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

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.

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.

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.

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.

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

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

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.

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*

(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).

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.

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.

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

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.

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.

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

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

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*

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

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

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.

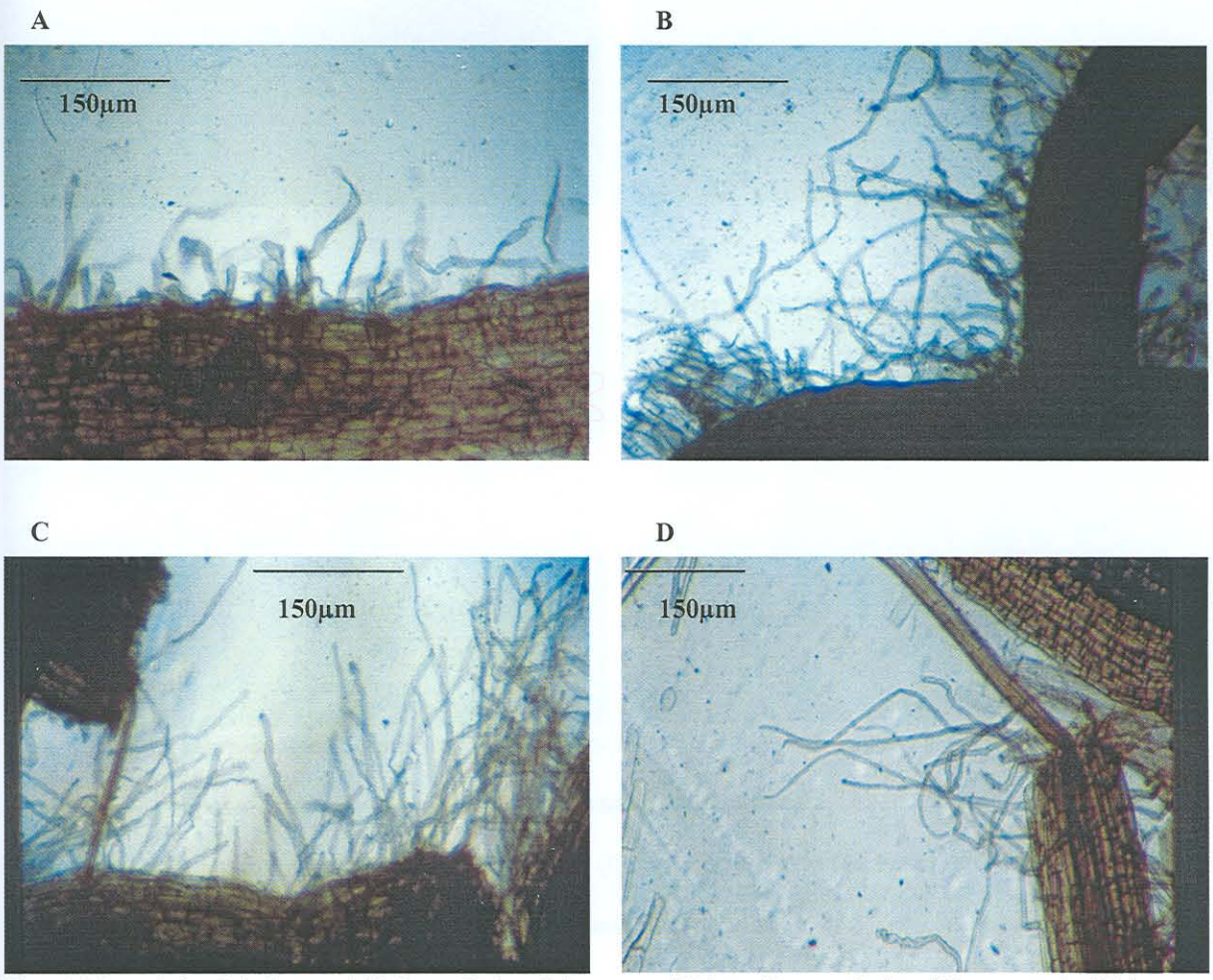


Plate 5.8 Root hairs (A) short and thick root hairs of *G. sepium* (B) long fine root hairs of *S. macrantha* (C) long fine and dense root hairs of *S. sesban* (D) long and fewer root hairs of *Z. mays*.

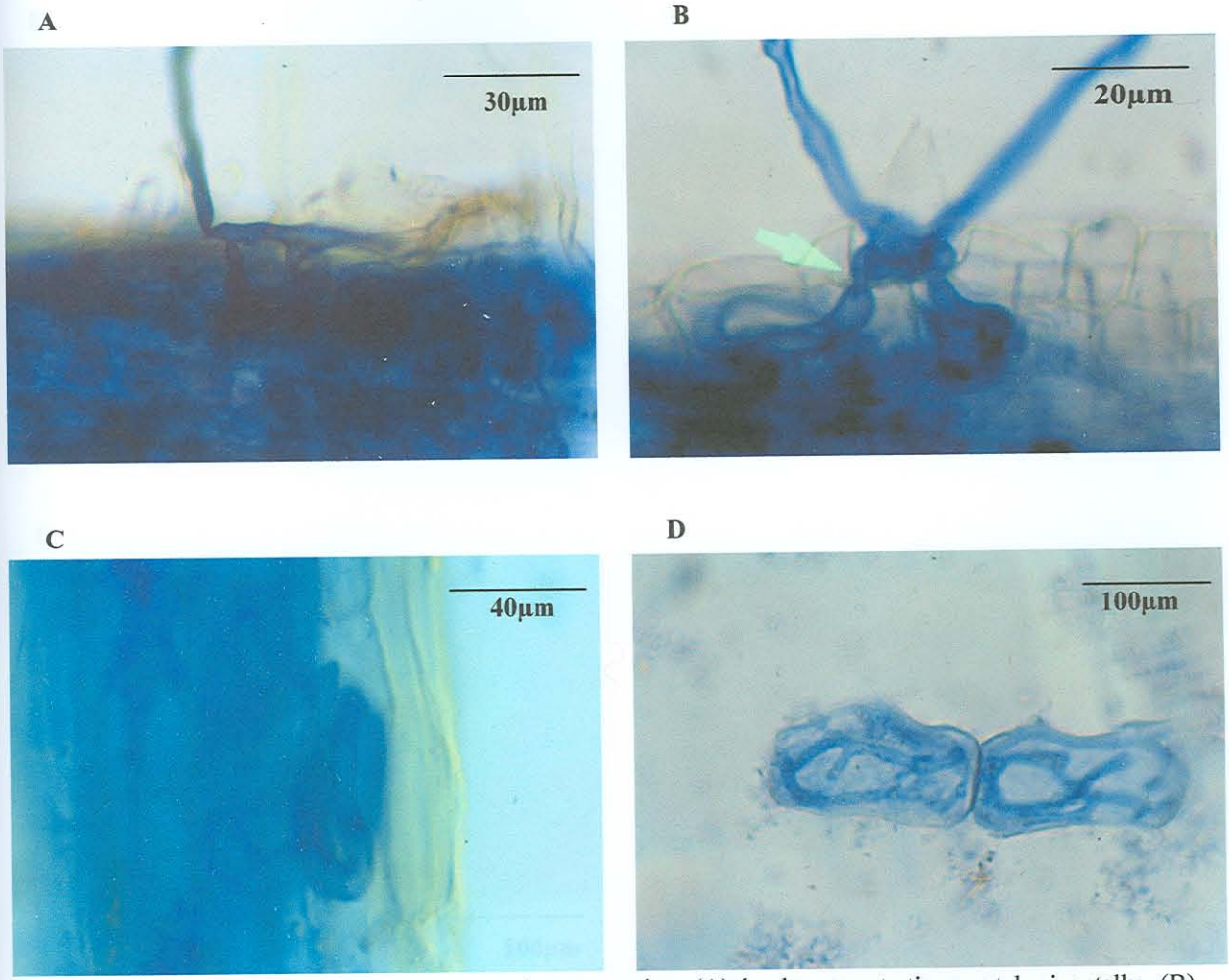


Plate 5.9 AMF morphological characters (A-B) Entry points (A) hyphae penetrating root horizontally. (B) Lumpy hyphae at point of penetration with hyphae penetrating root vertically.

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.

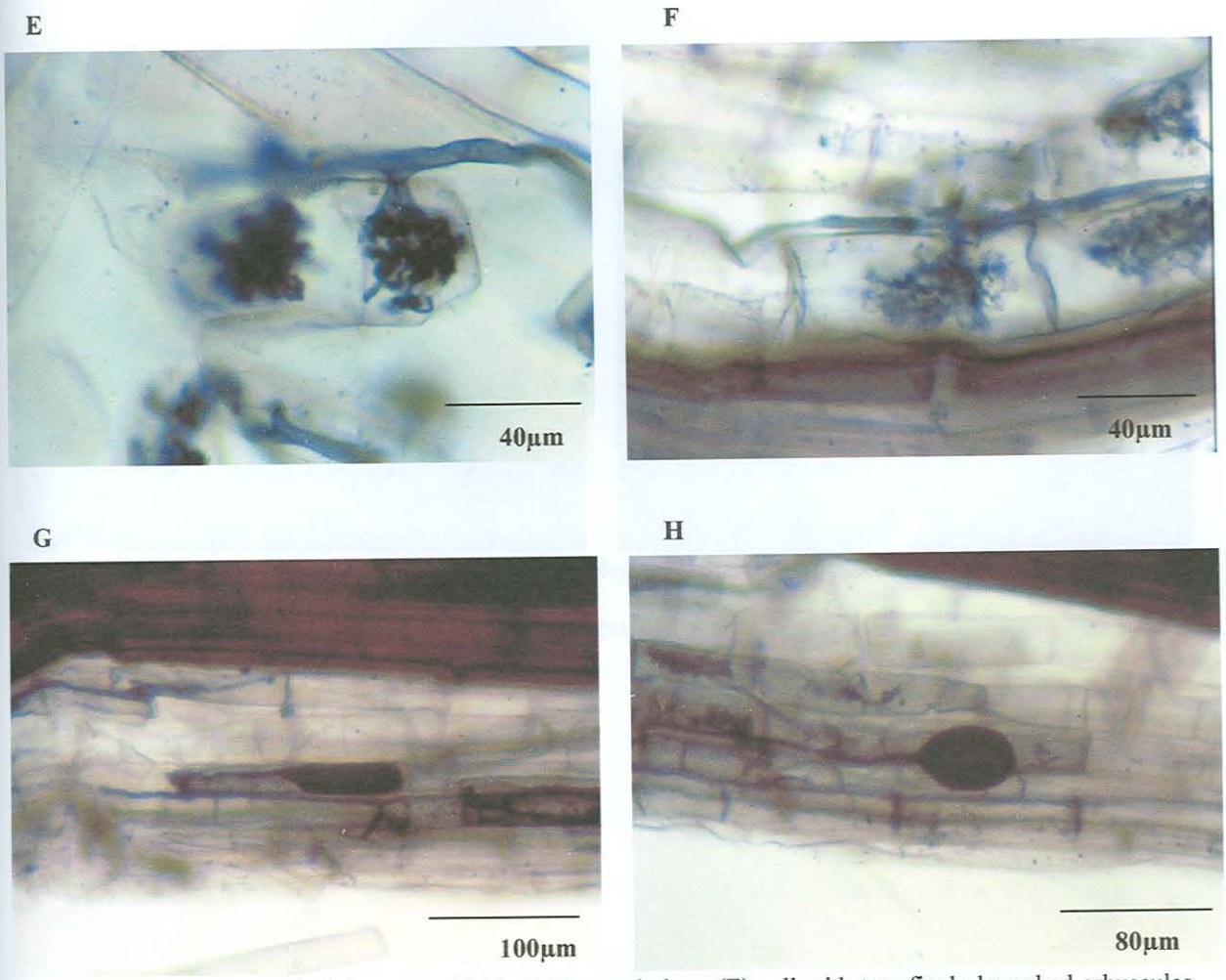
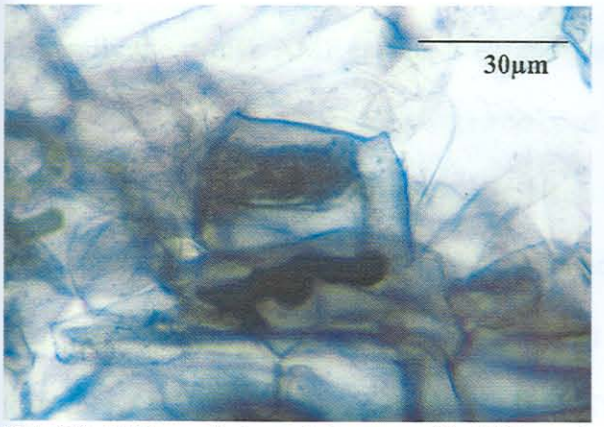


Plate 5.9 AMF morphological characters (E-F) AMF morphology (E) cell with two finely branched arbuscules (F) finely branched arbuscule from a hyphae along the cell wall (one arbuscule per cell)

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

I



J

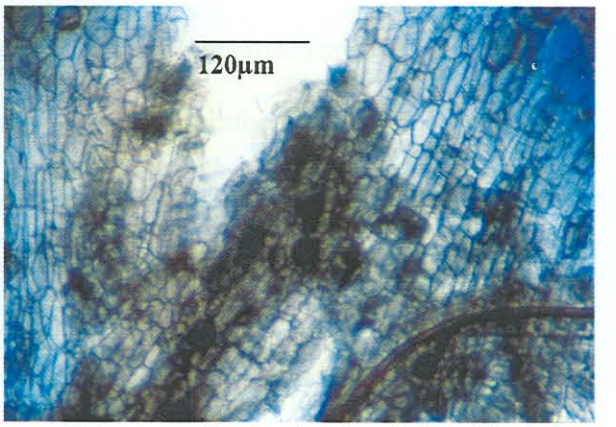


Plate 5.9 AMF morphological characters (I) AMF morphology at higher magnification showing thickened hyphal lump *in G. sepium* root (J) AMF morphology at lower magnification in *G .sepium*