Indigenous arbuscular mycorrhizal fungi of a tropical agroforestry system and their assocation with the intercrop, *Zea mays* L.

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CONTENTS

Abstract

Zea mays L. is an important food crop and comon intercrop species grown in semi-arid agroforestry systems in Kenya, East Africa. A preliminary study of spore populations of indigenous Arbuscular Mycorrhizal Fungi (AMF), was carried out for an alley cropping system with component species, *Senna siamea* Lam. and *Z. mays*. A series of experiments were established to examine the effects of inoculating *Z. mays* with tree and alley soil (containing AMF infective propagules) from fallow and cropped areas of the system on plant growth and nutrition. Further studies were established to determine the effects of soil type, inoculum type, quantity of inoculum, plant variety, soil volume and soil phosphorus content (adjusted with rock phosphate fertilizer) on the growth and nutrient uptake of inoculated and uninoculated plants.

The preliminary field study showed that AMF spores occurred in particularly high number in this system compared with that reported for other less disturbed ecosystems. The same spore types occurred in tree and alley soil, however spore number and composition were variable, between host species, in fallow and cropped areas of the system and between wet and dry seasons. At the end of the dry season (February, 1991), fallow areas had significantly higher spore numbers than cropped areas of the system. Lower numbers of spores were found in soil in the dry season than in the wet season, when spore numbers were significantly higher in tree soil in the cropped area than in soil from other areas.

The first experiment was established to determine the influence of host and fallowing on the effectiveness of AMF populations occurring in this system on mycorrhizal formation, growth and nutrition of plants. These results showed that plants inoculated with tree soil from the cropped area of the system had significantly higher mycorrhizal infection formation in roots, however this was not reflected in the growth and nutrient uptake of plants which were unaffected by AMF inoculation.

The effects of soil type, soil volume and soil phosphorus were thought to have influenced the response of plants to inoculation in the previous experiment and so the influence of these factors on growth and nutrition of inoculated and uninoculated plants

were investigated. Results from this experiment showed that plants had significantly different mycorrhizal infection in roots when grown in different soils. Mycorrhizal infection was higher in plants grown in smaller pots, decreasing significantly with incremental increases (1, 4 and 10 litre pots) in soil volume. Adding rock phosphate to soil increased mycorrhizal infection compared to that in unamended soil. However, at high amounts (300 mg l^{-1} soil), mycorrhizal infection was reduced relative to that at low amounts (150 mg l^{-1} soil). As found in previous experiments plant growth was unaffected by inoculation with AMF. However, the growth of plants was affected by soil type, in 10 litre pots only. Soil volume increased the growth of plants in unamended soils, while in low P soil, plant growth was highest in 4 litre pots and in high P soil, growth of plants was greatly reduced and unaffected by soil volume. The reduced growth of plants in response to inoculation with AMF may have been a pathological effect, however the results from a final experiment showed that this was highly unlikely, although these results were indicative rather than conclusive.

This study showed that agronomic practices had an important effect on spore number and population composition of AMF in agroforestry systems. It is suggested that high spore numbers found in the cropped area in association with the tree is directly related to effect of pruning on tree physological processes which indirectly affect AMF spore populations. This is therefore considered important and justifies further study. The failure of plants to respond favourably to inoculation in this experiment was probably because the conditions in which the symbiosis was studied were not conducive to proper functioning of the mycorrhiza. It is suggested that as soil volume was limited and as maize has quite an extensive root system that uninoculated plants were able to take up available P in the limited soil volume without the aid of the mycorrhiza. A favourable growth response of plants to AMF inoculation would therefore be expected to occur in the field.

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List of Tables

Table 5.6. Significance of F-values in the analysis of variance for root N, P, K, Mg and Ca content of inoculated and uninoculated *Z. mays* grown in 1, 4 and 10 litres of soil A and soil B.

Table 5.7. Significance of F-values in the analysis of variance for root N, P, K, Mg and Ca content of inoculated and uninoculated *Z. mays* grown in 1, 4 and 10 litres of unamended soil B, low P soil and high P soil.

Table 6.1. Significance of F-values in the analysis of variance for mycorrhizal infection, total dry weights, shoot dry weights, root dry weights and root : shoot ratios of two varieties of inoculated and uninoculated *Z. mays* plants harvested 60 d.a.i. inoculated with two types of inoculum (soil or spores).

List of Figures.

Figure 4.5. Effect of inoculating *Z. mays* with soil from different treatment areas of the RHI system at Machakos, Kenya on the shoot, root and total dry weights and root : shoot ratios of plants 120 d.a. i. Mean +S.E (n=5).

Figure 4.6. Effect of inoculating *Z. mays* with tree and alley soil from fallow and cropped areas of the RHI system on mean shoot N, P, K, Mg and Ca concentrations (% of dry weight) 60 d.a.i. Mean +S.E (n=5).

Figure 4.7. Effect of inoculating *Z. mays* with tree and alley soil from fallow and cropped areas of the RHI system on mean root N, P, K, Mg and Ca concentrations (% of dry weight) 60 d.a.i. Mean +S.E (n=5).

Figure 4.8. Effect of inoculating *Z. mays* with tree and alley soil from fallow and cropped areas of the RHI system on mean shoot N, P, K, Mg and Ca concentrations (% of dry weight) 120 d.a.i. Mean +S.E (n=5).

Figure 4.9. Effect of inoculating *Z. mays* with tree and alley soil from fallow and cropped areas of the RHI system on mean root N, P, K, Mg and Ca concentrations (% of dry weight) 120 d.a.i. Mean +S.E (n=5).

Figure 4.10. Effect of inoculating *Z. mays* plants with infected root material taken from plants previously inoculated with tree and alley soil from fallow and cropped areas of the RHI system in experiment 1a on the formation of mycorrhizal infection in roots. Mean +S.E (n=5).

Figure 4.11. Effect of inoculating *Z. mays* plants with infected root material taken from plants previously inoculated with tree and alley soil from fallow and cropped areas of the RHI system in experiment 1a on total dry weight, shoot dry weight, root dry weight and root : shoot ratios of inoculated and uninoculated plants harvested 120 d.a.i.. Mean +S.E (n=5).

Figure 4.12. Effect of inoculating *Z. mays* plants with infected root material taken from plants previously inoculated with tree and alley soil from fallow and cropped areas of the RHI system in experiment 1a on N, P, K, Mg and Ca concentrations (% dry weight) in shoots harvested 120 d.a.i.. Mean +S.E (n=5).

List of plates.

CHAPTER ONE
INTRODUCTION

1.1 Background

A substantial amount of research has been focussed on a group of soil borne fungi, the Arbuscular Mycorrhizal Fungi (AMF), since it was found that they could improve the mineral acquisition of plants, especially with regard to phosphorus (Brundrett, 1991). AMF associations occur in more than 80 % of all plant species (Bonfante-Fasolo, 1984) distributed across a wide geographical range (Brundrett, 1991).

Interactions between host and endophyte are such that some combinations are more effective than others in promoting plant growth. The 'effectiveness' of a particular host-endophyte association is not only determined by the species involved but by soil and environmental factors in which the association is formed (Medeiros *et al.*, 1994; Katiyar *et al.*, 1995). In agroecosystems, changes in the soil and environment caused by agronomic practices are a regular occurrence. Of those agronomic practices which have been studied, some (including fallowing and crop rotations with non-host species) have detrimental effects on the effectiveness of AMF populations (Thompson, 1987), while others (including pre-cropping with certain plant species) enhance indigenous AMF populations for subsequent crops (Dodd *et al.*, 1990a, 1990b).

Management of indigenous AMF to promote crop growth has been successful using agronomic practices known to enhance effective AMF species within populations. Studies in tropical (Sieverding, 1991; Dodd *et al.*, 1990a, 1990b; Douds *et al.*, 1993) and temperate monoculture agroecosystems (Black and Tinker, 1979; Daft, 1991) have shown that manipulating AMF in the field through the use of agronomic practices has great potential. Inoculation of plants with AMF from cropped soil has been found to overcome the deleterious effects of long term fallowing (> 12 months) (Thompson, 1994). Similarly, direct inoculation of plants with highly effective AMF species is reported to be effective (Sieverding, 1991), but is limited by the problems associated with culturing AMF.

In many tropical areas, despite the high potential for plant production in relation to light and temperature, soils are generally deficient in nutrients, particularly phosphorus, and water is often limiting. Population expansion is exerting great pressure on the carrying capacity of land. In such areas, low-input agroforestry systems are practised widely by subsistence farmers who have limited access to expensive single and triple super phosphate fertilizers. In this situation, management of indigenous AMF may be a 'low-tech' way of improving crop yields in soils which are low in P and in systems which are often not sustainable (Sanchez, 1987).

The present study was conducted in an agroforestry system situated in Kenya, Eastern Africa, in which the potential for increasing plant productivity in agroforestry systems was investigated by examining the occurrence and distribution of AMF spore populations associated with tree and crop components in agroforestry systems under different types of management (fallowing and intercropping). The effect of mixed indigenous AMF populations on the growth of the intercrop species was studied and further experimental work was carried out to determine the effect of rock phosphate (an inexpensive, slow release fertilizer) application on the effectiveness of the crop-AMF symbiosis.

In this chapter, the life cycle, functioning and ecology of AMF is discussed with reference, where possible to research in the tropics. The potential for exploiting indigenous AMF populations in tropical agroforestry systems is discussed referring where possible to examples of tropical studies.

1.2 Introduction to the Arbuscular Mycorrhizal Fungi (AMF)

In 1885, Professor A.B. Frank (cited in Nicolson, 1967) first used the term 'mycorrhiza' to describe the mutualistic associations between a particular group of fungi and the roots of most higher plants (Allen, 1991). Today, mycorrhizal fungi are divided into two main groups, ectomycorrhizas and endomycorrhizas. Ectomycorrhizas are characterised by intercellular hyphal penetration of the root cortical cells, forming what is known as the 'Hartig net' and a thick hyphal mantle or sheath covers the root surface resulting in morphological changes to the root system. By contrast, endomycorrhizas have inter- and intracellular hyphal penetration of the root cortical tissues forming structures such as hyphal coils, arbuscules and vesicles (see section

1.4). There is little change in the root appearance, although in some plant species such as maize, infected roots may be yellow in colour compared with white uninfected roots (Daft and Nicolson, 1986). The name 'vesicular arbuscular mycorrhizal fungi' (VAMF) was given to the endomycorrhizas because it was thought that arbuscules and vesicles were present in mycorrhizas formed by all species. However, it has since been discovered that one Family, Gigasporaceae (genera; *Gigaspora* and *Scutellospora*), never form vesicles and so the group was recently renamed 'arbuscular mycorrhizal fungi' (AMF) (Morton and Benny, 1990).

1.3 Occurrence and distribution of AMF

Of the two major groups of mycorrhizas, AMF are most common. Surveys of mycorrhiza have shown that members of certain plant families form AMF associations rarely. AMF are largely absent from families which form ectomycorrhizal associations including the Dipterocarpaceae, Pinaceae, Fagaceae, Betulaceae, Myrtaceae and Salicaceae (Newman and Reddell, 1987), although there are some reports of mycorrhizal formation in most of these families. They are absent from plant families which form the two other types of endomycorrhiza: ericoid and orchidaceous, formed by species of the Ericales and Orchidaceae (Hayman, 1982). There are well documented cases of trees belonging to taxa which normally have ectomycorrhiza also forming AMF especially as seedlings (Vozzo and Hacskaylo, 1974). Such dual associations are common in Australian arid communities (Warcup and McGee, 1983). AMF have a worldwide distribution, occurring on plants in dense tropical rainforest (Janos, 1983; Högberg, 1982; Redhead, 1977), savanna (Abbott and Robson, 1977; Redhead, 1977) heaths, moorlands (Sward *et al.* 1978), salt marshes (Sengupta and Chaudhuri, 1990), sand dunes and semi desert (Khan, 1974; Giovannetti and Nicolson, 1983). They are the mycorrhizal associates of all short rotation agricultural crop species, except those that are non-mycorrhizal and, with the exceptions of the ectomycorrhizal trees listed earlier, form mycorrhizal associations with many economically and ecologically important tree species.

Recognition of the occurrence and distribution of mycorrhizas in tree, crop and other plant species is important for AMF management in natural and agronomic ecosystems. The role of AMF and the effects of various soil, plant and environmental factors that affect them in natural ecosystems and agronomic practices affecting AMF in cultivated areas will be discussed later in this Chapter.

1.4 Life cycle of AMF

Before working with AMF in the field or in designed experiments, an understanding of the fungus' life cycle is required. Plant roots infected with arbuscular mycorrhizal fungi show little external modification in root morphology. However, infection can be observed under a light microscope when roots are stained with chemicals such as chlorazol black E, trypan blue, acid fuchsin or aniline blue WS (Brundrett *et al.*, 1984). Infection is located in the epidermis and cortical parenchyma of the root (Bonfante-Fasolo, 1984). Extramatrical mycelium, reproductive structures (either chlamydospores or azygospores asexually produced by the fungus), or infected root material serve as propagules of infection. Spores germinate in soil under certain conditions, producing germ tubes. They also germinate readily *in vitro* on water agar, but hyphal growth is restricted to a few centimetres (Smith and Gianinazzi-Pearson, 1988). Germination of spores in soil is usually influenced by soil physical and chemical factors (Sieverding, 1991). When germ tubes do not contact plant host roots within a few days to several weeks the infection potential of the fungus is lost (Sieverding, 1991).

The fungus penetrates the root between the epidermal cells and often forms an appressorium in the first cell layers. The appressoria connect infection inside the root with the external mycelium. After root penetration, hyphae grow inter- and intra-cellularly. Arbuscules are often formed within cells shortly (2-5 days) after penetration. These are produced by strong branching of a hypha after penetrating a cortical cell wall. The branching of the hyphae offers increased surface area for the bidirectional transfer of metabolites and nutrients to and from the fungus. The arbuscule life cycle is limited to 4-5 days (Cox and Tinker, 1976), after which the arbuscule branches deteriorate and collapse. In nature and older plants senescent arbuscules are more frequently observed than active ones which are often found within young mycorrhizal roots or in roots of plants grown under more controlled conditions. Arbuscules at different stages of development can be seen in the infected root at any one time. This is due to dynamic nature of spore germination over time (Bonfante-Fasolo, 1984).

At the same time or shortly after arbuscule formation, inter- and intra-cellular vesicles are formed. These are apical and intercalary swellings of hyphae which contain lipids and function as the storage organs of the fungus. As mentioned previously, vesicles are not formed by species of *Gigaspora* and *Scutellospora*. Instead these genera produce auxillary cells in the root extramatrical mycelium (Morton and Benny, 1990; Sieverding, 1991). The characteristic morphology of vesicles formed by *Glomus* spp. is elliptical, formed inter- or intracellularly (Abbott, 1982). *Acaulospora laevis* has been found to form lobed or irregular intracellular vesicles (Abbott and Robson, 1978), while *Acaulospora trappei* is known to form smaller unlobed ones (Gerdemann and Trappe, 1974).

Once the fungus becomes established within the root, hyphae grow out of the root and rhizosphere into the soil. This extramatrical mycelium is very important for elemental nutrient uptake from soil solution and for transport of nutrients to the root. This will be discussed in detail in section 1.5.2. Arbuscular mycorrhizal hyphae form three distinct types of extramatrical hyphae, the 'penetration hyphae', the 'runner hyphae' and the 'absorbing hyphae'. Penetration hyphae are hyphae produced when AMF hyphae come in contact with the root surface in order to facilitate entrance of hyphae into the root cortex. Runner hyphae are thick walled, larger hyphae that fix roots into the soil or grow through the soil in search of additional roots. Absorbing hyphae develop from runner hyphae, forming a dichotomously branched hyphal network which appears to be the component of the fungus that absorbs nutrients from the soil for transport to the host (Allen, 1991). Several reports suggest that there are 100 cm of absorbing hyphae per root penetration (see Read, 1984). Hyphal growth away from the root has been found to occur over distances ranging from 7 cm (Rhodes and Gerdemann, 1975b) to 11 cm (Jakobsen *et al.*, 1992). Recent research examining external mycelial development in a rhizobox using image analysis reported interspecific differences between plant and fungal species in relation to the quantity of mycelia produced per centimetre of colonised root (Green *et al.*, 1994).

Other mycorrhizal structures external to the root include the 'reproductive propagules' of the fungus. External mycelium produces structures known as resting spores, the morphology and anatomical features of which vary with different fungal species. Size of spores range from approximately 15 µm to 800 µm (Sieverding, 1991). These structures enable the fungus to survive periods of environmental stress and may remain in soil as fungal propagules until conditions adequate for growth occur.

1.5 Functioning of AMF

1.5.1 Effects on plant growth

A great deal of work has been reviewed (Barea and Azcón-Aguilar, 1983; Harley and Smith, 1983; Abbott and Robson, 1984; Hayman, 1980; Smith and Gianinazzi-Pearson, 1988) which shows that arbuscular mycorrhizal fungi usually enhance plant growth. Focussing on economically important crops, particularly tropical species, Burckhardt and Howeler (1985) obtained improved growth of *Manihotis* species by inoculating with indigenous and selected (highly effective) inocula in a pot experiment. In similar pot experiments, AMF improved the growth of various plants, including tropical forage legumes (Arias *et al.*, 1991; Saif, 1987), *Cajanus cajans* (Diederichs, 1990), *Zea mays* (Jackson *et al.*, 1972; Mosse, 1977a; Fabig *et al.*, 1989; Asmah, 1995), *Vicia faba* (Kucey and Paul, 1983), *Cicer arietinum* (Weber *et al.*, 1992), *Sorghum bicolor* (Fabig *et al.*, 1989; De Miranda *et al.*, 1989), *Glycine max* (Bethlenfalvay *et al.*, 1985), species of *Avocado* (Menge *et al.*, 1978; Jaizme-Vega and Azcón, 1995), *Theobroma cocao* (Cuenca *et al.*, 1990; Azizah Chulan and Martin, 1992), and many tree species, including numerous *Acacia* species (Borges and Chaney, 1988; Michelsen, 1993), *Caesalpinia eriostachys*, *Cordia alliodra*, *Pithecellobium mangense* (Huante *et al.*, 1993) , *Albizia falcataria* and *Parkia speciosa* (Alexander *et al.*, 1992).

In field experiments the effects of inoculating plants with AMF are variable. The choice of control in these experiments varies also and this is particularly important as it affects the results obtained and their interpretation. In pot experiments, most inoculated plants are grown in sterilized soil to which AMF have been added and these are compared with plants grown in sterilized soil without AMF. However, in field experiments it is less common for soil to be sterilized and furthermore extremely difficult to exclude AMF from the soil completely. In many cases plants are inoculated with AMF in pots and then transplanted to the field where plant growth is compared between pre-inoculated plants and those which are infected with indigenous AMF alone. Singh and Tilak (1989) observed improved growth of *Cicer arietinum* in a field experiment in Central

India in which control plants were grown in soil containing indigenous AMF (Singh and Tilak, 1989). In contrast, Weber *et al.* (1992) reported improved growth of *Cicer arietinum* plants grown in pots in sterilized soil, but failed to achieve this in the field. The poor mycorrhizal response in the field was attributed to the competitiveness of indigenous mycorrhizal fungi resulting in the development of highly effective mycorrhiza in control plants. By contrast, Kucey and Paul (1983) observed increases in the growth of inoculated *Vicia faba* in glasshouse and field trials despite the presence of indigenous mycorrhizal fungi in field soil.

Reduced or negative growth response of plants to AMF inoculation have been observed under a variety of environmental conditions. Huante *et al.* (1993) observed a negative growth response in AMF inoculated *Ipomoea wolcottiana*, a pioneer species of a tropical deciduous forest in Mexico. Inoculation of the leguminous tree species, *Erythrina berteroana* resulted in a 16 % decrease in shoot biomass of AMF inoculated cuttings (Copperband *et al.*, 1994). The responsiveness of plants to AMF was thought to reflect the cost-benefit relationship between plant host and fungal symbiont for energy and nutrient reserves. An inoculated plant may often exhibit poorer growth relative to an uninoculated plant because of the carbohydrate demand of the fungus. Less efficient AMF species may also contribute to reductions in the growth of inoculated relative to uninoculated plants when P concentrations in soil are low. Similarly, different plant species may have different responses to inoculation with the same AMF species or population. Inoculation of *Acacia tortilis* and *Prosopis juliflora* in the nursery improved survival and initial growth of trees in the field, while other species (*Terminalia spinosa* and *Terminalia brownii*) did not benefit to the same extent (Wilson *et al.,* 1989). Huante *et al.* (1993) found that late sucession trees from the mature part of a semi-deciduous forest in Mexico were more dependent on the mycorrhizal association than those in more disturbed environments. The symbiotic associations between plant and AMF have been found to be affected by host species (Wilson *et al.* 1989), fungal species in inoculum and status of the indigenous mycorrhizal population (Weber *et al.* 1992) as well as other factors (*e.g.* soil fertility, moisture etc.). It is difficult to make general conclusions as many factors affect the symbiosis and it is essential that these are understood if AMF populations are to be managed successfully.

1.5.2 Uptake of phosphorus

The beneficial effect of AMF on plant growth has been attributed to an increase in nutrient uptake, particularly that of phosphorus (P) (Abbott and Robson, 1977; Tinker, 1978). Several studies have shown that AMF infected plants are unable to extract P from soil solution in forms other than those already available to non-mycorrhizal roots (Barrow *et al.*, 1977; see Cooper, 1984). However, there are studies which report that mycorrhizal plants can use P adsorbed to iron hydroxides or in particulate iron phosphates (Bolan *et al.*, 1984).

Various mechanisms have been suggested for the increased P uptake of mycorrhizal plants, including increased soil volume explored by AMF hyphae, faster movement of P into mycorrhizal hyphae and solubilization of soil P (Bolan, 1991). Diffusion of available P through soil is the rate limiting and major pathway for movement of P to the root, particularly in dry conditions (see Mason and Wilson, 1994). The uptake of minerals (P and others) that have a slow rate of diffusion (Cooper, 1984) can be improved by extramatrical fungal hyphae and in dry conditions, the ability of mycorrhizal fungi to access P is particularly important, as the diffusion coefficient for phosphate in soil decreases linearly with increasing soil dryness (Fitter, 1985). Phosphorus is present in small amounts in soil solution as it becomes bound to soil colloids or fixed as iron and aluminium phosphate (Tinker, 1975), a situation very common in tropical ultisols and oxisols (Sanchez, 1987). AMF hyphae grow beyond nutrient depletion zones increasing the soil volume exploited for P uptake (Rhodes and Gerdemann, 1975a; Rhodes and Gerdemann, 1975b). The major site for transfer of P from the fungus to the plant occurs in root cells containing arbuscules although root internal hyphae can also release P to the host. As a result of increased P uptake, growth of inoculated plants is increased compared to uninoculated plants where P was limiting growth.

1.5.3 Uptake of other nutrients

It is likely that AMF also enhance the uptake of other immobile nutrients that move to the plant roots primarily by diffusion. However, to demonstrate this unequivocally it is essential that the supply of all other nutrients except that under test are not limiting, a condition that is difficult to meet because of the dynamic state of supply and demand for plant nutrients. Increasing P supply often reduces AMF infection levels which may reduce the uptake of other nutrients and so significantly affect the results obtained in experiments. Additionally, lower concentrations of a particular nutrient in a mycorrhizal plant may simply reflect increased plant growth (by relief of P deficiency) and dilution of nutrients under study.

Sieverding and Toro (1988) working in K deficient soil found increases in K uptake in mycorrhizal plants. However, in some instances, this was thought to be the indirect effect of alleviation of P deficiency in soil. Micronutrients such as zinc (Zn), copper (Cu), sulphur (S), boron (B) and molybdenum (Mo) are also taken up in increased quantities by mycorrhizal plants.

In a recent experiment AMF inoculation increased growth of *Z. mays* and reduced cadmium (Cd), Cu, Zn and manganese (Mn) concentrations in plants, suggested a protection against metal toxicity. Also, inoculating *Z. mays* with *G. mosseae* decreased shoot and root Cu concentrations compared to that achieved with mixed species inoculum. Significantly higher root colonization was achieved with mixed species inoculum than *G. mosseae* (31.7 and 19.1 % respectively) suggesting its higher metal tolerance. Zn shoot concentrations were higher in both mycorrhizal treatments and lead (Pb) concentrations, particularly in the roots, also tended to increase with mycorrhizal colonisation (Weissenhorn *et al.*, 1995). These results indicated that AMF influenced the uptake of metals in *Z. mays*, but that this depended on the growth conditions, on the fungal partner and on the metal, and could not be generalized (Weissenhorn, *et al.*, 1995).

1.5.4 Interaction with other microorganisms

A topic which has received much attention is that of the interactions between AMF and N fixing rhizobium found in the roots of leguminous plant species. The possibility of mycorrhizal infection increasing N concentration in plants by improving the effectiveness of rhizobium was suggested by Ross and Harper (1970) when they found that *G. max* plants inoculated with AMF had higher nitrogen concentrations than uninoculated controls. In 1974, Crush provided direct evidence that AMF increased nodulation and growth of legumes. He found that AMF had a positive influence on both parameters in *Centrosema pubescens*, *Stylosanthes guyanensis*, and *Trifolium repens*. Michelsen and Sprent (1994) found that AMF inoculation of non-nodulated *Acacia nilotica* (normally found to nodulate) increased mycorrhizal infection and shoot dry weight, and appeared to enhance N_2 fixation.

Studies show that in soils with low available P (predominant in tropical soils) the effectiveness of rhizobium is increased by mycorrhizal infection. Legumes (*e.g.*, French bean, peanuts, soybean, lucerne, cowpea and pigeon pea) inoculated with rhizobium and AMF, grown in low P soils, and fertilized with either bonemeal or rock phosphate (slow P release fertilizers) had higher N and P tissue content, leaf number, plant height and above- and below-ground biomass compared to non-mycorrhizal counterparts (see Bagyaraj, 1984). Pacovsky *et al.* (1986) found that soybean inoculated with AMF and rhizobium had dry weights 18% greater than nodulated non-mycorrhizal plants and that N fixation was increased significantly within the tripartite symbiosis. It has been shown that legumes are unable to make efficient use of the rhizobium symbiosis when plants are not mycorrhizal (Barea and Azcòn-Aguilar, 1983).

It appears that in addition to effects on phosphorus nutrition, mycorrhiza aid other processes involved in nodulation and nitrogen fixation. Such potentially limiting factors may include supply of photosynthate (Pate, 1976), trace elements (Daft and Hacskaylo, 1977; Bagyaraj and Manjunath, 1980) and plant hormones (produced directly by AMF or indirectly by free living bacteria in the rhizosphere stimulated by mycorrhiza) (Bagyaraj and Menge, 1978; Bagyaraj *et al.*, 1980). AMF have been shown to stimulate the production of phytohormones directly (Barea and Azcòn-Aguilar, 1983). Inoculation with AMF can result in growth inhibition of the legume hosts when

P is limiting, but N is not (Bethlenfalvay *et al.*, 1982). In *Acacia abyssinica*, nodulation and N_2 fixation were reduced in AMF inoculated plants compared to controls. This was explained as an effect of the carbon drain of the fungus, (a temperate isolate), which may have been of no benefit to the host under the nursery conditions prevailing (Michelsen, 1993). The synergistic interactions between microsymbionts suggests that response of the host to dual colonization is complex and depends on a balance between the three members of the symbiosis.

Similar to the interaction with rhizobium, AMF can have a positive effect on the N_2-fixing association between actinomycetes (*Frankia*) and non-leguminous plant species (see Gianinazzi-Pearson and Diem, 1982). Also, a positive influence of AMF on the 'phosphate-solubilizing' microorganisms (e.g. *Pseudomonas* spp., *Agrobacterium* spp., *Bacillus circulans*, *Aspergillus niger*, *Penicillium funiculosum*) in the rhizosphere is reported (Bagyaraj, 1984). It is probable that bacteria can affect plant growth by hormonal and vitamin synthesis, however the beneficial effect of AMF on the rhizobacteria is likely to be indirect, caused by an increase in growth and photosynthetic rates which in turn benefit the bacteria through increased root exudation. Azizeh *et al.* (1995) found three- to sixfold increases in the amounts of carbohydrates, amino acids and phenolics on agar sheets treated with antibiotics, to exclude rhizoplane bacteria, when plants were inoculated with AMF compared to those which were not.

AMF have been found to increase tolerance of plants to root and collar rots, often caused by fungal species such as *Phytophthora*, *Pythium*, or *Rhizoctonia*, to wilt diseases, and to attack by nematodes (Mosse *et al.*, 1981). Schonbeck and Dehne (1979) found that mycorrhizal cotton plants could withstand the stress of infection by the fungal pathogen *Thielaviopsis basicola* better than non-mycorrhizal plants. Liu (1995) observed increased cotton wilt tolerance of plants infected with AMF and *Verticillium dahliae*. Root colonization by both pathogen and symbiont were reduced, as were the number of germinable microsclerotia in soil. However, there were no effects on AMF spore numbers and cotton seedling growth was significantly improved in AMF inoculated plants. By contrast, AMF appear to make many plants more susceptible to leaf pathogens (Schonbeck and Dehne, 1979) and to viruses (Daft and Okusanya, 1973).

1.5.5 Water relations in AMF plants

As with other plant stresses, AMF colonization has been found to improve drought resistance of plants. They have been shown to reduce susceptibility to wilting and transplant shock in tropical trees (Menge *et al.*, 1978). Studies by Menge *et al.* (1978) and Sieverding (1981) found that mycorrhizal plants were capable of tolerating water stress better than non-mycorrhizal counterparts. Several glasshouse studies show that AMF infection increases drought resistance of cultivated crops, including soybean (Safir *et al.*, 1972) and maize (Subramaian *et al.*, 1995). Inoculation of maize with AMF was found to increase the retention of sugars and proteins, particularly in a drought sensitive cultivar relative to uninoculated plants. This was suggested to be of physiological importance aiding the plants resistance to drought (Subramanian and Charest, 1995). Another experiment emphasised the importance of AMF fungi in the establishment, growth, water relations and nutrition of cacti in arid tropical regions (Rincón *et al.* 1993). In contrast, the findings of some studies suggest that drought resistance is unaffected or reduced by AMF (Allen and Boosalis, 1983; Simpson and Daft, 1990).

The proposed mechanisms whereby AMF increase drought resistance or improve water flow are still uncertain. Mycorrhizal soybean plants were found to have lower root resistance to water flow compared to non-mycorrhizal plants and in unfertilized soils AMF infection reduced resistance of roots to water flow by 40% in soybean (Safir *et al.*, 1972). The results of most investigations suggest that improved water relations in plants inoculated with AMF are an indirect effect of improved plant nutrition, a positive effect on phytohormones, stomatal regulation or a more branched root system. Improved plant nutrition of AMF inoculated plants can influence drought resistance as plant growth increases and an extensive deeper root system is developed which can extract water more efficiently (Ellis *et al.*, 1985; Kothari *et al.*, 1991), or as water use efficiency (*i.e.* dry matter production per unit available water) is improved (Sieverding, 1991). In tropical areas it is important that plants are able to survive short periods of water stress (Ellis *et al.*, 1985). Similarly, another important function of AMF may be that due to faster and increased growth, plants can make better use of short periods (2-3 months; growing season) of optimum climatic conditions (Sieverding, 1991).

1.5.6 Other benefits

AMF affect soil stability directly by producing fungal hyphae in soil which aggregate soil particles and serve to improve the physical structure of soil. Sutton and Shepard (1976) showed that AMF hyphae in sand dunes improved the soil structure by such a mechanism. Erosion losses result in detrimental effects on chemical, physical and microbial properties of soil. Stabilization of soil in areas where soil erosion degrades land is particularly important for land reclamation, indeed it has also been suggested that in areas of high rainfall this mechanism of soil aggregation by AMF hyphae improves the plants efficiency at absorbing nutrients which would otherwise be lost through leaching, and is therefore responsible for the efficient nutrient cycling observed in tropical rainforests (Went and Stark, 1968).

1.6 AMF-plant symbiosis

1.6.1 Specificity and effectiveness of AMF plant symbiosis

Certain fungal species such as *Acaulospora scrobiculata* and *Acaulospora myriocarp* are found in the rhizosphere of many plant species under a wide range of conditions (Sieverding, 1991). Louis and Lim (1987) surveyed indigenous mycorrhizal populations of four plant species at two sites in a lowland tropical rainforest. There were no differences in AMF species found in association with plants at both sites but there were differences in the proportions of AMF species within populations. Until recently, it was generally considered that plants and AMF species lacked specificity under favourable conditions (Harley and Smith, 1983), however evidence to the contrary is beginning to increase. *Acaulospora splendida* has been found in the rhizosphere soil of 2 and 3 year old *Quercus costaricensis* but not on adjoining strawberry plants and peas, furthermore, culture of *A. splendida* using sorghum as a baiting plant was unsuccessful because of incompatibility between host and AMF species (Sieverding, 1991). Struble and Skipper (1988) encountered similar problems when propagating 4 *Glomus* and 1 *Gigaspora* species on soybean plants. Root infecting hyphae of AMF species have been examined for specificity. McGonigle and Fitter (1988) found that *Holcus lanatus* was predominately infected by *Glomus tenue*, while the roots of three other herbaceous species were colonized by other mycorrhizal symbionts.

In addition to specificity between AMF and plants, there is increasing evidence which suggests that there are differences in the efficiency of different endophyte-plant associations. Agricultural crops such as *G. max* (Schenck and Kinloch, 1980) and *Z. mays* (see Mosse, 1975) were reported to have consistently higher infection levels than others such as potato (Kruckelmann, 1975). Indicators used to assess effectiveness include the ability to colonize roots, produce spores, stimulate plant growth, increase host numbers in a natural ecosystem, or in a physiological comparison, the nutrient transfer per unit of carbohydrate utilized by AMF. These parameters for evaluating effectiveness of AMF vary according to the research interest and plant host investigated. The majority of studies however tend to use growth parameters of the host as indicators of AMF effectiveness.

Many studies have shown that AMF species and isolates differ in their ability to stimulate plant growth. Aggangan and Lorilla (1990) found that *G. etunicatum* and *G. macrocarpum* were more effective than *G. fasciculatum* and *G. mossae* in increasing height, diameter, biomass and P uptake of *Acacia auriculiformis*. In a field trial, Rao *et al.* (1989) found differences in the effect of 3 AMF isolates on the biomass, grain yield and P content of *Eleusine coracana*. Medeiros *et al.* (1994) found that certain fungal species were more effective than others at promoting growth in *Sorghum bicolor*, an effect which was consistent over a range of pH (4-7). AMF effectiveness trials have been carried out on a range of plant hosts including *Leucaena leucocephala* (Bagyaraj *et al.*, 1989), *Liquidambar styraciflua* (Kormanik *et al.*, 1981), *Acacia auriculiformis* (Aggangan and Lorilla, 1990), troyer citrange (*Poncirus trifoliata* x *Citrus sinensis*) (Graham *et al.*, 1982), *Ipomea batatas* (cv. White Star) and napier grass (*Pennisetum americana* x *P. purpureum* hybrid triploid PI 300086) (Hung *et al.*, 1990). Results suggest that AMF-plant associations vary considerably in effectiveness, in these examples depending on the inoculant AMF species. Conversely, the effects of a range of host species on a certain fungal species have also been investigated. These studies report differences in the effect of different plant species on AMF sporulation (Hetrick and Bloom, 1986) and root length infected by individual AMF species (Toth *et al.* 1984;1990). Daft and Hogarth (1983) found that spores produced by *Glomus clarum* were 3 times greater in number on *Z. mays* compared to onion plants. In a similar experiment the effect of plant species on AMF spore production was compared using corn (*Z. mays* cv. Pioneer 3369A), *Paspalum notatum*, *G. max* and *Sorghum vulgare* (var. sudanense (piper hitch)). Spores of *Glomus claroideum*, *G. etunicatum*, *G. mosseae* and *G. macrocarpum* were greater in number with *P. notatum* than with corn and *S. vulgare. G. max*, on the other hand, was not a suitable host for spore production with any of these AMF species (Struble and Skipper, 1988).

These studies show that inter- and intraspecific variation is common in host endophyte associations. In addition, other factors, such as pH, moisture, light and nutrients influence the effectiveness of the symbiosis. These effects will be discussed in detail later in this chapter.

1.6.2 Mycorrhizal dependency

It has already been established that mycorrhiza play a major role in the plants' acquisition of soil soluble P. Plant species and cultivars vary in their need for and response to phosphate therefore their dependence on the mycorrhizal condition varies also. Almost 2 decades ago, the term "Mycorrhizal Dependency" (MD) was defined as the degree to which a plant species is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given soil fertility (Gerdemann, 1975). Mycorrhizal dependency is an intrinsic property of a plant species (Janos, 1983). Plants may be non-mycotrophic, faculatively (weakly) mycotrophic or obligately (strongly) mycotrophic according to their ability to grow with and without mycorrhiza at different levels of soil fertility (Janos, 1983). Mycorrhizal dependence is not to be confused with responsiveness which is dependant on soil fertility, plant host, AMF species and other ecological conditions (temperature, light, soil moisture, etc.). Habte and Manjunath (1991) illustrated the difference between dependency and responsiveness during an experiment with *Brassica nigra* and selected species of *Leucaena* and *Sesbania.* Plants were grown in substrates with different concentrations of soluble P, inoculated with *G. aggregatum* or left uninoculated. They found that the responsiveness of the hosts to inoculation, (measured as the extent to which the plant depended on mycorrhiza for dry matter production), decreased with increasing P, but that the magnitude of decrease was species dependent. Habte and Byappanahalli (1994) examined the response of cassava to inoculation with AMF when grown from large and small vegetative cuttings. They found that cassava plants grown from smaller cuttings were more responsive to AMF inoculation than those grown from larger cuttings. This experiment showed that even strongly dependent species such as cassava, produced a range of responsiveness to inoculation depending on environmental conditions. Table 1.6 shows the mycorrhizal dependency of some crop plants.

Table 1.6. Mycorrhizal dependency of some crop plants

Obligate	Faculative	
Strong dependency	Medium dependency	Weak dependency
Cassava	Maize	Wheat
Onion	Sorghum	Barley
Citrus	Bahia grass	Cotton
Leek	Soybean	Sweet potato
Avocado		Rice
Cowpea		Papaya
Asparagus		
Mango		
Coffee		

(Sieverding, 1991)

1.7 Effect of environmental and agronomic factors on the AMF-plant symbiosis

1.7.1 pH, metals, salinity, pollutants

The development and consequences of AMF infection in plants is strongly influenced by the growth medium. Most interest has centred on the supply of phosphorus in the soil supporting the dual association (Hayman and Tavares, 1985). The effects of soil pH on the symbiosis are important and several authors have studied them with reference to the effect of pH on spore germination (Hepper, 1983; Daniels and Trappe, 1979) and spore production (Kruckelmann, 1975) or work on the mycorrhizal effect on growth and phosphorus uptake (Hayman and Mosse, 1972). Effects of soil pH are particularly difficult to evaluate since many chemical properties of the soil vary with changes in pH. Invariably the effects of pH are most exclusively due to metals such as, aluminium, iron and manganese. Acid tolerance can therefore be seen as a specialized aspect of metal tolerance. Spores of *G. mosseae* and *Gigaspora margarita* have not been found to occur naturally in tropical soils with pH less than 5.5 and *Entrophospora columbiana* has not been observed in soils with pH more than this. However, other species have a wide tolerance to pH as reported for *Acaulospora scrobiculata*, *A. morrowae*, *A. longula*, *A. spinosa*, *A. myriocarpa*, *G. aggregatum*, *G. versiforme*, and *Scutellospora pellucida* which have been identified in soils with pH ranging from 3.8 to 8.0 (Sieverding, 1991). The effect of metal ions on AMF plant relationships have been examined in a number of studies. Hepper (1979) found that Mn and Zn at concentrations common in soil severely reduced the germination of *G. mossae* and *G. caledonius* on agar paper.

There is increasing evidence that species of AMF are adapted to narrow range of soil pH, thereby affecting the occurrence and distribution of some AMF species. In Western Australia, Abbott and Robson (1977) found that yellow vacuolate spore types (*G. mosseae*) were absent from acid soils where they were replaced by *A. laevis*.

The effects of salinity on the AMF-plant symbiosis appear conflicting. More work is clearly required in this area particularly in the tropics where build up of soil salinity can occur quite easily due to high evaporation rates. AMF infection was found in the roots of pioneer salt marsh species, including two species of Chenopodiaceae at the terminal Gangetic delta of India. Five AMF species were found in the rhizosphere of plants and bioassays determined a low inoculum potential of these species (Sengupta and Chaudhuri, 1990). Increased growth of bell pepper and onion was demonstrated in saline soil by inoculation with AMF (Hirrel and Gerdemann, 1980). A recent review of the effects of soil salinity on AMF reported that, there is clear evidence that the germination of spores and subsequent hyphal growth in some AMF species are reduced by increasing concentrations of salts (Juniper and Abbott, 1993). However, it would appear that more work is required to distinguish between effects on different phases of the fungus life cycle in experiments which are designed to separate the direct effects of salinity from plant-mediated influences on fungal growth and reproduction (Juniper and Abbott, 1993).

There is also evidence that increases in soil salinity changes the species distribution among AMF populations occurring naturally in soil. One of the effects of salinity on plants is to produce physiological drought in plants which cope with high salinity by excluding salts (which removes the problem of ion toxicity but increases the problem of water deficiency). Other plants tolerate salts (which can lead to toxicity and imbalance) (see Baker, 1993). For plants which become deficient in water due to the exclusion of salts, mycorrhizal activity might be expected to ameliorate this affect. Baker (1993) found that, although large differences were not reported above 0 mol m^{-3} NaCl, young *P. juliflora* trees tended to have greater dry weight, increased nodulation, increased acetylene reduction and increased nitrogen accumulation when AMF were present.

1.7.2 Temperature and light

The effects of soil temperature on AMF species distribution, development and function have been investigated. Most tropical soils remain at a relatively constant temperature and here the effects may be lessened compared with those due to seasonal fluctuations in temperate areas. However, in tropical areas, opening of the forest canopy or other loss of vegetation cover results in increases of several degrees of temperature (see Musoko, 1991). Increasing soil temperature normally hastens development of AMF infection (Furlan and Fortin, 1973). Pot experiments have shown mycorrhizal infection development to be strongly temperature dependent. AMF may also exhibit physiological adaptation to different climatic conditions. This is suggested by the work of Schenck *et al.* (1975) who found that isolates of *Glomus* from Florida germinated best at around 34°C, whereas ones from Washington germinated at around 20°C. Since most AMF species have a worldwide distribution, it seems likely that temperature adaptation is common.

Pot experiments have shown that mycorrhizal development is greatly influenced by above ground light incident as well as temperature (Mosse *et al.*, 1981). Light has also been found to influence the effectiveness of plant-AMF relationships. Hayman (1974) found that both light and temperature influenced the development of mycorrhiza and growth of onions. Infection increased in longer day length and higher light intensities with 18 hours at 25,000 lux. By contrast, Furlan and Fortin (1977) reported the converse, that infection developed faster under low light intensities but that spore production increased with higher light intensity.

1.7.2 Soil moisture

Soil moisture similarly has been studied with respect to its' effect on mycorrhizal development and function. Soil water conditions may select for certain species of AMF. Saif (1986) showed that the bulbous reticulate spore type (*Gigaspora* sp.) was only prevalent in soils which had a constant 50-60% water holding capacity. Sieverding (1981) investigated the effects of different water regimes on the development of mycorrhiza, growth and water utilization of mycorrhizal and non-mycorrhizal plants in two soil types. The beneficial effect of mycorrhiza on plant growth was increased in drier water regimes, particularly under conditions of restricted P uptake. The relationship between soil water content and availability of phosphorus for plants demonstrated by Sieverding (1981) reinforces the points discussed in section 1.5.2. on the importance of AMF to plants in dry P deficient soils.

1.7.3 Soil nutrients, fertilizer addition and organic matter

Numerous pot experiments have established that phosphate fertilizer in particular can reduce root infection by AMF (Daft and Nicolson, 1969; Jasper *et al.,* 1979; Smith, 1982; Miranda *et al.*, 1989). Krishna and Bagyaraj (1981) found that infection levels and spore production in sorghum were reduced as concentrations of P increased. In contrast, Miranda *et al.* (1989) found that small additions of phosphorus increased spore numbers and external hyphae in sorghum grown in a phosphorus deficient Brazilian Red Latosol. They found that in the high P treatment the effect on percentage mycorrhizal infection and spore production was detrimental although total length of mycorrhizal root was unaffected. Soedarjo and Habte (1993) showed that fresh organic matter applied to soil could increase mycorrhizal infection and the effectiveness of the symbiosis in *L. leucocephala* by setting off the detrimental effects of aluminium toxicity in soil. A recent study found that plants had lower mycorrhizal infection when farmed conventionally compared with those farmed organically (Ryan *et al.*, 1994). Furthermore, pot experiments indicated that lower mycorrhizal infection was caused by continuous use of phosphorus fertilizer which, in the long- term, had a negative effect of the soil inoculum potential by maintaining high levels of available phosphorus (Ryan *et al.*, 1994).

Most, but not all pot trials and field surveys show that AMF infection is sensitive to added nutrients. However, in a survey by Hayman *et al.* (1976) nutrient levels were found to be unimportant in affecting AMF infection in plants. Host species will react differently however, more importantly is that fact that fungal species and strains occur which are adapted to particular levels of nutrients. The mechanism by which infection is reduced has been investigated and there is strong evidence that root internal P rather than soil P control AMF infection development (Menge *et al.*, 1978) and that nutrients act by controlling the soluble sugar content of the root (Jasper *et al.*, 1979).

1.7.4 Disturbance

Several growth chamber studies have examined the effect of soil disturbance on plants with and without AMF. Those in which plants were grown in disturbed and undisturbed cores of field soil reported higher shoot P absorption and mycorrhizal colonisation in plants in undisturbed soil than those in disturbed soil (O' Halloran *et al.*, 1986; Evans and Miller, 1988). Other studies began with disturbed soil in pots in which plants were grown in a sequence of four cycles with either soil disturbance or not (Fairchild and Miller, 1988; 1990). Findings from these experiments were in agreement with those of O'Halloran *et al* (1986) and Evans and Miller (1988). Studies by Mc Gonigle *et al.* (1990) suggest that frequency of disturbance is important in determining differences in the development of mycorrhizal infection. The effect of disturbance on shoot P was consistent, decreasing with increasing disturbance, irrespective of whether mycorrhizal colonisation was altered. Recent work in which the intensity of disturbance was varied, showed that at high disturbance, shoot dry weights of plants were reduced, mycorrhizal infection in roots decreased and there was a delay in initiation of colonisation (Bellgard, 1993b). Damage to the external network of mycelium was suggested to be an important factor contributing to the loss in infection in plants and that the delay in initiation of colonisation was due to the time required for hyphae to grow from other AMF propagules. The effects of disturbance in the field have also been investigated. Reduced tillage has been shown to favour nutrient uptake by mycorrhizal plants, but other factors may compound these effects. Mulligan *et al.* (1985) found that tilling and traffic (ie: soil compaction by machinery, animals etc.) increased bulk densities of soil and decreased plant growth and root development of *Phaseolus vulgaris.*

1.7.6 Crop rotation, irrigation, weed control, pesticide application

Crop rotation and intercropping are age-old techniques for growing two or more crop species in the same plot. The effect of cropping sequences on mycorrhizal infection and spore populations have been studied and there is a growing amount of evidence to suggest that AMF sporulation is significantly affected by host plant (Dodd *et al.*, 1990b; Black and Tinker, 1979; Schenck and Kinloch, 1980). An *et al.* (1993) found that a stable AMF population was established with continuous cropping of soybean, and that crop rotation disrupted AMF spore populations. Whether this is beneficial to succeeding crops has been investigated in a number of studies.

Dodd *et al.* (1990b) showed that precropping with a tropical grass, cassava, the tropical forage legume kudzu and *Sorghum* sp. significantly increased AMF infection and yield in cowpea and *Stylosanthes capita* in the following season, compared with both crops sown in native savanna. Similarly, AMF infection in cassava was increased when grown in rotation with legumes and a similar effect was achieved by intercropping these two species (Sieverding and Leihner, 1984). Contrary to this, precropping nursery soils with corn, sorghum or millet was effective for increasing mycorrhizal inoculum but ineffective for improving the growth of sweetgum seedlings compared to those seedlings grown in unfumigated soils (Kormanik *et al.*, 1980).

The carry over effect of AMF inoculation after 12 months was reported on the growth of *Lucerne* (Mosse *et al.*, 1981). Yet the carry over effect of introduced AMF on the growth of *Zea mays* (cv. Pagasa) was found to diminish on successive crops planted immediately after *Vigna radiata* (Buwalda *et al.*, 1985; Khadge *et al.*, 1992). Growing AMF host and non host plant species together or in rotation usually results in depression of mycorrhizal inocula for associated or subsequent host species (Hayman *et al.*, 1976; Iqbal and Qureshi, 1976; Harinkumar and Bagyaraj, 1988). Harinkumar and Bagyaraj (1988) showed that a non host species decreased mycorrhizal propagules by 13% whereas fallowing caused a 40% decrease in mycorrhizal propagules in soil. The reason for this is most likely due to the fact that roots were present with the non-host compared to the fallow although whether the fallow treatment was kept clear of plants was not mentioned. Thompson (1987) described a condition he termed 'Long fallow disorder' where crops, such as sunflower and linseed, grown after more than 12 months of fallow had low root infection levels and poor growth compared to those grown after short fallow in the North East belt of Australia.

Other changes in soil caused by treatments such as soil fumigation and biocide application are common practices in agronomic systems. Many treatments kill mycorrhizal fungi and reduce mycorrhizal inoculum potential, while others may be quite harmless depending on the treatment type, dosage and product (Mosse, 1986; Hayman, 1986). It is obvious that the effects of cultural practices on crop response to AMF can differ in each specific case and therefore general conclusions are difficult to formulate. Further study is necessary as results obtained so far in each of the above areas of research are by no means complete.

1.8 The potential of AMF in tropical agroforestry systems

In sub-Saharan Africa, population expansion exerts pressure on marginal arid and semi arid land where plant growth is limited by water stress and soil related constraints. Around 43 % of land in the tropics is deficient in plant nutrients (N, P, K, Ca, Zn, B) toxic in others (Al, Mn) and have high P-fixing capacity (at pH < 5.3 and > 7.5) (Sanchez and Salinas, 1981). Agroforestry systems, (in which woody perennial species (trees, shrubs etc.) are grown in association with herbaceous plants (crops, pasture) and/or livestock in a spatial arrangement and/or rotation), are thought to have great potential for subsistence "low-input" farming in marginal areas. Agroforestry research over the last 15 years has concentrated on screening and selection of multipurpose trees, component- and system management trials, component interaction studies, and prototype evaluation of trials (Nair, 1993).

One of the main tenets of agroforestry is that trees maintain soil fertility. This hypothesis is based on the assumption that efficient transfer of litter to trees observed in natural ecosystems (Vitousek and Sanford, 1986), will likewise occur between trees and crops in agroforestry systems. Observations of higher crop yield near trees (Vandenbelt, 1992), or where trees have been recently removed (Nye and Greenland,

1960) provides evidence to support this hypothesis. The effect of trees on crop growth is actually due to a combination of factors such as water and temperature relations, soil structure, soil organic matter, in addition to recycling of nutrients. A large number of screening and alley cropping trials in different climate-soil environments indicate that prunings of several tree species contain sufficient nutrients to meet the demand of the crop, with the notable exception of P. In addition, less than 20 % of nutrients released from prunings are captured by the crop as indicated in field trials with many tree species (Palm, 1995). In order to meet the P requirements of the crop, more prunings must be added or the concentration of P in prunings must be increased. Both options are limited by the fact that many of the soils in the tropics are P limited (Sanchez, 1976). There is an obvious need to channel research efforts to increasing P cycling and availability in agroforestry systems.

Until recently, agroforestry research has largely ignored the role of AMF on plant nutrition and growth. However, there have been many studies which have shown that agroforestry multipurpose tree and crop species form associations with arbuscular mycorrhizal fungi which are required for efficient uptake of P (Aziz and Sylvia, 1991). A collaborative effort between the two disciplines is now necessary, in which information obtained separately can be used to build a comprehensive research programme for manipulating AMF in agroforestry systems for increased P cyclng and availability as well as improved or sustained growth of component host species. The management of AMF involves either the introduction of more efficient AMF species or the enhancement of the effectiveness of indigenous AMF populations via specific cultural practices. In order to manage indigenous mycorrhiza or introduce a more efficient AMF species into a agroecosystem, evaluation of the current AMF status within a system must be carried out. Research into the effectiveness of the AMF populations or certain AMF species alongside investigations into the effects of cultural practices on indigenous mycorrhiza is a necessary prerequisite to AMF management.

1.9 The present study

This study examines the role of host species and fallow periods on the distribution and abundance of AMF spore populations in a tropical agroforestry system in Kenya. To examine whether changes in AMF spore populations (if found) would have subsequent effects on the growth of *Zea mays*, pot experiments were carried out in which maize was inoculated with soil (containing AMF) from the field site. Results from this experiment (Chapter 4) led to an investigation of the effect of soil type, soil volume and rock phosphate fertilizer on mycorrhizal formation, growth and nutrition of inoculated and uninoculated plants. A few smaller experiments were also established at intervals throughout this study to examine the effect of quantity and type of inocula and two tropical maize varieties on mycorrhizal formation, growth and nutrition of inoculated and uninoculated *Z. mays*. Experimental chapters are set out in a format similar to that of scientific papers, each containing an introduction, materials and methods, results and discussion sections. General materials and methods used throughout the study are described in chapter 2. There is a general discussion and overview with respect to future research in chapter 7.

1.9.1 Hypotheses and Objectives

Hypotheses of the thesis

1. AMF spore population composition is affected by agronomic practices (fallowing compared with intercropping), and component species in a tropical agroforestry system.

2. The potential effectiveness of AMF populations is dependent on component species and agronomic practice under which the AMF originate.

As a consequence of testing these hypotheses, a further series of hypotheses were formulated and tested, that

3. The host endophyte interactions investigated in pot experiments are influenced by soil type, soil volume (pot size) and rock phosphate (RP) fertilizer.

4. The host endophyte interactions investigated in pot experiments are influenced by a possible pathogen introduced through soil inoculum.

5. The host endophyte interactions investigated in pot experiments are influenced by the variety of *Z. mays* L used in experiments.

1.9.2. Objectives of the thesis

1. To compare tree and crop arbuscular mycorrhizal spore populations from fallow and intercropped areas of a tropical agroforestry system (Hypothesis 1).

2. To assess the effectiveness of indigenous mycorrhizal fungi on *Z. mays* using inoculum from tree and alley in fallow and intercropped areas of the tropical agroforestry system (Hypothesis 2).

3. To assess the effect of soil type, volume and applied RP fertilizer on the effectiveness of the symbiosis between *Z. mays* and indigenous mycorrhizal fungi (Hypothesis 3).

4. To access the effect of two local varieties of *Z. mays* on the symbiosis when inoculated with two types of inoculum; a) spores alone (no pathogen) and b) soil (containing AMF spores and possible pathogen) (Hypotheses 4 and 5).

1.9.3 Experimental strategies

The initial part of the thesis looks at the effect of component host species and fallowing on indigenous spore populations of an agroforestry system in Kenya. This involved sampling of AMF spore populations during wet and dry seasons. Experimental work was conducted in tropical glasshouses at the Institute of Terrestrial Ecology, near Edinburgh in the UK. These experiments investigate the effects of inoculating *Z. mays* plants with indigenous AMF from tree and alley in fallow and cropped areas of the Rotational Hedgerow Intercrop (RHI) system. The latter part of the thesis looks at the effects of soil type and volume as well as RP fertilizer on the effectiveness of indigenous AMF on *Z. mays*. This was carried out in order to investigate crop responsiveness as well as to elucidate how glasshouse experiments could be improved.

CHAPTER TWO
MATERIALS AND METHODS

2.1 Substrate preparation and nutrient application

Two soil mixtures were prepared and used as growing media in experiments. Soil mix A contained horticultural vermiculite (medium grade 'vermiperl', Silvaperl Products, Harrogate), sand, grit and loam mixed in the ratio 8:1:1:2 (v/v), and soil mix B was prepared by mixing sand, grit and loam in the ratio 1:1:1 (v/v). Loam used in the preparation of both soil mixtures came from a field near the Institute of Terrestrial Ecology (I.T.E.) in Edinburgh, Scotland. Field soil from Kenya which was collected on two occasions in 1991 for examination of indigenous spore populations in selected agroforestry systems, and loam used in pot soil mixtures used in experiments were chemically analysed, and the results are presented in Chapter 4.

Mycorrhizal populations occurring naturally in soil mixtures were eliminated when necessary by autoclaving at a temperature of 121°C and pressure of 1.03×10^5 Nm^{-2} for two hours. This method of soil sterilization has been tried and tested through years of mycorrhizal research carried out at the I.T.E. research station. In certain experiments various quantities of modified Ingestad's (Ingestad and Lund, 1986) nutrient solution (Table 2.1) were added once weekly to pots according to the experimental requirements.

In the modified nutrient solution, phosphorus ($K_2H_2PO_4$) was removed from the standard Ingestad's nutrient solution to give a basal solution containing all other nutrients except phosphorus. Plants receiving this treatment were grown in media with the only source of phosphorus being the small quantity contained in the loam of the soil mix. To compensate for the parallel reduction in potassium, K_2SO_4 was increased from 14.0 g l^{-1} in conventional Ingestads' to 40.4g l^{-1} which resulted in an increase in sulphur of 16 ppm in solution (Table 2.1).

Table 2.1 Modified Ingestad's Nutrient Solution applied to soil mixes in experiments 4 and 5

CHEMICAL	CONCENTRATION g l^{-1}
NH_4NO_3	140.2
KNO_3	37.2
K_2SO_4	40.4
HNO_3	1.6
H_3BO_3	0.57
$Fe_2(SO_4)_3$	2.5
$Ca(NO_3)_2.6H_2O$	20.58
$Mg(NO_3)_2.6H_2O$	44.92
$MnSO_4.4H_2O$	0.81
$CuCl_2.2H_2O$	0.043
$ZnSO_4.7H_2O$	0.064
$Na_2MoO_4.2H_2O$	0.008

2.2 Plant material

Zea mays L. is a widely distributed food crop commonly grown in alley cropping systems in Kenya, Eastern Africa. Seed of *Z. mays* (local variety, Katumani composite and Hybrid 11) were obtained from the International Centre for Research in Agroforestry (I.C.R.A.F.) field station at Machakos in Kenya. Katumani composite seed was used in earlier experiments (experiments one, two and three). In experiment four seed of Hybrid 11 was used due to a shortage in supply of Katumani composite seed. The local variety, Katumani composite grown in the field at Machakos is a short duration variety, highly adapted to the short growing season. Hybrids being genrally longer in their duration are not grown in the Machakos area. Experiment five contained each of the two seed varieties. Seed was stored at I.T.E. in a cold room at a temperature of 4°C. Maize seed was germinated in seed trays or individual pots (5 cm diameter) filled with the soil mix to be used in the experiment. Seven to 12 days after germination, seedlings of similar height were selected and inoculated with mycorrhizal fungi according to experimental requirements.

Plants were grown in the glasshouse where a mean temperature of 25°C (range 16°C-34°C) was maintained by automatic heating and cooling systems. A minimum day length of 12 hours was simulated using high pressure mercury vapour lamps (400 W MCFR/U). Approximately two weeks before experiments were set up, the glasshouse floor was sterilized with a dilute solution (7 ml l^{-1} H_2O) of Jeyes fluid (Jeyes Ltd. Thetford, Norfolk, England). Pots and saucers were surface sterilised by soaking in a solution (2 g 4.5 l^{-1} H_20) Bruclens sterilizer (Brewmaker plc., Southampton : chlorine 7%w/w) solution for 24 hours.

2.3 Production of inoculum

AMF inoculum collected from field sites in Kenya at the beginning of the rainy season during November, 1991 was used to establish trap cultures and inoculate plants in experiments. Inoculation was carried out under semi-aseptic conditions on benches in the glasshouse. To establish a trap culture, 100g of field soil (containing spores, roots and mycelium) was placed as a layer beneath a *Z. mays* (var. Katumani composite) seedling in a 10 cm diameter pot (1 litre) filled with autoclaved soil mix (vermiculite, sand, grit and loam). Trap cultures were positioned on benches separate from other glasshouse experiments with between-pot spacing of 10 cm. Watering was carried out carefully in order to prevent cross contamination between pots. Additional trap cultures were established every 16-20 weeks using soil (containing root, mycelium and spores) taken from previous cultures.

2.4 Plant harvests

Destructive harvesting was carried out at various times within each experiment (see materials and methods : Chapters 4, 5 and 6). Shoots were removed at the root collar, placed in labelled paper bags and dried in an oven at 80°C for about 3 days depending on sample weight. To aid the loosening of soil around the root systems, pots containing soil and root systems were submerged in water for 5 - 10 minutes, then washed through a sieve with mesh size 250 μm or through two sieves with mesh sizes 2000 μm and 250 μm respectively, depending on the volume of soil. To comply with regulations governing the import of soil to the U.K. and soil used in experiments, soil collected in each sieve was bagged and autoclaved before disposal. Washed root systems were stored in sealed polythene bags for a maximum of 3 days before sub-sampling and drying.

2.5 Mycorrhizal infection assessment

Roots were randomly sampled. Clearing and staining of root samples for mycorrhizal assessment was carried out following the procedure outlined by Koske and Gemma (1989). A modification of a slide technique described by Giovannetti and Mosse (1980) was used to quantify mycorrhizal infection. A wet weight was obtained from the washed root system which was then cut into 1 cm length root pieces. These were placed in water and dispersed, then transferred to a tray on which 100 random dots had been marked. Roots overlying dots on the tray were removed and weighed. The remaining root was dried at 80°C until a constant dry weight was recorded. Estimated total root dry weights were obtained using the total wet weight, the sub-sample wet weight and the dry weight of the root system after sub-sampling.

Root samples were stored in distilled water in the cold room for a maximum of 3 days before staining. Roots were covered with 2.5% potassium hydroxide (KOH) and autoclaved for 3 minutes at 121°C and 1.03×10^5 Nm^{-2}. KOH was removed by washing samples in water. Roots were bleached using alkaline hydrogen peroxide (3 ml of 30% ammonia, 10 ml H_2O_2 and 587 ml H_20) for 30 minutes, washed with water, then soaked in 1% hydrochloric acid (HCl) for 1 hour. HCl was drained off and roots were stained in 0.05% trypan blue in acidic glycerol (500 ml glycerol, 50 ml 1% HCl and 450 ml water) by autoclaving samples for 3 minutes. Root samples were left in the stain for at least 12 hours and then stored in acidic glycerol. After staining, the root samples were mounted on slides, 20 per slide. Roots were examined using a compound microscope (X 150 magnification) fitted with a calibrated eye piece graticule. Each graticule unit represented 0.1 cm of root length. The total length of root infected with mycorrhizal hyphae, and/or arbuscules and/or vesicles was recorded and expressed as a percentage of the whole root length. The presence of vesicles and arbuscules was also recorded.

2.6. Assessment of mycorrhizal spores

Spores occurring in soil around the roots of *Z. mays* and *S. siamea* were extracted for identification of the dominant species in the field. Identification of mycorrhizal species requires much time and experience in the area of fungal taxonomy. Because this particular research project was primarily concerned with plant-mycorrhizal growth responses in the glasshouse environment, time spent on spore assessment was limited. Where possible, mycorrhizal species producing high numbers of spores in the field and glasshouse were identified to the species level but those with lower spore numbers could only be identified to genus.

2.6.1 Spore extraction

Spore populations were extracted from soil taken from agroforestry sites in Kenya. Soil was sampled in the field (See Chapter 3 for details) and further subsampled (50 g soil sample) for spore extraction and drying. Soil was dried so that spore numbers could be expressed as the number 50 g^{-1} dry soil.

One of the earliest methods of spores extraction is the wet sieving and decanting method devised by Gerdemann and Nicolson (1963). It involves the use of different sized sieves (710 µm - 45 µm) to collect spores from known quantities of soil and tap water. Spore numbers are easily underestimated using this technique, as spores are often obscured from view by debris. However the spores are not damaged and remain viable for use in starter pot cultures.

Another method, the plate method of Smith and Skipper (1979) is slow, but more accurate for soils with high spore numbers (> 20 gm^{-1} soil) (Daniels and Skipper, 1982). The adhesion flotation method (Sutton and Barren, 1972), where a mixture of soil and water is poured through a separating funnel and spores adhere to the sides has the problem that larger spores which sink in water may be lost leading to an underestimate of spore numbers. Another extraction method (sedimentation differentiation) suitable for small samples and developed by Mosse and Jones (1968) involves the use of gelatin columns in which spores settle differentially depending on spore size and gradient.

A more efficient method of spore extraction, developed by Furlan *et al.* (1980), is known as the density gradient centrifugation method. It uses sucrose gradients where a layer of 50% sucrose is placed in a 50 ml centrifuge tube and another layer of 25% sucrose is placed upon the 50% layer. A soil sieving suspension is added carefully to the centrifuge tube and spun at 3100 rpm for 5 minutes. Spores collected in the 25% layer are removed using a syringe and transferred to a fine sieve then washed with tap water. This method gives clear spore suspensions for easy and accurate enumeration. It is noted however, that sucrose exerts an osmotic shock on the spores which could be damaged especially if they remain in sucrose for long periods. Furlan *et al.* (1980) suggested the use of radiopaque media to reduce osmotic shock, which Verkade (1988) substituted with a low cost silica solution (Ludoc, T.M. Manufactured by E.I. Dupont).

A method developed in 1964 by Jenkins known as the sucrose centrifugation method is similar to that of the layer density gradient centrifugation (Furlan *et al.*, 1980) but uses a single sucrose concentration (45 - 50%) instead. Larger quantities of soil (100 - 500 cm^3) are wet sieved and decanted through 2 sieves (710 µm and 45 µm respectively). The large sieve collects large debris ie., roots and small stones and the smaller sieve traps spores while allowing fine silt and sand to go through. The residue collected in the fine sieve is placed in centrifuge tubes and pelleted. Pellets are mixed with the sucrose solution and centrifuged again. Spores are suspended in the supernatant which is collected in a 45 µm sieve and washed with tap water. For the purpose of this study methods such as wet sieving and decanting, the plate method, differential sedimentation and adhesion flotation are time consuming and would (because of debris in samples) be likely to introduce error into the spore counts. As samples used for spore counts would not be used in experiments the choice was between the two centrifugation methods. The less time consuming of the two (sucrose centrifugation), was used in this study as it employed only one sucrose gradient.

Sucrose centrifugation method

Soil (50g) was placed in a 4 litre container with water and stirred vigorously to bring spores into suspension. The solution was allowed to stand for 15 seconds to let soil particles settle out of the water column. Water (containing spores) was decanted through sieves of mesh sizes 710 μm and 45 μm and the whole process was repeated four times (established by preliminary test) to ensure that a maximum number of spores was collected. Soil collected in the 45 μm mesh was rinsed into centrifuge tubes and placed in a horizontal rotor centrifuge for 5 minutes at 1750 rpm (revolutions per minute). The supernatant was decanted off while the plugged soil containing spores was resuspended in sucrose solution (227 g sucrose in 500 ml water). This mixture was centrifuged at 1750rpm for 15 seconds. The supernatant containing suspended spores was poured through a 45 μm sieve. Sucrose solution was removed by rinsing with water and the spore sample was collected in a petri dish for assessment.

2.6.2 Spore identification

Spores were placed under a dissecting microscope and examined at x 6-50 magnification. Spores were described according to a list of characteristics (eg: spore colour, diameter (μm), hyphal attachment : see Chapter Three for details) and similar spore types were grouped together. Whenever possible, 50 spores were taken from within each spore group and mounted in polyvinylactoglycerol (PVLG) with and without Meltzer's Reagent. Spore characteristics were examined under the compound microscope at x 150-1500 magnification. Spores were crushed and the spore wall was examined under oil immersion (x 1500 magnification).

Spore counts taken from extracted samples have been carried out in a number of different ways. Some methods involve spore counts of the whole population (Perez, 1987; Walker, 1979) while others count only a portion of the total number of spores in order to estimate a total (Daniels and Skipper, 1982; McKenny and Lindsey, 1987). Perez (1987) counted total spore numbers in a round petri dish (5.5 cm diameter) scored into parallel lines and systematically scanned under a dissecting microscope. Walker (1979) made similar counts in a petri dish scored into squares after which he removed a subsample of 50 spores (< 100 μm) and mounted them in lactophenol to make closer examination of each spore type. This enabled an estimation of the total number of spores of each type to be made. With the eelworm counting slide, Daniels and Skipper (1982) made counts of spores from 1 ml spore suspensions. The number of spores in 1 ml was used to estimate the total number of spores in the population from which the subsample was taken. McKenny and Lindsey (1987) used 20 ml spore suspension, placed on a 0.45 μm membrane filter marked with square grids and pulled under vacuum to give even spread before counting.

Similar, subsampling trials were carried out when testing potential methodologies for use in this study. Spore and water suspensions were sub-sampled using a plunger sampling pipette (Henson), normally used for plankton sub-sampling in the laboratories of Institute of Freshwater Ecology (I.F.E). Subsampling spore suspensions resulted in the loss of larger spores from the sample which sink rapidly in the water column. Of all the methods described above, that by Perez (1987) was used in this study because it would enable one to examine the whole spore population, and was thought to be the most accurate and simple technique. Spore samples were examined in a Petri dish containing a small quantity of water. Spores were categorized as "Live" or "dead" according to the following characteristics. Live spores were identified by their smooth shiny appearance and globular oily contents. Dead spores were identified by their degraded appearance and lack of inner contents. Dead spores were either empty or contained an air bubble in place of the usual translucent liquid contents of a fresh live spore. The majority of dead spores, however, floated to the surface of the water and adhered to the sides of the Petri dish. These were included in the dead spore count.

2.7 Plant and soil nutrient analysis

Plant and soil nutrient analysis was carried out at the University of Edinburgh laboratories. K, Ca and Mg cation concentrations in solution were measured using a Unicam 919 atomic absorption spectrophotometer with flame emission. N and P concentrations were determined using a flow injection analysis using a 5023 Fia Star Spectrophotometer (Injector L 100 l, 30 μl - chemifold type V) with a 5020 analyzer and 5007 sampler.

Shoot and root material was dried, ground and stored in sealed envelopes inside boxes in a dry area. Immediately prior to chemical analysis, samples were dried at 80˚C for 24 hours and remained in a desiccator until weighing for digestion. Approximately 0.1 g samples were weighed accurately. Samples were digested in 2 ml concentrated 18 M sulphuric acid (H_2SO_4) and 3 ml (2 x 1.5 ml aliquots) 100 vol hydrogen peroxide (H_2O_2) at 350˚C for 6 hours. Samples were made up to 50 ml with distilled water and transferred to 50 ml volume flasks following the methods of Allen (1974).

Soil was air dried and passed through a sieve with mesh size 2 mm. 5 g soil samples were weighed and placed in 250 ml bottles. 100 ml of 2% acetic acid (CH_3COOH) was added to each soil sample. Two blanks were prepared with extractant only. Samples were shaken for 2 hours and then filtered under vacumn through Whatman paper No. 44 into polythene sample tubes. The first 15 ml of each sample was rejected. Standard solutions were prepared with the 3.6 ml H_2SO_4 and with KCl, NH_4Cl, KH_2PO_4, Ca and Mg with the following concentrations.

Plant	**Soil**
N 15, 30, 45, 60 mg l^{-1}	NH_4-N 2, 4, 6 mg l^{-1}
P 1.5, 3.0, 4.5, 6.0 mg l^{-1}	NO_3-N 1, 2, 3 mg l^{-1}
K 10, 20, 30, 40 mg l^{-1}	P 1, 2 mg l^{-1}
Ca 0.5, 1.0, 2.0, 4.0 mg l^{-1}	K 10, 20, 30 mg l^{-1}
Mg 2.0, 4.0, 6.0 mg l^{-1}	Mg 4, 8, 12 mg l^{-1}
	Ca 10, 20, 30 mg l^{-1}

2.8 Data analysis and computer programs

Experiments were set up as randomised block designs and were analysed using one-way and two-way analysis of variance (ANOVA). When necessary, data were transformed to produce a normal distribution. Angular transformations on percentage mycorrhizal infection and root:shoot ratio data, and square root transformations on spore counts were used. The suitability of data for ANOVA (fulfilment of the assumptions of normal distribution and homogeneity of variances) was tested using applications within the Genstat program used for statistical analysis. Least significant differences were calculated to test differences between means when the Fishers F-test value indicated differences to be significant. Genstat 5 (Lawes Agricultural Trust, Rothamsted) was used for analysis for ANOVA. Sigmaplot and Stanford Graphics software were used to produce graphics.

CHAPTER THREE

AMF SPORE POPULATIONS IN AN AGROFORESTRY SYSTEM AT ICRAFS' FIELD RESEARCH STATION IN KENYA, EASTERN AFRICA.

3.1 Introduction

There are many reports of AMF inoculation increasing plant growth by enhancing the uptake of phosphorus (Gianinazzi-Pearson and Gianinazzi, 1983). In the field, elimination of the indigenous AMF populations by soil sterilization and introduction of highly effective AMF species has been found to increase the growth of plants such as, maize, cowpea (Islam and Ayanaba, 1981) citrus (Menge *et al.*, 1977) and cassava (Howeler *et al.*, 1982). However, introduction of highly effective AMF when indigenous AMF populations are present in soil has been less successful at improving plant growth (Khan, 1972; Saif and Khan, 1977).

Many studies report that different AMF species or strains have different effects on the growth of individual plant species. As well, different plant species, varieties or cultivars have been found to respond to and affect associated AMF differently (see section 1.6.1). AMF are affected by many other environmental factors (see section 1.7) and in agroecosystems, practices such as logging, fertilizer application, pesticide application, crop rotation, fallowing, burning, cover cropping etc., have been found to significantly alter AMF spore populations in soil. Changes in plant growth have been observed following changes in AMF spore populations (Dodd *et al.*, 1990a; Dodd *et al.*, 1990b). Many agronomic practices such as long fallow and logging have detrimental effects on AMF and subsequent plant growth (Thompson, 1987; Musoko, 1990) however, with this knowledge, such practices can be modified or avoided altogether. Other agronomic practices which enhance AMF populations (such as, pre-cropping with selected favourable species) could result in the successful management of AMF populations for improved plant growth (Dodd *et al.*, 1990a; Dodd *et* al., 1990b).

In the tropics, soil erosion, laterisation, desertification, salinity and deforestation affect the stability of tropical agroecosystems (Mosse, 1986). At present, forests are extensively exploited and cleared to meet demands for forest products (wood, fibre energy) and for agricultural, urban and industrial expansion (Evans, 1982) all of which have accelerated as populations and their rates of consumption increase.

Population pressure causes continuous conversion of forests to agricultural lands and the undesirable consequences of this are that highly productive land is degraded, floods increase in number and intensity, desertification accelerates, soil fertility is reduced and there is loss of genetic resources as well as loss of wildlife. In 1986, the FAO (Food and Agriculture Organization) forecast that by the year 2000, 2% of the agricultural land available since 1986 will have become unstable due to desertification, erosion and salinity. Consequently, food production in developing countries where 87% of the world's population will shortly be living, is a major problem.

One strategy to improve this is to intensify farming only on suitable land, another is to increase subsistence farming over an extended area including marginal land, where for climatic or edaphic reasons production cannot reach above 20 - 40% of maximum yield (Mosse, 1986). In such areas, sustainable land use systems such as agroforestry are receiving much attention. It is vital that agroforestry research programmes incorporate AMF research, in marginal areas with low P soils where the greatest effects of AMF on plant growth can be achieved. There is relatively little known about the ecology of AMF in tropical agroforestry systems compared to that in temperate ecosystems. The objectives of this study were to examine the effects of host species and fallowing on the indigenous AMF spore populations in a tropical agroforestry system.

3.2 Materials and Methods

Field surveys of AMF often involve examination of spores which are relatively easy to count but more importantly are the only stage of the life cycle of AMF which can be used to identify species. Recent developments could enable the use of highly sophisticated techniques to identify AMF species at the molecular level (Tuinen *et al.*, 1994). The use of spores as indicators of AMF populations in any ecosystem is associated with several disadvantages. Firstly, not all AMF species sporulate (Read *et al.*, 1976; Sparling and Tinker, 1978) secondly, a high level of expertise and experience is required in proper identification of AMF spores, and thirdly, spore characteristics used to identify species are often very variable (Morton, 1988). Attempts to develop techniques to distinguish between species of AMF using extramatrical or infecting hyphae (Morton, 1988; Abbott *et al.*, 1984; Kough and Lindermann, 1986) have been less successful. AMF hyphae in contrast to spore material have proved more difficult to quantify and identify. However, recent rhizobox studies have successfully quantified AMF hyphae from different species (Green *et al.*, 1994). Many spore extraction techniques have been developed and are easily adopted (Furlan *et al.*, 1980; Porter, 1982). These have been reviewed (Verkade, 1988; Walker, 1983, 1986; Berch and Koske, 1986; Morton, 1988; Schenck and Perez, 1987) and are discussed later in this chapter. After considering these factors, it was concluded that a survey of AMF spore distribution and abundance provided the best available option for studying AMF in the field.

This survey of AMF spore populations was carried out to test the hypothesis that AMF spore populations in tropical agroforestry systems are affected by agronomic practices and component host species. Spores of AMF were used as **indicators** of changes in the distribution and abundance of AMF populations within and between agroforestry systems. It is recognised that other propagules of AMF exist apart from spores, however, as previously discussed, these are more difficult to quantify and identify. Therefore, a preliminary study of AMF spores was carried out as a working investigation necessary in order to justify the experiments which were proposed to follow. It was not the intention to produce a detailed taxonomic spore survey which, although desirable, would have required more expertise, time and additional visits to Kenya for voucher specimens.

3.2.1 Site description

Soil samples (containing indigenous AMF spores) were taken from the Rotational Hedgerow Intercrop agroforestry system at the field research station of the International Center for Research in Agroforestry (ICRAF). The station is 70 Km southeast of Nairobi and 7 km south of Machakos town, in the Machakos district of Eastern Kenya. It is situated 1600 m above sea level in a semi-arid to sub-humid area at a latitude of 1°33'S and longitude of 37°14'E (see Figure 3.1). Average annual rainfall (740 mm) is distributed between two rainy seasons occurring from 20 March to 31 May (345 mm) (long rains) and 20 October to 20 December (315 mm) (short rains). The predominant soil type at the site is a well-drained, dark reddish-brown sandy clay. Soil pH is 6.0-6.5 with low to moderate levels of organic matter (top soil organic carbon: 1-1.5%), nitrogen and phosphorus and moderate amounts of other nutrients (Rao, 1990). The field research station has agroforestry trials and demonstrations with different tree and crop species combinations. Soil was sampled from the Rotational Hedgerow Intercrop system containing *Senna siamea* and *Zea mays*. (Plate 3.1).

The Rotational Hedgerow Intercrop (RHI) system

The RHI system was established at Machakos field station in October, 1986 on a plot 31 m in length and 4 m wide. It was planted with *Senna (Cassia) siamea* Lam. and intercropped with cowpea (*V. unguiculata*) for the first 5 growing seasons until April, 1988. *S. siamea* trees were planted in rows (4 m in length) with 4 m inter-row spacing. They were maintained as hedgerows (1 m high) by pruning on four occasions in each year. The first pruning was at the beginning of each rainy season and the second pruning mid-season about 6 to 8 weeks after crop sowing. Prunnings from the first harvest were incorporated into the soil manually while those from the second harvest were spread on the soil surface in the alleys of the system. No other fertilizers were used in this system.

In April 1988, trees in one half of the plot were intercropped with *Z. mays* and maintained as hedgerows. In the other half of the plot, trees grew unchecked as a tree fallow system in which alleys were colonized by pioneering plant species such as grasses and other weed species. In this half of the plot, trees grew tall reaching up to 3 to 4 meters during the fallow phase. This growth of trees spread into the alley areas, eventually smothering weed growth. After 3 growing seasons at the end of 1989, the practices in each end of the system were reversed. Sampling of the site for the purpose of this study was carried out following a further 3 growing seasons in February, 1991 (dry season) and after another growing season (April - October) at the start of the long rainy season in November, 1991. On both sampling occasions soil was removed from hedgerow and alley areas in both the fallow and intercropped ends of the plot.

At the time of sampling in February, 1991, maize plants were mature, having grown for approximately 110 days. Maize require 100 days to reach maturity in the field in this area. In the fallow area, trees were approximately 2 m in height and in alleys weed and grass species occurred at this time, although these were generally wilted and sparsely distributed.

In November, 1991, in the cropped area of the system, trees were recently pruned (at end of the dry season) to 1 m in height and in alleys, young maize plants (14 days after sowing) were growing vigorously. In the fallow area, trees remained unpruned, were growing vigorously (approximately 4 m in height) and were beginning to smother weed growth in alleys at this time (See Plate 3.1).

The fallow and cropping phases were maintained for two years in each end of the system. Leaf litter and standing foliar biomass at the harvest of the fallow after the two years was incorporated into soil on pruning the trees for cropping. Sennas are by and large evergreen and do not loose leaves as a distinct phenological character. However, in the fallow end of the system trees did loose leaves due to water stress and normal senescence. The water stress in Machakos is greatest in the second dry period of the year from June to mid-October. Trees do not loose leaves when maintained as hedgerows.

Plate 3.1. Rotational Hedgerow Intercrop system, November, 1991. Beginning of wet season.

Intercropped area

Fallow area

3.2.2 Field sampling

Prior to sampling careful consideration was given to the type of sampling technique which was to be used. Heterogeneity of spore distribution has been reported in numerous tropical studies (Redhead, 1968; Alexander *et al.*, 1991; Musoko, 1991) and to minimise the effects of this it was proposed that a large number of samples were taken. However, this conflicted with the practical considerations of transportation to the UK so, to overcome this, the number of replicates were reduced and bulking of composite samples followed by subsampling was carried out. Bulking, despite causing a reduction in the statistical information about the spatial distribution of propagules (Southwood, 1966; Green, 1979) at each plant, was carried out because loss of this information was less important than information gained about the overall distribution of the AMF population and its response to host species and fallowing.

Factors such as soil depth, season and associated vegetation were considered when sampling. AMF spores are concentrated in the top horizons (20 cm) of the soil profile (Sutton and Barron, 1972) where fine roots are abundant. Seasonal changes in AMF spore populations have been widely reported (Hayman, 1970; Koske and Halvorsen, 1981; Walker *et al.*, 1982; Giovannetti, 1985). Therefore it was important to sample more than once and during different seasons or at several stages in the life cycle of the botanical composition of the site (e.g. Period of active root and shoot growth and periods of flowering and seed production). Finally, associated vegetation is found to have an affect on the spore populations which occur. Therefore soil was sampled within the RHI system from tree and alley areas in fallow and cropped areas. In cropped areas *Z. mays* grew in alleys, while in fallow areas, which were not 'clean-fallows', pioneer species were beginning to colonize the alley area in the dry season and were vigorously growing in this area in the wet season of the same year (see Plate 3.1). Sampling was carried out at this site on both these occasions.

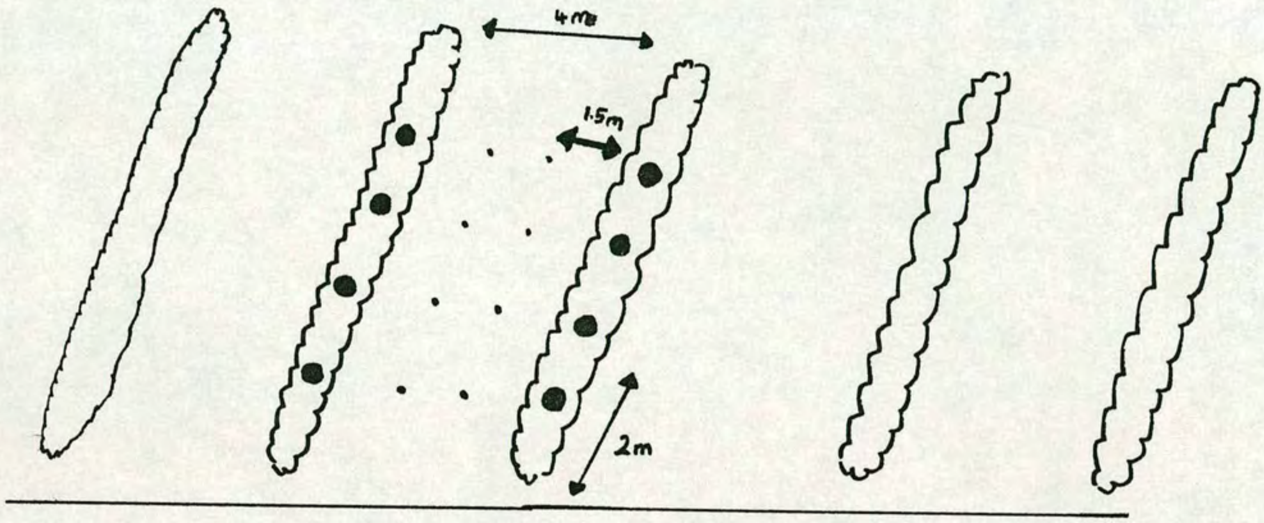
Mycorrhizal spore populations were sampled using a design of field sampling which would enable comparative statistical analysis between treatments at each site. On both sampling occasions the field sample layout was similar, but replication varied. Samples were taken along transects across the middle alley of fallow and intercropped areas within the R.H.I system. The first transect was positioned 2 m away from the end of the alley. Each of the remaining transects were spaced 2 m from each other along the alley coinciding with the planting points of tree or crops. Soil samples were collected from 0-20 cm depth in the soil profile and a distance of 10 cm from either side of the tree or crop stem. Two 50 g samples were removed from either side of the plant stem were placed together in plastic bags which were sealed and labelled. Samples were kept in the shade until they were transported to the National Museums of Kenya (NMK) where they were stored at room temperature.

Soil samples were mixed thoroughly before two 50 g (fresh weight) samples were removed. One sample was air-dried then weighed while the other was used for the extraction of spore propagules. The dry soil weight was then used to convert 'wet' spore numbers to number of spores 50 g^{-1} dry soil.

Soil was transported under the soil importation licence No. IP/MISC/4/91, The Scottish Office Agriculture and Fisheries Department Plant Health Act 1967 and The Plant Health (Great Britain) Order 1987.

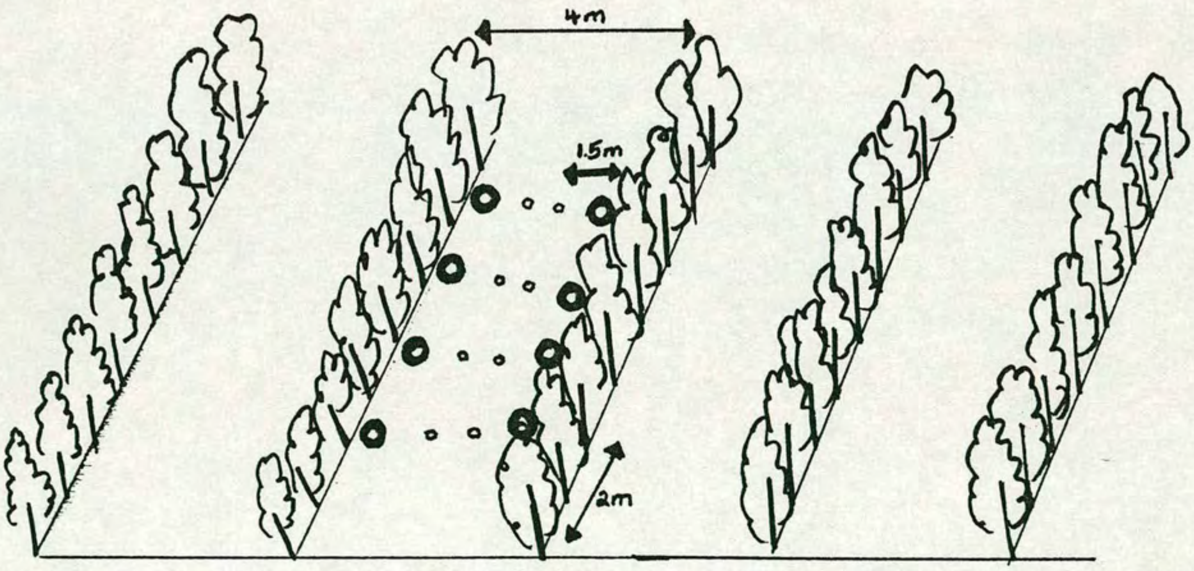
Spore extractions were carried out at NMK and returned to the U.K and examined under the microscope in the mycorrhiza laboratory at I.T.E . Fresh soil samples (100 g remaining after subsampling) were used as inoculum in glasshouse experiments. Soil remaining from field samples collected in March, 1991 was bulked and air-dried. This soil was passed through a 2 mm sieve and used for chemical analysis. Soil nutrient analysis was carried out in the Chemical Analysis Laboratory at the Institute of Ecology and Resource Management, University of Edinburgh.

Field design and soil sampling layout in the RHI system at Machakos, Kenya.



Cropped end

- TC soil sample
- AC soil sample



Fallow end

- TF soil sample
- AF soil sample

3.3 Results

3.3.1 AMF spore numbers. February, 1991. (End of dry season).

Analysis of mean spore numbers using square root transformations and percentage spore numbers using angular transformations as recommended by Sokal and Rohlf (1980) were carried out. There was a significant ($P = 0.011$) interaction effect between host and management on total spore numbers in soil in February, 1991 (End of dry season). The total number of spores in fallow areas was significantly higher than that in cropped areas at this time (Table 3.2; Figure 3.1a). A significant ($P = 0.007$) interaction effect between host and management on number of live spores was found (Table 3.2). Live spore numbers were also significantly higher in fallow than in cropped areas of the system. However, in the cropped areas live spore numbers were found to be significantly higher in alley than in tree soil (Figure 3.3b). Dead spores were also significantly ($P < 0.001$) higher in number in fallow than in cropped areas of the system (Table 3.2; Figure 3.1c). The proportion of dead spores represented as a percentage of the total number of spores was similar in all samples (Table 3.2; Figure 3.1d).

3.3.2 AMF spore numbers. November, 1991 (Beginning of wet season).

In November, 1991, there were significant interaction ($P < 0.001$) effects between host species and management on total and live spore numbers. Total number of spores were significantly higher in tree soil in the cropped area than in all other soil samples (Table 3.2; Figure 3.2a). In the cropped area, alley soil had significantly higher total spore numbers than alley soil in the fallow area (Figure 3.2a). Live spore numbers were significantly higher in tree soil from the cropped area of the system compared to all other samples (Table 3.2; Figure 3.2b). The number of dead spores was similar in all soil samples (Figure 3.2c), however the proportion of dead spores in soil was significantly lower in tree soil in the cropped area compared to all other areas (Table 3.2; Figure 3.2d).

Table 3.1. Significance of F-values in the analysis of variance in total (live+ dead), live, dead and % dead spore numbers in soil from the Rotational Hedgerow Intercrop system sampled in February (dry season) and November (wet season), 1991.

Treatment	Total	Live	Dead	%Dead
February 1991				
Host	ns	ns	ns	ns
Management	***	***	***	ns
Host.Manage	*	**	ns	ns
November 1991				
Host	***	***	ns	**
Management	ns	**	*	**
Host.Manage	***	***	ns	**

*** P < 0.001 ** P < 0.05 * P < 0.01 ns not significant

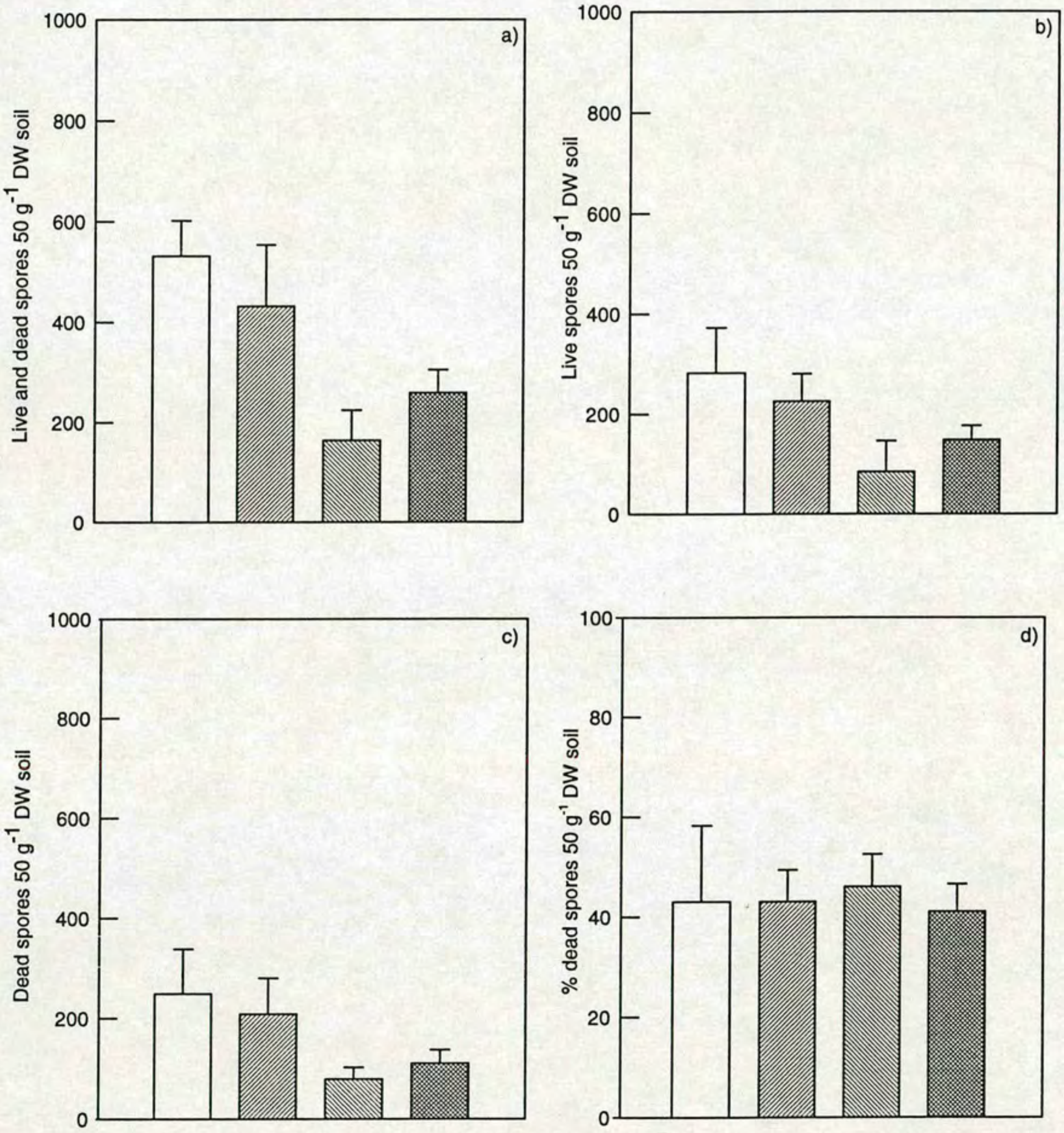


Figure 3.1. Effects of host species and fallowing on the mean number of a) total, b) live, c) dead and d) % dead spores 50 g^{-1} of dry soil from the Rotational Hedgerow Intercrop system at ICRAF's field station, Machakos. February, 1991(dry season).
Means of 8 soil samples in each area. Bars = + S.E. (P = 0.05). ▭ Tree zone / fallow area ▨ Alley zone / fallow area ▧ Tree zone / cropped area ▩ Alley zone / cropped area. (Note different scales for graphs)

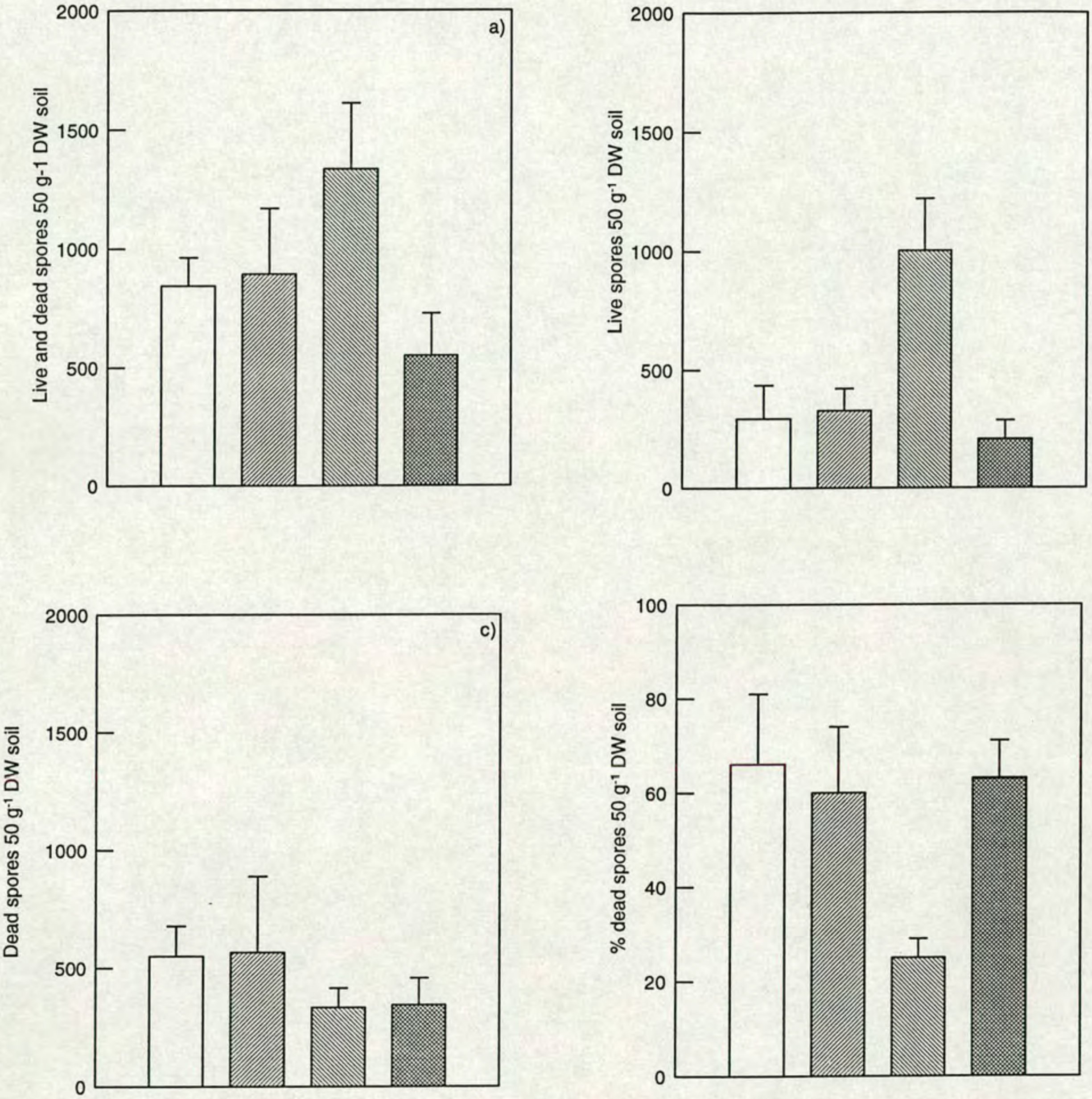


Figure 3.2. Effects of host species and fallowing on the mean number of a) total, b) live, c) dead and d) % dead spores 50 g^{-1} of dry soil from the Rotational Hedgerow Intercrop system at ICRAF's field station, Machakos. November, 1991 (wet season). Means of 5 soil samples in each area. Bars = + S.E. (P = 0.05). Tree zone / fallow area. Alley zone / fallow area. Tree zone / cropped area. Alley zone / cropped area. (Note different scales for graphs)

3.3.3 Seasonal differences in AMF spore numbers

Total number of AMF spores found in soil sampled at the end of the dry season ranged from 150 to 560 50 g^{-1} of dry soil. At this time, live spore numbers ranged from 85 to 283 g^{-1} of dry soil and dead spore numbers from 79 to 249 g^{-1} of dry soil. Between 41 % and 46 % of the spore populations found in soil at this time were dead.

At the start of the wet season in November, 1991, the number of spores in soil were higher than at the earlier sampling time. Total spore numbers ranged from 551 to 1335 50 g^{-1} of dry soil, live spore numbers from 207 to 1002 50 g^{-1} of dry soil and dead spore numbers from 333 to 566 50 g^{-1} of dry soil. The increase in spore number between seasons was greatest in tree soil in the cropped area of the system where total, live and dead spore numbers increased.

The total number of spores in alley soil in this area increased between seasons, but to a lesser degree from 200 to 400 50 g^{-1} of dry soil, as did tree (560 to 800 50 g^{-1} of dry soil) and alley (400 to 800 50 g^{-1} of dry soil) soil in the fallow area. Live spore numbers in these areas changed very little and in the fallow alley decreased a little between seasons. In February, the proportion of dead spores in soil samples was lower than that found in soil in November. At this time, the proportion of dead spores in soil was more than 60% in all but those samples taken from tree soil in the cropped area where the percentage of spores which were dead was only 25 %. Between season statistical analysis was not carried out as the two sample collections were not comparable due to sampling layout designs with unequal replication and blocking structure between sampling occasions.

3.3.4 Identification of the dominant spore types

A range of spores varying in morphology were recovered from soil in the RHI system in February and November, 1991. Four spore types, belonging to the genus *Glomus* (types t3, t10a, t10b) and genus *Acaulospora* (type t24), representing more than 87% of live spores, were recovered from the RHI system in February, 1991. The most dominant spore type, *Glomus* t3, represented 40 % of the live spore population, while *Glomus* t10a, *Glomus* t10b and *Acaulospora* t24 represented 28 %, 10 % and 10% respectively. Spores of the genus *Scutellospora* were also found in soil although in very small numbers.

Spores of *Glomus* t3 similar to the species *Glomus etunicatum* Becker and Gerdemann are shown in plate 3.2. They were 100-120 µm in size, globose to subglobose and sometimes irregular in shape, formed singly in soil, pale yellow to sienna in colour, oily globular contents with a roughened surface. Subtending hyphae were found attached, were lighter in colour than the spore, 8.0 µm thick, 100-120 µm long. The hyphal wall was thicker at the base of the spore (7.0 µm) becoming thinner and reaching a uniform thickness (1.0 µm) at a distance along the hyphal attachment. There were two wall groups, the composite thickness of which was 6.25 µm. The wall became thicker at the hyphal connection (10.0 µm).

Spores of *Glomus* t10a were 170-220 µm in diameter, globose, formed singly in soil, red-brown to dark brown in colour, opaque, with oily to globular spore contents. Subtending hyphae were found attached to spores, thicker at the base of the spore (20.0 µm), becoming thinner away from the spore (15.0 µm). The spore wall consisted of two wall groups, an outer laminated wall (5.0 µm) and inner membranous wall (0.5 µm) Spores of *Glomus* t10b were comparable in most of t10a characteristics except for the darker colouration. It is possible that types t10a and t10b are infact spores of the same species at different stages of maturity as illustrated in Plate 3.3. Top photograph shows spores of *Glomus* t10a. Below is a photograph showing a spore of *Glomus* t10b.

Spores of *Acaulospora* t24 resembled closely the species *Acaulospora scrobiculata* Trappe and are shown in Plate 3.4. They were 125-150 µm in size, globose to subglobose or ellipsoid in shape with a rough looking outer wall. This was evenly pitted with circular to elliptical pits (1.5-3.0 µm in diameter). Positive reaction to Meltzers. Composite wall thickness of spore, 2.5-5.0 µm consisting of an outer thick rigid wall with pits. A second wall group, a membranous wall, separates from the outer wall and an inner wall group consisting of a beaded membranous wall. The bud Scar on the spore surface is clearly visible (See Plate 3.4).

Plate 3.5 shows two unidentified *Acaulospora* species which occurred in spore populations in low number. The above photograph shows clearly the attached sacule on this *Acaulospora* species. The photograph below shows this *Acaulospora* species to be one of the group having an ornamented outer wall. Plate 3.6 shows a *Scutellospora* species, similarly found in low number in soil samples. The outer wall of this spore type shows that this is one of the species of *Scutellospora* which have an outer ornamented wall.

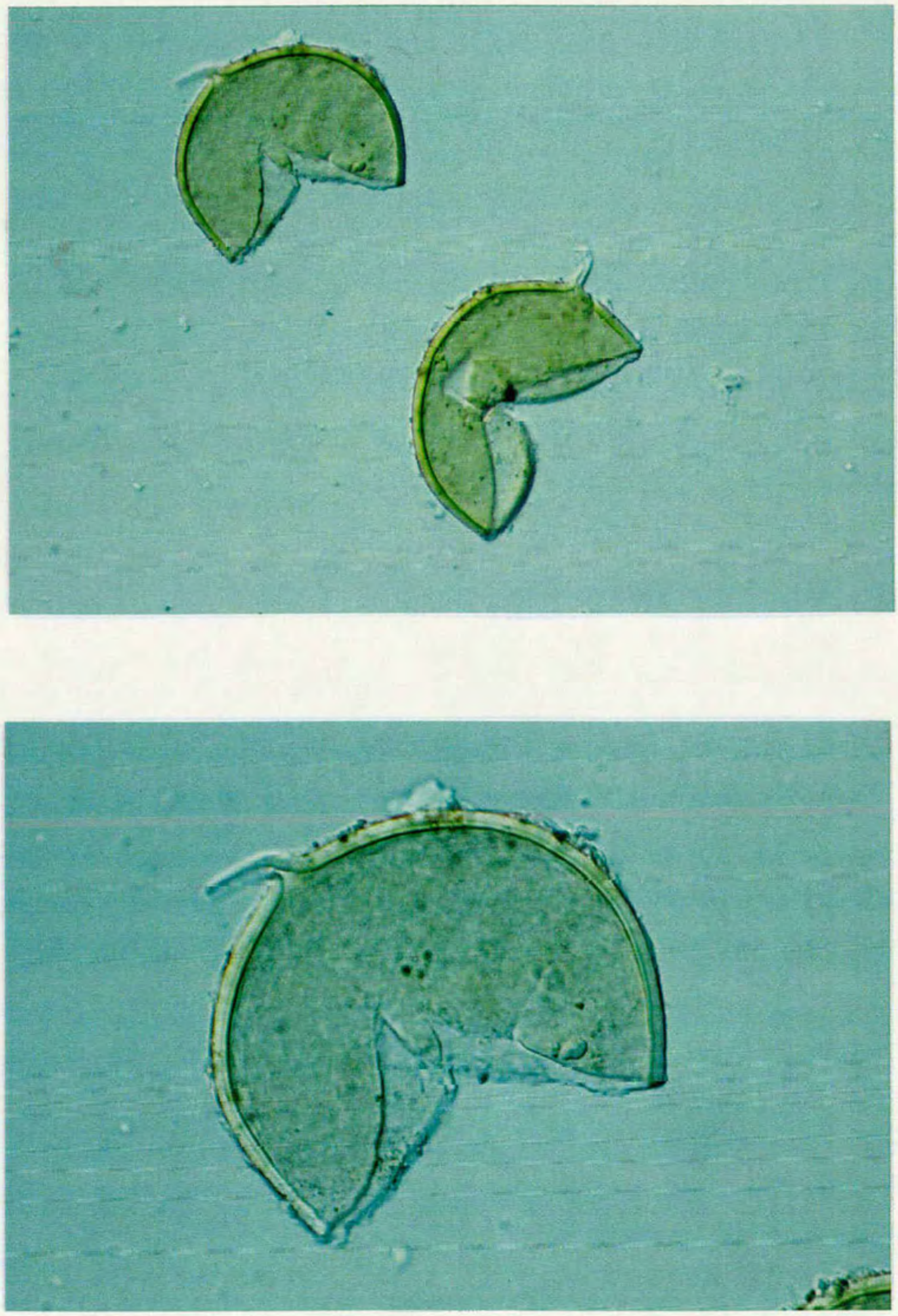
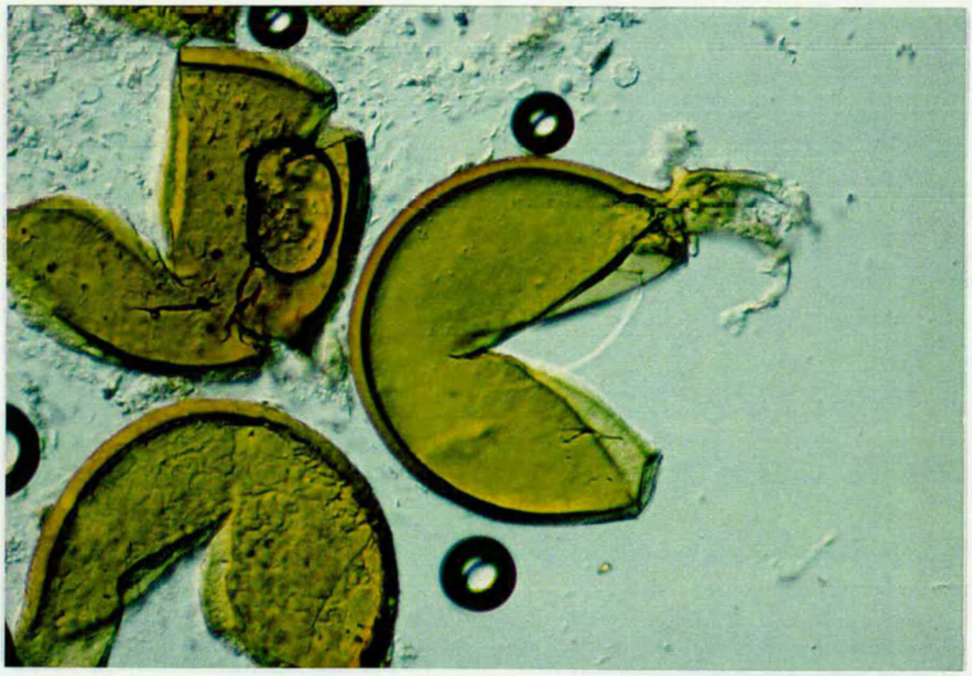


Plate 3.2 Spores of *Glomus* t3 (*Glomus etunicatum* Becker and Gerdemann)



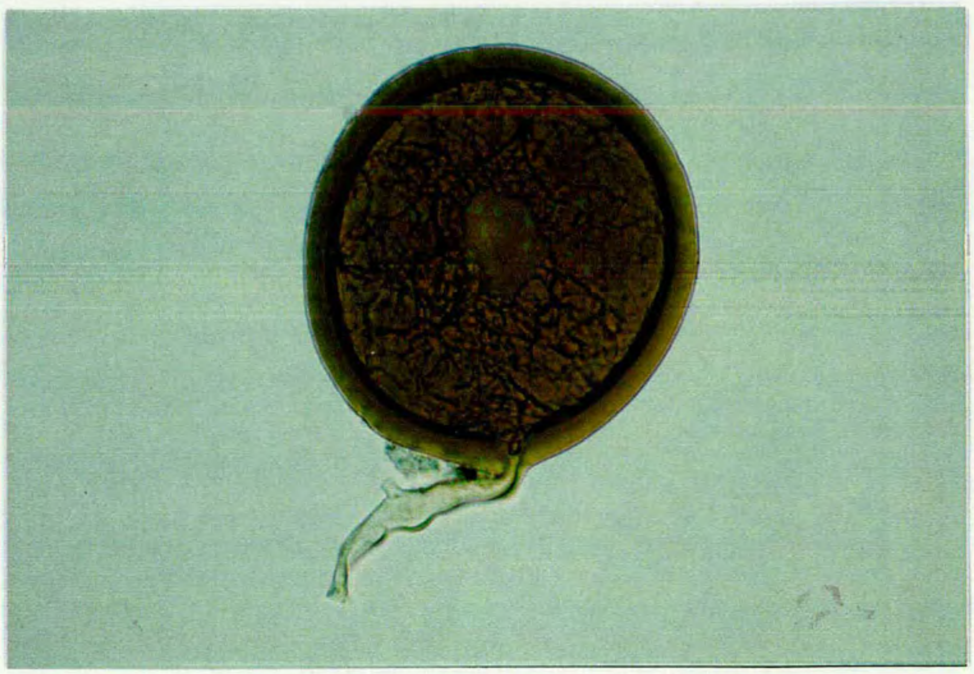


Plate 3.3 Spores of *Glomus* t10 [t10a (above) t10b (below)]

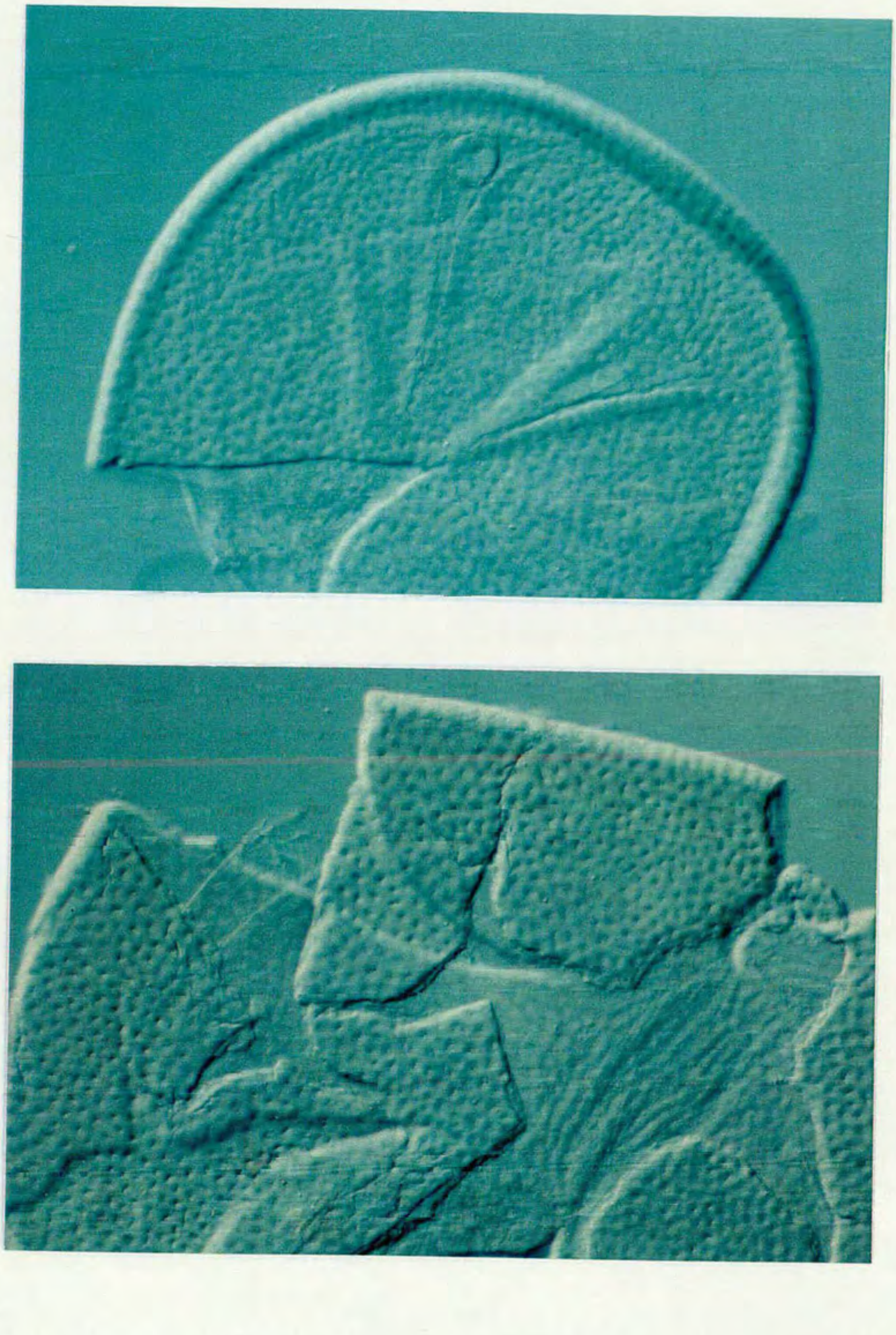


Plate 3.4 Spores of *Acaulospora* t24 (*Acaulospora scrobiculata* Trappe)

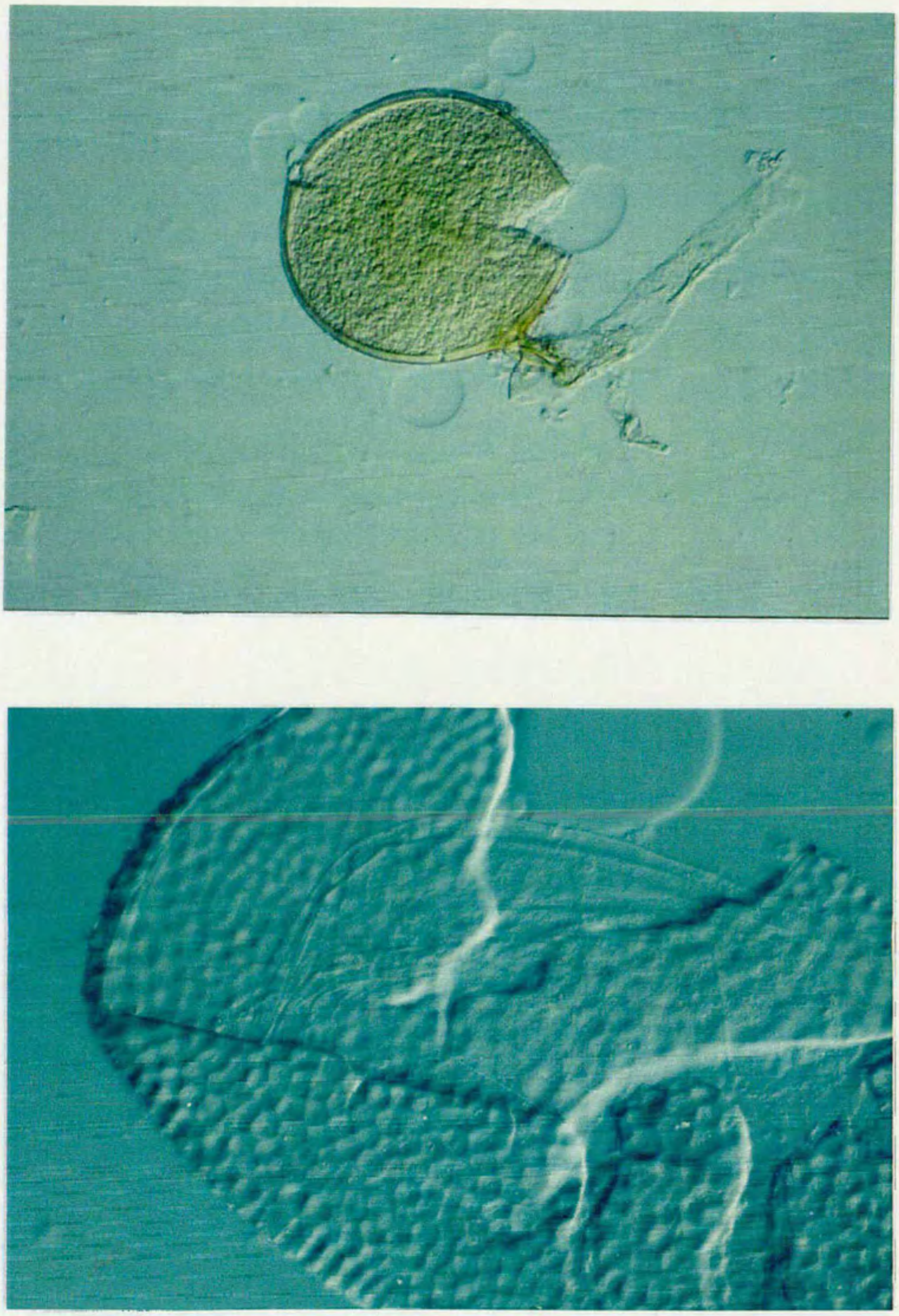


Plate 3.5 Spores of two species of *Acaulospora* found in low numbers in soil from the RHI system

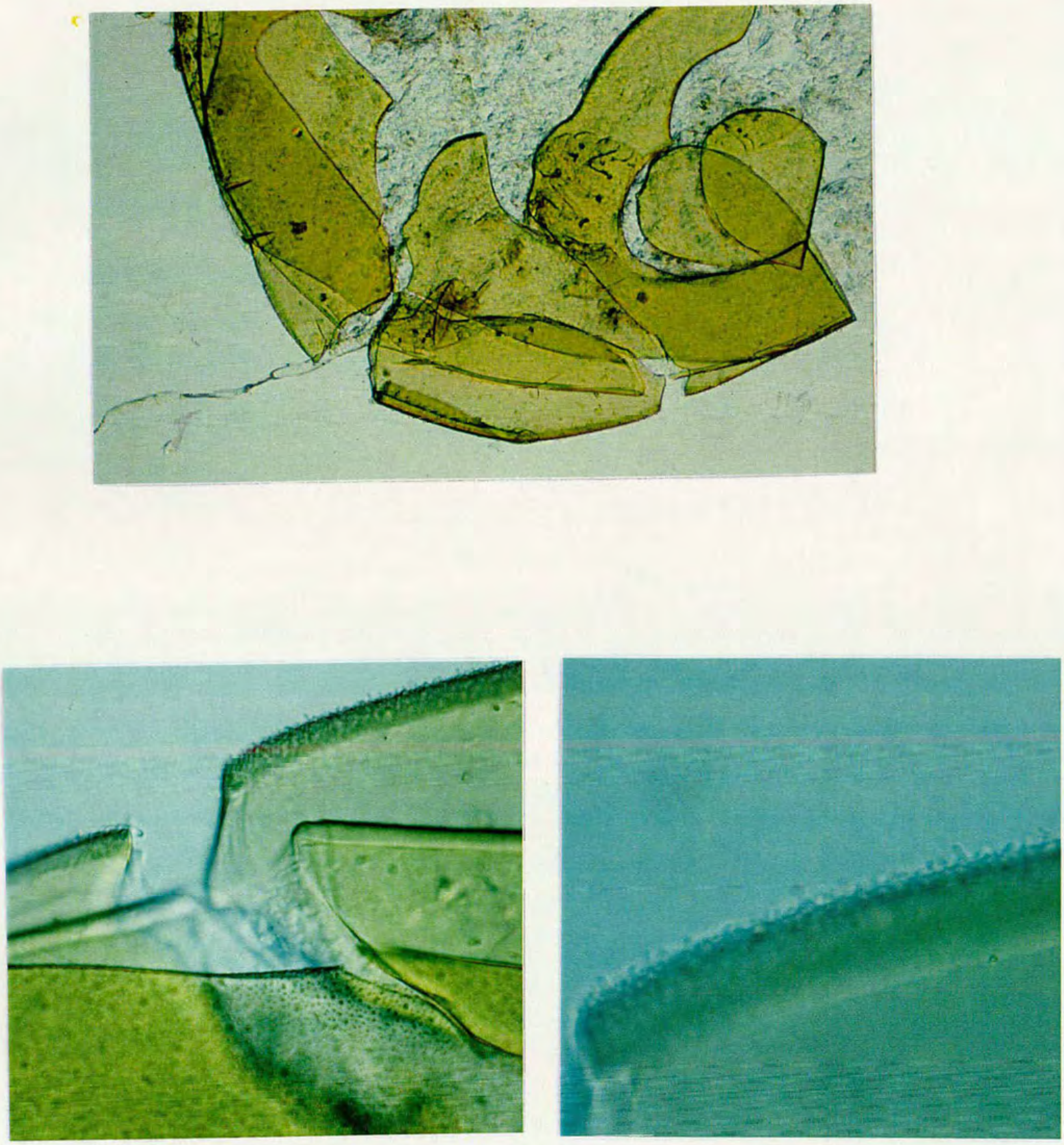


Plate 3.6. Spore of a *Scutellospora* species found in low numbers in soil from the RHI system

3.3.5 Distribution and abundance of the dominant spore types

At the end of the dry season, spores of *Glomus* t3 were found in significantly higher number in the fallow than in the cropped areas of the system (Table 3.3; Figure 3.3). There was a significant ($P < 0.001$) effect between host and management on the number of t10a spores in soil, such that they were found in significantly higher number in tree soil in the fallow area, compared to that in soil from elsewhere in the system. Spore type t10b were found in significantly lower number in tree soil than in alley soil irrespective of management (Table 3.3; Figure 3.3). There was a significant ($P < 0.001$) interaction effect between host and species on type 24 spore numbers such that, they were significantly higher in number in soil from the fallow alley of the system compared to elsewhere (Table 3.3; Figure 3.3).

At the start of the rainy season, three of the four pre-dominant spore types were found in February were also found to pre-dominate AMF spore populations in November, however, the distribution of abundance within the soil had changed since the earlier sampling at the end of the dry season in the same year. There was a significant ($P < 0.001$) interaction effect between host and management on spore type t3 numbers, such that they were significantly higher in tree soil in the cropped area of the system compared to elsewhere (Table 3.3; Figure 3.4). Spores of t10a were found in significantly ($P < 0.001$) higher number in alley soil than in tree soil in both fallow and cropped systems. There was a significant ($P = 0.09$) interaction effect between host and management on the number of spores of type t24 which were found in significantly higher number in tree soil in the fallow area of the system compared to elsewhere (Table 3.3; Figure 3.4).

3.3.6 Seasonal changes in the dominant spore types

Spores of *Glomus* t3 declined between February and November, 1991 in all soil samples, except in tree soil from the cropped area of the system where they increased dramatically from 40 50 g^{-1} dry soil to 834 50 g^{-1} dry soil and were pre-dominant in AMF spore populations in this area. Spores of *Glomus* t10a were similar in number between seasons all samples except those from alley soil in the fallow area, where they increased from 24 50 g^{-1} dry soil to 108 50 g^{-1} dry soil between sampling times.

Spores of *Glomus* type t10b occurred in low numbers in soil at both sampling times, however by November, 1991, numbers of spore of this type were not found in soil. Spore numbers of *Glomus* type t24 increased between sampling times. They occurred in higher number in fallow than in cropped areas on both sampling occasions, however at the first sampling they were more abundant in alley soil than tree soil in the fallow area, while at the second sampling they were greater in number in tree soil than in alley soils in the same area of the system.

Table 3.2. Significance of F-values in the analysis of variance of spore numbers for spore types t3, t10a, t10b and t24 in soil from the Rotational Hedgerow Intercrop system sampled in February (dry season) and November (wet season), 1991.

Treatment	*Glomus* t3	*Glomus* t10a	*Glomus* t10 b	*Glomus* t24
February 1991				
Host	ns	ns	*	***
Management	***	**	ns	***
Host.Manage	ns	***	ns	***
November 1991				
Host	***	***	nd	**
Management	***	*	nd	ns
Host.Manage	***	ns	nd	**

*** P < 0.001 ** P < 0.05 * P < 0.01 ns not significant nd not determined

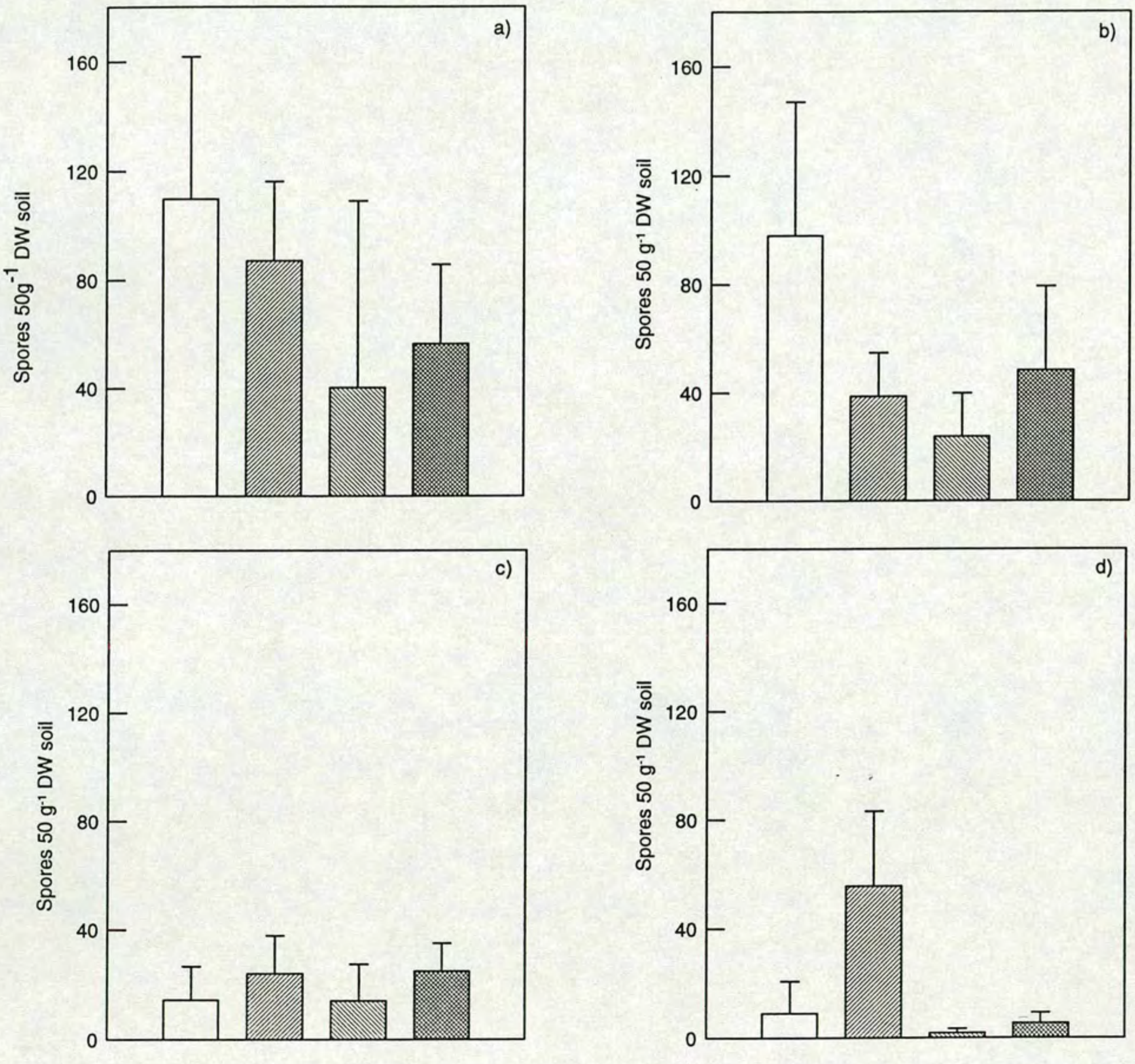


Figure 3.3. Effects of agronomic practice and plants species on the mean number of spores of a) t3, b) t10a, c) t10b and d) t24 collected from the Rotational Hedgerow Intercrop system February, 1991 (dry season). Means of 8 samples for each zone. Bars=+S.E. (P=0.05).
Tree sample/ fallow area. Alley sample/ fallow area.
Tree sample/ cropped area. Alley sample/ cropped area.

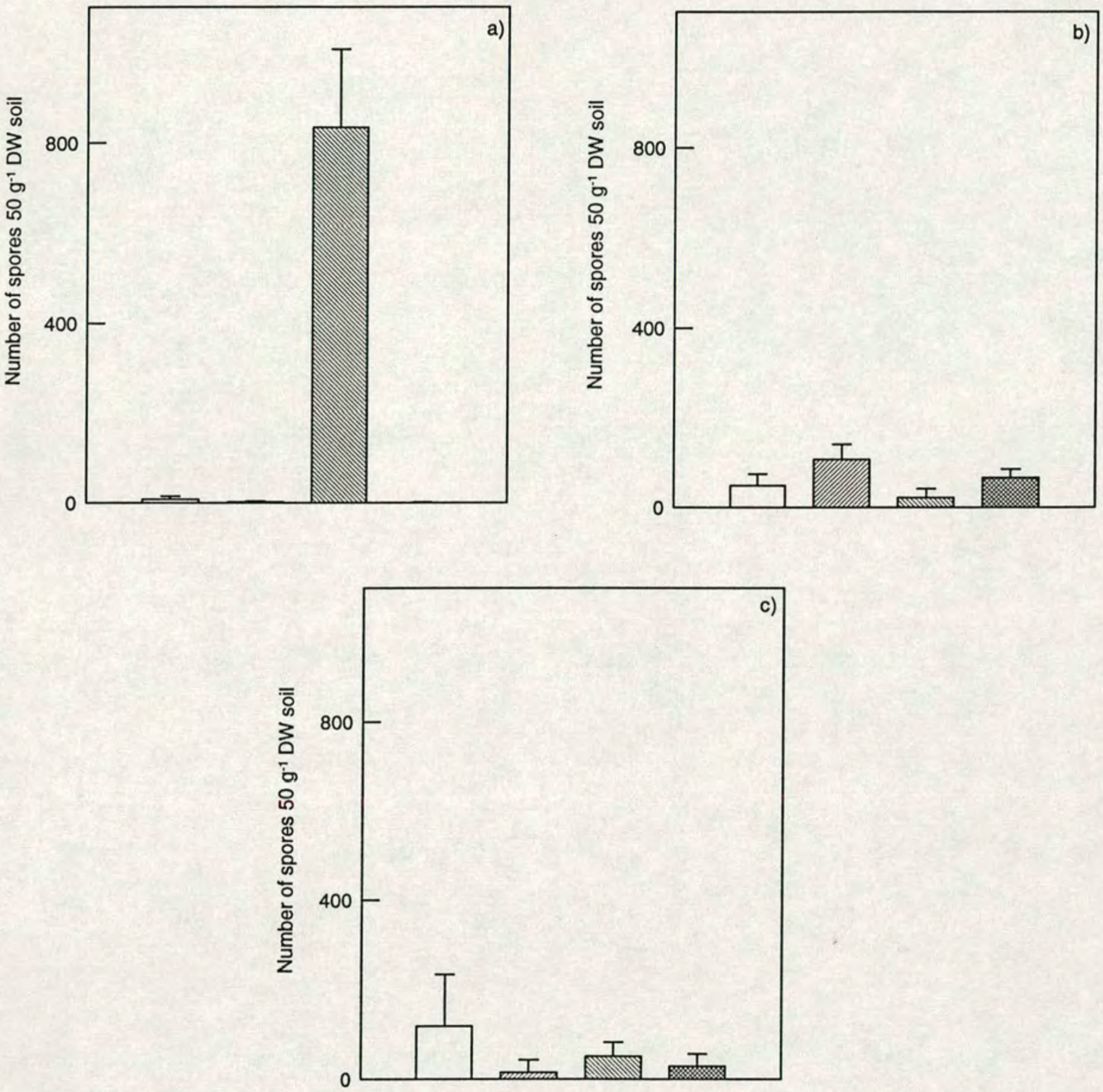


Figure 3.4. Effects of agronomic practice and plant species on the mean number of AMF spores of a) t3 b) t10a and c) t24 collected from the Rotational Hedgerow Intercrop system, November, 1991 (wet season). Means of 5 samples for each zone. Bars=+S.E. (P=0.05).

Tree zone / Fallow area   Alley zone / fallow area

Tree zone / cropped area   Alley zone / cropped area

3.4 Discussion

Spore numbers

Live spore numbers occurring in soil sampled in February, 1991 ranged from 85-283 50 g^{-1} dry soil. This is comparable to that reported by Abbott and Robson (1977). In soil from agricultural areas in Australia and New Zealand spore numbers ranged from 1-205 spores 50 g^{-1} dry soil. Likewise, Hayman and Stovold (1979) found a range in spore number from 24-232 spores 50 g^{-1} dry soil in wheat grown at different sites in New South Wales. Ahmad (1989) found 1-32 spores 50 g^{-1} dry soil in a lowland tropical rainforest. While, Musoko (1991) found in an undisturbed area of forest in Cameroon 131 spores 50 g^{-1} dry soil beneath species of *Terminalia*. These values although comparatively high for natural forest are still considerably lower than spore numbers occurring in this agroforestry system and in monoculture cropping systems studied elsewhere. The results from this study tend to support the generally accepted view that cultivated soils contain higher AMF spore numbers than soil from natural undisturbed ecosystems (Sieverding, 1989).

Within the RHI system, higher spore numbers were found in the fallow area than in the cropped area of the system (Figure 3.3.1). These results are contradictory to those usually reported for the effects of fallowing. Thompson (1987) found a higher number of spores and infected root propagules in soil from systems cropped with maize, sorghum and sunflower compared to one which had been fallow for 3 years. Similarly, in a three year field study, Black and Tinker (1979) found higher spore densities in fields that had been sown to barley a year previous to sampling compared with those which had been fallow the previous year. Most studies of fallowing involve the absence of crop plants (Black and Tinker, 1979; Kucey and Paul, 1983; Thompson, 1987). In this study, however, the fallow similarly involved the absence of crops, but within fallow alleys natural succession was allowed to proceed. This accounts for the inconsistencies in spore numbers between the results from this study and those of 'clean' fallows in the literature.

In the RHI system, fallowing has been carried out in a two year rotation system since 1986 when the system was first established. The first sampling occasion in February, 1991 occurred a year after a rotation, such that the area cropped at the time of sampling was fallow the previous year. An effect known as the 'carry-over' effect occurs when the effects of previous crops are found in soil at a particular time. Dodd *et al.* (1990a) observed significant increases in spore number in soil under a cowpea crop grown in plots previously cropped with Kudzu compared to those been previously cropped with cassava and a savanna control plot. Similarly, Black and Tinker (1979) reported low spore numbers in barley grown in plots previously fallowed or cropped with Kale compared to plots previously cropped with barley. In the fallow area of the RHI system higher numbers of live spores found in the fallow area towards the end of the dry season, 1991, may have been due to 'carry-over' effects from the previous crop. Likewise, the low number of spores found in tree soil in the cropped area may have been due to the 'carry-over' effect in soil from the previous fallow period. The need to sample systems in which plant hosts and agronomic practices are frequently changed is highlighted by these results.

In the study area, the occurrence of two dry seasons in every year each lasting approximately 3 months is most likely to have an effect on spore numbers. The proportion of non-viable spores in samples was at least 40% at the first sampling occasion. The proportion of dead spores in samples removed from the system during the same period in 1992 (Mbuthia, 1992) was 96%. Although sampling was carried out at approximately the same time on both occasions, the 1991 sample collection (28 February) was earlier than Mbuthias' collection in 1992 (10 March). It would therefore appear that as that dry season progresses survival of AMF spores is significantly reduced. Dodd *et al.* (1990a) in a savanna ecosystem of the Eastern plains of Colombia found a pronounced decline in spore numbers as the 4 month dry season progressed. At high temperatures survival of different AMF structures particularly spores are known to be reduced (Gianninazzi-Pearson and Diem, 1982). The prolonged exposure to high temperatures, drought conditions and consequent reduced root growth obviously contribute considerably and result in the loss of very high percentages of viable spores.

The number of viable spores found in soil in November, 1991 ranged from 207-1002 50 g^{-1} dry soil. Spore numbers in tree samples from the cropped area of the system were significantly higher than those in all other samples, and in general significantly greater than spore numbers found in natural and agroecosystems reported in the literature (Khan, 1971; Abbott and Robson, 1977; Hayman and Stovold, 1979).

Live spore numbers in the fallow area of the system were comparable between tree and alley soil, while in the cropped area live spore numbers were significantly higher in tree than in alley soil. In November, 1991, samples were removed from this system during the very early stages of maize development (2-3 weeks after sowing) (see Plate 3.1). Low spore numbers in soil around the roots of young maize plants may result from higher spore germination in response to the presence of vigorously growing roots. Giovannetti (1985) and Saif and Khan (1975) found that spore numbers decreased when root growth was greatest and increased at the end of the growing period, usually coinciding with a reduction in root growth. This could explain low spore numbers in alley soil in the cropped area of the system and may account for the greater differences in spore numbers between tree and alley samples in the cropped area compared to those in the fallow area at this time.

During the wet season soil contained higher total (live + dead) spore numbers than that which was found in soil during the dry season. Number of live spores in the fallow area were comparable at each sampling time with only a small increase in spore number in alley samples later in the year. In the cropped area live spore numbers increased dramatically in tree soil and a small increase in alley soil did occur. The number of non-viable spores was greater in all soil samples in November compared to that in February, 1991. The proportion of dead spores was greater in November with the exception of tree soil in the cropped area where the proportion of spore which were dead represented 25% of the total spore population recovered from soil samples. High number of dead spores in wet season samples may be due to the build up of dead spores during the previous dry season. Giovannetti (1985) and Saif and Khan (1975) reported increases in the number of spores towards the end of the growing season. The increase in both live and dead spores may result from the propagation of spores as root growth declines (Giovannetti, 1985). The results from this study show how important increasing the frequency of sampling in such systems which incur changes in spore population composition during short time spans. Kucey and Paul (1983) similarly, found that the number of spores in a range of Saskatchewan ecotypes were higher in virgin than in cultivated soils by 1.1-33.5 (Average 4.8) times. In the same study they reported no increase in spore numbers in virgin soils during the growing season, whereas, those in cultivated soil (wheat) increased once the crop had become established and eventually surpassed population levels found in adjacent virgin soils. This type of continual change with time in spore populations must be monitored in order to fully understand the mycorrhizal population dynamics conclusively.

Spore population composition

Live spores found in cropped and fallow alley samples in the RHI system in February, 1991 were comparable in number. However the population composition was different in each area. Spores of *Glomus* t3 were significantly higher in number in soil from the fallow end of the plot. Spores of *Glomus* t24 were significantly higher in number in alley soil in the fallow area compared to that in all other samples. The number of spores of *Glomus* t10a were highest in number in tree soil in the fallow area.

Plant species can exert a selective effect on the abundance of AMF species within a mixed indigenous population. Schenck and Kinloch (1980) found that more than 90% of an indigenous spore population associated with soybeans were species of the *Gigaspora* while in maize and peanuts this genus accounted for only 66% and 60% of the spore population respectively. For maize, the proportion of spores of *Glomus* species was greater than that for either peanuts or soybeans, whereas for peanuts the proportion of spores of *Acaulospora* species was greater that for maize or soybeans. Sieverding (1989) reported an immediate loss of *Sclerocysistis* species when native systems were taken into agronomic use as a result of the introduction of fertilizer and lime application within the system. Variation in spore population composition observed here could be the result of preferential associations between plant and AMF species when change in the vegetation cover of fallow alleys occur within the system. Similar compositional differences in AMF spore populations were found between tree and invading herbaceous vegetation following clearance of natural forest in Cameroon (Musoko, 1991).

In November 1991, although no significant differences in number of spores occurred between tree and alley samples in the fallow area, distinct differences were found in the abundance of different fungal species in both tree and alley samples. In tree samples in the cropped area fungal species *Glomus* t3 dominated spore populations accounting for the large overall number of spores which were recorded, while in alley samples from the cropped area relatively fewer spores of this species were found Here, another species dominated spore populations, *Glomus* t10a was found to account for 35% of the spore population in cropped alley samples, representing only 2% of the tree fungal populations in this area. Differences in dominance of fungal species between tree and crop in the fallow area are also apparent. *Glomus* t24 was higher in number, representing a higher proportion of spore populations in tree soil in the fallow area.

At the beginning of the wet season, tree soil in the cropped area had 1002 live spores 50 g^{-1} dry soil compared to a significantly lower number of live spores in tree soil in the fallow area (294 50 g^{-1} dry soil). Tree soil in the cropped area in the dry season had lower spore numbers (85 50 g^{-1} dry soil) than tree soil in the same area in November, 1991. Numbers of live spores in tree soil were only found to be significantly lower than alley soil in the dry season in the cropped end of the plot. In the wet season spore numbers on trees were either significantly greater than, or comparable to those in alley soil.

The results from this study agree with an increasing number of studies which show that trees maintain higher numbers of live spores compared to either the crop in an agroforestry system (Wilson *et al.*, 1990; 1991; Mbuthia, 1992) or surrounding vegetation in a natural ecosystem (Musoko, 1991). The similar spore types found in association with both tree and crop found here, has also been reported between trees and surrounding plant species in natural forest (Musoko, 1991). This would indicate that trees and crops in agroforestry systems share mycorrhizal fungal species and that trees introduced into natural or agroecosystems, by increasing live spore numbers around their roots, may have the potential to improve that inoculum for the intercrop and hence increase productivity in such systems. The influence of the tree component in agroforestry on the inoculum potential of soil has rarely been investigated, however evidence from a study carried out in this RHI system by Mbuthia (1992) shows significant increases in the number of spores in soil beneath maize plants growing nearest (0.5 m) the tree compared to those further from the tree (2 m). Similarly, infectivity of soil inoculum (i.e. infection produced in the roots of maize plants) was greater in plants inoculated with soil collected 0.5 m from the tree than at a distance of 2 m from the tree. In the present study, the number of live spores found were significantly higher in tree soil samples than in alley samples collected during the wet season. Mbuthia (1992) collected soil from this area in March, 1992 (Dry season) and found similar effects of trees on spore numbers and infectivity of soil. This study, in agreement with those mentioned previously, shows the potential for the use of trees in agroecosystems or in degraded marginal areas in order to maintain AMF fungal populations which could increase production in systems in these areas. Evidence that trees in natural and agroforestry ecosystems maintain greater spores populations has been provided by studies in tropical rainforest (Musoko, 1991) and semi arid agroforestry systems of Kenya (Wilson et al., 1990; 1991; Mbuthia, 1992). In fallow areas high spore populations occurring on trees may provide a source of inocula for subsequent crops grown in alleys. However, indications of changes in the mycorrhizal spore populations in fallow alleys recolonized by grasses and shrub vegetation may affect the overall effectiveness of the mycorrhizal population for the crop. It is important that changes in the mycorrhizal populations and their effects are understood so that proper management of the system can be practised in order to optimize crop and tree productivity in such areas.

CHAPTER FOUR

EFFECTS OF INOCULATING *Zea mays* L. WITH INDIGENOUS AMF FROM DIFFERENT HOST SPECIES WITHIN FALLOW AND INTERCROPPED AREAS OF A TROPICAL AGROFORESTRY SYSTEM

4.1 Introduction

Many studies have shown that changes in spore numbers or spore population composition can occur as a result of agronomic practices such as fallowing or crop rotation (Collins *et al.*, 1991; Saif, 1986; Saif and Khan, 1975; Sutton and Barron, 1972; López-Sánchez and Honrubia, 1992). Dodd *et al.*, (1990a; 1990b) found that changes in the indigenous AMF populations in a tropical monocropped agroecosystem affected the extent or efficiency of mycorrhizal infection and had a subsequent effect on plant growth. Such studies emphasise the need to understand the causes and effects of changes in AMF populations order to harness their potential.

In tropical areas, most studies examining the effects of agronomic practices on AMF populations have been carried out within monocropped agroecosystems. There are very few comparative studies of AMF in tropical agroforestry systems despite the growing interest in agroforestry as a type of land-use system and the potential for managing AMF populations for increased or sustained plant productivity. A preliminary study (Chapter 3) of AMF spore populations in a tropical agroforestry system showed that spore numbers and population composition were significantly altered by host species (tree or crop) and fallow periods. In the first of the following experiments, the effects of inoculating *Z. mays* with soil (containing indigenous AMF spore populations) from tree and alley areas of an agroforestry system under different agronomic practices (fallowed or intercropped) were investigated by observing the consequent effects on the development of mycorrhizal infection, growth and nutrition of maize.

At the first harvest of experiment 1, (60 d.a.i.) another (experiment 2) was established to determine which AMF spore types at this time were infecting the roots of plants. In addition, this provided an indication of the effects of using different types of inocula on the development of mycorrhizal infection, growth and nutrition of maize.

Although pot trials allow for better control within experiments, the study of AMF host relationships in pots presents many difficulties when extrapolating results to the field. For this reason, an important aspect of this study is to investigate how pot conditions within the glasshouse affect the results obtained. In this, and many other pot trials, most plants are grown in sterile soil with a small amount of field inoculum added. At the very least, it was suspected that the rate of spread of mycorrhizal infection may be different to that which occurs in the field such that a significant treatment effect in pots, would not occur in the field where the actual number of propagules is greater. Therefore, experiment 3 was established in which the effects of different amounts of soil inoculum (50 g, 100 g and 150 g) on the development of mycorrhizal infection, growth and nutrition of maize were examined.

4.2 Materials and Methods

Experiment 1 : to determine the effects of inoculating Zea mays plants with tree and alley soil (containing AMF spore populations) from fallow and intercropped areas of the RHI system.

Approximately two weeks before establishing experiments the glasshouse floor was sterilized using a dilute solution (7 ml l^{-1} H_2O) of 'Jeyes fluid' (Jeyes Ltd. Thetford. Norfolk. England). Maize seeds (*Zea mays* L. Katumani composite var. Machakos, Kenya) were germinated in the glasshouse in sterilized pot soil A (see Chapter 2). Pots of 10 cm diameter (1 litre volume) were sterilized by soaking in a solution of 'Bruclens sterilizer' (Brewmaker plc. Southampton). To prevent contamination, uninoculated and inoculated treatments were prepared separately, washing hands and equipment in between. Field soil inoculum (100 g per pot) was placed as a band at a depth of 5 cm in pots containing soil mix A. The roots of two, seven day old maize seedlings were placed directly into the inoculum band and plants were secured with soil mix leaving a depth of 1 cm from the top of the pot for watering. Seven days later, one seedling (the smaller of the two) was removed from each pot. Pots were positioned on benches 10 cm apart (see Plate 4.1), and plants were watered sparingly, while ensuring that plants were kept alive. Great care was taken to avoid contamination during preparation of experimental treatments and throughout the duration of the experiment, especially when watering plants.

A randomised block experimental design was used, testing the effects of 6 inoculation treatments (4 inoculated and 2 uninoculated), of which the 4 inoculated treatments consisted of plants inoculated with a) tree soil from cropped areas (TC), b) alley soil from cropped areas (AC), c) tree soil from fallow areas (TF) and d) alley soil from fallow areas (AF) of the system. One of the two uninoculated treatments consisted of plants grown in sterilized soil with 100 g of autoclaved soil inoculum (Control A (CA)). The other consisted of plants grown in sterilized soil only (Control B (CB)) and was introduced into the experiment to test for incoming contamination. The experiment was laid out in 5 randomised blocks with 2 replicates per treatment per block. Two destructive harvests were carried out 60 and 120 days after inoculation (d.a.i.). At each harvest one of two replicates per treatment in each block were selected randomly and removed. Shoots were severed at the root collar and root systems washed, then stored in a cold room (4°C) for less than 3 days before they were sampled and stained for mycorrhizal infection assessment. At the first harvest of this experiment in addition to removing a root sub-sample for determination of mycorrhizal infection, 10 g (wet weight) of root material was also removed to inoculate plants in experiment 2. The weight of both these sub-samples was recorded and used to estimate the total dry weight of the plant's root system as described previously (see section 2.5).

Before sampling root systems for determination of percentage mycorrhizal infection, two preliminary trials were conducted, one to determine the effect of different sample sizes (number of root pieces) on the percentage infection values obtained, and the other to look at the distribution of infection in plant root systems at the time of the first harvest. One of 5 replicate plants harvested from each treatment was used for each preliminary trial. For determination of infection at different sample sizes, a total of 250 root pieces was removed from each plant and mycorrhizal infection was determined as root length occupied with hyphae, arbuscules or vesicles using samples containing 50, 100, 150, 200 and 250 root pieces. There was little difference between percentage infection values obtained for each sample size except that determined for the 50 root pieces sample (Figure 4.1). The 100 root pieces sample was therefore used to determine mycorrhizal infection for this and all subsequent experimental harvests.

For determination of the effects of soil depth on mycorrhizal infection, 100 root pieces were removed from the plant root system at 3 depths (0-5 cm ; 5-10 cm ; 10-15 cm) in each pot. With the exception of one treatment, % mycorrhizal infection was significantly greater in roots sampled from the top 5 cm of soil compared with those deeper in the pot (Figure 4.2). Plants inoculated with alley soil from the cropped area of the system (Figure 4.2d) had lower % mycorrhizal infection in roots sampled from a depth of 5-10 cm compared with those sampled above and below this depth (Table 4.1; Figure 4.2). For each treatment, % mycorrhizal infection values for these replicate plants was determined by taking the average of the 3 depth values. This value was then used along with those from the other replicates in the analysis of variance of mycorrhizal infection. Percentage mycorrhizal infection of other replicates in each treatment, estimated root dry weight, shoot dry weight, shoot and root N, P, K, Ca and Mg concentrations were determined as described in chapter 2. After the first harvest, pots were rearranged so that plant density remained constant throughout the duration of the experiment.

Experiment 2 : to determine the effects of inoculating Zea mays plants with mycorrhizal roots of plants previously inoculated with soil from the RHI system.

Maize seeds were germinated and grown under conditions similar to those described for the previous experiment. Infected chopped root material (10 g) taken from each of the 4 inoculated treatments in experiment 1 were used to inoculate plants in this experiment. A completely randomized block design was used, with 6 fungal treatments (4 inoculated and 2 uninoculated) and 5 blocks each containing 1 replicate plant per treatment. All plants were harvested 120 d.a.i. This period of time was thought to be adequate for mycorrhizal formation and sporulation of infecting AMF. It is acknowledged that formation of mycorrhizal infection varies widely between different AMF and plant species. Abbott and Robson (1981a) found 80 % infection in subterranean clover plants inoculated with AMF just 56 d.a.i., while, Thompson (1987) found that mycorrhizal infection peaked (80 %) in sunflower plants 72 d.a.i. Obviously, sporulation will similarly vary between AMF and plant species, however, due to both time and space constraints it was difficult to continue this experiment for a longer period. The same assessments were carried out as those described for experiment 1. In addition, 4 (25 g) soil samples were removed from each the base of the stem of inoculated plants. Soil samples were bulked and two 50 g subsamples were taken, one for extraction and identification of AMF spores the other for determination of soil dry weight.

Experiment 3 : to determine the effects of inoculating Zea mays plants with different quantities of soil containing AMF populations from the RHI system.

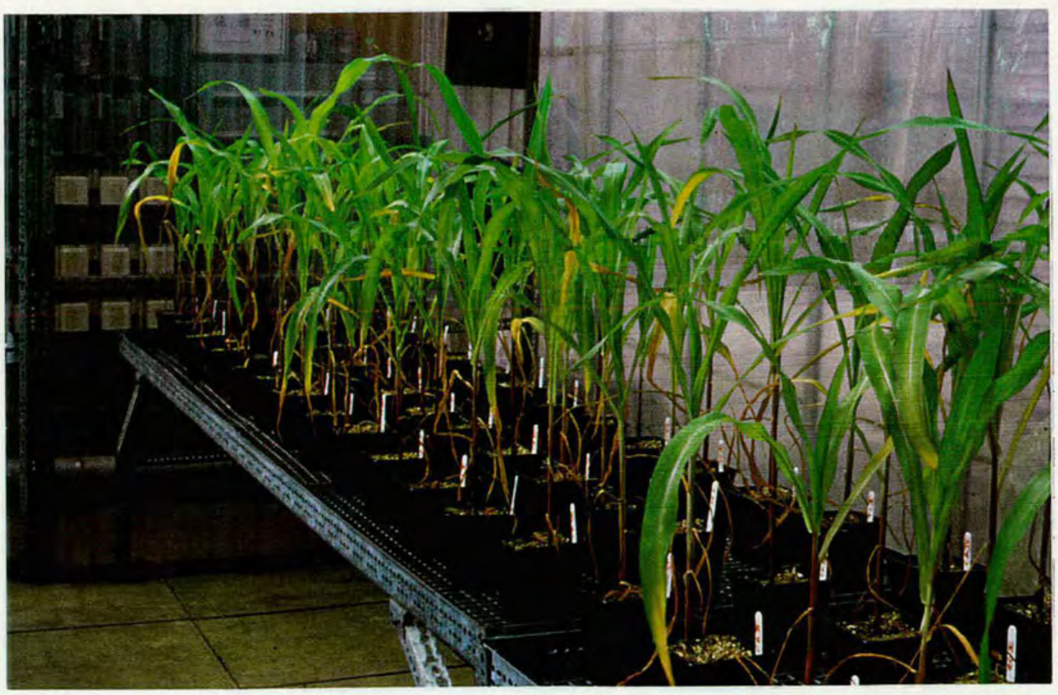
Maize seeds were germinated and grown under the same conditions as described for experiments 1 and 2. The experiment consisted of 4 fungal treatments (3 inoculated and 1 uninoculated), the 3 inoculated treatments consisted of plants inoculated with different amounts of field soil (50 g, 100 g and 150 g of soil inoculum (containing 950 spores 50 g^{-1} dry soil) in 1 litre pots. Soil inoculum from trees in the cropped area (collected November, 1991) was used because it contained a greater number of spores than that found in alley soil and would therefore increase the probability of mycorrhizal infection formation in plants. Control plants were grown in sterile soil A with 100 g of autoclaved soil inoculum. The reason why only one control was possible was due to the limited quantity of field soil available. In addition, plants grown in sterile soil only were placed in rows (3 plants per row) between plants to test for incoming contamination. There were 4 blocks each containing 2 replicates per treatment. Plants were destructively harvested 90 d.a.i and the same assessments carried out as those described for experiment 1. Experiment 1, 2 and 3 were carried out during the period May - August, 1992.

Table 4.1: Nitrogen, phosphorus, potassium, magnesium and calcium content of field soil and pot soil mix used in experiments 1, 2 and 3.

	Nutrient content (mg l^{-1})						
	pH	$N0_4$-P	NO_3-N	NH_3-N	K	Mg	Ca
Soil A	6.7	1.1	2.4	1.7	31	1.7	20.0
Field soil	5.9	0.1	5.7	1.6	27	1.5	14
Loam	5.8	0.9	5.3	1.4	10	1.4	24

Soil A : Loam : sand : grit : vermiculite (2:1:1:8 v/v)

Plate 4.1: The layout of experiment 1 prior to harvest 1 (60 d.a.i)



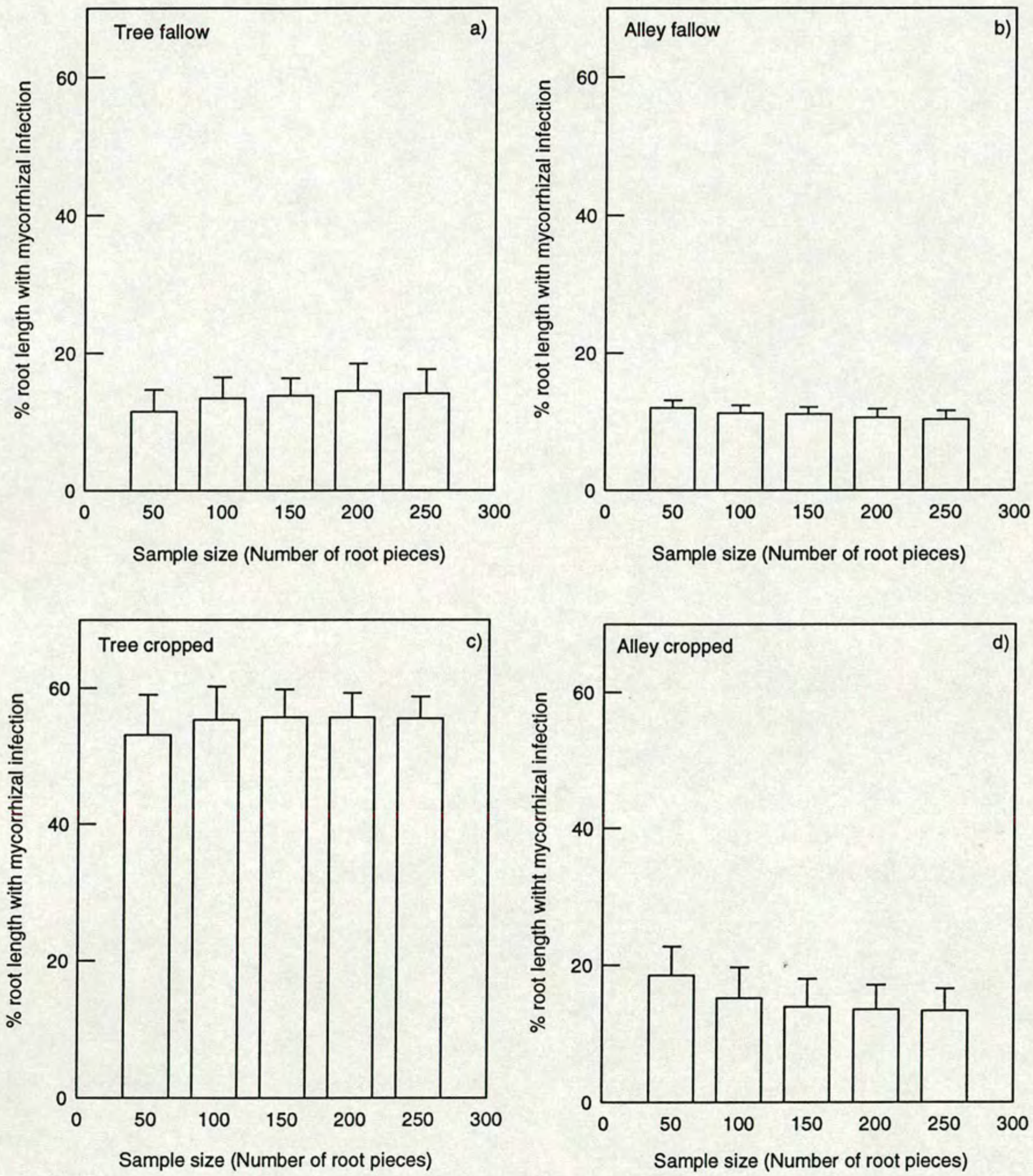


Figure 4.1. Effect of sample size (number of 1 cm root pieces) on % mycorrhizal infection determined for *Zea mays* L. plants 60 days after inoculation with soil from different treatment areas of the RHI system at Machakos, Kenya. Bars +S.E. (n= 50, 100, 150, 200, 250).

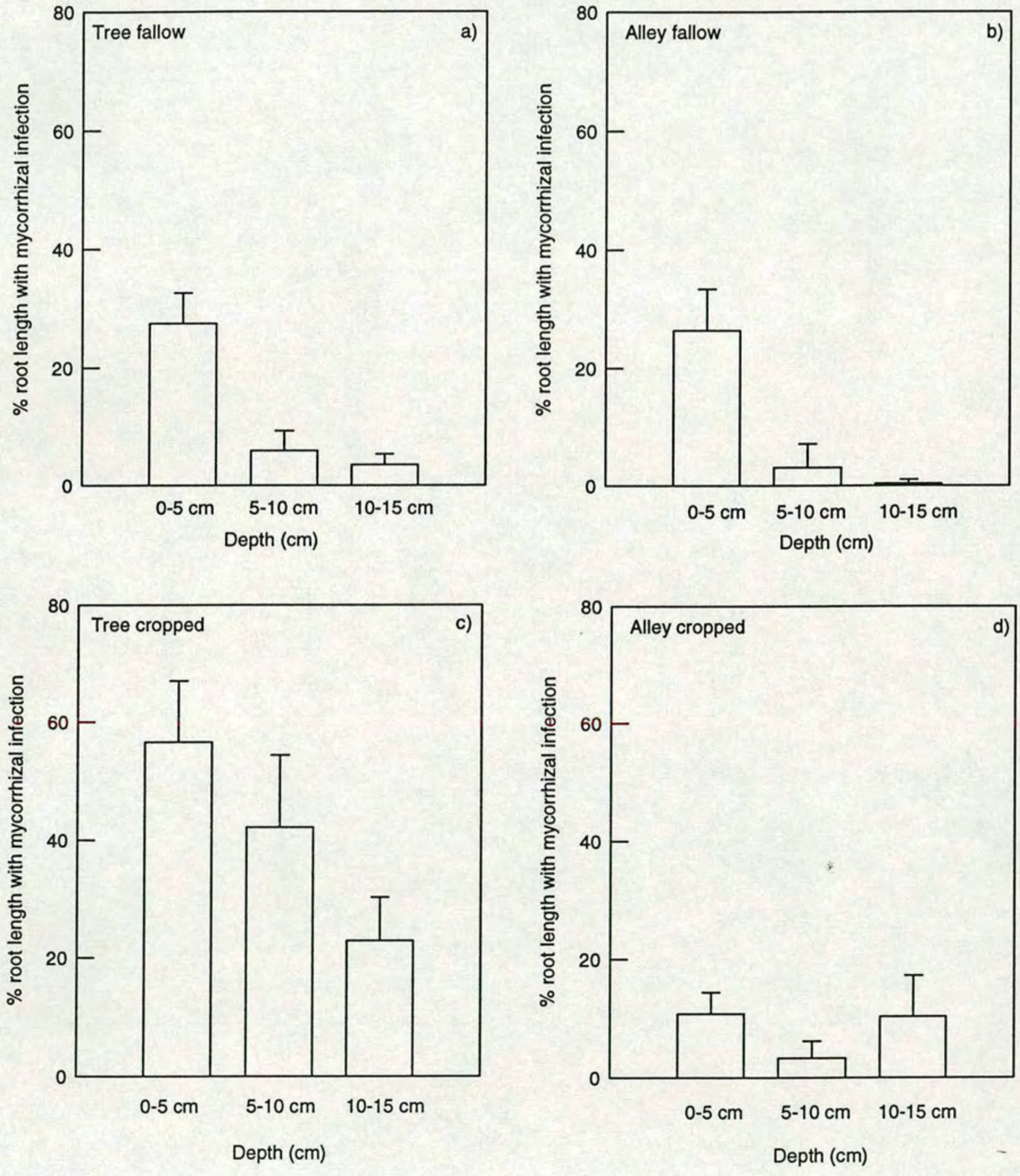


Figure 4.2. Effect of soil depth on mean % root length mycorrhizal infection in *Zea mays* L. plants 60 days after inoculating with soil from different treatment areas of the RHI system at Machakos, Kenya. Bar +S.E. (n=100).

4.3 Results

4.3.1 Experiment 1 : to determine the effects of inoculating Z. mays with tree and alley soil (containing AMF spore populations) from fallow and intercropped areas of the RHI system.

Mycorrhizal formation

At the time of the first harvest (60 d.a.i.), there was a significant interaction effect ($P < 0.001$) between species (tree and crop) and treatment (fallow and cropped) on mycorrhizal infection in *Z. mays.* This was such that a significant difference in mycorrrhizal infection occurred between plants inoculated with soil from different host species in the cropped area, but not the fallow area of the system (Table 4.2; Figure 4.3a). Plants from treatment TC had 47 % infection, while those from treatments AF, TF and AC had 9 %, 10 % and 12 % infection, respectively (Figure 4.3a). Mycorrhizal infection was further defined as the proportion of root infection containing hyphae, vesicles or arbuscules. Hyphal infection represented a high proportion of infection in plant roots. In treatment TC, approximately 50 % of infection contained hyphae and arbuscules. A similar proportion of the root infection consisted of hyphae and vesicles. In all other treatments, (i.e. those with relatively lower infection), the occurrence of vesicles in roots was less than 8 % of the total infection, while the proportion of infection containing arbuscules ranged from 19 % (AC) to 69 (TF).

At the end of the experiment (120 d.a.i), there was a significant interaction effect ($P = 0.027$) between species and treatment on mycorrhizal infection in *Z. mays.* The effect was consistent with that which had occurred at the time of the first harvest (Table 4.2). Percentage infection ranged from 60% to 90% (Figure 4.3) with plants from treatment TC having significantly higher mycorrhizal infection than all other inoculated plants (Figure 4.3b). As observed at harvest 1, hyphae represented a large proportion of the mycorrhizal infection recorded in plant root systems harvested at this time. In all treatments, the proportion of infection containing vesicles was low, while the proportion of infection containing arbuscules ranged from 86 - 100 %. There was no mycorrhizal infection detected in plants not inoculated with AMF. Plate 4.2 shows mycorrhizal infection in the roots of maize plants showing the different infection structures, vesicles, arbuscules and coiled hyphae. Tables of the results of analysis of variance for mycorrhizal infection, total, shoot and root dry weights and nutrition are presented in full in Appendix B.

Growth of plants

At the time of the first harvest (60 d.a.i.), there were no significant effects of AMF inoculation on plant total dry weights, estimated root dry weights or root : shoot ratios (Table 4.3). At this time plants were approximately 1 m in height and were just beginning to become pot bound. Apart from this they showed no other signs of stress. Inoculated plants had similar shoot dry weights irrespective of treatment, with mean shoot dry weights ranging from 13.0 g to 13.5 g. Uninoculated plants from treatment CA, with a mean shoot dry weight of 15 g, had significantly ($P < 0.05$) greater shoot dry weights than inoculated plants. Mycorrhizal plants from treatments TF and AC were larger but not significantly different than other mycorrhizal plants (Figure 4.4a). Shoot dry weights were similar between uninoculated plants from treatments CA and CB (Figure 4.4a).

Plants showed little increase in growth between the two harvests. By the time of the second harvest plants were heavily pot bound. The effects of inoculation on shoot dry weights of plants had diminished by the end of the experiment (120 d.a.i.) (Table 4.3; Figure 4.5a). However, root and total dry weights were now significantly ($P < 0.05$) different between treatments (Table 4.3). The root dry weights of uninoculated plants from treatment CA were significantly greater than inoculated plants, except for those in treatment TC (Figure 4.5b). Total dry weights of uninoculated plants were significantly greater ($P < 0.05$) than inoculated plants by the end of this experiment (Figure 4.5c). The root shoot ratios of plants were not significantly different at this time (Table 4.3). At the end of this experiment, plant shoot dry weights had changed very little since the first harvest. Those from treatment TC had the largest increase (2 g) in shoot dry weight between harvests. Root dry weights increased more than shoots between harvests. Uninoculated plants had the greatest increase (4 g) between harvests and those from treatment TC had the greatest increase (3 g) among inoculated plants.

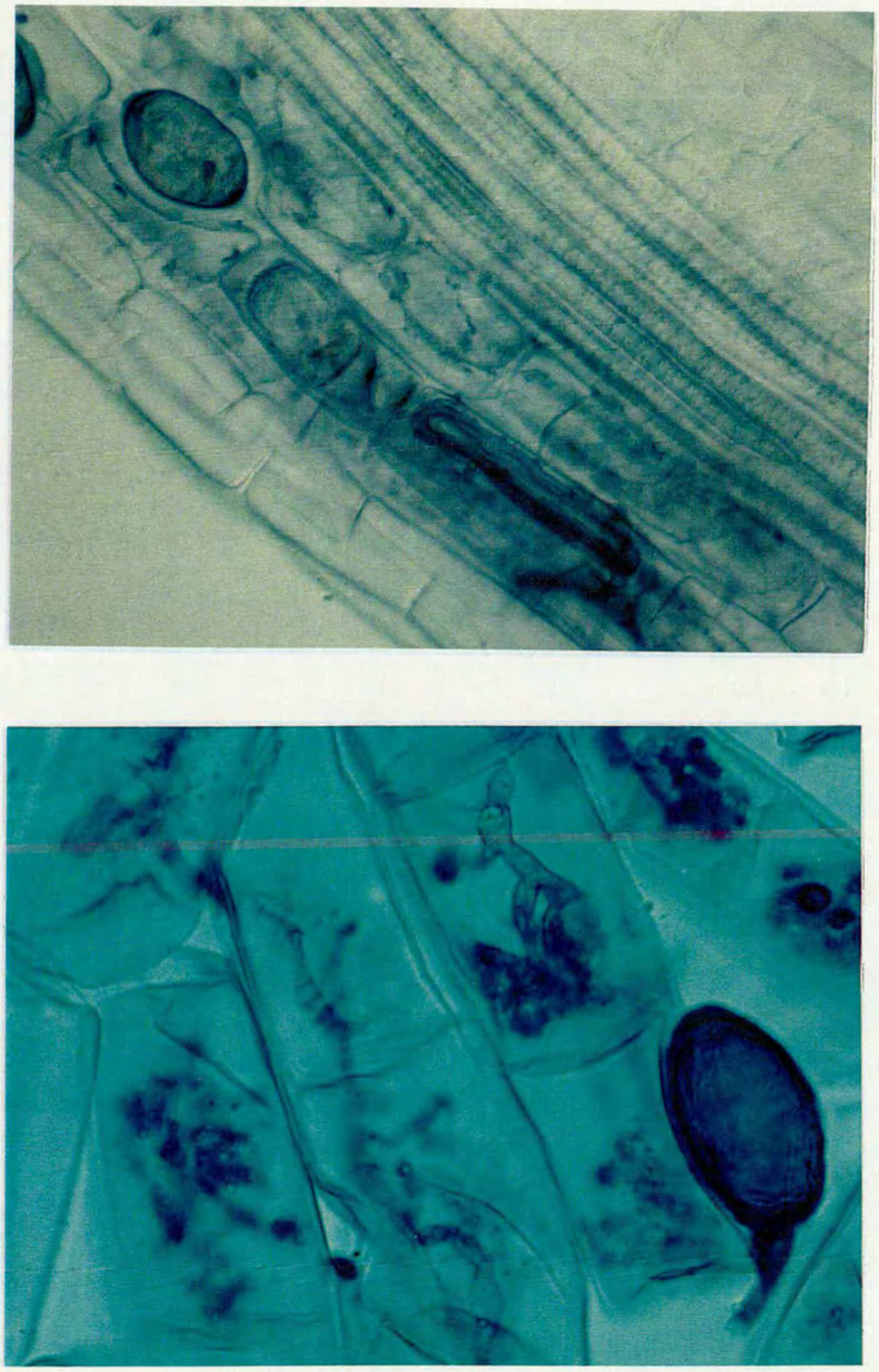


Plate 4.2. Mycorrhizal infection in the roots of *Z. mays*.

Table 4.2. Significance of F-values in the analysis of variance for mycorrhizal infection of *Zea mays* L. harvested 60 and 120 d.a.i.

	MYCORRHIZAL INFECTION	
	60 d.a.i.	120 d.a.i.
Species	***	**
Treatment	***	ns
Species.Treatment	***	*

* P < 0.01 ** P < 0.05 *** P < 0.001 ns not significant

Table 4.3. Significance of F-values in the analysis of variance of total dry weight, shoot dry weight, root dry weight, root : shoot ratio of inoculated and uninoculated *Zea mays* L. harvest 60 and 120 d.a.i.

	TDW	SDW	RDW	R : S ratio
Treatment effects (60 d.a.i)	ns	*	ns	ns
Treatment effects (120 d.a.i.)	*	ns	*	*

* P < 0.01 ** P < 0.05 *** P < 0.001 ns not significant

Nutrition

Shoot phosphorus content (Figure 4.6b), was found to be significantly (P < 0.001) greater in inoculated plants from treatments TC and TF and uninoculated plants from treatment CA compared to all other plants. Shoot magnesium content (Figure 4.6d), was significantly lower in plants from treatment CB compared to all other plants. Except for these, there were no other differences in shoot nutrient content at this time (Table 4.4). The root nutrient content was not significantly different in plants from different experimental treatments, except for root calcium content (Table 4.4; Figure 4.7e), which was significantly (P < 0.05) greater in uninoculated plants from treatment CA.

Shoot nitrogen content (Figure 4.8a) was found to be significantly (P < 0.001) greater in inoculated compared to uninoculated plants at the end of this experiment. A small but significant (P < 0.005) increase in shoot phosphorus content in inoculated compared to uninoculated plants was found also (Table 4.4; Figure 4.8b). The root nutrient content was not significantly different between plants from different treatments 120 d.a.i. (Table 4.4; Figure 4.9).

Table 4.4. Significance of F-values in the analysis of variance for shoot and root % N, % P, % K, % Mg and % Ca content of inoculated and uninoculated *Zea mays* L. harvested 60 d.a.i. and 120 d.a.i.

Nutrients	% N	% P	% K	% Mg	% Ca
Treatment effects Shoot (60 d.a.i)	ns	***	ns	***	ns
Treatment effects Root (60 d.a.i.)	ns	ns	ns	ns	**
Treatment effects Shoot (120 d.a.i.)	***	**	ns	ns	ns
Treatment effects Root (120 d.a.i)	ns	ns	ns	ns	ns

* P < 0.01 ** P < 0.05 *** P < 0.001 ns not significant

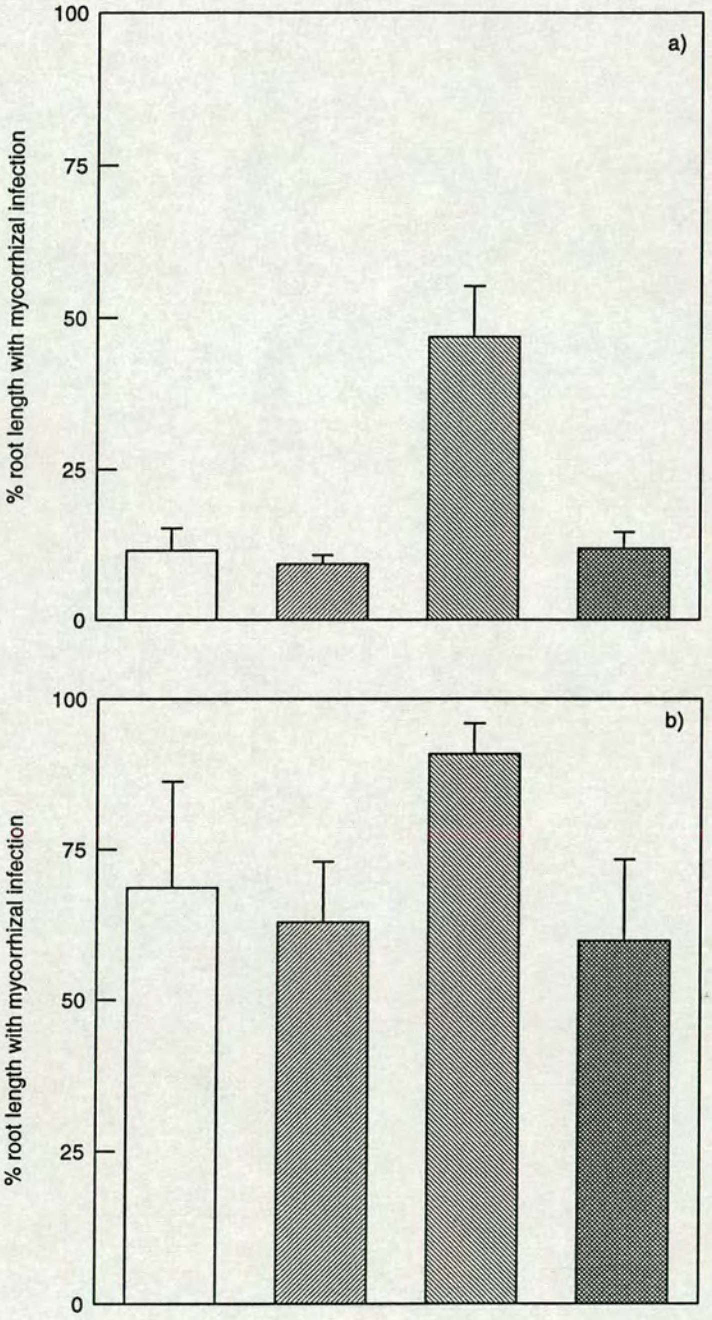


Figure 4.3. Effects of inoculating *Zea mays* L. with tree and alley soil from fallow and intercropped areas of the RHI system at Machakos, Kenya on the formation of mycorrhizal infection in roots, a) 60 d.a.i. and b) 120 d.a.i. Bars + S.E. (n=5).
Tree fallow inoculum, Alley fallow inoculum, Tree cropped inoculum, Alley cropped inoculum.

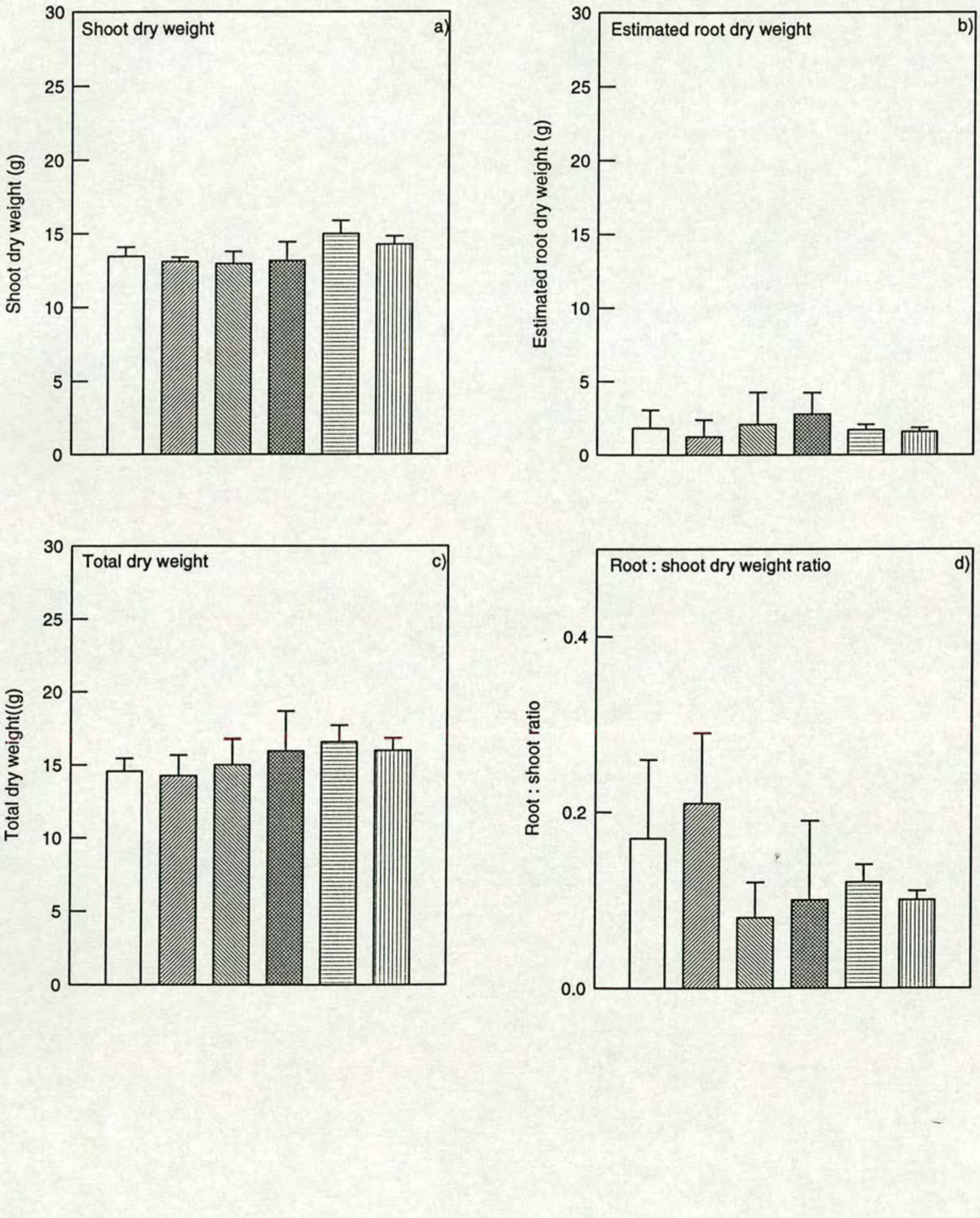


Figure 4.4. Effect of inoculating *Zea mays* L. with soil from different treatment areas of the RHI system on shoot, root and total dry weights and root : shoot ratios 60 d.a.i. Mean +S.E. (n=5).
Tree fallow inocula, Alley fallow inocula, Tree cropped inocula
Alley cropped inocula, Non-mycorrhizal CA, Non-mycorrhizal CB

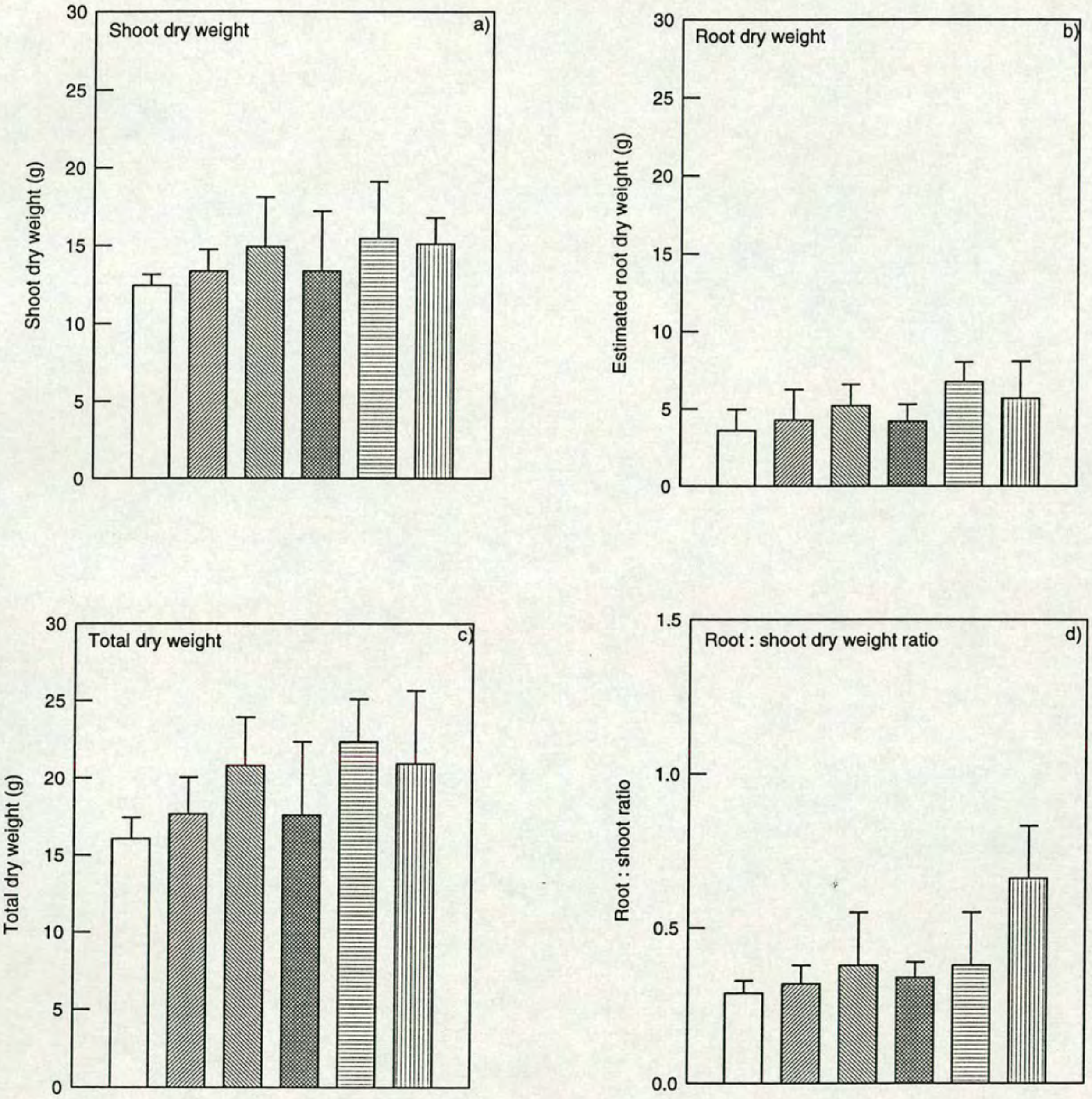


Figure 4.5. Effect of inoculating *Zea mays* L. with soil from different treatment areas of the RHI system on shoot, root and total dry weights and root : shoot ratios 120 d.ai. Mean +S.E (n=5).
Tree fallow inocula, Alley fallow inocula, Tree cropped inocula
Alley cropped inocula, Non-mycorrhizal CA, Non-mycorrhizal CB.

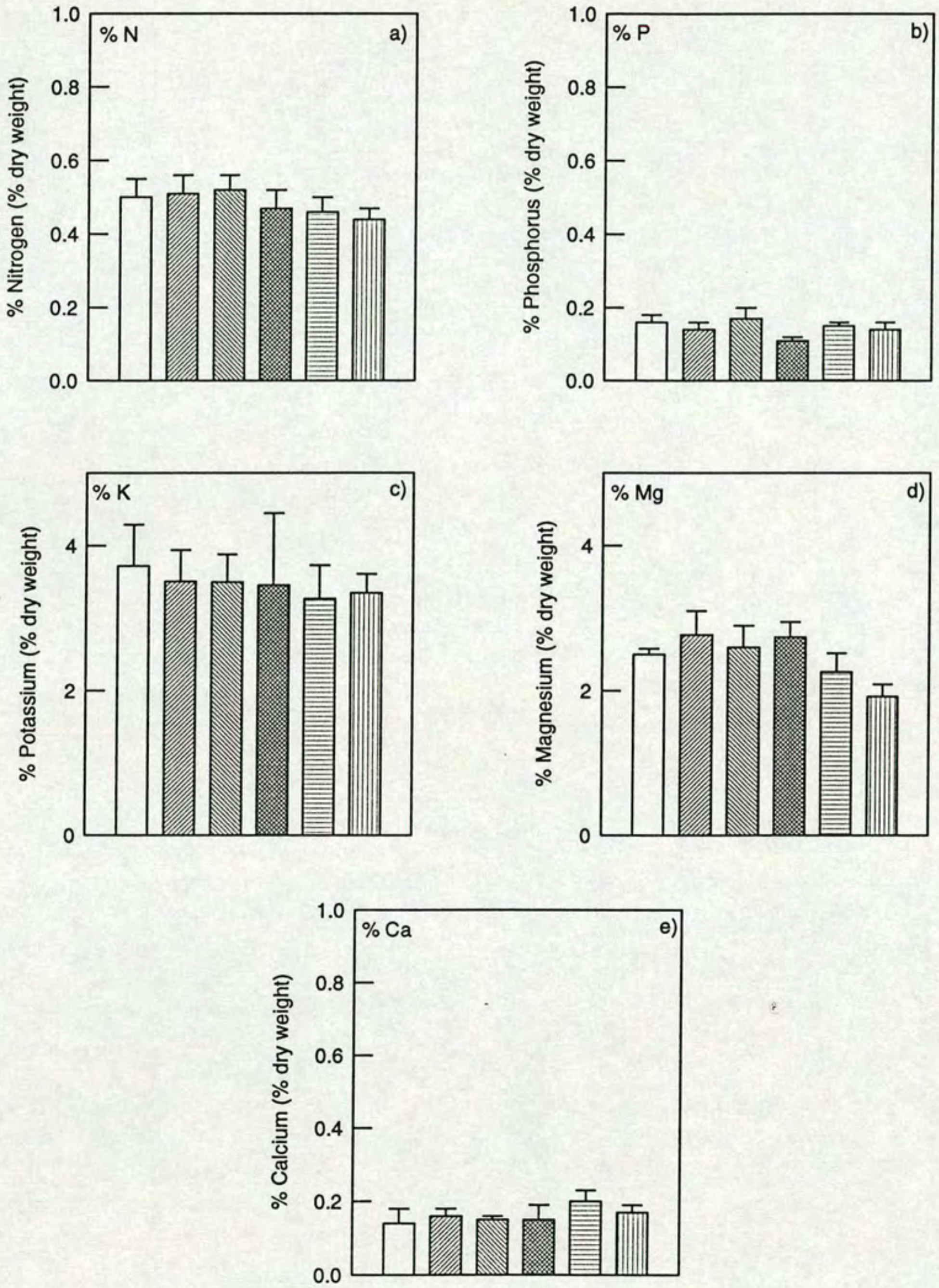


Figure 4.6. Effect of inoculating *Zea mays* L. with tree and alley soil from fallow and cropped areas of the RHI system on mean shoot N, P, K, Mg and Ca concentrations (% of dry weight) 60 d.a.i. Mean +S.E (n=5). Tree fallow inocula, Alley fallow inocula, Tree cropped inocula, Alley cropped inocula, Non-mycorrhizal CA, Non-mycorrhizal CB

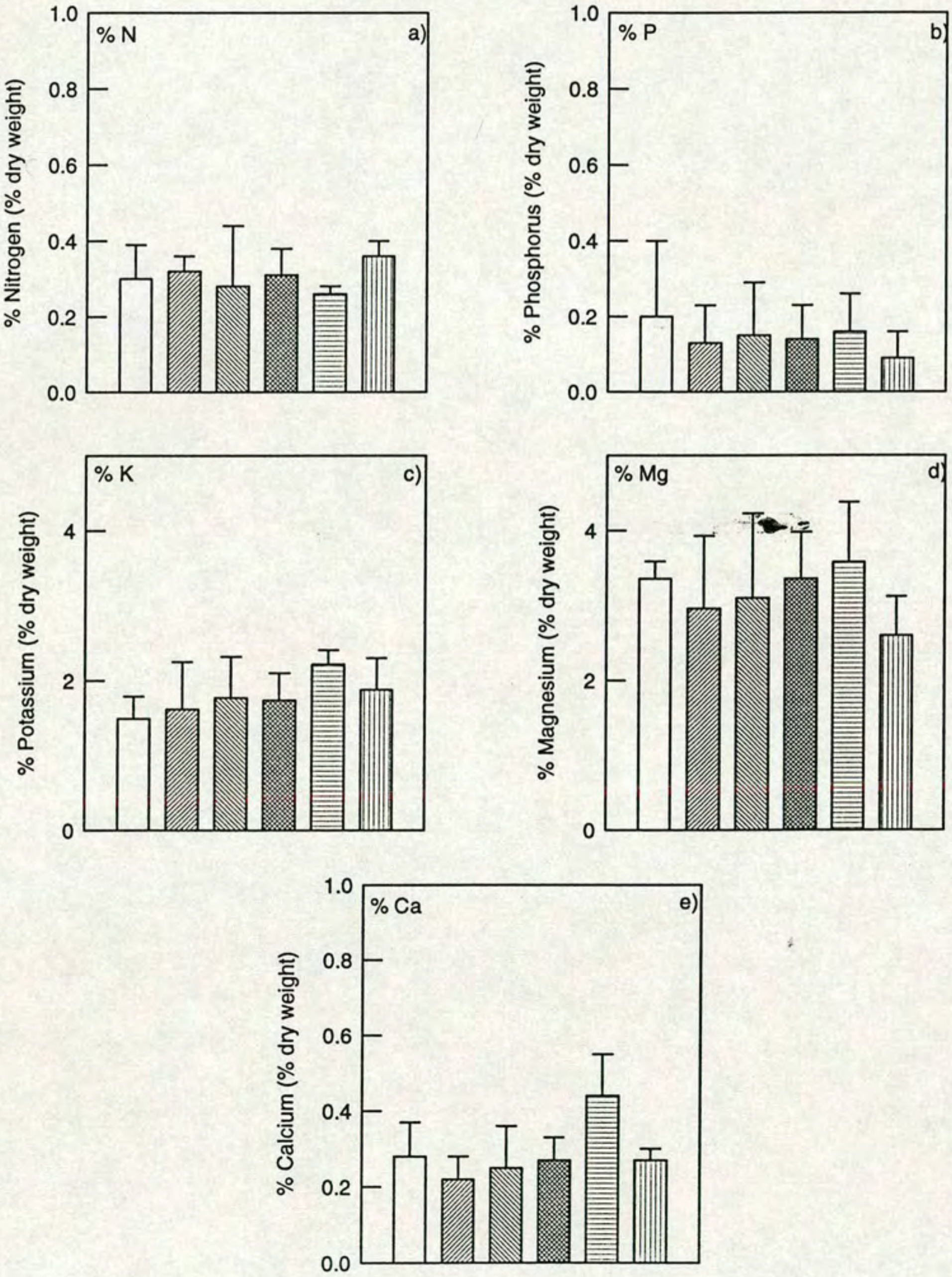


Figure 4.7. Effect of inoculating *Zea mays* L. with tree and alley soil from fallow and cropped areas of the RHI system on mean root N, P, K, Mg and Ca concentrations (% dry weight) 60 d.a.i. Mean =S.E (n=5). Tree fallow inocula, Alley fallow inocula, Tree cropped inocula, Alley cropped inocula, Non-mycorrhizal CA, Non-mycorrhizal CB.

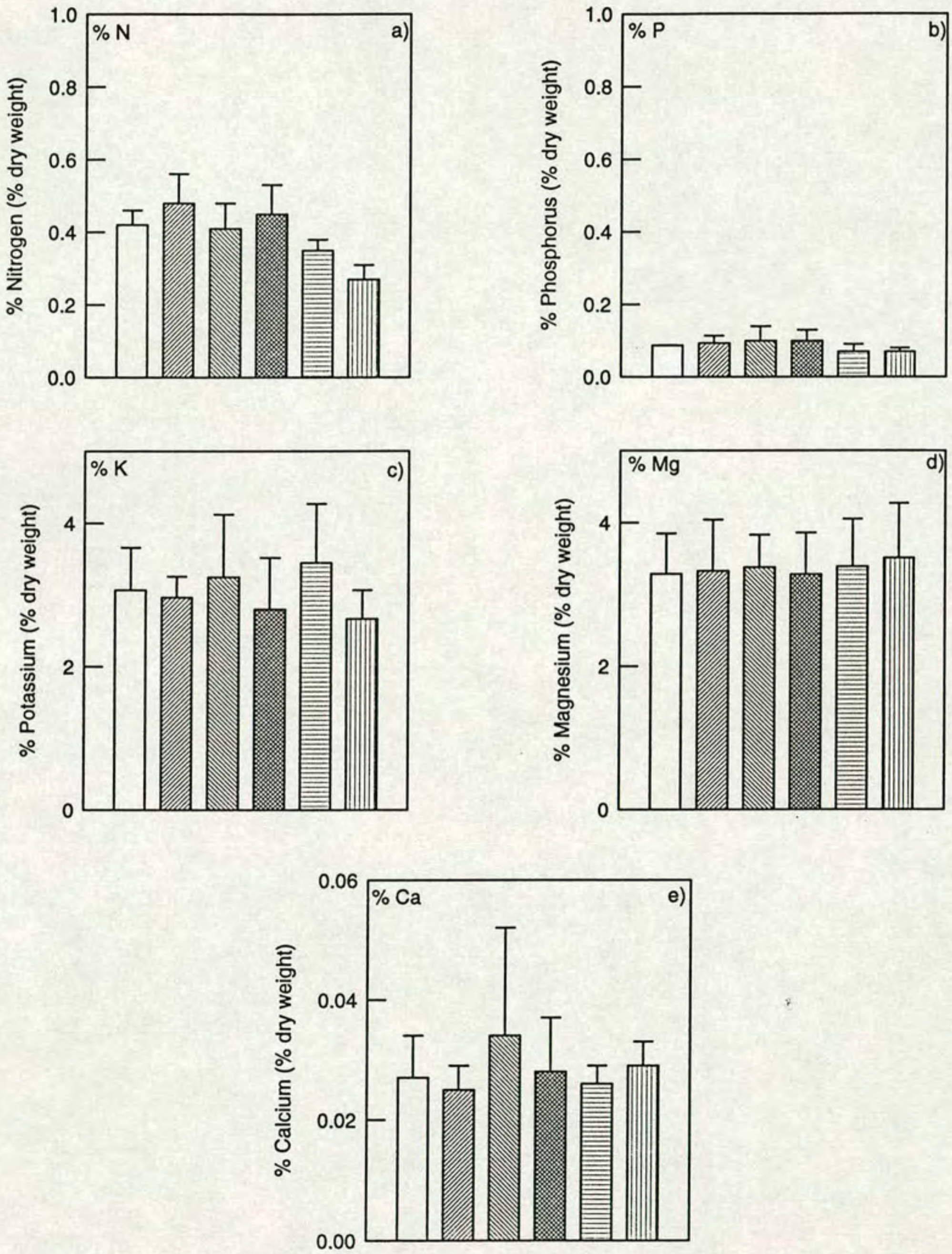


Figure 4.8. Effect of inoculating *Zea mays* L. plants with tree and alley soil from fallow and and cropped areas of the RHI system at Machakos, Kenya on mean shoot N, P, K, Mg and Ca concentrations (% of dry weight) 120 d.a.i. Mean +S.E. (n=5). ▭ Tree fallow inocula ▨ Alley fallow inocula ▧ Tree cropped inocula ▩ Alley cropped inocula ▤ Non-mycorrhizal CA ▥ Non-mycorrhizal CB.

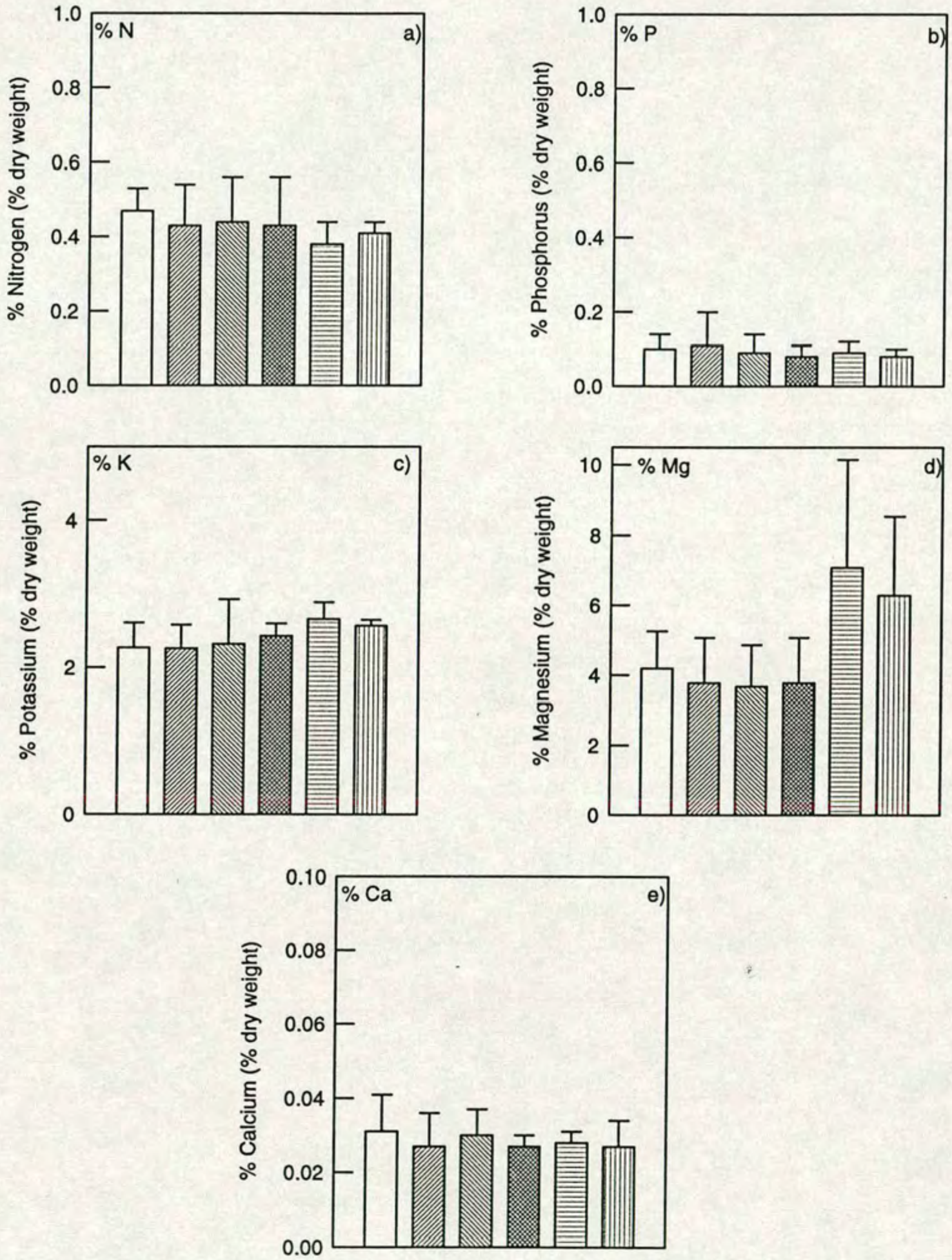


Figure 4.9. Effect of inoculating *Zea mays* L. plants with tree and alley soil from fallow and cropped areas of the RHI system at Machakos, Kenya on mean root N, P, K, Mg and Ca concentrations (% of dry weight) 120 d.a.i. Mean +S.E. (n=5). ▭ Tree fallow inocula ▨ Alley fallow inocula ▧ Tree cropped inocula ▩ Alley cropped inocula ▤ Non-mycorrhizal CA ▥ Non-mycorrhizal CB.

4.3.2 Experiment 2 : to determine the effects of inoculating <u>Zea mays</u> plants with infected root material from plants inoculated with soil from the RHI system in experiment 1a.

Spore counts

Spore numbers were found in significantly ($P < 0.001$) higher numbers (1408 spores 50 g^{-1} soil) in soil for plants inoculated with infected roots in treatment TC compared to the other inoculated treatments in this experiment (Table 4.6). Between 57 % and 82 % of spores occurring in soil were identified as the *Glomus* sp. type 3 (Table 4.5.). This was the predominant spore type in the field, occurring in significantly higher numbers in tree soil in the cropped area (TC) compared to elsewhere in the system. In the present experiment, the number of spores of type t3 as a proportion of the total was significantly ($P < 0.001$) lower in treatment TF than in all other treatments. Significantly higher ($P < 0.001$) numbers of dead spores occurred in treatments inoculated with roots from treatments TC and TF. The number of dead spores as a proportion of total spore numbers was low (14 % - 23 %), with the exception of treatment TF where the proportion of dead spores was 42 % (Table 4.5). The remaining unidentified live spores represented less than 4 % of spores extracted from most soil samples. In treatment TC the proportion was significantly greater (10 %). Because of small numbers, these spores could not be identified to species level and were classified as 'other' (Table 4.5; Table 4.6). All spores found belonged to the genus *Glomus*.

Mycorrhizal formation

There was a significant interaction effect ($P < 0.001$) between species and treatment on mycorrhizal infection, such that infection was significantly different between species in the cropped area but not the fallow area of the system (Table 4.7; Figure 4.10). Mycorrhizal infection ranged from 23% to 94% at the end of this experiment (120 d.a.i.), and was significantly greater in plants inoculated with infected root material from treatment TC in experiment 1. In treatment TC, a high proportion of infection consisted of hyphae and vesicles, while in all other treatments, this proportion ranged between 17 and 32 %. Few observations of arbuscules were made.

Table 4.5. Number of spores (and proportion of total) in soil sampled from around the roots of *Z. mays* plants 120 d.a.i with mycorrhizal root material of *Zea mays* plants inoculated with soil from tree and alley sites in cropped and fallow areas of the RHI system 60 d.a.i. in experiment 1a.

	Number of spores 50 g^{-1} soil and proportion of total						
	Total	Dead	*% Dead	t3	*% t3	Other	*% Other
TC	1408a	291a	21b	973a	69ab	144a	10a
AC	459b	106b	23b	336bc	73b	17b	4b
TF	380b	159a	42a	216c	57c	5b	1b
AF	583b	82b	14b	480b	82a	20b	4b

Values in the same column followed by the same letter are not significantly different at P < 0.05.
* calculated as a percentage of total spore number

Table 4.6. Significance of F-values in the analysis of variance for number of spores occurring in pot soil around the roots of inoculated *Zea mays* L. harvested from experiment 2 60 d.a.i.

Source of variation	Total	dead	t 3	other	% t 3	% dead	% other
Species	***	**	ns	*	**	**	ns
Treatment	**	***	ns	**	ns	ns	*
Spec.Trea	ns	***	***	***	***	***	*

* P < 0.01 ** P < 0.05 *** P < 0.001 ns not significant

Table 4.7. Significance of F-values in the analysis of variance for mycorrhizal infection in *Zea mays* harvested from experiment 2 60 d.a.i.

	Mycorrhizal infection (120 d.a.i.)
Species	***
Treatment	***
Species.Treatment	***

* P < 0.01 ** P < 0.05 *** P < 0.001 ns not significant

Growth of plants

At the end of this experiment plants were slightly larger than those in experiment 1 harvested at this time. In this experiment, significant differences occurred for all growth parameters between plants from different treatments (Table 4.8). The total dry weights of inoculated plants from treatment TF were significantly ($P < 0.001$) lower relative to all other plants in this experiment. Shoot dry weights of plants from treatment CA were significantly greater ($P < 0.01$) than that of inoculated plants from treatments AC and TF (Figure 4.11). Inoculated plants from treatment TF had significantly ($P = 0.05$) lower root dry weights than all other inoculated plants and along with TC had significantly lower root dry weights than uninoculated plants from CA (Table 4.8; Figure 4.11). The root : shoot ratio of inoculated plants from treatment TF was significantly ($P < 0.001$) lower than those of all other plants in this experiment.

Nutrition

Shoot and root nutrient content (% of dry weight) was not significantly different between plants from different treatments with the exception of root nitrogen content which was found to be significantly higher in inoculated compared to uninoculated plants (Table 4.9; Figure 4.12; Figure 4.13).

Table 4.8. Significance of F-values in the analysis of variance for total dry weight, shoot dry weight, root dry weight, and root : shoot ratio of inoculated and uninoculated *Zea mays* harvested from experiment 2 120 d.a.i.

	TDW	SDW	RDW	R : S ratio
Experimental treatments	***	**	*	***

* P < 0.01 ** P < 0.05 *** P < 0.001 ns not significant

Table 4.9. Significance of F-values in the analysis of variance for shoot and root % N, % P, % K, % Mg and % Ca of inoculated and uninoculated *Zea mays* harvested from experiment 2 120 d.a.i.

	% N	% P	% K	% Mg	% Ca
Shoot nutrient content	ns	ns	ns	ns	ns
Root nutrient content	***	ns	ns	ns	ns

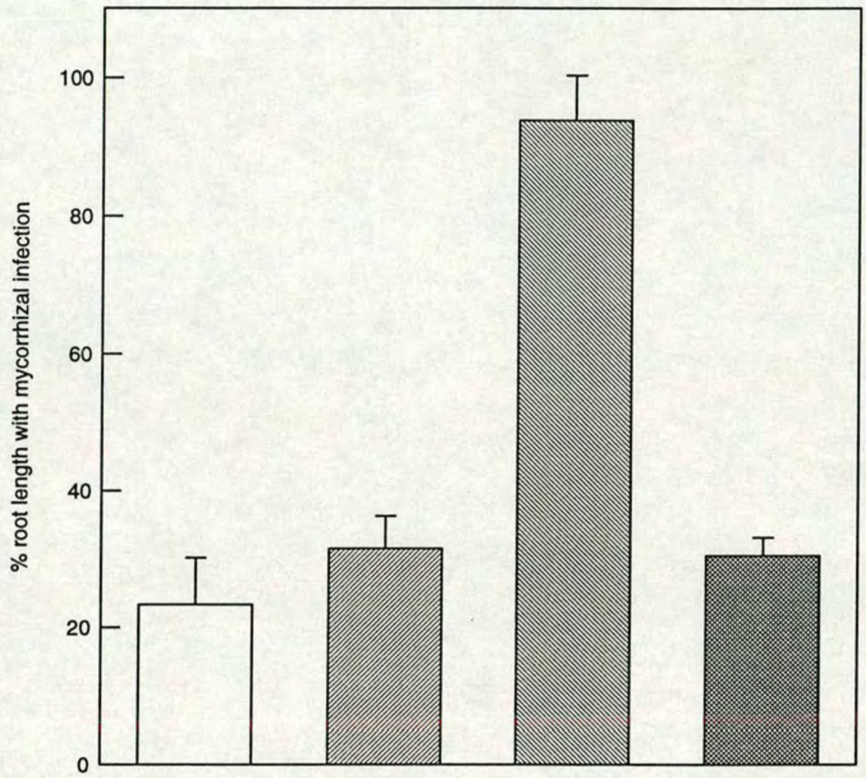


Figure 4.10. Effects of inoculating *Zea mays* L. plants with infected root material taken from plants previously inoculated with tree and alley soil from fallow and cropped areas of the RHI system in experiment 1a. Mean +S.E. (n=5). Tree fallow inocula Alley fallow inocula Tree cropped inocula Alley cropped inocula

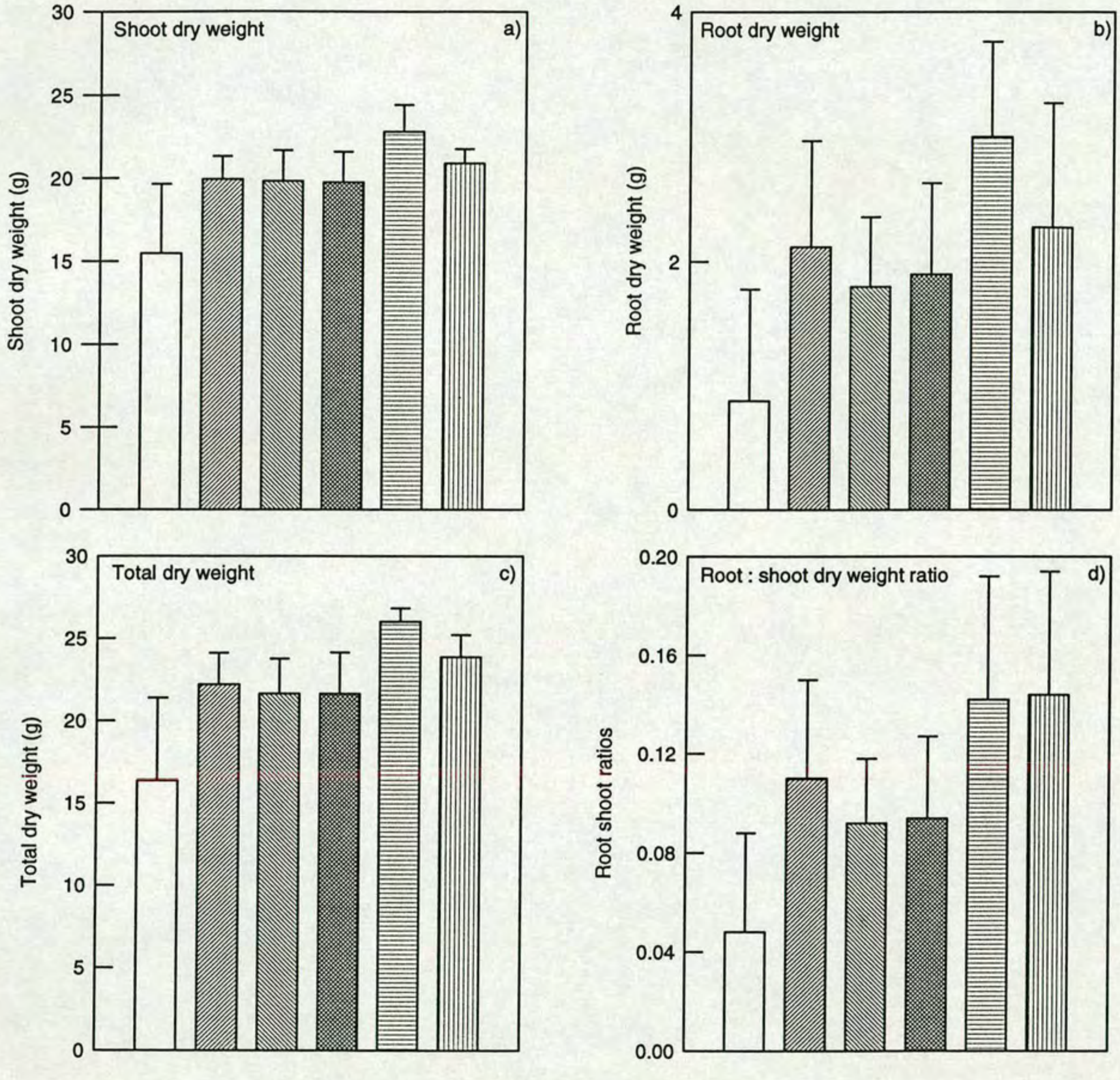


Figure 4.11. Effect of inoculating *Zea mays* L. plants with infected root material taken from plants previously inoculated with tree and alley soil from fallow and cropped areas of the RHI system in experiment 1a. Mean +S.E. (n=5). ▭ Tree fallow inocula ▨ Alley fallow inocula ▧ Tree cropped inocula ▩ Alley cropped inocula ▤ Non-mycorrhizal CA ▥ Non-mycorrhizal CB

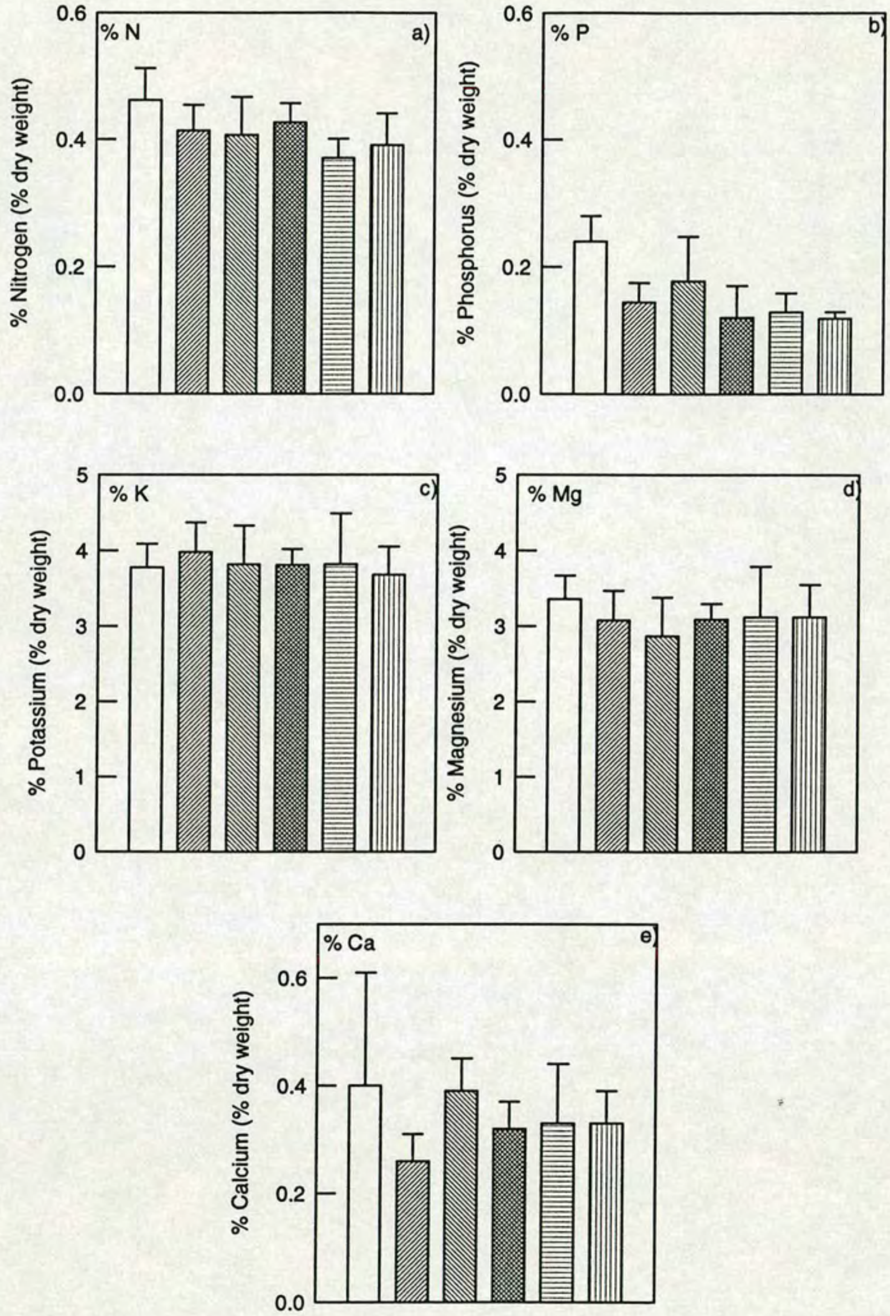


Figure 4.12. Effects of inoculating *Zea mays* L. with infected root material taken from plants previously inoculated with tree and alley soil from fallow and cropped areas of the RHI system in experiment 1a on % N, P, K, Mg and Ca concentrations (% dry weight) in shoots 120 d.a.i. Means +S.E. (n=5). ▭ Tree fallow inocula ▨ Alley fallow inocula ▧ Tree cropped inocula ▩ Alley fallow inocula ▤ Non-mycorrhizal CA ▥ Non-mycorrhizal CB.

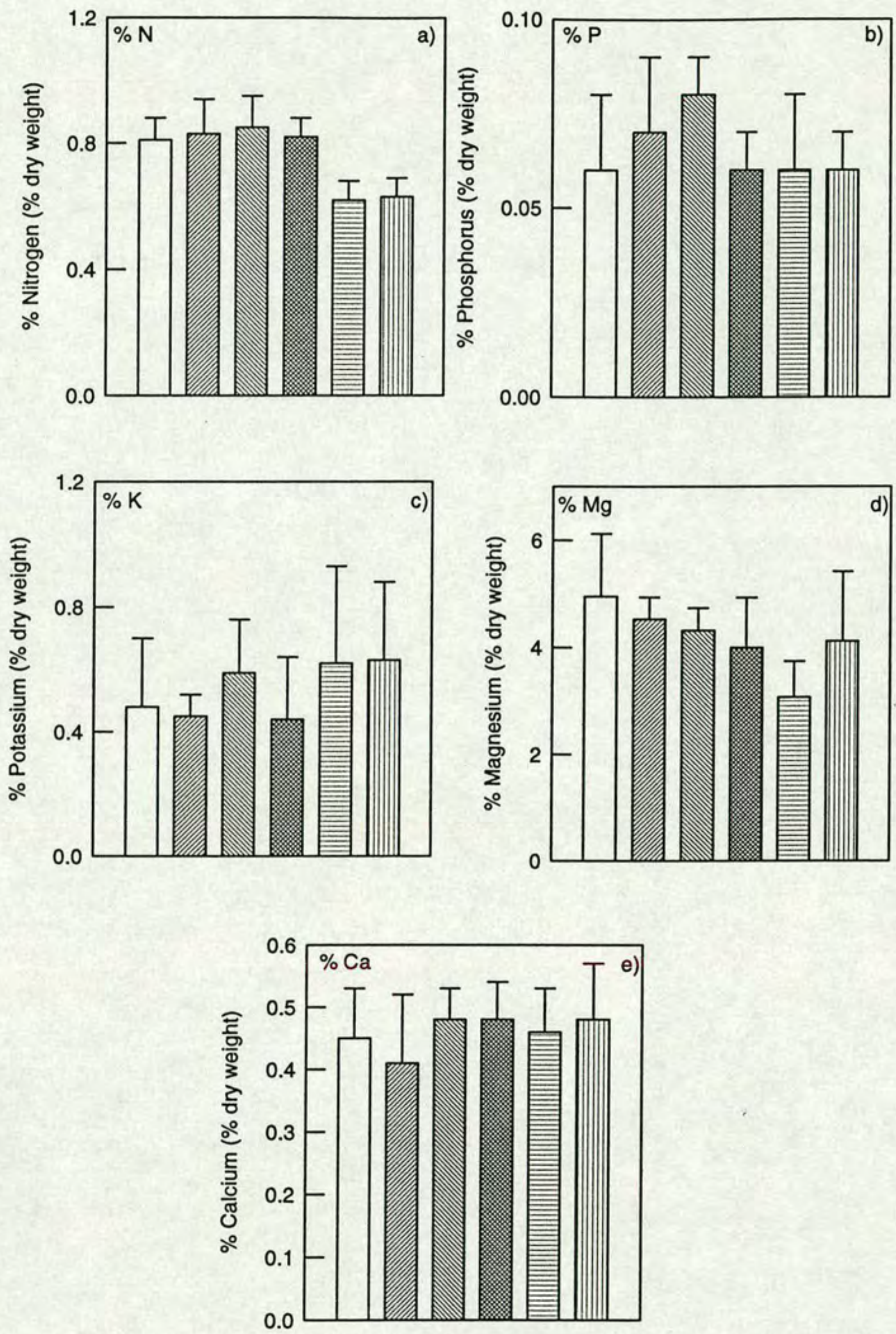


Figure 4.13. Effects of inoculating *Zea mays* L. plants with infected root material taken from plants previously inoculated with tree and alley soil from fallow and cropped areas of the RHI system on % N, P, K, Mg and Ca concentrations (% dry weight) in roots 120 d.a.i. Means +S.E. (n=5). ▭ Tree fallow inocula ▨ Alley fallow inocula ▧ Tree cropped inocula ▩ Alley fallow inocula ▤ Non-mycorrhizal CA ▥ Non-mycorrhizal CB.

4.3.3 Experiment 3 : to determine the effects of inoculum concentration on mycorrhizal infection, growth and nutrition of Zea mays plants.

Mycorrhizal formation

Mycorrhizal infection at the time of harvest (90 d.a.i.) ranged from 24 % to 80 % between treatments increasing significantly with incremental increases in the amount of inoculum (Table 4.10; Figure 4.14). Plants inoculated with 50 g of soil had a mean percentage mycorrhizal infection of 24 %, whereas those inoculated with 100 g and 150 g of soil had 55 % and 80 % mycorrhizal infection respectively (Figure 4.14). In all treatments a high proportion of the infection consisted of hyphae and vesicles. Few observations of arbuscules were made. There was no mycorrhizal infection detected in the roots of plants not inoculated with AMF.

Growth of plants

Plants harvested 90 d.a.i. in this experiment were similar in size and dry weight to those in experiment 1 (60 and 120 d.a.i.), but had lower dry weights than plants grown in experiment 2 and harvested at 60 d.a.i. Despite significant differences in the extent of mycorrhizal infection found in plants grown in pots containing different quantities of soil inoculum no significant differences occurred between treatments in relation to growth (Table 4.10; Figure 4.15).

Nutrition

Plant nutrient content was also similar between treatments for all nutrients analysed (Table 4.10; Figure 4.16).

Table 4.10. Significance of F-values in the analysis of variance for mycorrhizal infection, TDW, SDW, RDW, R:S ratios, shoot and root % N, % P, % K, % Mg and % Ca of inoculated and uninoculated *Zea mays* harvested from experiment 1c 90 d.a.i.

Source of variation	AMF infection	TDW	SDW	RDW	R:S ratio
Treatment effects	***	ns	ns	ns	ns
	% N	% P	% K	% Mg	% Ca
Treatment effects Shoot	ns	ns	ns	ns	ns
Treatment effects Root	ns	ns	ns	ns	ns

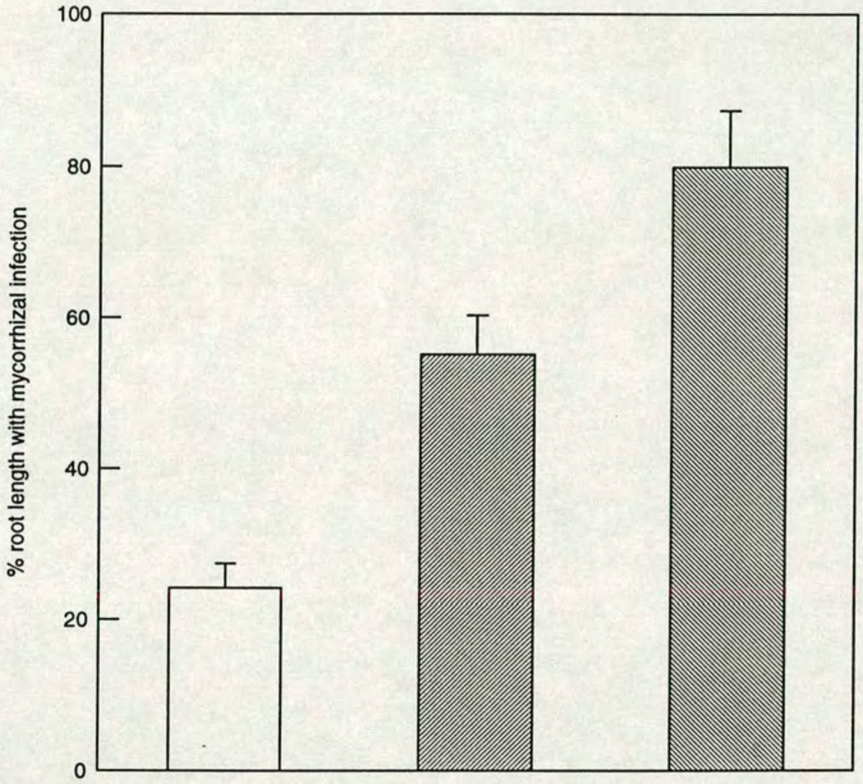


Figure 4.14. Effect of inoculating *Zea mays* L. with 50 g [white box], 100 g [hatched box] and 150 g [hatched box] of soil inoculum taken from the RHI system at Machakos, Kenya in November, 1991 on mean % mycorrhizal colonization of roots.

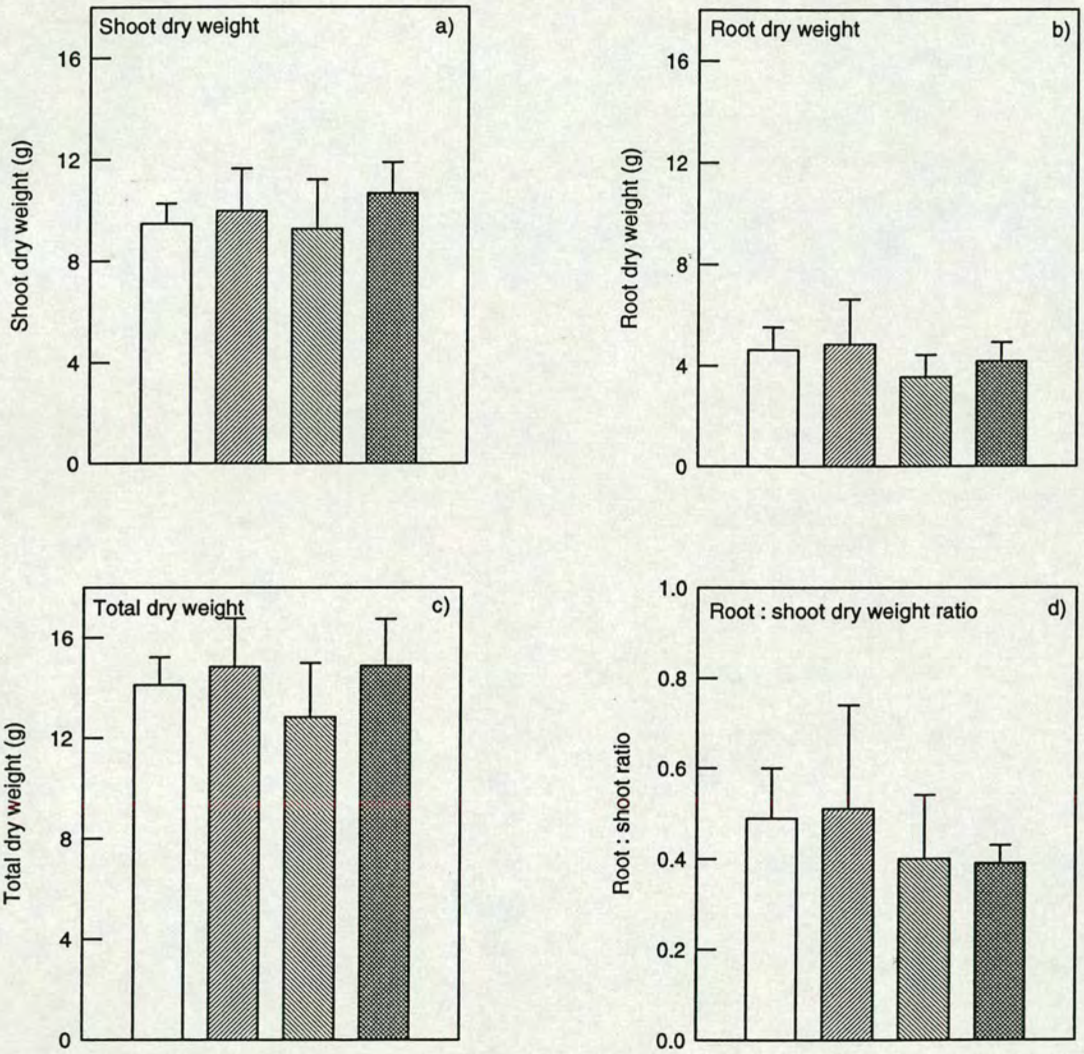


Figure 4.15. Effects of inoculating *Zea mays* L. with 50 g, 100 g, 150 g or not inoculating with soil taken from the RHI system at Machakos, Kenya in November, 1991 on shoot, root, total dry weights and root shoot ratios. Mean +S.E. (n=8). ☐ 50 g inoculated ▨ 100 g inoculated ▧ 150 g inoculated ▩ Uninoculated.

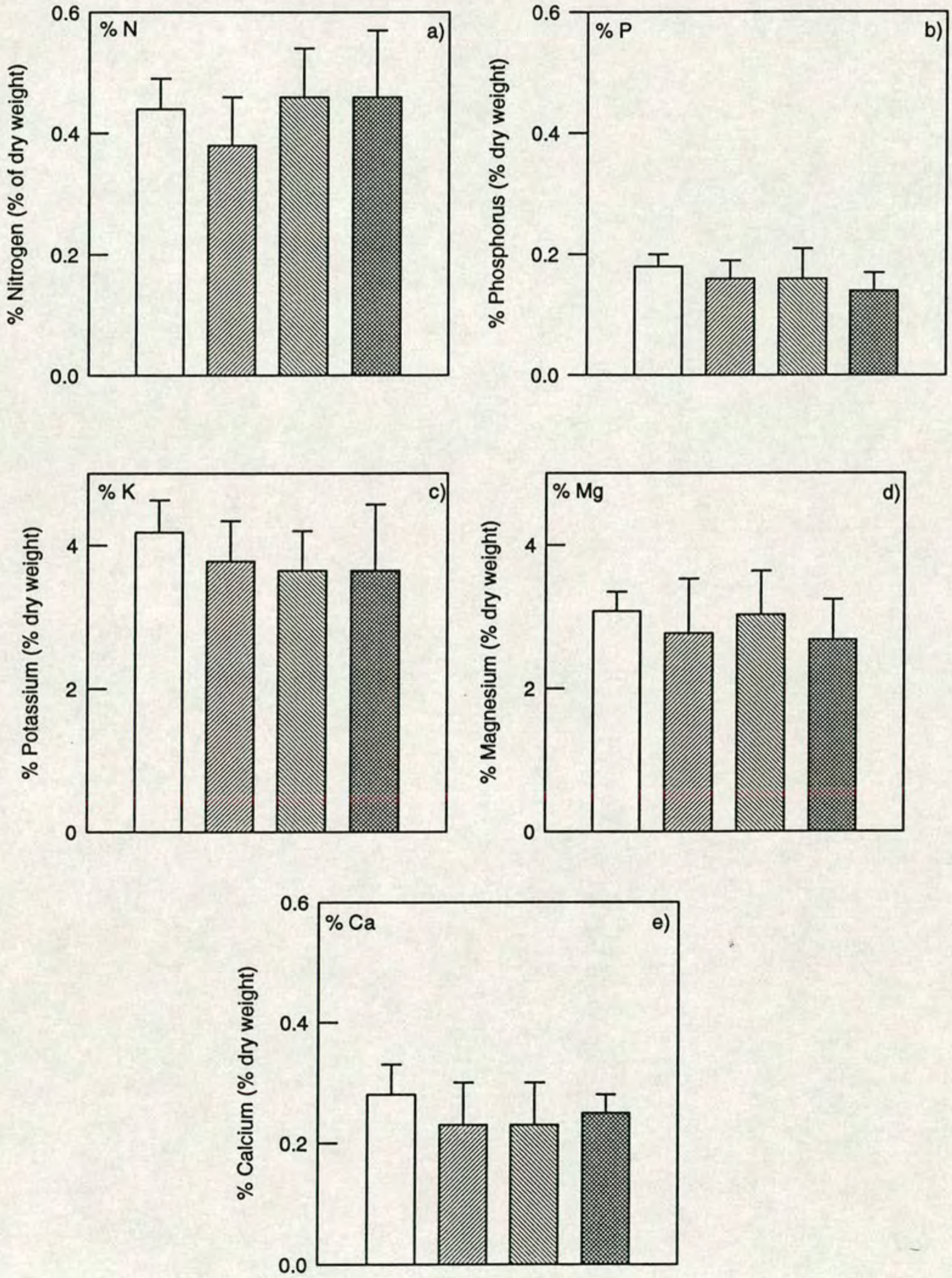


Figure 4.16. Effects of inoculating *Zea mays* L. plants with 50 g, 100 g, 150g or not inoculating with soil taken from the RHI system at Machakos, Kenya in November, 1991 on N, P, K, Mg and Ca concentrations in shoots. Mean +S.E.(n=8). ☐ 50 g inoculated ▨ 100 g inoculated ▧ 150 g inoculated ▩ uninoculated.

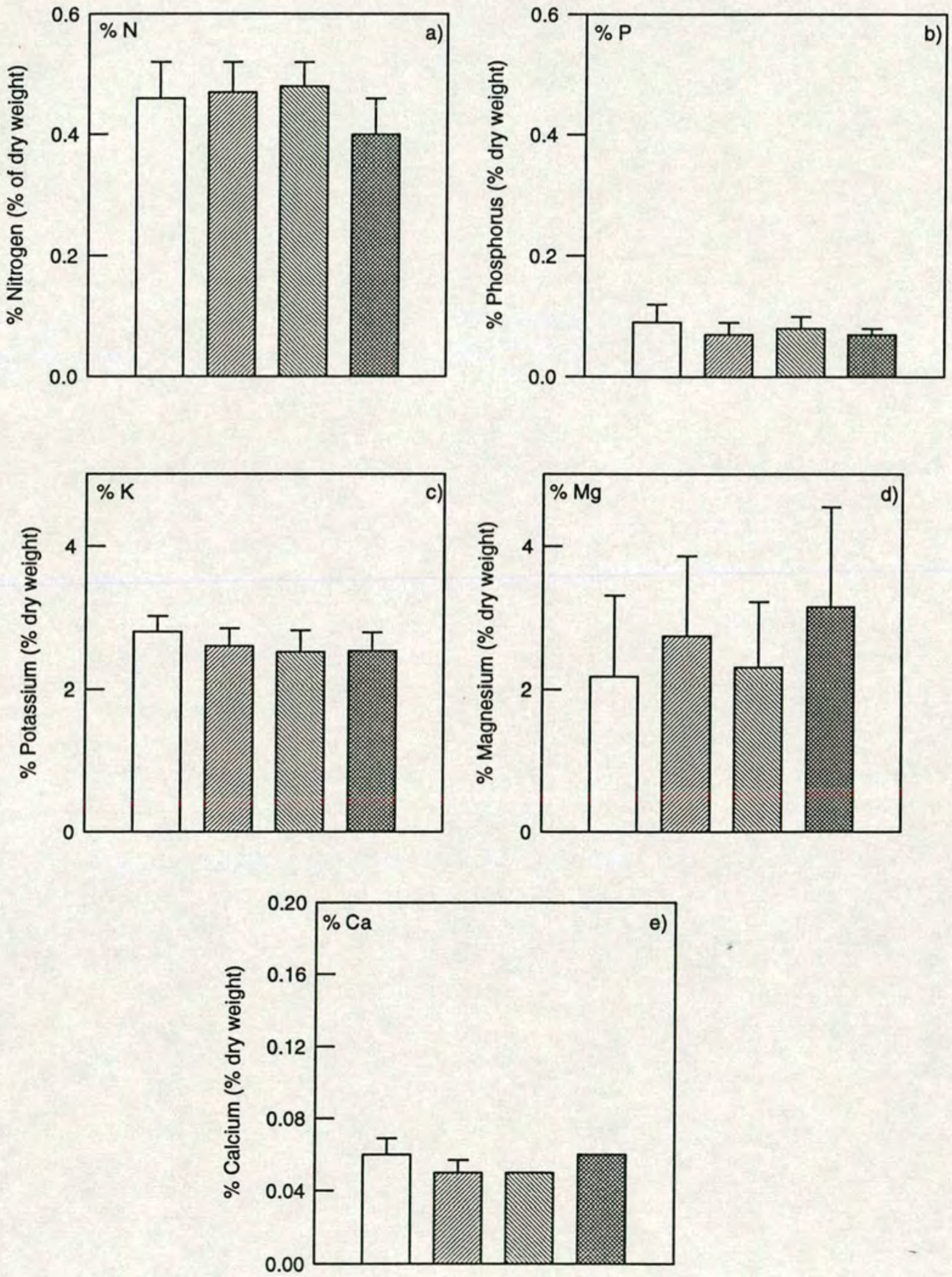


Figure 4.17. Effects of inoculating *Zea mays* L. plants with 50 g, 100 g, 150g or not inoculating with soil taken from the RHI system at Machakos, Kenya in November, 1991 on N, P, K, Mg and Ca concentrations in roots. Mean +S.E.(n=8). ▭ 50 g inoculated ▨ 100 g inoculated ▧ 150 g inoculated ▩ uninoculated.

4.4 Discussion

The effects of inoculating plants with AMF on mycorrhizal formation, growth and nutrition have been studied extensively. The majority of these studies have focussed on the effects of inoculating crop species, common in temperate agroecosystems, with exotic or indigenous AMF species or species mixtures. Comparatively little research has been carried out in the tropics, particularly in agroforestry systems. In experiment 1, the effects of inoculating *Z. mays* with AMF (from a *S. siamea*, *Z. mays* alley-crop system) on mycorrhizal formation in plant roots were obvious, while the effects on plant growth and nutrition were less marked.

At both harvests (60 and 120 d.a.i.), mycorrhizal infection was higher in plants inoculated with tree soil from the cropped area of the RHI system. A survey of AMF spore populations in this system (see section 3.3.2) found significantly higher number of live spores in tree soil in the cropped area than in soil around the roots of *Z. mays* in alleys, or tree and alley soil in the area left fallow.

There have been many reports that the number of spores in soil inoculum influences the degree of mycorrhizal infection resulting in plants (Khan, 1972; Mishra *et al.*, 1981). However, Hayman and Stovold (1979) found no relationship between spore numbers and mycorrhizal infection in plants and attributed this to differences in populations of non-sporing AMF and to differences in amount of non-sporing propagules such as hyphae and mycorrhizal root fragments. There are conflicting results from experiments attempting to relate the extent of mycorrhizal infection to growth and nutrient uptake in plants. Some studies have found that growth and nutrient uptake increase as mycorrhizal infection in roots increase (Khan, 1972), while others have not (Mc Gonigle et al., 1990a). More studies are required to elucidate the importance of the extent of infection in plants roots, the density of AMF hypha outside the root and the resultant nutrient uptake and growth of plants.

Apart from a few studies on the effects of disturbance on the AMF hyphal network (Evans and Miller, 1990; Mc Gonigle and Fitter, 1993; Bellgard, 1993b), there has been little research comparing directly the effects of different AMF propagules on the growth of host plant. This is due to difficulties encountered when working with AMF mycelium. These involve difficulties with the extraction of AMF hyphae from soil due to microscopic size of hyphae aswell as the fact that as yet, easily adopted techniques to distinguish AMF species using hyphae havee not been developed. However, a recent study using image analysis to quantify AMF mycelium found significant differences in density of mycelium produced by different AMF species (Green *et al.*, 1994).

It has been reported that external hyphae of AMF are involved in the mechanism by which disturbance decreases mycorrhizal colonisation in plants (Jasper et al, 1989b; 1989c; Evans and Miller, 1990). Bellgard (1993b) found that high soil disturbance delayed the initiation and extent of mycorrhizal colonization and decreased plant shoot dry weight relative to those at low and intermediate soil disturbance. He suggested that high disturbance damaged the external hyphal network and root fragments, thereby delaying initiation of colonisation due to the time required for hyphae to growth from other AMF propagules. In disturbed areas, the role of AMF spores as propagules of mycorrhizal infection could be particularly important. In disturbed agrecosystems, spore numbers are often found in higher number than in undisturbed areas (see section 3.1).

The results from this study showed that soil from the tree in the cropped area of the system produced significantly higher mycorrhizal infection in plants. Such an effect would be expected to occur in the field, although mycorrhizal infection would be slightly higher than that achieved in pots. This is because of the effect which disturbance of soil during field sampling and transportation is likely to have had. However, such an effect is not expected to be large as the contribution of AMF hyphae to soil infectivity in a frequently disturbed system would be expected to be relatively low. Soil used to inoculate plants was handled similarly and it is unlikely that this had any deleterious effects, other than to reduce mycorrhizal infection in all treatments equally. In the field, the actual number of spores is greater than that in pots and may be such that the treatment effects observed in experiment 1 would not occur in the field. However, it is argued that differences in mycorrhizal infection occurring at harvest 1 would not have been sustained for the duration of the experiment (section 4.3) had the differences in AMF infectivity been the result of some other factor influencing mycorrhizal formation in the experiment.

Plants in experiment 1 were inoculated with soil placed as a band 5 cm beneath the soil surface. Jackson *et al.* (1972) found this method of inoculum placement to be most effective for the development of mycorrhizal infection in the root system. The results from this experiment showed that mycorrhizal infection in plants was not evenly distributed throughout the root system, but was significiantly higher in the area of the root system closest to the inoculum band. In this area infection was lower than that determined for whole root systems, but followed a similar trend in relation to treatment effects. In agreement with these findings, Sieverding (1985) found that cassava root systems became infected with AMF as they passed through inoculum and that infection spread in the direction of root growth.

Despite significant differences in mycorrhizal infection, growth of inoculated plants was similar between different treatments and unexpectedly, control plants (CA) had higher shoot dry weights than inoculated plants. At first, there appeared to be a small, but significant mycorrhizal effect on P uptake in plants inoculated with tree soil however, as control plants (CA) had similarly high shoot P, this was not thought to be a mycorrhizal response, but rather the effect of higher nutrient content of tree soil inoculum. Campbell *et al.* (1993) reported an increase in the fertility of soil resulting from increases in organic matter and litter in the area beneath trees in an agroforestry system.

Following the first harvest, growth of shoots decreased, and there was little difference in the shoot dry weight of plants between 60 and 120 d.a.i. At the end of the experiment, control plants (CA) had significantly higher total dry weights compared to inoculated plants. This was due to an increase in root dry weight of plants particularly in controls. Numerous studies report that inoculation reduces the root dry weight and increases the root : shoot ratios of plants. The smaller root system is thought to be the result of a decrease in carbohydrate allocated to the root which is already extended by the external fungal hyphae (Arias *et al.* 1991; De Miranda *et al.* 1989).

The greater increase in root dry weight than shoot dry weight between harvests suggests that soil nutrients were limiting growth of plants at this time and that resources were allocated to the root in order to increase root size and thereby increase nutrient uptake. At the end of the experiment, uninoculated plants had significantly lower shoot nitrogen and phosphorus than inoculated plants, which may indicate a mycorrhiza effect on nutrient uptake. Although, many studies have found that inoculated and uninoculated plants appear to utilize the same form of P (Barrow *et al.*, 1977; see Cooper, 1984; Borie and Barea, 1981), there are some which report that mycorrhizal plants can use P adsorbed to iron hydroxides or in particulate iron phosphates, whilst non-mycorrhizal plants are unable to do so (Bolan *et al.* 1984). However, as the differences between inoculated and uninoculated plants in relation to nitrogen and phosphorus content were small, it is more likely that the significantly lower nitrogen and phosphorus content of uninoculated plants was due to a dilution effect, as these plants had significantly greater shoot dry weights than plants inoculated with AMF at the end of the experiment.

The effects of inoculation reported here on uptake of phosphorus and plant growth are unexpected as research in the last few decades has established that AMF inoculation can improve plant growth and uptake of phosphorus, particularly in soils of low fertility (Azcón-Aguilar *et al.*, 1982). The improved growth of control plants was most likely due to the absence of a carbohydrate drain by the fungal partner, suggesting that conditions under which plants were grown were not conducive to the symbiosis.

Poor growth response of AMF inoculated plants have been observed (Huante *et al.* 1993; López-Sánchez and Honrubia, 1992), although rarely. Mbuthia (1992) inoculated *Z. mays* with field soil from ICRAF's field station at Machakos and similarly reported poor growth of inoculated plants. He found evidence of pathogenic infection in plant roots and concluded that this was responsible for the poor mycorrhizal response in plants. However, in our experiment, permanent slides of stained root pieces were checked for pathogenic infection and it was not detected.

Mycorrhizal response of plants is greatest in poor soils, particularly those which have a low phosphorus content (Azcón-Aguilar *et al.* 1986). Mycorrhizal hyphae increase P uptake in plants by extending the root system through hyphal growth beyond the root depletion zone and into surrounding (non-depleted) soil. Hyphae have been found at a distance of 11 cm from the root system (Jakobsen *et al.*, 1992). If soil volume is limited (as in this experiment), then irrespective of P conditions, the mycorrhizal condition would be unable to benefit the plant.

In this experiment, the results for plant growth and nutrition show that mycorrhiza were ineffective. Chemical analysis (see section 4.3) of pot soil showed that the concentration of P at 2 mg l^{-1} was higher than that of field soil (1 mg l^{-1}) but lower than that in other experiments where improved growth of plants in response to AMF inoculation were observed. An experiment to test the effects of different levels of available P in soil on the mycorrhizal response was proposed. As it would have been unrealistic to increase soil P levels significantly above that which could be achieved in the field, it was decided to establish an experiment which would increase soil P using a slow release fertilizer, rock phosphate.

There have been many reports of significant differences in the growth response of plants to inoculation with different AMF (Aggangan and Lorilla, 1990; Medeiros *et al.*, 1994). This has been attributed to differences in the effectiveness of AMF species. Others have found that dependency of the plant species on AMF is important (Habte and Manjunath, 1991). The AMF species infecting plants at the first harvest of experiment 1 were identified by inoculating plants in experiment 2 with infected roots of plants from this experiment. The trend in mycorrhizal infection found in experiment 1 was continued in experiment 2. Approximately, 60 % to 90 % infection occurred in the roots of plants inoculated with 100 g of soil (containing AMF spore propagules) 120 d.a.i. in experiment 1, while that achieved using 10 g of root inoculum in experiment 2, ranged from 23 % to 94 %. These results suggest that infection in plant roots was increased in plants inoculated with highly infected root material compared to that achieved using spore inoculum. It is probable that attached hyphae around the roots of highly infected root material was able to infect plant roots immediately and thereby increase mycorrhizal infection in roots at 90 d.a.i. compared to those achieved 120 d.a.i. with spore inoculum in experiment 1. Bellgard (1993b) suggested that the observed delay in the initiation of colonisation in plants when soil was disturbed was due to the time required for propagules other than hyphae to produced hyphal growth for root infection.

The results from these experiments are in agreement with those which suggest that infected root material could provide an equally, if not more effective type of field inoculum. Currently, technologies to improve the handling of AMF inocula, decrease the cost of producing inocula and improve the quality (propagule density) of inocula are being developed. Sylvia and Jarstfer (1992) found that shearing roots proved to be an excellent method to prepare viable root inocula of small and uniform size, allowing for more efficient and effective use of limited inoculum supplies.

Glomus sp. type t3 spores were the predominant spore type recovered from soil in which plants inoculated with infected roots were grown. The effect of pot cultures is that in the majority of cases the number of species is reduced such that one species, best adapted to the host and conditions tends to become dominant. In this study, experiments described in chapters five and six used inoculum containing spores of this species, as it was not possible to obtain mixed populations from the field in Kenya. Whether other species infect *Z. mays* in the field needs to be determined. It could also be possible for non-sporing AMF species to exist in the roots or for AMF species to have infected roots but failed to sporulate under the glasshouse conditions. The species of AMF infecting plants is most likely a highly competitive species as it is the pre-dominant species in the field and continued to be in the glasshouse. This species may be highly competitive but ineffective and this would explain the poor growth response of inoculated plants. Screening of indigenous AMF species at different levels of soil P in relation to their effect on plant growth is necessary before AMF populations could be managed to their full potential for this system.

Z. mays is described as being facultatively dependant on AMF (Sieverding, 1991) (see section 1.). This would explain how under the conditions in this experiment those plants which were not inoculated grew well relative to those which were mycorrhizal. This further supports the explanation that the conditions in the experiment were not appropiate for an optimal mycorrhizal response. It may also indicate that the response to AMF in the field will occur only if P levels in soil are sub- optimal for plant growth.

In experiment 3, the extent of mycorrhizal infection was directly related to the number of spores in soil inoculum which in turn related to the quantity of inoculum in pots. The increased mycorrhizal infection in plants grown in soil with more inoculum and hence higher spore numbers was probably due to an increased probability of plant roots encountering germinating spores. Differences in root density may also affect this however in these experiments such differences, as represented by root dry weights, were not significant between mycorrhizal plants. Increasing the amount of inoculum had no significant effects on the growth or nutrient uptake of *Z. mays*. The effect on mycorrhizal infection of different amounts of soil inoculum was not unexpected although higher spore numbers are not always found to result in increased mycorrhizal infection in roots as discussed previously. Although, the degree of mycorrhizal infection is similarly, not always related to the growth response in plants, it was totally unexpected and difficult to explain why such variation in mycorrhizal infection, as seen in experiment 3, had no significant effect on plant growth.

The findings of all three experiments reported here show that the extent of mycorrhizal infection in roots does not ultimately determine the growth response of maize. If, as many studies indicate (section 1.7.4) the mechanism by which AMF benefit the plant is disrupted in some way, then the mycorrhizal effect is reduced. It is suggested that for experiments 1, 2 and 3, proliferation of fungal hyphae in soil necessary to achieve a mycorrhizal response in a facultatively dependant plant was limited by the volume of soil (pot size) in which plants were grown and that the soil nutrient content may not have been appropiate for optimal growth. The results from experiment 3 stress the need for experiments examining the effect of soil volume and soil phosphorus content on the mycorrhizal effect in *Z. mays.*

CHAPTER FIVE

EFFECTS OF DIFFERENT SOIL VOLUME, SOIL TYPES AND DIFFERENT LEVELS OF ROCK PHOSPHATE FERTILIZER ON MYCORRHIZAL FORMATION, NUTRITION AND GROWTH OF *Zea mays* L. PLANTS WITH OR WITHOUT AMF.

5.1 Introduction

In experiment 1, inoculation of *Z. mays* with soil (containing AMF spore populations) from different areas of an agroforestry system influenced the degree of mycorrhizal infection which developed in plant roots. However, the growth response of plants to this 'natural' inoculation was unexpected and unrelated to the differences in mycorrhizal formation (Section 4.3.3.). Many studies have found that AMF inoculation can significantly improve plant growth and nutrition (see section 1.5.1). Of these, some have found that the effect on plant growth is proportional to the degree of mycorrhizal infection in roots (Kormanik, 1985a; 1885b; Arias *et al.*, 1991), while others have not (Guttay and Dandurand, 1989; Mosse, 1972; Habte and Turk, 1991; Fabig *et al.*, 1989).

Studies which have observed poor growth responses in plants to inoculation with AMF, have often attributed this to infection by highly competitive but ineffective AMF species (Jasper *et al.*, 1979), or to the degree of dependency of the plant on mycorrhizal fungi for growth and nutrient uptake (Janos, 1980). Environmental factors have been found responsible for the variation in mycorrhizal growth response of plants in both field and glasshouse experiments (Lambais and Cardoso, 1993; Medeiros *et al.*, 1994; Fabig *et al.*, 1993). Differences in levels of available phosphorus, other nutrients and micronutrients in soil, soil pH, ambient and soil temperature as well as light conditions have all been found to affect the symbiosis (see section 1.7). The volume of soil in which plants are grown, particularly in pot experiments, has been found to influence plant-AMF interactions. This is primarily due to the effect of soil volume on the growth of plant roots and fungal hyphae (Bääth and Hayman, 1983; Fabig *et al.*, 1989).

In experiment 1, uninoculated plants had significant higher dry weights than plants inoculated with AMF and grown in 1 litre pots. It was suggested that in this volume of soil, uninoculated plants were able to exploit available soil P as efficiently as inoculated plants and had significantly greater dry weights because they did not have the carbohydrate drain of the fungal partner (Amijee *et al.*, 1993). The effect of available P levels in soil (Soil A contained 1.1 mg P l^{-1} soil) on the response of plants to inoculation were also discussed.

As a result, an experiment was set up to study whether soil type, soil volume or soil P levels affect the nutrient uptake and growth of inoculated and uninoculated *Zea mays* L. Rock phosphate (RP), a cheap and readily available fertilizer in some tropical areas, was used to alter soil P levels.

5.2 Materials and Methods

AMF inoculum was produced in the glasshouse on maize plants grown in 1 litre pots containing sterilized soil A and 100 g of soil inoculum from the field (collection: R.H.I system; November, Wet season). Pot cultures were maintained for 120 days before use. Maize seeds (*Zea mays* Katumani composite var. Machakos, Kenya) were germinated in two soils (Soil A and soil B). Chemical analysis of soil A (Table 4.1; Table 5.1) showed that high levels of K (possibly linked with high concentrations in vermiculite) occurred and this increased the root K content of plants in previous experiments. Vermiculite was also found to contain high levels of P (Table 5.1), To eliminate these variables as possible factors affecting results, soil B (without vermiculite), was made up for comparison. Plants were grown under the same conditions as those described for previous experiments. Maize seedlings were transplanted into soils A and B, in pots of different size (28 cm, 20 cm and 11 cm diameter ; 10 L, 4 L and 1 L volume respectively), with different quantities of RP fertilizer added, with and without mycorrhizal inoculation. The RP used was an unground form (amount passing through 0.15 mm sieve 30.0%) known as "Scotphos" (Scotphos) with 29.5% RP (12.9% P) of which 15.0% (6.5% P) is soluble in formic acid. This was mixed with pot soil B prior to planting and inoculation of seedlings. Low P soil mix contained 150 mg RP l^{-1} and high P soil mix had 300 mg RP l^{-1} equivalent to 22.5 mg soluble P l^{-1} and 45 mg soluble P l^{-1} respectively. Selected chemical properties of unamended soil A and B, and amended soil B, vermiculite, sand and rock phosphate are listed in Table 5.1.

Soil from pot cultures containing roots, spores and mycelium was mixed thoroughly in plastic bags and 100 g placed as a layer in each pot beneath the root system of each maize seedling. The same quantity of starter inoculum was therefore available to all seedlings at the beginning of the experiment. Pots were placed on the floor of the glasshouse and raised above ground level to prevent contamination of uninoculated plants through seepage water. Upturned pots, drip trays and small blocks were used to raise the upper surface of pots of different size to the same level ensuring that all plants developed under similar light conditions. Plants were grown at uniform density with wider gaps between smaller pots.

Initially, plants were watered daily or twice daily as required using the same quantity of water per pot. Seedling size began to differ between treatments three weeks into the experiment at which point the quantity of water given to plants was dependent on the volume of soil in which plants were grown. Basal nutrient solution without P (see section 2.1) was given to plants weekly to ensure that other nutrients did not become limiting to growth in this experiment. Both water and the basal nutrient solution were supplied in proportion to soil volume such that, 1 litre pots received 50 ml, 4 litre pots, 200 ml and 10 litre pots, 500 ml of water or nutrient solution. The amount of water and nutrient solution given to plants was therefore the same per unit volume of soil in each pot. Plate 5.1 shows the layout of the experiment in the glasshouse.

An unbalanced factorial experimental design was used, testing the effects of presence or absence of mycorrhizas, 4 soil types (unamended A and B plus amended B with 150 mg RP l^{-1} and amended B with 300 mg RP l^{-1}) and 3 soil volumes (1, 4 and 10 litres). The experiment was laid out in six randomised blocks with one replicate per treatment per block. At regular intervals in rows between blocks one and two, three and four and five and six another uninoculated treatment was included with plants grown in sterilized soil B only (without added autoclaved soil inoculum). These controls were included in the experiment for screening of incoming contaminant fungi, but not used for data collection. They experiment was established in May, 1993. One harvest was carried out 90 d.a.i. when height differences between treatments were detected.

Harvest assessments were the same as those carried out in previous experiments, however, due to time constraints only 4 randomly selected replicate plants from each treatment were analysed for shoot and root N, P, K, Mg and Ca.

Results in this chapter are presented in sections relating to the analysis of the experimental data which compare, 1) Soil type and soil volume (A and unamended B) 2. Soil volume and RP fertilizer additions (soil B only).

Table 5.1: Nitrogen, phosphorus, potassium, magnesium and calcium content of rockphosphate, sand, vermiculite, and soil A, and unamended and amended soil B with and without rockphosphate fertilizer added as used in experiment 4.

	Nutrient content (mg l^{-1})						
	pH	PO_4-P	NO_3-N	NH_3-N	K	Mg	Ca
Soil A	6.7	1.1	2.4	1.7	31	1.7	20.0
Soil B	6.2	1.03	-	1.26	1.95	2.07	29.4
Soil B RP1	6.6	43.5	-	1.08	44.6	6.02	14.6
Soil B RP2	6.0	143	-	0.05	80.6	20.9	4.67
Sand	-	1.23	-	0.06	15.5	58.8	15.3
Vermiculite	-	113.3	-	0.06	230	42.8	708
RP	-	1.24	-	0.05	1.17	0.90	12.4

RP0: no RP; RP1: 150 mg RP l^{-1} soil ; RP2 300 mg RP l^{-1} soil

Plate 5.1. Layout of experiment 4 in the glasshouse

5.3 Results

5.3.1 Effects of soil type and volume on mycorrhizal formation and growth of plants with and without AMF

Mycorrhizal formation

Plants grew in all treatments. There was no mycorrhizal infection in the roots of uninoculated plants. Plants had significantly higher mycorrhizal infection in unamended soil A than in unamended soil B (Table 5.2). The difference in percentage mycorrhizal infection between plants in different soil types was greater for those in larger (4 and 10 litre) pots (Figure 5.1a). The effect of pot size on mycorrhizal infection was the same for plants in both soils (Table 5.2). In the largest pots plants had least mycorrhizal infection, while infection was similar in plants grown in 1 and 4 litre pots (Figure 5.1a). A high proportion of infection in all plants consisted of hyphae and arbuscules together. The proportion of infection consisting of hyphae and vesicles greater in plants grown in soil B (approximately 15 - 20 %) than in soil A (< 1 %). Results of analysis of variance for mycorrhizal infection, total, shoot and root dry weights, and nutrition are given in full in Appendix C.

Plant growth

There was a significant interaction ($P < 0.001$) between pot size and soil type on each growth parameter (Table 5.2) Plants in 10 litre pots containing soil B had significantly reduced total and shoot dry weights compared to those grown in the equivalent amount of soil A. In smaller pots soil type had no effect on plant growth above ground (Table 5.2; Figure 5.2a; Figure 5.3a).

There was a significant interaction ($P = 0.02$) between soil type and inoculation on shoot dry weights, such that, in soil A, uninoculated plants had significantly higher shoot dry weights than plants inoculated with AMF. Shoot dry weights of those grown in soil B were unaffected by inoculation (Table 5.2; Figure 5.3a; Figure 5.3b). Overall, total dry weights were significantly greater in non-mycorrhizal compared to mycorrhizal plants in both soils (Figure 5.2a; Figure 5.2b).

There were a number of interaction effects between soil type, pot size and inoculation with respect to root dry weight (Table 5.2). Generally, in 1 litre pots there was no difference in the root dry weight of all plants. In 4 and 10 litre pots, uninoculated plants had significantly higher root dry weights than plants inoculated with AMF. In 4 litre pots, root dry weights were significantly higher in plants in soil A than in soil B, although this difference was small. In 10 litre pots, plants in soil B had higher root dry weights than those in soil A, although a significant ($P < 0.046$) interaction between soil type and inoculation, such that this difference in root dry weight was only significant for inoculated plants (Figure 5.4a; Figure 5.4b).

There was a significant interaction ($P = 0.003$) on root : shoot ratios between soil type and pot size, such that there was a decline in root : shoot ratios as pot size increased except in soil B where they were similar between plants in 4 and 10 litre pots. In addition, plants in soil B in 10 litre pots had greater root : shoot ratios than those in soil A (Figure 5.5a; Figure 5.5b).

The effect of pot size on all growth parameters for plants grown in unamended soils was highly significant ($P < 0.001$). All growth parameters increased with incremental increases in soil volume (Table 5.2; Figure 5.2; Figure 5.3; Figure 5.4), except root : shoot ratios which decreased significantly ($P < 0.001$) with incremental increases in soil volume (Table 5.2; Figure 5.5).

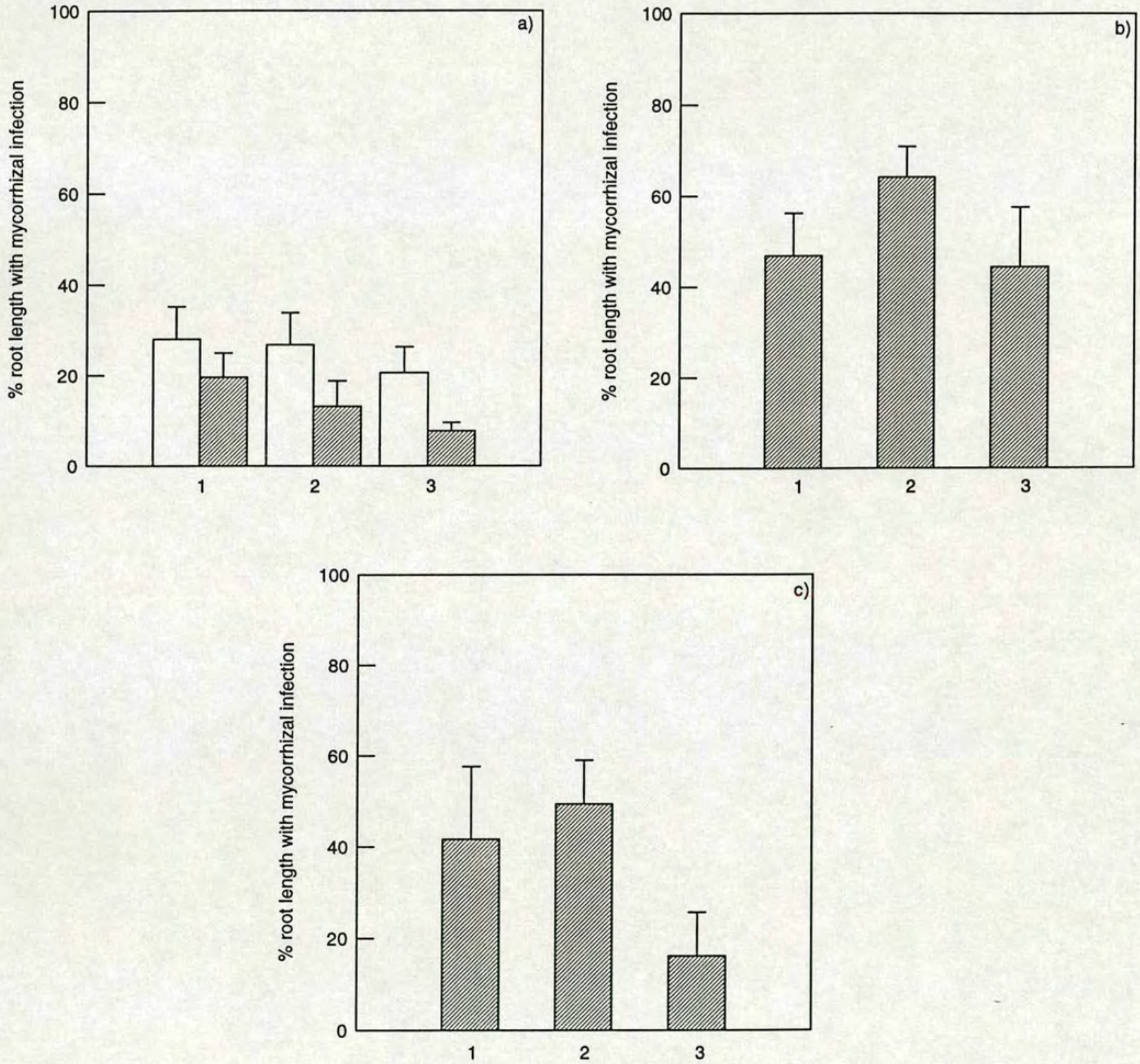


Figure 5.1: Effect of pot size on mean % root length mycorrhizal infection in inoculated *Zea mays* L. plants grown in soil A ▭ and soil B ▨ with a) No P, b) Low P and c) High P. 90 d.a.i. Bars are + S.E. (P = 0.05). 1=1 litre pot; 2=4 litre pot; 3=10 litre pot.

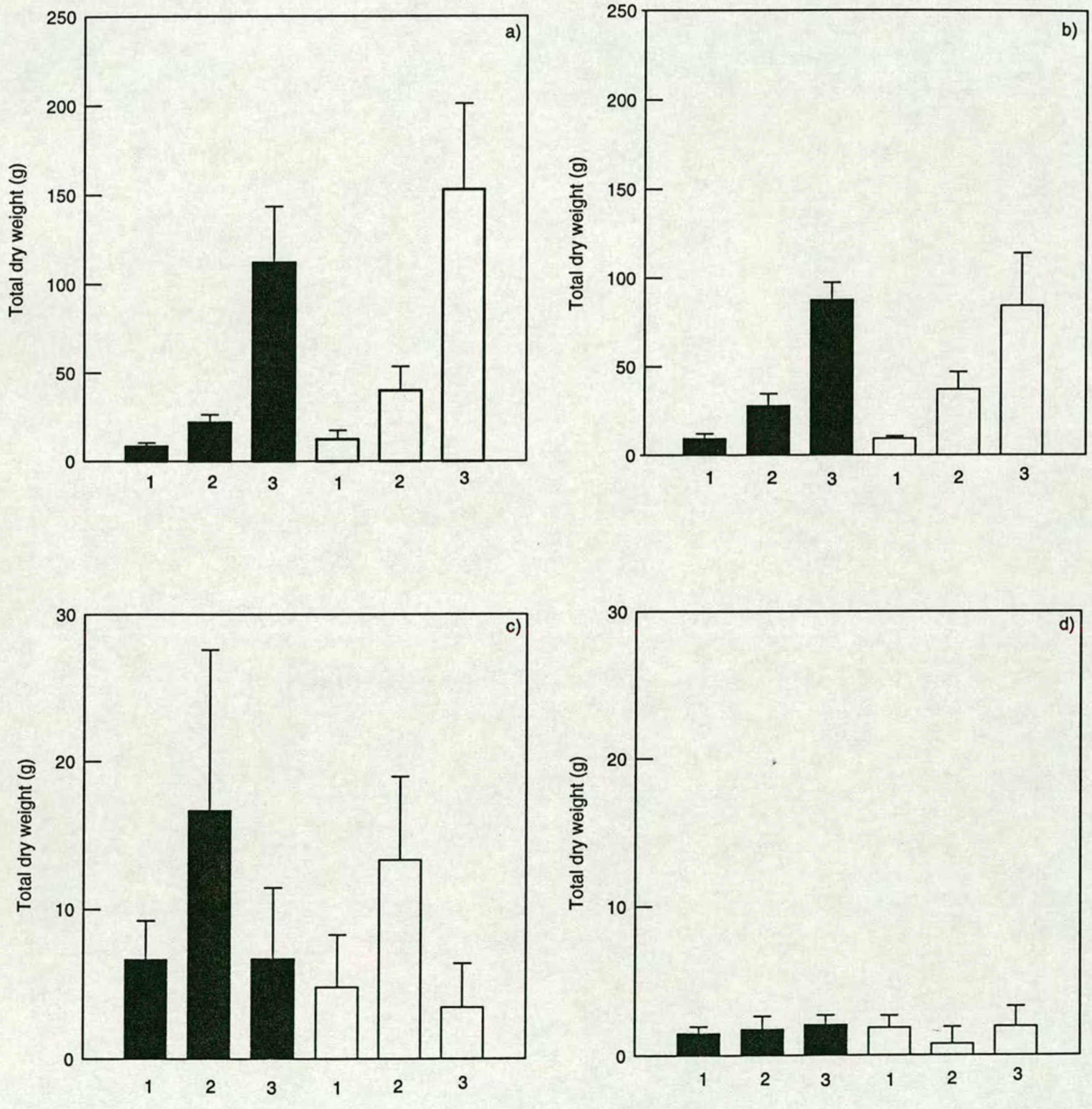


Figure 5.2: Effect of pot size on total dry weight of inoculated ▬ and uninoculated ▭ *Zea mays* L. plants in a) unamended soil A, b) unamended soil B, c) low P soil B and d) high P soil B. Bars are +S.E (P = 0.05). 1= 1 litre pot; 2= 4 litre pot; 3= 10 litre pot.
(Note different scales for graphs)

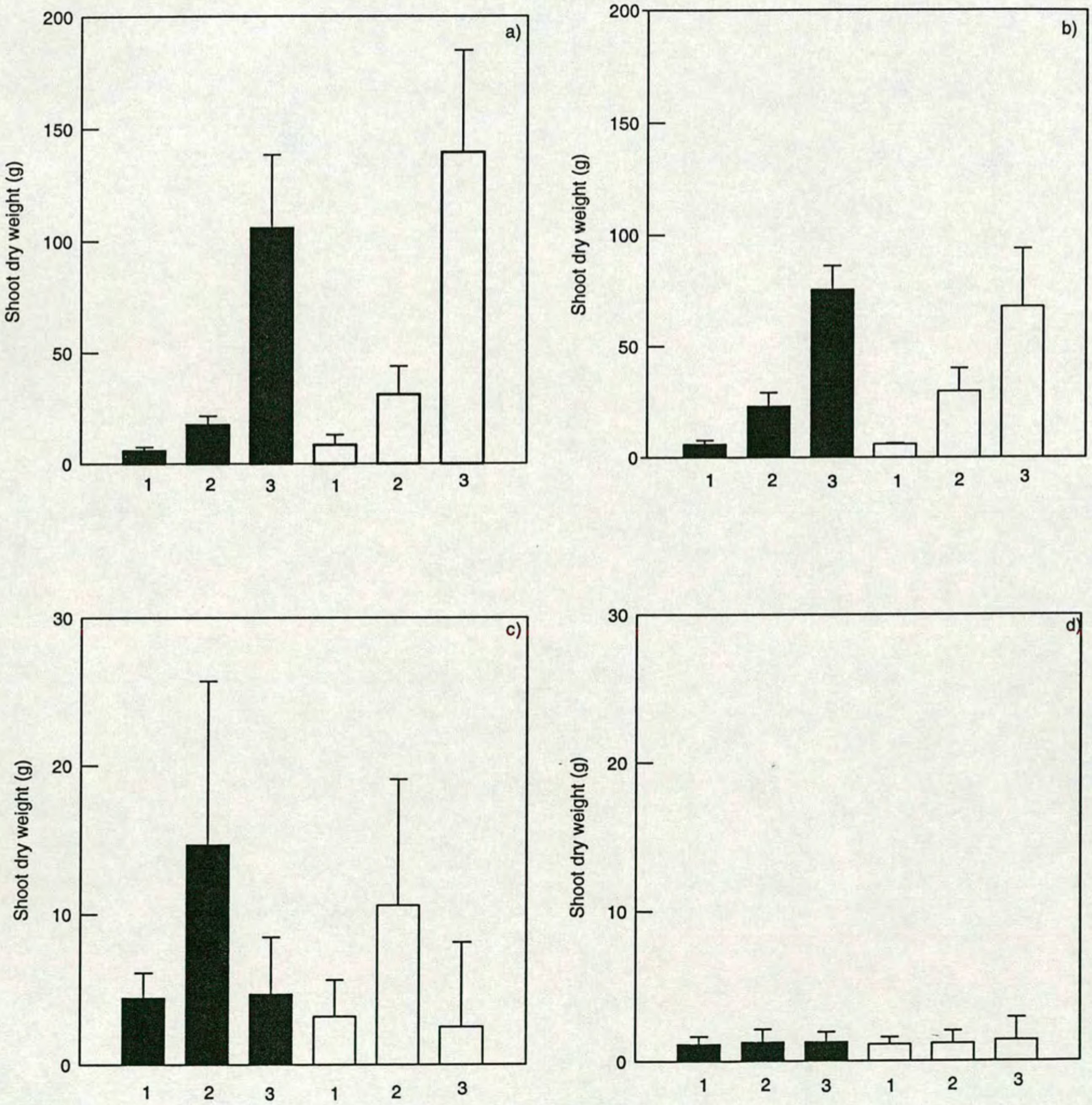


Figure 5.3: Effect of pot size on shoot dry weight of inoculated ■ and uninoculated □ *Zea mays* L. plants in a) unamended soil A, b) unamended soil B, c) low P soil B and d) high P soil B. Bars are +S.E (P=0.05)1= 1 litre pot; 2= 4 litre pot; 3= 10 litre pot.

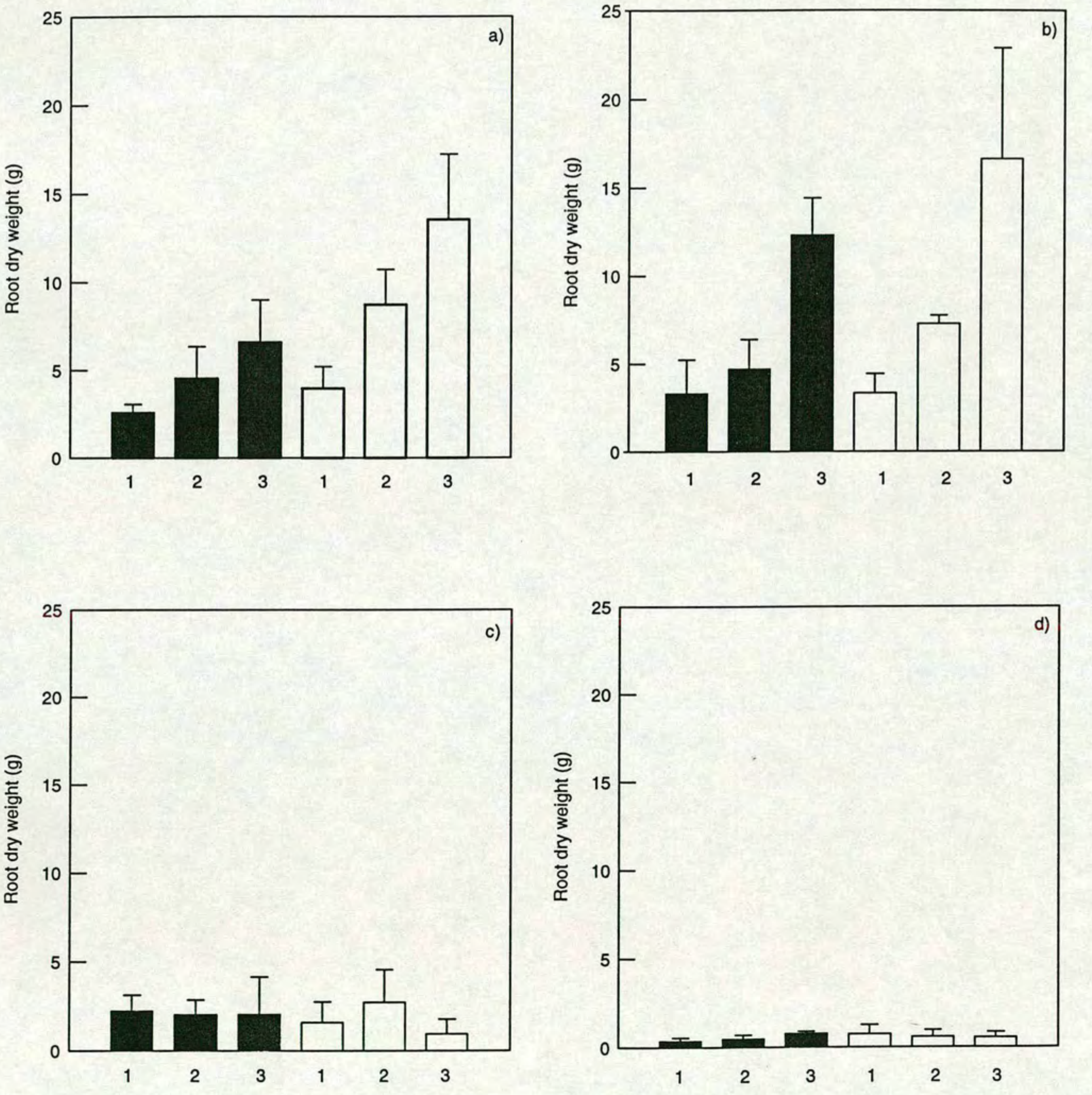


Figure 5.4: Effect of pot size on root dry weight of inoculated ■ and uninoculated □ *Zea mays* L. plants in a) unamended soil A, b) unamended soil B, c) low P soil B and d) high P soil B. Bars are + S.E (P = 0.05)1= 1 litre pot; 2= 4 litre pot; 3= 10 litre pot.

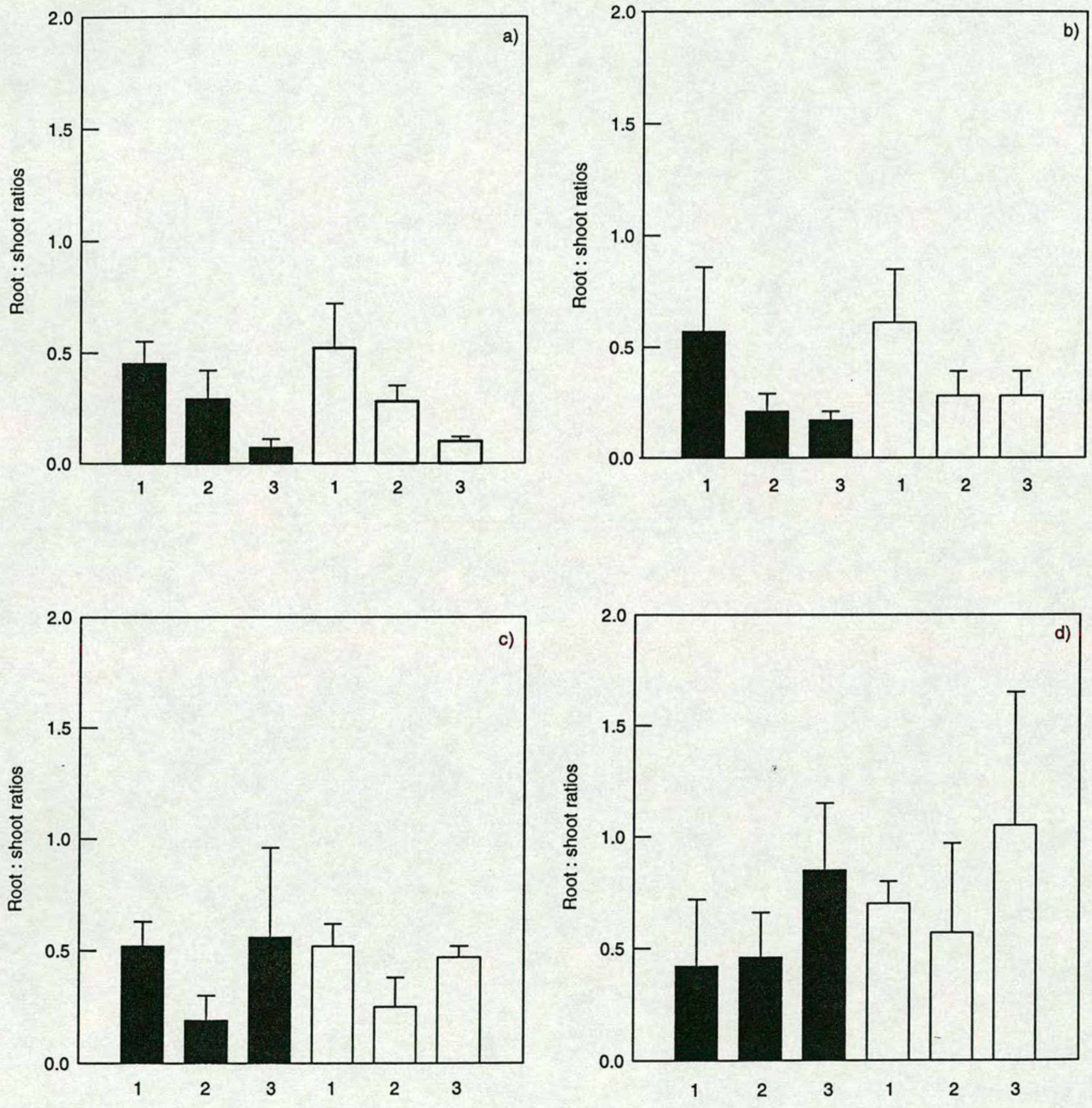


Figure 5.5: Effect of pot size on root : shoot ratios of inoculated ▬ and uninoculated □ *Zea mays* L. plants in a) unamended soil A, b) unamended soil B, c) low P soil B and d) high P soil B. Bars are +S.E.(P = 0.05).1= 1 litre pot; 2= 4 litre pot; 3= 10 litre pot.

5.3.2 Effects of RP fertilizer application to soil B and soil volume on mycorrhizal formation and growth of plants

Mycorrhizal formation

There was a significant interaction (P < 0.004) between pot size and RP on mycorrhizal infection (Table 5.3). In unamended soil, there was no pot size effect on mycorrhizal infection, while in low P soil, plants had significantly greater infection in 4 litre (64.3 %) than in 10 litre (44.4 %) or 1 litre pots (47.0 %). In high P soil, plants in 1 and 4 litre pots had similar infection (42 % and 49 % respectively), while in 10 litre pots mycorrhizal infection was significantly lower (15 %) (Figure 5.1). Mycorrhizal infection was significantly higher in plants in low P soil, irrespective of pot size. Similarly, plants in high P soil in 1 and 4 litre pots had higher mycorrhizal infection than those in low P soil. In 1 litre pots containing low and high P soils mycorrhizal infection in plants was similar. Whereas, in 4 and 10 litre pots, plants had significantly higher mycorrhiza infection in low P soil than in high P soil (Figure 5.1). Generally, infection consisting of hyphae and arbuscules was greater than that consisting of hyphae and vesicles. Addition of P to soil increased the proportion of hyphae and arbuscule infection in roots in soil B, while at high P, hyphae and vesicle infection appear to be reduced relative to low P and no P treatments. This reduction in hyphae and vesicle infection was more pronounced in larger pots.

Plant growth

There was a significant interaction (P < 0.001) effect between pot size and RP on total and shoot dry weight of plants. In unamended soil, total and shoot dry weights increased significantly with incremental increases in pot size, while in low P soil total and shoot dry weights were significantly greater in 4 litre pots than in larger or smaller pots. Pot size had no effect on the dry weight of plants in high P soil. Adding RP to soil reduced total and shoot dry weights of plants in all pots (Figure 5.2). This was particularly marked for plants grown in soil with RP added at the higher concentration. Growth of plants in this treatment was severely reduced. There was no effect of inoculation on total and shoot dry weights (Table 5.3). A significant interaction occurred between inoculation, pot size and RP (P < 0.001) on plant root dry weights such that, they decreased with incremental decreases in pot size in unamended soil. Root dry weights were higher in inoculated plants in this soil, except for those in 1 litre pots. In low and high P soil there was no significant effect of pot size or inoculation. Adding RP to soil reduced root dry weight incrementally, except for plants in 10 litre pots where root dry weights in low and high P soil were similar. (Table 5.3; Figure 5.4). There was a significant interaction ($P < 0.025$) between pot size and RP on root : shoot ratios. Plants had similar root : shoot ratios, except for those in high P soil in 10 litre pots which had significantly higher root: shoot ratios than those in the equivalent quantity of soil without RP or those in low P soil in 4 litre pots.(Figure 5.5).

Table 5.2: Significance of F-values in the analysis of variance for mycorrhizal infection, total dry weights, shoot dry weights, root dry weights and root : shoot ratios of inoculated and uninoculated *Zea mays* L. grown in 1, 4 and 10 litres of soil A and B.

Source of variation	AMF infection	TDW	SDW	RDW	R:S
A. Inoculation	-	*	*	***	*
B. Soil	***	*	***	ns	*
C. Pot size	***	***	***	***	***
A X B	-	ns	*	*	ns
A X C	-	ns	ns	*	ns
B X C	ns	***	***	**	**
A X B X C	-	ns	ns	ns	ns

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ ns not significant

Table 5.3: Significance of F-values in the analysis of variance for mycorrhizal infection, total dry weights, shoot dry weights, root dry weight and root : shoot ratios of inoculated and uninoculated *Zea mays* L. grown in 1, 4 and 10 litres of unamended soil B, low P soil and high P soil.

Source of variation	AMF infection	TDW	SDW	RDW	R:S
A. Inoculation	-	ns	ns	*	ns

B. Pot size	***	***	***	***	*
C. Rock phos	***	***	***	***	**
A X B	-	ns	ns	***	ns
A X C	-	ns	ns	ns	ns
B X C	**	***	***	***	*
A X B X C	-	ns	ns	***	ns

* P < 0.05 ** P < 0.01 *** P < 0.001 ns not significant

5.3.3. Effect of soil type and volume on plant nutrition

Analysis of plant nutrient content produced many significant effects and interactions between experimental treatments. In order to simplify the discussion of results, only the most important effects are discussed in detail.

There was a significant interaction ($P < 0.001$) between soil type and pot size on shoot nitrogen content, such that, in soil A, plants in 10 litre pots had significantly less shoot nitrogen content than those in smaller pots. In addition, plants in 10 litre pots had significantly less shoot nitrogen content in soil A than in soil B, while in 1 litre pots, plants had significantly higher shoot nitrogen content in soil A than soil B (Table 5.4; Figure 5.6). Root nitrogen content was significantly greater in plants in soil B and increased incrementally with increasing pot size (Table 5.6; Figure 5.7). Shoot phosphorus content was significantly higher in inoculated than uninoculated plants. There was a significant ($P= 0.037$) interaction between inoculation and soil type on root phosphorus content, such that inoculated plants in soil A had significantly higher root phosphorus content than those in soil B, whereas, uninoculated plants in soil A had significantly lower root phosphorus content than those in soil B (Table 5.6; Figure 5.6; Figure 5.7). There was a significant ($P = 0.02$) interaction between inoculation and soil type on shoot potassium content, however, soil type had the main effect, such that shoot potassium content was significantly greater in soil A than in soil B (Table 5.4; Figure 5.6). There were significant ($P = 0.006$; $P < 0.01$) interactions between soil type and pot size on shoot and root magnesium content, respectively. Plants had significantly greater shoot and root magnesium content in soil A in 10 litre pots compared to all other plants (Table 5.4; Figure 5.6; Figure 5.7). A significant interaction

(P = 0.038) effect between inoculation and soil type on shoot calcium content was such that inoculated plants in soil A had significantly higher shoot calcium than uninoculated plants in the same soil type (Table 5.4; Figure 5.6). There was a significant interaction (P = 0.026) between inoculation, soil type and pot size on root calcium content of plants, however the most important effect was that plants had higher root calcium content in soil B than in soil A (Table 5.6; Figure 5.7).

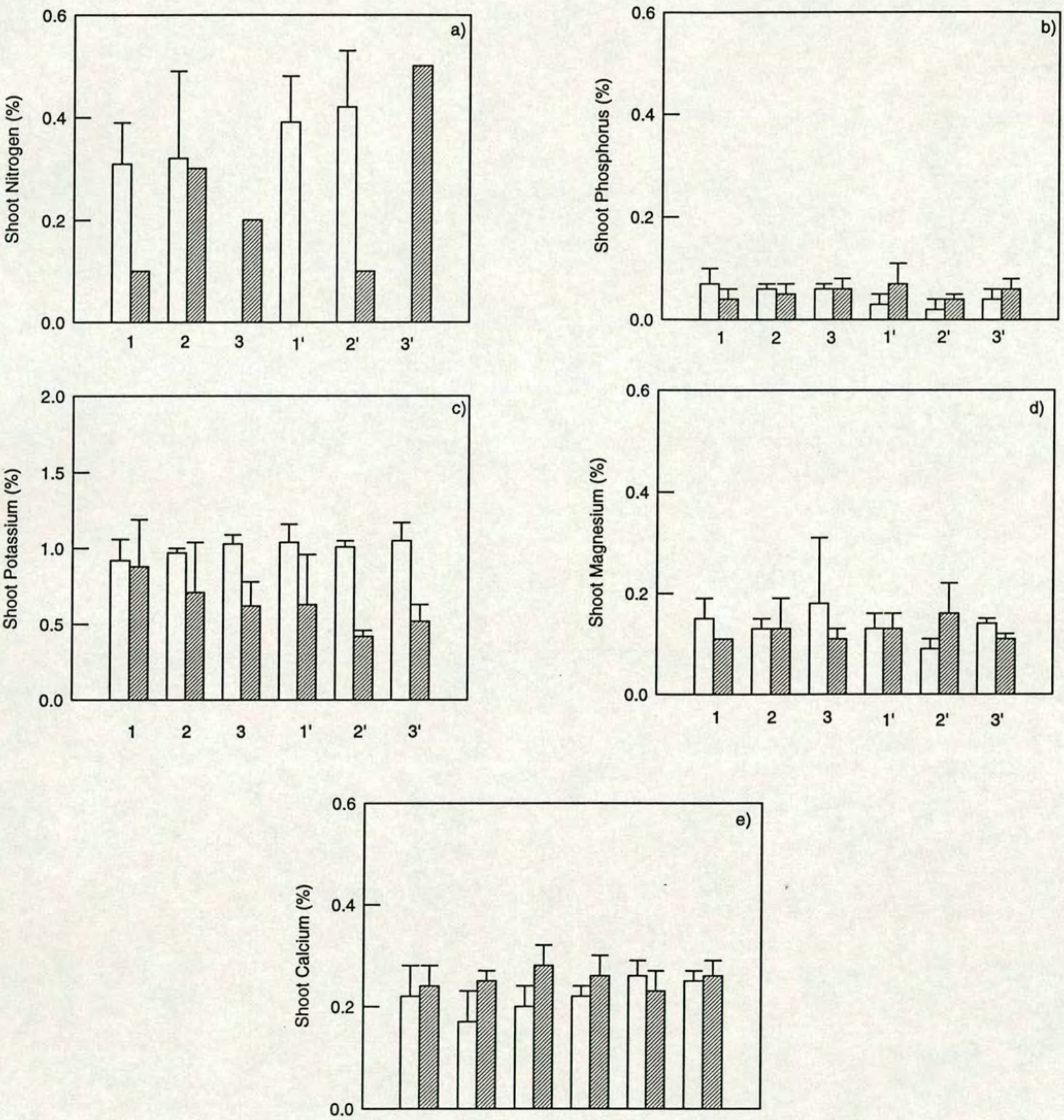


Figure 5.6: Effect of pot size (1=1litre pot; 2=4 litre pot; 3=10 litre pot) on shoot N, P, K, Mg and Ca concentrations (%) of *Zea mays* L. plants in soil A ▭ and soil B ▨ .
1= 1 litre pots (+M) ; 1'= 1 litre pots (-M); 2= 4 litre pots (+M) ; 2'= 4 litre pot (-M)
3= 10 litre pots (+M); 3'= 10 litre pots (-M); +M= Inoculated; -M= uninoculated
Bars are + S.E.(P = 0.05)(Note different scales for graphs)

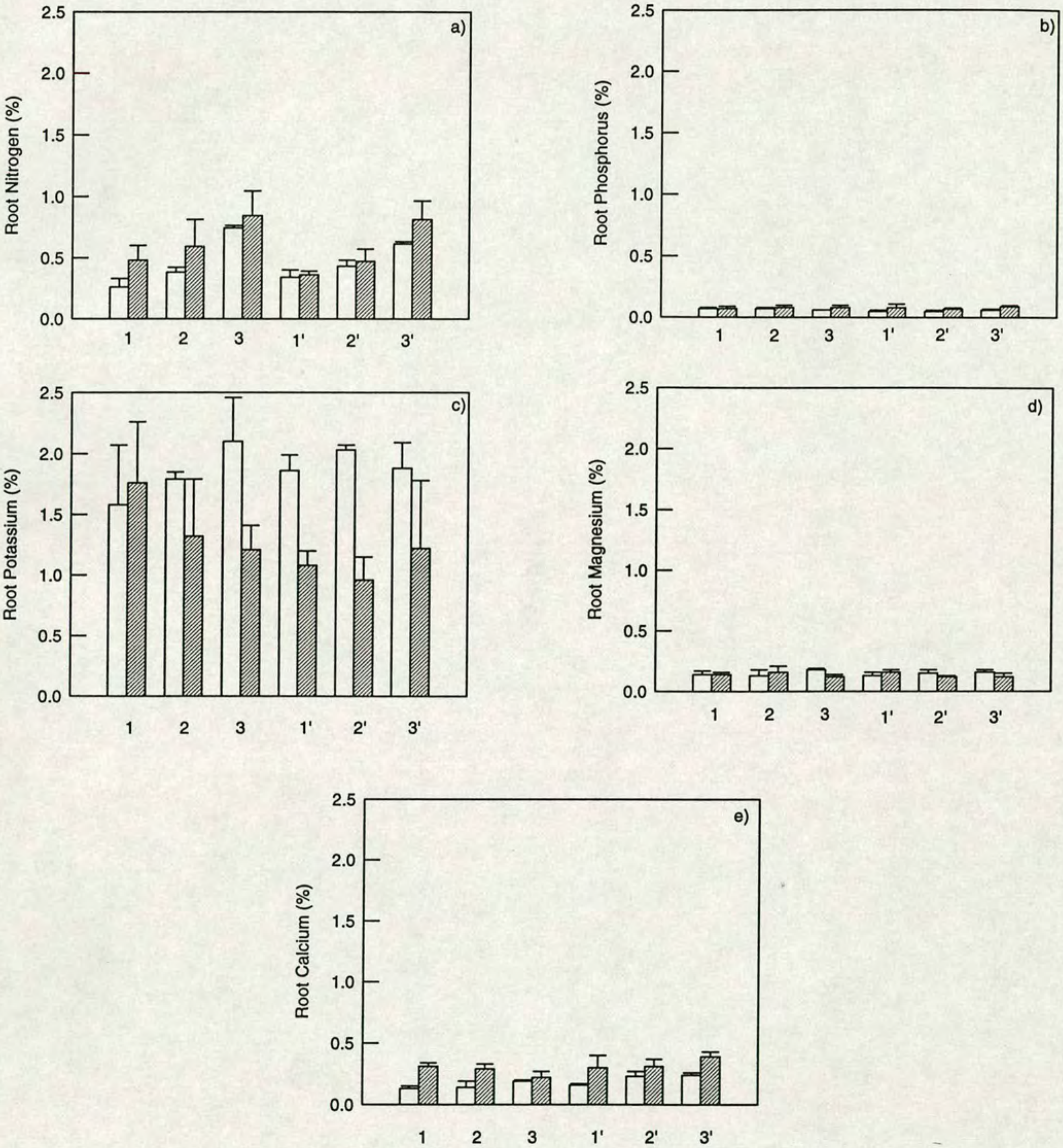


Figure 5.7: Effect of pot size (1=1litre pot; 2=4 litre pot; 3=10 litre pot) on root N, P, K, Mg and Ca concentrations (%) of *Zea mays* L. plants in soil A [ ] and soil B [hatched] .

1= 1 litre pots (+M) ; 1'= 1 litre pots (-M); 2= 4 litre pots (+M) ; 2'= 4 litre pot (-M)

3= 10 litre pots (+M); 3'= 10 litre pots (-M); +M= Inoculated; -M= uninoculated

Bars are +S.E.(P = 0.05)

5.3.4. Effect of soil volume and RP on nutrition of plants with and without AMF.

There was a significant interaction ($P = 0.008$) between inoculation and pot size on shoot nitrogen content of plants in soil B with and without RP added (Table 5.5). The main effect was a decrease in shoot nitrogen content in plants grown in soil without RP. In addition, plants in 1 litre pots had significantly lower nitrogen content in shoots than those in larger pots (Figure 5.8). A significant interaction ($P = 0.001$) on root nitrogen content between pot size and RP was such that plants in 10 litre pots with high P soil had significantly lower root nitrogen content compared to those in smaller pots. Also, in soil without RP, root nitrogen content of plants was significantly lower in 1 litre compared to 4 and 10 litre pots (Table 5.7; Figure 5.9). There was a significant interaction ($P = 0.041$) between pot size and RP on shoot phosphorus content. However, the main effect was that shoot phosphorus content of plants was higher in inoculated than in uninoculated plants (Table 5.5; Figure 5.8). There were no significant differences in root phosphorus content between plants (Table 5.7; Figure 5.9). A significant interaction ($P = 0.037$) between inoculation and pot size on shoot potassium content of plants was such that, plants in soil without RP had significantly ($P < 0.001$) less shoot potassium content than those in soil with added RP (Table 5.5; Figure 5.8). There was a significant interaction ($P < 0.001$) between inoculation, pot size and RP on root potassium content of plants. However, the main effect was that adding RP to soil significantly lowered root potassium content in plants compared to those in soil without RP (Table 5.7; Figure 5.9). A significant interaction ($P = 0.032$) between inoculation and RP on shoot magnesium occurred, such that, in unamended and low P soil, shoot magnesium content was significantly higher in inoculated than uninoculated plants. In high P soil, inoculated plants had significantly lower shoot magnesium content than uninoculated plants in the same soil or inoculated counterparts in low P and unamended soil (Table 5.5; Figure 5.8). Root magnesium content was significantly lower in plants in 10 litre pots than in smaller pot sizes, and decreased in plants when RP was added to soil (Table 5.7; Figure 5.9). Shoot calcium content was significantly higher in uninoculated than in inoculated plants (Table 5.5; Figure 5.8). There was a significant interaction ($P = 0.008$) between inoculation, pot size and RP on root calcium content of plants. However, the main effect was due to RP addition, such that plants grown in soil with RP added had significantly lower root calcium content than those in plants without RP added (Table 5.7; Figure 5.9).

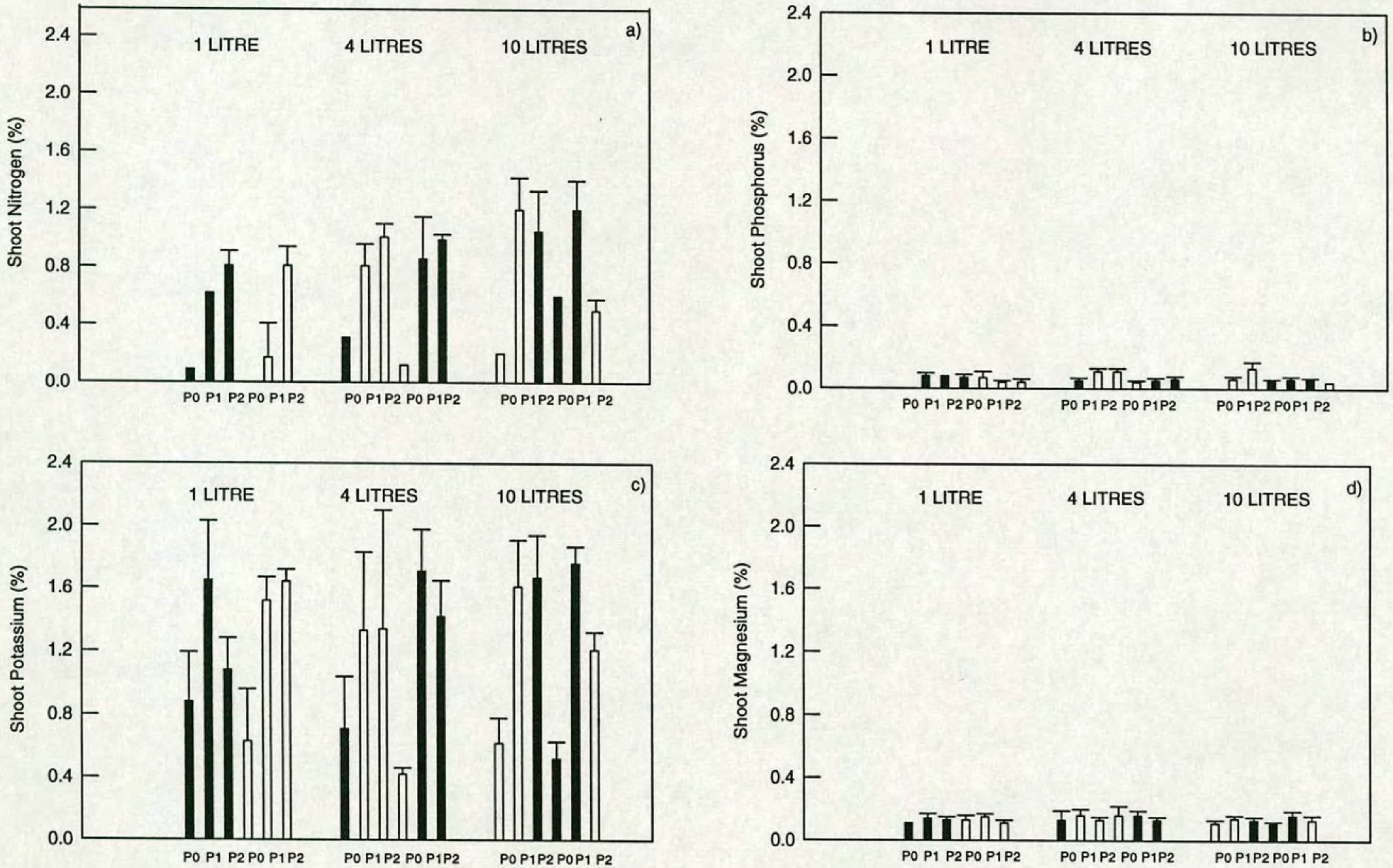


Figure 5.8: Effect of inoculation, pot size and RP on shoot a) N, b) P, c) K, d) Mg and e) Ca concentrations (%) inoculated ■ and uninoculated □ *Zea mays* L. plants grown in soil B.

Bars are + S. E. (P=0.05)

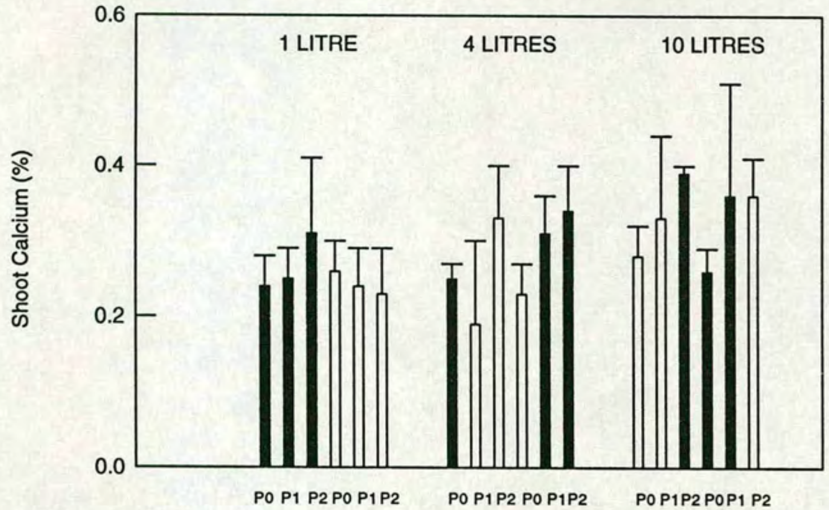


Figure 5.8 (continued) : Effect of inoculation, pot size and RP on shoot a) N, b) P, c) K, d) Mg and e) Ca concentrations (%) of mycorrhizal ▇ and non mycorrhizal ☐ *Zea mays* L. plants grown in soil B.

Bars are +S.E. (P = 0.05) (Note different scales for graphs)

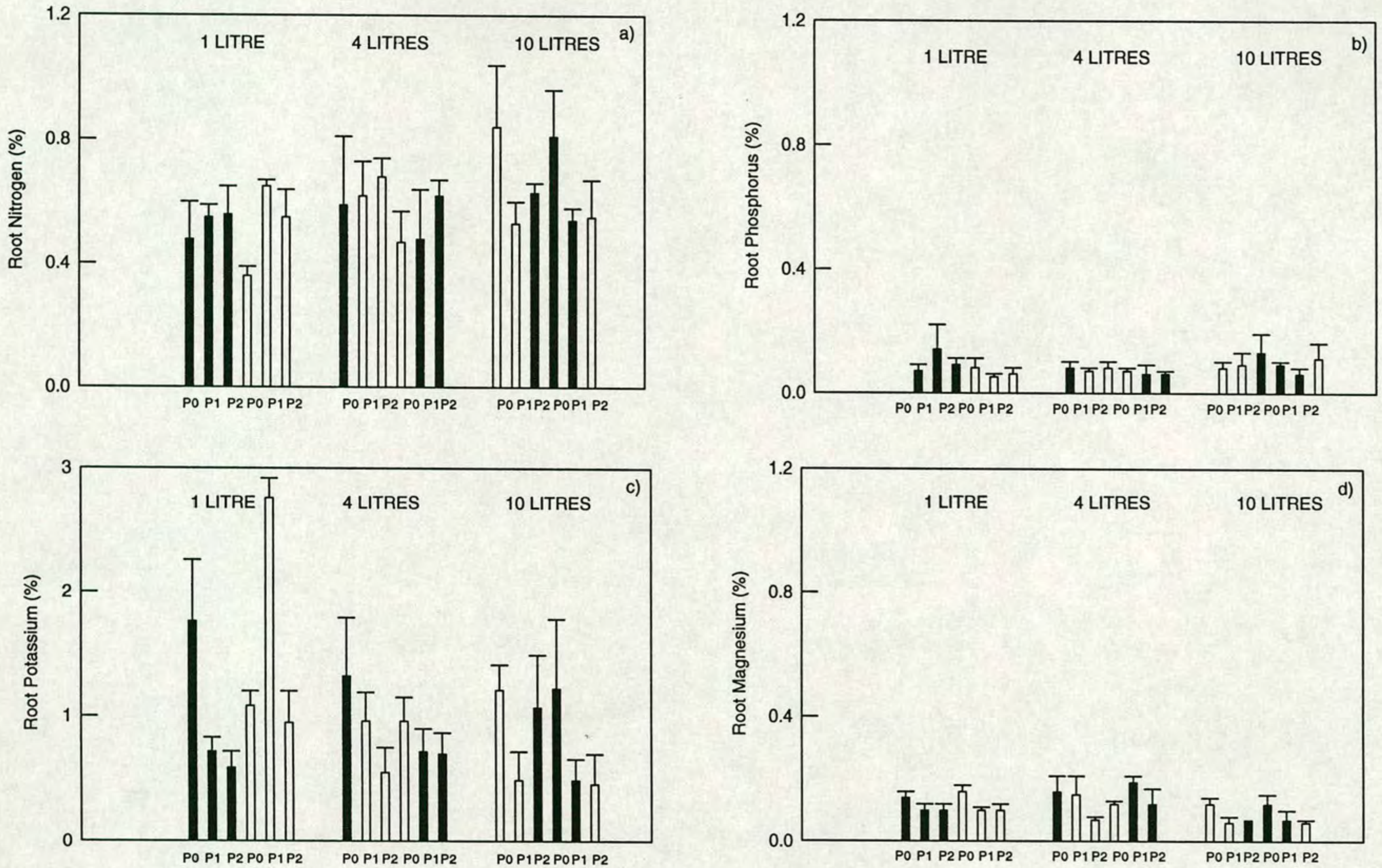


Figure 5.9: Effect of inoculation, pot size and RP on root a) N, b) P, c) K, d) Mg and e) Ca concentrations (%) of mycorrhizal ▬ and non mycorrhizal ☐ *Zea mays* L. plants grown in soil B. Bars are +S.E. (P = 0.05) (Note different scales for graphs)

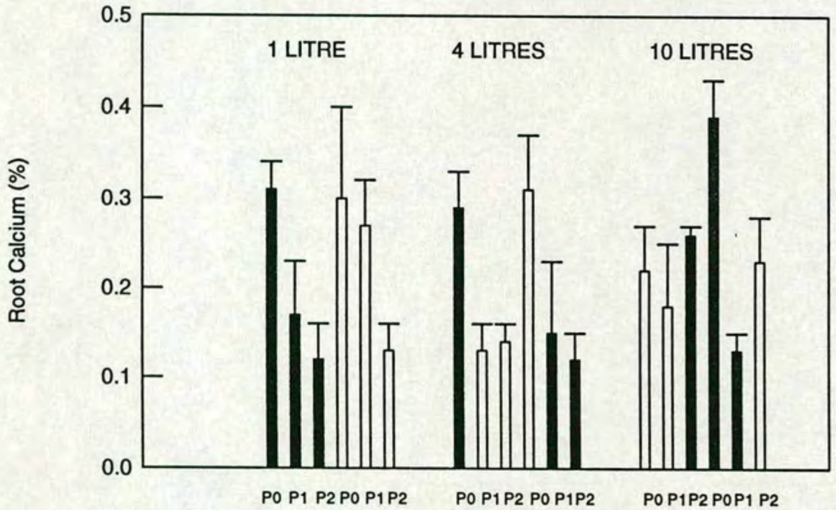


Figure 5.9 (continued) : Effect of inoculation, pot size and RP on root a) N, b) P, c) K, d) Mg and e) Ca concentrations (%) of mycorrhizal ■ and non mycorrhizal □ *Zea mays* L. plants grown in soil B.

Bars are +S.E. (P = 0.05) (Note different scales for graphs)

Table 5.4 : Significance of F-values in the analysis of variance for shoot N, P, K, Mg and Ca content of inoculated and uninoculated *Zea mays* L. grown in 1, 4 and 10 litres of soil A and B.

Source of variation	% N	%P	%K	%Mg	%Ca
A. Inoculation	ns	*	ns	**	*
B. Soil	ns	ns	***	ns	**
C. Pot size	ns	ns	ns	ns	ns
A X B	ns	ns	*	ns	*
A X C	ns	ns	ns	ns	ns
B X C	***	ns	ns	**	ns
A X B X C	ns	ns	ns	ns	ns

Table 5.5: Significance of F-values in the analysis of variance for shoot N, P, K, Mg and Ca content of inoculated and uninoculated *Zea mays* L. grown in 1, 4 and 10 litres of unamended soil B, low P soil and high P soil.

Source of variation	shoot % N	shoot % P	shoot % K	shoot % Mg	shoot % Ca
A. Inoculation	ns	**	ns	ns	**
B. Pot size	***	ns	ns	ns	ns
C. Phosphorus	***	ns	***	ns	ns
A X B	**	ns	ns	ns	ns
A X C	ns	ns	*	*	ns
B X C	ns	*	ns	ns	ns
A X B X C	ns	ns	ns	ns	ns

Table 5.6: Significance of F-values in the analysis of variance for root N, P, K, Mg and Ca content of inoculated and uninoculated *Zea mays* L. grown in 1, 4 and 10 litres of soil A and B.

Source of variation	% N	%P	%K	%Mg	%Ca
A. Inoculation	ns	ns	ns	ns	***
B. Soil	***	**	***	ns	**
C. Pot size	***	ns	ns	ns	ns
A X B	ns	*	ns	ns	ns
A X C	ns	ns	ns	ns	*
B X C	ns	ns	*	**	*
A X B X C	ns	ns	ns	ns	*

Table 5.7: Significance of F-values in the analysis of variance for root N, P, K, Mg and Ca content of inoculated and uninoculated *Zea mays* L. grown in 1, 4 and 10 litres of unamended soil B, low P soil and high P soil.

Source of variation	%N	%P	%K	%Mg	%Ca
A. Inoculation	ns	ns	ns	ns	*
B. Pot size	ns	ns	***	**	ns
C. Phosphorus	ns	ns	***	***	***
A X B	ns	ns	*	ns	ns
A X C	ns	ns	**	ns	ns
B X C	**	ns	ns	ns	ns
A X B X C	ns	ns	***	ns	**

5.4 Discussion

5.4.1 Effects of inoculation, soil type and soil volume on the growth and nutrition of plants in unamended soils

In this study, plants had significantly higher mycorrhizal infection in soil A than in soil B and, except in one treatment, plant dry weights were similar in different soil types. However, there was a significant increase in dry weight of plants in soil A compared to those in soil B in 10 litre pots, but this effect was similar for inoculated and uninoculated plants and unrelated to higher mycorrhizal infection in this soil. Different soils in the field (Mosse, 1972; Islam, 1980; Saif, 1986) and in pots (Mosse, 1972; Islam, 1980; Biermann and Lindermann, 1983) have been found to significantly affect the plant-AMF symbiosis. Islam *et al.* (1980) found significant differences in the growth of cowpea in different soils in the field and in pot trials. The largest response of plants to inoculation occurred in soil with the lowest soluble P content. There was no difference in the soluble P content of soil A and unamended soil B in this study. However, there were differences in the soils physical properties, due to the absence of vermiculite in soil B. This could partially explain the lower infection in plants in soil B and the lower dry weight of plants in 10 litre pots containing this soil. It would appear that soil type had a significant effect on mycorrhizal formation in plants and that soil volume influenced the effect of soil type on plant growth.

Plant root growth was similar in different soil types and between inoculated and uninoculated plants in 1 litre pots, while, in 4 litre pots, root dry weights were significantly higher in plants grown in soil A than soil B. This effect was similar to that observed for total and shoot dry weights of plants. In 10 litre pots, however, the effect of soil type on root dry weight was such that inoculated plants had significantly higher root dry weights in soil B than in soil A. This effect may reflect an adaptation by plants to the growing conditions in soil B, and this, coupled with the additional carbohydrate drain by the fungal partner, could explain why only root dry weights of inoculated plants were increased in this soil.

Plants inoculated with AMF in both soils had significantly lower total dry weights than uninoculated counterparts. Cooper (1975) found that when availability of soil P was not sufficiently limiting, then the mycorrhizal inoculation effect was to depress plant growth. Similar effects of inoculation on plant growth were observed by Singh and Singh (1993) when they added RP to soil. Parasitic effects of AMF infection have been reported during initial stages of AMF formation (Cooper, 1975) at high P availability (Bethlenfalvay *et al.*, 1983) and under conditions when photosynthesis was limited (Daft and El Giahmi, 1978). In the present experiment, although levels of soil P were low relative to that used in many AMF inoculation studies, compared to field soil, these levels were relatively high and could have been too high for the strains of indigenous AMF species which occurred in the field environment. There is increasing evidence that AMF can develop strains adapted to the environment in which they originate (see section 1.7). However, in order to elucidate whether this particular species is ineffective or rendered ineffective by the conditions under which this experiment was executed, further studies would be required.

Inoculated plants had lower root dry weights and consequently lower root : shoot ratios than uninoculated plants in 4 and 10 litre pots. Bääth and Hayman (1989) also observed a reduction in root dry weight and root : shoot ratios of inoculated plants compared to uninoculated plants in a glasshouse experiment. A reduction in root dry weight of plants inoculated with AMF is widely reported and is thought to occur as a result of increased efficiency of the mycorrhizal root system (see section 4.4). The decreased root mass of inoculated plants is thought to be functionally substituted by the external hyphal network of AMF (Michelsen and Rosendahl, 1990).

In this study, soil volume had a significant effect on mycorrhizal formation and growth of plants. Mycorrhizal infection decreased as the volume of soil in which plants grew increased. These findings are in contrast to those of Bääth and Hayman (1984), who found less infection in roots of onion seedlings grown at higher compared to lower densities, or grown in smaller (200 cm^3) than in larger pots (900 cm^3). However, as suggested by Daft and Nicolson (1969), if a measure of mycorrhizal infection accounted for the size of the root system then a more meaningful value would be obtained. In this experiment for example, plants in 10 litre pots of soil B had the lowest value of percentage root length with mycorrhizal infection, but the greatest root dry weights. Calculation of the actual infection (ie. percentage infection values expressed as a percentage of plant root dry weight) gives values of 0.84, 1.35 and 1.4 in 1,4 and 10 litre pots of soil A, respectively. In soil B, lower values of 0.60, 0.70 and 0.96 are obtained for plants in 1, 4 and 10 litre pots, respectively. This representation of mycorrhizal infection suggests that there were small increases in infection in plants as soil volume increases and this would agree with the findings of Bääth and Hayman (1984). The effect of soil type on these values is similar to that found for percentage root length with mycorrhizal infection. Bääth and Hayman (1984) suggested that mycorrhizal infection development is regulated by plant internal P levels and that those in smaller pots had less infection because plants take up P more efficiently from small pots than from large ones.

Hayman (1984) observed increases in dry weight and mycorrhizal growth response of plants with increasing pot size. Daft (1991) similarly found that the growth response of alfalfa and maize to inoculation with AMF when grown in increasing volumes of soil was improved. He found that increasing the density of plants per unit substrate reduced the dry weight of plants such that the effect of AMF inoculation was negligible. In our experiment, inoculation had a negative effect on total dry weight of plants in both soils and a negligible effect on shoot dry weights of plants in soil B. Although a positive mycorrhizal response of plants was not obtained, the differences in total and shoot dry weights between inoculated and uninoculated plants were larger in plants in soil A in larger 10 litre pots. The negative response of plants to inoculation tended to increase with increasing soil volume, but only in soil A. This is thought to be a response to higher infection in plants in this soil and increases in the actual infection in plants in larger pots. In soil B, differences between inoculated and uninoculated shoot dry weights of plants were negligible. As pointed out before, it would appear that in this soil, conditions were less conducive to growth than in soil A and hence the effect of inoculation was not expressed.

Experiment 4 was destructively harvested 90 d.a.i. and yet plant dry weights in 1 litre pots containing soil A were lower than those in experiment 1 harvested 60 d.a.i. In each of these experiments, mycorrhizal infection had a detrimental effect on plant growth however, the detrimental effect of inoculation would initially appear to have been greater in experiment 4. Soil inoculum used in this experiment was obtained from trap cultures which are known to be susceptible to pathogenic contamination. However, in this and experiment 1, there was no evidence of pathogenic infection in roots. One explanation for the higher growth rates observed in experiment 1 is that plants were positioned on benches closer to the light source, while in experiment 4, plants were positioned on the floor of the glasshouse at a greater distance from the overhead lights. Additionally, as plants grew those in larger pots may have produced a shading effect on smaller plants.

In addition, the variety of *Z. mays* grown in each experiment differed and this could have affected the growth of plants. Intra-specific variation in plant growth and in the response of plant cultivars and varieties to inoculation with AMF have been observed in many studies (Hajabbasi and Schumacher, 1994; Toth *et al.*, 1984; Daft, 1991). The final experiment carried out in this study was established, in part, to examine whether there were differences in the growth of these *Z. mays* varieties. The results from this experiment are presented in the following chapter.

Inoculation of plants with AMF is frequently accompanied by increases in the P content of plant tissues (Krishna and Dart, 1984; Guttay and Dandurand, 1989; López-Sánchez *et al.*, 1992; Singh and Singh, 1993). Likewise, inoculation of plants in this study significantly increased the P content in shoot tissue. However, as uninoculated plants had higher shoot dry weights than inoculated plants, then it is possible that shoot P content of uninoculated plants was reduced as a result of a dilution effect. López-Sánchez *et al.* (1992) found that inoculated plants had higher P content in tissues than uninoculated plants and at the highest soil P levels, they found that uninoculated plants had higher growth than mycorrhizal plants, although the possibility of a dilution effect was not discussed, probably because of the large differences in shoot P content of plants, unlike that found here.

Shoot and root potassium content of plants was higher for plants in soil A than soil B. This was probably due to the much higher potassium content of soil A (Table 4.1; Table 5.1). Plants grown in soil A had greater shoot magnesium content than those in soil B and in larger pots they also had greater root magnesium content, yet soil B contained higher concentrations of this nutrient. Calcium content of soil A was particularly higher than that in soil B yet shoot and root content of plants did not reflect this.

In this study the negative effect of inoculation on plant dry weights was increased when plants were grown in larger pots containing soil A. Percentage mycorrhizal infection (and therefore the carbohydrate drain) was lower in 10 litre pots whereas, values of actual mycorrhizal infection increased as soil volume increased. Another reason for increases in the negative mycorrhizal growth response of plants could be related to the extramatrical hyphae, which in greater soil volumes could have had a higher density and a subsequent greater carbohydrate drain. In soil B, a negative effect of inoculation on total dry weight was similarly found in plants grown in larger pots, although shoot dry weights of plants were not significantly affected by soil volume. It is probable that soil B growth conditions were less conducive to the proliferation of external AMF hyphae than that in soil A. It is reasonable to expect that the physical structure of soil, altered by the removal of vermiculite in soil B, was such that the porosity or water holding capacity of soils differed significantly. It is possible that the water content in soil B was greater than that of the more freely draining soil A and that this had a significant effect on development of mycorrhizal infection, hyphal growth and subsequent nutrient content and growth of plants. It is suggested that limited growth of external AMF hyphae and overall inoculation effect was reduced in plants in soil B.

5.4.2 Effects of inoculation, soil volume and rock phosphate fertilizer on the growth and nutrition of plants

Adding RP to soil B at the low rate of application (RP1; 150 mg l^{-1} soil) significantly increased mycorrhizal infection in plants in all volumes of soil compared to mycorrhizal infection in plants in soil without RP fertilizer (RP0) or in soil with RP added at the high rate of application (RP2; 300 mg l^{-1} soil). These results do not agree with those that which report that AMF root infection decreases when P in soil increases (Gianinazzi-Pearson, 1985). However, some authors reported that small additions of P to the soil increase AMF root infection when the supply of P is extremely deficient for plant growth (López-Sánchez *et al.*, 1992; Asmah, 1995).

In a pot experiment, mycorrhizal infection was reduced at higher P levels (0.07, 0.36 and 0.60 mg l^{-1}) in both *Sesbania* and *Leucaena* species. In the lower mycorrhizal dependent *Sesbania* sp., AMF inoculation reduced the mycorrhizal growth response of plants at high P, while in the highly dependent species of *Leucaena*, there was a mycorrhizal growth response even at the highest levels of available P in soil (Manjunath and Habte, 1992). The reduction in dry matter yield of the marginally dependant species was thought to be caused by utilization of photosynthate by AMF fungi.

In this study, growth of plants was severely reduced when RP was added to soil at the highest concentration. The effect of pot size was not consistent when plants were grown in soil with different levels of RP added. In treatment RP0, total and shoot growth increased with increases in soil volume, while, in treatment RP1, they increased between 1 and 4 litre pots but decreased in 10 litre pots. In treatment RP2, plant growth was unaffected by soil volume. Root dry weight of plants were unaffected by soil volume in all RP treatments. These results may have resulted from a number of soil chemical related factors. For example, when RP was added to soil the levels of available P increased, and this was particularly high and not proportionate to the added RP in the RP2 treatment. Potassium and magnesium concentrations in soil increased in soil when RP was added, while calcium concentrations decreased. This decrease in soil calcium content may have been expected to have reduced the pH of soil, and thereby explain the observed reduction in plant growth, however changes in soil pH were not observed (Table 5.1).

These results showed there to be no correlation between mycorrhizal infection and plant growth in response to increases in soil RP content. The effects of adding RP to soil here are unexpected in comparison to the findings of other studies which show that the growth promoting effect of adding phosphorus to soil is similar to that achieved with AMF inoculation in soil without added phosphorus (Michelsen and Rosendahl, 1990) and that when plants are inoculated, phosphorus applications to soil increase the growth of plants to a lesser extent than when plants are uninoculated (López-Sánchez *et al.*, 1992).

It has been found that at high soil soluble P plants inoculated with AMF have reduced growth compared to uninoculated counterparts (Singh and Singh, 1993). In plant species which are less dependent on the mycorrhizal condition for growth then the mycorrhizal growth response can be lost at lower P levels and can be less relative to that of a more AMF dependent species (Manjunath and Habte, 1993). In this experiment, it is probable that soil P was increased greatly above the optimal levels required for this particular species AMF to be effective. However, this effect was probably confounded by the fact that the soil type in which plants were grown was found to be a relatively poor growth substrate compared to soil A. In addition, the application of RP may not only have affected the soil chemical properties but soil physical properties. This makes it difficult to separate effects of increasing soil P levels from the physical and chemical changes in the soil itself due to the application of RP.

Instances have been reported where AMF have been effective with the application of phosphate from one source and ineffective with other sources (Jehne, 1980; Fabig *et al.*, 1989; Asmah, 1995). Soluble single and triple superphosphate fertilizers provide readily available forms of P to plants while less soluble rock phosphates release P into soil slowly. Asmah (1995) found that a low rate of a soluble P source significantly increased mycorrhizal infection relative to plants in soil with a high rate of P application. Plants in the treatment with no P added had similar mycorrhizal infection to those in the high P treatment. Adding rock phosphate to soil at high and low rates significantly increased mycorrhizal infection compared to controls and that in plants from the high soluble P treatment. With the exception of plants with the rock phosphate applied at low rates, yields of plants were significantly greater with all P sources than the controls. In the present study, inoculation increased shoot P uptake, although the differences in shoot P were very small and no difference in root P content was found. Pot size appeared to have an effect on the N content of plant shoots which were significantly reduced in plants grown in 1 litre pots compared to those in larger pots. Conversely, root K, Mg and Ca were all increased in the roots of plants grown in smaller pots although the same effects were not apparent in shoots.

These results indicate that soil types, soil volume and RP all significantly affect the growth of plants although there was no evidence of a mycorrhizal growth response in plants. The effects of soil volume and RP application are difficult to assess as changes in these variables not only affect the volume of soil available and the amount of available phosphorus in soil, but they also alter the physical structure of soil to such an extent that it is difficult to isolate the effects of soil physical and chemical changes on the growth of plants. Few studies involving addition of RP to soil in pots have discussed the effects which adding RP has on the physical properties of pot soil. This may be less important in the field, but makes it difficult to extrapolate results in pots to the field situation. This could be overcome by using a soluble form of phosphate in pot experiments as used in many studies. However, as the mycorrhizal effectiveness is usually reduced when the availability of phosphate in soil increases the use of soluble phosphate may not always be appropiate. More studies of the effects of RP (a cheap and readily available fertilizer in many areas of the tropics) on the mycorrhizal response are necessary, and would probably be more viable if carried out in the field.

The determination of mycorrhizal infection can be crucial to the interpretation of results from experiments. It is argued that at the very least, the size of the root system must be taken into account when assessing the effects of AMF inoculation on plant growth or nutrient uptake. Even with this knowledge, infection inside the root cortex is not always a good indicator of the plants growth response as hyphal growth external to the plant root plays an important role in plant nutrient uptake (Mc Gonigle *et al.*, 1990; Bellgard, 1993b). Also, these results could reflect the ineffectiveness of the AMF species involved. If so, as this species was found to be dominant in the field, it could be that inoculation of trees in the nursery with highly efficient AMF species, or inoculation of maize directly in the field would be necessary. However, to determine this, field and pot trials comparing the responsiveness of component species to inoculation with different indigenous, and perhaps some exotic AMF species (highly effective in crops under similar field conditions) would be required before AMF populations could be managed successfully in the field. If, as suspected here, experimental variables are affecting the mycorrhizal growth response, further work could make clear the effects of host and agronomic practice, such that, management of AMF in the field could be carried out through manipulation of agronomic practices. Further trials, similar to those described here, but carried out in pots containing field soil and under field conditions (where the volume of soil available to the plants is quite different to that obtained in a pot) are necessary.

CHAPTER SIX

EFFECT OF TWO TYPES OF INOCULUM ON MYCORRHIZAL FORMATION AND GROWTH OF TWO VARIETIES OF *Zea mays* L.

6.1 Introduction

A local variety of *Z. mays* (Katumani composite var.) was used in experiments 1, 2 and 3 (see chapter 4). In experiment 4 (see chapter 5) seed of this variety was not available. Instead, another local variety of *Z. mays* (Hybrid II var.) was obtained from ICRAF and used in this experiment. Interspecific variation between plants in response to AMF inoculation are well documented (Mosse, 1975; Kruckelman, 1975) and increasingly more studies report intraspecific variation between plant varieties and cultivars in response to AMF inoculation (Hall, 1978; Mercy *et al.*, 1990).

In previous experiments, AMF inoculation had detrimental effects on plant growth. In experiments, 1, 2 and 3 this was suspected to be due to the limited soil volume or P content of soil in which plants were grown. Experiment 4 was therefore established to test the effects of soil volume, soil P and different soil types on the growth and nutrition of plants. The results of this experiment were similarly unexpected, with detrimental effects of inoculation on plant growth, therefore the expected mycorrhizal growth responses of plants in different soil types and soil volumes were not observed.

Although there was no evidence of pathogenic infection in plant roots, this could not be excluded as a factor affecting results in all previous experiments. There are two ways in which a pathogen could have been introduced into the experiment, either in soil inoculum, or in pot soil. It is extremely unlikely that the pathogen was introduced through pot soil which was autoclaved before use. If the process of autoclaving had been ineffective, then mycorrhizal infection would have developed in uninoculated plants. Uninoculated plant roots were specifically checked for presence of mycorrhizal infection and this was not detected.

The following study investigated the effects of AMF inoculation on the development of mycorrhizal infection and growth of two local *Z. mays* varieties (Katumani composite and Hybrid II var.). Two types of AMF inoculum were used in this experiment, a) soil from pot cultures of AMF from the field b) spores extracted from pot cultures. Additionally, there were two types of control used in this experiment, a) Plants grown in autoclaved soil B and b) Plants in autoclaved soil B and 100 g of autoclaved soil inoculum .

6.2 Materials and Methods

A randomised block experimental design was used, testing the effects on two varieties of *Z. mays* (Katumani composite and Hybrid II.) 4 inoculation treatments (2 inoculated and 2 uninoculated), of which the 2 inoculated treatments consisted of plants inoculated with a) soil from pot cultures b) spores extracted from an equal volume of pot culture soil. One of the 2 uninoculated treatments consisted of plants grown in autoclaved soil and autoclaved soil inoculum (Control A). The other consisted of plants grown in autoclaved soil (Control B). The experiment was laid out in 5 blocks with one replicate per treatment per block.

Six days after sowing, seedlings of each variety were randomly selected and planted in pots (4 L) containing either, autoclaved soil mix and 100 g of soil inoculum (from pot cultures) or autoclaved soil mix and spore extracts (from 100 g of pot culture soil) (see section 2.6.1). Plants inoculated with spores had no pathogen, but neither had they infected root material or AMF mycelium present in soil inoculum. Therefore in order to exclude the effect of these propagules, soil was sieved. Although this would not remove fungal mycelium, the disturbance of soil will have reduced the infectivity of the AMF hyphae considerably, as observed by Jasper *et al.* (1989b; 1989c). Plants were randomly allocated to two uninoculated treatments. Control A (CA) contained sterile soil with 100 g of autoclaved soil inoculum. This was used in statistical comparisons with inoculated plants. Control B (CB) contained sterile soil only and was used to check for incoming contamination. Plants were grown in conditions similar to those described for previous experiments. The experiment was established in August, 1993. Plants were destructively harvested 60 d.a.i. At harvest, mycorrhizal infection and growth assessments were carried out for plants as described in earlier experiments.

6.3 Results

6.3.1 *Mycorrhizal formation and growth of plants*

There were significant effects ($P < 0.001$) of both plants variety and inoculum type on the development of mycorrhizal infection in plants. Plants of the same variety in pots containing soil inoculum had significantly higher mycorrhizal infection than those in pots containing spore inoculum. In each inoculum treatment hybrid II var. plants had developed significantly higher mycorrhizal infection than Katumani composite var. plants (Table 6.1; Figure 6.1).

At this harvest plants were approximately 1.5 m in height and had larger dry weights than plants grown in experiments 1, 2 and 3, harvested at this time and later, but grown in 1 L pots. There were significant interactions ($P = 0.043$; $P = 0.002$) between inoculation and inoculum type and inoculation and plant variety respectively, on the total dry weight of plants. Uninoculated plants had significantly higher total dry weights than inoculated plants and those in pots containing soil inoculum had significantly higher total dry weights than those in pots containing spore inoculum (Table 6.1; Figure 6.2a). Also, the total dry weight of uninoculated Hybrid II var. plants was significantly greater than those of Katumani composite var. plants. There were significant ($P < 0.001$) effects of inoculation, inoculum type and plant variety on the shoot dry weight of plants, such that the effect of inoculation and inoculum type were similar to those found for plant total dry weights. Hybrid II var. plants had significantly higher shoot dry weight than Katumani composite var. plants (Table 6.2; Figure 6.2b).

Similarly, there was a significant interaction effect ($P = 0.002$) between inoculation and plant variety on root dry weights, such that root dry weights of hybrid II plants were significantly greater than those of Katumani composite var. plants when inoculated with AMF. The effects of inoculum type were similar to those found for shoot dry weight and total dry weight (Table 6.1; Figure 6.2). This resulted in a significant interaction effect ($P = 0.044$) between inoculation and plant variety on root : shoot ratios, such that they were significantly higher in hybrid II var. compared to Katumani composite var. plants but only when plants were inoculated with AMF. Also, the root : shoot ratios of plants were significantly higher in uninoculated compared to inoculated Katumani composite var. plants but not in hybrid II var. plants (Table 6.1; Figure 6.2).

Table 6.1. Significance of F-values in the analysis of variance for mycorrhizal infection, total dry weights, shoot dry weights, root dry weights and root : shoot ratios of two varieties of inoculated and uninoculated Zea mays L. plants harvested 60 d.a.i . with two types of inoculum (soil or spores).

Source of variation	AMF infection	TDW	SDW	RDW	R:S
A. Inoculation	-	***	***	***	***
B. Inoculum type	***	***	***	***	ns
C. Plant variety	***	***	***	***	ns
A X B	-	*	ns	ns	ns
A X C	-	**	ns	**	*
B X C	ns	ns	ns	ns	ns
A X B X C	-	ns	ns	ns	ns

* P < 0.05
** P < 0.01
*** P < 0.001
ns not significant

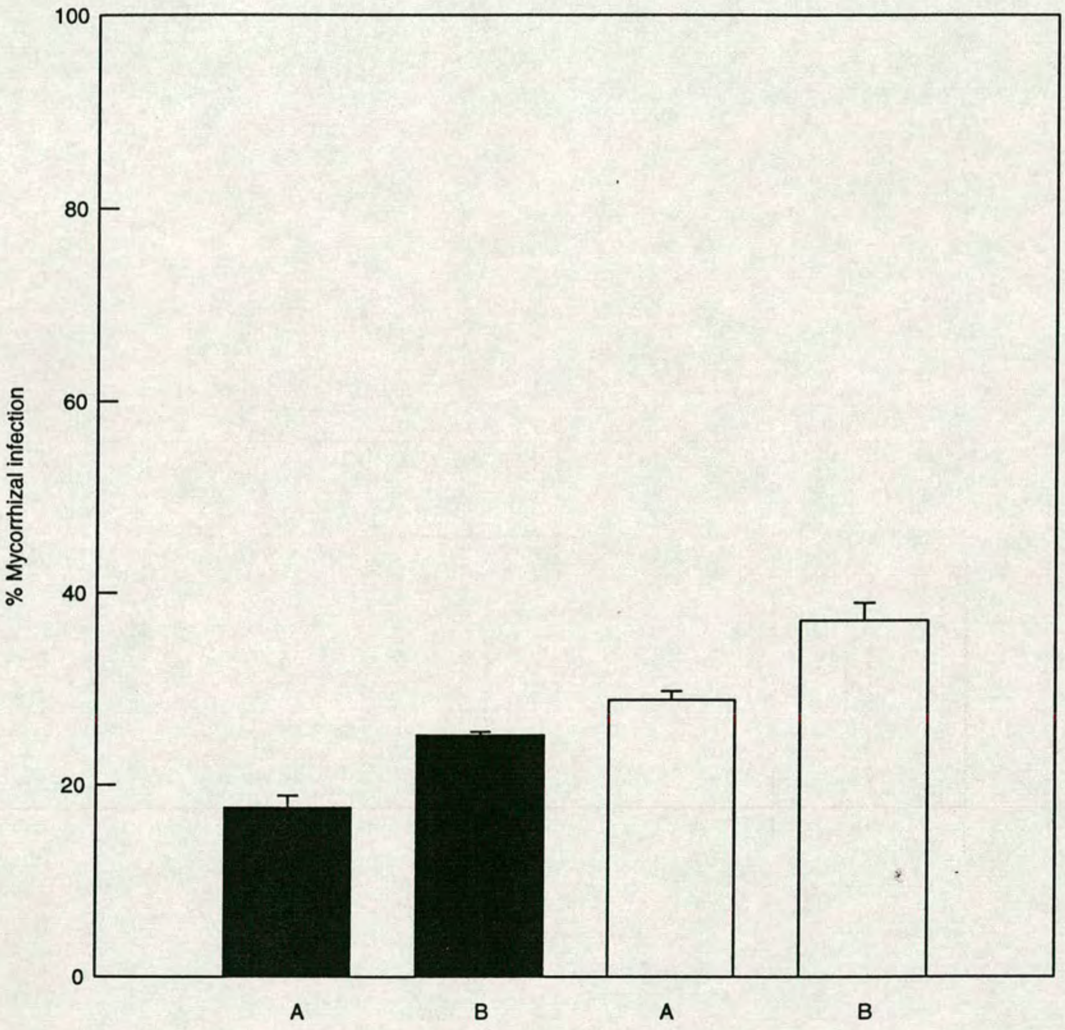


Figure 6.1: Effect of inoculating two varieties of *Zea mays* L. ■ Katumani composite var. and □ Hybrid II var. with different types of AMF inoculum on mycorrhizal infection in plant roots.

A) Spore inoculum B) Soil inoculum

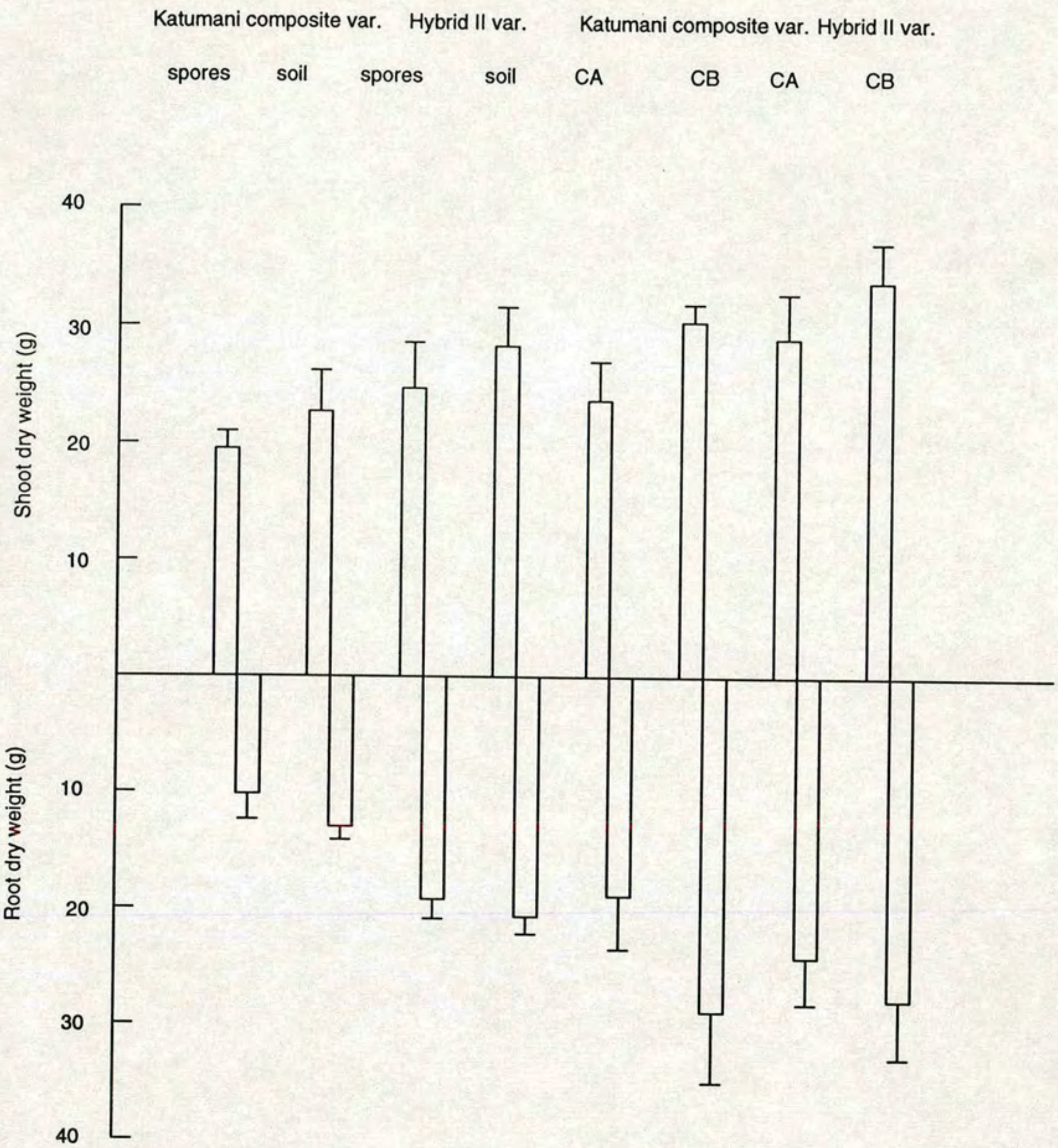


Figure 6.2: Effect of inoculating two varieties of *Zea mays* L. with different types of AMF inoculum on shoot and root dry weights of inoculated and uninoculated plants.

6.4 Discussion

In agreement with the findings of Mercy *et al.* (1990) and Hall (1978) there were significant differences in the degree of mycorrhizal development in the roots of the two maize varieties. Similarly, mycorrhizal formation was significantly higher when plants were inoculated with soil rather than spore inoculum. Despite attempts made to remove infected root pieces and reduce the infectivity of soil AMF by sieving soil inoculum, the findings, consistent with those from other experiments, showed that the infectivity and effectiveness of soil was generally greater than that of spores alone. This is most likely due to the AMF infected root and hyphal content of soil (Porter, 1979). Obviously, the degree of soil disturbance was not high enough, or the inoculum potential of pot culture soil so great, that disturbance effect was not realised. The fact that soil inoculated plants, had higher growth than spore inoculated plants, and were not found to have pathogenic infection in roots would lead us to believe that soil inoculum did not contain pathogenic organisms. Steam sterilization has been found to provide and environment conducive to the growth of contaminant micro-organisms (Abbott and Robson, 1984). If the autoclaving process used to eliminate AMF from soil in this experiment had been ineffective, then the presence of mycorrhizal infection in plant roots would have been detected even if pathogenic infection was overlooked.

The results from this experiment showed significant reductions in the growth of inoculated compared to uninoculated plants, despite the absence of pathogen infection in plant roots and despite having been grown in 4 L pots. This effect has been found in all experiments in this study and as stated before is likely to have occurred as a result of the experimental conditions. In previous experiments th limited soil volume in which plants were grown may have reduced the mycorrhizal effect which in a larger pot would otherwise have been possible. The mechanism by which the mycorrhizal root increases nutrient uptake has been discussed previously (see section 4.4). It was not expected that such an effect would have occurred in this experiment.

Plant growth was significantly different between the two plant varieties, although, the total dry weight of inoculated plants and root dry weight of uninoculated plants were similar. These findings would indicate an intrinsic difference between plant varieties in relation to growth. Hall (1978) found significant differences between growth of different plant varieties inoculated with AMF.

Uninoculated plants of Katumani composite var. plants had significantly higher root : shoot ratios than inoculated plants, while inoculated and uninoculated plants of Hybrid II var. has similar root : shoot ratios. This is consistent with many studies which report increases in the R:S ratios of uninoculated plants (see section 4.4). It is suggested that inoculated plants allocated less resources to the growth of the root system already extended by the external hyphal matrix.

These results indicate that there are intrinsic intra-specific differences between *Z. mays* plants as indicated by higher dry weights of Hybrid II var. plants compared to Katumani composite var. plants. Although, a negative mycorrhizal response to inoculation with AMF was found, this was thought to be due to the limited volume of soil in which plants were grown and would be expected to disappear in the field where there is a larger volume of soil available for proliferation of AMF hyphae. The effects of inoculating plants with soil inoculum rather than spore inoculum increased the formation of mycorrhizal infection in roots of these plants. There was similarly, improved growth of plants of each variety when they were inoculated with soil inoculum rather than spore inoculum. This would appear to indicate that contaminant micro-organisms were absent from soil inoculum. However, it is also possible that the effectiveness of AMF in soil inoculum was greater than that of spores alone, despite attempts made to reduce this, and that this masked the detrimental effects which contaminant microorganisms may have produced. The use of AMF inoculum of the highest purity is particularly important as stressed by Koide and Schreiner (1992).

CHAPTER SEVEN
DISCUSSION

7.1 Evaluation of experimental approaches and methods

In this section, problems encountered in the field and experiments are discussed, as well as possible ways of overcoming them in future work. In subsequent sections the main findings of this study are outlined and the implications for understanding of AMF ecology and design and management in agroforestry systems are discussed. Finally, proposals for further research are given.

The distribution and abundance of AMF can be investigated using spores, hyphae or infected roots in soil. Spores are usually easier to extract from soil, quantify and identify compared to AMF hyphae or infected roots, therefore spore surveys are frequently used to indicate AMF fungal distribution and abundance (Redhead, 1977; Diem *et al.*, 1981; Koske, 1981; Walker, 1982; Louis and Lim, 1987; Musoko *et al.*, 1994). There are limitations associated with the use of spores as indicators of AMF distribution and abundance. Some species do not sporulate, while others produce many spores. The ability to produce spores and the effectiveness of a particular AMF species are not necessarily related.

As stated before, other AMF propagules such as infected roots and particularly, AMF hyphae, are often difficult to study. AMF hyphae are difficult to extract from soil, however, recent developments by Green *et al.* (1994) have enabled AMF hyphae of different species to be examined in "rhizobox" studies. Information on the quantity of AMF hyphae produced and the effectiveness of hyphae produced by different AMF species could assist in the interpretation of experimental results in the future. Dodd *et al.* (1983) found differences in infection produced by different AMF species. However, identification of AMF species using infected roots is hampered by the fact that infection produced by most AMF species is similar with the exception of species belonging to the genera, *Gigaspora* and *Scutellospora*, which never form vesicles in the root (Morton and Benny, 1990).

Careful consideration was given to the type of sampling technique used in this study, for sampling AMF spores. Heterogeneity of spore distribution has been found in many tropical soils (Alexander, 1991; Musoko, 1991). Therefore, the accuracy of AMF spore recovery depends on the intensity of sampling within the area. However, this conflicted with the practical considerations of tansportation to the UK, so to overcome this, the number of replicates was reduced and bulking of composite samples followed by subsampling was carried out. The survey of AMF spores in this system was therefore preliminary and used to show whether there were host or fallowing effects on AMF spore populations and to provide information about AMF spore populations occurring in soil used as inoculum in experiments.

The identification of AMF species from spores requires that single spore cultures are established from which fresh spores can be obtained. These can be problematic to establish and maintain. Additionally, not only is spore identification a specialized area of expertise, but it requires a lot of time which was unavailable within the framework of this study. Detailed identification of AMF spores to species level would have been advantageous, however the objectives of this study, to examine what, if any effects hosts and fallowing had on AMF populations in soil, would not have been realised.

In experiments 1, 2 and 3, plants were inoculated with field soil (100 g pot^{-1}) containing spores, hyphae and infected root material in 1 litre pots of autoclaved soil A (see section 2.1). Uninoculated plants were grown in autoclaved soil A only. The type of controls used in mycorrhizal experiments varies between studies, however the use of appropriate controls is important. In these experiments, a more appropriate control would have included 100 g of autoclaved field soil (to include additional nutrients) and soil leachate from 100 g of field soil (to include soil rhizosphere organisms). However, the use of available soil for replication in these experiments was considered more important to the design of experiments. The increased nutrients available to inoculated plants from added field soil was expected to be small, as the nutrient content of field soil, particularly P was low. However, in experiment 4, the addition of autoclaved soil inoculum was included in the experimental design. The findings are discussed in the following section. The addition of soil rhizosphere microorganisms was not carried out because of the limited quantity of available soil inoculum.

Inoculated plants were grown in a more dilute concentration of inoculum in pots than that which occurs in the field. As experimental work was carried out in the UK, it was not possible to obtain the quantities of soil necessary to grow plants in pots containing field soil. The reduced concentration of AMF spores in soil in which inoculated plants were grown may have affected results, and to elucidate this, experiment 3 was established. The findings are discussed in the following section.

It is expected that the infectivity of AMF hyphae and infected root may have been reduced by the disturbance of field soil during sampling before use in experiments, thus favouring infection by spores. However, this was difficult to avoid and as all samples were handled in the same way, this is unlikely to have altered treatment effects. Despite attempts made to use soil in experiments with similar chemical properties, particularly pH and available P, to field soil, it is likely that there were differences between soils in relation to the physical structure. This makes extrapolation of experimental results to the field difficult.

The effects of inoculation of plants with AMF are achieved largely through the effect of AMF on the size of the root system (see section 1.5.2). In the field, the volume of soil available for the proliferation of the mycorrhizal root is much greater than that which can be used in pot experiments involving different treatment effects. To overcome this problem either pot size has to be increased, as carried out in experiment 4, or alternatively future experiments should be more field orientated, however this makes the containment of root systems for data collection more difficult.

Establishment of experiments connected to field sites in other countries is not an ideal situation. Although, there are many problems encountered in the tropics with respect to establishing experiments, it is felt that these could be easily overcome, and the advantages of carrying out AMF studies in the country of origin, such as access to fresh spore material and field soil for use in pot experiments are likely to greatly out-weigh the problems, given time. Further field and pot studies examining the effects of host and fallowing on AMF inoculum in agroforestry systems in their country of origin are required, in which there is easy access to the field to provide AMF spores for pot cultures and proper identification. In addition, disturbance of field soil for use in pot experiments can be minimised, the effects of pot size on plant growth and mycorrhizal response can be reduced by establishing field trials and the effects of RP application on the mycorrhizal growth response of plants can be examined without affecting the physical properties of soil to the same degree as that which occurs in limited soil volumes. In experiment 4 and in pot experiments in general, use of soluble P fertilizers may have overcome this problem, but this would have caused other problems because P would have been freely available.

Although it is believed that experiments in this and other studies would be improved if established close to field sites, results from this study did indicate that AMF spore populations in agroforestry systems are altered by management practices. Such variation in spore populations significantly altered the development of mycorrhizal infection in plant roots. The negative growth response of plants inoculated with AMF in this study appeared to be attributable to the experimental conditions rather than the ineffectiveness of the inoculant fungi. It is suggested that future experimental work in pots should take into consideration findings of this study relating to the experimental conditions, such as choice of pot soil, volume of soil used for a particular plant species, as well as soil nutrient status. If taken into account, future experiments would be expected to show either a positive plant growth response to AMF inoculation, or confirm the findings of this study. In this case, it is possible that the inoculant fungi are ineffective for the particular crop species, which is not highly dependent on AMF. Perhaps other crop species would have been more responsive. Screening and effectiveness trials may be required in the future so that highly effective AMF inoculants can be introduced into the field in order to improve the sustainability of this particular agroforestry system.

7.2. Implications of field and experimental results

The main findings of the AMF spore survey have been;

1. AMF spores occurred in higher number in the RHI system relative to that reported for natural undisturbed systems

2. The management practice of fallowing had a significant effect on AMF spore numbers in this system.

3. In the dry season, fallowing significantly increased live spore numbers, however this effect was small.

4. In the wet season, spore numbers in all soil samples were significantly higher than in the dry season.

5. In the wet season, the number of live spores was significantly higher in the cropped area of the system, particularly in tree soil.

6. The dominant AMF spore types found were similar in tree and alley areas.

7. The population composition of AMF was found to change between season, between fallow and cropped areas and between host species.

AMF spore numbers in soil in the RHI system were high compared to that reported for less disturbed ecosystems (Sieverding, 1989). This may indicate that, in highly disturbed ecosystems, spores are more important for initiation of infection than other infective propagules of AMF in soil. This is based on findings from studies which have shown that the infectivity of AMF hyphal networks and infected roots are reduced by disturbance (O' Halloran et al., 1986; Evans and Miller, 1988; Mc Gonigle et al., 1990a; Bellgard, 1993b). The rate of increase in density of AMF hyphae or quantity of infected root material in soil after disturbance has never been studied. Such information would be required for systems which are frequently disturbed and would help researchers evaluate the relevance of spore surveys.

In the dry season, spores were found in higher number in the fallow area compared to cropped area of the system, while in the wet season the opposite effect was observed. The seasonal variation in spore numbers in tree soil in the fallow area was not as great as that observed for tree soil in the cropped area. This increase in spore number in tree soil in the cropped area could be due to a factor other than fallowing or host species. In November, pruning of hedgerows is carried out before planting the crop and is highly likely to significantly affect the physiological processes of trees which in turn indirectly effect AMF spore numbers and composition.

Fallowing in monoculture agroecosystems has been found to produce detrimental effects on AMF spore populations and subsequent growth of plants (Thompson, 1987). In this study, there were no detrimental effects of fallowing on spore number. Density of vegetation cover in fallow alley areas increased between sampling occasions (see Plate 3.1) and this would explain how spore numbers were initially maintained and then increased in soil in November. No details of species composition in fallow alleys was obtained at sampling times, however it is likely that population composition of AMF spores in fallow alleys changed between season, due to effects of changes in the vegetation cover, species composition and biomass. This has significant implications as a management strategy in tree fallow systems, particularly if the population composition of AMF associated with colonizing species in fallow alleys is not effective for the next crop.

Spore numbers were lower in soil at the end of the dry season than at the start of the following wet season in the same year. Temporal changes in AMF spore populations have been observed in temperate ecosystems, however in tropical ecosystems, observations are conflicting, with some studies observing seasonal variation and others reporting little or no seasonal variation (see Chapter 3). At the field site in this experiment the semi-arid conditions were probably largely responsible for the high numbers of dead spores found in soil. Dead spore numbers were greater in soil in the wet season sample compared to that found in soil in the dry season. More than 65 % of AMF spores recovered from soil in the wet season were non-viable, except in soil taken from trees in the cropped area. Here, only 25 % of the spore population were non-viable. This is thought to be due to a build up in the number of dead spores as the dry season preceeding this sampling occasion progressed. Evidence to support this comes from a comparison of the results of this study and another carried out by Mbuthia (1992) in the same system. Mbuthia (1992) sampled soil at the end of the dry season in early March, 1992, and found that 96 % of spores in soil were dead. In this study, sampling of the system was carried out in late February, the previous year and the percentage of dead spores in all soil samples was less than 46 %. These findings would indicate that as the dry season progresses the number of dead spores in soil increases. To confirm this AMF spore populations would need to be monitored at frequent intervals throughout the year.

Although, spore types were not identified to species level, the same types were found associated with trees and the intercrop, however the abundance of each of the four dominant spore types varied greatly between hosts within each site and between sites for the same host species. Such results are often interpreted as a lack of host specificity which has always been regarded as a characteristic of AMF. However, compositional changes in AMF populations indicate possible differences in the competitive ability of different AMF species associated with different hosts or between species under the conditions existing in fallow or cropped sites. This is particularly true for spore type t3 which in the wet season, was found in particularly higher numbers in tree soil from the cropped area of the system, than elsewhere (see section 3.3). Spore type t24 was found in higher number in fallow areas, particularly in tree soil in the wet season. Spores of t10a were high in number in tree soil in the fallow area in the dry season and greater in alley areas in the wet season.

It would appear that the distribution of spore types t24 and t10a is different to that of spore type t3. While, spore types t24 and t10a are more abundant in the fallow area those of t3 are more abundant in the cropped area. The implications of these results are that different host species and agronomic practices can enhance the relative abundance of one spore type over another and that this could result in significant alteration in plant growth if for example, crops which favour different AMF species are rotated, or as in sequential agroforestry systems, trees and crops occupy the same piece of land, but at different times (Palm, 1995). Whether such effects would be of benefit to plant growth requires investigation so that the management or design of agroforestry systems can be improved.

In alley cropping systems, roots of *S. siamea* have been found to extend tens of metres from the trunk (Schroth, 1995). When hedgerow spacing is only a few metres, the whole alleys are usually permeated by tree roots (Hauser, 1993). This could have great implications for the distribution and abundance of AMF in the field and the way in which AMF are studied in agroforestry systems. It may also explain how little variation in spore numbers was found between host species within fallow and cropped areas, with the exception of the high number spores in tree soil in the cropped area during the wet season sample collection. This is thought to be explained by the results of a study which reports that the density of tree roots of *S. siamea* decreased logarithmically with increasing distance from the tree (Schroth *et al.*, 1995). As already suggested, pruning may also have produced root die back and this stress may have resulted in the increases in AMF spore numbers observed. Sanginga *et al.* (1994) observed that pruned *S. siamea* trees distributed approximately 118 % more total P to branches and had a higher physiological phosphorus-use efficiency than unpruned plants. The implications of this for AMF would appear to be great. Perhaps the increase in sporulation observed beneath pruned trees was a response to the stress of the sink of P from the tree branches.

It is clear from the results of the spore survey carried out in this study that fallowing had a greater effect than host species on the number and population composition of AMF spores. In addition, these findings agree with the majority of those in agroecosystem which report a similar range in spore numbers, greater than that occurring in natural undisturbed soil. The possible effect of pruning on spore numbers as indicated by the results from this study requires further investigation to confirm this. Preliminary indications of possible effects of host and fallowing on the spore population composition could have important implications for design and management aspects in agroforestry systems. Spores of the four dominant types were found in tree and alley soil. However, the spore population composition varied between tree and alley soil in fallow and cropped areas at different sampling times.

Results from this preliminary investigation of AMF spore populations indicate that a more detailed study of AMF spores would be worthwhile. Screening and effectiveness trials could then be conducted to examine the compatibility between indigenous AMF species at this site and a range of tree and crop host species. How a range of management regimes affect AMF populations could also be examined.

The main findings concerning inoculation of Z. mays with AMF from experiments in this study have been:

1. Plants inoculated with tree soil from the cropped area of the RHI system had significantly higher mycorrhizal infection than those inoculated with soil from other areas (experiment 1), however the growth and nutrition of plants was largely unaffected by inoculation with AMF in this experiment.

2. Plants (in experiment 2) inoculated with highly infected roots (of plants from experiment 1) had significantly higher mycorrhizal infection and produced higher spore numbers in soil than plants inoculated with less infected root material. The majority of spores produced in soil were *Glomus*, t3 spore type predominant in the field.

3. There was a significant difference in mycorrhizal infection formation in plants inoculated with 50 g, 100 g and 150 g of field soil, infection being higher in plants grown in pots with greater soil inoculum, however inoculum amounts had little effect on the growth and nutrition of plants. (see experiment 3)

4. Plants grown in soil A (experiment 4) had significantly higher mycorrhizal infection than those in unamended soil B. Percentage mycorrhizal infection decreased incrementally as the volume of unamended soil A and B increased. Adding RP to soil produced significantly higher mycorrhizal infection in plants compared to those in unamended soil.

8. In 10 litre pots of unamended soil A, growth of plants was significantly higher than that of plants in 10 litre pots of unamended soil B. Plant growth was significantly increased with incremental increases in soil volume. Adding RP to soil B significantly reduced the growth of plants compared to those in unamended soil B. Shoot P content of plants was significantly higher in inoculated than uninoculated plants.

9. Intra-specific variation between two varieties of *Z. mays* were found with respect to the formation of mycorrhizal infection and growth of plants. Plants of each variety inoculated with soil had significantly higher mycorrhizal infection and growth than those inoculated with spores. Generally, inoculated plants had significantly lower growth than plants left uninoculated (see experiment 5).

Reduced growth of plants in response to inoculation observed in all experiments in this study was unexpected. However, results similar to those found here are consistent with those of Mbuthia (Mbuthia, 1992) working on AMF inoculation of *Z. mays* using soil from the same field site as in this study. Detrimental effects of AMF inoculation on plant growth have been reported in other studies (Koide, 1985; Abbott and Robson, 1984; Cooper, 1984). However, the majority of studies show increases in the growth of plants in response to AMF inoculation (De Miranda *et al.*, 1989; Asmah *et al.*, 1995). This is thought to be achieved primarily by increased uptake of P by plants (see section 1.5.2), although AMF have been found to be beneficial to plants under other stresses, such as drought (Sieverding, 1981) salinity (see Baker, 1993) or pathogenic infection (Menge, 1982).

In this study, the degree of mycorrhizal infection inside the root cortex was found to be unrelated to the resultant plant growth. Other studies have reported similar findings, in which mycorrhizal infection has not been related to growth improvements (Clarke and Mosse, 1981; Jensen, 1982). This raises the question of how mycorrhizal infection in the root cortex of plants is related to the overall functioning of the mycorrhiza. The influence of AMF hyphae in this relationship cannot be ignored. Determination of mycorrhizal infection in the root tells us nothing about the AMF hyphae outside the root, as a relationship between the two has never been established. Until recently, studies have been hampered by the difficulties involved in extracting, quantifying and identifying AMF species using hyphae. However, recent studies of AMF hyphae by Green *et al.* (1993) would appear to indicate that this can be achieved, with the right equipment, and hopefully such work can be incorporated into future studies of AMF plant interactions.

There are several possible reasons for the reduced growth of mycorrhizal plants observed in these studies. One mechanism by which P uptake is increased in mycorrhizal plants is through the external hyphae which extend the root system beyond nutrient depletion zones and into the surrounding (non-depleted) soil (see section 1.5.2). If conditions in this experiment were not appropriate for this to occur, then the ability of the mycorrhiza to function properly would have been affected and the reduced growth of inoculated plants observed here, not surprising. Competition between the host and fungus for photosynthate has been indicated as a reason for this reduced growth observed in plants inoculated with AMF in some experiments (Huante *et al.* 1993; López-Sánchez and Honrubia, 1992).

Another reason for the reduced growth could relate to the effectiveness of the infecting AMF species. Compatibility between host species and AMF species can significantly affect the growth response of plants (Mosse, 1975; Kruckelmann, 1975; Aggangan and Lorilla, 1990; Medeiros *et al.*, 1994). This aspect of AMF ecology is critical to the success of AMF inoculation programmes or management of indigenous AMF populations in the field. As mentioned before, experiments examining the effectiveness of AMF species are a necessary pre-requisite before AMF can be properly managed in the field.

The presence of pathogens has been reported to reduce the growth of plants (Cooper, 1984). In his study, Mbuthia (1992) observed an organism in the stained root material of plants grown in his experiment which he suggested could have been pathogenic. He suspected that this had been introduced in the field soil inoculum as pot soil was sterilised. However, in this study pathogenic infection was not found in the plant roots. It was possible that this had been overlooked, however, this aspect of the study was investigated further in another experiment (experiment 6), the findings of which are discussed later in this section. Mbuthia (1992) also indicated that the soil sterilisation process may have failed to have fully eradicated any contaminants. However, had the autoclaving of soil been unsuccessful then mycorrhizal infection if not pathogenic infection would have been detected in the roots of uninoculated plants in this and Mbuthias study. The use of AMF inoculum of the highest purity was stressed by Koide and Schreiner (1992) and ways of achieving this should be carefully thought out in all AMF studies.

Soil type had a significant effect on the mycorrhizal development. The growth of plants was affected but only in the largest pot size (10 litres). Differences between soil physical and chemical properties have been found to affect the distribution and abundance of AMF spores in the field (Mosse, 1972; Islam, 1980; Saif, 1986), as well as the mycorrhizal formation in roots and growth of plants in pot experiments (Biermann and Lindermann, 1983). In this experiment, the potassium content of soil varied between different soil types, being much higher in soil A than soil B. This could have had an effect on plant growth as shoot and root potassium content of plants reflected this increase in soil, although differences in potassium content of tissues of plants in different soils was very small. This was suspected as a factor causing the reduced growth observed in plants in the first experiment (see Chapter 4), and was a major factor influencing the decision to remove vermiculite from soil in experiment 4. The use of vermiculite in soil would appear to solve more problems than it creates as found in this study.

The significant increase in growth of plants in response to incremental increases in the volume of soil available to the plants was not unexpected. However, the absence of a mycorrhizal response, even in the largest soil volume, was surprising. These findings conflict with those of Bääth and Hayman (1984) who found that both the growth and growth response of plants to inoculation were significantly increased in larger soil volumes or as the density of plants was reduced in pots. One possible explanation for such discrepancies between the findings of this and the present study is related to the species of plant involved and its soil volume requirements. Bääth and Haymans' study examined the response of onion plants to increased soil volume and mycorrhizal inoculation. However, in the experiments of this study, *Z. mays* was the host plant and the extensive root system of these plants may have been such that uninoculated plants were able to exploit the soil without the aid of AMF in 10 litre pots. A better mycorrhizal response may have been achieved if a plant species with a less extensive root system had been used instead. Likewise, a species with a higher mycorrhizal dependency may have shown a greater mycorrhizal response.

Changes in chemical and physical properties of soil may have affected the growth of plants in different soil types and in soil amended with RP, to such an extent that the treatment effects were masked. When RP was added to soil, as well as increases in the available P levels found, there were also changes in the concentration of other soil nutrients. The increase in available P content of soil in treatment RP2 was particularly high and not proportional to the amount of RP added to soil in this treatment. This may explain the particularly poor growth of plants in this treatment. However, there were also parallel increases in soil potassium, magnesium and a reduction in calcium content as higher amounts of RP were added to soil. The reduced calcium content of soils in treatment RP2 were not reflected in soil pH readings. Therefore, it is unlikely that soil pH had any significant effects on plant growth in plants in amended soils. However, the effects of changes in soil chemistry could have been masked by the physical changes in soil due to differences in the quantity of RP in each pot. In soil without vermiculite, the porosity of soil would have been expected to have changed and hence differences in the water holding capacity may have resulted in poorer growth of plants in a poorly drained soil. The differences in water holding capacity were undetected during the experiment, however perhaps the experiment could have been improved if additional plants had been available for 'look-see' examinations. This would have enabled watering procedures to be modified and such problems, which were probably exacerbated by adding RP to soil, could have been controlled.

7.2 Future work

The mycorrhizal growth response of plants in the field is of ultimate interest in AMF research. In tropical areas much more research is necessary if ecosystems, natural or agronomic are to be maintained or improved in ways which are sustainable.

The results from this experiment showed that spore numbers in tree soil were significantly increased, probably in response to physiological changes in the tree as a result of pruning. Direct evidence for this could be obtained in future studies specifically designed to examine the effects of tree pruning on AMF spores in parallel pot and field studies. Examination of spore populations in future studies would need to be carried out more frequently throughout the year if seasonal variation in spore numbers are to be fully understood. However, it must be emphasised that AMF spore propagules do not represent the whole population of infective propagules in soil. It is becoming clear that the role of external hyphae must be taken into account in studies of AMF. The effects of disturbance, particularly in agroecosystems in which the AMF hyphal network is disrupted frequently, must be investigated outside the growth chamber. It would appear from our results and those of other AMF spore surveys that spores are particularly higher in number in more disturbed environments. In addition to studies such as those already mentioned, it would also be of vital importance that the time required for a disrupted AMF hyphal network to recover after soil disturbance be determined and that the contribution to the functioning of AMF by spores, hyphae and infected root material be elucidated.

The poor growth response of plants to inoculation observed in these experiments may indicate that the inoculant fungi from the field site are ineffective. Further work is required in which AMF species found in the field are screened for their effectiveness on a range of hosts. The need to confirm the results of screening trials in the field is also important as highlighted by the results of experiments in this study which showed how different experimental variables, from soil type to soil volume can affect the growth of plants. It is speculated that poor growth responses of plants which occurred in these experiments would disappear in the field where the volume of soil for exploitation is large. In addition, the effects of adding RP to soil could be examined properly without the accompanying changes in soil physical properties which occurred in this study when plants were grown in limited soil volumes. In the field, this would not be expected to occur as the RP fertilizer would be applied to field sites rather than individual plant pots. Despite the problems, the need for field evaluations cannot be stressed enough, there are too many factors which influence the AMF symbiosis in the field which cannot be simulated in pots.

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APPENDIX A

Table 1. Analysis of variance (for summary Table 3.2) of mean number of live, dead and total spore numbers in soil taken from the RHI system at ICRAFs research station at Machakos, Kenya in February, 1991.

Source **TOTAL**	d.f	s.s	m.s	v.r	f.prob
Block	3	4.599	1.533	0.19	
Species	1	3.327	3.375	0.41	0.530
Treatment	1	435.601	435.601	53.06	(0.001
Spec.Trea	1	61.479	61.479	7.49	0.011
Residual	25	205.229	8.209		
Total	31	710.235			
LIVE					
Block	3	4.908	1.636	0.29	
Species	1	5.625	5.625	0.98	0.331
Treatment	1	223.393	223.393	39.11	(0.001
Spec.Trea	1	50.170	50.170	8.78	0.007
Residual	25	142.812	5.712		
Total	31	426.908			
DEAD					
Block	3	13.427	4.476	0.99	
Species	1	0.166	0.166	0.04	0.849
Treatment	1	220.242	220.242	48.83	(0.001
Spec.Trea	1	17.168	17.168	3.81	0.062
Residual	25	112.765	4.511		
Total	31	363.768			
% DEAD					
Block	3	143.11	47.70	1.60	
Species	1	59.79	59.79	2.01	0.169
Treatment	1	0.23	0.23	0.01	0.930
Spec.Trea	1	67.20	67.20	2.26	0.146
Residual	25	744.26	29.77		
Total	31	1014.59			

Table 2. Analysis of variance (for summary Table 3.2) of mean total (live+dead), live and dead number of spores and proportion of dead spores in soil samples taken from the RHI system at ICRAFs research station at Machakos, Kenya in November, 1991.

Source **TOTAL**	d.f	s.s	m.s	v.r	f.prob
Block	3	9.92	14.96	1.20	
Species	1	195.34	195.34	15.02	⟨0.001
Treatment	1	1.24	1.24	0.10	0.757
Spec.Trea	1	237.74	237.74	19.13	⟨0.001
Residual	14	174.01	12.43		
Total	19	638.26			
LIVE					
Block	2	6.59	3.29	0.29	
Species	1	327.06	327.06	28.35	⟨0.001
Treatment	1	147.89	147.89	12.82	0.003
Spec.Trea	1	430.81	430.81	37.35	⟨0.001
Residual	14	161.49	11.53		
Total	19	1073.83			
DEAD					
Block	2	16.21	8.10	0.53	
Species	1	0.01	0.01	0.00	0.982
Treatment	1	123.66	123.66	8.12	0.013
Spec.Trea	1	0.19	0.19	0.01	0.913
Residual	14	213.26	15.23		
Total	19	353.33			
% DEAD					
Block	2	10.75	5.38	0.11	
Species	1	484.71	484.71	9.47	0.008
Treatment	1	669.23	669.23	13.08	0.003
Spec.Trea	1	835.16	835.16	16.32	0.001
Residual	14	716.23	51.16		
Total	19	2716.08			

Table 3. Analysis of variance (for summary Table 3.3) of mean number of spore types t3,t10a,t10b and t24 in soil taken from the RHI system at ICRAFs research station at Machakos, Kenya in February, 1991.

Source **T3**	d.f	s.s	m.s	v.r	f.prob
Block	3	24.519	8.173	1.26	
Species	1	2.360	2.360	0.36	0.552
Treatment	1	94.476	94.476	14.59	<.001
Spec.Trea	1	19.119	19.119	2.95	0.098
Residual	25	161.928	6.477		
Total	31	302.402			
T10a					
Block	3	10.046	3.349	0.84	
Species	1	4.477	4.477	1.13	0.299
Treatment	1	38.8633	38.8633	14.68	0.004
Spec.Trea	1	58.380	58.380	14.68	<.001
Residual	25	99.450	3.978		
Total	31	211.216			
T10b					
Block	3	13.668	4.556	2.48	
Species	1	13.810	13.810	7.51	0.011
Treatment	1	0.010	0.010	0.01	0.942
Spec.Trea	1	0.253	0.253	0.14	0.714
Residual	25	45.998	1.840		
Total	31	73.740			
T24					
Block	3	9.465	3.155	1.92	
Species	1	58.705	58.705	35.81	<.001
Treatment	1	72.297	72.297	44.10	<.001
Spec.Trea	1	29.984	29.984	18.29	<.001
Residual	25	40.981	1.639		
Total	31	211.432			

Table 4. Analysis of variance (for summary Table 3.3) of mean number of spore types t3,t10a and t24 in soil taken from the RHI system at ICRAFs research station at Machakos, Kenya in November, 1991.

Source **T3**	d.f	s.s	m.s	v.r	f.prob
Block	2	8.944	4.472	2.02	
Species	1	1049.336	1049.336	474.86	<.001
Treatment	1	816.717	816.717	369.60	<.001
Spec.Trea	1	865.050	865.050	391.47	<.001
Residual	14	30.937	2.210		
Total	19	2770.983			
T10a					
Block	2	5.909	2.955	0.98	
Species	1	65.399	65.399	21.59	<.001
Treatment	1	25.964	25.964	8.57	0.011
Spec.Trea	1	0.109	0.109	0.04	0.852
Residual	14	42.406	3.029		
Total	31	139.787			
T24					
Block	2	16.87	8.44	0.84	
Species	1	103.56	103.56	10.29	0.006
Treatment	1	2.14	2.14	0.21	0.652
Spec.Trea	1	33.42	33.42	3.32	0.090
Residual	14	140.96	10.07		
Total	19	296.95			

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APPENDIX B

Table 1: Analysis of variance (for table 4.1) of mean % root length mycorrhizal infection in *Zea mays* L. plants 60 and 120 d.a.i with tree and alley soil from fallow and intercropped areas of the RHI system, Machakos, Kenya. Angular transformations were made on mean values

Source 60 d.a.i	d.f	s.s	m.s	v.r	f.prob
Block	4	23.04	5.76	0.47	
Species	1	786.62	786.62	63.60	<0.001
Treatment	1	833.55	833.55	67.40	<0.001
Spec.Trea	1	556.07	556.07	44.96	<0.001
Residual	12	148.42	12.37		
Total	19	2347.70			
Source 120 d.a.i					
Block	4	300.61	75.15	1.13	
Species	1	889.44	889.44	13.32	0.003
Treatment	1	269.83	269.83	4.04	0.067
Spec.Trea	1	420.44	420.44	6.30	0.027
Residual	12	801.09	66.76		
Total	19	2681.40			

Table 2: Analysis of variance (for table 4.2) of mean shoot dry weights of inoculated and uninoculated *Zea mays* L. plants harvested 60 and 120 d.a.i.

Source 60 d.a.i	d.f	s.s	m.s	v.r	f.prob
Block	4	0.6980	0.1745	0.24	
Treatment	5	16.0190	3.2038	4.39	⟨ 0.05
Residual	20	14.5860	0.7293		
Total	29	31.3030			
Source 120 d.a.i					
Block	4	50.461	12.615	2.07	
Treatment	5	39.271	7.854	1.29	0.309
Residual	20	122.063	6.103		
Total	29	211.795			

Table 3: Analysis of variance (for table 4.2) of mean estimated root dry weights of inoculated and uninoculated *Zea mays* L. plants harvested 60 and 120 d.a.i.

Source 60 d.a.i	d.f	s.s	m.s	v.r	f.prob
Block	4	3.285	0.821	0.52	
Treatment	5	9.419	1.884	1.19	0.349
Residual	20	31.651	1.583		
Total	29	44.355			
Source 120 d.a.i					
Block	4	6.671	1.668	0.83	
Treatment	5	33.799	6.760	3.38	< 0.05
Residual	20	40.013	2.001		
Total	29	80.483			

Table 4: Analysis of variance (for table 4.2) of mean total dry weights of inoculated and uninoculated Zea mays L. plants harvested 60 and 120 d.a.i.

Source 60 d.a.i	d.f	s.s	m.s	v.r	f.prob
Block	4	3.195	0.799	0.29	
Treatment	5	19.611	3.922	1.41	0.262
Residual	20	55.509	2.775		
Total	29	78.315			
Source 120 d.a.i.					
Block	4	83.145	20.786	3.84	
Treatment	5	139.571	27.914	5.16	< 0.05
Residual	20	108.187	5.409		
Total	29	330.903			

Table 5: Analysis of variance (for table 4.2) of mean root:shoot ratios of *inoculated and uninoculated Zea mays* L. plants harvested 60 and 120 d.a.i. Angular transformations were made on mean root: shoot ratio values.

Source60 d.a.i.	d.f	s.s	m.s	v.r	f.prob
Block	4	0.9736	0.2434	0.64	
Treatment	5	3.1131	0.6226	1.63	0.197
Residual	20	7.6237	0.3812		
Total	29	11.7104			
Source 120 d.a.i					
Block	4	1.1524	0.2881	0.32	
Treatment	5	5.2159	1.0432	1.17	0.360
Residual	20	17.8873	0.8944		
Total	29	24.2555			

Table 6: Analysis of variance (for table 4.3) of mean shoot P and Mg and root Ca concentrations (% of dry weight) for inoculated and uninoculated *Zea mays* L. plants harvested 60 d.a.i. Angular transformations were made on mean % of dry weight values.

Source	d.f	s.s	m.s	v.r	f.prob
Shoot % Phosphorus					
Block	4	0.0754	0.01885	0.99	
Treatment	5	0.62128	0.12426	6.55	〈 0.001
Residual	20	0.37942	0.01897		
Total	29	1.07610			
Shoot % Magnesium					
Block	4	1.3907	0.3477	2.13	
Treatment	5	9.5618	1.9124	11.73	〈 0.001
Residual	20	3.2614	0.1631		
Total	29	14.2138			

Root % Calcium

Source	d.f	s.s	m.s	v.r	F.prob
Block	4	1.8823	0.4706	3.44	
Treatment	5	3.7691	0.7538	5.51	〈 0.05
Residual	20	2.7366	0.7368		
Total	29	8.3880			

Table 7: Analysis of variance (for table 4.3) of mean shoot N and P concentrations (% of dry weight) in inoculated and uninoculated *Zea mays* L. plants harvested 120 d.a.i. Angular transformations were made on mean % dry weight values.

Source %N	d.f	s.s	m.s	v.r	f.prob
Block	4	0.54410	0.13603	2.18	
Treatment	5	3.27983	0.65597	10.51	⟨ 0.001
Residual	20	1.24883	0.06244		
Total	29	5.07277			

Source %P	d.f	s.s	m.s	v.r	f.prob
Block	4	0.26771	0.06693	1.45	
Treatment	5	0.70839	0.14168	30.6	⟨ 0.05
Residual	20	0.92564	0.04628		
Total	29	1.90174			

Table 8: Analysis of variance (for Table 4.6) of mean % mycorrhizal infection in *Zea mays L.* plants 120 d.a.i. with infected root material from maize previously inoculated with tree and alley soil from fallow and cropped areas of the RHI system in experiment 1a. Angular transformations were made on % values before analysis.

Source	d.f	s.s	m.s	v.r	f.prob
Block	4	78.99	19.75	0.82	
Species	1	1828.00	1828.00	75.76	<0.001
Treatment	1	2861.31	2861.31	118.58	<0.001
Spec.Trea	1	3009.13	3009.13	124.71	<0.001
Residual	12	289.56	24.13		
Total	19	8066.99			

Table 9: Analysis of variance (for table 4.7) of shoot dry weights of *Zea mays* L. plants 120 days after inoculating with infected root material taken from plants previously inoculated with tree and alley soil from fallow and cropped areas of the RHI system in experiment 1a.

Source	d.f	s.s	m.s	v.r	f.prob
Block	4	11.531	2.883	0.55	
Treatment	5	144.471	28.894	5.49	⟨ 0.01
Residual	20	105.313	5.266		
Total	29	261.315			

Table 10: Analysis of variance (for Table 4.7) of root dry weights of *Zea mays* L. plants 120 days after inoculating with infected root material taken from plants previously inoculated with tree and alley soil from fallow and cropped areas of the RHI system in experiment 1a.

Source	d.f	s.s	m.s	v.r	f.prob
Block	4	3.20563	0.8013	1.47	
Treatment	5	12.0067	2.4013	4.41	0.05
Residual	20	10.8867	0.5443		
Total	29	26.0987			

Table 11: Analysis of variance (for tabled 4.7) of total dry weights of *Zea mays* L. plants 120 days after inoculating with infected root material taken from plants previously inoculated with tree and alley soil from fallow and cropped areas of the RHI system in experiment 1a.

Source	d.f	s.s	m.s	v.r	f.prob
Block	4	32.479	8.120	1.19	
Treatment	5	258.962	51.792	7.62	⟨ 0.001
Residual	20	1325.953	6.798		
Total	29	427.394			

Table 12: Analysis of variance (for table 4.7) of root:shoot ratios of *Zea mays* L. plants 120 days after inoculating with infected root material taken from plants previously inoculated with tree and alley soil from fallow and cropped areas of the RHI system in experiment 1a. Angular transformations were made on root shoot ratio values.

Source	d.f	s.s	m.s	v.r	f.prob
Block	4	1.1513	0.2878	2.49	
Treatment	5	3.4912	0.6982	6.04	⟨ 0.001
Residual	20	2.3137	0.1157		
Total	29	6.9562			

Table 13: Analysis of variance (for table 4.8) of root N concentration (% of dry weight) of *Zea mays* L. plants inoculated with infected roots material taken from plants previously inoculated with tree and alley soil from fallow and cropped areas of the RHI system in experiment 1a. Angular transformations were made on % values.

Source Root % N	d.f	s.s	m.s	v.r	f.prob
Block	4	0.084847	0.021212	3.52	
Treatment	5	0.276777	0.055355	9.17	⟨ 0.001
Residual	20	0.120673	0.006034		
Total	29	0.482297			

Table 14: Analysis of variance (for table 4.9) of mean mycorrhizal infection in roots of *Zea mays* L. plants inoculated with 50 g, 100 g and 150 g of soil inoculum taken from the RHI system at Machakos, Kenya in November, 1991.

Source	d.f	s.s	m.s	v.r	f.prob
Block	3	41.78	13.93	0.97	
Treatment	2	4731.35	2365.68	165.36	⟨ 0.001
Residual	18	257.52	14.31		
Total	23	5030.65			

APPENDIX C

Table 1: Analysis of variance (for table 5.2) of mean % root length mycorrhizal infection in root systems of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing 2 soil types and harvested 90 d.a.i. Angular transformations were made on % root length infection values.

Source of variation	d.f	s.s	m.s	v.r	f.pr
Block	5	74.25	14.85	0.79	
Soil type	1	698.95	698.95	36.99	<0.001
Pot size	2	344.94	172.47	9.13	0.001
Soil type. potsize	2	45.32	22.66	1.20	0.318
Residual	25	472.41	18.90		
Total	35	1635.89			

Table 2 : Analysis of variance (for table 5.2) of total dry weight of mycorrhizal and non- mycorrhizal *Zea mays* L. plants grown in 1, 4 and 10 litre volume pots containing 2 soil types, harvested 90 d.a.i. Square root transformations were made on total dry weights.

Source of variation	d.f	s.s	m.s	v.r	f.pr
Block	5	12.495	2.499	1.41	
Inoculation	1	12.198	12.198	6.86	0.011
Soil type	1	11.287	11.287	6.35	0.015
Pot size	2	586.121	293.060	164.92	<0.001
Inoc.Soil type	1	4.254	4.254	2.39	0.128
Inoc.Pot size	2	2.684	1.342	0.76	0.475
Soil type.Pot size	2	34.169	17.084	9.61	<0.001
Inoc.Soil.Pot size	2	0.492	0.246	0.14	0.871
Residual	55	97.735	1.777		
Total	71	761.434			

Table 3 : Analysis of variance (for table 5.2) of shoot dry weight of mycorrhizal and non- mycorrhizal *Zea mays* L. plants grown in 1, 4 and 10 litre volume pots containing 2 soil types, harvested 90 d.a.i. Square root transformations were made on total dry weights.

Block	5	12,8778	2.5756	2.78	
Inoculation	1	5.7868	5.7868	6.25	0.015
Soil type	1	12.3769	12.3769	13.38	<0.001
Pot size	2	630.0325	315.0163	340.48	<0.0011
Inoc.Soil type	1	5.3004	5.3004	5.73	0.020
Inoc.Pot size	2	1.6713	0.8357	0.90	0.411
Soil type. Pot size	2	27.1593	13.5796	14.68	<0.001
Inoc.Soil.Pot size	2	2.3778	1.1889	1.29	0.285
Residual	55	50.8863	0.9252		
Total	721	748.4692			

Table 4: Analysis of variance (for table 5.2) of root dry weights for mycorrhizal and non- mycorrhizal *Zea mays* L. plants grown in 1, 4 and 10 litre volume pots containing 2 soil types, harvested 90 d.a.i. Square root transformations were made on total dry weights.

Source of variation	d.f	s.s	m.s	v.r	f.pr
Block	5	0.2967	0.0593	0.34	
Inoculation	1	6.0132	6.0132	34.78	<0.001
Soil type	1	0.6684	0.6684	3.87	0.054
Pot size	2	32.8373	16.4136	94.95	<0.001
Inoc.Soil type	1	0.7172	0.7172	4.15	0.046
Inoc.Pot size	2	1.2757	0.6379	3.69	0.031
Soil.Pot size	2	2.1481	1.0740	6.21	0.004
Inoc.Soil.Pot size	2	0.0849	0.0424	0.25	0.783
Residual	55	9.5079	0.1729		
Total	71	53.5394			

Table 5: Analysis of variance (for table 5.2) of root : shoot ratios of mycorrhizal and non- mycorrhizal Zea mays L. plants grown in 1, 4 and 10 litre volume pots containing 2 soil types, harvested 90 d.a.i. Square root transformations were made on total dry weights.

Source of variation	d.f	s.s	m.s	v.r	f.pr
Block	5	3.7441	0.7488	1.93	
Inoculation	1	1.7628	1.7628	4.55	0.037
Soil type	1	2.3346	2.3346	6.03	0.017
Pot size	2	47.5029	23.7514	61.35	<0.001
Inoc.Soil type	1	0.1648	0.1648	0.43	0.517
Inoc.Pot size	2	0.2565	0.1282	0.33	0.719
Inoc.Soil.Pot size	2	5.1552	2.5776	6.66	0.003
Residual	55	21.2947	0.3872		
Total	71	82.3286			

Table 6: Analysis of variance (for table 5.3) of % root length mycorrhizal infection in *Zea mays* L. grown in 1, 4 and 10 litres of pot soil B containing different quantities of rock phosphate fertilizer (0 mg l^{-1}; mg l^{-1}; mg l^{-1}) harvested 90 d.a.i. Angular transformations were made on % root infection values.

Source of variation		s.s	m.s	v.r	f.pr
Block	5	93.10	18.62	0.49	
Potsize	2	1447.19	723.59	19.12	<0.001
Phosphorus	2	5748.65	2874.33	19.12	<0.001
Potsize.Phos	4	682.71	170.68	4.51	0.004
Residual	40	1514.01	37.85		
Total	53	9485.66			

Table 7 : Analysis of variance (for table 5.3) of total dry weight of mycorrhizal and non- mycorrhizal Zea mays L. plants grown in 1, 4 and 10 litre volume pots containing soil B with and without RP fertilizer, harvested 90 d.a.i. Square root transformations were made on total dry weights.

Source of variation	d.f	s.s	m.s	v.r	f.pr
Block	5	1.5715	0.3143	0.58	
Inoculation	1	0.4025	0.4025	0.74	0.392
Pot size	2	77.7632	38.8816	71.64	<0.001
Phosphorus	2	412.1322	206.0661	379.68	<0.001
Inoc.Pot size	2	1.1686	0.5843	1.08	0.345
Inoc.Phosphorus	2	2.5727	1.2864	2.37	0.100
Pot size.Phosphorus	4	173.3679	43.3420	79.86	<0.001
Inoc.Pot size.Phos	4	1.0347	0.2587	0.48	0.753
Residual	85	46.1326	0.5427		
Total	107	716.1458			

Table 8 : Analysis of variance (for table 5.3) of mean shoot dry weight of mycorrhizal and non-mycorrhizal *Zea mays* L.plants grown in 0 P, low P and high P in pots containing 1,4 and 10 litres of soil type B, harvested 90 d.a.i. Square root transformations were made on shoot dry weights.

Source of variation	d.f	s.s	m.s	v.r	f.pr
Block	5	0.9996	0.1999	0.38	
Inoculation	1	0.7517	0.7517	1.42	0.236
Phosphorus	2	341.9078	170.9539	323.75	<0.001
Pot size	2	73.7602	36.8801	69.84	<0.001
Inoc.Phosphorus	2	1.4152	0.7076	1.34	0.267
Inoc.Pot size	2	0.8577	0.4288	0.81	0.447
Phosphorus.Pot size	4	160.5721	40.4130	76.02	<0.001
Inoc.Phos.Pot size	4	1.3439	0.3360	0.64	0.638
Residual	85	44.8839	0.5280		
Total	107	626.4922			

Table 9: Analysis of variance (for table 5.3) of mean root dry weight of mycorrhizal and non-mycorrhizal *Zea mays* L.plants grown in 0 P, low P and high P in pots containing 1,4 and 10 litres of soil type B, harvested 90 d.a.i. Square root transformations were made on root dry weights.

Source of variation	d.f	s.s	m.s	v.r	f.pr
Block	5	0.7680	0.1536	0.88	
Inoculation	1	1.0747	1.0747	6.16	0.015
Phosphorus	2	19.8206	9.9103	56.77	<0.001
Pot size	2	6.8772	3.4386	19.70	<0.001
Inoc.Phosphorus	2	52.8032	26.4016	151.23	<0.001
Inoc.Pot size	2	0.0697	0.0349	0.20	0.819
Phosphorus.Pot size	4	7.8456	1.9614	11.24	<0.001
Inoc.Pot size.Phos	4	12.3774	3.0943	17.72	<0.001
Residual	85	14.8392	0.1746		
Total	107	116.4755			

Table 10: Analysis of variance (for table 5.3) of root : shoot ratios of mycorrhizal and non- mycorrhizal Zea mays L. plants grown in 1, 4 and 10 litre volume pots containing soil B with and without RP fertilizer, harvested 90 d.a.i. Square root transformations were made on total dry weights.

Source of variation	d.f	s.s	m.s	v.r	f.pr
Block	5	33.405	6.681	3.05	
Inoculation	1	1.710	1.710	0.78	0.379
Pot size	2	19.310	9.655	4.41	0.015
Phosphorus	2	22.200	11.100	5.07	0.008
Inoc.Pot size	2	0.330	0.165	0.08	0.927
Inoc.Phosphorus	2	1.605	0.803	0.37	0.694
Pot size.Phosphorus	4	25.663	6.416	2.93	0.025
Inoc.Pot size.Phos	4	2.413	0.603	0.28	0.893
Residual	85	186.006	2.188		
Total	107	292.641			

Table 11: Analysis of variance (for table 5.4) of mean % N in shoots of Zea mays L. grown in 1, 4 and 10 litre volume pots containing 2 soil types 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	14.369	4.790	2.19	
Myco	1	0.320	0.320	0.15	0.704
Soil	1	1.969	1.969	0.90	0.349
Potsize	2	7.794	3.897	1.78	0.184
Myco.Soil	1	0.137	0.137	0.06	0.804
Myco.PS	2	8.443	4.221	1.93	0.161
Soil.PS	2	62.994	4.221	1.93	<0.001
Myc.Soil.PS	2	7.016	3.508	1.60	0.216
Residual	33	72.134	2.186		
Total	47	175.175			

Table 12: Analysis of variance (for table 5.4) of mean % P in shoots of Zea mays L. grown in 1, 4 and 10 litre volume pots containing 2 soil types 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	1.3318	0.4439	1.28	
Myco	1	1.8232	1.8232	5.26	0.028
Soil	1	1.0025	1.0025	2.89	0.098
Potsize	2	0.6730	0.3365	0.97	0.389
Myco.Soil	1	0.4802	0.4802	1.39	0.247
Myco.PS	2	0.5931	0.2966	0.86	0.434
Soil.PS	2	0.4203	0.2101	0.61	0.551
Myc.Soil.PS	2	0.2135	0.1067	0.31	0.737
Residual	33	11.4322	0.3464		
Total	47	17.9698			

Table 13: Analysis of variance (for table 5.4) of mean % K in shoots of *Zea mays L.* grown in 1, 4 and 10 litre volume pots containing 2 soil types 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	2.5367	0.8456	1.43	
Myco	1	1.4890	1.4890	2.53	0.122
Soil	1	29.1095	29.1095	49.37	<0.001
Potsize	2	0.3953	0.1977	0.34	0.718
Myco.Soil	1	3.4093	3.4093	5.78	0.022
Myco.PS	2	0.2334	0.1167	0.20	0.821
Soil.PS	2	0.0325	0.0163	0.03	0.973
Myc.Soil.PS	2	0.7334	0.3667	0.62	0.543
Residual	33	19.4590	0.5897		
Total	47	57.3982			

Table 14: Analysis of variance (for table 5.4) of mean % Mg in shoots of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing 2 soil types 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	0.07248	0.02416	0.38	
Myco	1	0.64142	0.64142	10.18	0.003
Soil	1	0.09664	0.09664	1.53	0.224
Potsize	2	0.08350	0.04175	0.66	0.522
Myco.Soil	1	0.00062	0.00062	0.01	0.922
Myco.PS	2	0.22883	0.11442	1.82	0.179
Soil.PS	2	0.74795	0.37398	5.93	0.006
Myc.Soil.PS	2	0.11264	0.05632	0.89	0.419
Residual	33	2.08002	0.06303		
Total	47	4.06410			

Table 15: Analysis of variance (for table 5.5) of mean % Ca in shoots of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing 2 soil types 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	0.23180	0.07727	1.43	
Myco	1	0.32613	0.32613	6.04	0.019
Soil	1	0.43571	0.43571	8.07	0.008
Potsize	2	0.11448	0.05724	1.06	0.358
Myco.Soil	1	0.25207	0.25207	4.67	0.038
Myco.PS	2	0.06547	0.03274	0.61	0.551
Soil.PS	2	0.00784	0.00392	0.07	0.930
Myc.Soil.PS	2	0.34665	0.17332	3.21	0.053
Residual	33	1.78093	0.05397		
Total	47	3.56109			

Table 16: Analysis of variance (for table 5.5) of mean % N in shoots of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing soil B with and without added rock phosphate 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	3.887	1.296	0.54	
Myco	1	0.142	0.142	0.06	0.809
Potsize	2	42.779	21.390	8.86	<0.001
Phos	2	177.267	88.633	36.73	<0.001
Myco.Potsize	2	25.401	12.700	5.26	0.008
Myco.Phos	2	5.789	2.895	1.20	0.310
Potsize.Phos	4	19.019	4.755	1.97	0.113
Myc.PS. Pho	4	11.357	2.839	1.18	0.332
Residual	51	123.075	2.413		
Total	71	408.717			

Table 17: Analysis of variance (for table 5.5) of mean % P in shoots of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing soil B with and without added rock phosphate 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	1.0321	0.3440	1.50	
Myco	1	2.1584	2.1584	9.41	0.003
Potsize	2	0.1209	0.0604	0.26	0.769
Phos	2	0.5818	0.2909	1.27	0.290
Myco.Potsize	2	0.3281	0.1641	0.72	0.494
Myco.Phos	2	0.7628	0.3814	1.66	0.200
Potsize.Phos	4	2.4793	0.6198	2.70	0.041
Myc.PS. Pho	4	0.5452	0.1363	0.59	0.668
Residual	51	11.6963	0.2293		
Total	71	19.7051			

Table 18: Analysis of variance (for table 5.5) of mean % K in shoots of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing soil B with and without added rock phosphate 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	10.368	3.456	2.67	
Myco	1	0.158	0.158	0.12	0.729
Potsize	2	0.114	0.057	0.04	0.957
Phos	2	119.457	59.729	46.08	<0.001
Myco.Potsize	2	0.272	0.136	0.11	0.900
Myco.Phos	2	9.130	4.565	3.52	0.037
Potsize.Phos	4	4.141	1.035	0.80	0.532
Myc.PS. Pho	4	3.756	0.939	0.72	0.579
Residual	51	66.109	1.296		
Total	71	213.506			

Table 19: Analysis of variance (for table 5.5) of mean % Mg in shoots of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing soil B with and without added rock phosphate 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	0.3561	0.1187	1.03	
Myco	1	0.0473	0.0473	0.41	0.524
Potsize	2	0.2519	0.1260	1.10	0.341
Phos	2	0.6143	0.3071	2.68	0.078
Myco.Potsize	2	0.4515	0.2257	1.97	0.150
Myco.Phos	2	0.8436	0.4218	3.68	0.032
Potsize.Phos	4	0.4845	0.1211	1.06	0.388
Myc.PS. Pho	4	0.2056	0.0514	0.45	0.773
Residual	51	5.8533	0.1148		
Total	71	9.1080			

Table 20: Analysis of variance (for table 5.5) of mean % Ca in shoots of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing soil B with and without added rock phosphate 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	1.8140	0.6047	2.31	
Myco	1	1.5339	1.5339	5.85	0.019
Potsize	2	0.4697	0.2349	0.90	0.415
Phos	2	1.2086	0.6043	2.31	0.110
Myco.Potsize	2	0.5602	0.2801	1.07	0.351
Myco.Phos	2	0.7322	0.3661	1.40	0.257
Potsize.Phos	4	0.8698	0.2175	0.83	0.513
Myc.PS. Pho	4	0.6431	0.1608	0.61	0.655
Residual	51	13.3705	0.2622		
Total	71	21.2020			

Table 21: Analysis of variance of mean (for table 5.5) % N in roots of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing 2 soil types 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	1.0951	0.3650	2.23	
Myco	1	0.2472	0.2472	1.51	0.228
Soil	1	3.1231	3.1231	19.08	<0.001
Potsize	2	19.9117	9.9558	60.82	<0.001
Myco.Soil	1	0.5363	0.5363	3.28	0.079
Myco.PS	2	0.0998	0.0499	0.30	0.739
Soil.PS	2	0.0148	0.0074	0.05	0.956
Myc.Soil.PS	2	0.09947	0.4973	3.04	0.062
Residual	33	5.4021	0.1637		
Total	47	31.4247			

Table 22: Analysis of variance (for table 5.6) of mean % P in roots of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing 2 soil types 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	0.05787	0.01929	0.80	
Myco	1	0.05107	0.05107	2.13	0.154
Soil	1	0.47558	0.47558	19.82	<0.001
Potsize	2	0.02307	0.1154	0.48	0.623
Myco.Soil	1	0.11392	0.11392	4.75	0.037
Myco.PS	2	0.05195	0.02597	1.08	0.350
Soil.PS	2	0.01117	0.00559	0.23	0.794
Myc.Soil.PS	2	0.04250	0.02125	0.89	0.422
Residual	33	0.79171	0.02399		
Total	47	1.61884			

Table 23: Analysis of variance (for table 5.6) of mean % K in roots of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing 2 soil types 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	1.9465	0.6488	0.91	
Myco	1	2.0768	2.0786	2.93	0.097
Soil	1	23.3228	23.3228	32.86	<0.001
Potsize	2	0.5145	0.2573	0.36	0.699
Myco.Soil	1	2.4976	2.4976	3.52	0.070
Myco.PS	2	0.4592	0.2296	0.32	0.726
Soil.PS	2	4.7026	2.3513	3.31	0.049
Myc.Soil.PS	2	2.9012	1.4506	2.04	0.146
Residual	33	23.4203	0.7097		
Total	47	61.8416			

Table 24: Analysis of variance (for table 5.5) of mean % Mg in roots of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing 2 soil types 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	0.15796	0.05265	1.03	
Myco	1	0.02076	0.02076	0.41	0.529
Soil	1	0.11625	0.11625	2.27	0.141
Potsize	2	0.03545	0.01772	0.35	0.710
Myco.Soil	1	0.00158	0.00158	0.03	0.862
Myco.PS	2	0.05033	0.02517	0.49	0.616
Soil.PS	2	0.61093	0.30547	5.97	0.006
Myc.Soil.PS	2	0.33544	0.16772	3.28	0.050
Residual	33	1.68916	0.05119		
Total	47	3.01786			

Table 25: Analysis of variance (for table 5.6)of mean % Ca in roots of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing 2 soil types 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	0.20956	0.06985	1.03	
Myco	1	1.28676	1.28676	19.05	<0.001
Soil	1	6.43313	6.43313	95.22	<0.001
Potsize	2	0.36601	0.18301	2.71	0.081
Myco.Soil	1	0.01148	0.01148	0.17	0.683
Myco.PS	2	0.61424	0.30712	4.55	0.018
Soil.PS	2	0.51383	0.25691	3.80	0.033
Myc.Soil.PS	2	0.54881	0.27441	4.06	0.026
Residual	33	2.22947	0.06756		
Total	47	12.21329			

Table 26: Analysis of variance (for table 5.7) of mean % N in roots of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing soil B with and without added rock phosphate 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	4.9402	1.6467	2.17	
Myco	1	0.5333	0.5333	0.70	0.406
Potsize	2	1.6530	0.8265	1.09	0.3451
Phos	2	1.1087	0.5544	0.73	0.487
Myco.Potsize	2	3.4567	1.7283	2.27	0.113
Myco.Phos	2	2.7008	1.3504	1.78	0.180
Potsize.Phos	4	16.3908	4.0977	5.39	0.001
Myc.PS. Pho	4	6.8314	1.7079	2.25	0.077
Residual	51	38.7844	0.07605		
Total	71	76.3993			

Table 27: Analysis of variance (for table 5.7) of mean % P in roots of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing soil B with and without added rock phosphate 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	0.2467	0.0822	0.37	
Myco	1	0.0688	0.0688	0.31	0.579
Potsize	2	0.0072	0.0036	0.02	0.984
Phos	2	0.1677	0.0839	0.38	0.686
Myco.Potsize	2	0.5554	0.2777	1.26	0.293
Myco.Phos	2	0.3427	0.1714	0.78	0.465
Potsize.Phos	4	0.1166	0.0292	0.13	0.970
Myc.PS. Pho	4	1.1985	0.2996	1.36	0.261
Residual	51	11.2503	0.2206		
Total	71	13.9541			

Table 28: Analysis of variance (for table 5.7) of mean % K in roots of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing soil B with and without added rock phosphate 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	5.280	1.760	1.14	
Myco	1	5.921	5.921	3.82	0.056
Potsize	2	30.377	15.188	9.80	<0.001
Phos	2	52.325	26.162	16.89	<0.001
Myco.Potsize	2	15.260	7.630	4.92	0.011
Myco.Phos	2	21.047	10.523	6.79	0.002
Potsize.Phos	4	11.141	2.785	1.080	0.144
Myc.PS. Pho	4	40.779	10.195	6.58	<0.001
Residual	51	79.015	1.549		
Total	71	261.144			

Table 29: Analysis of variance (for table 5.7) of mean % Mg in roots of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing soil B with and without added rock phosphate 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	1.1815	0.3938	1.69	
Myco	1	0.5194	0.5194	2.23	0.142
Potsize	2	3.1715	1.5858	6.80	0.002
Phos	2	3.9824	1.9912	8.54	<0.001
Myco.Potsize	2	0.1179	0.0590	0.25	0.777
Myco.Phos	2	0.5726	0.2863	1.23	0.301
Potsize.Phos	4	1.8814	0.4704	2.02	0.106
Myc.PS. Pho	4	0.8147	0.2037	0.87	0.486
Residual	51	11.8903	0.2331		
Total	71	24.1317			

Table 30: Analysis of variance (for table 5.7) of mean % Ca in roots of *Zea mays* L. grown in 1, 4 and 10 litre volume pots containing soil B with and without added rock phosphate 90 d.a.i. Angular transformations were made on % N, P, K, Mg and Ca.

Source	d.f	s.s	m.s	v.r	f.pr
Bloc	3	1.0612	0.3537	1.04	
Myco	1	1.9217	1.9217	5.64	0.021
Potsize	2	0.0949	0.0475	0.14	0.870
Phos	2	16.2603	8.1302	23.88	<0.001
Myco.Potsize	2	1.0349	0.5174	1.52	0.229
Myco.Phos	2	0.0222	0.0111	0.03	0.968
Potsize.Phos	4	0.3393	0.0848	0.25	0.909
Myc.PS. Pho	4	5.2905	1.3226	3.88	0.008
Residual	51	17.3659	0.3405		
Total	71	43.3909			

APPENDIX D

Table 1. Significance of F-values in the analysis of variance (for summary Table 6.1) of mycorrhizal infection levels in 2 varieties of Zea mays L. inoculated with AMF.

Source of variation	d.f	m.s	s.s	v.r	f.pr
Block	4	2.1350	0.5337	0.83	
Inoculation	1	134.3147	134.3147	207.88	<0.001
Variety	1	281.5722	281.5722	435.79	<0.001
Inoc.var	1	0.030	0.030	0.05	0.833
Residual	12	7.7535	0.6461		
Total	19	425.8054			

Table 2. Significance of F-values in the analysis of variance (for summary Table 6.1) of total dry weight of 2 varieties of *mycorrhizal and non mycorrhizal Zea mays* L. plants inoculated with either AMF spores or AMF spores, mycelium and infected root material.

Source of variation	d.f	m.s	s.s	v.r	f.pr
Block	4	0.50208	0.12552	1.35	
Inoculation	1	11.78758	11.78758	126.80	<0.001
Inocula type	1	4.45706	4.45706	47.94	<0.001
Variety	1	6.18045	6.18045	66.48	<0.001
Inoc.Inoc type	1	0.41890	0.41890	4.51	0.043
Inoc.variety	1	1.10774	1.10774	11.92	0.002
Inoc type.variety	1	0.39078	0.39078	4.20	0.050
Inoc.Inoc type. var	1	0.09994	0.09994	1.08	0.309
Residual	28	2.60294	0.09296		
Total	39	27.54747			

Table 3. Significance of F-values in the analysis of variance (for summary Table 6.1) of shoot dry weight of 2 varieties of *mycorrhizal and non mycorrhizal Zea mays* L. plants inoculated with either AMF spores or AMF spores, mycelium and infected root material.

Source of variation	d.f	m.s	s.s	v.r	f.pr
Block	4	0.44725	0.44725	1.22	
Inoculation	1	2.74187	2.74187	30.02	<0.001
Inocula type	1	1.98930	1.98930	21.78	<0.001
Variety	1	2.25644	2.25644	24.71	<0.001
Inoc.Inoc type	1	0.10508	0.10508	1.15	0.293
Inoc.variety	1	0.05196	0.05196	0.57	0.457
Inoc type.variety	1	0.02767	0.02767	0.30	0.586
Inoc.Inoc type. var	1	0.03159	0.03159	0.35	0.561
Residual	28	2.55732	0.09133		
Total	39	10.20848			

Table 4. Significance of F-values in the analysis of variance (for summary Table 6.1) of root dry weight of 2 varieties of *mycorrhizal and non mycorrhizal Zea mays* L. plants inoculated with either AMF spores or AMF spores, mycelium and infected root material.

Source of variation	d.f	m.s	s.s	v.r	f.pr
Block	4	0.5720	0.1430	0.92	
Inoculation	1	10.7342	10.7342	69.42	<0.001
Inocula type	1	2.4325	2.4325	15.73	<0.001
Variety	1	4.3836	4.3836	28.35	<0.001
Inoc.Inoc type	1	0.3987	0.3987	2.58	0.120
Inoc.variety	1	1.7195	1.7195	11.12	0.002
Inoc type.variety	1	0.5509	0.5509	3.56	0.069
Inoc.Inoc type. var	1	0.1070	0.1070	0.69	0.412
Residual	28	4.3298	0.1546		
Total	39	25.2282			

Table 5. Significance of F-values in the analysis of variance (for summary Table 6.1) of root shoot ratios of 2 varieties of *mycorrhizal and non mycorrhizal Zea mays* L. plants inoculated with either AMF spores or AMF spores, mycelium and infected root material.

Source of variation	d.f	m.s	s.s	v.r	f.pr
Block	4	0.06552	0.01638	1.58	
Inoculation	1	0.15527	0.15527	14.97	<0.001
Inocula type	1	0.00100	0.00100	0.10	0.758
Variety	1	0.04035	0.04035	3.89	0.059
Inoc.Inoc type	1	0.00047	0.00047	0.05	0.833
Inoc.variety	1	0.04595	0.04595	4.43	0.044
Inoc type.variety	1	0.02144	0.02144	2.07	0.162
Inoc.Inoc type. var	1	0.00140	0.00140	0.13	0.717
Residual	28	0.29047	0.01037		
Total	39	0.62188			