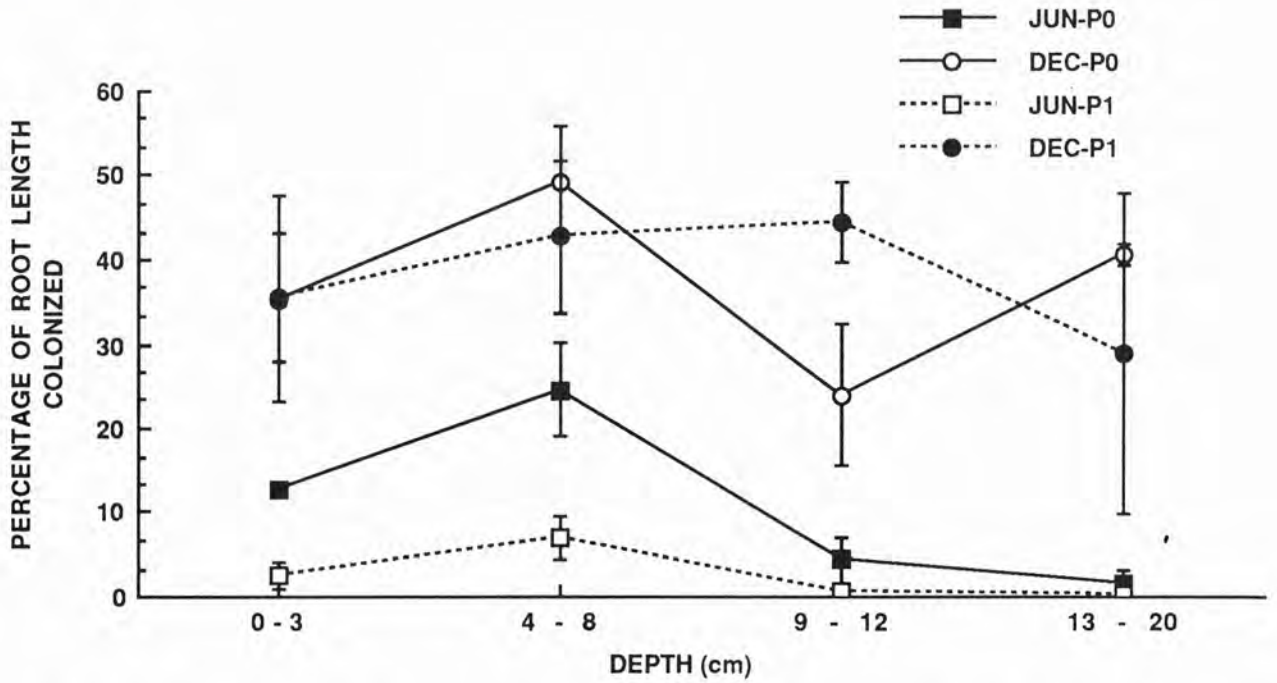

Figure 3.5. Effect of soil depth and phosphorus on formation of VA mycorrhizas in seedlings of *Acacia pulchella* grown in soil with two levels of phosphorus application (P_0= no phosphorus added; P_1= 33μgP/g soil); soil was collected in winter (June) and early summer (December). Values are average of three replicates and bars are standard errors of means (Experiment 2).

level of colonization decreased with phosphorus application in soil collected during winter.

Root length colonized by VA mycorrhizal fungi was greater in soil collected in December than in June (Fig.3.6). Soil depth and application of phosphorus had different effects according to time of soil collection. Soil from the 4-8cm layer had the greatest root length colonized in June. There was no difference in root length colonized among soils from the other three layers, at that time. On the other hand, root length colonized in soil collected in December tended to increase with increasing soil depth. Application of phosphorus did not affect root length colonized for seedlings grown in soil from the 4-8cm layer, when collected in June. There was no effect of phosphorus application on root length colonized for the other layers at this collection time. Application of phosphorus to soil collected in December almost doubled the root length colonized by VA mycorrhizal fungi, regardless of soil depth.

Root length produced by *A. pulchella* seedlings was greater in soil collected in June than for soil collected in December (Fig.3.7). There was no difference in root length produced as an effect of soil depth or phosphorus application in plants grown in soil collected in June. On the other hand, plants grown in soil collected in December showed increased root length with increased soil depth and with increased phosphorus application.

Glomus was by far the predominant genus of VA mycorrhizal fungi colonizing roots of seedlings of *A. pulchella* grown *in soil from jarrah forest in this experiment. Glomus was responsible* for 70-100% of the colonization formed, regardless of season, soil depth or application of phosphorus (Fig.3.8). *Gigaspora* was present within roots most of the time but generally formed only 10-20% of the

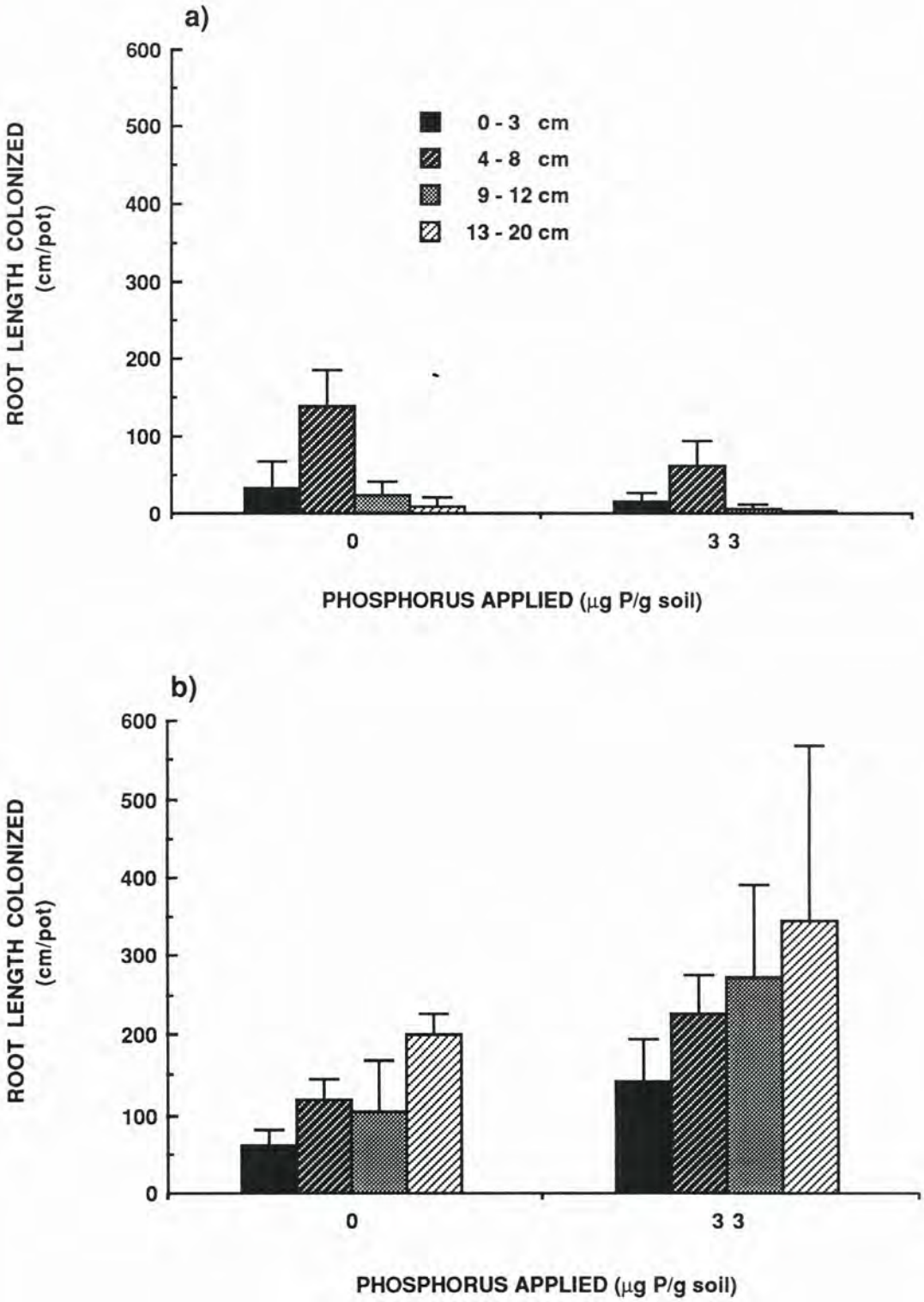

FIGURE 3.6. Root length of seedlings of *Acacia pulchella* grown in soil from four increasing depths in the top 20cm of the soil profile, collected in (a) June and (b) December as affected by phosphorus application. Each point is a mean of three replicates and bars are standard error of means (Experiment 2).

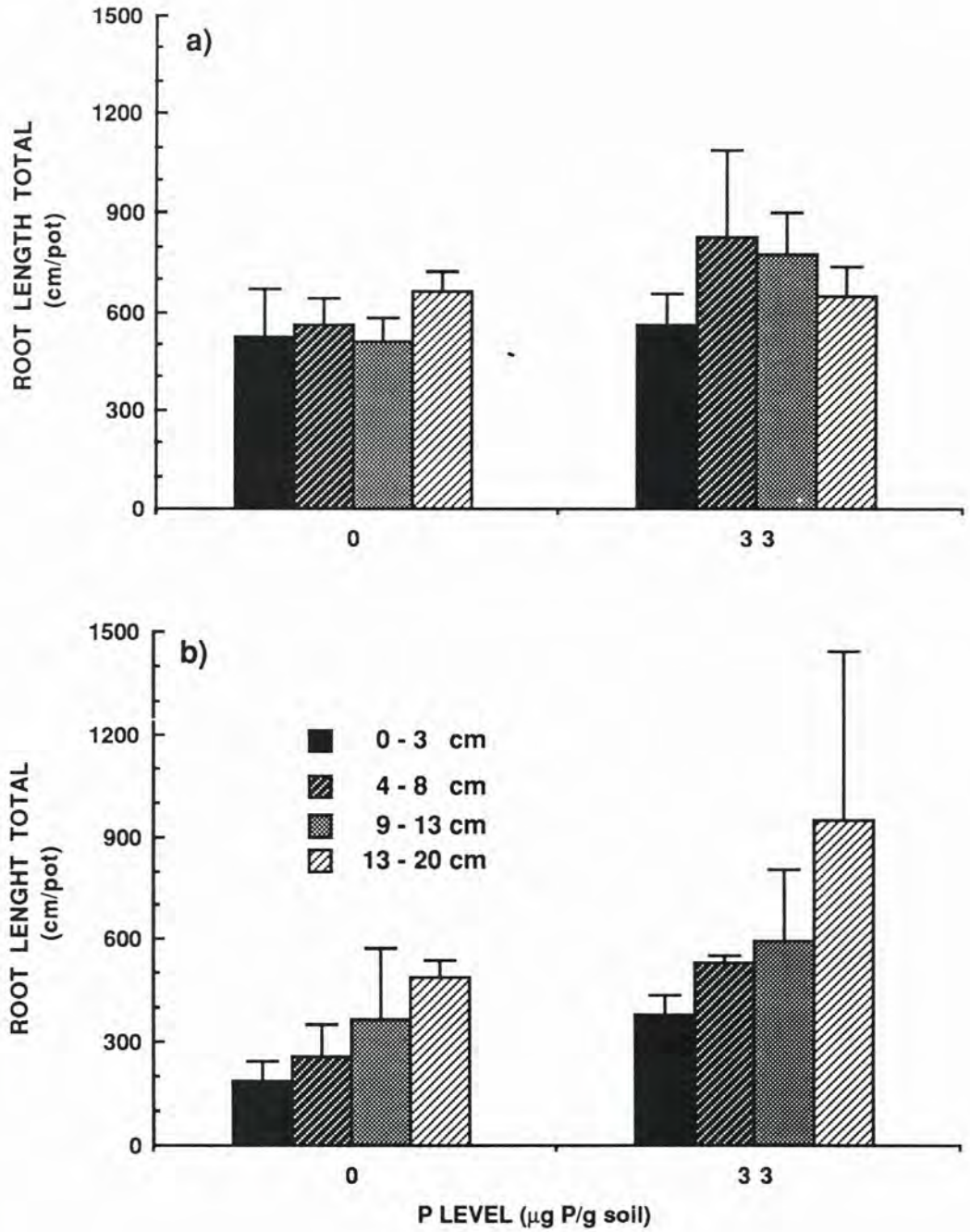

FIGURE 3.7. Effect of phosphorus application on total root length of *Acacia pulchella* seedlings grown in soil from increasing depth in the top 20cm of a jarrah forest, collected in (a) June and (b) December. Each point is a mean of three replicates and bars are standard errors of means (Experiment 2).

colonization. *Acaulospora* and fine endophyte occurred only occasionally. Occurrence of *Glomus* decreased in the top 3cm in soil collected in December when compared to soil collected in June. There was no effect of phosphorus application to soil from the top 8cm on the proportion of colonization formed by *Glomus* , but this proportion increased with phosphorus application in soil from 9-20cm depth collected during winter (Fig. 3.8).

Gigaspora was more common in roots of plants grown in soil collected in December than in soil collected in June (Fig.3.8). There was an effect of phosphorus application on colonization by *Gigaspora* in the top 8cm of the soil, but the proportion of colonization by *Gigaspora* was higher in P-amended soil than in soil which had received no phosphorus, for depths lower than 9cm. *Acaulospora* only contributed a significant proportion of root colonized in soil collected between 9-12cm depth with no P application, when collected in June. Fine endophyte was only present within roots grown in soil collected from the top 3cm in June. Application of phosphorus doubled the proportion of roots colonized by fine endophyte for this soil collection.

3.4. DISCUSSION

The seasonal trend in percentage of root length colonized by VA mycorrhizal fungi in plants of *Acacia pulchella* grown in undisturbed cores collected from natural forest followed the pattern of rainfall distribution in this region. The decrease in infectivity during summer (January) coincided with the dry period when root growth

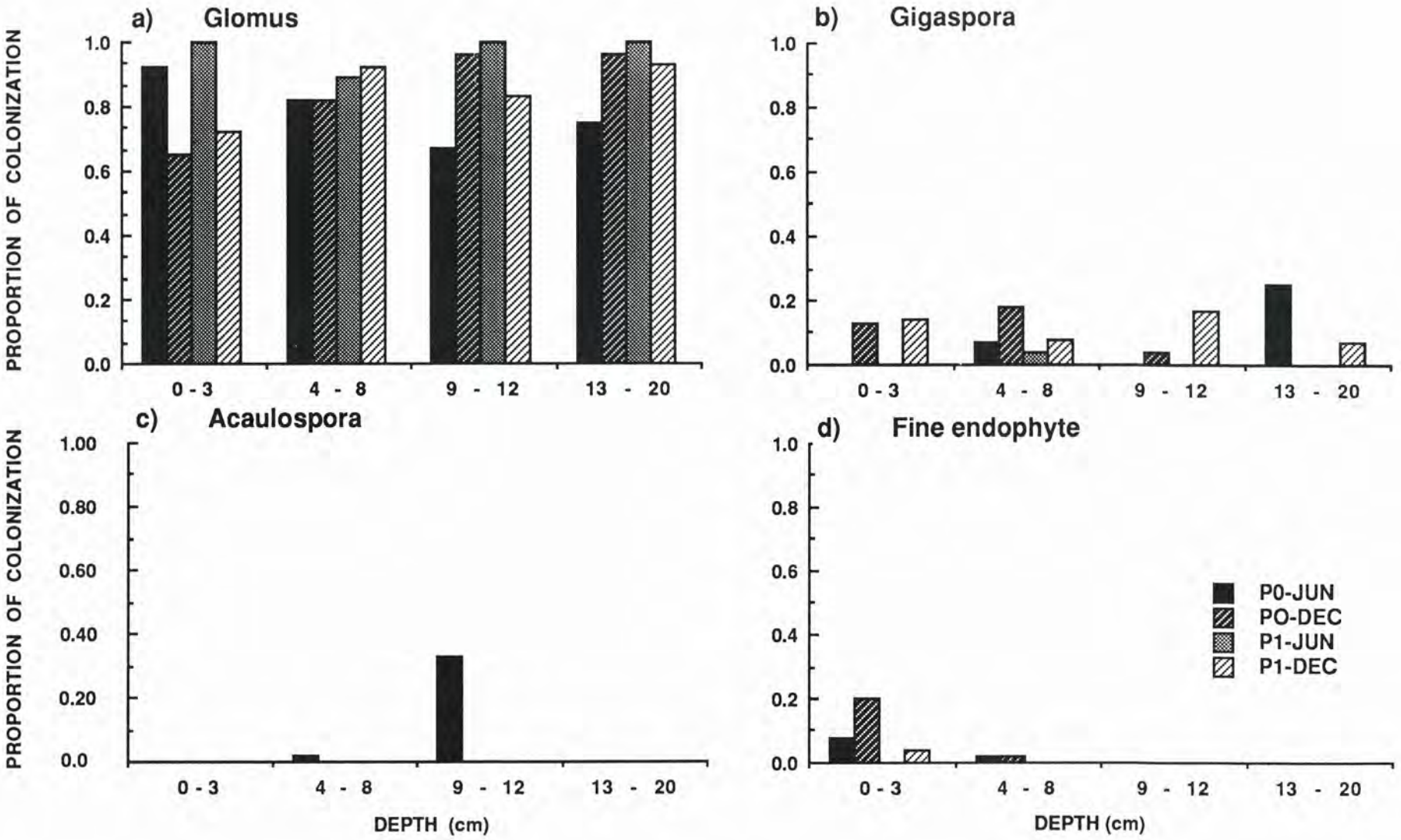
a) Glomus
b) Gigaspora
c) Acaulospora
d) Fine endophyte
PROPORTION OF COLONIZATION
DEPTH (cm)
0 - 3
4 - 8
9 - 12
13 - 20
P0-JUN
PO-DEC
P1-JUN
P1-DEC

FIGURE 3.8. Proportion of total length of infected root colonized by each of the four genera of VA mycorrhizal fungi as affected by soil depth, time of soil collection (June or December), and phosphorus application (P0 = no P applied; P1 = 33 μg P/g soil). Each value is a mean of three replicates (Experiment 2).

stops in the forest. With the beginning of the winter rainfall, in April/May, new root growth commences, and the fungi increase their activity as shown by the sharp increase in the level of colonization in seedlings of *A. pulchella* grown in glasshouse conditions in cores collected in May, and remaining at the same level up to November. Rochell (cited by Lamont, 1982a) found that trees of *E. marginata* stopped new root production during winter. If this is the case for the forest as a whole, it seems to have no effect on the infectivity of VA mycorrhizal fungi, perhaps because a decline in new root growth does not necessarily mean a decline in root activity. Actually at this stage, roots are likely to be at maximum activity because they accumulate nutrients for the flowering period that occurs for most of the species in the jarrah forest between July and October. Maximum nodule activity in *Acacia* species also occurs between June and October (Hansen *et al.*,1987). Dell and Wallace (1979) reported two peaks in new root production in *E.marginata* in autumn and spring. Autumn is the season prior to the flowering period, and late spring is when shoot growth occurs. The increase in new root growth just prior to flowering and the resumption of shoot growth is timed to correspond with the onset of these energy demanding processes.

On the other hand, restored areas did not show any trend with season in levels of colonization; these levels remained low throughout the year (Fig.3.2). The lower levels of colonization in restored areas were expected because soil disturbance is known to decrease the capacity of VA mycorrhizal fungi to colonize plants (Jasper *et al.*, 1987). Surprisingly, with increasing age of the stand, an increase in infectivity was also expected but this did not happen. In the one year of the experiment the plants had grown bigger, promoting better cover of the soil and supposedly decreasing variation in day/night

temperature and water losses. Nevertheless, there was no increase in percentage of root length colonized in plants grown in soil collected from restored areas. Gardner and Malajczuk (1988) working in a similar area, examined plants in revegetation areas with different ages and found that the number of plants colonized by VA mycorrhizal fungi increased with increasing age of the stand but the levels of colonization were always less than 5%. Their experiment assessed colonization in field-grown plants and there were some problems with the process of clearing the roots for mycorrhizal assessment that may have affected their results. In another study, Miller (1987b) worked on areas disturbed by coal mining in the United States and found that inoculum potential of VA mycorrhizal fungi was decreased during the first five years of restoration but started to recover from the sixth year. In his study there was a predominance of non-mycorrhizal plants during the early stages of soil rehabilitation, and the increase in infectivity of mycorrhizal fungi was associated with the return of mycorrhizal plants to the area. That does not seem to be the case in my experiment because all the plants in the rehabilitation area were potentially mycorrhizal (*Eucalyptus* spp., and legumes).

An altered belowground environment was considered to be the reason for delayed recovery of infectivity of VA mycorrhizal fungi in the early stages of rehabilitation of areas disturbed by coal mining in Wyoming (Stahl *et al.*,1988). These authors suggested that because of changes in the soil environment due to mining processes, the mycorrhizal fungi were unable to form an extensive network of external hyphae into the soil, therefore the hyphae could not follow the expansion of the root system. Nevertheless, no explanation was given of what those changes could be. In my study, nutritional levels of soils from natural and restored areas were quite similar (Table 3.2). Organic

carbon and mineral nitrogen levels were the only two parameters with higher levels in natural forests than in restored areas but there is no evidence, so far, that any of these factors can significantly regulate the amount of colonization caused by VA mycorrhizal fungi or their rate of spread. It is interesting to notice though that some cores from restored areas did produce levels of colonization similar to natural forest but these were isolated cases and the fungi seemed unable to spread in the surrounding areas. This was also observed by Gardner and Malajczuk (1988) and may indicate that the "altered belowground environment" proposed by Stahl *et al.* (1988) could force the mycorrhizal fungi to make some ecological adaptations before they start to spread in the area or at least stay in a "stand by" situation until microclimatic conditions are more suitable for fungal development.

No seasonal trend was observed either for natural or restored areas in the occurrence of genera of VA mycorrhizal fungi. *Glomus* was by far the most common genus in the natural forest with very little colonization by the other genera. Similar results were attained by Scheltema *et al.* (1987) working with annual pastures in this region. They suggested that differences in the extent of infection formed by different genera of fungi may be related to differences in the biology of those fungi (eg. spore dormancy, ability to form infection from dead pieces of roots).

In restored areas on the other hand there was a more even distribution of genera with *Glomus, Gigaspora,* and fine endophyte occurring in both sites. *Acaulospora,* as in natural forest, was present at very low levels. There was variation in predominance of different genera with time but that was not consistent, changing according to site. The large variance in proportion of genera between harvests and sites (as evidenced by large error bars) reflects the lack of

uniformity of inoculum distribution in the area. In natural forest, it was possible to identify differences in characteristics of internal hyphae within roots of plants grown in a single core (although the classification was made to genus level) indicating a diversity of fungal species, most of them belonging to *Glomus* . In restored areas just one genus was present, and the characteristics of hyphae were always the same for a single core, indicating that all the colonization came from only one fungal species. Hence, double colonization was almost absent in restored areas, but in the few times it was observed to occur one of the fungi involved was fine endophyte. The low propagule density in restored areas may have eliminated the factor that favoured *Glomus* species in natural forest allowing a more uniform distribution of genera within the area.

In experiment 2, the percentage of root length colonized in plants grown in forest soil that had been mixed and sieved was higher in December than in June. Similar results were obtained by Bolan and Abbott (1983) working with soil from a different jarrah forest site; no colonization was observed in spring and higher colonization was observed in summer. This apparently contrasts with the results from experiment 1 where the percentage of colonization was lowest during summer (Jan). In experiment 1 undisturbed cores were used but in experiment 2, and in Bolan and Abbott's experiment the soil was mixed and dried before starting to grow plants. It is likely that hyphae growing in the soil was the main type of propagule in June; this may have been affected by soil disturbance causing the very low levels of infection . For example, disruption of the hyphae network in the soil decreased the frequency of infection by 56% due to loss of vigour of the mycelium inoculum following its fragmentation and detachment from supplies of carbon (Read and Birch,1988). In the same way, in a split-

root experiment, Jasper *et al.* (1989b) observed an 85% decrease in root colonization after disturbance of the hyphal mycelium in the soil.

Spores and hyphae inside the root are likely to be the main types of propagule in south-western Australian forests in summer (December). Spores are very resistant to adverse conditions and have been reported to survive under temperatures as high as 75°C in semi-arid conditions (McGee,1989). Thus spores would be the type of inoculum most likely to be found in these dry conditions. In my experiment, the number of spores per 100g of soil averaged between 1 and 3 throughout the year. Reasons for this low number of spores are not known. Spores may not have been counted because they were too small or were present in sporocarps that are difficult to recover. Because most of the colonization, especially in natural forest, was formed by *Glomus* species, the first alternative is unlikely because this genus forms spores which are mostly bigger than 60µm. Thus these spores are large enough to be recovered in the smallest sieve used (53µm). On the other hand, some *Glomus* species do form sporocarps and some of those can be lost during the sampling and sieving process because they are not evenly distributed in the soil or they are not recovered from the organic matter. But the most likely explanation for the low number of spores is that in forest ecosystems not many spores are formed (Baylis,1969). The root framework is always present and the fungi can survive inside the root and sporulation may not be stimulated.

Hyphae of many fungi have been reported to be able to survive inside dry pieces of roots (Rives *et al.*, 1980; Tommerup and Abbott,1981) and become infective when conditions are favorable. The main type of propagule during summer may be hyphae inside the roots. Colonized roots are also present in June but infectivity of propagules

dropped markedly at that time with soil disturbance. The difference is that hyphae are probably growing actively in June and were not resistant to the sudden disturbance and desiccation of the soil. In contrast, the soil sampled in December had been gradually losing moisture and the fungi had had a chance to make the necessary physiological adaptation to survive during summer, and act as a propagule when conditions become favourable.

It is interesting to note that the levels of colonization for the top 12cm in the no-phosphorus added treatment in experiment 2, in December (36%), and in the experiment of Bolan and Abbott (1983) in February (27%) were very similar to the levels of colonization in natural forest sites, in experiment 1 (undisturbed cores), in January (38%). This occurred although different species of fungi were present within roots in each case. The contrasting levels of mycorrhizal formation from experiments 1 and 2 in my study when soil was collected in winter highlight the necessity of specifying the soil conditions in studies of seasonal variation in infectivity of those fungi, and raises the question about the validity of using disturbed cores to assess the infectivity of VA mycorrhizal fungi in field conditions.

CHAPTER 4

ROOT GROWTH AND MYCORRHIZAL DEVELOPMENT IN *ACACIA PULCHELLA* SEEDLINGS GROWN IN A RECONSTRUCTED SOIL PROFILE FROM THE JARRAH FOREST

4.1. INTRODUCTION

Root architecture and density are important factors affecting the colonization of roots by VA mycorrhizal fungi (Miller, 1987a). However there has been very little study of the growth and distribution of roots for forest species, especially studies that consider the relationship between root growth and colonization by VA mycorrhizal fungi. Soils in south-western Australia are generally very infertile and plants growing in this region have developed a series of mechanisms that enable them to cope with such conditions (Lamont, 1982a). Most of the understorey species in the jarrah forest concentrate their roots in the top 15cm of soil (Lamont, 1982a), and it is likely that most of the mycorrhizal activity also occurs in that layer of the soil. Some studies have been done by Shea and Dell (1981) and Dell and Wallace (1983) on seasonality of root production for *Eucalyptus marginata* but this species has a different pattern of root distribution from understorey legumes.

Acacia pulchella has a shallow root system (Dodd *et al.*, 1984) and a "magnolioid" type root (Barrow,1977) leading to a low ability to explore the soil. At the same time it forms an association with VA mycorrhizal fungi (Malajczuk *et al.*,1981), and it has been suggested that it depends strongly on this association for uptake of phosphorus (Jasper *et al.*,1989c). This association is likely to be most important during seedling establishment and early stages of plant development but this is poorly understood. In this study (experiment 3) I have tried to get a better understanding of root dynamics and colonization by VA mycorrhizal fungi during the early stages of plant development by following root growth and spread of VA mycorrhizal

fungi with soil depth and plant age in seedlings of *A. pulchella* from seed germination until root activity decreased during summer.

4.2. MATERIALS AND METHODS

Experiment 3

It is difficult to recover roots from individual plants growing in the field. This is because (1) these roots are easily broken and detached from the main root which make it difficult to distinguish between roots from different species, and (2) as soil depth increases root density decreases. Moreover, individual plants in the forest have different ages and stage of development which make it difficult to compare the results from successive samplings. To overcome some of those difficulties I worked with reconstructed profiles placed in a nursery environment, as described below.

Soil was collected at depths of 0-3cm, 3-10cm, 10-20cm, 20-35cm and 35-50cm from a jarrah forest site where *A. pulchella* was one of the main understorey species. Soil from each layer was homogenized and placed into wooden boxes (50cm high x 20cm width x 40cm length) in the same order as they were in field. Chemical analysis of the soil collected from the different depths is shown in Table 4.1. The wooden boxes were put into the soil and eighty pre-germinated *A. pulchella* seeds were sown per box. Seeds were boiled in water for 60 seconds to decrease hard seededness, and then kept in aerated water until the start of germination. Seedlings were thinned to 16 after germination. As the time and percentage of germination of seeds was very variable, the germination was followed daily. When the number of seedlings reached 16, in three consecutive days, the plants

Table 4.1: Chemical analysis of soil used in the study on root development of *Acacia pulchella*.

Depth (cm)	pH 1:5 water	Organic Carbon (%)[1]	Mineral Nitrogen (µg/g) NO_3^-	Mineral Nitrogen (µg/g) NH_4^+	Extractable Phosphorus (µg/g)[2]	Exchangeable Potassium (µg/g)[3]
0 - 3	5.86	3.34	3.5	6.5	13.0	52
3 - 10	6.01	2.62	3.0	6.0	6.5	28
10 - 20	6.07	1.17	3.0	3.5	3.0	21
20 - 35	6.15	0.71	2.0	3.5	3.5	26
35 - 50	6.24	0.53	2.0	4.0	2.5	19

[1]Walkley (1947); [2]Colwell (1963); [3]Pratt (1965)

that germinated before or after that period were removed. The second day was considered as the start of germination, therefore seedlings could be only one day older or younger than each other. This procedure was used to minimize the variation of stage of plant development, especially at the early stages of the experiment. Six boxes were set up in total, and plants were grown from 20th of April, 1988 until 15th of February,1989. Boxes were harvested at 2, 6, 13, 29, 38, and 43 weeks after germination. At each harvest there was no replication. Rainfall and temperature were recorded throughout the experiment (Fig.4.1). At the last two harvests the moisture content of the soil was 2.4, 3.4, 3.4, and 3.6% in the layers 0-10cm, 10-20cm, 20-35cm and 35- 50cm, respectively.

Roots were carefully washed free of soil at each harvest, and roots from each layer of the soil were collected separately. Roots from the top layer (0-3cm) were bulked together with the roots from the second layer (3-10cm). This was necessary because at the first harvest, it was not possible to collect the two layers separately due to low root density. Fresh shoot and root weights were measured at each harvest. Total root length, percentage of root length colonized, total root length colonized, and identification of fungi were determined in the same way as in the seasonal variation study (Chapter 3). The procedure for clearing and staining the roots was also similar to that used in Chapter 3 but as the roots from the last two harvests were already suberized, these roots were heated for 24 hours in KOH, at 75°C, instead of for 10-12 hours.

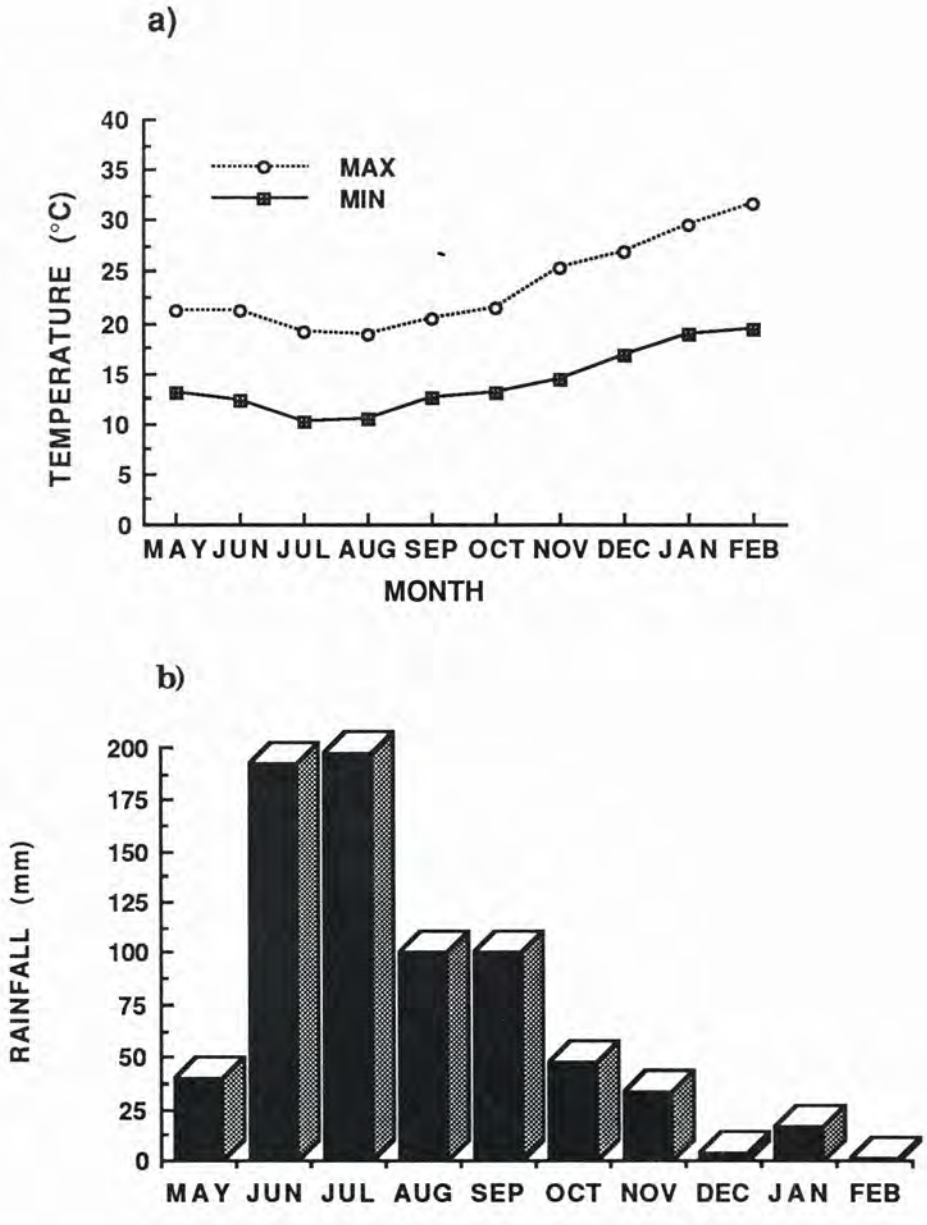

Figure 4.1: Monthly average maximum and minimum temperature (°C), and rainfall (mm) during the period plants were grown in wooden boxes.

4.3. RESULTS

a) Soil Properties

The highest concentration of nutrients was in the top 3cm of the soil. The concentration of organic carbon and phosphorus decreased exponentially with depth (Fig.4.2 and Fig.4.3). Potassium was located especially in the top 3cm of the soil, decreasing to about half of that concentration in deeper layers. The soil pH increased steadily with increasing soil depth (Table 4.1).

b) Growth

Root length of *A. pulchella* seedlings grown in a rebuilt soil profile down to 50cm depth, had a slow increase during the first 13 weeks after seed germination. The biggest increment in root production occurred between the 13^{th} and 29^{th} weeks (Fig.4.4). This period corresponds to the spring season when both temperature and soil moisture are ideal for plant growth. From then until the end of the experiment (43 weeks) there was a decrease in total root length, probably due to death of fine roots associated with the dry and hot environmental conditions. At any time, at least half of the total root length was in the top 10cm of the soil. Increment in root length in this layer followed the same pattern as for total root length, increasing up to the 29^{th} week, and then decreasing until the end of the experiment. However, roots grown in deeper layers of the soil had a different pattern

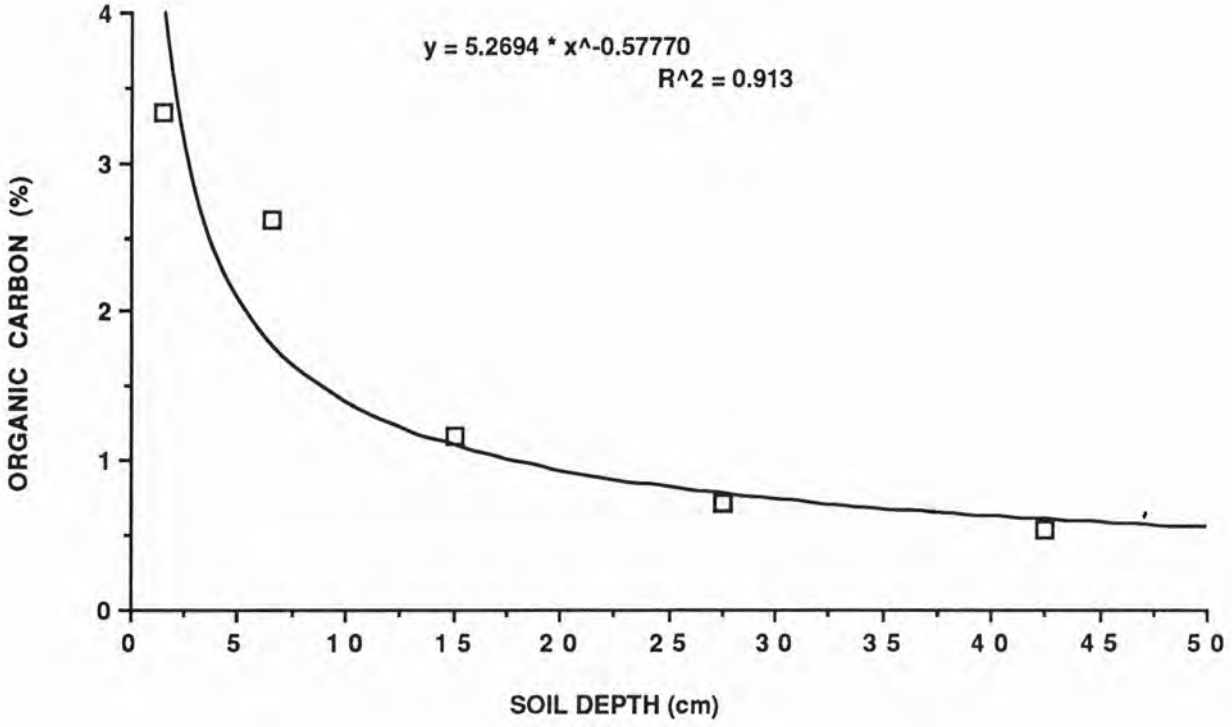

Figure 4.2. Distribution of organic carbon in the soil profile (Experiment 3).

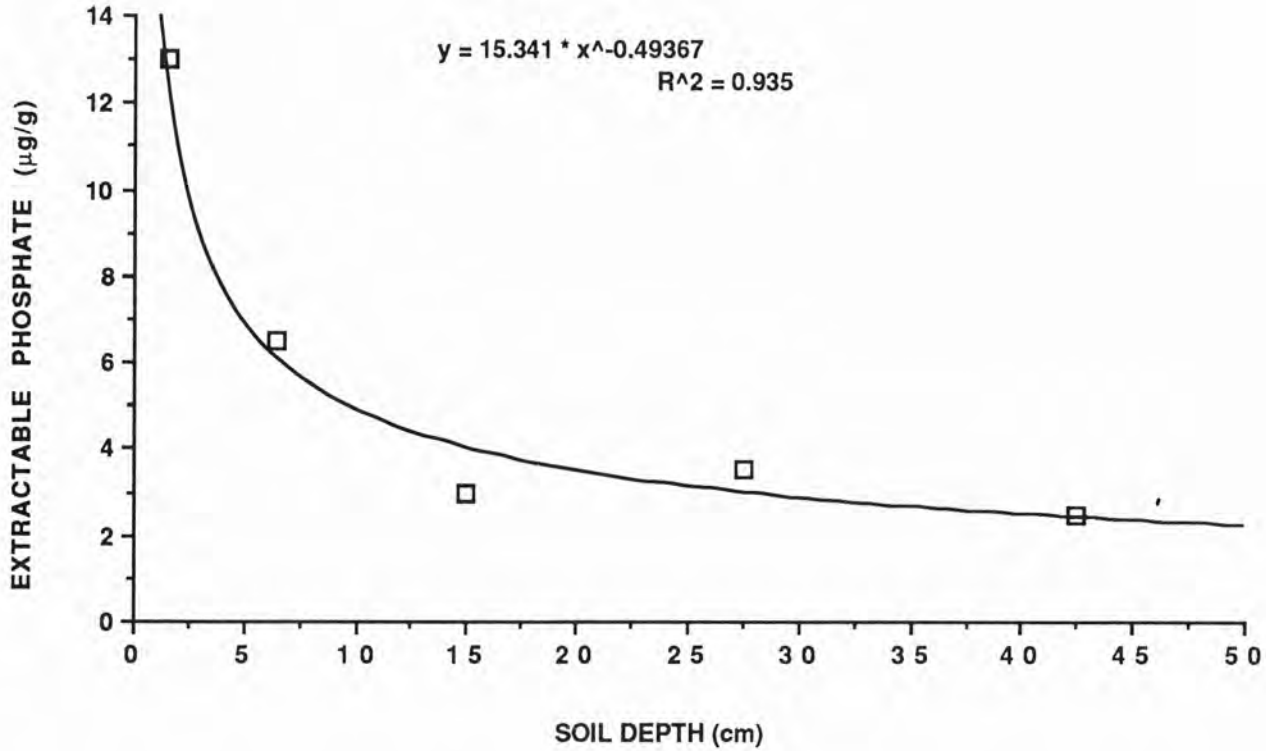

Figure 4.3. Distribution of $NaHNO_3$ extractable phosphate in the soil profile (Experiment 3).

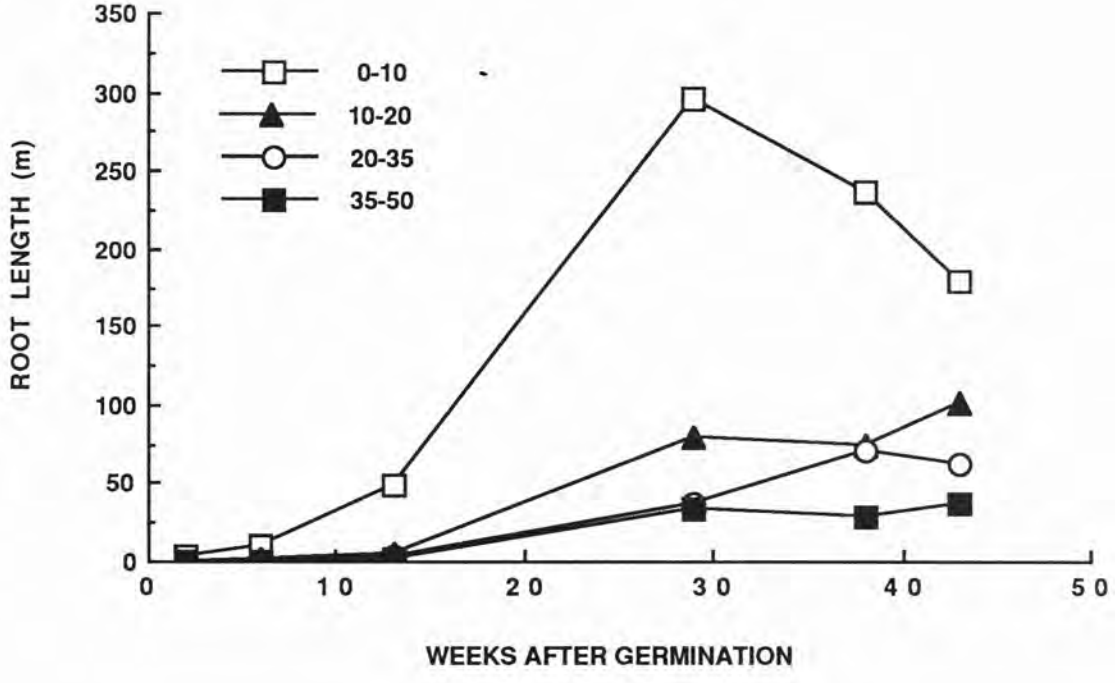

FIGURE 4.4: **Total root length (m box^{-1}) produced by *Acacia pulchella* seedlings grown in a reconstructed soil profile.**

of growth to that of the shallower roots. Roots which grew between 10 and 20cm depth increased in length continously until the last harvest in mid-summer, when one quarter of the total root length was in that layer of the soil. Root length produced between 20 and 35cm depth increased until early summer (38 weeks) and remained at the same level until the end of the experiment. On the other hand, roots grown in the bottom layer seemed to reach their maximum length by late spring (29 weeks), and maintained that amount of root until the last harvest. By the end of the experiment, one sixth of the total root length was in the layer between 20 and 35cm, and one tenth in the bottom layer.

The length of the tap root, and the number and length of lateral roots were recorded at the first harvest when seedlings were two weeks old (Table 4.2). There was no difference between length of tap roots and length of lateral roots in two week old seedlings. Only first order branching was present in two week old seedlings. It was not possible to record either the number of lateral roots or the length of the tap and lateral roots from the second harvest onwards because the roots were too long to be washed free of soil without being broken.

As for total root length, shoot fresh weight increased slowly during the first 13 weeks and increased sharply between then and the 29th week (Fig. 4.5 and Fig. 4.6). However, the relative growth rate (RGR) was constant at about 0.13 g/g/week from seed germination until the 29th week. Fresh shoot weight continued to increase until the end of the experiment throughout the dry season, although at a slower rate than the increment between the 13th and 29th weeks. The increase in fresh root and shoot weight as shown by the root/total fresh weight ratio (Fig.4.7) was similar during the first 6 weeks after seed

TABLF 4.2. Characteristics of roots of 16 individual seedlings of *Acacia pulchella* two weeks old, grown in a reconstructed soil profile.

Plant number	Number of lateral roots	Length of the tap root (mm)	Maximum length of lateral roots (mm)
1	9[b]	24[b]	40
2	9	60	59
3	11[b]	26[b]	62
4	7[b]	15[b]	45
5	7	28	30
6	14	85	42
7	3[b]	18[b]	18[b]
8	4[b]	12[b]	49
9	8	42	48
10	6[b]	25[b]	37
11	4	19	22
12	8	56	44
13	5	42	40
14	9	36	36
15	13	52	53
16	6[b]	27[b]	13[b]

[b]: broken

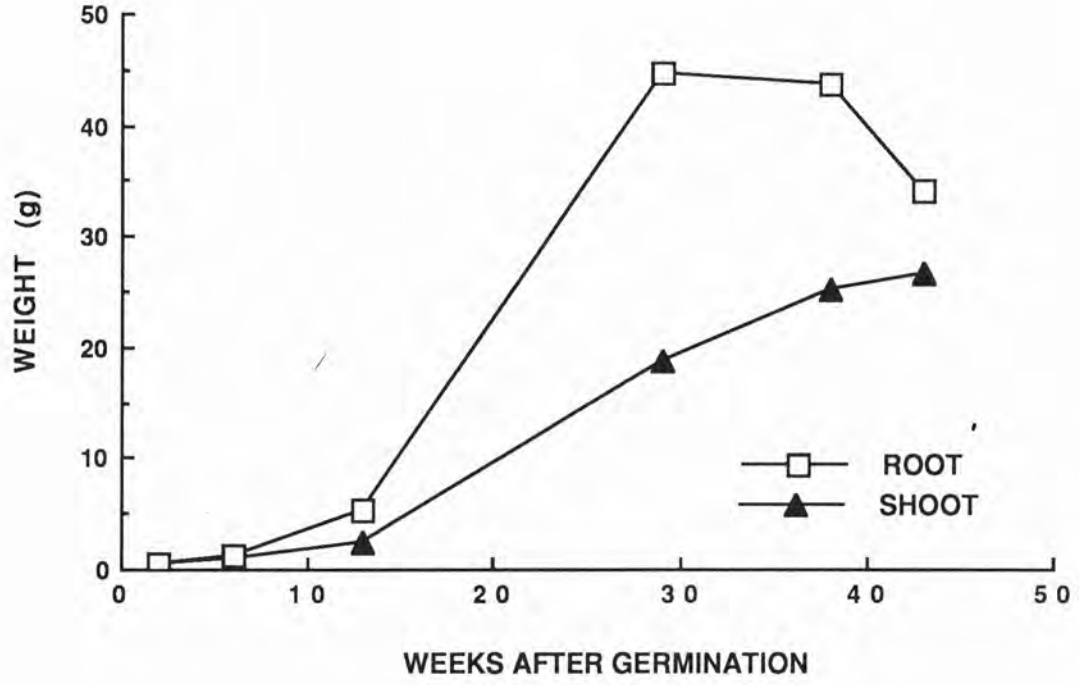

FIGURE 4.5: **Fresh weight of shoots and roots (g box^{-1}) of *Acacia pulchella* seedlings grown in a reconstructed soil profile.**

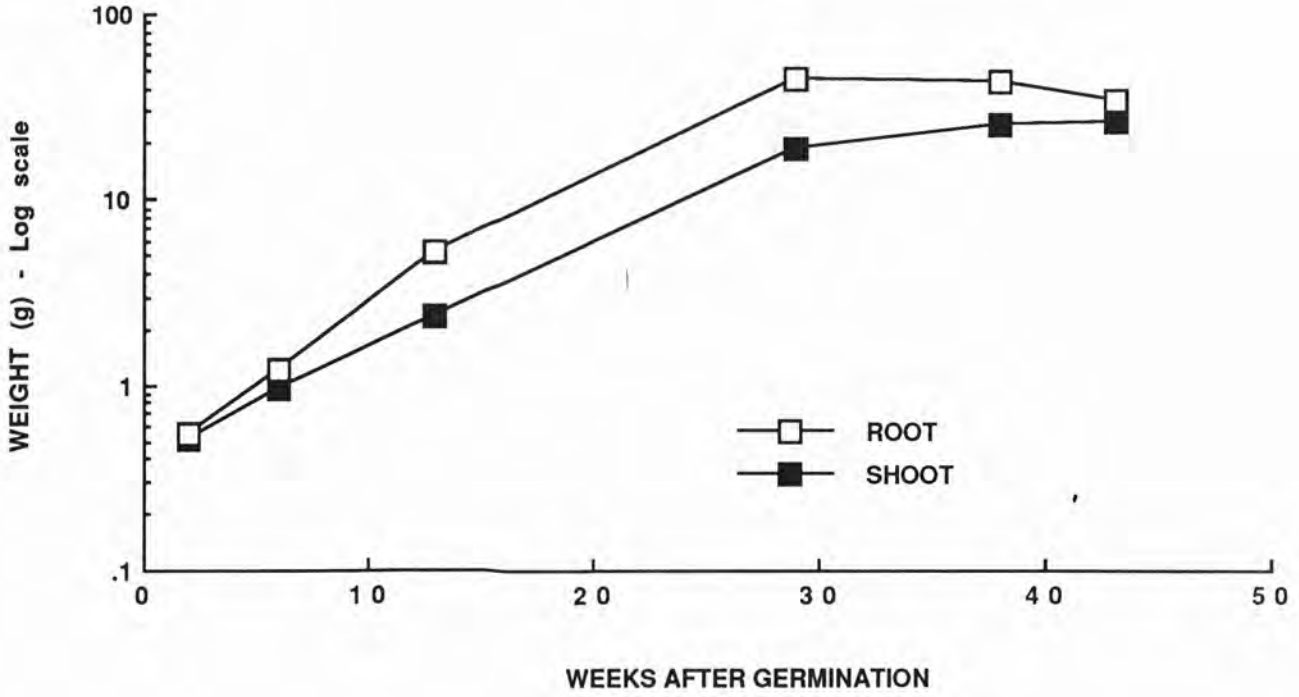

FIGURE 4.6. Fresh weight of shoot and root (g box^{-1}) (Log_{10} scale) of *Acacia pulchella* seedlings grown in a reconstructed soil profile (Experiment 3).

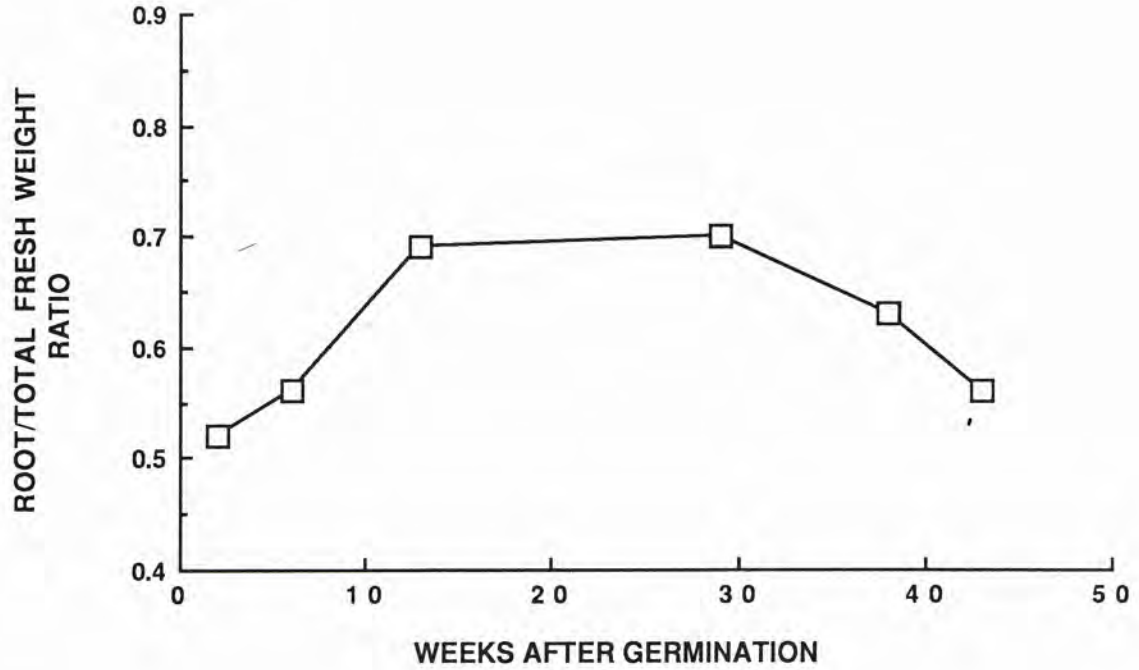

Figure 4.7. **Root/total fresh weight ratio of *Acacia pulchella* seedlings grown in wooden boxes under nursery conditions.**

germination. From then until the 13th week, root weight increased about twice as fast as shoot weight up until the 29th week. Root weight had a RGR of 0.16. At that stage, root length started to decrease due to the dry soil conditions and the root/total fresh weight ratio dropped to about 0.5.

c) Mycorrhizal Colonization

The percentage root length of *A. pulchella* colonized by VA mycorrhizal fungi decreased with increasing soil depth (Fig.4.8). The percentage of root length colonized in roots grown in the top 10cm of the soil increased sharply until the 13th week after germination to a maximum of approximately 70%, then decreased steadily until the last harvest when only approximately one third of the root length was colonized by VA mycorrhizal fungi. In a similar way, in roots which formed in the layer 10 to 20cm deep in the soil, the percentage of root length colonized increased up to the 13th week when it seems to have reached a plateau. Roots present between 20 and 35cm were first observed to contain VA mycorrhizal fungi at the 29th week harvest, although roots had been present in this layer of the soil from the 6th week. Little change in the level of colonization occurred in that layer between the 29th and 38th week with a further increase to approximately 20% at the last harvest. At that stage, both root length and percentage of root length colonized had declined in the top layer; this was probably due to the dry soil conditions. Only about 2% of the roots were colonized in the bottom layer at the end of the experiment.

In the same way as percentage of root length colonized, the length of root colonized was mostly concentrated in the

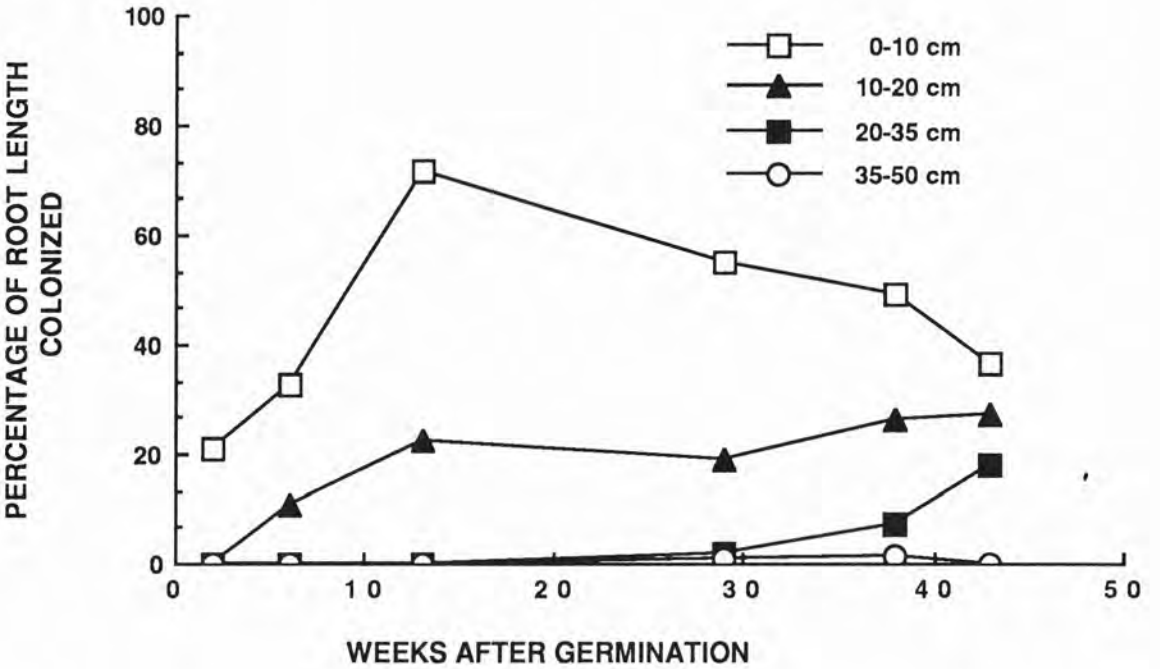

Figure 4.8: Percentage of root length of seedlings of *Acacia pulchella* colonized by VA mycorrhizal fungi at increasing soil depths (Experiment 3).

top 10cm of the soil. The top 10cm had at least 80% of the total root length colonized until the 38th week harvest but this percentage decreased to approximately 60% at the last harvest (Fig. 4.9). Root length colonized in the layer between 10 and 20cm increased gradually until the last harvest when about one quarter of the total root length infected was in that layer of soil. Roots grown between 20 and 35cm started to become colonized by the 4th harvest (29 weeks) but represented less than 1% of the total root length colonized at that stage. However, one sixth of the total root length colonized was in that layer by the end of the experiment. Less than one metre of roots grown in the bottom layer were infected by VA mycorrhizal fungi at the last harvest.

The proportion of the total length of mycorrhizal roots colonized by each of four genera of VA mycorrhizal fungi in seedlings of *A. pulchella* was affected by soil depth and time after seed germination (Fig. 4.10 and Table 4.3). *Glomus* and *Gigaspora* were the main genera present at all times and at all depths, but the diversity of genera increased with increasing age of the plants. *Gigaspora* was predominant during the first 13 weeks of seedling growth, and the only genus present in the top 10cm of the soil two weeks after germination (Fig. 4.10a). The proportion of colonization formed by *Gigaspora* in the top 10cm decreased steadily until the 29th week to less than one fifth and then kept this level until the end of the experiment. *Glomus* first colonized roots in the top 10cm of the soil at the 6 week harvest. *Acaulospora* and fine endophyte were first recorded at the fourth harvest (29 weeks). *Acaulospora* was responsible for very little of the root length colonized in the top layer at the last three harvests but fine endophyte was responsible for about 25% of the total root length colonized in plants 43 weeks old.

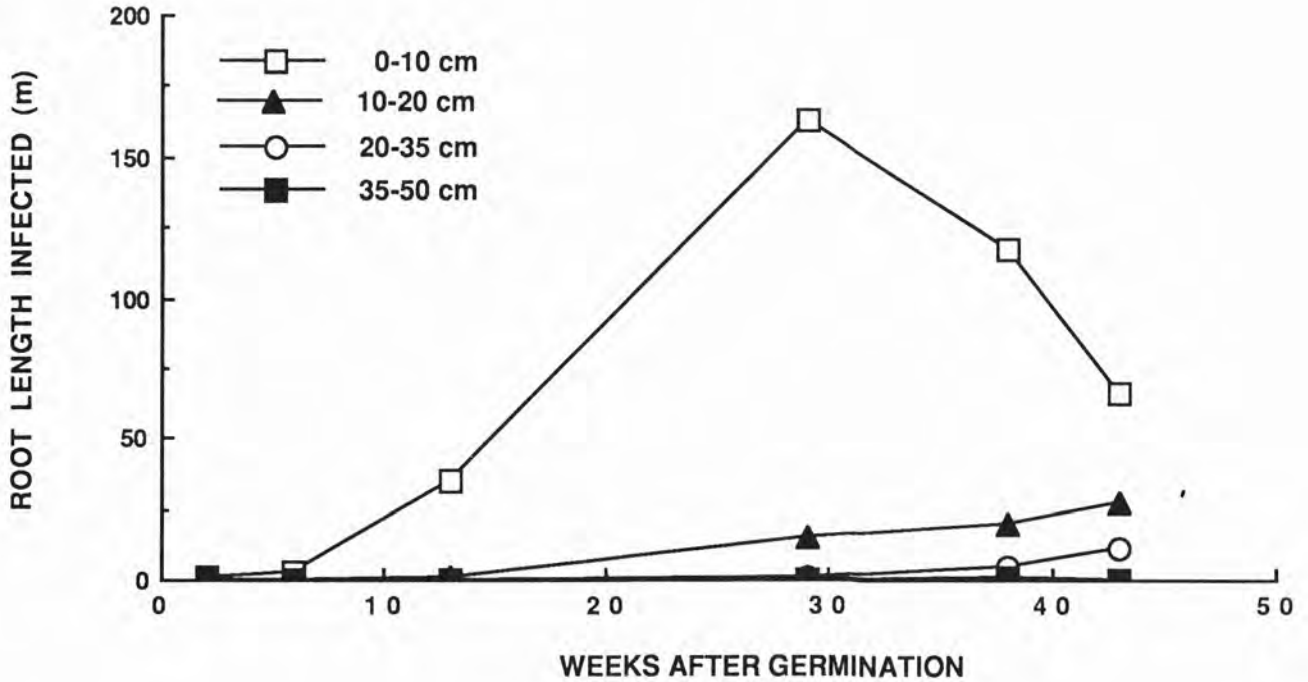

FIGURE 4.9: Length of root (m box^{-1}) of seedlings of *Acacia pulchella* colonized by VA mycorrhizal fungi at different soil depths and times from germination (Experiment 3)

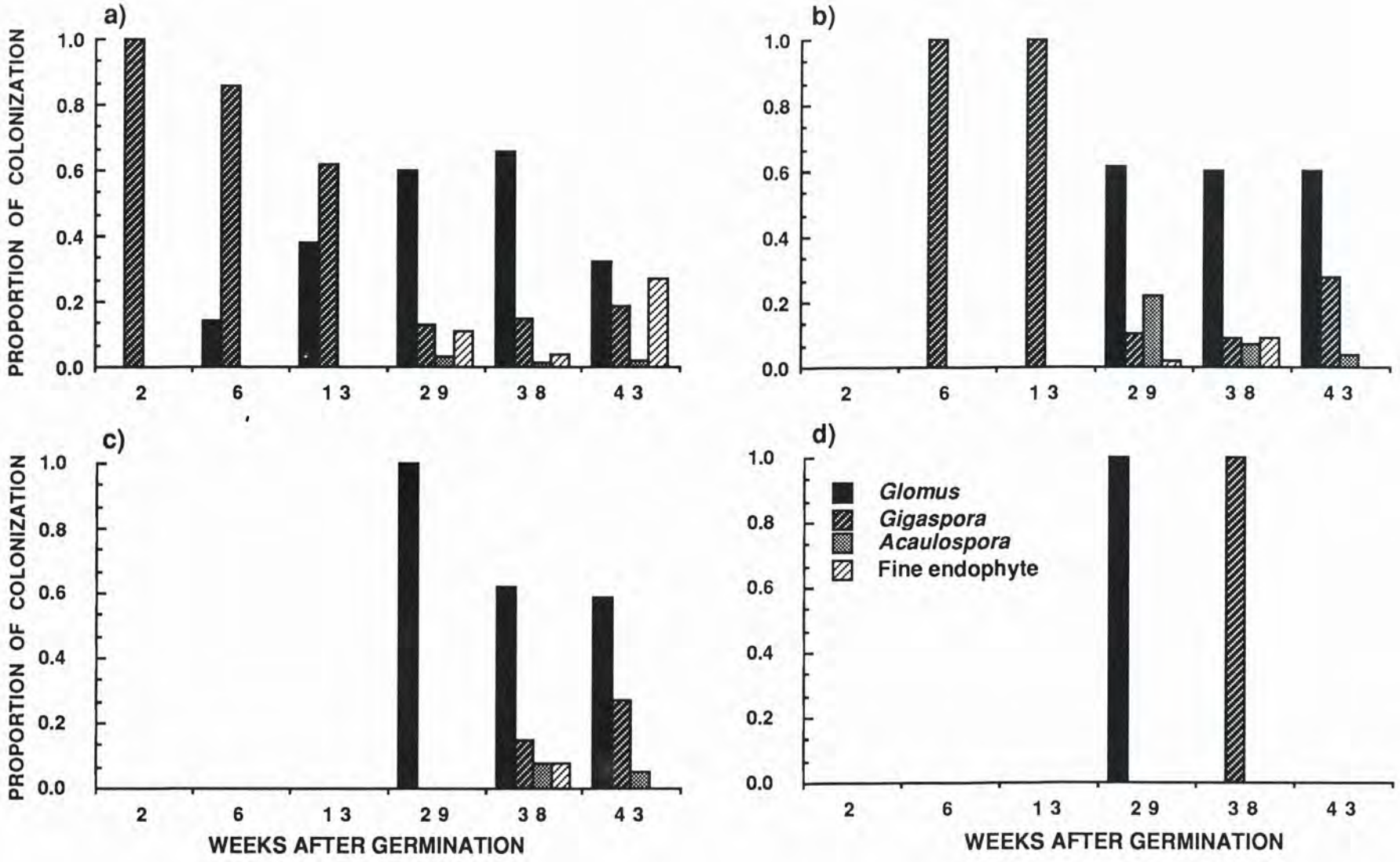
a)
b)
c)
d)
PROPORTION OF COLONIZATION
1.0
0.8
0.6
0.4
0.2
0.0
2
6
13
29
38
43
WEEKS AFTER GERMINATION
Glomus
Gigaspora
Acaulospora
Fine endophyte

Figure 4.10. Proportion of total length of colonized root containing structures formed by particular genera of VA mycorrhizal fungi in roots of seedlings of *Acacia pulchella* as affected by time after seed germination and soil depth (a: 0-10cm; b: 10-20cm; c: 20-35cm, and d: 35-50cm).

Table 4.3. Root length (cm/box) colonized by each of four genera of VA mycorrhizal fungi in seedlings of *Acacia pulchella*. (Experiment 3)

Depth (cm)	Fungi Genera	Time after germination (weeks)					
		2	6	13	29	38	43
0-10	*Glomus*	0	45	1334	9754	7773	2119
	Gigaspora	60	275	2176	2113	1766	1258
	Acaulospora	0	0	0	487	117	132
	Fine endophyte	0	0	0	1788	471	1788
10-20	*Glomus*	0	0	0	903	1182	1650
	Gigaspora	0	20	120	148	177	770
	Acaulospora	0	0	0	325	138	110
	Fine endophyte	0	0	0	29	177	0
20-35	*Glomus*	0	0	0	64	318	653
	Gigaspora	0	0	0	0	77	298
	Acaulospora	0	0	0	0	41	55
	Fine endophyte	0	0	0	0	41	0
35-50	*Glomus*	0	0	0	32	0	0
	Gigaspora	0	0	0	0	48	0
	Acaulospora	0	0	0	0	0	0
	Fine endophyte	0	0	0	0	0	0

Similar to the distribution of VA mycorrhizal genera in the top 10cm, *Gigaspora* was the predominant genus colonizing roots of seedlings up to 13 weeks old in the layer 10 and 20cm deep (Fig. 4.10b). *Glomus* become dominant from the 29th week harvest. *Acaulospora* formed relatively little infection in this layer and was only observed from the fourth harvest onwards. Fine endophyte also formed a minor component of the mycorrhizal root.

Roots present in the layer between 20 and 35cm were first colonized at the 29 week harvest (Fig. 4.10c). *Glomus* was responsible for all the colonization formed at 29 weeks. *Gigaspora* was present in roots of the last two harvests; fine endophyte and *Acaulospora* colonized roots to a limited extent only at these last two harvests. Root density and level of infection were very low in this bottom layer which may have enhanced the chance of variation in species colonizing these roots.

4.4. DISCUSSION

The shoot growth of seedlings of *Acacia pulchella* increased exponentially during the first 29 weeks after germination. A similar pattern was attained by Hansen and Pate (1987), Hingston *et al.*, (1982), and Monk *et al.*,(1981) for field grown plants. Hansen and Pate (1987) found an exponential growth in seedlings of *A. pulchella* from germination in July until January when shoot growth stopped due to the dry summer conditions. However, Hingston *et al.* (1982) observed exponential shoot growth until late summer. Bell and Stephens (1984) considered that water availability was the only parameter controlling shoot growth of *A. pulchella* in mature field plants, although

temperature was the main factor controlling floral bud development. The time of the decrease in the rate of shoot growth in my experiment coincided with the decrease in soil moisture content due to the summer season. *A. pulchella* was classified as a spring-summer grower species by Bell and Stephens (1984). In their study, *A. pulchella* started shoot growth in spring, even if levels of moisture in the soil were capable of supporting growth long before that, and maintained growth well into summer as long as roots could reach water either in the surface zone or at depth. Their assessment was based on pattern of shoot growth, and it is likely that during the period between the onset of rainfall and the start of shoot growth the plants were expanding their root system. New root growth in *E. marginata* occurred mainly in spring and in autumn (Shea and Dell,1981). It is interesting to note that even if root growth started well before shoot growth it did not decrease with the start of the period of shoot growth.

Similarly to shoot growth, root growth also increased exponentially during the first 29 weeks after germination but decreased as soon as soil started to dry off. Very little rainfall was recorded between December and February, and maximum air temperature averaged around 30°C. Root growth of *E. marginata* (Shea and Dell,1981) and nodule activity in field-grown seedlings of *A. pulchella* (Hansen and Pate,1987) also decreased during summer. In both cases this was attributed to a decrease in water availability during the dry summer. However, a decrease in root growth in my experiment occurred only at the top layer (10cm) and roots deeper in the soil profile continued to grow during summer. The higher concentration of roots in the top 15cm of the soil is a common feature of plants growing in this region (Lamont,1982a). Most of the nutrients and organic matter are in this layer of the soil and the concentration of roots in that layer

contribute to a maximization of nutrient cycling in the forest floor. However, continued root growth at depth may be responsible for maintaining shoot growth when roots in the surface zone are dry.

Distribution of VA mycorrhizas in the profile followed a similar pattern to root growth. The higher concentration of VA mycorrhizas in the top layer was probably due to (1) higher concentration of nutrients and organic matter, (2) greater root density, and (3) greater propagule density. Organic matter may control the distribution of fine roots that can become mycorrhizal (Mosse *et al.*,1981). Root density and root architecture are some of the more important factors controlling VA mycorrhizal spread inside the root (Miller,1987a). The colonization of roots of *Trifolium repens*, *Festuca ovina* (Warner and Mosse,1982) and *Trifolium subterraneum* (Abbott and Robson,1984b) increased with increasing root density.

Despite the abundance of VA mycorrhizas being lower with increasing depth, colonization of roots increased gradually with time. This may be due to a combination of low root and inoculum density at deeper layers in the early stages of plant growth. Increased colonization by VA mycorrhizal fungi has been associated with increased inoculum density (Hetrick,1984) and its interaction with host, soil, and climatic factors (Miller,1987a). Maybe of greater relevance, is that the twofold increase in percentage of colonization and root length colonized at depth occurred when levels of colonization in the top layer were decreasing. The maintenance of fungal activity between 10 and 20cm and its increase between 20 and 35cm during the summer season may be of great ecological importance for the plant. Roots grown deeper in the soil are considered important in supplying water for the plants during a period when the temperatures are very high, and there is no decrease in shoot production which means that the plant continues to

have a high rate of transpiration. Improved water relations in mycorrhizal plants are considered to be an indirect effect of the fungi due to a better nutritional status of the plants and it is unlikely that VA mycorrhizal fungi are directly involved in uptake of water (Nelsen, 1987). If this is the case, we can hypothesize that the mycorrhizal fungi are taking up nutrients and supplying them to the plant when the nutrients in the surface layers are unavailable due to the dry conditions. Levels of nutrients in those deeper layers are considerably less than in the top layer but even small inputs of nutrients during summer may be crucial for meeting the needs of the plants.

The relative abundance of VA mycorrhizal genera within roots was greatly affected by age of the plant. The initial dominance of *Gigaspora* at the early stages of plant development is surprising because *Glomus* has been reported as a common genus colonizing roots in jarrah forests (Jasper *et al.*,1987). It may be that each genus had a different form of propagule at the time that the soil was collected from the field and that progagules of *Glomus* were either more affected by disturbance of the soil or slower to germinate than those of *Gigaspora*. The soil was collected in mid-April, just after the break of the rainfall season. It is possible that propagules of *Glomus* present in the field germinated earlier than those of *Gigaspora* and therefore were more susceptible to soil disturbance at the time of soil collection. The major type of propagule of these fungi in jarrah forests at the end of the summer is not known. The number of spores recovered from jarrah forest is generally low and may not account for the amount of colonization formed. Hyphae in the soil and within the roots are likely to be a major type of propagule in this environment (Jasper *et al.*,1989c). Hyphae of *A. laevis* can survive for up to 36 days in soil, in glasshouse conditions, at a matric potential of -21MPa (Jasper *et*

al.,1989b); other VA mycorrhizal species can survive in dead pieces of roots (Tommerup and Abbott,1981), or perennate inside long-lived roots (Brundrett and Kendrick,1989). However, it remains to be confirmed, in field conditions, the extent to which hyphae either in the soil, and the inside the roots can survive, and the mechanisms used by the fungi to survive the long, dry and hot summer in the mediterranean environment of south-western Australia.

Gigaspora produced almost five times more external hyphae than *Glomus* when growing in association with subterranean clover in glasshouse conditions (Abbott and Robson,1985). On the other hand, *Glomus* was more efficient in colonizing roots than *Gigaspora* because *Gigaspora* relies on new points of colonization to spread while *Glomus* spreads more extensively within the root (Wilson,1984). It may be that the higher hyphal production by *Gigaspora* favoured this genus at the early stages of seedling development but once established, *Glomus* was more efficient than *Gigaspora* in colonizing the new roots.

CHAPTER 5

CONCLUDING REMARKS

5. CONCLUDING REMARKS

The seasonal variation in infectivity of VA mycorrhizal fungi from natural jarrah (*Eucalyptus marginata* Donn. ex Sm.) forests and restored areas after bauxite mining, and the development of seedlings of *Acacia pulchella* R.Br. were studied in a series of three experiments using glasshouse and nursery conditions.

The infectivity of VA mycorrhizal fungi was always much higher in undisturbed soil cores from natural jarrah forest than in cores from restored areas after bauxite mining. The infectivity of VA mycorrhizal fungi, measured as a percentage of the root length colonized or the length of colonized roots, decreased during summer for cores from natural jarrah forest but there were no seasonal changes for cores from restored areas. The decrease in infectivity for cores from natural jarrah forest in summer coincided with the time when water availability had decreased and fine roots would have died in the surface soil layers. There was no difference in infectivity for cores from jarrah forest when water was not a limiting factor. The lack of seasonal changes in cores from restored areas is probably due to the low levels of inoculum present in these areas. These results are in agreement with my hypothesis that there is a difference in seasonal variation in infectivity of VA mycorrhizal fungi if plants are grown in cores from natural or restored areas.

There was no seasonal trend in predominance of particular genera of VA mycorrhizal fungi infecting roots of seedlings grown in undisturbed soil cores either from natural jarrah forest or from restored areas after bauxite mining. However, there was a greater diversity of genera in soil from restored areas than from natural jarrah forest. *Glomus* was by far the dominant genus

colonizing roots of plants grown in soil cores from natural jarrah forest with very little colonization formed by the other genera of VA mycorrhizal fungi. On the other hand, there was a more even distribution of genera of VA mycorrhizal fungi in soil cores from restored areas. *Glomus*, *Gigaspora*, and fine endophyte occurred in similar proportion throughout the year in soils from restored areas. *Acaulospora* was present in low proportions in soil cores from both natural jarrah forest and restored areas.

There was a different trend in seasonal variation in infectivity of VA mycorrhizal fungi when the soil was collected as disturbed cores. In this situation, infectivity was higher in soil collected in December, and least in soil collected in June. However, the levels of colonization of roots of plants grown in disturbed cores in December were very similar to the levels in plants grown in undisturbed cores in January. This indicates that the VA mycorrhizal propagules present in summer were more tolerant of soil disturbance than propagules growing in winter. Hyphae in the soil are likely to be the major type of propagule during winter and these are easily damaged by disturbance. The contrasting results from experiments using disturbed and undisturbed soil cores collected in winter highlight the necessity of specifying the soil conditions in studies of seasonal variation in infectivity of VA mycorrhizal fungi.

The infectivity of VA mycorrhizal fungi was higher in soil from the top 8cm than in soil from 9-20cm deep, collected in June. However, there was no difference in infectivity of VA mycorrhizal fungi in soil collected in the top 20cm, in December. There was a decrease in infectivity of VA mycorrhizal fungi with phosphorus application for 0-3 and 4-8 cm depths in the June sampling.

The distribution of roots of seedlings of *Acacia pulchella* grown in a reconstructed profile followed the distribution of nutrients in the soil. At least half of the total root length was in the top 10cm of the soil at any time. Root length in the top 10cm of the soil, increased exponentially from germination but decreased when levels of moisture decreased during summer. However, the length of roots formed deeper in the profile remained at the same level throughout the summer. VA mycorrhizal colonization was also concentrated mainly in the top 10cm. The spread of mycorrhizal fungi at depth was slow but continued to increase even when moisture content of the soil decreased during summer.

Gigaspora was the main genus of VA mycorrhizal fungi colonizing roots during the first 13 weeks of seedling growth. *Glomus* was slow to become established but it was responsible for most of the colonization from the 29th week after seedling germination.

My results suggest that it is important to identify the form of propagules present in forest ecosystems at different times of the year and to identify the mechanisms used by the fungi to survive the long, dry and hot summer of south-westen Australia. The identification of the different types of propagules occurring in different seasons could allow the definition of strategies for collecting soil for assessment of infectivity of VA mycorrhizal fungi in natural conditions. For example, there was no difference in infectivity of VA mycorrhizal fungi in my experiment, when soil was collected in summer either as disturbed or undisturbed cores. However, there were marked changes in the results obtained for plants grown in soil collected as disturbed or undisturbed cores during winter. This is likely to reflect differences in the type of propagules with season. Propagules occurring in summer seem not to be affected by soil

disturbance. Therefore, the assessment of infectivity in summer could probably be assessed using both disturbed or undisturbed soil with similar results. However, the assessment of infectivity in winter should consider the minimization of soil disturbance to avoid damage to potential fungal propagules.

Nevertheless, the simultaneous growth of plants in glasshouse and field conditions may give a measurement of the accuracy of using plants grown in glasshouse to predict mycorrhizal formation in field-grown plants. Although the establishment of seedlings in field during summer may be a problem, because of the dry conditions, the data from the wet months may give an indication of the accuracy of the prediction of VA mycorrhizal colonization in the field using plants grown in glasshouse conditions.

REFERENCES

Abbott,L.K. 1982. Comparative anatomy of vesicular-arbuscular mycorrhizas formed on subterranean clover. *Aust. J. Bot.* **30,** 385-399.

Abbott,L.K. and Robson,A.D. 1977. The distribution and abundance of vesicular-arbuscular endophytes in some Western Australian soils. *Aust. J. Bot.* **25**, 515-522.

Abbott,L.K. and Robson,A.D. 1981. Infectivity and effectiveness of five endomycorrhizal fungi: Competition with indigenous fungi in field soils. *Aust. J. Agric. Res,* **32**, 621-630.

Abbott,L.K. and Robson,A.D. 1984a. The effect of mycorrhizae on plant growth. In: *VA Mycorrhiza*. (Ed.by C.Ll.Powell and D.J.Bagyaraj). CRC Press. Boca Raton. pp. 113-130.

Abbott,L.K. and Robson,A.D. 1984b. The effect of root density, inoculum placement and infectivity of inoculum on the development of Vesicular-Arbuscular mycorrhizas. *New Phytol.* **97**, 285-299.

Abbott,L.K. and Robson,A.D. 1985. Formation of external hyphae in soil by four species of vesicular-arbuscular mycorrhizal fungi. *New Phytol.* **99**, 245-255.

Abbott,L.K. and Robson,A.D. 1989. Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. *Agric., Ecos. and Environ.*. In press

Adams,M.A. and Attiwill,P.M. 1984a. Role of *Acacia* spp. in nutrient balance and cycling in regenerating *Eucalyptus regnans* F.Muell. forests.I. Temporal changes in biomass and nutrient content. *Aust. J. Bot.* **32**, 205-15.

Adams,M.A. and Attiwill,P.M. 1984b. Role of *Acacia* spp. in nutrient balance and cycling in regenerating *Eucalyptus regnans* F.Muell forests.II.Field studies on acetylene reduction. *Aust. J. Bot.* **32**, 217-223.

Allen,M.F. 1988. Belowground structure: A key to reconstructing a productive arid ecosystem. In: *Reconstruction of Disturbed Arid*

Ecosystems. (Ed.by E.B.Allen). Westview Press. Boulder, Colorado. pp. 113-135.

Ashton,D.H. 1975. The root and shoot development of *Eucalyptus regnans* F.Muell.. *Aust. J. Bot.* **23**, 867-887.

Barea,J.M. and Azcón-Aguilar,C. 1983. Mycorrhizas and their significance in nodulating nitrogen-fixing plants. *Adv. Agron.* **36**, 1-54.

Barley,K.P. 1970. The configuration of the root system in relation to nutrient uptake. *Adv. Agron.* **22**, 159-201.

Barrow,N.J. 1977. Phosphorus uptake and utilization by tree seedlings. *Aust. J. Bot.* **25**, 571-584.

Baylis,J.T.S. 1969. Host treatment and spore production by *Endogone*. *N. Z. J. Bot.* **7**, 173-174.

Baylis,J.T.S. 1975. The magnolioid mycorrhiza and mycotrophy in root systems derived from it. In: *Endomycorrhizas*. (Ed.by F.E.Sanders, B.Mosse, and P.B.Tinker). Academic Press Inc.. London. pp 373-89.

Beard,J.S. 1983. Ecological control of the vegetation of Southwestern Australia: Moisture versus Nutrients. In: *Mediterranean-Type Ecosystems: The role of nutrients*. (Ed.by F.J.Kruger, D.T.Mitchell, and J.U.M.Jarvis). Springer-Verlag. Berlin, 66-73.

Bell,D.T. and Stephens,L.J. 1984. Seasonality and phenology of Kwongan species. In: *Kwongan - Plant Life of the Sandplain*. (Ed.by J.S.Pate and J.S.Beard). University of Western Australia Press. Nedlands. pp.205-222.

Birch,C.P.D. 1986. Development of VA mycorrhizal infection in seedlings in semi-natural grassland turf. In: *Physiological and Genetics Aspects of Mycorrhizae*. (Ed.by V.Gianinazzi-Pearson and S.Gianinazzi). INRA. Paris. pp.233-237.

Bolan,N.S. and Abbott,L.K. 1983. Seasonal variation in infectivity of vesicular-arbuscular mycorrhizal fungi in relation to plant response to applied phosphorus. *Aust. J. Soil Res.* **21**, 207-210.

Bowen,G.D. 1981. Coping with low nutrients. In: *The biology of Australian native plants.* (Ed.by J.S.Pate and A.J.McComb). Univ. of Western Australia Press. Perth, pp. 33-64.

Bowen,G.D. 1987. The biology and physiology of infection and its development. In: *Ecophysiology of VA mycorrhizal plants.* (Ed.by G.R.Safir). CRC Press. Boca Raton. pp 27-57.

Brundrett,M.C. and Kendrick,B. 1988. The mycorrhizal status, root anatomy, and phenology of plants in a sugar maple forest. *Can. J. Bot.* **66**, 1153-1173.

Brundrett,M.C. and Kendrick,B. 1989. The roots and mycorrhizae of herbaceous woodlands plants. I. Quantitative aspects of morphology. *New Phytol.* (in press).

Cannon,W.A. 1949. A tentative classification of root systems. *Ecology* **30**, 542-548.

Carbon,B.A.; Bartle,G.A; Murray,A.M. and MacPhearson,D.K. 1980. The distribution of root length, and the limits to flow of soil water to roots in a dry sclerophyll forest. *For. Sci.* **26**, 656-64.

Clarke,C.A. 1978. Requirements for germination and growth of VA mycorrhizal spore. *Rothamsted Exp. Station Rep.*, pp. 233-234.

Colwell,J.D. 1963. The estimation of phosphorus fertilizer requirements of wheat in southern New South Wales by soil analysis. *Aust. J. Exp. Agric. An. Husb.* **3**, 190-197.

Cooper,K.M. 1984. Physiology of VA mycorrhizal association. In: *VA Mycorrhiza.* (Ed.by C.Ll.Powell and D.J. Bagyaraj). CRC Press. Boca-Raton. pp.155-186.

Cornet,F. and Otto,C. 1985. Nitrogen fixation by *Acacia holosericea* grown in field-simulating conditions. *Acta Oecologica - Ecol. Plant.* **6**, 211-218.

Daniels,B.A. and Trappe,J.M. 1980. Factors affecting spore germination of the vesicular-arbuscular mycorrhizal fungus, *Glomus epigaeus. Mycologia* **72**, 457-71.

Dell,B. and Wallace,J.M. 1983. Periodicity of fine root growth in jarrah (*Eucalyptus marginata* Donn. ex Sm.). *Aust. J. Bot.* **31**, 247-254.

Dodd,J.; Heddle,G.M.; Pate,J.S. and Dixon,K.W. 1984. Rooting patterns of sandplain plants and their functional significance. In: *Kwongan- Plant Life of the Sandplain*. (Ed.by J.S.Pate and J.S.Beard). University of Western Australia Press. Nedlands. pp 146-77.

Francis,R. and Read,D.J. 1984. Direct transfer of carbon between plants connected by vesicular-arbuscular mycelium. *Nature* **30**, 420-422.

Furlan,V. and Fortin,J.A. 1973. Formation of endomycorrhizae by *Endogone calospora* on *Allium cepa* under three different temperature regimes. *Naturaliste Canad.* **100**, 467-477.

Gardner,J.H. and Malajczuk,N. 1988. Recolonization of rehabilitated bauxite mine sites in Western Australia by mycorrhizal fungi. *Forest Ecology and Management* **24**, 27-42.

Gemma,J.N. and Koske,R.E. 1988. Seasonal variation in spore abundance and dormancy of *Gigaspora gigantea* and in mycorrhizal inoculum potential of a dune soil. *Mycologia* **80**, 211-216.

Gemma,J.N.; Koske,R.E. and Carreiro,M. 1989. Seasonal dynamics of selected species of V-A mycorrhizal fungi in a sand dune. *Mycol. Res.* **92**, 317-321.

Giovannetti,M. and Mosse,B. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.* **84**, 489-500.

Giovannetti,M. 1985. Seasonal variations of vesicular-arbuscular mycorrhizas and endogonaceous spores in a maritime sand dune. *Trans. Br. Mycol. Soc.* **84**, 679-684.

Gould,A.B. and Liberta,A.E. 1981. Effects of topsoil storage during surface mining on the viability of vesicular-arbuscular mycorrhizas. *Mycologia* **73**, 914-922.

Graham,J.H.; Linderman,R.G. and Menge,J.A. 1982. Development of external hyphae by different isolates of mycorrhizal *Glomus spp.* in relation to root colonization and growth of Troyer citrange. *New Phytol.* **91**, 183-189.

Graham,J.H.; Syvertsen,J.P. and Smith Jr.,M.L. 1987. Water relations of mycorrhizal and phosphorus fertilized non-mycorrhizal *Citrus* under drought stress. *New Phytol.* **105**, 411-420.

Grove,T.S. 1987. Nutrient uptake and growth of overstorey trees and understorey shrubs in developing stands of karri (*Eucalyptus diversicolor* F.Muell.) forest. Ph.D. Thesis. Univ. of Western Australia. 363pp.

Hansen,A.P. and Pate,J.S. 1987. Comparative growth and symbiotic performance of seedlings of *Acacia* spp in defined pot culture or as natural understorey components of a eucalypt forest ecosystem in S.W. Australia. *J. Exp. Bot.* **38**, 13-25.

Hansen,A.P.; Pate,J.S.: Hansen,A. and Bell,D.T. 1987. Nitrogen economy of post-fire stands of shrub legumes in Jarrah (*Eucalyptus marginata* Donn.ex Sm.) forest of S.W. Australia. *J. Exp. Bot.* **38**, 26-41.

Harley,J.L. and Smith,S.E. 1983. *Mycorrhizal symbiosis.* Academic Press Inc..London. 483pp.

Harris,J.A.; Hunter,D.; Birch,P. and Short,K.C. 1987. Vesicular-arbuscular mycorrhizal populations in stored topsoil. *Trans. Br. Mycol. Soc.* **89**, 600-603.

Hayman,D.S. 1970. *Endogone* spore number in soil and vesicular-arbuscular mycorrhiza in wheat as influenced by season and soil treatment. *Trans. Br. Mycol. Soc.* **54**, 53-63.

Hayman,D.S. and Stovold,G.E. 1979. Spore populations and infectivity of vesicular-arbuscular mycorrhizal fungi in New South Wales. *Aust. J. Bot.* **27**, 227-233.

Hetrick,B.A.D. 1984. Ecology of VA mycorrhizal fungi. In: *VA Mycorrhiza*. (Ed. by C.Ll.Powell and D.J.Bagyaraj). CRC Press. Boca-Raton. pp.35-55.

Hingston,F.J.; Malajczuk,N. and Grove,T.S. 1982. Acetylene reduction (N_2-fixation) by Jarrah forest legumes following fire and phosphate application. *J. Appl. Ecol.* **19**, 631-645.

Hopper,S.D. and Maslin,B.R. 1978. Phytogeography of *Acacia* in Western Australia. *Aust. J. Bot.* **26**, 63-78.

Huang,R.; Smith,W.K. and Yost,R.S. 1985. Influence of vesicular-arbuscular mycorrhiza on growth, water relations, and leaf orientation in *Leucaena leucocephala* (Lam.)De Wit.. *New Phytol.* **99**, 229-243.

Jakobsen,I. and Nielsen,N.E. 1983. Vesicular-arbuscular mycorrhiza in field-grown crops. I. Mycorrhizal infection in cereals and peas at various times and soil depths. *New Phytol.* **93**, 401-413.

Jasper,D.A.; Abbott,L.K. and Robson,A.D. 1989a. Soil disturbance reduces the infectivity of external hyphae of vesicular-arbuscular mycorrhizal fungi. *New Phytol.* **112**, 93-99.

Jasper,D.A.; Abbott,L.K. and Robson,A.D. 1989b. Hyphae of vesicular-arbuscular mycorrhizal fungus maintain infectivity in dry soil, except when the soil is disturbed. *New Phytol.* **112**, 101-107.

Jasper,D.A.; Abbott,L.K. and Robson,A.D. 1989c. The loss of VA mycorrhizal infectivity during bauxite mining may limit the growth of *Acacia pulchella* R.Br.. *Aust. J. Bot.* **37**, 33-42.

Jasper,D.A.; Robson,A.D. and Abbott,L.K. 1979. Phosphorus and the formation of vesicular-arbuscular mycorrhizas. *Soil Biol. Biochem.* **11**, 501-505.

Jasper,D.A.; Robson,A.D. and Abbott,L.K. 1987. The effect of surface mining on the infectivity of vesicular-arbuscular mycorrhizal fungi. *Aust. J. Bot.* **35**, 641-652.

Jasper,D.A.; Robson,A.D. and Abbott,L.K. 1988. Revegetation in an iron-ore mine - nutrient requirements for plant growth and the potential role of vesicular-arbuscular (VA) mycorrhizal fungi. *Aust. J. Soil Res.* **26**, 497-507.

Kobayashi,N. 1988. Factors affecting the germination of spores of *Gigaspora margarita. Soil Microorganisms* **31**, 13-28.

Koide,R.T. and Mooney,H.A. 1987. Spatial variation in inoculum potential of vesicular-arbuscular mycorrhizal fungi caused by formation of gopher mounds. *New Phytol.* **107**, 173-182.

Koske,R.E. 1981. *Giagaspora gigantea:* Observations on spore germination of a VA mycorrhizal fungus. *Mycologia* **73**, 288-300.

Koske,R.E. 1982. Evidence for a volatile attractant from plant root affecting germ tubes of a VA mycorrhizal fungus. *Trans. Br. Mycol. Soc.* **79**, 305

Lamont,B. 1981. Specialized roots of non-symbiotic origin in heathlands. *In* : Heathlands and related shrublands of the world; B. Analytical studies. Ed. R.L. Specht, Elsevier Scientific Publishing Company, Amsterdam.

Lamont,B. 1982a. Mechanisms for enhancing nutrient uptake in plants, with particular reference to mediterranean South Africa and Western Australia. *Bot. Rev.* **48**, 597-689.

Lamont,B. 1982b. Specialised roots in the Genus *Acacia:* A Review. *An. Rep. Mulga Res. Centre* **5**, 9-11.

Lamont,B. 1983. Strategies for maximizing nutrient uptake in two Mediterranean ecosystems of low nutrient status. In: *Mediterranean-Type Ecosystems: The role of nutrients.* (Ed. by F.J.Kruger, D.T.Mitchell and J.U.M.Jarvis). Springer-Verlag. Berlin. 246-273.

Lamont,B. 1984. Specialized modes of nutrition. In: *Kwongan- Plant Life in the Sandplain.* (Ed.by J.S.Pate and J.S.Beard). University of Western Australia Press. pp.126-145.

Lambert,D.H. and Cole,Jr. H. 1980. Effects of mycorrhizae on establishment and performance of forage species in mine spoil. *Agron. J.* **72**, 257-260.

Langkamp,P.J. and Dalling,M.J. 1982. Nutrient cycling in a stand of *Acacia holosericea* A.Cunn. ex G.Don..II. Phosphorus and endomycorrhizal associations. *Aust. J. Bot.* **30**, 107-119.

Langkamp,P.J.; Swinden,L.B. and Dalling,M.J. 1979. Nitrogen fixation (Acetylene reduction) by *Acacia pellita* on areas restored after mining at Groote Eylandt, Northern Territory. *Aust. J. Bot.* **27**, 535-561.

Lawrie,A.C. 1981. Nitrogen fixation by native Australian legumes. *Aust. J. Bot.* **29**, 143-157.

Loree,M.A.J. and Williams,S.E. 1987. Colonization of western wheatgrass (*Agropyron smithii* Rydb.) by vesicular-arbuscular mycorrhizal fungi during the revegetation of a surface mining. *New Phytol.* **106**, 735-744.

Malajczuk,N.; Linderman,R.G.; Kough,J. and Trappe,J.M. 1981. Presence of vesicular-arbuscular mycorrhizae in *Eucalyptus spp.* and *Acacia* sp., and their absence in *Banksia* sp. after inoculation with *Glomus fasciculatus. New Phytol.* **87**, 567-572.

Malajczuk,N.; Molina,R. and Trappe,J.M. 1982. Ectomycorrhiza formation in *Eucalyptus*. I. Pure culture synthesis, host specificity and mycorrhizal compatibility with *Pinus radiata. New Phytol.* **91**, 467-482.

McGee,P.A. 1987. Alteration of growth of *Solanum opacum* and *Plantago drummondii* and inhibition of regrowth of hyphae of vesicular-arbuscular mycorrhizal fungi from dried roots by manganese. *Plant and Soil* **101**, 227-233.

McGee,P.A. 1989. Variation in propagule numbers of vesicular-arbuscular mycorrhizal fungi in a semi-arid soil. *Mycological Research* **92**, 28-33.

Menge,J.A. 1984. Inoculum production. In: *VA Mycorrhiza.* (Ed.by C.Ll. Powell and D.J. Bagyaraj). CRC Press. Boca Raton. pp 187-203.

Menge,J.A.; Steirle,D.; Bagyaraj,D.J.; Johnson,G.L.V. and Leonard,R.T. 1978. Phosphorus concentration in plants responsible for inhibition of mycorrhizal infection. *New Phytol.* **80**, 575-578.

Miller,R.M. 1987a. The ecology of vesicular-arbuscular mycorrhizae in grass- and shrubland. In: *Ecophysiology of VA mycorrhizal plants.* (Ed.by G.R.Safir). CRC Press. Boca Raton. pp.135-170.

Miller,R.M. 1987b. The management of VA mycorrhizae in semi-arid environments. In: *Mycorrhizae in the Next Decade - Practical Applications and Research Priorities*, 7ºNACOM. (Ed.by D.M.Sylvia, L.L.Hung and J.H.Graham). IFAS, Gainesville. pp.139-141.

Moorman,T. and Reeves,F.B. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid west. II. A bioassay to determine the effect of land disturbance on endomycorrhizal populations. *Amer. J. Bot.* **66**, 14-18.

Monk,D.; Pate,J.S. and Loneragan,W.A. 1981. Biology of *Acacia pulchella* R.Br. with special reference to symbiotic nitrogen fixation. *Aust. J. Bot.* **29**, 579-592.

Mosse,B. and Bowen,G.D. 1968. The distribution of *Endogone* spores in some Australian and New Zealand soils and in an experimental field soil at Rothamsted. *Trans. Br. Mycol. Soc.* **51**, 485-492.

Mosse,B.; Stribley,D.P. and Le Tacon,F. 1981. Ecology of mycorrhiza and mycorrhizal fungi. In: *Advances in Microbial Ecology*, vol.5. (Ed. by M. Alexander). Plenum Press. New York. pp. 137-210.

Nadarajah,P. and Nawawi,A. 1987. Effect of temperature on germination and growth of vesicular-arbuscular mycorrhizal fungi. In: *Mycorrhizae in the Next Decade: Practical Applications*

and Research Priorities. 7ºNACOM.(Ed.by D.M.Sylvia, L.L.Hung and J.H.Graham). IFAS, University of Florida. Gainesville. p.214.

Nambiar,S.E.K. 1981. Ecological and physiological aspects of the development of roots: From nursery to forest. In: *Australian Forest Nutrition Workshop: Productivity in Perpetuity.* CSIRO. Canberra. pp 117-29.

Nelsen,C.E. 1987. Water relations of the vesicular-arbuscular mycorrhizal system. In: *Ecophysiology of VA mycorrhizal plants.* (Ed.by G.R.Safir). CRC Press. Boca-Raton. pp.71-91.

New,T.R. 1984. *A biology of acacias.* Oxford University Press. Melbourne. 153pp.

Newman,E.I. 1966. A method of estimating the total length of root in a sample. *J. Appl. Ecol.* **3**, 139-145.

Nichols,O.G.; Carbon,B.A.; Colquhoun,I.J. and Murray,N.J. 1985. Rehabilitation after bauxite mining in south-western Australia. *Landscape Planning* **12**, 75-92.

Nye,P.H. and Tinker,P.B. 1977. *Solute movement in the soil-root system.* Blackwell Scientific Publications. Oxford. 342pp.

Phillips,J.M. and Hayman,D.S. 1970. Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **55**, 158-160.

Porter,W.M. 1979. The "Most Probable Number" method for enumerating infective propagules of vesicular arbuscular mycorrhizal fungi in soil. *Aust. J. Soil Res.* **17**, 515-519.

Powell,C.Ll. 1976. Development of mycorrhizal infections from *Endogone* spores and infected root segments. *Trans. Br. Mycol. Soc.* **60**, 439-445.

Pratt,P.F. 1965. Potassium. In: *Methods of soil analysis*, Vol.2. (Ed.by C.A. Black). American Agronomy Society. pp.1022-1030.

Read,D.J. and Birch,C.P.D. 1988. The effects and implications of disturbance of mycorrhizal mycelia systems. *Proc. of the Royal Soc. of Edinburgh.* **94B**, 13-24.

Read,D.J.; Francis,R. and Finlay,R.D. 1985. Mycorrhizal mycelia and *nutrient cycling in plant communities. In: Ecological Interactions in Soil*, Special Publication nº4 of the British Ecological Society. (Ed. by A.H.Fitter). London. pp. 193-217.

Reeves,F.B.; Wagner,D.; Moorman,T. and Kiel,J. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid west. I. A comparison of incidence of mycorrhizae in severely disturbed vs. natural environments. *Amer. J. Bot.* **66**, 6-13.

Rives,C.S.; Bajwa,M.J.; Liberta,A.E. and Miller,R.M. 1980. Effects of topsoil storage during surface mining on the viability of VA mycorrhiza. *Soil Science* **129**, 253-257.

Roldan-Fajardo,B.E., Barea,J.M.; Ocampo,J.A. and Azcon-Aguilar,C. 1982. The effect of season on VA mycorrhiza of the almond tree and of phosphate fertilization and species of endophyte on its mycorrhizal dependency. *Plant and Soil* **68**, 361-367.

Rose,S.L. and Youngberg,C.T. 1981. Tripartite associations in snowbrush (*Ceanothus velutinus*) effect of vesicular-arbuscular mycorrhizae on growth, nodulation, and nitrogen fixation. *Can. J. Bot.* **59**, 34-39.

Saif,S.R. and Khan,A.G. 1975. The influence of season and stage of development of plant on *Endogone* mycorrhiza of field-grown wheat. *Can. J. Microbiol.* **21**, 1020-1024.

Saif,S.R. 1981. The influence of soil aeration on the efficiency of vesicular-arbuscular mycorrhizae.I. Effect of soil oxygen on the growth and mineral uptake of *Eupatorium odoratum* L. inoculated with *Glomus macrocarpus. New Phytol.* **88**, 649

Sas,A. 1988. The infectivity of VA mycorrhizal fungi from revegetated and undisturbed forest soils in the south-western Australia. Honours Thesis. University of Western Australia.

Scheltema,M.A.; Abbott,L.K. and Robson,A.D. 1987. Seasonal variation in the infectivity of VA mycorrhizal fungi in annual pastures in a mediterranean environment. *Aust. J. Agric. Res.* **38**, 707-715.

Schenck,N.C.; Graham,S.O. and Green,N.E. 1975. Temperature and light effect on contamination and spore germination of vesicular-arbuscular mycorrhizae fungi. *Mycologia* **67**, 1189-1192.

Schenck,N.C. and Schroder,V.N. 1974. Temperature response of *Endogone* mycorrhiza on soybean roots. *Mycologia* **66**, 600-605.

Schwab,S.M.; Menge,J.A. and Leonard,R.T. 1983. Quantitative and qualitative effects of phosphorus on extracts and exudates of sudangrass roots in relation to vesicular-arbuscular mycorrhizal formation. *Plant Physiol.* **73**, 117-119.

Schwab,S.M. and Reeves,F.B. 1981. The role of endomycorrhizae in revegetation practices in the semi-arid West. III. Vertical distribution of vesicular-arbuscular (VA) mycorrhiza inoculum potential. *Amer. J. Bot.* **68**, 1293-1297.

Shea,S.R. and Dell,B. 1981. Structure of the surface root system of *Eucalyptus marginata* Sm. and its infection by *Phytophthora cinnamomi* Rands.. *Aust. J. Bot.* **29**,49-58.

Shea,S.R.; McCormick,J. and Portlock,C.C. 1979. The effect of fires on regeneration of leguminous species in the northern jarrah (*Eucalyptus marginata* Sm.) forest of Western Australia. *Aust. J. Ecol.* **4**, 195-205.

Smith,T.F. 1978. A note on the effect of soil tillage on the frequency and vertical distribution of spores of vesicular-arbuscular endophytes. *Aust. J. Soil Res.* **16**, 359-361.

Specht.R.L. 1979. Heathlands and related shrublands of the World. In: *Ecosystems of the World, vol.9. Heathlands and related shrublands. Descriptive studies*. (Ed.by R.L.Specht). Elsevier, Amsterdam. pp.1-19.

Stahl,P.D.; Williams,S.E. and Christensen,M. 1988. Efficacy of native vesicular-arbuscular mycorrhizal fungi after severe soil disturbance. *New Phytol.* **110**, 347-354.

St John,T.V. and Coleman,D.C. 1983. The role of mycorrhizae in plant ecology. *Can. J. Bot.* **61**, 1005-1014.

Sutton,J.C. and Barron,G.L. 1972. Population dynamics of *Endogone* spores in soil. *Can. J. Bot.* **50**,1904-1914.

Sylvia,D.M. and Schenck,N.C. 1983. Germination of chlamydospores of three *Glomus* species as affected by soil matric potential and fungal contamination. *Mycologia* **75**, 30-35.

Thomson,B.D. 1987. Phosphorus supply and formation of vesicular-arbuscular mycorrhizas with particular reference to differences among fungal species. PhD Thesis. University of Western Australia. 186 pp.

Tommerup,I.C. 1983a. Spore dormancy in vesicular-arbuscular mycorrhizal fungi. *Trans. Br. Mycol. Soc.* **81**, 37-45.

Tommerup,I.C. 1983b. Temperature relations of spore germination and hyphal growth of vesicular-arbuscular mycorrhizal fungi in soil. *Trans. Br. Mycol. Soc.* **81**, 381-387.

Tommerup, I.C. and Abbott,L.K. 1981. Prolonged survival and viability of VA mycorrhizal hyphae after root death. *Soil Biol. and Biochem.* **13**, 431-433.

Walkley,A. 1947. A critical examination of a rapid method for determining organic carbon in soils - effects of variations in digestion conditions and of inorganic soil constituents. *Soil Sci.* **63**, 251-264.

Warcup,J.H. 1980. Ectomycorrhizal associations of Australian indigenous plants. *New Phytol.* **85**, 531-535.

Warner,A. 1983. Re-establishment of indigenous vesicular-arbuscular mycorrhizal fungi after top-soil storage. *Plant and Soil* **73**, 387-394.

Warner,A. and Mosse,B. 1982. Factors affecting the spread of vesicular mycorrhizal fungi in soil. I. Root density. *New Phytol.* **90**, 529-536.

White,J.A.; Munn,L.C. and Williams,S.E. 1989. Edaphic and reclamation aspects of vesicular-arbuscular mycorrhizae in Wyoming Red Desert soil. *Soil Sci. Soc. Am. J.* **53**, 86-90.

Wilson,J.M. 1983. Introductory survey of rehabilitated bauxite mine for recolonization by vesicular-arbuscular mycorrhizal fungi. *A Report for Alcoa of Australia.* Pinjarra, Western Australia.

Wilson,J.M. 1984. Comparative development of infection by three vesicular-arbuscular mycorrhizal fungi. *New Phytol.* **97**, 413-426.

Zajicek,J.M.; Hetrick,B.A.D. and Owensy,C.E. 1986. The influence of soil depth on mycorrhizal colonization of forbs in the tallgrass prairie. *Mycologia* **78**, 316-320.