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THE TAXONOMY AND ECOLOGY OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) IN AGROFORESTRY SYSTEMS IN MALAWI

BY

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DECLARATION

I hereby declare that the thesis submitted for the degree of PhD (Botany) at the University of Pretoria is my work and has not previously been submitted by me for a degree at any other university or institution of higher education.

DEDICATION

I dedicate this work to my late father who was my source of inspiration and motivator in my life.

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ABSTRACT

Decline in soil quality and removal of subsidies on fertilizers has led to use of biological based farming practices such as agroforestry. Soil management and conservation practices have not used a holistic approach and have often overlooked the fact that soil is a dynamic system. Agroforestry improves soil fertility, but the effect on soil organisms has not been established. Knowledge of soil organisms in Africa is also scarce, yet soil organisms are crucial in soil processes and are greatly affected by farming practices. The Arbuscular Mycorrhizal Fungi (AMF) (*Glomeromycota* C. Walker & Schuessler) which form an interface between plant and soil systems and are sensitive to changes in plant and soil conditions can be used as indicators of changes in fertility.

A study was conducted in southern Malawi in agroforestry and maize monocrop systems to evaluate AMF species composition, diversity and frequency of occurrence as measured by spore occurrence, and AMF species effectiveness. Southern Malawi is a region prone to drought conditions and has nutrient deficient soils. The mycorrhizal symbiosis could be harnessed to improve root functions. The effectiveness of mycorrhizal symbiosis is highly dependent on AMF composition and inoculum potential of soils. In drought prone areas, spore propagules that persist longer under dry conditions are the best sources of inoculum. Two experiments were investigated for the AMF symbiosis, *Gliricidia sepium* (Jacq.) Walp/maize intercrop and maize monocrop system and *Sesbania sesban* (L) Merr./maize intercrop and *Sesbania macrantha* E Phillips & Hutch/maize intercrop and maize monocrop systems. A bioassay experiment was conducted under greenhouse conditions to evaluate colonization and effectiveness of selected AMF species from this site in plants.

The objective of the study was to determine AMF species using their spore morphological characters. A further aim of studying the agroforestry system with *Gliricidia sepium* was to determine the effect of agroforestry system on the diversity of AMF species and their frequency of occurrence. The *Sesbania* species experiment was used to establish whether two agroforestry systems would differ in their effects. In both agroforestry systems studies, it was determined whether the effect of the farming systems would be modified by application of inorganic nitrogen and phosphorus fertilizer and seasonal changes. Three AMF species extracted from the study site were evaluated for colonisation and effectiveness on growth of four host plants.

Soils were sampled and the spores extracted, fully described and taxon determined. The spore abundance of each individual species was quantified. Species relative diversity indices were computed from Shannon-Weinner and Simpson dominance diversity indices and the data was subjected to ANOVA. The Shannon-Weinner diversity Index was used to discuss the results. The species frequency of occurrence data measured by spore occurrence was analysed using logistic regression. The greenhouse bioassay experiment was subjected to ANOVA followed by comparisons of means by Least Square Means (LSM).

Twelve AMF species were extracted from the agroforestry sites, with four positively determined and three species affiliated to specific taxon. Out of the twelve AMF species only nine were successfully pot cultured. *Glomus etunicatum* Becker & Gerdemann and *Gigaspora margarita* Becker & Hall have been recorded from other parts of Africa. *Acaulospora rehmii* Sieverding & Toro, and *Scutellaspora cerradensis* Spain & Miranda were recorded for the first time in Africa. AMF species, namely *Archaeospora* sp. lacked

sufficient materials to diagonise it to species level while *Acaulospora* sp. 1, *Acaulospora* sp. 2, *Glomus* sp. and *Gigaspora* sp. had morphological features that strongly overlapped with closely related species. The study shows that 50 % of the species described from the agroforestry site in Malawi exist in other biogeographical regions of the world outside Africa. Two species, *A. rehmii* and *S. cerradensis* have been described only from South America. The study showed the mature stage of *A. rehmii, G. etunicatum, G. margarita* and *S. cerradensis* to have consistently similar morphological features over wider biogeographical regions. Additional morphological characters were observed in young spores of *A. rehmii* while some features observed in *Glomus* sp., which closely resembles *Glomus globiferum* Koske & Walker in most aspects, were not described in the type specimen description of *G. globiferum*, but were described in the type specimen description of *Glomus tortuosum* Schenck & Smith and *Glomus claroideum* Schenck & Smith. The possibly in young spores of *A. rehmii*, mature spores of *Acaulospora* sp 1, *Acaulospora* sp. 2, *Glomus* sp. and *Gigaspora* sp. described from spore cultures could not be distinguished easily from closely related allies.

In the *Gliricidia*/maize intercrop and maize monocrop systems, species diversity tended to be significantly ($p \leq 0.05$) higher in the wet season than the dry season in all treatments except for NP in agroforestry system. There was significant ($p \leq 0.05$) interaction between season, farming systems and fertility regimes. AMF species diversity was higher in agroforestry plots under conditions of low fertility and no addition of inorganic fertilizer than monocropped plots, although this was not significant ($p \leq 0.05$). Application of inorganic fertilizer modified the effects of farming systems, with inorganic fertilizer reducing species diversity in agroforestry plots. The fertilizer effect in agroforestry systems was more evident with N in the dry season and combination of N and P in the wet

season. Fertilizer effect showed AMF species diversity to be consistently low in all plots with a combination of inorganic N and P. Variable fertilizer effects were observed in maize monocrop in the two seasons. The effect of fertilizer in maize monocrop system was evident only in the dry season with species diversity higher in plots with P than plots with both N and P. The fertilizer effects in maize monocrop were more evident with P. There was no trend observed in species occurrence by members of the same family or genera. AMF species frequency of occurrence varied with season, farming systems and fertility regimes. The spores of five species occurred most in the maize monocrop system and the remaining seven were not affected. Spores of five species occurred most in the wet, three in dry season and four were not affected. The sporulation of five out of the twelve species decreased in plots with inorganic fertilizer, three species were enhanced, three species were not affected and spores of one species were variably affected.

In the two *Sesbania* spp./maize and maize monocrop systems, analysis of variance data showed farming systems to significantly ($p \leq 0.05$) affect AMF species diversity, species diversity being reduced by agroforestry systems. Plots with *Sesbania macrantha* intercropped with maize had significantly ($p \leq 0.05$) lower species diversity than maize monocrop but were not significantly different from plots with *S. sesban* intercropped with maize. Inorganic nitrogen fertilizer had the greatest effects on AMF species diversity measured by spore occurrence, species diversity being significantly ($p \leq 0.05$) enhanced by inorganic nitrogen fertilizer. Studies on frequency of occurrence of spores of AMF species showed season to have the most significant ($p \leq 0.05$) effect, nine species being prevalent in the dry season and three in the wet season. Farming systems exerted selective pressure on the occurrence of spores of four AMF species; *Gigaspora* sp. and *Scutellospora dipurpurascens* Morton & Koske were significantly ($p \leq 0.05$) affected and *Acaulospora* sp.

and *Glomus* sp. were slightly ($p \leq 0.1$) affected. Inorganic fertilizer also showed spores of *A. rehmii*, *Archaeospora* sp. and *S. cerradensis* to be significantly ($p \leq 0.05$) affected and *G. etunicatum* and *S. dipurpurascens* slightly ($p \leq 0.1$) affected.

The bioassay experiment showed AMF species from similar habitats to differ in their effects on plant growth. The closely related *Glomus aggregatum* Schenck & Smith and *G. etunicatum* showed preference for *G. sepium* while *A. delicata* showed preference for the closely related *S. sesban* and *S. macrantha*. There was no correlation between root colonisation levels and the three growth parameters in the three tree species, although colonisation levels were negatively correlated with the growth of maize. This study shows AMF species from the same site to differ in their effects on plant growth while AMF species effective on tree growth not necessarily improving the growth of maize.

Observations made in this study show little divergence of AMF species, some species to have a wide biogeographical range of distribution and the morphological characters inadequate diagnostic tools for separating closely related species. The effects of agroforestry systems are site specific and highly dependent on the fertility regimes and AMF species diversity seemed to respond most to the manipulation of the most deficient nutrient N. Root hairs seemed to play a vital role in determining host response to AMF association and the association of host plants with AMF species seemed to be a hereditary trait. The spores of AMF species from the same site differed in their adaptations with few species showing consistency and most changing with changes in environmental conditions. These results have great implication on management of AMF symbiosis and inoculation programs.

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CHAPTER 1: INTRODUCTION

1.1 INTRODUCTION

The pressure from a rapidly increasing population in Africa is causing an increase in demand for land used for settlement and food production. Traditional farming methods such as a ten year natural vegetation fallow followed by slash and burn practices have been replaced by the use of fertilizers and chemicals because of the unavailability of arable land that can be left fallow for long periods (Kamara *et al.* 1993). These soil amendment methods are expensive for African farmers and are non-sustainable and environmentally unfriendly (Kumwenda *et al.* 1995; Parks & Seaton 1996). Over-utilisation of agricultural land is leading to a decline in soil fertility and an overall decline in environmental quality.

Solutions for soil fertility problems rely on improvement of soil chemical and physical properties, but often the role of biological properties is overlooked (Lee 1994). The soil biota has evolved and is widely established to govern soil processes that naturally maintain soil fertility such as soil nutrient cycling and soil aggregate stability processes both of which are influenced positively by fungi (Gupta *et al.* 1990; Hawksworth 1991; Kennedy & Smith 1995). Application of fertilizers and pesticides greatly alter the soil environment under which these microbes function. Their efficiency may thus be altered, possibly leading to a total elimination of vital bio-components before their potential is realized. The effects of the application of fertilizers and chemicals are a short-term solution and they do not accommodate the living nature of soil that constantly requires the replenishment of organic input to sustain life in it.

Awareness in environmental degradation by conventional agricultural methods has ushered in more environmentally friendly biologically based farming practices (Bull 1996; Parks & Seaton 1996). The use of biological methods to improve soil fertility includes farming practices that use less fertilizer and chemical inputs. Agroforestry is one such practice with multiple benefits to the soil, the environment and the farmer (Young 1997). Agroforestry, the deliberate integration of trees or shrubs into crop and livestock production systems (Young 1997; Sanchez 1995) has multiple benefits. Trees are known to have a different impact on soil properties than annual crop because of their longer residence time, larger biomass accumulation and longer lasting more extensive root system (Sanchez *et al.* 1996). The ability to improve soil fertility by acquisition of nutrients and improving soil structure has been verified with these methods (Young 1997; Sanchez *et al.* 1997). Few studies have, however, been undertaken on the effects of these practices on soil biota, particularly in Africa.

A healthy soil maintains a balance of a wide range of organisms that have mutual benefit for the system that supports them. Soil organisms maintain soil pH with production of organic acids, fixing of atmospheric nitrogen and providing for the enzymatic breakdown of the more complex carbon molecules (Atlas & Bartha 1986; Paul & Clark 1989). Alterations of the soil ecosystem may result in changes in populations and functions of the soil biota.

The soil biota is considered a long-term investment in sustainable systems and may not have a direct and immediate impact on the soil quality. However, monitoring changes in

distribution and quantity of soil organisms may serve as indicators of directional changes in processes such as recycling and solubilization of mineral nutrients, organic acids which are plant growth promoters and disappearance of organisms antagonistic to potential pathogens. There is a wide range of soil organisms known for decades as vital in natural soil nutrient cycling and homeostasis maintenance for good growing conditions. Among these organisms is the Arbuscular Mycorrhizal Fungi (AMF) in the phylum *Glomeromycota* C. Walker & Schuessler, phylum nov. (Shüßler *et al.* 2001), that form a mutually beneficial association with plants and are keystone to soil processes (Harley & Smith 1983; Hawksworth 1991; Power & Mills 1995; Bull 1996). The interface between the soil and plants created by these fungi is affected by both plant and soil conditions.

The AMF are globally widespread and associate with most plant species (Harley & Smith 1983). The potential of AMF fungi in agriculture and forestry is well established and its potential for Africa has been postulated (Sanginga *et al.* 1992; Sieverding 1990). Most parts of Africa still remain unexplored and hence AMF communities are not known. The use of AMF is hampered by inadequate information on the diversity of species, global and local distribution and conditions of survival and effective functioning.

The AMF symbiosis in agroforestry is a reasonable point to initiate the evaluation of mycorrhizal functioning in a system that approaches the management ideal of low input sustainable agriculture. Agroforestry trees have great potential to improve soil properties in association with mycorrhizal fungi. This, however, demands knowledge of the best conditions required for survival of the mycorrhizal symbiosis. Hence the type of fungi that

thrives best under agroforestry conditions and their relationship with agroforestry trees and crops will form a basis for the development of utilisation strategy. Biologically based sustainable farming systems such as agroforestry widely used in resource-poor countries of Africa, Asia and South America (ICRAF 1996), may enhance diversity of AMF. Trees primarily enhance soil fertility through processes of nutrient capture, improving nutrient levels and protecting soil from soil erosion and are recorded to improve maize yield in highly nutrient depleted soils of Malawi (Maghembe & Prinns 1994). Agroforestry trees improve soil nutrients, particularly nitrogen (Kwesiga & Coe 1994; Ikerra *et al.* 1999) and other nutrients such as phosphorus and potassium (Mwiinga *et al.* 1994) through incorporation of leaf biomass. Trees, through the root system and organic inputs, improve soil physical properties by enhancing structure, porosity, permeability and water holding capacity (Yamoah *et al.* 1986).

Studies relating to soil organisms in agroforestry systems have focused on nitrogen fixing bacteria, as nitrogen is the nutrient required in large quantities for maize production. Agroforestry was reported to improve soil nitrogen levels (Kwesiga & Coe 1994). It was also observed that non-nodulating leguminous species accumulated high nitrogen levels (Ladha *et al.* 1993; Tomlinson *et al.* 1995) with mechanism speculated to be mycorrhizal.

The root system is an important organ of the plant that supports the shoot, enhances nutrient replenishment, nutrient uptake, aggregate formation and it forms 30–50 % of the plant biomass (Young 1997). Associations with organisms, particularly AMF, may enhance the efficiency of the root system and maximize root functions. Nutrient capture may be

enhanced by a highly mycelial root system that would provide a large surface area and aggregate stability may also be improved by an extensive mycelial network (Sutton & Sheppard 1976). Root-knot nematode disease in *Sesbania sesban* could be reduced by mycorrhizal symbiosis.

Incorporation of leaf biomass from agroforestry trees into the system may provide favourable conditions and support a high diversity of AMF. Agroforestry trees may differ in their effects on fungal species diversity and occurrence. Agroforestry practice was proved to be suitable for the highly nutrient depleted soils of southern Malawi that occasionally experience severe droughts and mild floods (Maghembe & Prinns 1994).

Studies on AMF symbiosis, particularly the diversity and occurrence of the indigenous communities in agroforestry systems, may form the initial basis for utilization. The use of AMF in agroforestry will depend on soil and plant conditions favourable for survival of the various fungal species. How the fungal species respond to seasonal changes may establish the best way to conserve and maintain a high AMF fungal diversity under severe conditions such as drought. The general and most significant test of the agroforestry systems is how it compares with the most intensive and purely organic management systems.

Commercial production and use of AMF inoculum will depend upon knowledge of species diversity, distribution and adaptation. Johnson and Menge (1982) saw the use of mycorrhiza as an alternative to fertilizer and hence a way of saving money. The mycorrhizal symbiosis to date is unable to substitute for fertilizers, though there is

increasing interest in the use of mycorrhizal fungi. This is evident in the increase in numbers of companies producing commercial formulations of AMF such as Vaminoc (Microbiodiv. Agricultural Genetics Company, Royston, England), Dr. Kinkon (Idemitsu Koskan Co. Ltd, Tokyo Japan), Mycorise (Premier Tech., cp 3500, Quebec, Canada) and Biovam (Washington, United States of America). Gaur *et al.* (1998), however, observed success with the formulation Mycorise and a mixed indigenous AMF culture on Capsicum and Polianthes species but not with Dr. Kinkon or Vaminoc. There is therefore need to study indigenous fungal communities before use of exotic species is recommended and also explore the prospects of developing commercial formulations in a more systematic and orderly way. The use of indigenous fungal communities will also eliminate the dangers of introducing root pathogens from exotic inoculum and the exotic species becoming hazardous.

The aim of the study is to establish the taxonomic diversity of AMF in an agroforestry system of Malawi and determine whether changes in farming systems, soil conditions and season will affect AMF species as well as plant growth.

1.2 OBJECTIVES

1. To establish the taxonomic groups of AMF from agroforestry systems of Malawi.
2. To determine species and ecological diversity of AMF fungi in *Gliricidia sepium* /maize intercrop and maize monocrop systems in the wet and dry season, and at four fertility levels.

3. To determine species and ecological diversity of AMF in *Sesbania sesban*/maize and *S macrantha*/maize intercrop and maize monocrop systems in the wet and dry season, and at four fertility levels.
4. To determine the effects of selected AMF species from the same site on growth of maize, *G. sepium, S. sesban* and *S. macrantha*.

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CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

Soil organisms play a crucial role in ecosystem functioning and potential benefits may be derived from an understanding of the biodiversity (Lee 1994). Knowledge of soil biodiversity will create opportunity to establish their functional significance. Only then can it be determined how to manipulate this biodiversity to increase and sustain production. Fungi are a major component of the soil biomass with considerable importance in regulating ecosystem processes (Hawksworth 1991; Fogel 1980), yet these fungi have received little attention (Hawksworth 1991). The mycorrhizal fungi are proposed as keystone organisms (Power & Mills 1995).

The AMF species associate with a wide range of hosts in diverse soil types and climatic regions of the world and are more widespread than any other symbiotic organisms (Newman & Reddell 1987; Chong-Kyun Kim *et al.* 1989; Allsopp & Stock 1993; Ragupathy & Mahadevan 1993). Members of the *Glomeromycota* (AMF) are obligate symbionts (Harley & Smith 1983) due to their necessity for an autotrophic symbiotic partner to supply their carbon needs, and function as absorptive organs for nutrient acquisition, particularly phosphorus. They are reported to have the ability to transfer nutrients from one host plant to another (Francis *et al.* 1986 and Haystead *et al.* 1988) in a source to sink relationship. The hyphae play an important role in the formation of soil aggregates greater than 240 µm diameter (Hamel *et al.*1997). AMF may also influence plant biodiversity (van der Heijden *et al.* 1998), help to control pests (e.g nematodes) and

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fungal pathogens (Azcon-Aguilar & Barea 1996) and affect fitness of plants in polluted environments (Hildebrandt *et al.* 1999). Information on taxonomic groups of AMF species in Africa is still scarce.

2.2 TAXONOMY

The essential foundation for utilisation of AMF depends on the determination of species, which is not adequately done in most studies undertaken in this group of fungi. The most prevalent problem with this is demonstrated in the lack of ability to replicate experiments. As a result, Morton (1988) and Walker (1992) both emphasized the importance of taxonomy in any experimentation using AMF as a controlled biological variable.

Classification of the *Glomeromycota* started as early as 1809 with the description of the genus *Endogone* Link:Fr. by Link (Gerdemann & Trappe 1974b). Tulasne and Tulasne (1845) first described the genus *Glomus* Tulasne & Tulasne and later transferred it to *Endogone*, when they failed to notice that the zygospores originated in a way distinctly different from two species originally placed in *Endogone*. Nicolson and Gerdemann (1968) included the endomycorrhizal forming fungi as non-sporocarpic members of the genus *Endogone*. In an endomycorrhiza symposium at the University of Leeds, Gerdemann and Trappe (1974a) acknowledged the difficulty in AMF taxonomy and recognized the likelihood of having to change the generic classification as more information became available. This was followed by an era of description of new taxa and the re-organization of classification of the known species. The genus *Endogone* was noted to be too large and species difficult to distinguish. A revision based on a formal Linnean classification was

made by Gerdemann and Trappe (1974b) with sporocarps and mycorrhizal infection used to classify the group into seven genera. They segregated sporocarpic chlamydospore-forming *Endogone* species into *Glomus*. The first identification key was compiled. They revised the genus *Endogone* by describing two new genera, *Acaulospora* Gerdemann & Trappe emend. Berch and *Gigaspora* Gerdemann & Trappe emend. and separated *Glomus* Tulasne & Tulasne (Walker & Sanders 1986).

Based on modes of spore formation, Ames and Schneider (1979) described a new genus *Entrophospora* Ames & Schneider emend. wu, separating it from the genus *Acaulospora*. Wall characteristics (Walker 1983) and the mode of spore germination and auxiliary cells were used (Walker and Sanders 1986) to describe a new genus *Scutellospora* Walker & Sanders separating it from *Gigaspora*. Until Pirozynsky and Dalpe (1989) noted that some genera formed vesicles and arbuscules, all the genera were placed in the family Endogonaceae, order Endogonales. Pirozynsky and Dalpe (1989) proceeded to separate six mycorrhizae forming genera into the family Glomaceae and a non-mycorrhizae forming family the Endogonaceae. Schenck and Perez (1990) produced a manual with species descriptions. Morton and Benny (1990) classified AMF to a new order *Glomales* (correctly referred to as *Glomerales*) based on patterns of morphological characteristics of somatic (hyphae, arbuscules and vesicles) and reproductive stages (spores), patterns of common descent, ontogeny and spore germination. They raised the taxonomic level on the basis of mycorrihizae formation to the order *Glomales* and created two new suborders *Glomineae* and *Gigasporineae*, two new families *Acaulosporaceae* and *Gigasporaceae* and they amended the family *Glomaceae*. A recent addition into the *Glomales* is the fossil genus

Glomites Taylor, Remy, Hass et Kerp. that was first described by Taylor *et al.* (1995). Morton and Benny's (1990) classification was followed by a description of a new class *Glomomycetes* with a brief latin diagnosis (Cavalier-Smith 1998), later corrected to *Glomeromycetes*, within the *Zygomycota*, containing *Glomerales* and *Endogonales* (Shüßler *et al.* 2001). The most recently revised classification is based on molecular techniques with a comprehensive SSU rRNA analysis. The group has been classified in a new monophyletic phylum, the *Glomeromycota* C. Walker & Schuessler, phylum nov. (Shüßler *et al.* 2001) with order *Glomales*, more correctly referred to as *Glomerales* and three new orders *Archaeosporales*, *Paraglomerales* and *Diversisporales* (Figure 2.1).

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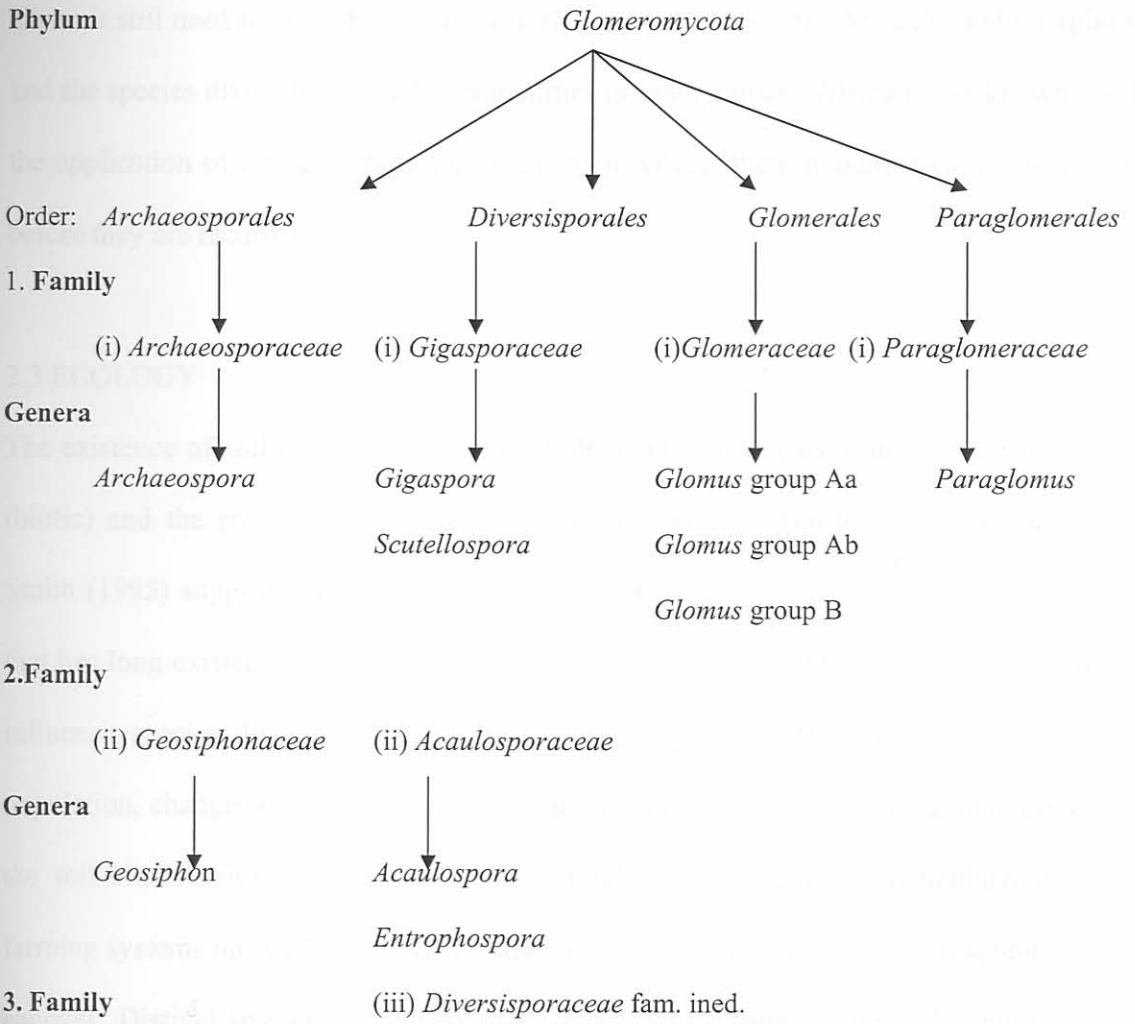


Figure 2.1. Classification of glomeromycotan fungi (Shüßler *et al.* 2001).

Comparative information concerning distribution and diversity of AMF species in different continents is still being assembled. Many species are being re-described and some species merged or assigned to new taxa. Description of new species continues. The International culture collection of Vesicular-arbuscular and Arbuscular Mycorrhizal fungi (INVAM) in the United States and the International Bank for the *Glomeromycota* at Dijon, France, are progressively working on collections from all over the world to bridge this knowledge gap.

There is still need to collect from the different parts of the world. Africa is under-explored and the species diversity of AMF communities in most parts of Africa is not known. With the application of intensive farming practices in Africa, there is danger of losing species before they are recorded.

2.3 ECOLOGY

The existence of soil organisms depends on their relationship with other living organisms (biotic) and the non-living (abiotic) environment (Atlas & Bartha 1986). Kennedy and Smith (1995) suggested that each soil type has an inherent community of soil organisms that has long existed in a state of equilibrium. Disturbance by human activities can greatly influence species diversity and functions of soil organisms. With the rapid increase in population, changes in landscape in Africa are drastic and may have irreversible effects on the soil biodiversity, hence it is crucial to establish the effects of particularly different farming systems on AMF species diversity and how the individual species respond to these changes. Distinct seasonal variations that control plant activities and soil conditions also play a major role in AMF species activities. Distinct seasonal patterns in Africa may therefore greatly affect AMF species.

The most useful method of evaluating the factors influencing a group of organisms in a habitat is to study their diversity. Kennedy and Smith (1995) stated the following as vital reasons for studying biodiversity: "to increase knowledge of the diversity of genetic resources and understand the distribution of diversity on earth, and to increase knowledge of the functional role of diversity and identify changes in diversity associated with

disturbance and management practices". However, diversity has many meanings to soil biologists. Morton *et al.* (1994) interpreted diversity in terms of species diversity, ecological diversity and the diversity of functional forms. This is a concept to be adapted for this study.

2.3.1 Species diversity

Species diversity is defined (Gaston 1996; Kennedy & Smith 1995; Morton & Bentivenga 1995) as the variety of species. The simplest measure of species diversity is a count of the number of different species termed "species richness" (Gaston 1996; Stiling 1996). The species concept in AMF is interpreted differently from most other organisms. Morton and Bentivenga (1995) conceptualized AMF species as "the smallest assemblage of reproductively isolated individuals or populations diagnosed by epigenetic, morphological or organizational properties of fungal spores that specify a unique genealogical origin based on the criterion of monophyly". This concept includes terms from evolutionary biology with limited practical importance. Li and Graur (1991) described taxonomic species concept as "a group of organisms resembling each other more than they resemble any other group". Rosendahl *et al.* (1994) considered Li and Graur (1991) concept more practical and rephrased it as "repeatedly recognizable fungus that can be defined by the morphological characteristics of its spores (allowing differences of gene expressions), and which is internally consistent if found in widely separated geographic locations". This concept is applied in this study and the spore is thus used as the morphological structure of reference.

The dispersal patterns and biogeographical ranges of most species are not fully established and fungal communities of most habitats particularly in Africa are not known. With the persistent destruction of habitats and expansion of intensive farming systems, there is need to evaluate changes in the diversity of fungal communities as affected by changes in environmental factors and human activities. Among the AMF communities are species with the potential to improve agriculture, forestry and restore degrading habitats. These may be lost.

2.3.2 Ecological diversity

Ecological diversity is observed as the partitioning of a niche in space and time for different species (Morton 1993). Diversity is observed in the distribution and occurrence of the AMF symbiosis in relation to soil nutrients, plant composition and season. Various investigations indicate that abiotic factors such as changes in seasons and soil conditions, biotic factors such as plants and man-made factors could greatly affect the mycorrhizal symbiosis. Depending on the changes in environmental conditions, the symbiosis might shift.

2.3.2.1 Seasonal effects

Distinct seasons determine plant activity and thus influencing the activity of mycorrhizal symbiosis. Plant phenology was observed (Giovannetti 1985; Brundrett 1991) to relate to seasonal changes that influenced the seasonal patterns of spore production. Recent studies by Guadarrama and Sanchez (1999) confirm season to be an important factor in determining the physiological changes of the host and the influence in species diversity and

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spore numbers. The highest number of species and spores occurred during the dry season with a marked decrease in the rainy season. These changes were linked to poor root growth in summer and dry season and active growth in spring and wet season in the temperate and tropical regions respectively. Ilag *et al.* (1987) also observed dry season rice to have higher populations of infective propagules than wet season rice. The dry season marks the end of optimum plant growth and it coincides with very slow root growth, absence of root growth or senescence of the host roots. Baylis (1969) suggested that intermittent root growth and drying might stimulate sporulation while adequate soil moisture favours actively growing roots rendering sporulation unnecessary.

Change in soil conditions, particularly moisture and nutrients, also follow a seasonal pattern and they are also used to explain changes in the symbiosis. Studies at the agroforestry site in Malawi noted seasonal fluctuations in levels of ammonium and nitrate in a *Gliricidia sepium* and maize intercrop (Ikerra *et al.* 1999). Rabatin (1979) recorded the highest infection levels in spring when phosphorus was deficient and moisture was low. Seasonal changes of phosphorus are not widely reported, however, seasonal changes in nitrogen are known to affect the balance between nitrogen and phosphorus. Root colonisation was reported to increase as nitrogen content increased if phosphorus levels were moderate and at higher levels of phosphorus, nitrogen application was inhibitory (Bevege 1972). The importance of changes in moisture was more evident under desert conditions as was noted by Jakobsen (1997) in her studies of spore abundance and fungal diversity of the Namibian desert.

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AMF species differ in tolerance to changes in seasonal conditions. Sylvia (1986) observed differences in spore numbers of AMF species with spore counts of *Glomus globiferum* increasing by more than 500 % from May to August in summer, while *Glomus aggregatum* Schenck & Smith emend. Koske spore numbers increased by less than 30 %. The majority of spores of *Gigaspora gigantea* (Nicol & Gerd.) Gerdemann & Trappe occurred in late summer and autumn (Gemma & Koske 1988). In a more recent study by Lee Pau-Ju and Koske (1994), healthy spores of *G. gigantea* were most abundant in winter and least in summer. Gemma *et al.* (1989) later reported spore abundance of *Acaulospora scrobiculata* Trappe and *G. gigantea* to be highest in October, spore abundance of *Scutellospora calospora* (Nicol & Gerd) Walker & Sanders was highest in August *and Scutellospora pellucida* (Nicol. & Schenck) Walker & Sanders and *Scutellospora persica* (Koske &Walker) Walker & Sanders were highest in May and October. These differences were linked to competition amongst individual fungi for limited nutrients during different stages of plant development (Sylvia 1986).

Studies on the effects of season on AMF in Africa are scarce, yet the continent experiences distinct severe droughts and sometimes floods. Prolonged drought may have devastating effects on the AMF symbiosis. Thompson (1987) noted a total decline in infective propagules in prolonged bare fallow with subsequent decline in crop productivity. The tolerance of AMF species to drought conditions in Africa is not known. Indiscriminate cutting of trees that could protect drought sensitive mycorrhizal species may lead to total loss of these species. Prolonged bare land under dry conditions would probably reduce the diversity of AMF species if not eliminate them completely. The use of perennial plants,

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particularly trees and shrubs as fallow in farming systems has proved to be effective fallows for the drought prone southern Africa region of Malawi and Zambia (Kwesiga & Beniest 1998). Trees and shrubs in farming systems compared with bare fallow may alleviate negative effects of extreme conditions on the fungal symbiosis. Based on the many farming interventions used in the African continent to improve crop production, it is important to evaluate their effects on mycorrhizal symbiosis. Improved tree fallow with *Sesbania sesban* and *S. macrantha* are effective fallows in the drought prone regions of eastern Zambia (Kwesiga *et al.* 1997). The effect of improved fallow on AMF symbiosis will be evaluated in this study.

2.3.2.2 Host effects

The host determines the boundary conditions within which the fungus is able to grow and reproduce (Morton 1993). Plant distribution in natural habitats is a reflection of the different demands for water and mineral nutrients from the soil. Each plant species has evolved mechanisms that facilitate uptake of water and nutrients within its habitat. These mechanisms range from physical modification of the root system to chemical exudates from the root system. The root structure and root exudates modify the root rhizosphere and affect the composition and functions of organisms at the rhizoplane and the rhizospheres of the plant (Atlas & Bartha 1986; Paul & Clark 1989). Plant roots are known to provide suitable habitat for the growth of microorganisms (Atlas & Bartha 1986).

Variation in response of host plants to mycorrhizal fungi is widely reported. Baylis (1970) linked differences in plant response to mycorrhizal symbiosis to the root structure. Fitter

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(1985) and Douds and Schenck (1990) linked variation in hosts to demands for water, nutrients and allocation of carbohydrates while Morinda *et al.* (1992) and Chabot *et al.* (1992) noted specific plant metabolites exuded by roots, the flavinoids, to also explain the variation in mycorrhizal symbiosis. Azcon and Ocampo (1981) showed wheat cultivars inoculated with *Glomus mosseae* (Nicol & Gerd) Gerdemann & Trappe to differ in mycorrhizal dependence and associated absence of infection with lack of exudation of sugar by roots.

McGonigle and Fitter (1990) observed the species *Holcus lanatus* L. to be predominantly colonized by *Glomus tenue* (Greenhall) Hall and the roots of three other herbaceous species to be predominantly colonised by other mycorrhizal endophytes. Gaur *et al.* (1998) also noted the distribution of mycorrhizal fungal species to differ in two tree species with *Glomus* dominant in *Terminalia arjuana* (Roxb. Ex DC.) Wight & Arn. plots while *Gigaspora* was abundant in a *Populus euphratica* Oliv. plot. Plant species *Agropyron smithii* Rydb. and *Bouteloua gracilis* (HBK) Lag. Ex Steud. affected the fungal community of *G. mosseae, Glomus fasciculatum sensu lato* (Thaxter) Gerd. & Trappe, *Glomus macrocarpum* Tulasne & Tulasne, *Entrophospora infrequens* (Hall) Ames & Schneider, and an unidentified species (Stahl & Christensen 1982).

The effect of host plants on the mycorrhizal symbiosis has been reported to vary widely with the type of crops. Kormanik *et al.* (1980) noted corn, millet and sorghum as effective cover crops at increasing inoculum density of *Glomus* species with sorghum the most effective and corn the least. The differences at increasing inoculum density of *Glomus*

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species was attributed to the differences in finer root system of sorghum and the coarser root system of corn. Dodd *et al.* (1990) also observed the increase in inoculum density to be dependent on the hosts that were previously planted. Studies in soybeans and bahia grass by Schenck and Kinlock (1980) showed the former to associate with *Scutellospora gregaria* (Schenck & Nicol.) Walker & Sanders, cited as *Gigaspora gregaria* Schenck & Nicol., and two *Gigaspora* species (*G. margarita* and *G. gigantea*), compared to the latter plant associating with numerous spores of *Glomus fasciculatum* and *Glomus clarum* Nicolson & Schenck. Khalil *et al.* (1992) also noted four *Glomus* species as most abundant around the rhizospheres of soybean, *Gigaspora* species as second most abundant and *Acaulospora* species the least. The different tree species used in agroforestry may also affect AMF species symbiosis differently. This study aims to evaluate the effects of agroforestry trees on AMF species.

Depending on the sites, the cropping history largely influenced the type of AMF species. Johnson *et al.* (1991) noted the occurrence of *G. aggregatum*, *Glomus leptotichum* Schenck & Smith now the glomoid morph of *Archaeospora leptoticha* (Schenck & Smith) Morton & Redecker and *Glomus occultum* Walker spores to be high with corn than soybean cropping history and *G. macrocarpum* to be high with soybean history at one site. At another site *G. aggregatum* was not affected by cropping history while three species *G. occultum, Glomus albidum* Walker & Rhodes and *G. mosseae* were more abundant in plots with corn history than soybean history. In a pot culture experiment with soils from a field site, Sanders and Fitter (1992) observed six plant species to cause differences in sporulation within a community of AMF species. The *Gliricidia*/maize intercrop and maize monocrop

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and the *Sesbania* spp./maize intercrop and maize monocrop experimental sites have different cropping history.

In agroforestry practices, trees and shrubs vary in their ability to improve soil fertility and to coexist with crops. Differences were noted in the decomposition, nitrogen and mineral contents of foliage in *Gliricidia sepium* and *Sesbania sesban* (Mwiinga *et al.* 1994). Variation in growth rates, capacity to fix nitrogen, litter quality, root decomposition and resistance to drought exist in *S. sesban*, *Sesbania macrantha* and *G. sepium* (Kwesiga & Beniest 1998). They also noted that *S. sesban* was more susceptible to insect pests and was highly infected by nematodes compared with *S. macrantha*. The effects of the two *Sesbania* species on AMF symbiosis will also be evaluated.

The differences in survival of agroforestry trees to water stress in Malawi was reported by Maghembe & Prinns (1994) and suggested the mycorrhizal symbiosis as a prerequisite for survival of some tree species. There is evidence in survival of trees inoculated with AMF in water stressed semi-arid lands of Kenya (Wilson *et al.* 1992a), hence mycorrhizal associations have the potential to alleviate water stress in agroforestry species. Variation in root functions in *G. sepium* and *Grevillea robusta* A. Cunn. were attributed to root structure (Odhiambo 1999) while there is a possibility of exudation of organic acids speculated to reduce fixation of phosphorus by some shrubs such as *Tithonia diversifolia* (Hemsl.) Gray (Palm and Rowland 1997). There are no studies undertaken to determine mycorrhizal symbiosis in different agroforestry practices and yet most factors known to vary in trees would influence the quality and the function of the mycorrhizal symbiosis in

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the system. *Sesbania sesban* and *S. macrantha* associate differently with the root-knot nematodes and differ in their effect on maize yield. Their effect on AMF symbiosis has not been determined.

2.3.2.3 Soil conditions

Soil is the part of the earth's crust that is a seat of biological activity (Russell 1969). Parks and Cousins (1995) conceived soil as a dynamic system and the core to life on earth maintaining primary producers. Kennedy & Smith (1995) described soil as a key to maintenance of plants and Sanchez (1995) linked insecurity and wars in Africa as a consequence of poverty resulting from a bad soil.

Most agricultural soils in Africa are deficient in nutrients and can hardly support optimum crop production. Nitrogen and phosphorus are the most limiting nutrients to crop production in the tropics (Sanchez 1976). In the study area, both nitrogen and phosphorus are deficient with nitrogen (0.09%) noted as the most limiting nutrient to plant growth (Ikerra *et al.* 1999). Optimum crop production in the region is therefore realized with inorganic fertilizers. The Government's removal of subsidies on fertilizer in Malawi has rendered it unaffordable to the majority of farmers. As a result low-input farming systems were encouraged (Kumwenda *et al.* 1995). Agroforestry farming system was recommended as a low-input practice with great potential for the region. Palm *et al.* (1997) noted the inadequacy of trees alone as a supplement in soil replenishment and recommended a complement with minimal inorganic fertilizers. Hence, agroforestry practices in the region

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are complemented with minimal inorganic input. The effect of the low-input agroforestry system on AMF symbiosis has not been established.

Brundrett (1991) regarded the soil environment to establish the initial conditions for mycorrhiza establishment, with the soil acting directly on the hyphal thallus during pre-mycorrhiza formation and on extraradical hypha during later establishment and growth phases. The efficiency of mycorrhizal symbiosis was observed as the function of a particular set of soil conditions (Lambert *et al.* 1980). Haas and Menge (1990) noted edaphic conditions to explain for the differences in fungal species composition in avocado orchards of two sites with similar Mediterranean climates.

There are contradicting reports on the effects of soil conditions on mycorrhizal symbiosis. Most studies report the AMF symbiosis to function effectively at low fertility. Douds *et al.* (1993) noted biological based farming systems to have more abundant AMF spores than conventional farming systems. They also noted *G. gigantea* to be more numerous in low-input systems than conventional systems. There are also reports on enhanced mycorrhizal symbiosis under high soil fertility. Crush (1975) noted an increase in colonisation levels of sown legumes in fertilized soils and Land & Schonbeck (1991), in studies of arable fertile land, observed a high spore number of *Glomus* species and concluded that it was more adapted to fertile soils. No fertilizer effect on the mycorrhizal symbiosis has been documented by Cooke *et al.* (1992) who noted fertilization of forest soil to have no effects on the incidence of fungi. The variation in response by different fungal species to soil conditions led Sieverding (1991) to classify AMF according to their adaptations to soil

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conditions. Classification of species according to adaptations has, however, not been confirmed.

Phosphorus is widely documented as the most important soil nutrient that explains the AMF symbiosis. The effects of phosphorus on the mycorrhizal symbiosis are variable, though generally it is associated with a reduction of the symbiosis. Nielsen *et al.* (1981) reported low numbers of mycorrhizal spores in all soils with high levels of phosphorus and Schwab *et al.* (1983) noted an increase in mycorrhizal formation in phosphorus deficient soils. Studies by Shukla and Vanjare (1990) confirm the declining trend of fungal population in oilseed crops with increase in phosphorus. In natural habitats, Allsopp and Stock (1994) noted a reduction in mycorrhizal colonisation with increase in soil phosphorus levels.

Response of AMF to phosphorus fertilization depends on the inherent phosphorus levels in the soil. Nielsen *et al.* (1981) observed the inherent soil phosphorus levels as a determining factor to mycorrhizal response to phosphorus fertilization. They observed no mycorrhizal response in soils with high phosphorus levels to phosphorus application. They also observed AMF species occurring in soils with low phosphorus status to decline to a greater extent with increasing phosphorus than endophytes from soils with high phosphorus status. This was confirmed by Katiyar *et al.* (1995) who noted a decrease in spore numbers with addition of low doses of phosphorus to P deficient soils.

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Jasper *et al.* (1979) noted variation in sensitivity of AMF species to different phosphorus levels. Abbott and Robson (1984) noted shoot growth to increase with inoculation with *G. fasciculatum* at the lowest rates of phosphorus. Schubert and Hayman (1986) noted differences among endophytes in stimulating plant growth at different phosphorus levels with some endophytes showing potential value at low, medium and/or high phosphorus levels. The study site in Malawi has inherently a low phosphorous level although the selected sites had different histories of phosphorus fertilization.

Nitrogen is the most limiting nutrient in the tropics and it was the most limiting at the study site. Contrary to phosphorus, the role of the mycorrhizal symbiosis in nitrogen nutrition has been unnoticed. Sundaresan *et al.* (1988) observed variations in the ability of twelve mycorrhizal species in reducing nitrate to nitrite. There are contrasting reports on the effects of nitrogen on the AMF symbiosis. Early studies by Hayman (1970) reported a decline in colonisation and spore number with addition of nitrogen fertilization while Baltruschat & Dehne (1989) noted general a decline in mycorrhizae formation. In most recent studies Shukla and Vanjare (1990) confirmed nitrogen fertilizer to have adverse effects with increase in nitrogen gradually decreasing fungal populations to zero. Contrasting reports by Furlan and Bernier-Cardou (1989) indicate that nitrogen stimulates root colonisation and spore production. Recent studies by Sreenivasa and Bagyaraj (1990) also observed an increase in the number of spores with nitrogen increase in the form of nitrate and ammonium.

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Balanced soil nutrition is acknowledged as a requirement for plant growth (Buresh & Smithson 1997). Sanchez *et al.* (1997) recommended replenishment of nitrogen to follow phosphorus replenishment in agroforestry systems. There are few studies on the effects of balanced soil nutrients on the mycorrhizal symbiosis. Hepper (1983) noted a dense colonisation at high levels of nitrogen compared to low nitrogen and suggested the ratio of nitrate to phosphate as the only explanation for the increase. Hall *et al.* (1984) also noted an increase in AMF symbiosis when phosphorus was combined with nitrogen and also attributed this to an increase in nitrogen:phosphorus ratio with application of nitrogen and suggested the possibility of nitrogen lowering plant phosphorus status. In most farming systems, application of both nitrogen and phosphorus fertilizer is recommended. In agroforestry systems, the two types of fertilizer are applied singly or/and in combination. The effects of combinations of the two fertilizer types on AMF symbiosis will be evaluated.

The advent of sustainable agriculture emphasizes use of organic supplements as soil amendments. The main thrust in agroforestry farming systems is the incorporation of leaf biomass into soil to enhance fertility. Organic inputs contain essential nutrients plus carbon, a source of energy for soil biota (Atlas & Bartha 1986) and thus supporting life in the soil and mediating all processes of nutrient replenishment. There is a decline in organic matter when natural vegetation is converted to cropland. In the miombo region of Zimbabwe, a higher soil macrofauna and microbial biomass was found in soils from the closed miombo than from a maize system (Campbell *et al.* 1998). Tree prunings are an important source of nitrogen for maize in the miombo ecozone (Mafongoya & Dzowela

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1998). Tree prunings are used in agroforestry systems to improve fertility. The effects of tree prunings on AMF symbiosis will be determined in this study.

Hayman (1982) noted better mycorrhizal development with addition of organic matter and St John and Coleman (1983) and Harinkumar and Bagyaraj (1988) observed addition of organic matter to increase the abundance of certain AMF species. These studies were recently confirmed by Verma and Arya (1998) who showed increase in mycorrhizal spore production, root colonisation, shoot phosphorus and height in tissue cultured-raised *Dendrocalamus asper* (Schult. & Schult. f.) Becker ex K. Heyne (bamboo) plantlets with organic manure amendments compared to a sand/soil medium. Negative effects on mycorrhizal symbiosis were reported by Nemec (1978) who noted spore numbers to negatively correlate with soil organic matter, and supported by Baltruschat and Dehne (1989) who noted green manure to have negative effects on inoculum potential in continuous monocropping barley. Whether the positive effects of the recommended agroforestry practice are also reflected on the mycorrhizal symbiosis, needs to be verified.

2.3.3 Functional diversity

Functional diversity is a recent concept and it has many meanings to biologists. Steele (1991) defined functional diversity as a variety of different responses to environmental factors while Zak *et al.* (1994) defined microbial functional diversity in terms of utilization of substrates.

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The management and use of AMF species will not only depend on their adaptations to environmental conditions but also in their ability to utilize nutrients and improve plant growth. The mycorrhizal fungi alter plant processes. They promote plant growth by increasing absorption and nutrient transfer (Francis *et al.* 1986; Fogel 1980), improve soil aggregation (Sutton & Sheppard 1976; Forster & Nicolson 1981) and prolong root life and protect roots against soil borne pathogens (Harley & Smith 1983).

Generalization about mycorrhizal functions masks considerable functional diversity in fungal organisms (Kothari *et al.* 1991; Graham *et al.* 1982; Sanders *et al.* 1977) and yet considerable variation exists. AMF species enhance growth in a number of field crops (Ross & Hepper 1970; Khan 1972) and benefits of the mycorrhizal symbiosis are reported in tree species, some of which are commonly used agroforestry species (Michelsen 1993; Sharma *et al.* 1996 and Atayese *et al.* 1993). Plant growth responses showed highly specific responses to different species of mycorrhizal fungi (Allen *et al.* 1995).

Commercial production and utilization of AMF will depend largely on knowledge of the nature of the symbiotic associations. It will also depend on knowledge of the symbiotic efficiency and compatibility with a wide range of host plants. This knowledge is lacking for most AMF species, particularly for species from a similar habitat. Most AMF species tested for efficiency are from different geographical regions.

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CHAPTER 3: MATERIALS AND METHODS

3.1 EXPERIMENTAL SITE

Soil samples were collected during July 1995 and March 1996 in the dry and wet season from an agroforestry site in Malawi to investigate the diversity and ecology of AMF. Studies were carried out in two agroforestry systems, *Gliricidia sepium* maize intercrop and maize monocrop system (Plate 3.1 A-D) and *Sesbania sesban* and *S. macrantha* maize intercrop and maize monocrop system (Plate 3.1 E & F). At the time of sampling the experiments had been in existence since 1992.

The soil is classified as a Ferric Lixisol (FAO) or Oxic Haplustalf (USDA). The topsoil (0–20 cm) is 52 % sand and 37 % clay; pH = 6.0 (1:2.5 soil:water suspension); organic C = 1.33 %; total N = 0.09 %; bicarbonate-EDTA extractable P = 5.1 mg kg^{-1}; KCl extractable Ca = 6.4 $cmol_c$ kg^{-1}, KCl extractable Mg = 1.7 $cmol_c$ kg^{-1} and bicarbonate-EDTA extractable K = 0.19 $cmol_c$ kg^{-1}. Nitrogen was the most limiting nutrient at the site (Ikerra *et al.* 1999).

3.1.1 Host description

3.1.1.1 *Gliricidia sepium* (Jacq) Walp.

This is a native of South America in the Family Fabaceae. It forms a tripartite symbiotic association with both rhizobia and AMF. *Gliricidia sepium* has multiple uses (Stewart, *et al.* 1996). In Malawi, *G. sepium* is used to enhance soil fertility. It is well adapted to environmental conditions and produces large amounts of biomass (Maghembe & Prinns

1994; Ikerra *et al.* 1999). The prunings from *G. sepium* rapidly release nitrogen (Mafongoya & Dzowela 1998), form coppices and hence do not need re-establishment. They form coppices readily during the rainy season and continue to grow into the dry season then cut before the end of the rains. *Gliricidia sepium* improved soil fertility and it enhanced maize production in southern Malawi (Appendix A). It is also valuable for fuel wood.

3.1.1.2 *Sesbania sesban* (L.) Merr. and *S. macrantha* E. Phillips & Hutch.

The *Sesbania* spp. belong to the Family Fabaceae. It is a pantropically distributed genus and forms a tripartite symbiotic association with rhizobia and AMF. *Sesbania* spp. are well recognized green manure, fodder and fuel-wood trees. *Sesbania sesban*, a native of Malawi and other parts of Africa, grows rapidly and vigorously, has high biomass, high quality litter and is easily propagated from seed (Kwesiga & Beniest 1998). Because of these qualities, it is widely recommended as an excellent tree for soil fertility improvement in short-duration improved fallow (Kwesiga *et al.* 1997). *Sesbania sesban* improved maize yield and this improvement was especially marked where fertilizer was not used (Appendix B).

Sesbania macrantha, a native of parts of Africa (Zambezi, Sudanian, Somalia-Masai and Lake Victoria phytochoria) (Lock 1989) was introduced after it was realized that *S. sesban* had many drawbacks. The production of foliage by *S. sesban* seemed to be reduced considerably when the rainy season was short, hence there was need to test faster growing *Sesbania* species like *S. macrantha*. *S. sesban* was susceptible to the leaf-defoliating beetle

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(*Mesoplatys ochroptera* (Stål) and the root-knot nematode (Karachi 1995), it has low calorific wood and it needs to be re-established after the soil-depleting cropping phase. The average maize yield at the Makoka research station in plots with non-fertilized *S. macrantha* was slightly higher than non-fertilized plots of *S. sesban* (Appendix B).

3.2. DESCRIPTION OF SAMPLED AGROFORESTRY EXPERIMENTS

3.2.1 *Gliricidia sepium*/maize and maize monocrop systems

3.2.1.1. Cropping history

Prior to the establishment of *Gliricidia*/maize intercrop in 1992, cotton was the crop until 1987, followed by a year fallow period in 1988; thereafter, a cassava crop was grown for a period of three years (1989-1991). *G. sepium* was established in 1992 together with a first crop of maize. The site has a history of high application of phosphorus fertilizer (Maghembe, J. A Programme leader, Makoka research station, ICRAF 1992-2000-Personal communication).

The field was cleared and ridged by a tractor in 1992. In subsequent years the re-ridging was done manually following farmers practices. This was to facilitate the incorporation of green manure into ridges before planting. The plots were kept free from weeds with all weeding done manually. Seedlings of *G. sepium* were germinated and inoculated with rhizobia liquid inoculum in nursery containers and allowed to grow for 12 to 16 weeks before planting. The *G. sepium* (Rhizobia strain GSKFR) inoculum was supplied by the Kenya Forestry Research Institute (KEFRI). The arrangement represented a population of 7400 trees ha^{-1}. During the first year in 1992, no maize was planted for easy establishment

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of the trees. All plots were planted with beans as a cover crop. The trees were allowed to grow until the end of August when trees in all plots were cut to form coppices. The trees were intercropped with maize with the fertilizer applied in 1993.

3.2.1.2. Experimental design and arrangement

The present study was undertaken from 1995 to 1996 in an agroforestry experiment (Plate 3.1 A-D). The experiment was a randomized complete block design with a 2 x 2 x 2 factorial treatment arrangement replicated three times, with up to 24 (2 x 2 x 2 x 3) plots sampled for the study. The treatment factors were farming systems maize monocrop or maize intercropped with *Gliricidia* trees and fertilizer regimes N fertilizer at 0 and 24 kg ha^{-1} and P fertilizer at 0 and 40 kg ha^{-1}. The recommended N rate for this region is 96 kg N ha^{-1}. Plot size was 6.75 m by 5.1 m, separated by 1-m wide walkways. *Gliricidia* plots consisted of 4 rows of *Gliricidia* planted in every other furrow at a spacing of 0.9 m within a row and 1.5 m between rows (7400 plants ha^{-1}). Maize hybrid NSCM 41 was planted on 0.3 to 0.4 m ridges at a spacing of 0.3 m within a row and 0.75 m between rows (44,400 plants ha^{-1}) in both monocropped and agroforestry plots. In order to minimize tree root encroachment into adjacent plots or outside the experimental area, iron sheets were installed around plots to a depth of 0.5 m. *Gliricidia* trees were pruned to about 0.3 m prior to maize planting and the prunings were incorporated into the ridges during land preparation. In addition, a 'pre-cut' occurred once or twice from July to October to remove old biomass and encourage new growth for later incorporation. The leaves from the pre-cut were also incorporated. Inorganic N applied as calcium ammonium nitrate (CAN) and P as triple super phosphate (TSP), were split-applied at two and six weeks after planting or

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when maize was 0.3 m tall. The *Gliricidia* trees were cut again in January, at about 6 weeks after planting, and the prunings incorporated into the maize ridges during hilling operations. The total *Gliricidia* biomass incorporated during the study period was 3.7 to 4.9 ton ha^{-1} $season^{-1}$. Nitrogen content of *Gliricidia* ranged from 2.9 % to 4.9 %, (average = 3.9 %), corresponding to 140 to 190 kg N ha^{-1} $season^{-1}$ (Ikerra *et al.* 1999). Maize was weeded twice by hand during the season and was harvested in April each year.

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Plate 3.1 A



Plate 3. 1 B



Plate 3.1. Site with farming system (A) maize monocrop with sunted yellow maize without inorganic fertilizer on the fore ground representing normal farmers practices and fertilized green maize monocrop at the background. (B) *Gliricidia sepium*/maize intercrop with no addition of inorganic fertilizer.

G = *Gliricidia*; N = Inorganic N fertilizer; P = Inorganic P fertilizer.

Plate 3.1 C

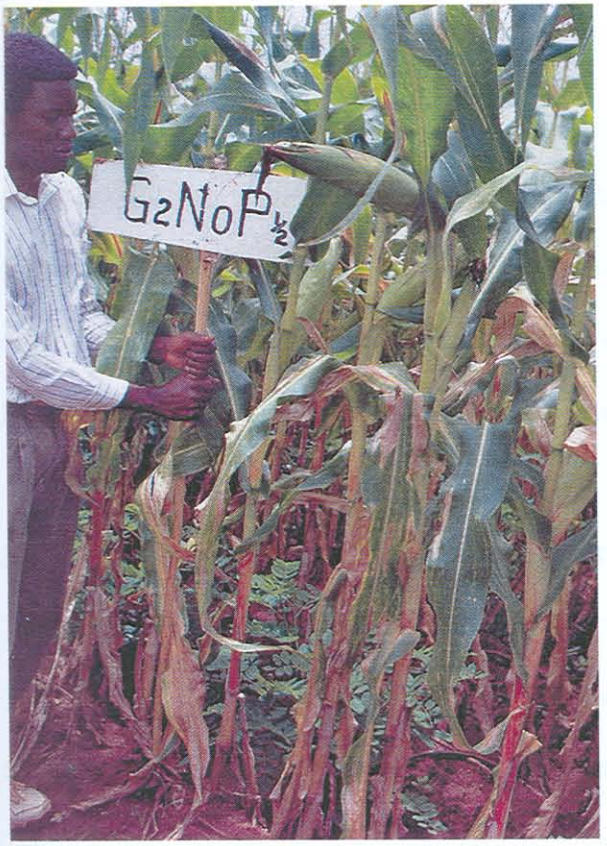


Plate 3.1 D

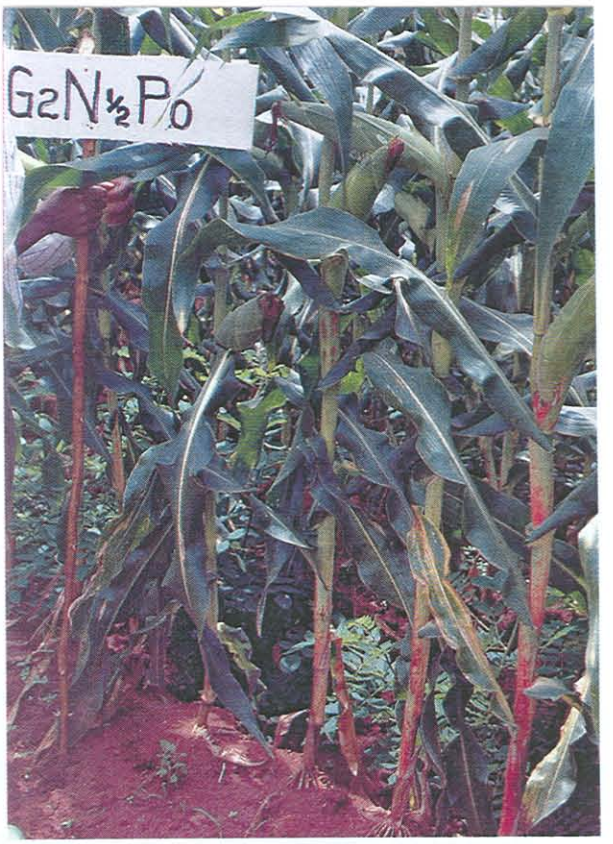


Plate 3.1. Site with farming system (C) *Gliricidia sepium*/maize intercrop with addition of inorganic P fertilizer. (D) *Gliricidia sepium*/maize intercrop with addition of inorganic N fertilizer.

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3.2.2 *S. sesban*/ maize, *S. macrantha*/maize and maize monocrop systems.

3.2.2.1 Cropping history

Until 1982 the site had been abandoned because of soil erosion. For three subsequent years (1983-1986), *Leucaena leucocephala* (Lam.) De wit was used for reclamation. This was followed by two years maize crop (1986-1988), thereafter two years *S. sesban* fallow (1989-1991). *S. sesban* and *S. macrantha* were first established with maize crop in December 1992 and left until maize was harvested in May 1993.

3.2.2.2 Experimental design and management (Appendix D).

The experiment was established in December 1992, and the present study began in March 1995 to July 1996. The sampled treatments were arranged in a three by two by two factorial design (3 × 2 × 2) with three tree treatments (0, *S. sesban* and *S. macrantha* at 7400 trees ha^{-1}), two inorganic N fertilizer rates (0 or 24 kg N ha^{-1}) applied as calcium ammonium nitrate (CAN) and two inorganic P fertilizer rates (0 or 40 kg P ha^{-1}) applied as triple super phosphate (TSP). Treatments were replicated three times with up to 36 (3 × 2 × 2 × 3) plots sampled for the study.

Maize hybrid NSCM 41 was planted on 0.3 to 0.4 m high ridges in December 1995 and 1996 at a spacing of 0.30 by 0.75 m (44,400 plants ha^{-1}) in both monocropped and agroforestry plots. Tree seedlings were germinated in seedling containers in a mixture of non-sterile forest soil and river sand. The forest soil was inoculated with the rhizobia liquid inoculum but not with AMF. The *Sesbania* spp. (Mesorhizobium strain KFR647) inoculum was supplied by the Kenya Forestry Research Institute (KEFRI). The seedlings were

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allowed to grow for approximately three months before planting out. After maize germination the two *Sesbania* species seedlings were planted on the same ridges at a spacing of 0.9 m by 1.5 m (7400 trees ha^{-1}). At the time of sampling for mycorrhizal studies, the tree seedlings had already been out planted hence they could not be assessed for mycorrizal colonisation. Inorganic N and P fertilizer were split applied at two and six weeks after planting. Plots were weeded manually twice. Maize was harvested in late April each year. After maize harvest each year the trees were allowed to grow during the dry season until October (Plate 3.1 E) when they were cut. The non-woody biomass and litter plus maize stalk were arranged in the open ridges and covered with soil during land preparation in October (Plate 3.1 F). The wood was removed as fuelwood. After the first rain in late November or early December maize and trees were planted again on the ridges. Plot sizes were 5.7 m by 11.25 m.

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Plate 3.1 E



Plate 3.1 F



Plate 3.1. Site with farming system (E) *Sesbania* spp/maize intercrop with dry season *Sesbania* tree fallow and maize stalk on the ridges. (F) *Sesbania* spp./maize intercrop site with dry season bare fallow and maize stalks on the ridges.

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3.3. DIVERSITY AND ECOLOGY OF ARBUSCULAR MYCORRHIZAL FUNGI

3.3.1. Soil sampling and processing

Soils were sampled in the dry season of July 1995, after maize harvest and the wet season of March 1996 during the cropping season. Soil samples were taken from all 24 plots at the *Gliricidia* site and 36 plots at the *Sesbania* site. For each plot, five sub-samples were collected to a depth of 25 cm from the surface horizon on a diagonal of the plot. The other diagonal was sampled in second season. One 50 g sub-sample was collected from each of the five points and pooled to make a composite sample (250 g). Soil samples were air-dried and stored at 4 °C until processing.

Arbuscular Mycorrhizal fungal (AMF) spores were extracted from five 50 g sub-samples retrieved from the composite sample. AMF population distribution strongly depends on the chance encounter with susceptible roots, hence are patchily distributed (Sylvia 1986). Comparisons between plots of non-mixed sub-samples may result to a high degree of variation, hence the mixing of sub-samples to make a composite sample. Since comparisons were to be made between treatments and not within plots, the composite sample seemed more appropriate. Spores were extracted from the soil by sucrose centrifugation method (Jenkins, 1964), modified by using 710 μm and 45 μm mesh sieves (Walker *et al.* 1982) and sucrose concentration of 50 % w/v. The study used spore morphological characteristics to ensure certain determination of AMF species

Spores were examined with a 40× stereomicroscope, aided by both reflected light and white background (specifically used for colour determination) and a transmitted light (to

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aid morphological character recognition). Only fresh spores were counted in small petri dishes. Fresh spores were recognized by the appearance of the oily contents as either a diffuse milky white substance or as multiple oil globules, or one of a few large oil droplets within the spore, and the absence of parasitism. Prior to examining each soil, preliminary identified morphotypes of intact spores were subsequently subjected to destructive examination. Morphotype characterization was done by using a compound microscope with the aid of Normarksi differential interference optics up to a magnification of 1000x under oil immersion. Spores were mounted on poly vinyl lactophenol glycerol (PVLG) and Melzer's reagent (5:1 v/v) (Koske & Tessier 1983; Walker 1983; Morton 1988).

Spore morphotypes, now fully characterized, were confirmed within the morphological characteristic ranges for the known Arbuscular mycorrhizae taxa successfully cultured from soils collected earlier in March 1995. The spore cultures are all preserved as live and slide specimens at the National Museums of Kenya (Plate 3.2 A-E). Species were determined using conventional methods (Morton 1988) and INVAM specimen description, publications on type specimen description and manual with species description (Schenck and Perez 1990). Reference collections at the University of Pretoria, Republic of South Africa and the National Museums of Kenya were used to confirm some of the species. The source of reference materials for Kenya is the University of Kent and Forestry Commission (Edinburgh) in the United Kingdom.

The presence or absence of spores of each species was recorded as + or – for each sub-sample and fresh spores of each species were counted in each sub-sample. The data were

subjected to diversity indices and species frequency of occurrence computation using Genstat 6.1.

3.3.2 Assessment of functional diversity of AMF

3.3.2.1 Experimental design and management

A greenhouse bioassay experiment was established to assess mycorrhizal functions of AMF species from the Malawi site. This comprised four experimental units, with a unit composed of a plant species tested against four fungal treatments (no fungi, *Acaulospora* sp. 1, *Glomus aggregatum* and *Glomus etunicatum*. Four plant species (Maize, *G. sepium*, *S. sesban* and *S. macrantha*) were used as test plants.

Statistical analysis was done separately for each experimental unit and comparisons of treatments made within a unit. To avoid contamination, plant species from the four different fungal treatments inoculated with the same fungal species were placed in the same block. Plant species were completely randomized within a block. The experimental units were established under similar greenhouse conditions.

3.3.2.2 Preparation of inoculum and substrate

The fungal inoculum for the bioassay was bulked up from single spore cultures of fungi isolated from the agroforestry sites and maintained in seven host plants (Plate 3.2 A-E). The fungal inoculum was prepared from a single pot with pure spore culture derived from an original single spore culture for each species. A voucher specimen of each fungal species is preserved at the National Museums of Kenya (NMK) herbarium. Two *Glomus*

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species and an *Acaulospora* sp. were selected for the study. The inoculum was produced by transferring a mixture of spores, roots and soil from planting tubes with pure spore cultures to larger pots with sorghum for a period of 6 weeks to bulk up the inoculum Munro *et al.* (1999) (Plate 3.3). To confirm the presence of mycorrhizae, the roots were stained and examined for colonisation, and soils were checked for mycelia and spores to confirm the species. The soil mixture with spores, finely chopped root fragments of less than 0.5 cm and mycelia were thoroughly mixed to homogenise the inoculum. Each plant received 50 cm^3 of inoculum.

Mineral soil substrate from the National Museums of Kenya nursery was crushed to less than 2mm particles and mixed with river sand at a ratio of 4:1 of sand:mineral soil and pasturized at 90 °C twice for one hour at intervals allowing two days between intervals. The phosphorus and carbon content of the mineral soil pH=7.1 was P=51.6 mg kg^{-1} and C= 3.66 %. The pots were half filled with a sterile substrate followed by a sandwich layer of 50 cm^3 inoculum, then topped up with the substrate in 250 mls pots. The pots were labeled according to the treatment.

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Plate 3.2A

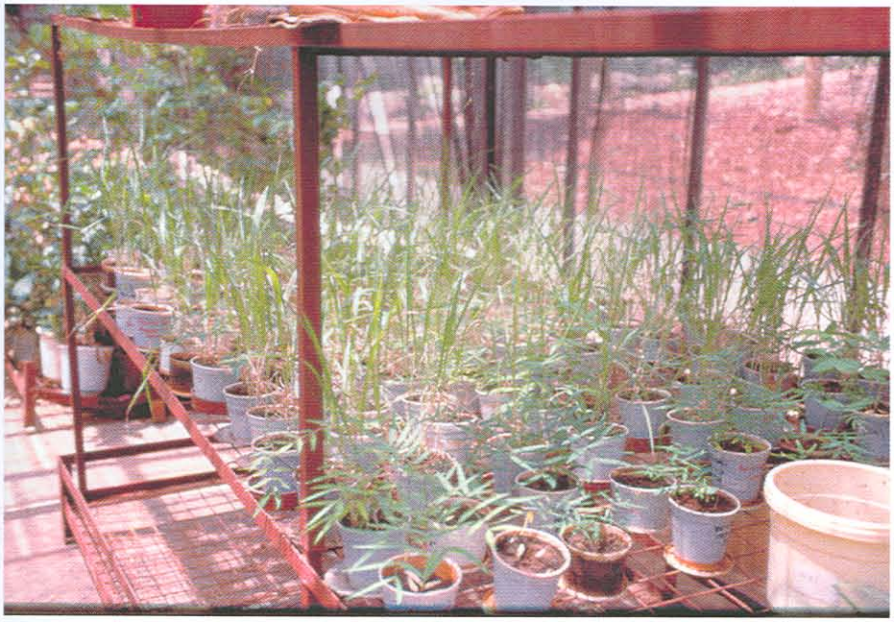


Plate 3.2 B



Plate 3.2 Soil and spore cultures (A) soil cultures with *Sorghum bicolour* (L.) Moenche and *Senna spectabilis* (DC) Irwin & Barneby. (B) spore cultures with *Senna siamea* (Lam.) Irwin & Barneby.

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Plate 3.2 C



Plate 3.2 D



Plate 3.2 Soil and spore cultures (C) spore cultures with *Senna spectabili*(DC) Irwin & Barneby (D) spore cultures with *Sorghum bicolour* (L) Moenche.

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Plate 3.2 E



Plate 3.2 Soil and spore cultures (F) spore cultures with *G. sepium, S. sesban* and *S. macrantha.*

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Plate 3.3



Plate 3.3. Inoculum production of *Acaulospora* sp. 1, *G. aggregatum* and *G. etunicatum*.

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3.3.2.3 Sowing and harvesting

Tree seeds were surface sterilized with 10 % domestic sodium hypochlorite (3.5 % active Na hypochlorite) for a period of 10 minutes. The seeds of the three tree species were pre-germinated on sterile sand. The seedlings were allowed to grow for a period of two weeks and transferred into 250 cm^3 pots. Maize seeds were washed and also surface sterilized for five minutes, and soaked in sterile water overnight. Thereafter the seeds were sown directly at three seeds per pot. After emergence, maize was thinned to one plant per pot. Each tree seedling was inoculated with 5 cm^3 liquid rhizobia one week after transplanting. The *Sesbania* spp. (Mesorhizobium strain KFR647) and *G. sepium* (Rhizobia strain GSKFR) inocula was supplied by the Kenya Forestry Research Institute (KEFRI)

Plants were carefully watered, a pot at a time, to avoid contamination. The plants were harvested after 16 weeks and the plant growth parameters, plant height, shoot dry weight, root dry weight and root colonisation assessed. At harvest, the total root weight was determined, the fresh root was chopped into 1cm pieces and 100 pieces sampled and stained for the assessment of colonisation. The remainder of the root was oven dried and the percentage root colonisation was expressed per gram dry weight.

3.3.2.4 Root clearing

Roots were placed in 10 % KOH (w/v) and put into an autoclave at 121 °C for three minutes. The cleared roots were rinsed with water and bleached with alkaline hydrogen peroxide (0.5 % NH_4OH v/v and 0.5 % H_2O_2 v/v in water). The duration in the bleaching reagent was determined by the intensity of the root pigment. The roots were rinsed with

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water before transferring them into the staining solution. The roots were stained with Chlorazol Black E (0.03 % w/v) stain in lactoglycerol solution (1:1:1 lactic acid:glycerol:water) (Brundrett *et al.* 1983). Roots were autoclaved for three minutes at 121 °C. The staining solution was slowly decanted through a 45 µm mesh sieve and the roots stored in a de-staining solution of 50 % lactic acid and glycerol solution prior to assessment.

3.3.2.5 Assessment of percentage root colonisation

Semi-permanent slides of not more than 100 pieces of 1 cm each were made with PVLG as the mounting medium. An estimate of the proportion of the root length colonised in each sample was done under a compound microscope using the technique as described in McGonigle *et al.* (1990). The percentage root colonisation was measured by making slides and viewing them with a compound microscope. Root fragments were arranged lengthwise on slide with forcep, a small drop of PVLG mountant was added at one end, then coverslip was slowly lowered. The mountant was allowed to flow around roots before gently tapping coverslip to flatten roots and remove air bubbles.

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3.4 COMPUTATIONS AND STATISTICS

Two indices of diversity, Shannon-Weinner index of species diversity and Simpson dominance index were computed for relative species diversity. The two indices are based on the proportional abundance of species and also take both evenness and species richness into account compared with using single number (Magurran 1988). Shannon-Weiner diversity index assumes that individuals are randomly sampled from an 'indefinately large' population (Pielou 1975) and it also assumes that all species are represented in the sample. The Simpson dominance index is reffered to as the dominance measure since it is weighted towards abundance of the commonest species rather than providing a measure of species richness. The Shannon-Weiner index was computed according to the formula: $\Delta_{Sh} = -\sum(X_i/X_0) \log (X_i/X_0)$ where X_i = the spore abundance for an individual species and X_0= the total spore abundance of the population (sum of spore abundance of all AMF species). The Simpson index was computed according to the formula: $\Delta_{Si} = 1 - \sum(X_i/X_0)^2$. Diversity indices were calculated for each plot of the experiment, in the wet and dry seasons. An analysis of variance of the diversity indices was conducted. The resulting ANOVA was used to determine differences in diversity between different treatments.

The frequency of occurrence of the binary data of dependent non-continuous variables was analysed using logistic regression. A logit transformation was done on the binary data so as to constrain the model to ensure that any estimated proportion cannot fall outside the range 0-1. For each species in a plot, occurrence was recorded in 0 to 5 subsamples. The resulting analysis of deviance table was used to determine significant treatment effects. The logistic model gave predicted (probabilities or proportions) values. Predicted values from fitted

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logistic regression models were used to examine the nature of various treatment effects. All calculations were done using Genstat 6.1.

The bioassay greenhouse experiment data was analysed by SAS (SAS Institute 1990). Normal data was analysed by parametric (ANOVA) methods followed by a paired comparison of means by the Least Square Means (LSM). Non-normal data was analysed by the non-parametric method of Kruskal-Wallis analysis of variance, followed by a chi-square test with multiple comparisons of means. Spearman correlation coefficient was used to assess the relationship between plant growth parameters and colonisation level.

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CHAPTER 4: TAXONOMY OF ARBUSCULAR MYCORRHIZAL FUNGI

Spores of twelve AMF species were recovered from the soil from the agroforestry and maize monocrop systems. Spores of seven species were determined as *Acaulospora rehmii* Sieverding & Toro, *Glomus* aff. *aggregatum, Glomus etunicatum, Glomus* aff. *geosporum*, *Gigaspora margarita, Scutellospora cerradensis* and *Scutellospora dipurpurascens*. Specimen represented as *Archaeospora* sp. was determined to genus level as it failed to establish in pot culture in this study, hence there was insufficient material to accurately diagnose the species. *G. margarita* and *G. geosporum* also failed to establish in pot culture, however, all morphological features of spores from the field enabled complete and accurate reference with the INVAM specimen and the type specimen description in Schenck and Perez (1990). Specimen represented as *Acaulospora* sp. 1, *Acaulospora* sp. 2, *Glomus* sp. and *Gigaspora* sp. although successfully established in pot culture, had some morphological characters that strongly overlapped with their allies, hence were also represented to genus level. Nine species were successfully pot cultured on *Senna siamea* (Lam.) Irwin & Barneby (syn. *Cassia siamea*); *Senna spectabilis* (DC) Irwin & Barneby (syn. *Cassia spectabilis*), *Sorghum bicolour* (L.) Moenche; *Zea mays* L., *Sesbania sesban*; *S. macrantha* and *Gliricidia sepium*.

4.1 ACAULOSPORA sp. 1. (Plate 4.1 A)

4.1.1 Spore characteristics

Spores found singly in soil; hyaline, white and cream (0-0-5-0 to 0-0-15-0) when young and yellowish cream (0-0-20-0 to 0-0-40-0) at maturity (INVAM Colour Chart & Colour

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Chart for Fungi, Edinburgh Botanic Garden 1969); globose to subglobose; 78-114 to 99-105 ×114-120 µm.

Spore wall consists of four layers (**L1-4**) in two groups (groups A and B). Group A with an outer granular uneven sloughing layer (**L1**) 1.5–2.5 µm thick; usually adherent to the middle layer; middle layer (**L2**) laminate; 3.0–6.0 µm thick; white to cream (0-0-5-0 to 0-0-15-0) in immature spores and yellowish cream to yellow (0-0-20-0 to 0-0-40-0) in mature spores. Group B with a bi-layer, outer sub-layer (**L3**) hyaline, thin, 0.5-1 µm thick, visible in mild and vigorously crushed spores, completely separating from L2. Inner sub-layer (**L4**) <1.0 µm thick, appearing wrinkled in crushed spores. Spore has a circular cicatrix 6-12 µm indicating the region of contact between spore and saccule neck during spore development. Some spores appear smooth with no indication of an attachment to a sporiferous saccule. L2, darkening to yellow (0-0-60-0) in Melzer's reagent other walls not reacting. The surface of spores glitters and the spore is fragile and breaks with light pressure on crushing (Plate 4.1A).

4.1.2 Discussion

Spores of this *Acaulospora* sp.1 were distinguished by the small size, hyaline, white to cream colour, smooth glittery surface and fragile spores. Hyphal attachments were absent and spores rarely had sporiferous saccules. A circatrix was present in some spores.

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The species from Malawi is compared with published reference literature (Schenck & Perez 1990) and INVAM specimen description. The spore colour, shape and surface appearance of the Malawi species are similar to *Acaulospora delicata* Walker, Pfeiffer & Bloss. The Malawi species record is however smaller than *A. delicata*. Spore size of the Malawi species range from 78-114 to 99-105 ×114-120 µm compared with 80-125(-150) × 80-110(-140) µm diam. of *A. delicata* from type specimen description but closer to size 80–120 µm of *A. delicata* specimen from INVAM. There are possibilities of errors in the interpretation of range of spore size because of non-statistical comparisons, hence it was suggested that it should not be weighted highly in species determination (Morton 1988). I therefore feel this size difference is not significant. The number of spore wall layers of the species from Malawi, are similar to the descriptions of *A. delicata* specimens from INVAM and the type description, however, the wall thickness differ slightly. The Malawi specimen differs slightly by a thicker outer layer (**L1**) (1.5–2.5 µm compared with 1 µm of type specimen description and 0.4-0.8 µm of the specimen from INVAM) and a thicker middle layer (**L2**) (3.0–6.0 µm compared with 2.5-3.5 µm of the type specimen description and 1.8-3.2 µm of the INVAM specimen). The inner wall group, L3 (0.5-1 µm compared with ±0.5 µm in the type specimen description and 0.5-0.8 µm in the INVAM specimen) and L4 (<1.0 µm compared with ±0.75-1.0 µm in the type specimen description and 0.6-0.8 µm in the type specimen description) are within the range of *A. delicata* description. The type and number of layers in spore wall is a much more stable character than sizes of the layers. The outer sub-layer of the inner wall group (L3) of the Malawi species never had ornamentation visible under light microscopy. This is contrary to observations made in *A. delicata*. The

beaded layer in the INVAM specimen tended to dislodge with applied pressure or became invisible after the specimen was mounted in PVLG for several months or longer. The orange red colour observed in the inner membranous layers of the type specimen description of *A. delicata* was never observed in the inner wall group of the Malawi species. The middle laminate layer (L2) however darkened to yellow as in L2 of *A. delicata* specimens. L2 of the INVAM specimen stained light pink to a slightly darker pink. The range of size of the circatrix (6-12 μm) is close to the size of the circatrix for the INVAM specimen (6.5-9.6 μm). At maturity, the spores of specimen from INVAM detached from the saccule and were sessile. The spore completely separated from the parent saccule neck and the spore wall had no break or opening region of attachment to the saccule neck. The circatrix in the species from Malawi was also not commonly found on spores.

Spore size and the thickness of layers are continuous variables that may be affected by environmental conditions. I do not therefore consider these differences taxonomically significant to classify *Acaulospora* sp. 1 differently from *A. delicata*. Staining of the inner membranous layer is considered taxonomically important (Morton 1988). However, reaction to Melzer's reagent was also not consistent in *A. delicata* type specimen and INVAM specimen. I therefore consider this species taxonomically close to *A. deilcata* but further comparisons need to be undertaken with *A. delicata* from pot cultures from ex-type material.

Only one record of *A. delicata* has been reported on the African continent. Ba *et al.* (1996) reported *A. delicata* in association with *Acacia holosericea* A. Cunn. ex G. Don. and

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Acacia mangium Willd. in Sudan. If the species is confirmed to be *A. delicata*, it will be a new record for Malawi.

4.2 ACAULOSPORA REHMII Sieverding & Toro, Angewandte Botanik 61: 217 – 223, 1987. (Plate 4.1 B-D).

4.2.1 Spore characteristics

Spores found singly in soil; borne intercalary on a sporiferous saccule (Plate 4.1 B); cream (0-0-5-0 to 0-0-20-0) and cinnamon (0-10-2-0-0 to 0-10-60-0) in young spores to rusty tawny (0-20-20-0 to 20-40-100-10), date brown (20-60-40-0 to 20-80-100-10), mouse gray and smoke gray (40-60-50-10 to 40-60-10010) in older spores (INVAM Colour Chart & Colour Chart for Fungi, Edinburgh Botanic Garden 1969); globose to subglobose; 74-90 × 153-177 µm.

Spore wall characteristics of four layers (**L1-4**) in three wall groups (A, B & C). Group A consists of outer ornamented layer (**L1**), cream (0-0-5-0 to 0-0-20-0), cinnamon (0-10-2-0-0 to 0-10-60-0), date brown (20-60-40-0 to 20-80-100-10) and mouse gray to smoke gray (40-60-50-10 to 40-60-100-10). L1 forms depressions 1–1.5 µm deep 0.5-1 µm wide, creating a network of ridges organized in a labyrinth pattern. The labyrinth pattern is distinctly variable with spore age with intensity increasing with age. The ornamentation on young spores is fine to thickly spiny and highly crowded (Plate 4.1 C) and sinusoidal in mature spores (Plate 4.1 D). Group B single, hyaline, rigid layer (**L2**), 3–4 µm, usually adhering to L1. Group C a bi-layer, outer sub-layer (**L3**) with warts, 0.5-1.5 µm; inner sub-

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layer (**L4**) hyaline, thin and wrinkling membranous layer. The inner sub-layer (**L4**) turns slowly to light pink in Melzer's reagent. Other layers do not react.

Sporiferous saccule with white contents; globose; 135 × 135 µm; wall of single hyaline smooth layer; distance from saccule to spore 69–75 µm. Spore consists of a circular cicatrix 15-21 µm.

4.2.2 Discussion

Spores of *A. rehmii* were separated from other species by the rusty brown colour in mature spores to cream and light brown in young spores. The spores are slightly large in size and surface is darl and granular in appearance. Most spores have sporiferous saccule or the remnant of a thick hyaline pedicel or a circatrix.

The specimen from Malawi resembles the type description and specimen from INVAM in size, shape and number of layers. Slight variations are observed in colour. The range of colours in the type description specimen is light yellow to brown with older spores often appearing brown to black and INVAM species is yellow-brown to orange-brown. The Malawi specimen differs in colour, being cream and cinnamon in young spores to rusty tawny, date brown, mouse gray and smoke gray in older spores. This colour differences in my opinion does not constitute sufficient reason for reclassifying this specimen. There are slight variations in L2, with adherence observed with only L1 in the species from Malawi and not to L1 and L3 as in the type specimen description. In the specimen from INVAM, L1 was described as a hyaline degrading layer that sloughs early in spore wall

differentiation so that it was usually absent in mature spores. The sloughing layer was not detected in the specimens from Malawi. The ornamented L2 of the INVAM species is therefore described as the ornamented L1 in the specimen from Malawi. As in the specimen from Malawi which has the ornamented L1 adherering to only L2, the ornamented L2 of the INVAM specimen adhered to L3. In the INVAM specimen, L3 consisted of three zones with the inner zone with sub-layers that separated collectively or only the innermost sub-layer separated and appeared as a distinct structure that resembled a flexible inner wall with two germinal wall layers (gw1 and gw2). The gw2 had granular excresences (or beads) that tended to become dislodged and float away with applied pressure. In the specimen from Malawi, the single hyaline rigid L2 was separated from the inner flexible group C bi-layer. This bi-layer comprised of two flexible sub-layers, L3 and L4. The inner sub-layer (L4) of the specimen from Malawi did not appear beaded. The beaded appearance was probably present but since no consideration was made on the degree of pressure applied to spores, it might have been dislodged. This difference is, however, minimal and not substantial enough to reconsider classification as a new species. A major observation of spores of *A. rehmii* from this site was the distinct variations in surface ornamentations at different stages of spore development. The range of ornamentation encompassed the types in *Acaulospora denticulata* Sieverding & Toro, *Acaulospora foveata* Trappe & Janos, *Acaulospora gerdemanii* Schenck & Nicolson and *Acaulospora lacunosa* Morton. Although this difference appears very distinct, the overall similarities in size and wall characteristics warrant identification of our specimen as *A. rehmii*. Except for *A. lacunosa* and *A. denticulata*, the remaining species were described before the concept of wall characteristics as diagnostic features was well understood and

overstressed (Walker 1983). I support the postulate role in wall layers having a significant role in species identification. The variation in different stages of development will be confirmed further by a series of single spore cultures of *A. rehmii*. Until the variation in surface ornamentation in the different stages of spore development is confirmed, it will be maintained as a possibility.

In the description of the type specimen of *A. rehmii*, similarities were noted with *Acaulospora gerdemanii* (Sieverding & Toro 1987) now referred to as *Archaeospora gerdemanii* (Rose, Daniels & Trappe) Morton & Redecker. Preliminary work on phylogenetic relationships amongst the AMF taxa placed *A. denticulata* and *A. rehmii* at the same level (Morton 1990). Similarities were also noted in in spore colour between *A. foveata* and *A. lacunosa* with distinctions made in only spore size, size of pits and reaction with Melzer's reagent (Schenck & Perez 1990). I am in support of maintaining distinctions for these species.

The species *A. rehmii* was first recorded in Colombia from a crop field in association with cassava, sorghum and *Crotolaria* species (Sieverding & Toro, 1987). The species was also found in pot culture with *Peuraria phaseoloides* (Roxb.) Benth. This species record from Malawi is a first record for the African continent. The species was recovered from agroforestry sites with *Gliricidia sepium*/maize intercrop and *Sesbania sesban*/maize intercrop, *S. macrantha*/maize intercrop and maize monocrop systems.

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4.3 ACAULOSPORA sp.2 (Plate 4.1 E-F)

4.3.1 Spore characteristics

Spores found singly in soil borne intercalary on a sporiferous saccule (Plate 4.1 E); hyaline, white, ivory, cream (0-0-20-0 to 0-10-20-0) to yellowish cream (0-10-40-0) at maturity; globose to subglobose 60-105 × 78-135 µm.

Spore wall characteristics of four layers (**L1-4**) in three wall groups (groups A, B and C) (Plate 4.1 F). Group A of single outer layer (**L1**); hyaline, white, ivory, cream (0-0-20-0 to 0-10-20-0) to yellowish cream (0-10-40-0); 3-5 µm; ornamented; ornamentation with circular to elliptical pits 1.0-1.5 µm apart, 1-1.5 µm deep and 1.5-2.0 µm wide. Group B of single hyaline thin layer (**L2**); 1-1.5 µm. Group C of bilayer with two adherent sub-layers; outer sub-layer (**L3**) beaded and inner sub-layer (**L4**) membranous. L4 slowly turning pink with Melzer's reagent (Plate 4.1 F). Sporiferous saccule subglobose; 108-114 µm; distance from saccule to spore is 27-75 µm; with white contents; circatrix circular; 7.5-12 µm.

4.3.2 Discussion

Spores of this *Acaulospora* sp. 2 appeared under the stereomicroscope as small white to cream spores distinguished from *Acaulospora* sp. 1 by the darl appearance and a fine granular surface. Spores with white sporiferous saccule or a hyaline collapsed sporiferous saccule were frequent (Plate 4.1 E).

This species from Malawi resembles *Acaulospora scrobiculata* Trappe and *Acaulospora undulata* Sieverding. The spore size of the species from Malawi (60-105 × 78-135 µm) is smaller than spores of type description of *A. scrobiculata* (100–240 × 100–220 µm) and INVAM specimen of *A. scrobiculata* (80-160 µm) but larger than spores of *A. undulata* (84 × 74–60 × 54 µm). The hyaline to white and cream to yellowish cream colour of the *Acaulospora* species from Malawi is within the range of colours of the type specimen description of *A. scrobiculata* (hyaline to light greenish yellow), INVAM specimen of *A. scrobiculata* (subhyaline, pale yellow to straw) and *A. undulata* (hyaline to subhyaline). The surface wall ornamentation of the species from Malawi showed discrete circular to elliptical pits 1.0-1.5 µm apart, 1-1.5 µm deep and 1.5-2.0 µm wide compared the type specimen description of *A. scrobiculata* with circular to elliptical occasionally linear to Y-shaped depressions, 1-1.5 × 1-3 µm separated by ridges 2-4 µm and INVAM specimen of *A. scrobiculata* with ovoid concave depressions 0.6-2.0 µm across and 0.5-1.4 µm deep with some merging together to form channels 5-12 µm long. *A. undulata* has similar ovulate or slightly irregular shaped pits. However, the space between the pits for *A. undulata* (4-9 ×4-9 µm) is wider than the species from Malawi (1.5-2.0 µm). The spore wall ornamentation of the species from Malawi is distinctly different from the description of type specimen and INVAM specimen of *A. scrobiculata*. The spore wall ornamentation of species from Malawi appears distinct from *A. scrobiculata*.

The spore walls of the species from Malawi and *A. scrobiculata* have four layers compared with three layers in *A. undulata*. The species from Malawi has three wall groups with

Group A of single ornamented outer layer, group B of single hyaline thin layer and group C of bi-layer with two adherent sub-layers of outer sub-layer beaded and inner sub-layer membranous and turning pink with Melzer's reagent. The type specimen description of *A. scrobiculata* has an outer ornamented layer, an adhering but separable, smooth, hyaline layer (L2), an adhering but separable smooth hyaline layer (L3) and a separated sometimes minutely roughened hyaline inner layer (L4) that turns deep red with Melzer's reagent. The *A. scrobiculata* specimen from INVAM has a hyaline layer (L1) degrading and sloughing early in spore wall differentiation and often absent, an ornamented L2 and L3 that is sometimes considered to be a sub-layer (lamina) of spore wall and two flexible germinal layers which may separate when each spore is broken. The outer layer of *A. undulata* has wall group A of bi-layer with outer sub-layer evanescent and appressed to ornamented L2, group B single layer of a hyaline, often minutely roughened unit layer (L3) often turning yellowish orange in Melzer's reagent. Based on the inner flexible layer (wall group) and the reaction to Melzer's reagent, the species from Malawi does not match the descriptions of *A. undulata* but has number of layers and reaction to Melzer's reagent matching that of *A. scrobiculata*. This species has characters that overlap with *A. scrobiculata* and *A. undulata*. The spore wall surface ornamentation and number of layers in a spore wall are significant diagnostic characters. I therefore consider *A. scrobiculata* and *A. undulata* different and also different from the *Acualospora* sp. 2 from Malawi. *Acaulospora paulineae* Blaszkowski, a spore similar to *A. undulata* could not be compared with the species from Malawi as there was neither published information nor reference specimen materials for this species. There is need to study the morphology of ex-type cultures of *A.*

scrobiculata, *A. undulata* and *A. paulineae* species and also use molecular techniques to verify the species.

A. scrobiculata has a wide distribution. It has been recorded in South America, USA, Japan and Australia (Schenck & Perez 1990). There are three records of this species from Africa. *A. scrobiculata* was isolated from Cameroon (Mason *et al.* 1992) and Ivory Coast (Wilson *et al.* 1992b) from *Terminalia ivorensis* A. Chev. plantation. In Kenya, it was found in association with *Leucaena leucocephala* and *Calliandra calothyrsus* Meissner (Shepherd *et al.* 1996). *A. undulata* has so far been extracted from the highlands of Zaire (Sieverding, 1988) while there is no record of *A. paulineae* from Africa.

4.4 ARCHAEOSPORA sp. (Plate 4.1 G-H)

4.4.1 Spore characteristics

Spore found singly in soil; white to pale cream (0-0-5-0 to 0-0-15-0); globose to sub-globose; 187-242 × 209-275 µm.

Spore wall characteristics of five layers (**L1-5**) in four groups (A, B, C, & D) (Plate 4.1 H). Group A with bi-layer; outer sub-layer (**L1**) with fissures, hyaline, rigid layer upto <1.5 µm. Inner bi-layer (**L2**) pale cream, laminated, brittle layer, 7.8-8.7 µm. Group B of single, thickly beaded layer (**L3**) separating from L2; 3-6 µm. Group C of single hyaline, unit layer (**L4**), 3-4.5 µm. Group D of single, membranous; hyaline layer (**L5**), 1 µm; circatrix circular, 6-7.5 µm (Plate 4.1 G).

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4.4.2 Discussion

The species looks like the acaulosporoid phase of a species in the genus *Archaeospora* Morton and Redecker possibly *Ar. leptoticha*. Spores of the species *Archaeospora* can easily be separated from other species by the white to pale cream colour and darl rough surface. The spores are much larger in size than the ornamented *Acaulospora* sp. 2 and some spores had remnants of sporiferous attachment that appeared as a circatrix.

Unlike *Acaulospora* species, the species has no evidence of a flexible layer and it has a spore wall consisting of at least two permanent layers; an outer layer varying in thickness and a thicker laminate layer. The species differs in the thick inner beaded L3 that has not been described in *Acaulospora* species with ornamented surface. The five layers in this species were also observed in *Acaulospora longula* Spain & Schenck (Schenck & Perez 1990). The layers in *A. longula* are in three wall groups with L1 described as a mucilaginous ephemeral outer layer compared with the hyaline, rigid, fissured L1 in the *Archaeospora* species from Malawi. L2 and 3 are inseparable in *A. longula* while in the specimen from Malawi L3 is a separate wall group. L4 and 5 of the *Archaeospora* sp. from this site are also separated unlike in *A. longula* and no reaction with Melzer's reagent was observed in L5 as was the case with *A. longula*. The size of *A. longula* (55-)90(-100) to 110-115 × 66-98 µm is significantlty smaller than the size 187-242 × 209-275 µm of the *Archaeospora* sp. from Malawi. The differences between *A. longula* and the *Archaeospora* sp. are substantial to maintain the two as separate species. The fissures observed on L1 of the *Archaeospora* sp. were observed in *Acaulospora nicolsonii* Walker, Reed & Sanders. Two wall groups with four layers were described in *A. nicolsonii* compared with four wall

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groups and five layers in the specimen from Malawi. Further differences exist between the two species (Schenck & Perez 1990). The description of the outer brittle L1 of *A. nicolsonii* that is first smooth but later roughened as it breaks up and sloughs leaving granular fragments attached to L2, resembles the characteristics of L1 of the *Archaeospora* sp. from Malawi. Both species display a distinct laminated L2. However, L3 of the *Archaeospora* sp. is thickly beaded, a characteristic not described in *A. nicolsonii*. The brittle unit L3 in *A. nicolsonii* that often separates when the spore is crushed was similar to unit L4 of *Archaeospora* sp. from Malawi. The inner membranous L5 in *Archaeospora* sp. from Malawi was similar to the innermost membranous L4 in *A. nicolsonii*, except for the lack of reaction with Melzer's reagent. The size, 99-198 × 109-218 µm of *A. nicolsonii* is within the range of the size 187-242 × 209-275 µm, of spores of the *Archaeospora* sp. The circatrix was distinct in the *Archaeospora* sp. Although the species from Malawi appears similar to *A. nicolsonii* in most aspects, the lack of the distinct beaded L3 is significant enough to maintain the two as separate species.

The *Archaeospora* sp. from Malawi could not be established in pot culture in this study, hence the materials were insufficient to fully describe the species. The specific epithet could not be confirmed as *Archaeospora leptoticha* as I had neither published nor pot culture reference materials for comparisons.

Plate 4.1 A

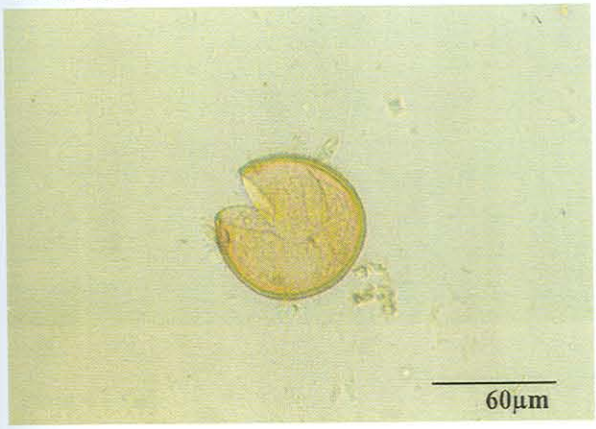


Plate 4.1 B

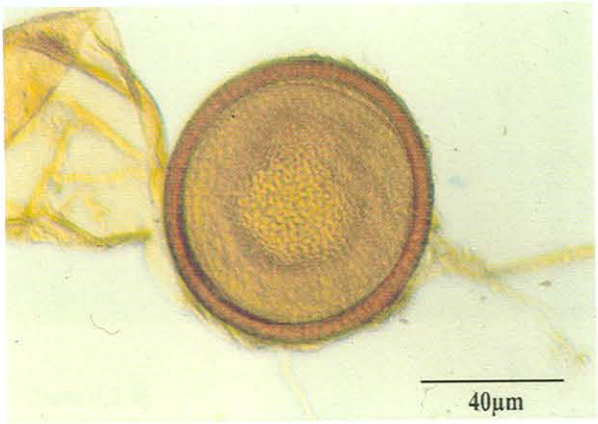


Plate 4.1 C

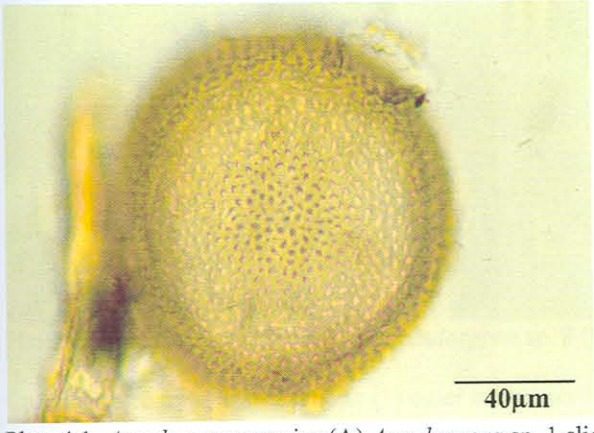


Plate 4.1 D

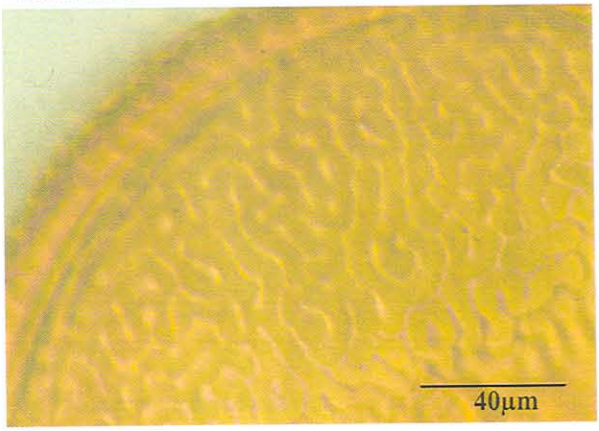


Plate 4.1. *Acaulospora* species (A) *Acaulospora* sp. 1 slightly crushed in Melzer's reagent. (B-D) *Acaulospora rehmii* (B) Spore with collapsed soporiferous saccule (C) Intermediate stage of spore with denticulate ornamentation (D) Mature spore with sinusoidal ornamentation.

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Plate 4.1 E

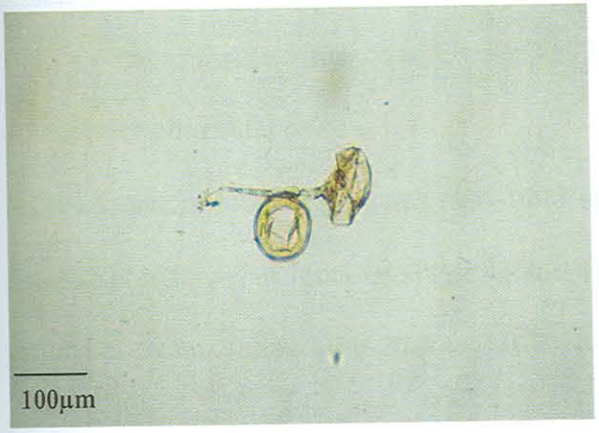


Plate 4.1 F

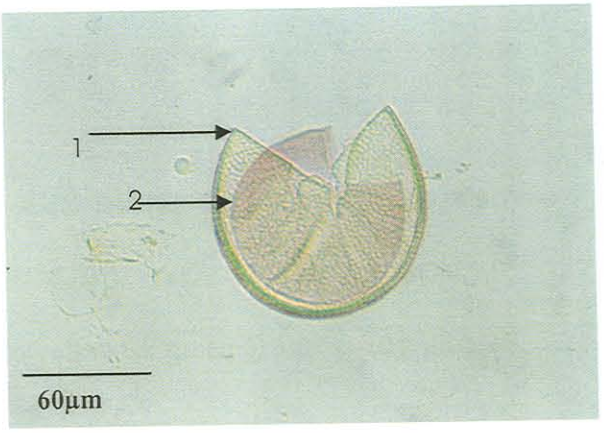


Plate 4.1 G

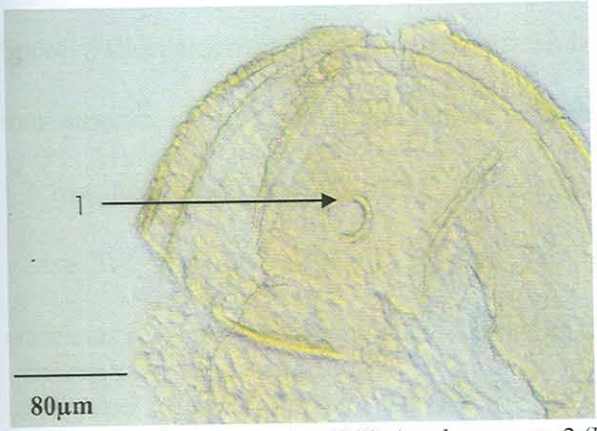


Plate 4.1 H

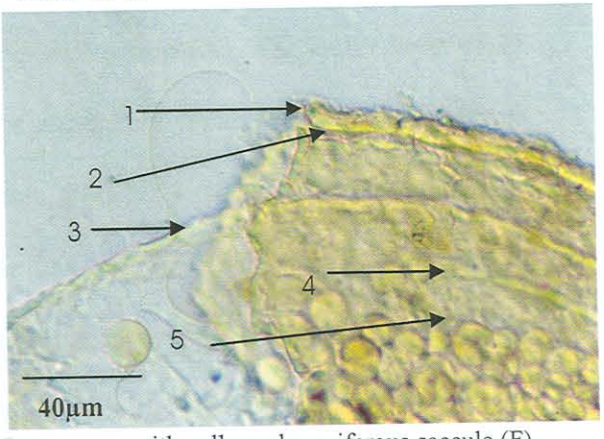


Plate 4.1 *Acaulospora* species (E-F) *Acaulospora* sp. 2 (E) Intact spore with collapsed sporiferous saccule (F) Crushed spore in Melzer's reagent (1) outer ornamented layer (2) inner membranous layer staining pink with Melzer's reagent.

Plate 4.1 *Achaeospora* species (G-H) *Achaeospora* sp. (G) Circatrix (H) Crushed spore with (1) outer layers with fissures (2) thick laminated layer 2 (3) beaded L 3 (4) unit L 4 and (5) flexible L 5 and a.

4.5 GLOMUS aff. AGGREGATUM Schenck & Smith emend. Koske, Mycologia 77: 619, 1985 = *Glomus fasciculatum* complex. Koske, R.E. (Plate 4.2A)

4.5.1 Spore characteristics

Spores found in loose aggregates, 300-1500 µm wide, lacking a peridium, in irregularly shaped sporocarps, in roots or single in soil (Plate 4.2A); cream (0-0-50-0 to 0-0-15-0); yellowish cream, yellow (0-0-20-0 to 0-0-70-10) to yellowish green (10-0-70-10); globose, subglobose to irregular; 25-75 × 33-120 µm.

Spore wall characteristics of three layers (**L1-3**) in one group (A). The outer layer (**L1**) thin, smooth, rigid, hyaline, sometimes with debris and rarely separating from layer (**L2**), 1.5 µm. Middle layer (**L2**), single, laminate, cream (0-0-50-0 to 0-0-15-0) to yellowish cream (0-0-20-0 to 0-0-40-0), 1.5-2.5 µm. Inner layer (**L3**), hyaline, thin, adhering to middle layer, rarely distinguished from the middle layer except at the point of hyphal connection to spore, <1 µm, the inner layer appears wrinkled in some spores. All three layers do not stain with Melzer's reagent.

Subtending hyphae straight; irregular and flared, cream tint (pale cream); width variable (3.0-14 µm); point of attachment with spore 3-12 µm wide. Hyphal wall of two layers, slightly thicker at the point of attachment; hyphal wall continuous with spore wall, pore open, 3.0-12 µm in young spores or closed by a thin septum that forms part of the inner spore wall.

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4.5.2 Discussion

The spores of this species are easily distinguished from others by the small size, cream to yellowish cream colour with a green tint and the smooth shiny surface. Unlike the spores of *Acaulospora* sp. 1, the spores are robust and do not break easily and may occur singly, with or without subtending hyphae or in groups interconnected by hyphae.

The spores from this site resemble description of *G. aggregatum* in most aspects and also bear resemblance to *Glomus fasciculatum*. The spores of both species are found in loose aggregates, single in soil or in sporocarps. Considering that the spores occur in loose aggregates, the size of the aggregate is variable. The sizes 25-75 × 33-120 µm of species from Malawi is within the range of sizes (30-)40-120(210) × (20-)40-85(-120) × (20-)40-85(-120) µm of the type description of *G. aggregatum*. The size range is a highly variable character and of no taxonomic significance. The species from Malawi displayed the very pale yellow and the greenish tint which is also described in the *G. aggregatum* type specimen description but did not display the yellow-brown to orange-brown colour observed in both *G. aggregatum* and *G. fasciculatum*. The composite wall of spores of the species from Malawi is thicker (4.5-9 µm) than *G. aggregatum* description (2-6)-10 µm) but less than composite wall of *G. fasciculatum* (5-12 µm). The species from Malawi has three layers with two of the layers continuous with the two layers of the subtending hyphae and the inner layer most visible as a thin septum occluding the pore and also appears as a wrinkled layer in some spores, slightly separating from L1 and L2. This layer was absent in young spores which lacked an occlusions. The pore occlusion also appears as an inner wall thickening in some spores. Spores with and without occluded pore were observed in the

same aggregate of spores. The spore wall of *G. aggregatum* has two layers with the innermost layer extending for a short distance down the subtending hypha. The inner spore layer of spores that have not reached full maturity collapses in mounting reagents such as polyvinyl alcohol (Walker 1983). Spores of *G. fasciculatum* have three layers. The differences in spore wall characteristics and colour in the species from Malawi, the type and INVAM description of *G. aggregatum* and *G. fasciculatum* are not distinct. However, reaction with Melzer's reagent distinguishes the species from Malawi and *G. aggregatum* from *G. fasciculatum*. The species from Malawi and *G. aggregatum* are distinguished from *G. fasciculatum* by the complete absence of reaction of spore wall with Melzer's reagent. An intense dextrinoid reaction of L2 and possibly spreading to L3 is observed in *G. fasciculatum*. However, staining of L2 is considered an unstable diagnostic character and inner membranous layer of taxonomic importance (Morton 1988). I therefore consider the species from Malawi to be affiliated to *G. aggregatum* species.

The species from Malawi and the *G. aggregatum* display distinct straight to cylindrical (curved) subtending hyphae with similar range of width, composite wall thickness and number of layers. Only one subtending hyphae was present compared with occasionally two in the type specimen description of *G. aggregatum*. The pore in the species from Malawi is variable with some spores occluded by the inner wall thickening, thin septa and some not occluded. The thin septa in the species from Malawi, is similar to the fragile septa formed by L3 in *G. fasciculatum*. The pore in *G. aggregatum* is plugged by a cytoplasmic plug or a thin curved septum. The species from Malawi resembles *G. aggregatum* in most aspects and I feel these similarities warrant determining the species from Malawi as *G.*

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aggregatum. This requires confirmation through comparisons with ex-type pot culture materials of *G. aggregatum*.

G. aggregatum has not been documented from the African continent. *G. aggregatum* was recorded from Canada (Schenck & Perez 1990), the USA as occupants of other dead spores (Koske 1984., Schenck & Perez 1990) and England from sand dunes (Mosse, 1959). The soil texture at the ICRAF Malawi site is sandy loamy. There are several records of *G. fasciculatum* from the African continent. *G. fasciculatum* has been recorded in Nigerian soils (Sani 1976), from the rhizosphere of *Faidherbia albida* (del.) A. Chev.(syn. *Acacia albida* Del.) in Senegal (Dalpe *et al.* 2000), in *Terminalia ivorensis* in Cameroon (Mason *et al.* 1992), in Egyptian soils (Mankarios & Abdel-Fattah 1994) and in Libyaan soils (El-Giahmi *et al.* 1976). Some of these species were recorded long before the species *G. fasciculatum* was revised.

4.6 GLOMUS ETUNICATUM Becker & Gerdemann, Mycotaxon. 6: 29, 1977.

= Glomus etunicatus sp. nov. Becker, W. N. and J. W. Gerdemann, 1974. (Plate 4.2 B-E)

4.6.1 Spore characteristics

Spores found singly in soil; white to cream (0-0-5-0 to 0-0-15-0) in young spores, pale ochraceous to ochraceous (0-10-20-0 to 0-10-40-0 and 0-20-60-0 to 0-20-100-0) and sienna (0-30-60-0 to 0-30-100-0 and 0-40-50-10 to 0-40-100-0) in mature spores; globose to sub-globose 60-100 × 77-153 µm.

Spore wall characteristics of three layers (**L1-3**) in one group. Outer layer (**L1**) rough, uneven, with debris adhering 1.5-3.0 µm. Outer sloughing layer variable with age (Plate 4.2 C & D). The middle layer (**L2**) does not separate from L1; laminate, white to cream (0-0-5-0 to 0-0-15-0) in young spores, pale ochraceous, ochraceous (0-10-20-0 to 0-10-40-0 and 0-20-60-0 to 0-20-100-0) to sienna (0-30-60-0 to 0-30-100-0 and 0-40-50-10 to 0-40-100-0) in mature spores; intensity of laminae varies with age of spore; 3-6 µm. The inner layer (**L3**) hardly distinguishable from L2, thin, hyaline < 0.5 µm. Outer layer (L1) staining patchily reddish purple and reddish orange with Melzer's reagent (Plate 4.2 D). Other layers do not stain (Plate 4.2 E).

Subtending hyphae cylindrical (Plate 4.2 B); one subtending hyphae per spore; pale cream; width at point of attachment 9-15 µm, away 3-6 µm. Wall structure of two layers, 3-6 µm at the point of attachment, <1.0 µm further away. Hyphal wall continuous with spore wall; pore plugged by thick curved septum formed by the inner layer.

4.6.2 Discussion

The spores of *G. etunicatum* are easily separated from other spores by the distinct cream to sienna colour, small size and the smooth glittery surface. Long curved subtending hyphae may be present in some spores.

The species from Malawi was found singly in soil. The colour and shape were similar to the type specimen and INVAM specimen description in all aspects. The size, 60-100 × 77-153 µm, of the species from Malawi was at the smaller end of the size range, 68-144(162)

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µm, of the type description and at the smaller end and also larger of the INVAM specimen (60-160 µm). Spores of *G. etunicatum* recorded from banana plantation soils in Uganda were 60-125 µm in diameter (Msiska 2001). The composite wall thickness, 4.5-9.5 µm for the species from Malawi was similar to the wall thickness, 4.5-9 µm for the species from Uganda and slightly thinner than the type description wall thickness of 4-13 µm and the INVAM wall thickness of 5-13.2 µm. Spores of the species from Malawi had one wall group with three layers with the inner layer (L3) indistinguishable from the middle laminate layer (L2). Except for the differences in perception of number of layers in the specimen from Malawi, the specimen from Uganda, the type description and the INVAM specimen description, the rough uneven outer layer and the middle laminate layer are consistent in all the three records. Sturmer and Morton (1997) observed an innermost sub-layer that slightly separated from the spore wall. In the species from Malawi, an innermost L3 was also observed.

The subtending hyphae of spores from Malawi are cylindrical in shape compared with flared in the type description and cylindrical and flared in the INVAM specimen description. The 9-15 µm width of the subtending hyphae at the point of hyphal attachment is less than the 30 µm width of the type specimen description and slightly larger than the 5-10.2 µm in the INVAM specimen. The pore in the species from Malawi is occluded by an inner wall thickening as is the case with the type specimen description. The pore in the specimen from INVAM is occluded by an innermost sub-layer of laminate layer of the spore wall in some spores with the bridging structure resembling a septum. The spore contents in the species from Uganda are separated by a bridge structure resembling a

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curved septum while mature spores display spore contents separated from hypha by inner spore wall thickening. The Malawi specimen agrees well with descriptions for the species from Uganda and INVAM. The pactchy reddish purple and reddish orange reaction to Melzer's reagent of outer layer (L1) of species from Malawi is similar to reaction of the INVAM mucilaginous outer layer (L1), having some plasticity with uneven outer surface, staining pink to reddish purple in Melzer's reagent. The outer layer (L1) degrading and sloughing as the spore matures, so that it may be present in patches usually detected in Melzer's reagent or appear as a granular layer. The spore morphology of the record from Malawi is similar with the two records. The differences in spore morphology, in my opinion do not constitute significant reason for re-classifying this species. I therefore consider the species from Malawi as *G. etunicatum* species.

There are six records of *G. etunicatum* from the African continent. The species was recorded from Uganda in association with *Musa paradisiaca* L. plants (Msiska 2001), in association with *Terminalia ivorensis* plantations in Cameroon (Mason *et al.* 1992) and Ivory Coast (Wilson *et al.* 1992b), in association with *Acacia nilotica* (L) Del. seedlings in Senegal (Ginwal *et al.* 1997), in the arid regions of Namibia and North America (Stutz *et al.* 2000) and in association with *Vangueria infausta* Berch. Subsp.*infausta* (Rubiaceae) from South Africa (Gaur *et al.* 1999).

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4.7 GLOMUS sp. (Plate 4.2 F-J).

4.7.1 Spore characteristics

Spores found singly, in pairs or triplets closely attached, covered with a peridium (Plate 4.2 F); spores also found singly without a peridium (Plate 4.2 G); spores covered by peridia may also have smaller spore emerging from the margin (Plate 4.2 J); peridium cinnamon (0-20-40-0 to 0-20-50-10) to date brown (0-30-50-10 to 0-30-70-10 and 0-40-50-10 to 0-40-70-10); spores without a peridium pale ochraceous to pale citrine and yellowish green (0-10-40-0 to 0-10-70-10); sporocarp globose to subglose 144-282 × 150-432 µm; spores without a peridium, globose to subglobose 120-258 × 135-360 µm.

Spore wall characteristics of three layers (**L1-3**) in one group; tightly adhering layers. Outer layer (**L1**) thin, < 0.5 µm, adhering to layer L2. Middle layer (**L2**), no distinct laminae; pale ochraceous to citrine and yellowish green (0-10-40-0 to 0-10-70-10); 3-6 µm; adhering to L3. Inner layer (**L3**), thin, hyaline, < 1.0 µm. The inner layer of spores without a peridium with projections extending into the spore contents, appearing as dark brown concentric discs in surface view of the spore (Plate 4.2 G). All three layers did not react with Melzer's reagent.

Peridium loose to compact, comprised of septate, sub-hyaline to pale cream, irregular hyphae, width of hyphae is variable, 3-15 µm (Plate 4.2 I). Spore-like structures, 63 × 66 µm, thin walled, 1.5-3.0 µm, sub-hyaline, pale cream to orange tint (0-20-60-0 to 0-0-20-0), attached to the peridium (Plate 4.2 I). The wall of the spore-like structures thickened (Plate 4.2 H).

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4.7.2 Discussion

The spores from Malawi were distinguished into spores with and without peridium. The spores with peridium are large, irregular shaped, covered with hyaline mycelia and soil particles adhering on to the mycelia, giving an impression of a soil particle at first glance. The spores with a peridium take the reddish brown colour of the soil and at first glance are easily mistaken for soil particles. Spores without a peridium are large, yellowish cream to citrine in colour and variable in shapes. Spores may or may not have subtending hyphae. When subtending hyphae is present the point of contact of the hyphal attachment and spore appears black.

The spores with a peridium from Malawi resemble *Glomus globiferum*, *Glomus tortuosum* Schenck & Smith and *Glomus mosseae*. Like the species from Malawi, *G. mosseae* and *G. tortuosum* also have spores without hyphal mantle. The description of the hyphal mantle for all the species is similar to the species from Malawi with variation found only in the number of spores per cluster. A cluster of 1-10 spores within hyphal peridia described in the INVAM species description of *G. mosseae* was noted to be unstable and of little taxonomic value. The hyphal mantle was also described as an unstable character in the species description of the INVAM specimen for *G. mosseae*. It was noted that some isolates of *G. mosseae* from the United Kingdom produced abundant sporocarps in the first culture circle, but then reverted to single spores with smooth surfaces in the third culture circle. The spores of *G. mosseae*, without peridia never reverted to sporocarpic nature. The species from Malawi has been in culture since 1995 and has been subsequently recultured until 2003. The species still produces spores with and without hyphal peridia. Contrary to

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the spores of *G. mosseae,* which did not revert to sporocarpic form, the culture for the species from Malawi was initiated from spores without hyphal peridia and resulted to a culture of spores with and without hyphal peridia. The spores of *G. tortuosum* are borne single but are occasionally in pairs with young spores without mantle and mature spores with a mantle of sinuous hyphae closely appressed to the spore and flattened. The description of sporocarp for *G. mosseae* by both INVAM and Schenck and Perez (1990) has 1-10 spores, measuring up to 1 mm. This is larger than the 1-3 spored sporocarps of *G. globiferum* (150-260 × 150-270 µm) and the 1-3 spored sporocrp from Malawi (144-282 × 150-432 µm). The size of spores without hyphal peridia for species from Malawi (120-258 × 135-360 µm) is larger than *G. mosseae* (100-260 µm) and *G. tortuosum* (94-180 × 112-230 µm). The size range, unless statistically analysed is a variable character. Spores without hyphal peridia were not described in the type specimen description of *G. globiferum* (Koske & Walker 1986a). There is considerable resemblance of *G. tortuosum*, *G. mosseae* and *G. globiferum* with the species from Malawi. The differences between *G. mosseae* and the *Glomus* sp. from Malawi are so distinct that I feel I should maiantain them as separate species. Except for the lack of spores without peridium in *G. globiferum*, the species from Malawi conformed more to the description of *G. globiferum*.

Thin-walled vesiculates representing the "spore-like" structures were found in the specimen from Malawi. It is postulated that these spore-like structures develop into spores. The vesiculates have so far been described in *G. globiferum* (Koske & Walker 1986a) and *G. tortuosum* (Schenck & Smith 1982). The species from Malawi has both thin-walled and thick-walled (Plate 4.2 H) spore-like structures that fit the description of the vesiculate of

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G. globiferum. Wall thickening in vesiculates was only suggested as a possibility by the authors (Koske & Walker 1986a). The smaller spore formed beside a larger spore was not described in *G. globiferum* as is seen in our specimen (Plate 4.2 J).

The spores without a peridium observed in the species from Malawi were not described in *G. globiferum* (Koske & Walker 1986a). The spores without a peridium have concentric disc-like features and colour that is similar to *Glomus clariodeum sensu stricto*. In *Glomus clariodeum* Schenck & Smith, the concentric discs were described as unstable parasitic structures of no taxonomic significance (Walker & Vestberg 1998). The spores of species from Malawi are pale ochraceous, pale citrine to yellowish green while the colour of *G. clariodeum sensu stricto* is hyaline when immature, becoming straw-coloured to ochracceous when mature. The size of the spores of the species from Malawi, 120-258 × 135-360 µm, are slightly larger than the size for *G. clariodeum* (95-)135-178(-220) × (95-) 130–180(-220) µm. The spores of species from this site have one wall group with three layers. Similarly *G. clariodeum* has one wall group with three layers. The spores from Malawi have a thin outer layer adhering to a middle laminate layer with no distinct laminae and a thin hyaline inner layer compared with hyaline outer unit layer and a laminated layer with the innermost laminae sometimes appearing to be a separate unit layer that often forms a septum at the base in *G. clariodeum*. L3 of *G. clariodeum* is membranous, very thin and tightly adherent to L2. The domed, scalloped ingrowths in *G. clariodeum* were observed in the spores of the species from this site. These are now regarded as of no taxonomic importance (Walker & Vestberg 1998). Since it is the concentric discs that linked the species from Malawi with *G. clariodeum,* other features variable, I therefore

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consider the spores from Malawi without a hyphal peridium to be different from *G. clariodeum*.

The type description of *G. globiferum* has hyphal wall of peridium and spore wall composed of one or two wall groups with 3 or 4 layers respectively. The characteristics of L1 and L2 are similar to spores of the species from Malawi while L3 and 4 are hyaline and membranous. The membranous nature of L3 and L4 was not observed in spores of the species from Malawi. The characteristics of the peridium of the species from Malawi are indistinguishable from the peridium of *Glomus tortuosum* (Schenck & Perez 1990), yet this was the only diagnostic feature separating the two species. The size of the hyphal width of the species from Malawi (3-15 µm) is less than the hyphal width of the *G. globiferum* (5-50 µm) and greater than the hyphal width of *G. tortuosum* (4-10 µm). Unless statistically anaylsed the differences in hyphal width may not not constitute enough reasons to separate the species. The spore-like structures (vesiculates) are described in *G. tortuosum* as swellings on a hyphal attachment. The size of the spore-like structures of the species from Malawi (63 × 66 µm) is within the range of the *G. globiferum* (12-65 × 12-75 µm) although larger than the diameter for the similar structures in *G. tortuosum* (10-20 µm). The size range of the species described from Malawi with peridium (144-282 × 150-420 µm) and excluding the peridium (120-258 × 135-360) encompasses the sizes of *G. tortuosum* (94-180 × 112-230 µm) and the size range of the *G. globiferum* (150-260 × 150-270 µm) excluding the peridium. These two species of *Glomus, G. globiferum* and *G. tortuosum* have strongly overlapping similar characteristics including size, colour and wall layer characteristics. There is a distinct possibility that these two names may be recognized as

synonyms for the same species but it is beyond the scope of this work to undertake such taxonomic revision.

The species from Malawi was successfully cultured in this study and the description done from fresh spore culture. It was proven mycorrhizal with five leguminous agroforestry tree species (*Senna siamea, S. spectabilis, Sesbania sesban, S. macrantha* and *Gliricidia sepium*) and two gramineae species (*Zea mays* and *Sorghum bicolour*). The spore culture was established from spores without peridia, and spores with and without peridia and some with sparse hyphal peridia were recovered from the culture. If the species is confirmed to be *G. globiferum*, this record from southern Malawi will be the first record for the African continent. There is only one record of *G. claroideum* from semi-arid areas of Senegal (Diallo *et al.* 1999) and there is still no record of *G. tortuosum* for Africa. *G. mossae* is widely reported in Africa from Nigerian soils (Sani 1976), in association with *Acacia nilotica*. in Senegal (Ginwal *et al.* 1997), in the arid regions of Namibia (Stutz *et al.* 2000), in Egyptian soils (Mankarios & Abdel-Fattah 1994), arid zones of Morocco (Meddich *et al.* 2000) and Libyan soils (El-Giahmi *et al.* 1976)

4.8 GLOMUS aff. GEOPSPORUM (Nicol. & Gerd.) Walker, Mycotaxon 15: 49-61, 1982. (Plate 4.2 K-L)

4.8.1 Spore characteristics

Spores found singly in soil; bay (40-60-60-0 to 40-60-100-0 and 60-80-100-0 to 60-80-100-10) to rusty tawny and chestnut (20-80-80-0 to 20-80-100-10 and 40-80-80-0 to 40-80-100-10); globose to subglobose 85-115 × 105-205 µm.

Spore wall characteristics of three layers (**L1-3**) in one group (Plate 4.2 L). Outer layer dull, thin, hyaline sloughing layer (**L1**); <1 µm. Middle layer (**L2**) laminate, drab (40-60-60-0 to 40-60-100-0 and 60-80-100-0 to 60-80-100-10) to rusty brown (20-80-80-0 to 20-80-100-10 and 40-80-80-0 to 40-80-100-10); 5-9 µm. Inner layer (**L3**), thin; hyaline; 1-2 µm; with parasitic protrusions extending into the cytoplasm; 9-15µm in length (Plate 4.2 K). All the three layers did not react with Melzer's reagent. Subtending hyphae flared, sub-hyaline, 14.5 µm wide; occlusion of the pore is by a thin septum.

4.8.2 Discussion

The spores of this *Glomus* species were separated from other species by the rusty brown colour and the flared subtending hyphae. It was distinguished from *A. rehmii* by the smooth shiny surface.

The colour, shape and wall characteristics of this *Glomus* species resemble *Glomus geosporum*.The spores of the specimen were described from field materials hence only rusty tawny and chestnut spores were described. The spores of the decribed specimen therefore resemble the dark red brown mature spores of *G. geosporum*. Three layers were described in the specimen from Malawi compared with the two layers plus an inner thin membranous layer, in the type description for *G. geosporum*. The species from Malawi had an outer dull thin sloughing layer, which in the type specimen description has been described as smooth, shiny or with dull appearance or roughened from adherent debris, which outer layer is observed easily in young spores and is sometimes absent in mature

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spores. The middle layer was distinctively laminate in both. The specimen described from Malawi appeared to have an inner thin hyaline layer. This was described as a yellow to yellow-brown inner wall (<0.1 μm) that appears membranous and forms a separate septum separating spore contents from the lumen of sub-hyphae.

The flared sub-hyaline subtending hyphae of the species from Malawi slightly differs from the type specimen description of straight to curved, simple to slightly funnel-shaped, yellow to dark yellow subtending hyphae. The pore of the species from Malawi is occluded by a thin septum while the pore of the type specimen forms a septum with the inner membranous wall. The type specimen was observed to have walls that became perforated probably by attacks by microorganisms during aging. In the species from Malawi, parasitic intrusions extending into the cytoplasm were observed. The *Glomus* species was not successfully cultured in this study.

The diagnostic features distinguishing this specimen is limited and are continuous variables that could easily be plastic characters dependent on biotic and abiotic factors. All attempts to culture the *Glomus* species failed thus limiting studies to field samples. The parasitized nature of the spores could explain the inability to establish pot cultures of this species. There are two records of *G. geosporum* from a *Terminalia ivorensis* plantation in Cameroon (Mason *et al.* 1992) and in association with *Acacia holosericea* and *Acacia mangium* in Sudan (Ba *et al.* 1996)

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Plate 4.2 A

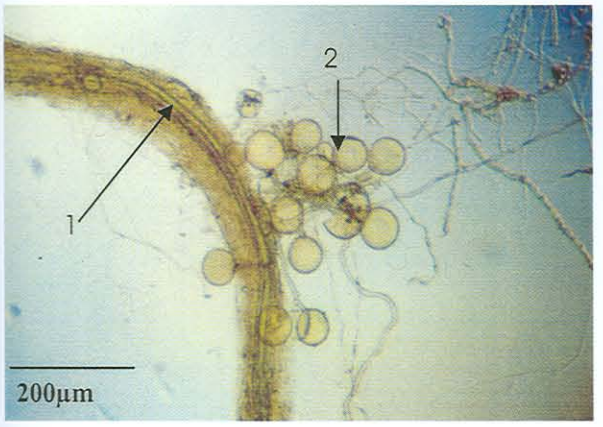


Plate 4.2 B

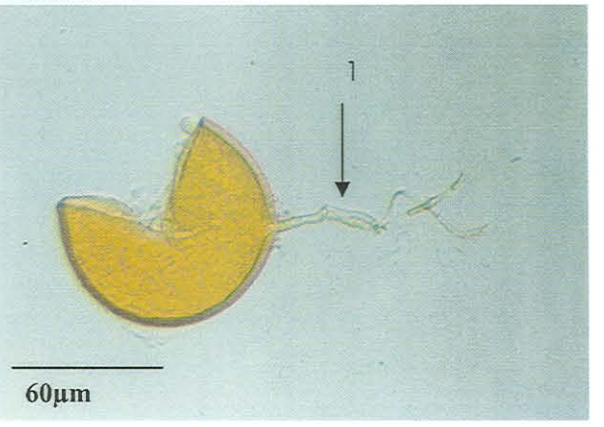


Plate 4.2 C

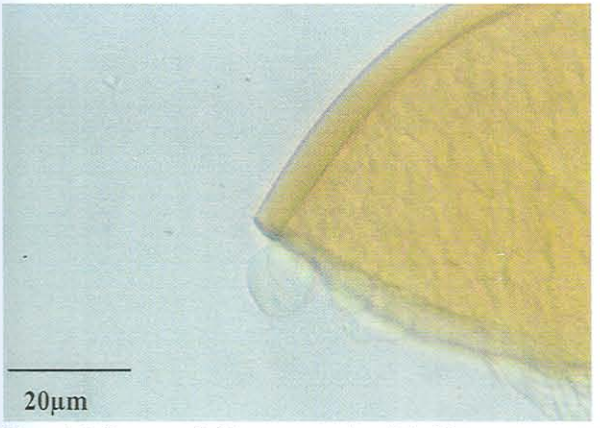


Plate 4.2 D

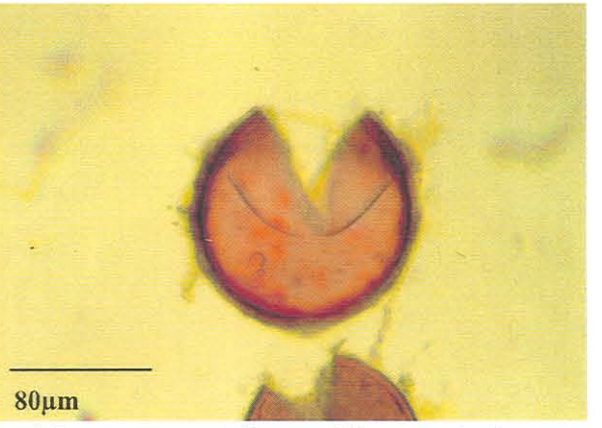


Plate 4.2 Spores of *Glomus* species (A) *Glomus aggregatum*. A loose aggregate of spores (1) a spore in the root (2) aggregate of spores. (B-D) *Glomus etunicatum*. (B) Slightly crushed mature spore with outer layer already sloughed (1) recurved cylindrical subtending hyphae (C) paler more immature spore where sloughing layer and laminae on middle layer has not yet developed (D) spore with sloughing layer patchily stained with Melzer's reagent.

Plate 4.2 E

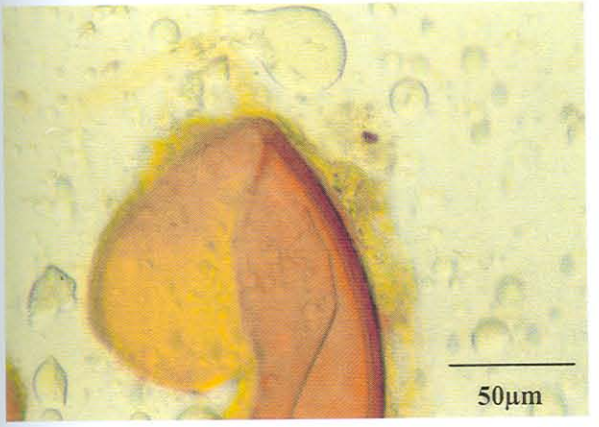


Plate 4.2 F

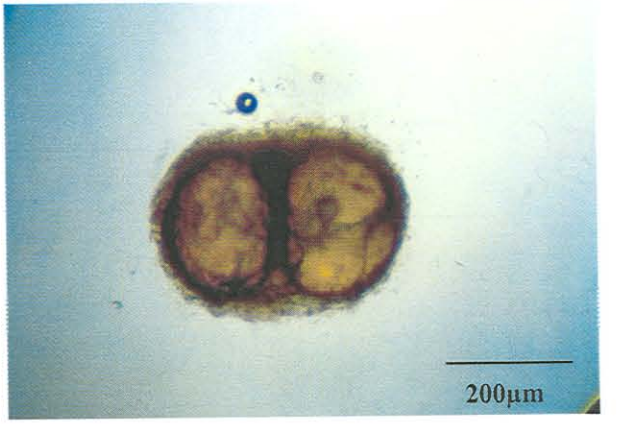


Plate 4.2 G

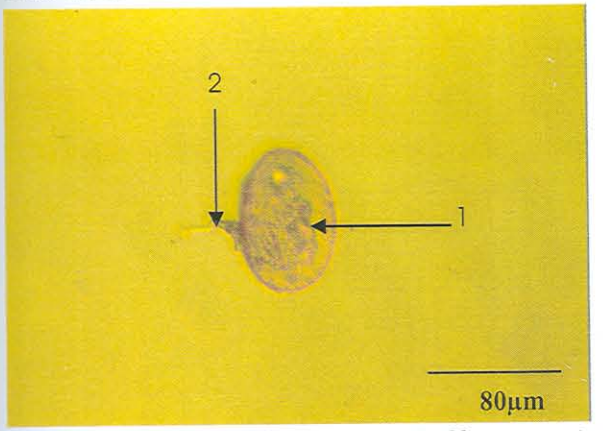


Plate 4.2 H

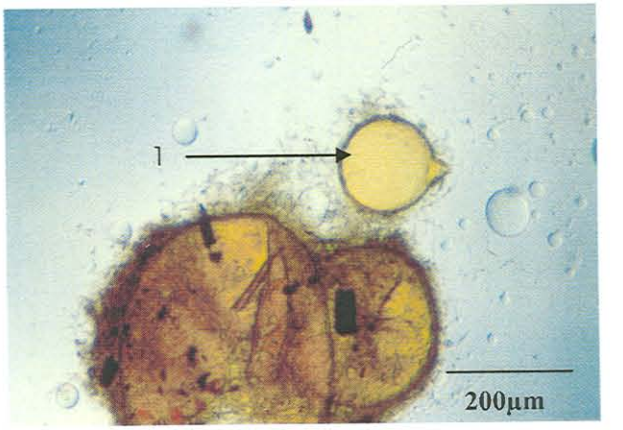


Plate 4.2 Spores of *Glomus* species (E) *Glomus etunicatum* crushed spore in Melzer's reagent showing a stained laminated layer (F-H) *Glomus* sp. (F) Two spores enclosed by a peridial hyphal mantle. (G) Spore with (1) concentric discs and (2) cylindrical hyphal attachment. (H) Vesiculate swelling with wall thickening, with (1) vesiculate spore-like structure appearing as immature spore.

Plate 4.2 I

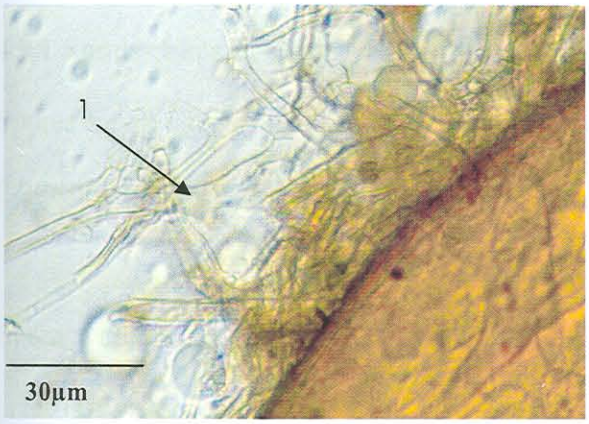


Plate 4.2 J

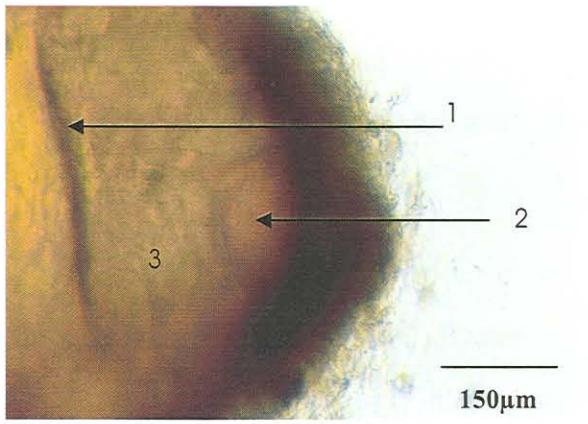


Plate 4.2 K

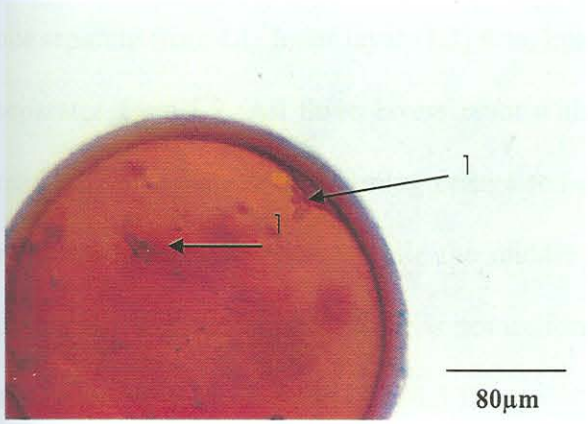


Plate 4.2 L

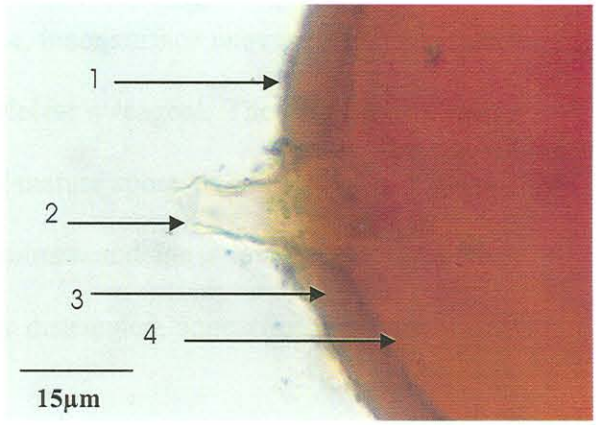


Plate 4.2 Spores of *Glomus* species (I-J) *Glomus globiferum* mantle with (1) septate hyphae (J) Spore with (1) crack in the middle (2) spore-like structure forming on the margin of a large spore.

(K-L) *Glomus geosporum*. (K) Parasitic invaginations. (L) Spore with (1) sloughing outer layer (2) flared subtending hyphae (3) thick middle layer and (4) thin inner layer enclosing the spore contents.

4.9 GIGASPORA sp. (Plate 4.3 A-G)

4.9.1 Spore characteristics

Spores found singly in soil, borne on a sporogenous cell (Plate 4.3 A), cream (0-0-5-0 to 0-0-20-0) to pale ochraceous (0-10-20-0 and 0-10-40-0), globose, 204-396 µm.

Spore wall characteristics of three layers (**L1-3**) in one group (Plate 4.2 B & C). Outer layer (**L1**) rigid, thin, pale yellow, 1.0-1.5 µm. Middle layer (**L2**) laminate, cream (0-0-5-0 to 0-0-20-0), pale ochraceous to pale yellow (0-10-20-0 and 0-10-40-0), 15-18 µm, does not separate from L1. Inner layer (**L3**) thin, hyaline, inner surface uneven, 0.5-3 µm, rarely separates from L2. All three layers react with Melzer's reagent. The intact spore reacts readily, with young spores turning orange red and mature spores reddish purple. The outer rigid layer reacts to orange while the middle laminate and inner layer reacts to reddish purple in older spores, the colour is not uniformly distributed, appearing as orange brown and reddish brown patches (Plate 4.3 D).

Sporogenous cell borne vertically, 36-48 µm wide, with two layers, outer layer hyaline and inner layer cream (0-0-5-0 to 0-0-20-0) to pale ochraceous (0-10-20-0 and 0-10-40-0); sporogenous cell wall continuous with outer two layers of the spore wall, cell wall at the point of connection with spore 2.5-4.5 µm gradually decreasing to 1.5-3 µm away from the point of attachment; subtending hyphae coenocytic, 15-30 µm wide close to the sporogenous cell and gradually decreasing to 3-9 µm wide away from the sporogenous cell; hyphal wall 3-4.5 µm gradually decreasing to 1-1.5 µm, occlusion closure by plug; colour same with the laminate layer.

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Auxiliary cells borne on curved and coiled aseptate hyphae, cells in clumps before development (Plate 4.3 E) and mature cells in aggregates of 4-12 cells; globose, subglobose to clavate, 27-30 × 33-36 µm, spines cover the entire surface of the cell (Plate 4.3 F), surface with spines 1.5-4.5 µm high, <1.5 µm wide at the base and 1.0-4.5 µm apart (Plate 4.3 G). Hyphae pale cream (0-0-5-0 to 0-0-15-0); 4.5-7.5 µm wide; wall with two layers, 0.5-1.5 µm thick.

4.9.2 Discussion

The spores of *Gigaspora* sp. were separated from other species by the large size, white to yellowish cream colour and the sporogenous cell. It is distigushed from the three members of *Gigasporaceae* from the site by the darl smooth surface. *Gigaspora* sp. from Malawi was distinguished from the synonym of *G. margarita* also found at the same site by colour, with the colour of the synonym of the *G. margarita*, the species *Gigaspora ramisporophora* Spain, Sieverding & Schenck, distinctly orange to saffron and the *Gigaspora* sp. cream to pale yellow.

The morphological features of *Gigaspora* sp. resembles spores of the true type description for *G. margarita* in colour (Schenck & Perez 1990) even though the spore sizes of the *Gigaspora* sp., (204-396 µm), ranges both smaller and larger than the true type description of *G. margarita* (260-280 µm) and smaller than INVAM specimen description of *G. margarita* (260-400 µm). The subglobose shape noted in the true type specimen description of *G. margarita* and INVAM specimen of *G. margarita* and the consistently globose shape of the *Gigaspora* sp. spores are plastic morphological characters that cannot be used to

distinguish species. The sizes were also overlapping and hence based on these three morphological characters, *Gigaspora* sp. could easily be considered a *G. margarita*. The spore wall of the *Gigaspora* sp. from Malawi has three distinct layers observed with composite wall size of 16.5-22.5 µm compared with the type specimen description of *G. margarita* composite wall of 5-24 µm and INVAM specimen of *G. margarita* of 14.6-33.4 µm. The spore wall characters for the new type for *G. margarita* spores described by Bentivenga and Morton (1995) are similar to the specimen from Malawi. The spores of the new type specimen description for *G. margarita* was also described to have three layers, with the outer layer < 1.0-2.5 µm thick, middle layer with variable number of laminae (5-) 14-25 µm and an inner papilliate layer 1-5 µm extending into the lumen of the spores. The papilliate appearance of the inner layer was observed as uneven surface in the specimen from Malawi. No multiple germ tubes were observed from the uneven surface of the specimen from Malawi. The spore wall characteristics of *Gigaspora* sp. are also similar to the type specimen description of *G. margarita* (Schenck & Perez 1990) and the newly revised description of *G. margarita* (Bentivenga & Morton 1995).

The sporogenous cell (36-48 µm) diam. and the wall thickness (2.5-4.5 µm) is within the range of the size of the type specimen description cell diam. (27-58 µm) and wall thickness (1-5 µm) of *G. margarita*. The colour of the subtending hyphae being cream to ochraceous and hyphae coenocytic in the *Gigaspora* sp. compared with hyphae being hyaline to pale brown and septate in the type specimen description of *G. margarita*. The hyphal wall thickness in the specimen from Malawi (1.5-4.5 µm) is within the range of the type specimen description (1-5 µm). The variations in sporogenous cell are not significant.

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The auxiliary cells of the *Gigaspora* sp. are also similar to descriptions of *G. margarita*. Auxilliary cells were described as vesicles formed in soil in the true type specimen description of *G. margarita* with up to 20-35 µm diam. becoming covered with warty projections up to 4 µm high and 5 µm wide. The auxiliary cells of the *Gigaspora* sp. (27-30 × 33-36 µm) with spines 1.5-4.5 µm high are within the range for the type description of *G. margarita* but the width, <1.5 µm is thinner than in the type specimen description. The characteristics of the spore wall, sporogenous cell and auxiliary cells of the *Gigaspora* sp. are not distinctly different from *G. margarita*.

The spore wall of the *Gigaspora* sp. from Malawi however, reacts differently to Melzers reagent from the spore walls of *G. maragarita* (Schenck & Perez 1990; Bentivenga & Morton 1995). Reactions to Melzer's reagent were observed in all three layers with variable staining intensity in the *Gigaspora* sp. specimen from Malawi compared with reaction of only the middle laminate L2 in the recently revised *G. margarita* specimen described by Bentivenga and Morton (1995) and no reaction was reported in the true type description of the *G. margarita* type specimen (Schenck and Perez 1990).

Bentivenga & Morton (1995) did not obviously consider Melzer's staining as distinctive because the true type of *G. margarita* did not stain, yet the description of *G. margarita sensu stricto* (which is not a new type) has L2 staining whereas the true type (Becker & Hall 1976) does not. The synonym of *G. margarita*, *Gigaspora ramisporophora*, has L1 and L2 staining with Melzer's, therefore does not conform to the true type specimen description of *G. margarita*. The fact that the *Gigaspora* sp. from Malawi and the true type

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of *G. margarita* and the synonym of *G. margarita*, *G. ramisporophora,* have completely different reactions to Melzer's reagent makes them distinct. However, Bentivenga and Morton (1995) felt differently because they synonymized *G. margarita* and *G. ramisporophora* and described a new type for *G. margarita* with layer (L2) that stains. Melzer's reagent appears to offer the greatest potential to aid in identifying AMF with the dextrinoid colour gradation relating to chemical composition of spore walls and the degree of polymerization of these compounds (Morton 1988). Variability in staining reactions was observed with spore ontogeny (Koske & Walker 1985). With the exception of the inner spore layer, the remaining layers were considered to be variable under different environmental conditions (Morton 1988). Morton and Bentivenga (1995) in their revision of *G. margarita* described reaction with Melzer's in only L2. This explains why they still considered the type specimen described by Becker and Hall (1976) as the same as their species, since staining of L2 is considered to be variable. However, the *Gigaspora* sp. from Malawi had all the three layers reacting with Melzer's reagent. I therefore consider wall staining of the inner layer of the *Gigaspora* sp. taxonomically distinct and hence the *Gigaspora* sp. is distinct from the newly described *G. margarita*.

4.10 GIGASPORA MARGARITA Becker and Hall, Mycotaxon 4: 155-160, 1976. (Plate 4.3 H-J) =*Gigaspora ramisporophora* Spain, Sieverding & Schenck, Mycotaxon 34: 667-677, 1989.

4.10.1 Spore characteristics

Spores found singly in soil, borne on sporogenous cell; saffron (0-30-40-0 to 0-30-100-10 and 0-40-60-0 to 0-40-100-10) to orange and apricot (0-60-60-0 to 0-60-100-10); globose to subglobose, spore size 187-444 × 396µm-456 µm.

Spore wall characteristics of three layers (**L1-3**) in one group (Plate 4.3 I & J). Outer layer (**L1**) thin, rigid, smooth, with debris adhering on the surface, 1-3 µm. Middle layer (**L2**) laminate, with 3-4 laminae, saffron to orange, 9-10 µm. Inner layer (**L3**) thin, hyaline, 1-2 µm, inner surface uneven with coalesced spore contents at a localized region (Plate 4.3 J). All spore layers not reacting with Melzer's reagent.

Sporogenous cell borne vertically, sub-hyaline, pale saffron, pale orange to apricot, 36-48 µm wide; cell wall 3-6 µm at point of attachment thinning gradually to 1-3 µm away from the point of attachment; sub-tending hyphae 12-18 µm, sparsely septate, thinning to 9-12 µm away from the sporogenous cell; two hyphal walls 3-4 µm thick gradually narrowing to <1.0 µm away from the sporogenous cell. Spore occlusion is by a plug.

4.10.2 Discussion

The spores of *G. margarita* were separated from other species by the large size, sporogenous cell and the distinct apricot to orange colour. The spores have a smooth shiny surface.

The description of this specimen was done entirely with spores from field soils hence it was not possible to describe the developmental characters and characters of the auxiliary cells. The specimen resembles the revised species *G. ramisporophora* in all aspects (Bentivenga & Morton 1995; Schenck and Perez 1990). The colour is distinctively orange to apricot (golden yellow to yellowish brown) in the specimen from Malawi as the synonym of *G. margarita*, *G. ramisporophora* (Spain *et al.* 1989). The spore shape and size (globose to subglobose; 187-444 × 396-456 µm) of the species from Malawi is within the range of the revised *G. margarita* globose (96-) 200-450(567) µm) to subglobose (143-)150-400 × 200-450(-501) µm) (Spain *et al.* 1989).

The spore wall of the specimen from Malawi and the synonym of *G. margarita, G. ramisporophora* have three layers comprising a slightly thinner outer layer of 1-3 µm compared with 1.4-4.0(-5.7) µm of the true type description and similar to 1.6-2.4 µm of the description of INVAM specimen, a middle laminate layer with 3-4 laminae and slightly thinner (9-10 µm) than the true type specimen description (4.0-28 µm) and the INVAM specimen description of 13-31 µm in PVLG and and 14-23 µm in Melzer's reagent. The thickness of inner layer 1-2 µm and the uneven inner surface with coalesced spore contents at a localized region are within the range of the true type description with thickness of L3 of 1.3-2.6 µm with papillae developing prior to germination, but slightly less than the L3 of the INVAM specimen description of warts 1.2-5 µm and 2.5-3 µm wide in germinating spores. In the species from Malawi, the layers did not react with Melzer's reagent, although the orange spore colour could have obscured the reaction, contrary to the reddish purple reaction of L1 and L2 in the type description. The fact that the synonym of *G. margarita*,

G. ramisporophora, the true type specimen description of *G. margarita* and the INVAM specimen of *G. margarita* stain differently makes them distinct. However, Morton (1988) considered reaction to Melzer's reagent of outer layers (L1 and L2) to be unstable and only the innermost layer (L3) to be stable. Hence based on Morton's observations on reaction of spore layers to Melzer's reagent, the species from Malawi and the synonym of *G. maragarita*, *G. ramisporophora* constitute sufficient evidence to maintain them as the newly described *G. margarita*.

The sporogenous cell was only vertically (apically) attached in the species from Malawi compared with both apical and sub-apically attached in the true type description. The sporogenous cell wall of three layers in both species with the sub-hyaline, pale saffron, pale orange to apricot colours correspond to the colours of the three spore layers. The size (36-48 µm) of the sporogenous cell of the specimen from Malawi is smaller than the true type specimen ((32-)40-60(-72) × (50-)60-80(-83) µm). Hyphal protrusions were not observed in the species from Malawi. This may not necessarily indicate that the hyphal protrusions were absent as the material used for description was from the field. The sub-tending hyphae of diameter 12-18 µm and sparse septae of the species from Malawi is from one sporogenous cell compared with the thinner sub-tending hyphae of the type description species with diameter 9.3-13.9 µm, wall thickness 1.4-2.8 µm and distance between septae variable and becoming more frequent close to the sporogenous cell with one or rarely two bulbous cells, some with protrusions. These differences are not sufficient to reclassify the species.

G. margarita has been recorded in association with *Vangueria infausta* subsp. *infausta* in the Republic of South Africa (Gaur *et al.* 1999), in association with *Faidherbia albida* in Senegal (Dalpe *et al.* 2000) and in association with *Acacia holosericea* and *Acacia mangium* in Sudan (Ba *et al.* 1996). This is the first record of *G. margarita* in Malawi.

4.11 SCUTELLOSPORA CERRADENSIS Spain & Miranda, Mycotaxon 60: 130, 1996. (Plate 4.4 A-E).

4.11.1 Spore characteristics

Spores found singly in soil and in roots as ovoid spores; borne on sporogenous cell (Plate 4.4 A & 4.4 B), hyaline, white, straw (0-0-5-0 to 0-0-15-0) to yellowish cream 0-0-20-0 to 0-0-40-0) in mature spores, globose to subglobose 126-246 × 186-300 µm.

Spore wall characteristics of five layers (**L1-5**) in three groups (groups A, B and C) (Plate 4.4 D). Spore wall breaks easily under slight pressure. Group A a bi-layer, outer sub-layer (**L1**) hyaline, thin, 1.5-3.0 µm, with warts, warts more conspicuous in young spores appearing highly spiny (Plate 4.4 B), 0.5-3.0 µm high and <1.0 wide and 0.5-3.0 µm apart. Inner sub-layer (**L2**), unit, rigid, straw to yellowish cream, 3-6 µm. Group B a bilayer, outer sub-layer **(L3)**, laminate, slightly separated from L4. Inner sub-layer **(L4)** a unit layer completely detached from L5. Group C of a single layer (**L5**), thin, membranous layer, <1.5 µm. L2 stains lightly to reddish orange, and L4 and L5 stains readily to purplish red in Melzer's reagent, other layers not reacting (Plate 4.4 D); sporogenous cell is borne laterally or vertically (Plate 4.4 A & 4.4 B), cinnamon (0-30-50-10 to 0-30-70-10) to pale brown (0-

40-50-10 to 0-40-70-10), cell 21-30 µm wide; cell wall two layers, 1.5-4.5 µm at the point of attachment with spore thinning to <1.0 µm, cell wall continuous with the spore outer layer; subtending hyphae pale brown (0-40-100-0 to 0-40-100-10), width at point close to sporogenous cell 15-25 µm thinning to 6-9 µm away from the cell, hyphal wall 0.5-1.5 µm; germination shield located beneath the sporogenous cell underneath L3, rusty tawny to date brown (20-60-60-0 to 20-80-100-10), subglobose; 21-52 × 30–60 µm, rough margin, 8 fissures with Y configuration, aperture present (Plate 4.4 A); auxiliary cells 5-9, borne on coiled hyphae (Plate 4.4 E), rusty tawny to date brown (20-60-60-0 to 20-80-100-10), hyphal wall <1.5 µm, cells lobed/knobbed, 15-24 µm, lobes 2-4 per cell, 9-12 µm wide, 3-6 µm high, sometimes lobes are so low that they are indistinguishable.

4.11.2 Discussion

The spores are distinguished from other species by the white, yellowish cream to straw colour, the sporogenous cells, large size, and the globose, subglobose to irregular shaped spores. The surface appearance of the spore is finely granular and the distinct brown germination shield is visible under the stereomicroscope.

The species from this site resemble the type specimen description in most aspects with very slight variations observed (Spain & Miranda 1996). The spore size (126-246 × 186-300 µm) of the specimen from Malawi is within the range of sizes (167-)230-363 of the type specimen and 220-380 µm of INVAM specimen description, though at the small end of the size distribution. Five layers in three groups, in PVLG and in PVLG and Melzer's reagent, were observed in the specimens from Malawi compared with four layers and two inner

walls in the type description and four layers and two bi-layered inner flexible walls in the INVAM specimen description. The type specimen has a laminate layer surrounded by two outer layers and an inner flexible layer that generally separates from the other layers at the point of attachment to sporogenous cell. In the specimen from Malawi the laminate layer (L3) is also surrounded by an outer bi-layer (L1 & L2) and a single unit layer (L4). The INVAM laminate layer (L3) is surrounded by an outer bi-layer (L1 and L2) and a thin hyaline layer (L4) that is easily mistaken for a separate flexible inner wall. The type specimen description does not mention L4. This unit layer (L4) could easily be mistaken for part of the laminate layer (L3). This could possibly have led to observation of four layers by Spain & Miranda (1996). L5 in the specimen from Malawi was observed as a wrinkled layer. The single layer of wall group C (L5) in the specimen from Malawi is comparable with the germinal membranous layers in the type specimen description and the INVAM specimen description with the possibility of the wrinkles obscuring the sub-layers observed in the type and the INVAM specimen description. In outer sub-layer (L3) of wall group B for the specimen from Malawi, two slightly separated laminae were observed and staining with Melzer's reagent showed non-staining of these laminae in L3 and heavily staining L4. In the type specimen description, the stained specimen was observed to have five layers. Reaction with Melzer's reagent in the type specimen was observed on the laminate layer (L3) (reddish yellow-reddish brown) and the outer of inner layer 1 (reacting weakly to pink tinge), inner layer 2 (pink), two layers of inner wall two (a light pink outer layer over a reddish purple inner layer). In the species from Malawi, reaction to Melzer's reagent was observed on rigid layer (L2) to reddish orange and L4 & L5 to reddish purple. Except for the differences in perception of layers by Spain and Miranda (1996), spore

reaction to Melzer's reagent and number of spore layers of the type specimen and the INVAM specimen agrees well with stained specimen from Malawi. The inner membranous layer stained pink to purplish red in the specimen from Malawi, INVAM and type specimen. There was no consistency in reactions to Melzer's reagent in the remaining layers. According to Morton (1988) staining of all layers except the innermost membranous layer is unstable and hence of no taxonomic importance, hence the differences in staining of the outer layer may be considered of no taxonomic importance. The difference in staining of outer layer is minimal and not substantial enough to reconsider classification.

The confusion in the perception of particularly the inner layers by Spain & Miranda (1996) was acknowledged in the INVAM specimen description. They confused the numerous folds of the highly plastic L2 of the germinal layer 2 as being a separate layer. The perception of the innermost L5 as one wrinkled layer could be explained by observations made in the INVAM specimen description. They observed both germinal walls (gw1 and gw2) not to separate easily even in vigorously crushed spores and thus resembling one very thick hyaline wall, much like found in *Scutellospora erythropa* (Koske & Walker) Walker & Sanders. The more pronounced warty ornamentations in the younger spores of specimen from Malawi were not mentioned in the type and INVAM specimen description.

The location of the shield was between germinal inner wall one (gw1) and two (gw2) in the type specimen desciption and on the germinal layer 2 in the INVAM specimen compared with the inner bilayer (L4 & L5) in the specimen from Malawi. The colour of the germination shield is consistently light brown to dark brown.

The yellowish-brown to brown sporogenous cell of the type specimen description and INVAM specimen resembles the cinnamon to pale brown colour of the specimen from Malawi. However, the emergence of hyphae from several pegs in the type specimen description was not observed in the specimens from Malawi. There was no evidence of two spores of variable sizes developing at the apex of sporogenous cell as was observed by Spain *et al.* (1989). These are however not significant diagnostic features. The yellowish brown coiled hyphae of the type specimen description corresponds with the rusty tawny to date brown colour of the specimens from Malawi. The size of sporogenous cells (15-24 µm) of the specimen from Malawi is smaller compared to the size (20-33 µm) of cells of the type specimen description and 32-45 µm of cells of the INVAM specimen description. I consider the sporogenous cells, location of germination shield and the auxiliary cells taxonomically similar. I therefore consider this species to be *S. cerradensis*.

The type specimen was recorded from Brazil from a dark red latisol, mostly clayey and poor in phosphorus availability (3.5 µg/g soil) and pH (water 1:2.5) 4.8-5.0. The conditions of soil from the collection site of Malawi specimen was that of a ferric lixisol with 52% sand and 32% clay, pH (water 1:2.5) 6.0 and phosphorus 0.51 µg/g soil. The species from Malawi were also extracted from soils without inorganic fertilizer and soils that recieved 40 kg P ha^{-1} fertilizer since 1992, implying tolerance in this species to high phosphorus as well as the predilection for low phosphorus soils. The type specimen site of collection progressed from predominantly native grass to pasture to soybeans and sites that had been left for a fallow period of five years followed by planting of different legumes for green manure. The type species was successfully cultured on *Tephrosia candida* (Roxb.) DC. In

my case, the natural vegetation of southern Malawi is predominatly ceasalpinoid species. My species was recoverd from the agroforestry systems with *Gliricidia sepium*/maize, *Sesbania sesban*/maize and *S. macrantha*/maize intercrops and maize monocrop. This too implies a lack of sensitivity to plant type and this soil condition. The species was successfully cultured on *G. sepium, S. sesban, S. macrantha, S. siamea, S. spectabilis* and *Sorghum bicolour*.

4.12 SCUTELLOSPORA *aff.* DIPURPURASCENS Morton & Koske., Mycologia 80 (4): 520-524, 1988. (Plate 4.4 F-I)

4.12.1 Spore characteristics

Spores found singly in soil, terminally on sporogenous cell, cream (0-0-5-0 to 0-0-15-0) to yellowish cream 0-0-20-0 and 0-0-40-0), globose 165-300 µm.

Spore wall characteristics of four layers (**L1-4**) in two groups (group A & B) (Plate 4.4 G & H). Group A, a bi-layer with outer sub-layer (**L1**) smooth, pale cream, rigid, breaks only under high pressure, <1.5µm, adhering to L2. Inner sub-layer (**L2**), laminate, 6-9 µm, cream (0-0-5-0 to 0-0-15-0) to yellowish cream (0-0-20-0 and 0-0-40-0), separates from an inner layer (**L3**). Group B, bi-layer of inner hyaline layer with two equal sub-layers (**L3 & L4**), two sub-layers separating at a point enclosing the germination shield, <1.0 µm each (Plate 4.4 G). Reaction to Melzer's reagent, L2 turning orange brown and inner membranous bi-layer (**L3 & L4**) turning purplish red (Plate 4.4 H).

Sporogenous cell borne vertically, sub-hyaline, 36-54 µm, cell wall of two layers 1-1.5 µm, outer layer continuous with the outer rigid spore layer (L1) and inner hyaline thin layer of cell continuous with middle spore layer (L2), peg 4.4-9 µm long, arising from sporogenous cell and projecting towards spore; subtending hyphae septate, 15-24 µm wide near sporogenous cell reducing to 6-9 µm at point of first septum, hyphal wall 1-1.5 µm.

Germination shield, pale cinnamon (0-30-50-10 to 0-30-70-10) (Plate 4.4 F), ovoid 105 × 117 µm, location of the germination shield underneath the sporogenous cell, below the laminate layer (L2), between the two inner sub-layers (L3 & L4), 5-10 germination tubes emerging from the shield.

Auxiliary cells in aggregates of 4-10 cells per coiled hyphae, hyaline to sub-hyaline; clavate; top margin of cell undulated with 4-5 lobes, 1.5-4.5 µm high, 3-9 µm wide, 3-6 µm apart, cells borne on a coiled sub-hayline to pale cinnamon (0-30-50-10 to 0-30-70-10) hyphae, 3-12 µm wide, with hyphal wall 0.5-1.5 µm wide (Plate 4.4 I).

4.12.2 Discussion

The spores of this Scutellospora species were distinguished from spores of other species by the large size, sporogenous cell and the yellowish cream colour. The spore is robust, the surface smooth, shiny and consistently globose.

The specimen resembles *S. dipurpurascens*, *Scutellospora calospora* and *Scutellospora fulgida* Koske & Walker in most aspects with slight variations noted. The spores from Malawi were consistently globose and larger in size (165-300 µm) compared with subglobose to oblong and sometimes irregular spores of INVAM specimen descriptions of *S. dipurpurascens* (140-240 µm) and *S. calospora* (120-220 µm), but was similar in shape of globose to subglobose spores and still larger than *S. fulgida* (120-240 µm). Four spore layers were recorded from the specimen from Malawi compared with two spore layers and one flexible inner germinal layer in *S. fulgida*, two spore wall layers and two hyaline flexible inner germinal layers in *S. calospora* and *S. dipurpurascens*. The descriptions of the first two layers (L1 & L2) were similar to the two layers that make up the spore wall in the INVAM specimen description of the three species. The inner flexible layers that enclose the germination shield in the species from Malawi are similar to the inner germinal layers in the three species. *S. fulgida* has one germinal layer with two layers that appear so adherent particularly in field collected spores and separates in pot culture spores with the outer layer appearing as folds from parts of the wall. The outer layer of the germinal layer is so thin and difficult to resolve without differential interference optics. *S. dipurpurascens* has two germinal layers with germinal layer 1 (gw1) with one hyaline layer often adherent to the laminate spore wall (L2) and it is difficult to resolve and an inner gw2 with two hyaline layers that are almost always adherent. *S. calospora* has two germinal layers each of two layers with gw1 with two layers that are usually adherent, and are positioned very close to spore wall, and thus are difficult to detect, and gw2 with two layers always adherent. It is difficult to separate these three species from the species from Malawi based on the inner flexible layers unless a higher resolution and differential intereferance optics is

used. However, reaction of spore layers to Melzer's reagent is able to separate the *Scutellospora* species from Malawi from some of the species. L2 of the species from Malawi turns orange brown and the inner flexible bilayer (L3 & L4) turns purplish red contrary to L2 of *S. fulgida* that is bright yellow to gold colour in Melzer's reagent, the inner flexible layers not reacting. Since the staining of the inner membranous layer is considered taxononmically diagnostic (Morton, 1988), the *Scutellospora* species from Malawi, *S. dipurpurascens* and *S. calospora* are distinct from *S. fulgida*. The reaction to Melzer's reagent for the species from Malawi is similar to *S. calospora* and *S. dipurpurescens*. No reaction to Melzer's reagent was detected in L2 of *S. dipurpurescens* and *S. calospora*, hence differing from the orange brown colour observed in the specimen from Malawi. In *S. dipurpurescens*, gw1 stained light pink in Melzer's reagent and in gw2, the outer layer stained a weak pink and the inner layer stained red-purple to dark red-purple. In *S. calospora*, reaction to Melzer's reagent is detected in only the gw2 with outer layer 1 producing a weak pink reaction in Melzer's reagent and inner layer 2 staining red-purple to dark red-purple in Melzer's reagent. It was difficult to separate the intensity of reaction to Melzer's reagent in the species from Malawi for L3 and L4 since they did not separate. Staining of the outer wall layers was noted to be unstable and hence of no taxonomic importance (Morton 1988), therefore the species from Malawi differs from *S. fulgida* but greatly resembles both *S. calospora* and *S. dipurpurascens*. A comparison of auxiliary cells places the species from Malawi closer to *S. dipurpurascens*. The species from Malawi has aggregates of 4-10 cells per coiled hyphae compared with 1-10 cells in *S. dipurpurascens* and only 1 cell in *S. calospora*.

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With the exception of *S. calospora* that was recorded from Libyan soils as *Gigaspora calospora* (Nicolson & Gerd.) Gerd. (El-Giahmi *et al.* 1976), There is no record of *S. fulgida* and *S. dipurpurascens* from the African continent. *S. fulgida* was decribed from the coastal sand dunes on the eastern North America seaboard in the root zones of *Ammophila breviligulata* Fern., *Solidago sempervirens* L., and *Uniola paniculata* L., but the plants were not proven mycorrhizal (Koske & Walker 1986b). *S. dipurpurascens* was first recorded in several unmanaged pastures and partially reclaimed surface coal mine in West Virginia (Morton & Koske 1988) and *S. calospora* was recorded in Washington in sand dunes, in east of the Cascade range in Oregon and in orchards, open pine forests, and semi-arid mountains (Gerdemann & Trappe 1974b) and the Forestry Commission in Scotland, sand dunes of California and Massachsetts, in lake dunes in New Jersey and beach dunes in Rhodes island (Koske & Walker 1986b). The natural vegetation of southern Malawi is predominatly ceasalpinoid species. The *Scutellospora* sp. was recoverd from agroforestry systems with *Gliricidia*/maize, *S. sesban*/maize and *S. macrantha*/maize intercrops and maize monocrop. The isolate from Malawi was successfully cultured on *G. sepium, S. sesban, S. macrantha, S. siamea, S. spectabilis* and *S. bicolour*. This implies lack of sensitivity to plant types.

Plate 4.3 A

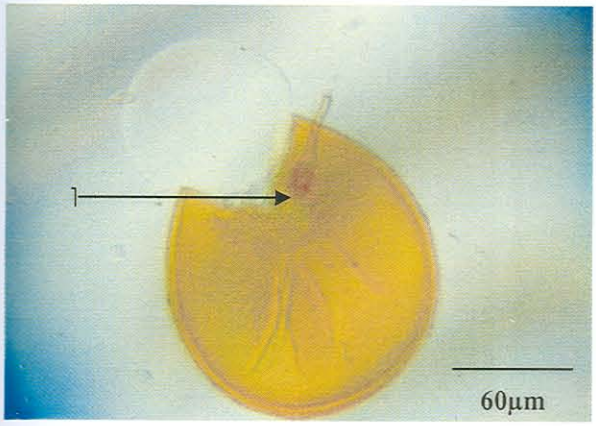


Plate 4.3 B

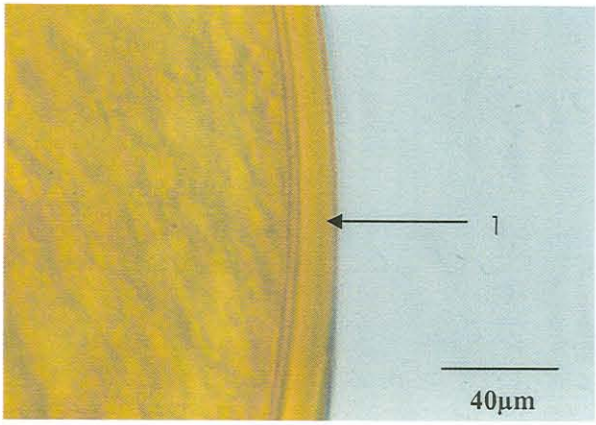


Plate 4.3 C

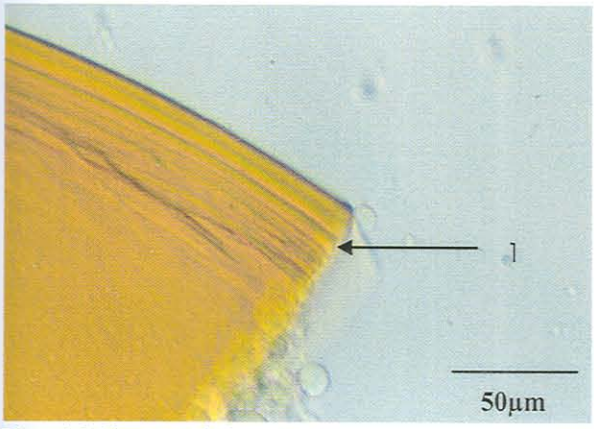


Plate 4.3 D

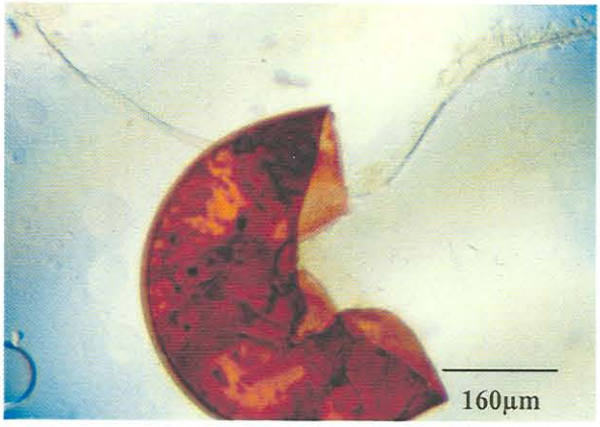


Plate 4.3 Spores of *Gigaspora* sp. (A-D) *Gigaspora* sp (A) (1) Spore with bulbous suspensor cell. (B) (1) Composite wall of young spore without laminations. (C) (1) Laminations in mature spore (D) Patchy staining with Melzer's reagent

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Plate 4.3 E



Plate 4.3 F



Plate 4.3 G

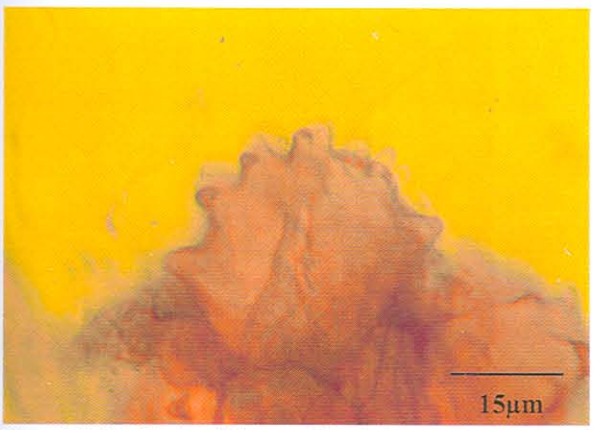


Plate 4.3 (E-G) Auxilliary cells of *Gigaspora* sp (E) clump before auxiliary cell development (F) auxiliary cells exposed (G) spiny auxilliary cell.

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Plate 4.3 H

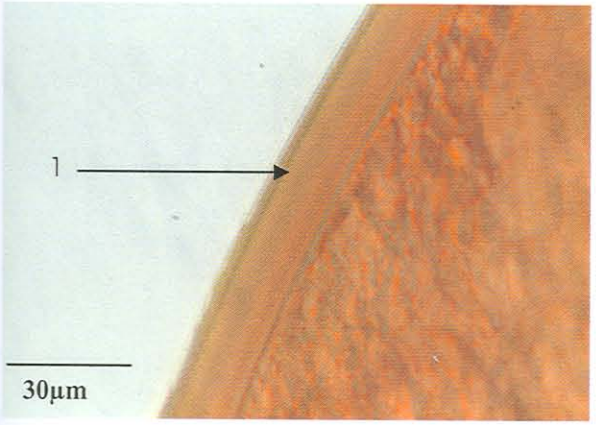


Plate 4.3 I

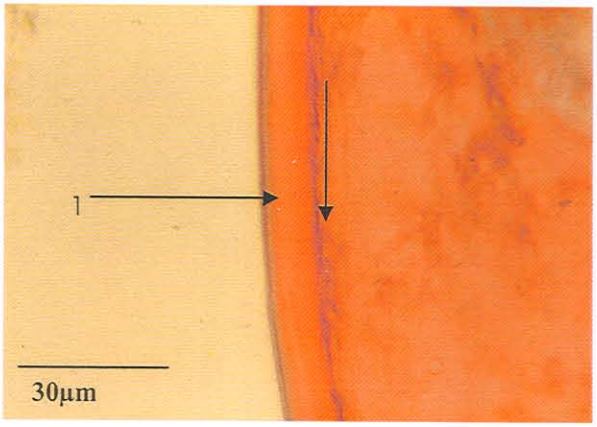


Plate 4.3 J

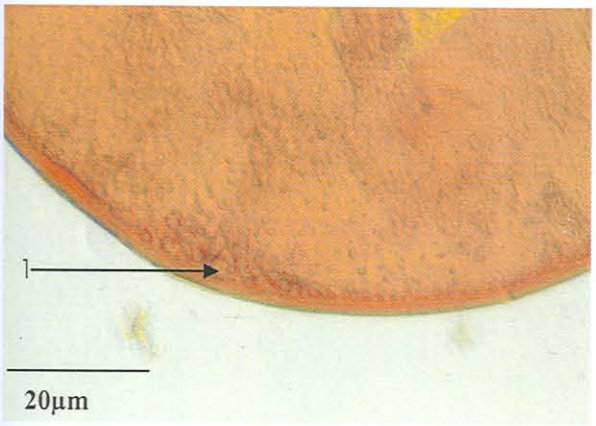


Plate 4.4 A

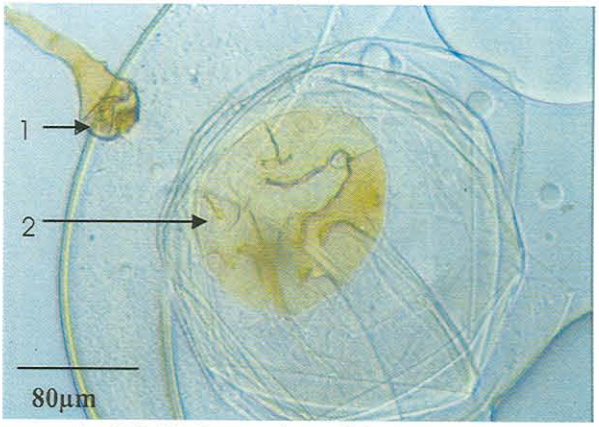


Plate 4.3 Spores of *G. margarita* (H-J) Spore of *Gigaspora margarita* (H) (1) Composite wall in mature spore (I) (1) Composite wall in young spore (J) (1) Germinal point with coalasced spore contents in a localized region.

Plate 4.4 Spores of *S. cerradensis* (A) *Scutellospora cerradensis* (A) Spore with (1) sporogenous cell (2) germination shield.

Plate 4.4 B

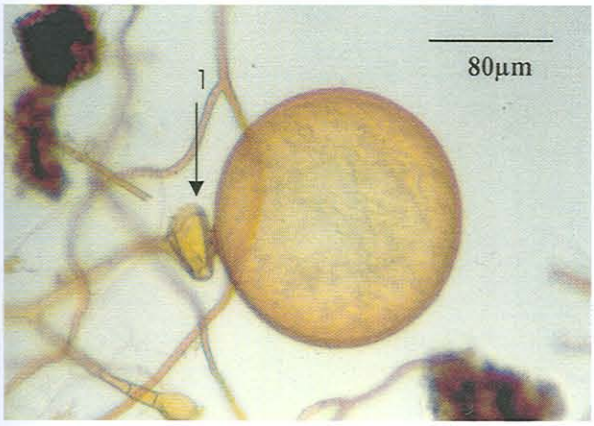


Plate 4.4 C

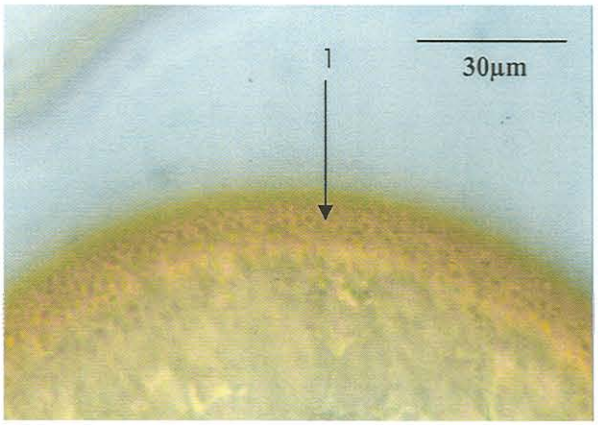


Plate 4.4 D

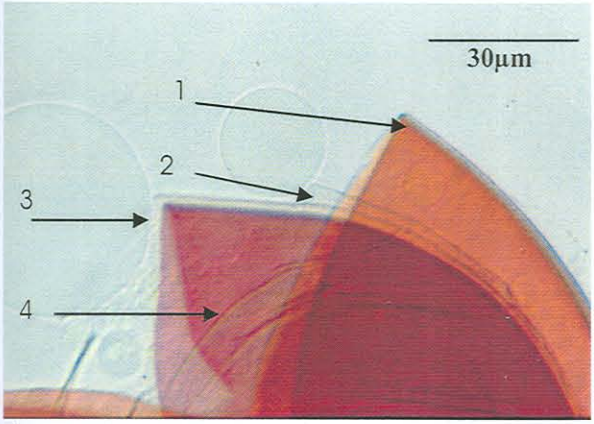


Plate 4.4 E



Plate 4.4 Spores of *S. cerradensis* and auxiliary cells (B-E) *Scutellospora cerradensis* (B) Young spore (1) with large sporogenous cell on hyphae (2) germination shield (C) young spore with outer papillate surface (D) pore resction with melzter's reagent (1) outer layer with two sub-layers with only inner sub-layer staining (2) L 2 staining lightly (3) inner layer with two sub-layers (L4 & L5) with inner membranous sub-layer staining heavily and (E) knobbed auxiliary cell.

Plate 4.4 F

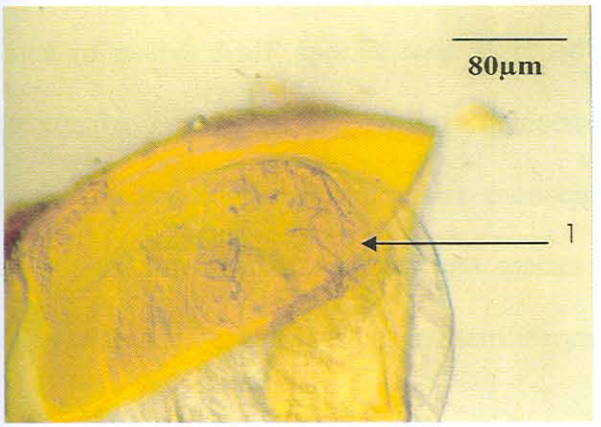


Plate 4.4 G

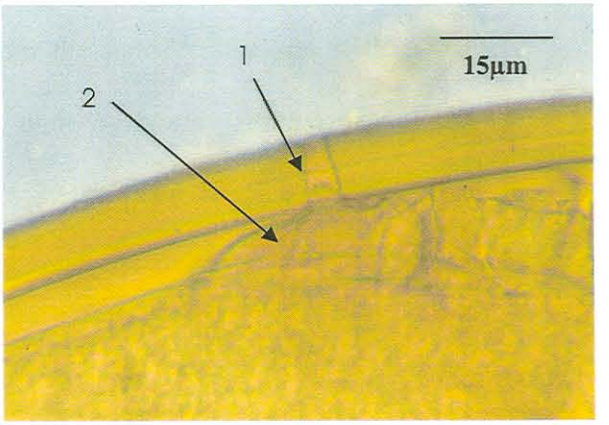


Plate 4.4 H

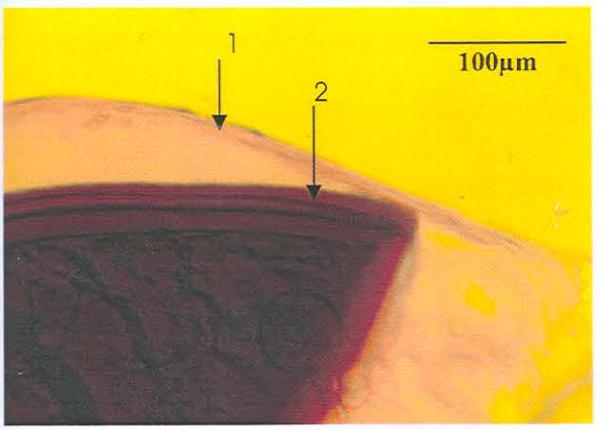


Plate 4.4 I

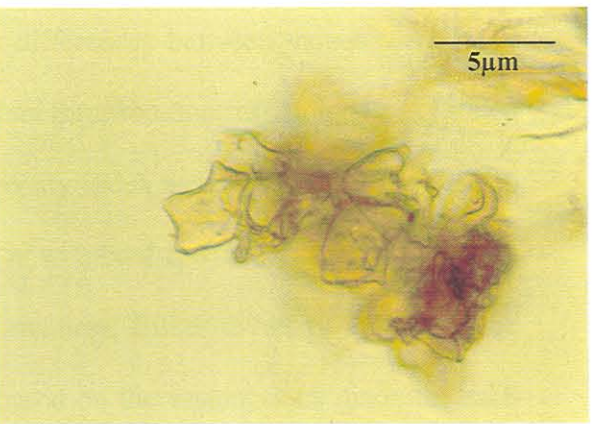


Plate 4.4 Spores of *Scutellopora dipurpurascens*. (F-I) *Scutellospora dipurpurascens*. (F) Spores with (1) pale brown germination shield. (G) Spore with (1) germination tube through wall from the (2) germination shield (H) Crushed spore with (1) outer layer stained lightly with Melzer's reagent and (2) inner layer stained heavily (I) knobbed auxiliary cells.

CHAPTER 5: RESULTS AND DISCUSSION

A total of twelve AMF species were recorded from the agroforestry site with *Gliricidia sepium*/maize intercrop and maize monocrop, and *Sesbania sesban*/maize and *S. macrantha*/maize intercrop and maize monocrop systems. The species are described in chapter four. The species diversity and species frequency of occurrence was evaluated in relation to season, farming system and fertilizer regime.

Similar trends in species diversity were observed with Simpson dominance diversity index and Shannon-Weinner diversity index although the differences between values were more pronounced with Shannon-Weinner diversity index than Simpson dominance index. As stated in the materials and methods, Shannon-Weinner diversity index assumes that all species are represented in the sample and Simpson dominance is weighted towards the most dominant species rather than providing a measure of species richness. Either diversity indice can be used to measure AMF species diversity. However, based on the assumptions made for each index and the need to emphasize on species richness, discussion of results will refer to the Shannon-diversity index

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5.1 *GLIRICIDIA SEPIUM*/MAIZE INTERCROP AND MAIZE MONOCROP

5.1.1 Species diversity

Analysis of variance showed differences in species diversity due to seasonal variation. There were interactions between farming systems, fertilizer and season. Tables of means of Shannon and Simpson diversity indices per 250g soils are presented (Table 5. 1).

Table 5.1. Means of species diversity indices per 250 g air-dried soil in two farming systems at four fertility regimes in the dry and wet season.

Seasonal variations		Dry season				Wet season			
		Fertility regimes				Fertility regimes			
Diversity indices	Farming systems	0	N	P	NP	0	N	P	NP
Shannon	Monocrop	1.01	1.07	1.29	0.81	1.70	1.89	1.72	1.65
	Agroforestry	1.25	0.72	0.84	1.07	1.79	1.55	1.76	1.12
Simpson	Monocrop	0.48	0.51	0.60	0.34	0.77	0.82	0.76	0.78
	Agroforestry	0.56	0.34	0.45	0.53	0.77	0.74	0.80	0.60

Shannon index: SED for comparing 2 treatments in the same season = 0.1902 and SED for comparing same treatment in the different seasons = 0.14. Simpson Index: SED for comparing 2 treatments in the same season= 0.08 and SED for comparing same treatment in different seasons = 0.07. Values with differences less than 2 SED are not significantly different. Each number represents the means of 3 observations.

There were significant ($p<0.05$) differences in mean species diversity between dry season and wet season, wet season species diversity being higher than dry season for all treatments

in both monocrop and agroforestry systems except for NP treatment in agroforestry (Table 5.1). Effects of the two farming systems on AMF species diversity varied with season and fertility regimes. In the absence of inorganic fertilizer, agroforestry plots had slightly higher AMF species diversity than monocrop system plots, however, the increase was not significant (Table 5.1). Inorganic fertilizer modified the effects of farming systems. This was more evident in the agroforestry system in both dry and wet season, species diversity being reduced by application of inorganic fertilizer (Table 5.1). Significant decline in species diversity was however evident in only plots with N and P applied singly in the dry season and plots with a combination of N and P in the wet season (Table 5.1). There were no significant differences between farming systems in the same season at all fertilizer treatments except for P treatments in the dry season and NP treatment in the wet season, where agroforestry systems had lower species diversity than maize monocrop systems.

Observations made in the monocrop system, showed inorganic fertilizer to increase species diversity in plots with N and P fertilizer applied singly and decrease species diversity in plots with a combination of N and P fertilizer (Table 5.1). Significant differences were, however, evident only in the dry season between plots with P and plots with a combination of N and P, the latter AMF species diversity being lower. AMF species diversity in plots with P were however not significantly different from plots without inorganic fertilizer and plots with N fertilizer.

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5.1. 2 Species frequency of occurrence

There were variations in species occurrence due to season, farming systems and fertility regimes. Season significantly ($p<0.05$) affected the occurrence of spores of eight AMF species (Table 5.2). Spores of *Acaulospora* sp. 1, *Archaeospora* sp., *G. aggregatum, Gigaspora* sp. and *S. dipurpurascens* occurred most in the wet season, *A. rehmii*, *G. etunicatum* and *G. margarita* occurred most in the dry season and *Acaulospora* sp 2, *Glomus* sp, *G. geosporum* and *S. cerradensis* were not affected by season.

Farming systems significantly ($p<0.1$) affected the frequency of occurrence of spores of five AMF species (Table 5.2). Spores of *A. rehmii*, *G. aggregatum, G. etunicatum*, *G. geosporum* and *Gigaspora* sp. occurred more in the monocrop system than the agroforestry system.

Table 5.2 Probability of frequency of occurrence of AMF species in two seasons and two farming systems.

	Season[1]			Farming systems[2]		
AMF species	Dry	wet	p-value[3]	Agroforestry	Monocrop	p-value[3]
Acaulospora sp. 1	0.14	0.67	0.002	0.39	0.42	0.607
Acaulospora rehmii	0.39	0.13	0.001	0.18	0.34	0.007
Acaulospora sp. 2	0.31	0.25	0.372	0.26	0.30	0.469
Archaeospora sp.	0.13	0.29	0.002	0.21	0.21	0.967
Glomus aggregatum	0.05	0.35	0.001	0.16	0.25	0.068
Glomus etunicatum	0.84	0.63	0.001	0.66	0.82	0.007
Glomus sp.	0.16	0.12	0.351	0.14	0.14	0.843
Glomus geosporum	0.04	0.03	0.971	0.01	0.06	0.019
Gigaspora margarita	0.17	0.02	0.001	0.11	0.09	0.611
Gigaspora sp.	0.14	0.36	0.001	0.17	0.33	0.003
Scutellospora cerradensis	0.52	0.64	0.054	0.56	0.61	0.418
Scutellospora dipurpurascens	0.17	0.39	0.001	0.28	0.28	0.889

Fungal species with p-value ≤ 0.05 have significantly different probability of frequency of occurrence

[1] Each number represents means of 15 observations (sub-samples), average of four fertility regimes and two farming systems. [2] Each number represents means of 15 observations (sub-samples), average of four fertility regimes and two seasons. [3] p-value is the test of hypothesis of no difference between treatments.

Inorganic fertilizer effect was observed on spores of nine species (Table 5.3), the occurrence of spores of *Acaulospora* sp. 1, *A. rehmii, G. aggregatum* being significantly low at $p \leq 0.05$ and *G. margarita* and *S. dipurpurascens* being significantly low at $p \leq 0.1$ in

plots with inorganic fertilizer, spores of *Archaeospora* sp., *G. geosporum* and *Gigaspora* sp. being significantly ($p \leq 0.05$) high and spores of *S. cerradensis* being variably affected (Table 5.3). Spores of *Acaulospora* sp., *G. etunicatum,* and *Glomus* sp. were also lower in plots with inorganic fertilizer but this was not significant (Table 5.3). Seven species had the least spore occurrence in plots with inorganic N and P applied in combination and nine species had the highest spore occurrence in plots without inorganic fertilizer. There were interactions observed but these were difficult to interpret.

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Table 5.3. Probability of frequency of occurrence of AMF species at four fertility regimes

Fertility regimes	0	N	P	NP	p-value
AMFspecies					
Acaulospora sp. 1	0.61	0.38	0.35	0.29	0.002
Acaulospora rehmii	0.37	0.30	0.22	0.13	0.026
Acaulospora sp.2	0.41	0.25	0.28	0.18	0.34
Archaeospora sp.	0.12	0.31	0.28	0.14	0.025
Glomus aggregatum	0.42	0.16	0.12	0.12	0.001
Glomus etunicatum	0.81	0.76	0.69	0.68	0.68
Glomus sp.	0.27	0.07	0.07	0.14	0.278
Glomus geosporum	0	0.07	0.02	0.05	0.004
Gigaspora margarita	0.17	0.003	0.14	0.05	0.078
Gigaspora sp.	0.15	0.27	0.40	0.17	0.008
Scutellospora cerradensis	0.63	0.56	0.67	0.46	0.027
Scutellospora dipurpurascens	0.45	0.26	0.31	0.31	0.108

Fungal species with p-value ≤ 0.05 have significantly different probability of occurrence. Inorganic N treatment is compared to treatments without N fertilizer, inorganic P treatment is compared to treatments without P fertilizer and inorganic NP is compared to treatments without fertilizer and either N or P.

[1] Each number represents means of 15 observations (sub-samples), an average of two farming systems and two seasons. [2] p-value is the test of hypothesis of no difference between treatments.

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5.1 3 Discussion

The study was undertaken to determine whether farming systems, fertilizer regimes and season affect AMF species, as measured by spores. The high AMF species diversity observed in the wet season contrasts with observations made in previous studies. In a tropical rain forest in Mexico, Guardarrama and Sanchez (1999) noted higher spore numbers and a greater species diversity in the dry season than in wet season and explained the differences with the seasonal effect on root senescence. In arid grassland in Namibia, Jakobsen (1997) associated high decline in spore production and species diversity with the high moisture content in the wet season, and linked the low species diversity in the wet season to increase in spore germination and root colonisation. The low species diversity in the dry season compared to wet season could also imply low seasonal sporulation of most species in dry conditions.

The study showed interactions among farming systems, season and fertility regimes. Generally farming systems affected species diversity. AMF diversity was higher in agroforestry plots without inorganic fertilizer than maize monocropped plots. Agroforestry systems incorporate leaf biomass (organic matter) into the soil. Verma and Arya (1998) noted increase in mycorrhizal spores and colonisation in the growth of micropropagated *Dendrocalamus asper* (bamboo) plantlets with organic amendments. Similarly agroforestry plots with organic ammendements of *Gliricidia* leaf biomass and without inorganic fertilizer had higher AMF species diversity compared to monocropped plots in the dry season. The increase in species diversity, as measured by spores, in the agroforestry plots without addition of inorganic fertilizer may be linked to the organic ammendements with *Gliricidia* leaves.

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Application of inorganic fertilizer modified the effects of farming systems, fertilizer effect being greater in agroforestry more than in the monocrop system. Decrease in species diversity with inorganic input was evident in agroforestry, in the dry and wet season. The effect of inorganic fertilizer in monocrop system varied with season; plots with P fertilizer having higher species diversity than plots with a combination of N and P in the dry season. The combination of N and P seemed to have the least species diversity irrespective of the farming system and season. The site has a history of high P application and this may explain why species were not sensitive to P application. Negative effect of inorganic fertilizer is widely documented. Shukla and Vanjare (1990) noted decline in mycorrhizal spore population in oilseed crop fields with application of phosphorus and nitrogen. The negative effects of inorganic fertilizer (P) were attributed to the threshold concentrations of the nutrient (P) in the roots that inhibited mycorrhizal colonisation (Douds & Chaney, 1986).

Although the three inorganic fertilizer regimes reduced species diversity in agroforestry plots, the decline was more pronounced in plots with N fertilizer in the dry season and a combination of N and P in the wet season. Previous studies showed high concentrations of inorganic nitrogen compounds to reduce mycorrhizal colonisation, and the reduction, generally greater with NH_4^+ than NO_3^- (Chambers *et al.* 1980a; Chambers *et al.* 1980b). The differences between the two sources of nitrogen were attributed to mycorrhizal preference for NH_4^+, which it readily utilizes than the highly mobile NO_3^- source which must be converted to NH_4^+ before it is utilized (Bowen & Smith 1981). Differential effects of types of fertilizer was reported by Sreenvasa and Bagyaraj (1990) who noted an increase in number of spores with increase in nitrogen in the form of nitrate and ammonium nitrate. The seasonal variation of

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effects of fertility regimes in the agroforestry plots may be explained by variation in the forms of nutrients in the two seasons. Nitrogen fertilizer seemed to affect root colonisation differently from spore production, with the former reduced (Chambers *et al.* 1980a; Chambers *et al.* 1980b) and the latter enhanced (Sreenvasa & Bagyaraj 1990). In this study, AMF species diversity measured as spores was also reduced. There is need to simultaneously investigate the effects of nutrients on root colonisation and spore production. A study on nutrient dynamics undertaken by Ikerra *et al.* (1999) at this same agroforestry and monocrop experiment showed *Gliricidia* plots to accumulate more ammonium-N during the dry season and nitrate-N remaining constant during the dry season but rising rapidly after the onset of rains. Seasonal variation in species diversity at the *Gliricidia* agroforestry plots may be linked to the changes in forms of nitrogen. Ammonium-N (NH_4^+), was higher in the dry season and this may explain the decrease in AMF species diversity in the dry season compared with the increase in the wet season when NO_3^- was the dominant nitrogen form. There is need to investigate the effect of different forms of N and P on AMF species in relation to climate and soil conditions.

In this study, high AMF species diversity was observed in maize monocropped plots with P and low species diversity in agroforestry plots with P in the dry season. A study that slightly contrast the observations made in the monocrop system is by Nielsen *et al.* (1981) who noted low spore numbers to be associated with high levels of phosphorus. The contrast in response to P between the *Gliricidia*/maize intercrop and maize monocrop systems may be explained by slightly similar observations made by Bevege (1972). He noted a delicate balance between N and P and found root colonisation to increase as nitrogen content increased if phosphorus

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levels were moderate and nitrogen application to be inhibitory at high levels of phosphorus. In the two farming systems, P content remained constant in both monocrop and agroforestry plots, however application of *Gliricidia* leaf biomass in agroforestry plots altered the N content (Ikerra *et al.* 1999). The variations in N content may explain the differences in response to P by AMF species in the two farming systems. The study site is deficient in nitrogen and this may explain the response of AMF to changes in nitrogen more than phosphorus. There is need to investigate the effects of different proportions of nutrients on mycorrhizal symbiosis, and also whether changes in the levels of the most deficient nutrient in the soil would affect mycorrhizal symbiosis more. Clark (1997) observed that in acid soils, minerals whose concentration is enhanced by AMF are those commonly deficient in the acid soils.

A study on the frequency of occurrence of spores of individual AMF species showed marked variations with season, farming systems and fertility regimes. There was no trend in occurrence of species in the same family or genera. The differential occurrence of species depicts variation in species tolerance to seasons. Spores of *A. rehmii*, *G. etunicatum* and *G. margarita* were more tolerant to dry season while the decline in occurrence of spores of *Acaulospora* sp. 1, *Archaeospora* sp., *G. aggregatum*, *Gigaspora* sp. and *S. dipurpurascens* may be linked with low tolerance to dry conditions. Spores of *Acaulospora* sp. 2, *Glomus* sp., *G. geosporum* and *S. cerradensis* were tolerant to dry and wet conditions. The occurrence of spores of species may also be linked with seasonal variation in sporulation and not necessarily tolerance, with spores of species higher in the dry season sporulating more under the dry conditions and spores higher in the wet season sporulating more under wet conditions.

Seasonal conditions favouring occurrence of *G. aggregatum* is supported by other studies. Sylvia (1986) observed spore counts of *G. globiferum* to increase more than 500 % from May to August, while *G. aggregatum* increased less than 30 %. The mechanisms explaining tolerance is not clear. However, spore wall characteristic is a possible character that might determine spore tolerance to seasonal environmental conditions in the soil. There is need to investigate the relationship between spore wall character and spore tolerance to different levels of moisture content in the soil with spore tolerance and also spore production by a species. This is a prerequisite particularly if AMF inoculation programs are to be undertaken in habitats prone to drought conditions, where spores would be the most favourable source of inoculum as they last longer in the soil than other propagules (Babara & Hetrick 1984).

Another factor that may also be linked to variation in species frequency of occurrence is the stage of plant growth. Maximal spore abundance of some species was observed to coincide with the end of the host growing cycle or reproductive cycle (Giovanneti 1985). Sylvia (1986) also reported that while the overall population of AMF increased as plants matured, sporulation by individual species was not synchronous. The study at the Malawi site did not evaluate species occurrence at different stages of plant growth.

There were variations in species frequency of occurrence between the two farming systems such that a fungus–farming system association was found. Spores of *A. rehmii*, *G. etunicatum*, *G. geosporum*, *G. aggregatum* and *Gigaspora* sp. were higher in monocrop systems. Dodd *et al.* (1990) observed the occurrence of AMF species to vary widely with type of crop, with spore occurrence implying species specific associations with the host plant.

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Fertility regimes had the greatest effect on species frequency of occurrence. Only three species, *Acaulospora* sp 2, *G. etunicatum* and *Glomus* sp. were not affected by changes in fertility. Except for *Archaeospora* sp., *G. geosporam* and *Gigaspora* sp. the frequency of occurrence of the nine species declined with application of inorganic fertilizer with N and P applied in combination having the greatest effect. This implies that few AMF species are tolerant to inorganic fertilizer and may also imply that sporulation of most AMF species is reduced by inorganic fertilizer. This may be linked with the inherent soil characteristics at the study site being deficient in nutrients hence sporulation of most AMF species from this site has evolved under nutrient deficient soils and might therefore be sensitive to changes. Only *G. etunicatum* seemed to have a wide range of adaptation to soil conditions. There is limited information relating individual AMF species tolerance to inorganic fertilizer. *G. aggregatum* and *G. etunicatum* are the only species reported to be tolerant to high input in tropical agricultural soils (Sieverding 1991). In this study, the spores of *G. aggregatum* were sensitive to inorganic fertilizer while *G. etunicatum* was not affected by changes in fertility. *G. etunicatum* has been isolated from a broad range of habitats (Shepherd *et al.* 1996; Gaur *et al.* 1999; Wilson *et al.* 1992b) in Africa. *G. etunicatum* was recorded in the P deficient soils of western Kenya in association with *Leucaena leucocephala* and *Calliandra calothyrsus* (Shepherd *et al.* 1996), with *Terminalia ivorensis* plantations in Cameroon (Mason *et al.* 1992) and Ivory Coast (Wilson *et al.* 1992b), banana plantation in Uganda (Msiska 2001), *A. nioltica* in Snegal (Ginwal *et al.* 1997), *Vangueria infausta* subsp. *infausta* (Gaur *et al.* 1999) and the arid regions of Namibia (Stutz *et al.* 2000). *G. margarita* has been reported in association with *Vangueria infausta* subsp. *infausta* (Gaur *et al.* 1999), *Faidherbia albida*

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(Dalpe *et al.* 2000) and *Acacia holosericea.* and *Acacia mangium* (Ba *et al.* 1996). This signifies a broad range of adaptations to soil and host plant conditions for *G. etunicatum* and *G. margarita*. The occurrence of *A. rehmii, G. aggregatum, S. cerradensis* and *S. dipurpurascens* are recorded for the first time in Africa.

5.2. TWO *SESBANIA* SPECIES/ MAIZE INTERCROP AND MAIZE MONOCROP.

5.2.1. Species diversity

Analysis of variance of diversity indices data showed farming systems and inorganic fertilizer to significantly ($p \leq 0.05$) affect AMF species diversity.

Farming systems had significant ($p \leq 0.05$) effects on the diversity of AMF species. Species diversity in the three farming systems differed, species diversity being lower in these agroforestry systems than maize monocrop system opposite to results of *Gliricidia*/maize intercrop vs. maize monocrop experiment (Table 5.4). The decline was more prominent in agroforestry system with *S. macrantha*/maize intercrop than with *S. sesban*/maize intercrop system (Table 5.4). Species diversity of the *S. macrantha*/maize intercrop was however not significantly ($p \leq 0.05$) different from *S. sesban*/maize intercrop system.

Table. 5.4 Main effects of farming systems, nitrogen and phosphorus fertilizer on species diversity of AMF.

Factors	Farming systems			Nitrogen (N)		Phosphorus (P)	
Levels	*S.sesban*	*S.macrantha*	monocrop	none	present	none	present
Diversity indices							
Shannon	1.43	1.30	1.53	1.33	1.51	1.43	1.42
Simpson	0.72	0.64	0.74	0.65	0.73	0.69	0.68

Values with differences greater than 2SED are significantly different. Shannon index: SED comparing three farming stsytems = 0.08, two nitrogen levels = 0.07 and two phosphorus levels = 0.07. Simpson index: SED for comparing three farming systems = 0.03, two nitrogen levels 0.02 and two phosphorus levels 0.024.

Fertilizer effect was evident in plots with inorganic N fertilizer, AMF species diversity being significantly ($p \leq 0.05$) higher in plots with inorganic N fertilizer than in plots without (Table 5.4). Inorganic P fertilizer had no significant ($p \leq 0.05$) effect on AMF species diversity (Table 5.5).

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Table 5.5 Mean effects of inorganic phosphorus fertilizer by season on species diversity of AMF.

Factors	Dry season		Wet season	
Phosphorus	none	present	none	present
Diversity indices				
Shannon	1.55	1.40	1.30	1.43
Simpson	0.72	0.66	0.66	0.71

Values with differences greater than 2SED are significantly different. Shannon index: SED for comparing two phosphorus levels = 0.09. Simpson index: SED for comparing two phosphorus levels = 0.03.

5.2.2 Frequency of occurrence of AMF

Season had the greatest effect on the frequency of occurrence of spores of AMF species. Season significantly ($p \leq 0.05$) affected the frequency of occurrence of all AMF species, spores of nine species being predominant in the dry season and three in the wet season (Table 5.6).

Table 5.6 Probability of frequency of occurrence of AMF species in two seasons and three farming systems.

	Season[1]			Farming systems[2]			
AMF species	Dry	wet	p-value[3]	monocrop	SS	SM	p-value[3]
Acaulospora sp. 1	0.14	0.27	0.002	0.21	0.22	0.19	0.89
Acaulospora rehmii	0.38	0.24	0.003	0.37	0.28	0.28	0.18
Acaulospora sp. 2	0.34	0.15	0.001	0.21	0.32	0.21	0.06
Archaeospora sp.	0.12	0.03	0.001	0.12	0.05	0.06	0.12
Glomus aggregatum	0.06	0.15	0.003	0.13	0.08	0.11	0.42
Glomus etunicatum	0.76	0.52	0.001	0.66	0.58	0.67	0.29
Glomus sp.	0.13	0.24	0.006	0.14	0.18	0.25	0.09
Glomus geosporum.	0.19	0.01	0.001	0.13	0.12	0.06	0.11
Gigaspora margarita	0.11	0.02	0.001	0.05	0.10	0.05	0.22
Gigaspora sp.	0.28	0.09	0.001	0.21	0.23	0.12	0.04
Scutellospora cerradensis	0.59	0.27	0.001	0.44	0.37	0.48	0.18
Scutellospora dipurpurascens	0.14	0.01	0.001	0.09	0.02	0.12	0.003

Fungal species with p-value ≤ 0.05 have significantly different probability of occurrence

[1]Each number represents means of 15 observations (sub-samples), average of four fertility regimes and three farming systems. [2]Each number represents means of 15 observations (sub-samples), average of four fertility regimes and two seasons. [3]p-value is the test of the hypothesis of no difference between treatments.

SS = *Sesbania sesban*/maize intercrop and SM = *Sesbania macrantha*/maize intercrop.

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Farming systems significantly ($p \leq 0.05$) affected the frequency of occurrence of *Gigaspora* sp. and *S. dipurpurascens* and slightly ($p \leq 0.1$) affected *Acaulospora* sp.2 and *Glomus* sp. (Table 5.6). The species differed in their association with farming systems, frequency of occurrence of spores of *Acaulospora* sp. 2 higher and *S. dipurpurascens* least in *S. sesaban*/maize intercrop system and *Glomus* sp. higher and *Gigaspora* sp. least in *S. macrantha*/maize intercrop system (Table 5.6). The remaining eight species were not affected by farming systems.

Effect of inorganic fertilizer was observed on few arburscular mycorrhizal species, four species significantly ($p \leq 0.05$) affected and two species slightly ($p \leq 0.1$) affected (Table 5.7). *A. rehmii* was significantly ($p \leq 0.05$) affected by N with the frequency of occurrence of spores higher in plots with N fertilizer. *Archaeospora* sp. was significantly ($p \leq 0.05$) affected by P with the frequency of occurrence of spores higher in plots with P fertilizer. The frequency of occurrence of spores of *S. cerradensis* were significantly ($p \leq 0.05$) affected by NP fertilizer, with the frequency of occurrence of spores in plots with NP highest. Both N & P slightly ($p \leq 0.1$) decreased the frequency of occurrenace of spores of *G. etunicatum*. The frequency of occurrence of spores of *G. etunicatum* was lower in plots with inorganic fertilizer N and P applied singly and in combination. *S. dipurpurascens* was also slightly ($p \leq 0.1$) affected by NP with frequency of occurrence of spores least in plots with N applied singly and highest in plots with inorganic P fertilizer.

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Table 5.7 Probability of frequency of occurrence of AMF species at four fertility regimes

	Fertility regimes				p-values		
AMF species	0	N	P	NP	N	P	NP
Acaulospora sp. 1	0.23	0.17	0.23	0.19	0.19	0.79	0.77
Acaulospora rehmii	0.23	0.33	0.27	0.41	0.01	0.25	0.74
Acaulospora sp. 2	0.26	0.27	0.21	0.26	0.54	0.54	0.69
Archaeospora sp.	0.06	0.03	0.08	0.13	0.54	0.03	0.20
Glomus aggregatum	0.09	0.09	0.11	0.12	0.86	0.38	0.88
Glomus etunicatum	0.70	0.53	0.66	0.67	0.12	0.38	0.08
Glomus sp.	0.19	0.18	0.20	0.19	0.79	0.79	1.0
Glomus geosporum.	0.16	0.10	0.07	0.09	0.60	0.11	0.25
Gigaspora margarita	0.06	0.08	0.06	0.08	0.4	1.0	1.0
Gigaspora sp.	0.19	0.17	0.17	0.22	0.68	0.68	0.35
Scutellospora cerradensis	0.48	0.44	0.30	0.50	0.11	0.24	0.02
Scutellospora dipurpurascens	0.10	0.01	0.11	0.08	0.02	0.15	0.07

Fungal species with p-value ≤ 0.05 have significantly different probability of frequency of occurrence. Each mean value represents means of 15 observations (sub-samples), an average of two farming systems and two seasons. p-value for N is the significant level for testing whether there is a main effect of N on species frequency of occurrence. p-value for P is the significant level for testing whether there is a main effect of P on species frequency of occurrence. p-value for N ×P is the significant level for testing for N by P interaction effect on species frequency of occurrence.

5.2.3 Discussion

The study was undertaken to determine whether agroforestry systems differ in their effects on AMF species, as measured by spores. The agroforestry system *S. sesban*/maize and *S. macrantha*/maize intercrop had similar effects on AMF species diversity. Both *S. sesban*/maize intercrop and *S. macrantha*/maize intercrop lowered the diversity of AMF species compared to the maize monocrop system. These observations however contradict findings at the same site in *Gliricidia*/maize intercrop system, which had higher species diversity than maize monocrop system. The lowering effect by *S. macrantha* agroforestry system was however more pronounced. *Sesbania sesban* and *S. macrantha* are closely related species, implying the possibility of plant species associations with mycorrhizal fungi being a hereditary trait. The lower species diversity in the two agroforestry systems with *Sesbania* species, compared to maize monocrop system, may be linked with root turnover. In temperate climates where root growth by perennial plants is more or less continuous, Baylis (1969) found that few spores were produced despite high levels of colonisation and suggested lack of evolutionary stimulus for spore production if root growth was not intermittent. Baylis (1969) also observed a New Zealand bush soil to have fewer spores compared to soils carrying native grasses or those of cultivation and suggested that the native grass soils were subjected to intermittent root growth and drying that stimulates sporulation. At the *Sesbania* spp. agroforestry site, trees were left longer in agroforestry plots as dry season fallow and hence tree roots lasted longer than maize roots. Maize plants were in active growth for a period of only four months, thereafter maize was harvested in the dry season, leaving maize roots to decay. Guadarrama and Sanchez (1999) noted high species and spores numbers in a tropical rain forest in the dry season compared to wet season and linked this to root senescence. Soil moisture content may also be a possible

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factor that affected sporulation in AMF species. In an alley cropping agroforestry system, soil moisture content under both *L. leucocephala* and *Flemigia macrophyla* (Wild.) Kuntze hedgerows was higher than under maize rows throughout the growing season (Chirwa *et al.* 1994). Baylis (1969) observed actively growing roots in a Newzealand bush soil with year-round adequate soil moisture and temperature to favour AMF species that do not necessarily sporulate. The moisture content in the two agroforestry systems with *Sesbania* species and maize intercrop was not evaluated. However, it is likely that soil moisture content was higher in the two agroforestry systems with *Sesbania* species than the maize monocrop system and hence the low species diversity in the two *Sesbania* agroforestry systems compared with the maize monocrop.

The observations made in the two agroforestry systems with *Sesbania* species however contrasted observations made in the *Gliricidia*/maize intercrop plots with no addition of inorganic fertilizer. Studies that may relate to the differences in response between agroforestry systems with *Sesbania* species and *Gliricidia* are by Ocampo *et al.* (1980) who demonstrated less colonisation due to high root biomass and vice versa. The root biomass in the agroforestry system with the two *Sesbania* species may be higher than in *Gliricidia* as evidenced by the large quantities of root hairs in the two *Sesbania* spp. compared with *Gliricidia sepium* (Plate 5.8). Baylis (1970) also observed plants with denser root hairs to associate less with mycorrhizal fungi. There is therefore a possibility that the two *Sesbania* spp. with denser root hairs than *Gliricidia sepium* could have associated less with AMF.

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The high species diversity in plots with inorganic N is close to observations made by Sreenvasa and Bagyaraj (1990) who noted increase in spore numbers with increase in nitrogen in the form of nitrate and ammonium nitrate. This contradicts observations made at the *Gliricidia*/maize intercrop site where inorganic N reduced AMF species diversity. Early studies showed contradicting effects with nitrogen fertilizer to not only reduce root colonisation but suppress spore formation as well (Hayman 1970). Chambers *et al.* (1980a) also and Chambers *et al.* (1980b) observed high concentrations of inorganic nitrogen compounds to reduce mycorrhizal colonisation, and the reduction was generally greater with NH_4^+ than NO_3. The differences between the two sources of nitrogen were linked to mycorrhizal preference for NH_4^+ than NO_3^- (Bowen & Smith 1981). The differences in response to N at the *Sesbania* species site compared with the *Gliricidia* site may be linked with differences in N levels at the two sites. The history of the *Sesbania* site differs from the *Glricidia* site with the *Gliricidia* having comparatively higher N levels than the *Sesbania* species site (Ikerra *et al.* 1999; Ikerra *et al.* 2001). The contradicting reports by other workers on the effects of N on AMF species may be linked to lack of specification of the source of nitrogen and the inherent nitrogen level in the soil. This study suggests that response of spores of AMF species to N fertilizer may depend on the existing nitrogen source and the inherent nitrogen levels in the soil. There is also a possibility that AMF species have threshold levels for each nutrient beyond which changes in the mycorrhizal symbiosis are observed. Inorganic P fertilizer had no effect on AMF species diversity. Similar observations were made at the *Gliricidia*/maize intercrop site, hence confirming the site history of high P application.

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A study on the frequency of occurrence of individual spores of AMF species, showed marked variations with season and slight variation with farming systems and fertility regimes. There was no trend in frequency of occurrence of spores of species in the same family or genus. Spores of most species seemed to occur in the dry season. Similar observations were made by Guadarrama and Sanchez (1999) who noted high species diversity and spore numbers in the dry season and linked this to root senescence. Wet season was associated with high mycorrhizal activity, root colonisation and spore germination followed by decline in spore production and species number in the arid grasslands of Namibia (Jakobsen 1997). The high frequency of occurrence of some species in the dry season followed by a decrease in the wet season could possibly be linked with low tolerance of spores to dry conditions or low sporulation. Two of the species, *Acaulospora* sp. 1 and *G. aggregatum*, prevalent in the wet season, exhibited similar occurrence in a *Gliricidia*/maize intercrop and maize monocrop experiment studied at the same site, prevalence of species in the wet season being possibly linked with spore wall morphology or high sporulation. Considering that southern Malawi is prone to drought, most species adapted to a dry season are likely to maintain high inoculum potential of soils. Spore wall morphology and/or high spore production are likely stratrgies of species survival under dry conditions as less sporulating species that are in the form of mycelia and root fragments become non-viable in prolonged dry conditions (Barbara & Hetrick 1984).

Studies on frequency of occurrence of spores of AMF species showed *Acaulospora* sp. 2 to associate most with *S. sesban*/maize intercrop and *S. dipurpurascens* to associate less. *Glomus* sp. associated most with *S. macrantha*/maize intercrop system and *Gigaspora* sp. associated

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less. There are numerous studies that have reported specific associations between AMF species and plant species/farming systems. Schenck and Kinloch (1980) observed *Gigaspora* species to associate with soybeans and *G. fasciculatum* and *G. clarum* with Bahia grass. Khalil *et al.* (1992) also noted four *Glomus* species to be most abundant around the rhizosphere of soybeans, *Gigaspora* species second most abundant and *Acaulospora* species the least. As with my own study, there were no generic or family trends observed in the association of spores of AMF with the plant species. The concept of host-species association needs to be investigated by techniques that would evaluate whether species found in abundance in the plant rhizosphere would also be the most dominant species in the roots of host plants. The occurrence of spores in the rhizosphere of the host plants implies AMF species association with a host plant although this does not necessarily indicate the level of colonization of the sporulating species in the root of the host plant. The use of spores to determine host/plant associations also excludes non-sporulating species that may be dominant colonizers in the root system.

The differential response in spore production of arbuscular mycorrhizal species to different types of fertilizer dismisses the generalization that inorganic fertilizers always have a lowering effect on all arbuscular mycorrhizal species. This study showed sporulation of few species not to be affected by inorganic fertilizer and disapproved the generalizations. Mosse *et al.* (1981) observed AMF to differ in their sensitivity and tolerance to applied P and Cooper (1978) reported mycorrhizal colonisation in well fertilized soils and linked this to AMF tolerance to high P regimes. The levels and types of nutrients under each environmental soil

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conditions should therefore be determined to establish the range of conditions for mycorrhizal survival and functioning for each species.

AMF species most sensitive to inorganic fertilizer application were *A. rehmii*, *Archaeospora* sp., *G. etunicatum*, *S. cerradensis* and *S. dipurpurascens*. Studies by Sieverding (1991) showed *G. etunicatum* to be more prevalent in soils with high inorganic input. At the *Sesbania* spp/maize intercrop and maize monocrop site the spores of *G. etunicatum* were less frequent in plots with inorganic fertilizer. A study undertaken at the *Gliricidia*/maize intercrop and maize monocrop site showed variable effects of inorganic fertilizer on the occurrence of spores of the four species. Spores of *Archaeospora* sp. which were frequent in plots with inorganic P at the *Sesbania* spp/maize intercrop experimental site were also found to be frequent in plots with inorganic P and also N in the *Gliricidia*/maize intercrop system. The spores of *A. rehmii* occurred most to soil conditions with inorganic N input, while *S. cerradensis* and *S. dipurpurascens* occurred most in soils with P fertilizer.

5.3 FUNCTIONAL DIVERSITY

The morphology of AMF colonisation of the four host plants by the three AMF species was characterized by entry points, coils, arbuscules and hyphae (Plate 5.9). Different types of AM morphological structures were observed in the host plants although characterization of the AMF morphological structures in different host plants was not undertaken in this study. Except for *G. sepium* that had diffused arbuscular-like structures, all the three plant species had AM morphological strucures described (Plate 5.9). There was no colonisation observed in

the control, hence suggesting an effective control. The open air glasshouse bench experimental system was therefore effective in this study.

5.3.1 *Gliricidia sepium*

Inoculation with AMF species affected the growth of *G. sepium* (Table 5.8). The effect was particularly evident on the root dry weight. Inoculation with all the three fungal species enhanced root dry weight. However, a significant ($p \leq 0.05$) increase in root weight was only observed following inoculation with *G. aggregatum* and *G. etunicatum*. There was a slight increase in height following inoculation with all the fungal species and a slight increase in shoot weight following inoculation with *G. aggregatum*. Inoculation with *G. aggregatum* produced the tallest plants and the greatest shoot and root dry weights. There was a significant ($p \leq 0.05$) positive correlation between shoot weight and plant height and shoot weight and root weight, but no significant ($p \leq 0.05$) correlation between plant height and root weight and between all plant growth parameters and level of colonisation (Table 5.9).

5.3.2 *Sesbania sesban*

The AMF species differed in their effects on plant growth and infection level (Table 5.8). *Acaulospora* sp. 1 produced the tallest plants with the greatest shoot and root dry weight and the highest colonisation level. *Acaulospora* sp. 1 seemed to be the most effective fungal species on the growth of *S. sesban*. *Acaulospora* sp. 1 produced significantly ($p \leq 0.05$) higher plant height than plants inoculated with *G. aggregatum*. Although *G. aggregatum* had negative effects on plant height and shoot weight, it slightly enhanced the root weight of *S. sesban*. This was however, not significant. *G. aggregatum* was significantly ($p \leq 0.05$) less

effective in improving shoot weight and height than *Acaulospora* sp. 1, while *G. etunicatum* was less effective on improving only the shoot dry weight compared to *Acaulospora* sp. 1.

Plants inoculated with *Acaulospora* sp. 1 and *G. aggregatum* had significantly ($p \leq 0.05$) higher colonisation levels than *G. etunicatum*. There was significant ($p \leq 0.05$) positive correlation between plant height and root weight, plant height and shoot weight, and shoot weight and root weight. There was however, no significant ($p \leq 0.05$) correlation between all plant growth parameters and root colonisation levels.

5.3.3 *Sesbania macrantha*

Inoculation with the AMF had no significant effects on the growth of *S. macrantha*, although the colonisation levels were significantly ($p=0.002$) different. *Acaulospora* sp. 1 had the highest colonisation level. There was a slight increase in height with inoculation though the increase was not significant. A slight increase in root dry weight was also noted with *G. aggregatum* and a decrease with *G. etunicatum*. Again these changes were not statistically significant. There was no change in shoot dry weight following inoculation with all the three fungal species. *Acaulospora* sp. 1 had significantly ($p \leq 0.05$) higher colonisation level than *G. aggregatum* and *G. etunicatum*. There was a significant ($p \leq 0.05$) positive correlation between root weight and shoot weight but both shoot and root weight had no significant ($p \leq 0.05$) correlation with plant height and all plant growth parameters and had no significant ($p \leq 0.05$) correlation with root colonisation levels (Table 5.9).

5.3.4 *Zea mays*

Inoculation of maize with AMF had slight negative effects on plant growth (Table 5.8). The effect was significant ($p \leq 0.05$) on only plant height with inoculation with *Acaulospora* sp. 1 and *G. etunicatum* having the most significant ($p \leq 0.05$) effect. *Acaulospora* sp. 1 had the highest root colonisation level. The colonisation levels of the three fungal species were not significantly different. There was a significant ($p \leq 0.05$) positive correlation between plant height and shoot weight and no significant ($p \leq 0.05$) correlation between both plant height and shoot weight and root weight. There was a significant ($p \leq 0.05$) negative correlation between the root weight and colonisation level (Table 5.9).

Table 5.8 Effect of inoculation with three AMF species on growth of four plant species and the percentage root colonization.

AMF species	Control	Ac	GA	GE	p-value	Distribution (Normality value)
G. sepium						
Height (cm)	6.5a	7.8a	8.8a	7.5a	0.34	0.43
Shoot weight (g)	0.29a	0.29a	0.35a	0.28a	0.20	0.007
Root weight (g)	0.03a	0.06ab	0.11b	0.07b	0.07*	0.0001
Colonisation (%)		43.9a	57.4a	66.0a	0.11	0.47
S. sesban						
Height (cm)	26.1ac	32.8b	23.8a	28.6bc	0.04*	0.08
Shoot weight (g)	0.19a	0.28b	0.18a	0.2a	0.02*	0.09
Root weight (g)	0.05a	0.10a	0.06a	0.05a	0.44	0.0001
Colonisation (%)		54.6a	46.4a	21.4b	0.0005*	0.94
S. macrantha						
Height (cm)	30.1a	32.0a	32.5a	34.8a	0.59	0.74
Shoot weight (g)	0.37a	0.37a	0.37a	0.38a	0.96	0.04
Root weight (g)	0.12a	0.12a	0.15a	0.10a	0.47	0.68
Colonisation (%)		72.2a	39.0b	47.3ab	0.002*	0.19
Z. mays						
Height (cm)	29.8b	20.2a	27.0ab	22.0a	0.21	0.44
Shoot weight (g)	0.92a	0.51a	0.67a	0.60a	0.29	0.09
Root weight (g)	0.23a	0.13a	0.16a	0.19a	0.42	0.11
Colonisation (%)		67.7a	56.8a	48.2a	0.33	0.97

Comparison of means is by Least Square Means (LSM). Values followed by the same letter on the horizontal axis are not significantly different at p = 0.05 and p = 0.1. p-values with asteriks show variables with values significantly different. Distribution represents the F-probability values with F values ≤0.05 not normally distributed.

Ac=*Acaulospora* sp. 1, GA=*Gl. aggregatum*, GE=*Gl. etunicatum*

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Table 5.9. The relationship between plant height, shoot dry weight, root dry weight and root colonisation analysed by the Spearman correlation coefficient

	Height	Shoot dry weight	Root dry weight	Colonisation
G. sepium				
Height	1.00	0.59*	0.16	0.085
Shoot weight	0.59	1.00	0.70*	0.13
Root weight	0.16	0.70*	1.00	0.06
Colonisation	0.08	-0.09	0.06	1.00
S. sesban				
Height	1.00	0.62*	0.50*	-0.10
Shoot weight	0.62	1.00	0.74*	0.22
Root weight	0.50*	0.74*	1.00	0.23
Colonisation	-0.10	0.22	0.23	1.00
S. macrantha				
Height	1.00	0.35	0.24	0.13
Shoot weight	0.35	1.00	0.74*	-0.04
Root weight	0.24	0.74*	1.00	-0.002
Colonisation	0.13	-0.04	-0.002	1.00
Z. mays				
Height	1.00	0.81*	0.33	-0.01
Shoot weight	0.81*	1.00	0.29	-0.26
Root weight	0.33	0.29	1.00	-0.66*
Colonisation	-0.01	-0.26	-0.66*	1.00

Values with asteriks have a significant correlation at $p \leq 0.05$

5.3.5 Discussion

Considerable variability in function exists among the effects of *Acaulospora* sp. 1, *G. aggregatum* and *G. etunicatum*. Differences were observed in fungal species preference for host plants. There is evidence that plants show highly specific growth responses to different species of mycorrhizal fungi (Allen *et al.* 1995). Cucumber (*Cucumis sativus* L.) and *Pueraria phaseoloides* used as test plants for mycorrhizal soil infectivity of three soils differed in their ability to determine mycorrhizal propagules in soils from a pot culture of AMF species,

temperate soil from an agricultural system in the United Kingdom and a tropical soils from an agroforestry site in Kenya (Jefwa 1991).

The most effective species on *G. sepium* was *G. aggregatum* and to a slight extent *G. etunicatum*. *G. aggregatum* was the most effective only in improving root dry weight, and to a slight extent plant height and shoot dry weight of *G. sepium*. Manjunath and Habte (1988) reported significant changes in dry matter yield on *L. leucocephala* after significant colonisation by *G. aggregatum*. Osonubi *et al.* (1992) also observed increased biomass of inoculated *F. albida* and *Acacia nilotica* compared to control plants. Although *G. etunicatum* had higher percentage colonisation compared to *G. aggregatum,* it was not the most effective on plant growth. Edriss *et al.* (1984) observed the greatest growth response of citrus with *G. fasciculatum* and the greatest mycorrhizal colonisation intensity with *G. etunicatum* and deduced that mycorrhizal colonisation intensity does not determine the efficiency of the symbiosis.

Acaulospora sp. 1, *G. aggregatum* and *G. etunicatum* had no effect on the growth of *S. macrantha*, not even *Acaulospora* sp. 1 with the highest colonisation level. This contrasts observations made by Michelsen (1993) on significant growth improvement of *Acacia sieberiana* DC. and *Acacia abyssinica* Benth. with high mycorrhizal colonisation. In *S. sesban*, *Acaulospora* sp. 1, which had the highest colonisation level and consistently improved height and shoot dry weight, had the least effect on root dry weight. In this study low efficiency of AMF species on maize growth was observed in inoculated plants compared to non-inoculated plants. The negative correlation between root weight and AMF colonization

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may explain the decrease in maize growth. This is supported by earlier observations on maize, which was considered to have low dependence on mycorrhiza (Howeler *et al.* 1987) with *Acaulospora bireticulata* Rothwell & Trappe low in its ability to improve maize growth (Myra & Varela 1995). Modjo *et al.* (1987) also reported negative correlation between height of tobacco plants and populations of spores of *G. macrocarpum*.

The effectiveness of *Acaulospora* sp. 1 at improving growth of *S. sesban* contrast with observations made on an *Acaulospora* sp. and *A. bireticulata* which were not effective on banana plantlets and maize respectively (Howeler *et al.* 1987; Sieverding 1991; Myra & Varela 1995; Jaizme-Vega & Azcon 1995).

Gliricidia sepium seemed to be the most affected host species by mycorrhizal inoculation. Baylis (1970) attributed differences in response to mycorrhizal inoculation to root hairs with species with numerous root hairs being less dependent. The root hairs of the four host plants differed in quantity and length (Plate 5.8), *G. sepium* having the least and shortest root hairs. This may explain its response to mycorrhizal inoculation. Root hair morphology may form a basis for determining requirements for AMF inoculation of agroforestry tree nursery seedlings.

In this study, AMF morphological structures were not characterized for each plant species. However, differences existed in the mode of hyphal penetration with branched (Plate 5.9 A & B) and lobed (Plate 5.9 C) type of appressoria observed. Wubert *et al.* (2003) in his studies on mycorrhizal status of indigenous tress in dry afromontane forest of Ethiopia observed

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appressoria with branched penetrating hyphae in *Podocarpus falcatus* (Thunb.) R. Br. Ex Mirb., *Prunus africana* (Hook. F.) Kalkman, *Ekebergia capensis* Sparrm., *Syzygium guineense* (Wild.) DC and *Hagenia abyssinica* (Bruce) JF Gmel. and the lobed appressoria in *Juniper procera* Hochst. Ex Endlicher and *Olea europaea* L ssp. *cuspidate*. Both Arum (Plate 5.9 E, F, G & H) and the Paris series of associations were observed. The AM morphological structures in *G. sepium* (Plate I & J) showed the Paris series of association. Wubert *et al.* (2003) observed plant families to differ in the type of AM morphological structures. Oval and elongate vesicles were associated with the genus *Glomus* (Wubert et al. 2003), hence the ovoid vesicles and ellipsoid vesicles observed in the study could be associated with *G. etunicatum* and *G. aggregatum*. Differences in AM morphological structures in plant species was also observed by Brundrett and Kendrick (1990) and Cavagnaro *et al.* (2001). AMF morphological structures of most tropical trees have not been established. There is need to evaluate mycorrhizal structures of most species. This will particularly form a basis for evaluating infective and effective species in the field. The differences observed in AMF morphological structures may be attributed to differences in root morphology. The roots of *G. sepium* are short and thick while the roots of the two *Sesbania* species and maize are variably longer and thinner (Plate 5.8), hence influencing the morphology and subsequent effectiveness of AMF symbiosis.

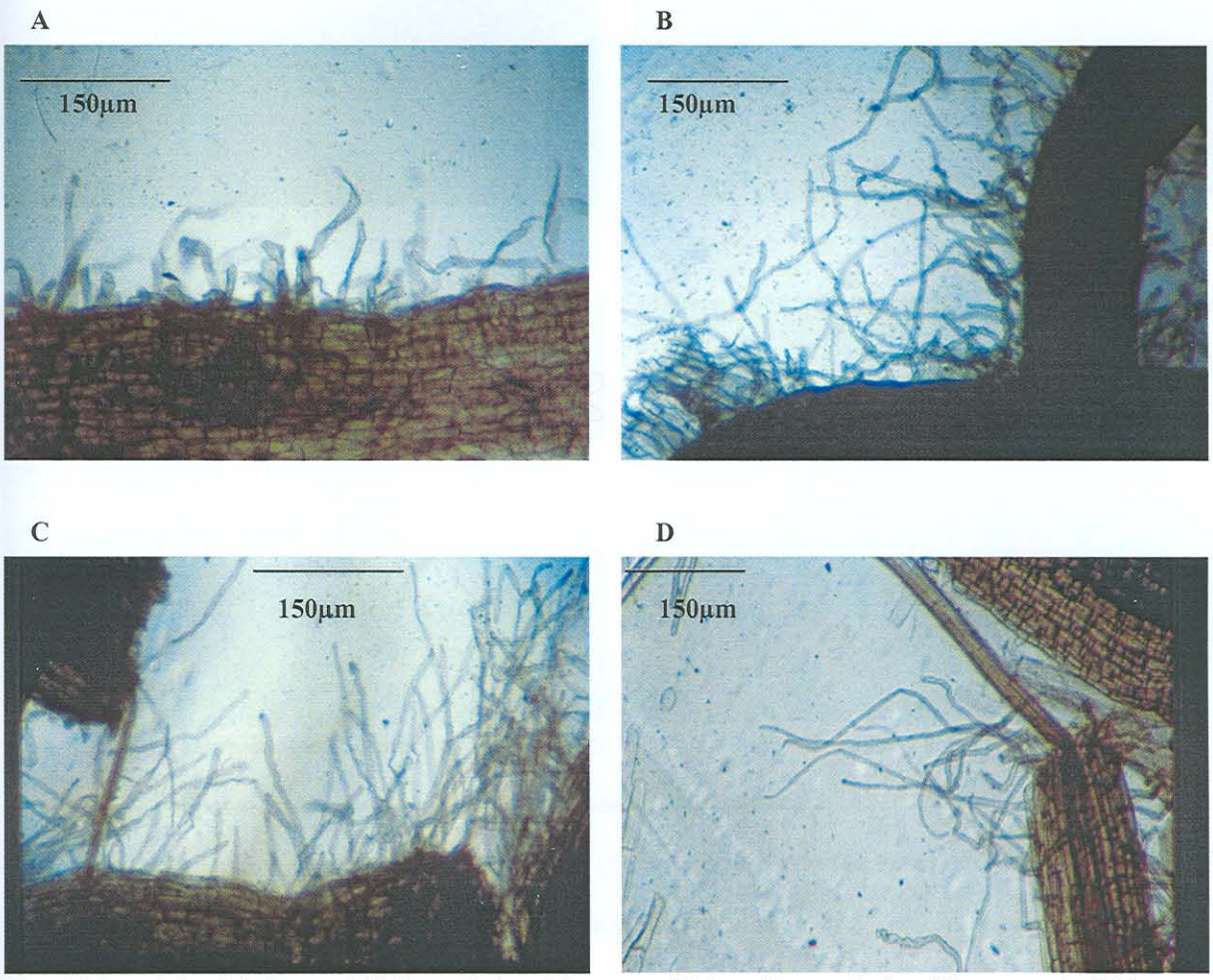


Plate 5.8 Root hairs (A) short and thick root hairs of *G. sepium* (B) long fine root hairs of *S. macrantha* (C) long fine and dense root hairs of *S. sesban* (D) long and fewer root hairs of *Z. mays*.

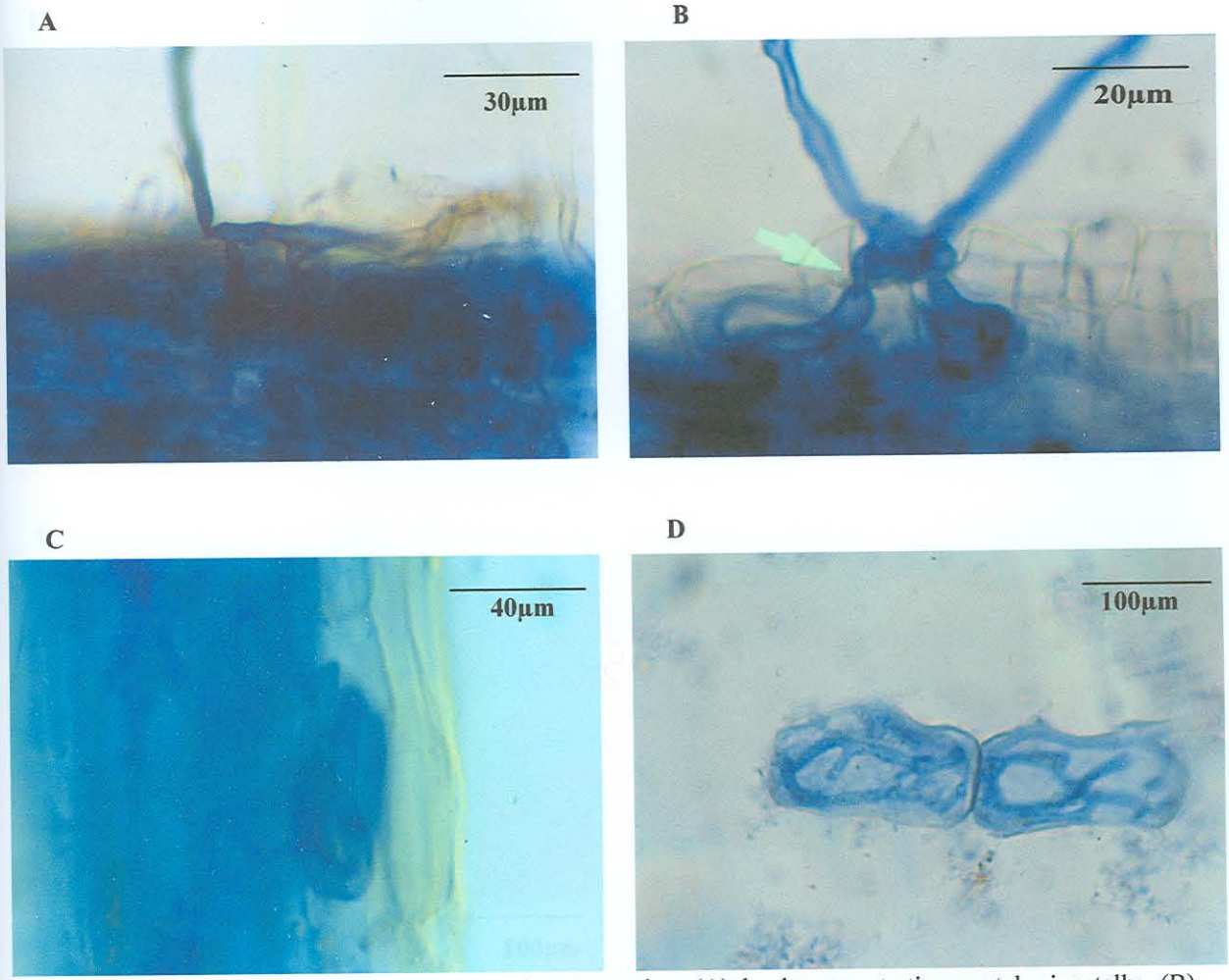


Plate 5.9 AMF morphological characters (A-B) Entry points (A) hyphae penetrating root horizontally. (B) Lumpy hyphae at point of penetration with hyphae penetrating root vertically.

Figure 5.9 AMF morphological characters (C-D) Coils (C) Coil formed at initial stages of development (D) Coil at advanced stage of development in the inner cortical tissue.

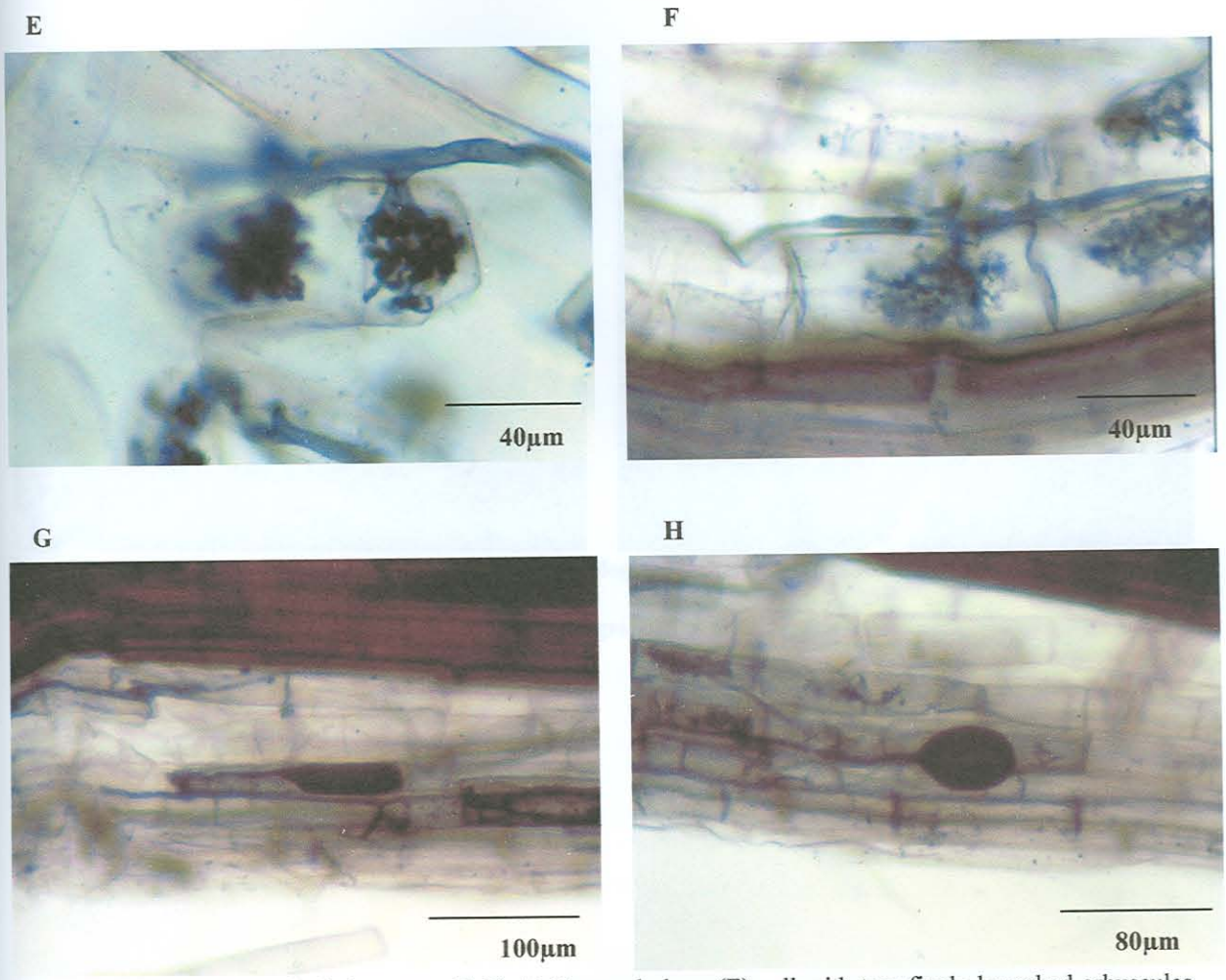
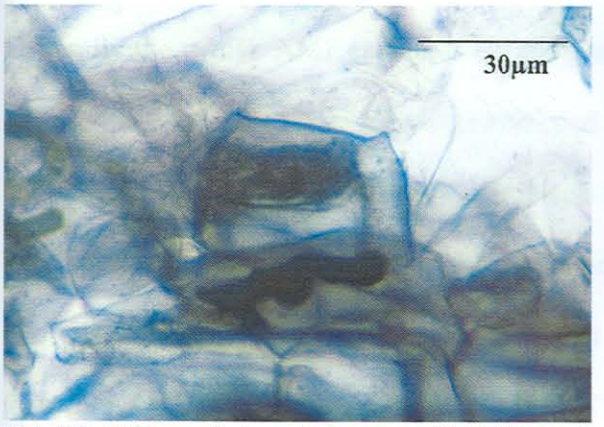


Plate 5.9 AMF morphological characters (E-F) AMF morphology (E) cell with two finely branched arbuscules (F) finely branched arbuscule from a hyphae along the cell wall (one arbuscule per cell)

Plate 5.9 AMF morphological characters (G-H) Vesicles (G) elongate intercellular vesicles (H) sub-globose intercellular vesicles.

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I



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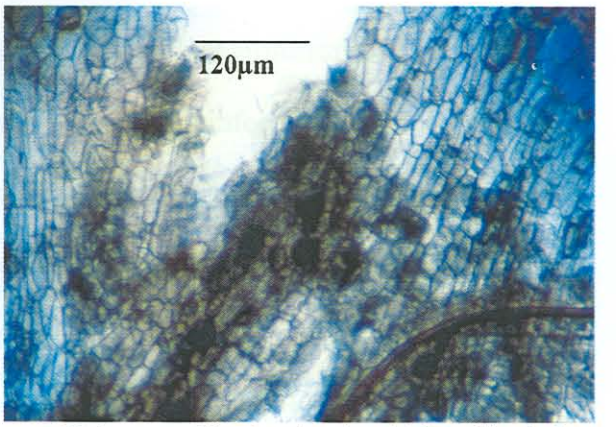


Plate 5.9 AMF morphological characters (I) AMF morphology at higher magnification showing thickened hyphal lump *in G. sepium* root (J) AMF morphology at lower magnification in *G .sepium*

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CHAPTER 6: CONCLUSION

Generally the twelve AMF species were comparatively low at the agroforestry site in Malawi. In other studies, over 40 species were recorded in a Cameroon rainforest (Mason *et al.* 1992) and the rhizosphere forest soils of Tamil Nadu in India (Ragupathy & Mahadevan 1993) and over 15 species were recorded in a farm with acid soils of farms in western Kenya (Shepherd *et al.* 1996). This supports the suggestion that agricultural soils have lower AMF diversity than forest soils (Schenck & Siqueira 1987). The sites in Malawi and in western Kenya are both agricultural systems. It is recognized that the estimate of species diversity is relative in that it is based on measures of numbers detectable by the method employed (Martini 1992). Baltana (1992) also suggested that small sub-samples were likely to underestimate actual species diversity. In this study, only three replicates each with five sub-samples were assessed. Visits to the sampling site were limited by cost although large samples and prolonged sampling is often recommended (Walker *et al.* 1982).

The comparatively high species number observed in the Cameroon rainforest and rhizosphere soils of Tamil Nadu in India sites could also be a result of overestimation resulting from lack of taxonomic skills. Lack of skills to clearly distinguish features may affect species discrimination and hence overestimate species number. Errors of classifying species as one, two species or more and errors of lumping species by classifying two or more as one, with the former inflating and the latter making species numbers small, are recognized (Gaston 1996). In the study in Malawi, prior to successful production of spore cultures, determination of AMF species from field materials resulted to over 50 spore types compared to identification from spore cultures that resulted to only the twelve species. Taxonomic skills, easily

distinguishable and defined morphological features and specimen from spore cultures are therefore vital in the study of AMF species. There is also need to constantly consult taxonomists and centres with living and preserved reference materials to confirm species. Overlap in morphological characteristics and inability to distinguish layers, particularly the inner membranous layers as was observed in spores of some species from Malawi and their allies, requires the use of microscopes with high resolutions and additional taxonomic diagnostic tools such as molecular techniques to verify closely related species. These facilities are lacking in most developing countries. Isoezyme analysis was able to confirm morphologically similar species *G. mosseae* and *G. coronatum* Giovannetti as two distinct groups (Dodd *et al.* 1996). The comparison of variety of morphologically similar species collected from wide range of geographical regions should continue to confirm species. This however demands for mandatory preservation of voucher specimen as evidence and also depositing materials of each species in other international herbaria and centres of culture collection.

Some species from Malawi occur in geographically different regions with some found in almost every continent with virtually little morphological variation. *Glomus etunicatum* and *Gigaspora margarita* have been recorded in many parts of the world while *S. cerradensis* and *A. rehmii* have so far been found only in South America and are here recorded for the first time in Africa. Only five species could not be matched with described species (Schenck & Perez 1990), while the spore morphology of three species was affiliated to *G. aggregatum, G. geosporum* and *S. dipurpurascens*. I support the proposal by Rosendahl *et al.* (1994) to do isoezyme ananlysis of morphologically species complex to verify the relationship among

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them. New species continue to be described from Africa (Sinclair *et al.* 2000). Comparisons on species distribution in different continents are still scarce and a more systematic search is necessary to establish trends. AMF species distribution has a strong implication for utilization of AMF species in different habitats. Species with a wider distribution would have greater advantages in utilization over a wider geographical range.

Spore morphology, the dominant diagnostic tool in this group, was sufficient to distinguish the twelve AMF species. However, some morphological features observed in *A. rehmii*, *Acaulospora* sp. 1, *Acaulospora* sp. 2. *Glomus* sp., *Gigaspora* sp. and *S. dipurpurascens* made it difficult to distinguish the species from closely related allies. Weak and continuous variable characters that are not easily distinguishable are used in delimiting species. Most morphological features used to separate species have no range and the degree of deviation from the typical features is not known. There is inconsistency in wall ornamentation in *A. rehmii* at different stages of spore development and it has similarities to the outer wall ornamentation and wall layers of *Acaulospora gerdemanii* now *Archaeospora gerdemanii* (Rose, Daniels & Trappe) Morton & Redecker and *Acaulospora elegans* Trappe & Gerdemann. A *Glomus* sp. from this site also displayed thick walled vesiculates, concentric discs-like features on spores without a hyphal mantle and a loose septate mantle, features that were not observed in the type descriptions. These features were similar to *G. globiferum*, *G. clariodeum* and *G. tortuosum*. Similarly, *Gigaspora* sp. has limited diagnostic features separating it from *G. margarita*. Morphological features separating young spores of *A. rehmii*, mature spores of *Acaulospora* sp. 1, *Acaulospora* sp. 2, *G. aggregatum, Glomus* sp., *Gigaspora* sp. and *S. dipurpurascens* from their allies are limited and not easily

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distinguishable. There is an overlap amongst allies in ornamentation and the inner membranous layers and their reactions to Melzer's reagent. Gabriel and Lynch (1992) recognized adaptive sets of phenotypes produced by different genotypes in different environments. Mallet (1996) noted morphological variation to be a good clue to genetic variation though the two did not correlate perfectly and much morphological variation, though genetically programmed depended on seasonal or maturity changes that affect many individuals regardless of the genotype. Morton (1988) considered reaction to Melzer's reagent of the outermost spore wall layers to be unstable and affected by environmental conditions, and hence of no taxonomic importance.

Phenotypic expressions can be distinguished into stage specific expressions as in the two phenotypic expressions in *A. rehmii* and plastic phenotypes where expression depends on environment (Mallet 1996). Plasticity of phenotypic expressions in AMF taxonomy is not a widely used phenomenon. The existence of environmentally plastic characters was suggested by Rosendahl *et al* (1994). Recently, Bentivenga & Morton (1995) transferred *Gigaspora ramisporophora* Spain, Sieverding & Schenck to *G. margarita*. The orange to saffron colour in *G. ramisporophora* was observed to be an unstable character that was not expressed in pot cultures and while re-classifying the species, the staining of the L2 was considered of no taxononmic importance. Possible cases of species synonyms based on close similarities of spore morphology and studies on iso-enzymes of the morphological species complex were proposed to verify the relationship among them (Rosendahl *et al*. 1994). Whether the similarities of *A. rehmii*, *Acaulospora* sp. *Glomus* sp., *G. aggregatum*, *Gigaspora* sp. and *S. dipurpurascens* with their allies are phenotypic expressions of the same genotypes under

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different environmental conditions needs to be verified. To verify the status of species and whether differences are related to environmental conditions, initially comparisons with ex-types followed by iso-enzyme and molecular analysis of closely related species is necessary. There is need to define plastic characters and the range for each morphological feature in an AMF species to avoid multiple description of the same species. As was suggested earlier by Rosendahl *et al.* (1994), due to the uncertainty of most important structures, there is a need to re-describe species to identify overlaps. It is recognized that the lack of consideration of phenotypic expressions of most features leads to species with large geographic ranges and high levels of morphological variation to be prone to being described multiple times (Gaston *et al.* 1995). Observations made in this taxonomic study support the need for revision of closely related species and redefining morphological features over wide geographic ranges to eliminate inconsistencies.

There is no intra-specific morphological variation amongst speciemens of some species from different geographical regions. Morphological features of *G. etunicatum, G. margarita* and *S. cerradensis* are stable over a wide geographical region. Species with stable morphological features if also physiologically stable could render advantages in commercial inoculum production for use over a wide geographical range. There is need to verify whether morphological simililarities amongst closely related allies are also genetically and physiologically similar.

Lack of knowledge of adaptations to environmental conditions is the main factor limiting utilization of AMF. Effects of season, farming systems and fertility are vital in predicting

survival, nutrient utilization and compatibility with host plant. In this study, there was no taxononmic trend observed in species adaptations with species from the same genera responding differently. *Acaulospora* sp. 1, *G. aggregatum, A. rehmii, G. margarita, Archaeospora* sp. and *G. etunicatum* were consistent in their response to seasonal variations irrespective of the site. The response of *Acaulospora* sp., *Glomus* sp., *G. geosporum*, *S. cerradensis, S. dipurpurascens* and *G. margarita* was site specific. Spores of species adapted to dry conditions or species with high spore production under dry conditions are likely to survive longer under drought conditions and also maintain high levels of spore inoculum. Spores survive longer under dry conditions compared to mycelia and hyphae in root fragments (Babara & Hetrick 1984), hence abundant spore production under dry conditions may be a favourable survival mechanism of AMF species for habitats prone to drought. Few AMF species showed specific associations to farming systems and there was no family or generic trends, contrary to observations made by Khalil *et al.* (1992).

Agroforestry systems affected AMF species with variation observed in different agroforestry tree species. In this study, the effect of farming systems was modified by fertility regimes and seasonal variations. Agroforestry systems with *Gliricidia*/maize intercrop seemed to favour AMF species in the absence of inorganic fertilizer. Agroforestry system with two *Sesbania*/maize intercrop seemed to have lesser effect on AMF species diversity. This implies that agroforestry trees differ in their effect on AMF species diversity, with closely related tree species having almost similar effects. Differences in effects of agroforestry trees may be linked with root morphology and/or the quality of organic leaf biomass and may also be a hereditary trait in plants. The *Gliricidia* species has fewer, shorter root hairs than the *Sesbania*

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species (Plate 5.8) and the leaf biomass from *Gliricidia* improves fertility more than the *Sesbania* species (Ikerra *et al.* 2001). Baylis (1970) associated the structure of root morphology with mycorrhizal symbiosis with species with fewer and shorter root hairs considered more mycorrhizal. In the greenhouse bioassay experiment on the effectiveness of AMF species from the agroforestry site in Malawi, *Gliricidia sepium* responded more to inoculation more than the two *Sesbania* species and maize. This supports the field observations on *G. sepium* having more effect of AMF spores than the two *Sesbania* species, hence the possibility that *G. sepium* is more dependent on the mycorrhizal symbiosis than the two *Sesbania* species and maize. Mycorrhizal symbiosis may also be a hereditary trait in host plants.

Agroforestry system with *Gliricidia*/maize intercrop was more highly modified by inorganic fertilizer compared to maize monocrop and *Sesbania* spp./maize intercrop systems. *Gliricida*/maize intercrop system, through tree leaf biomass incorporation, was reported to enhance soil nutrients more than the maize monocrop and *Sesbania* spp/maize intercrop systems (Ikerra *et al.* 1999; Ikerra *et al.* 2001). Leaf biomass from different agroforestry trees differ in their nutrient levels (Mwiinga *et al.* 1994), hence contribute differently to soil fertility and may therefore have different effects on mycorrhizal symbiosis. Guttay (1983) observed quality of compost as important for optimum AMF formation. Howard (1943) reported positive effect of compost on AMF and linked this to improved soil aeration and AMF development. He also observed positive influence in plant residue return (weeds and pruning fron tea) on AMF colonisation in the tea plantation. Sieverding (1987) in Zaire confirmed Howard's observations. He reported cassava roots to be more intensively colonized

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by AMF when compost was applied. Application of compost however did not alter the diversity and relative spore composition of AMF fungal community.

Although *Sesbania sesban* and *S. macrantha* seem not to differ in their effects on AMF species diversity, the two plants vary in their susceptibility to the root-knot nematodes, *S. sesban* being susceptible and *S. macrantha* not (Karachi 1995). The high species diversity in maize monocrop systems compared to *Sesbania* spp./maize intercrop system could be linked with root turnover. Root growth in *Sesbania* spp. plants is more or less continuous while growth in maize roots is intermittent. Baylis (1969) associated spore production with lack of stimulus for spore production if root growth was not intermittent. Soil mositure content (Chirwa *et al.* 1994; Baylis 1969), root biomass (Ocampo *et al.* 1980) and root hair density (Baylis 1970) are possible attributes explaining differences in species diversity amongst the farming systems. The contrasting results of *Sesbania* spp./maize intercrop, *Gliricidia*/maize intercrop and maize monocrop farming systems may be explained by differences in root hair density and root biomass of the agroforestry tree species and possibly differences in quality of the organic matter. Greenhouse bioassays evaluating the effects of leaf biomass from different agroforestry trees on AMF species colonization and subsequent spore production can be undertaken to verify these factors.

Seasonal variations on AMF species diversity in a farming system were evident at the *Gliricidia*/maize intercrop system. A study on nutrient dynamics undertaken by Ikerra *et al.* (1999) at this same agroforestry and monocrop experiment showed *Gliricidia*/maize intercrop plots to accumulate more ammonium-N during the dry season and nitrate-N remaining

constant during the dry season but rising rapidly after the onset of rains. There is a possibility that in nitrogen deficient soils AMF symbiosis may be affected by the different forms of nitrogen and their interaction with phosphorus.

Utilization of AMF species will depend upon species that associate with a broad range of soil fertility and host plants and species that can survive extreme environmental conditions. Commercial production of inoculum will only be economical if the inoculum can be used in a wide range of habitats, with a broad range of hosts and linked to efficiency in mycorrhizal functions and plant growth. Hence, conditions favourable for the survival of AMF species in their habitats of origin needs to be verified prior to large scale production of inoculum and also before manipulation of effective AMF species through farming systems is recommended.

Observations made in this study showed variation in response of individual species to season, host plants and soil fertility. The occurrence of most AMF species was site specific. However, there were few species that were consistent in response to changes in season irrespective of the site.The spores of *A. rehmii*, *G. margarita*, *Archaeospora* sp. and *G. etunicatum* were frequent in the dry season and hence likely to survive drought conditions compared to *Acaulospora* sp. 1 and *G. aggregatum,* which were frequent in the wet season. The mechanism for drought resistance is probably physical with wall characteristics playing a major role or it could be associated with spore reproduction. Spore morphological characters and ability to produce abundant spores under dry conditions may be one criterion for selection of AMF species particularly for drought prone areas. *G. aggregatum* is also found in dead roots, indicating possible inherent mechanisms for protection against drought.

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The effect of fertilizer on the occurrence of AMF species was more evident in the *Gliricidia*/maize intercrop system. As was observed with species diversity of *Gliricidia*/maize intercrop system, these changes may be linked to the greater effects of soil fertility by the *Gliricidia* tree (Ikerra *et al.* 2001). The effect of fertilizer on AMF species seemed to range from low, high to neutral with the occurrence of most species being site specific. Nitrogen fertilizer seemed to have the greatest effects at both sites. The site is deficient in nitrogen and the response to changes in nitrogen may dispute the generalization that mycorrhizal symbiosis is important in mostly phosphorus deficient soils. The study implies that mycorrhizal fungi respond more to manipulation of the inherently deficient nutrient.

Functional diversity exists in different AMF species from the same habitat. The different AMF species showed preference for plant species and also affected some plant growth parameters more than others. Variation in infectivity and effectiveness on *G. sepium*, *S. sesban*, *S. macrantha* and *Z. mays* was observed following inoculation with *Acaulospora* sp. 1, *G. aggregatum* and *G. etunicatum*. Fungal species most infective were not necessarily most effective. The study showed AMF species from the same genera to prefer the same host and also tree species belonging to the same genera to prefer the same AMF species. This implies that the prediction for association with specific fungal species could be a heritable character. Variations are evident in colonisation and effectiveness of AMF species from the same habitat. The fungal species effectiveness on plant growth also differed in the plant growth parameters evaluated, with *G. aggregatum* more effective on root dry weight increase in *G. sepium* and *Acaulospora* sp. 1 on shoot growth of *S. sesban*. This implies functional diversity

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exists amongst AMF from the same habitat. The scientific basis for this may provide the need to use mixed cultures in inoculation programmes, as is often recommended.

The study also showed fungal species frequently associated with the *G. sepium, S.sesban* and *S. macrantha* trees not to necessarily have preference for the maize crop. If AMF species are to be manipulated by trees to benefit the subsequent crop, it is important that the fungi effective on tree growth be also effective on the crop. The species may adapt to a wide range of soil conditions but may fail to associate effectively with the host plant. Hence it is essential to test across all the AMF species discovered at a site if AMF species are to be considered for manipulation. Not all AMF species found at the site were tested in this study. Root hairs could be important in determing plant species dependency on mycorrhizal symbiosis. *G. sepium* with shorter and fewer root hairs seemed to respond more to inoculation than the two *Sesbania* species and maize, with relatively longer numerous root hairs (Plate 5.8).

This study has shown AMF spores to differ in their adaptation to season, host plant and soil conditions. These conditions determine the range of distribution and subsequently range of utilization of the species. Companies producing AMF inoculum commercially should take into consideration the adaptive conditions of a species. Previous inoculation programmes overlooked AMF species adaptation to environmental conditions as criteria for AMF selection and concentrated on the effectiveness of AMF species on plant growth. Hence, most inoculum produced, though efficient under greenhouse conditions, has frequently failed to have positive results under field conditions. There is no record on the range of conditions suitable for commercially produced AMF, yet they are sold and distributed worldwide. If fertilization is to

be minimized and the benefits of mycorrhizal symbiosis maximized, then there is no short cut but to establish the basis by understanding the organisms in their own environment and the range of distribution globally. Based on the three studies, commercial formulations can be made for specific groups of host plants, soil conditions and climatic regions. More studies need to be undertaken relating mycorrhizal functions to adaptations to environmental condition and mapping of global distribution of AMF so as to facilitate utilization.

This study did not examine the soil inoculum potential of the different management practices, hence it cannot be concluded that decrease in species diversity leads to decline in mycorrhizal inoculum potential. Absence of spores of a species does not necessarily indicate absence of species. Other methods that can detect presence of species in mycelial form are necessary to adequately quantify AMF species diversity and species frequency of occurrence. The study could, however, verify the effects of the farming systems on AMF spore production. Spores are important infective propagules that remain longer in the soil than mycelia or infected root fragments. The greater ability of spore inoculum to withstand harsh environmental conditions was suggested (Hall 1979). The spore propagule was also suggested as the primary propagule for survival during intermittent root growth or under field conditions in the absence of host plants except under very dry conditions (Babara & Hetrick 1984). Hence, spores have long-term implications on the inoculum potential of soils, particularly in this region of southern Malawi, that experience prolonged dry seasons, and is prone to drought.

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CHAPTER 8.0: APPENDICES

APPENDIX A

The mean maize yield in *Gliricidia sepium*/maize intercrop and maize monocrop system

Tree	Phosphorus 48 kg ha^{-1}	Nitrogen 20 kg ha^{-1}	Grain yield tons ha^{-1}
none	0	0	1.93
none	100 %	0	2.07
none	0	50 %	4.08
none	100 %	50 %	3.96
Tree	0	0	6.96
Tree	100 %	0	6.77
Tree	0	50 %	8.02
Tree	100 %	50 %	8.12

None = Maize monocrop, Tree = *G. sepium* agroforestry practice, 0 = no phosphorus and no nitrogen, 100 % full recommended dose of phosphorus, 50 %= half recommended dose of nitrogen.

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APPENDIX B

The mean maize yield in *Sesbania sesban*/maize, *S macrantha*/maize intercrop and maize monocrop systems

Tree	Phosphorus 48 kg ha^{-1}	Nitrogen 20 kg ha^{-1}	Grain yield tons ha^{-1}
None	0	0	1.67
None	100 %	0	1.91
None	0	50 %	4.00
None	100 %	50 %	4.16
S. sesban	0	0	2.00
S. sesban	100 %	0	2.90
S. sesban	0	50 %	4.42
S. sesban	100 %	50 %	4.37
S. macrantha	0	0	2.47
S. macrantha	100 %	0	3.13
S. macrantha	0	50 %	3.84
S. macrantha	100 %	50 %	4.36

None = maize monocrop, 0 = no phosphorus and no nitrogen, 100 % = full recommended dose of phopshorus, 50 % = Half recommended dose of nitrogen

APPENDIX C

Experimental layout for *Gliricidia sepium*/maize intercrop and maize monocrop system.

Nitrogen rates: zero rate (0), quarter recommended dose (25 %) of 20 kg ha^{-1}, half recommeded dose (50 %) of 40 kg ha^{-1}

Phosphorus rates: zero rate (0), half recommended dose (50 %) of 24 kg ha^{-1}, full recommended dose (100 %) of kg 48 ha^{-1}
C = Tree prunning regimes: C1 = no pruning, C2 = prunned once, C3 = prunned twice

The plots in bold were sampled for glomeromycotan species assessment.

Layout of Replicate l (Block 1)

PLOT	PRUNING	NITROGEN	PHOSPHORUS
plot1	**c1**	**0**	**100**
plot 2	c3	0	100
plot 3	c2	25	100
plot 4	**c2**	**50**	**100**
plot 5	**c1**	**50**	**100**
plot 6	c1	50	50
plot 7	c3	50	100
plot 8	c1	0	50
plot 9	c3	50	50
plot 10	c3	0	50
plot 11	c2	50	50
plot 12	c3	0	0
plot 13	**c1**	**0**	**0**
plot 14	**c2**	**50**	**0**
plot 15	c3	50	0
plot 16	**c1**	**50**	**0**
plot 17	c3	25	0
plot 18	c1	25	0
plot 19	c1	25	100
plot 20	c3	25	100
plot 21	c2	25	0
plot 22	c1	25	50
plot 23	c2	25	50
plot 24	c3	25	50
plot 25	c2	0	50
plot 26	**c2**	**0**	**0**
plot 27	**c2**	**0**	**100**

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Layout of replicate 2 (Block 2)

PLOT	PRUNING	NITROGEN	PHOSPHORUS
plot1	c2	0	50
plot 2	c3	50	100
plot 3	**c1**	**50**	**100**
plot 4	c3	0	100
plot 5	**c1**	**0**	**100**
plot 6	c3	50	50
plot 7	**c2**	**50**	**0**
plot 8	c1	50	50
plot 9	**c2**	**0**	**0**
plot 10	c3	0	0
plot 11	**c1**	**0**	**0**
plot 12	c2	50	50
plot 13	c3	50	0
plot 14	**c1**	**50**	**0**
plot 15	c1	0	50
plot 16	c3	0	50
plot 17	c2	25	0
plot 18	c3	25	50
plot 19	c3	25	100
plot 20	c1	25	50
plot 21	c1	25	100
plot 22	c3	25	0
plot 23	c1	25	0
plot 24	**c2**	**50**	**100**
plot 25	c2	25	50
plot 26	c2	25	100
plot 27	**c2**	**0**	**100**

Layout of rep 3 (Block 3)

PLOT	PRUNING	NITROGEN	PHOSPHORUS
plot1	**c2**	**50**	**0**
plot 2	c3	50	50
plot 3	c1	50	50
plot 4	c1	0	50
plot 5	**c1**	**0**	**0**
plot 6	c3	0	50
plot 7	c3	0	0
plot 8	**c2**	**0**	**0**
plot 9	**c2**	**0**	**100**
plot 10	**c1**	**0**	**100**
plot 11	c2	50	50
plot 12	c3	0	100
plot 13	**c2**	**50**	**100**
plot 14	c3	25	0
plot 15	c1	25	0
plot 16	c3	50	100
plot 17	**c1**	**50**	**100**
plot 18	c2	0	50
plot 19	c3	50	0
plot 20	c3	25	50
plot 21	**c1**	**50**	**0**
plot 22	c2	25	50
plot 23	c1	25	50
plot 24	c2	25	0
plot 25	c3	25	100
plot 26	c1	25	100
plot 27	c2	25	100

APPENDIX D

Experimental layout for *Sesbania sesban*/maize and *S. macrantha*/maize intercrop and maize monocrop systems.

Nitrogen rates: zero rate (0), quarter recommended dose (25 %) of 20 kg ha^{-1}, half recommended dose (50 %) of 40 kg ha^{-1}

Phosphorus rates: zero rate (0), half recommended dose (50 %) of 24 kg ha^{-1}, full recommended dose (100 %) of kg 48 ha^{-1}

Trees: none = no tree, sesban = *S. sesban*, macrantha = *S. macrantha.*
Tree density: 0 trees ha^{-1}, 7400 trees ha^{-1} and 14800 trees ha^{-1}.

The plots in bold were sampled for assessment of Glomeromycotan fungi.

Layout of replicate 1 (Block 1)

Plot	Tree species	Tree density	Nitrogen	Phosphorus
Rep1; Block 1				
plot 1	sesban	14800	50	100
plot 2	**macrantha**	**7400**	**50**	**100**
plot 3	**macrantha**	**7400**	**50**	**0**
plot 4	sesban	14800	0	0
plot 5	**sesban**	**7400**	**0**	**0**
plot 6	**none**	**Void**	**50**	**0**
plot 7	**sesban**	**7400**	**50**	**100**
Rep 1; Block 2				
plot 1	macrantha	14800	50	100
plot 2	none	Void	100	100
plot 3	**sesban**	**7400**	**50**	**0**
plot 4	Macrantha	14800	0	0
plot 5	**none**	**Void**	**0**	**0**
plot 6	**none**	**Void**	**0**	**100**
plot 7	sesban	14800	0	100
Rep 1; Block 3				
plot 1	**sesban**	**7400**	**0**	**100**
plot 2	**none**	**Void**	**50**	**100**
plot 3	macrantha	14800	50	0
plot 4	sesban	14800	50	0
plot 5	Macrantha	14800	0	100
plot 6	**macrantha**	**7400**	**0**	**0**
plot 7	**macrantha**	**7400**	**0**	**100**

Layout of replicate 2 (Block 2)

Plot	Tree species	Tree density	Nitrogen	Phosphorus
Rep 2; Block 1				
plot 1	sesban	14800	0	100
plot 2	sesban	14800	50	0
plot 3	**sesban**	**7400**	**0**	**100**
plot 4	**macrantha**	**7400**	**0**	**100**
plot 5	sesban	14800	50	100
plot 6	sesban	14800	0	0
plot 7	macrantha	14800	0	0
Rep 2; Block 2				
plot 1	none	void	100	100
plot 2	**macrantha**	**7400**	**0**	**0**
plot 3	**none**	**void**	**0**	**100**
plot 4	**none**	**void**	**50**	**100**
plot 5	**sesban**	**7400**	**50**	**0**
plot 6	**none**	**void**	**50**	**0**
plot 7	**macrantha**	**7400**	**50**	**100**
Rep 2; Block 3				
plot 1	macrantha	14800	0	100
plot 2	macrantha	14800	50	100
plot 3	**sesban**	**7400**	**0**	**0**
plot 4	macrantha	14800	50	0
plot 5	**none**	**void**	**0**	**0**
plot 6	**macrantha**	**7400**	**50**	**0**
plot 7	**sesban**	**7400**	**50**	**100**

Layout of replicate 3 (Block 3)

Plot	Tree species	Tree density	Nitrogen	Phosphorus
Rep 3; Block 3				
plot 1	none	void	100	100
plot 2	**sesban**	**7400**	**0**	**0**
plot 3	sesban	14800	0	100
plot 4	**none**	**void**	**50**	**100**
plot 5	sesban	14800	50	100
plot 6	macrantha	14800	0	100
plot 7	sesban	14800	50	0
Rep 3; Block 2				
plot 1	**sesban**	**7400**	**50**	**100**
plot 2	**none**	**void**	**50**	**0**
plot 3	macrantha	14800	0	0
plot 4	macrantha	14800	50	100
plot 5	**none**	**void**	**0**	**100**
Plot 6	**macrantha**	**7400**	**0**	**0**
Plot 7	**sesban**	**7400**	**0**	**100**
Rep 3; Block 3				
Plot 1	**macrantha**	**7400**	**50**	**0**
Plot 2	**sesban**	**7400**	**50**	**0**
Plot 3	**macrantha**	**7400**	**50**	**100**
Plot 4	macrantha	14800	50	0
Plot 5	**macrantha**	**7400**	**0**	**100**
Plot 6	**none**	**void**	**0**	**0**
Plot 7	sesban	14800	0	0