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

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

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

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*

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

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.

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

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.