Research Article

Arbuscular mycorrhizal status of *Cordia africana* and *Millettia ferruginea* trees in traditional agroforestry land use systems of Sidama Regional State, southern Ethiopia

Zebene Asfaw [1] and Håkan Hultén[2]

Abstract

Arbuscular mycorrhizal fungi (AMF) enhance plant growth and productivity through nutrient acquisition, organic matter decomposition, improved soil health, increased resilience and stress tolerance. A higher percentage of root colonisation by AMF indicates a stronger symbiotic relationship and potentially greater benefits to the plant. A high abundance of AMF spores reflects their overall potential to colonise plant roots. The aim of this study was to determine the number of spores and root colonisation potential of AMF in the soil beneath Cordia and Millettia trees grown in enset and maize fields, and to estimate the extent of AMF colonisation of Millettia and maize seedling roots grown on these field soils. Six tree-crop combinations, making 36 plots, and two open maize plots in the traditional agroforestry systems of Sidama. At the field level, the study was conducted under the canopy of *Cordia africana* and *Millettia ferruginea* trees in different plots. To assess the relationship, root colonisation and spore counts of field soils sampled from different Cordia and Millettia trees in enset and maize plots, maize and Millettia plants were grown in the nursery. The tree-crop combinations induced higher spore counts and higher colonisation levels than in the open maize field. Significantly, lower numbers of spores were observed in soils under Cordia and Millettia trees grown in maize plots than under trees grown in enset coffee and enset plots. The order of colonised roots was: tree enset coffee > tree enset > tree maize for Cordia trees and tree enset > tree enset coffee > tree maize for Millettia trees. At the nursery level, a significantly higher level of root colonisation was observed for maize plants grown on soil from under tree-enset-coffee and enset plots than for those grown on soil from tree-maize and open maize plots. The percentage of AM colonised maize roots was significantly positively correlated with spore counts for field soils. Both maize and Millettia plants with high root colonisation achieved higher fresh weight. The presence of spore counts and root colonisation in the plants studied suggests a contribution of native AMF in improving plant growth and productivity.

Key words: Number of spores, Open-maize plot, Root colonizations, Tree-enset,Tree-maize

1. Introduction

Mycorrhizal association can contribute to agricultural sustainability through improved mineral uptake, especially of low mobile ions (e.g. Sieverding, 1991), and biological N fixation (Ibijbijen et al., 1996). Mycorrhizal association can also improve nutrient cycling and soil structure quality (Hamel, 1996), plant community diversity and plant tolerance to biotic and abiotic stresses (Hamel 1996; Smith and Read, 1997). Studies of some agroecosystems (Sieverding, 1991) and natural forests in the tropics (Janos, 1996) suggest that the presence of mycorrhizal associations can maintain plant diversity. Mycorrhizal association is also helpful in explaining the relative abundance of plant species in a given habitat (Reader, 1998).[1] In tropical agroecosystems, AMF is the most common

[1] [1]Wondo Genet College of Forestry and Natural Rresources, P.O. Box 128, Shashamane, Ethiopia. Corresponding author: zebeneasfaw@gmail.com
*****Corresponding author**:** zebeneasfaw@gmail.com

[2]Swedish University of Agricultural Sciences, Department of Forest Management and Products, P.O. Box 7060, SE 750 07, Uppsala, Sweden.
Received 3 April 2024
Accepted 30 June 2024

mycorrhizal symbiosis (Sieverding, 1991). In managed ecosystems, however, these contributions may be influenced by management practices. The most important management practices influencing AM associations are the intensity of agricultural, high and low input practices.

High-input farming practices in this context refer to the use of artificial fertilisers, intensive tillage, weed control with herbicides, chemical control of insects and diseases, and monoculture with high-yielding crop varieties, while low-input farming practices refer to mixed cropping, low fertiliser application, mainly of organic origin, hand weeding and no pesticide application (Sieverding, 1991). The effects of high-input farming practices include (i) reduction in the diversity and effectiveness of native mycorrhizal populations (Sieverding, 1991; Schreiner and Bethlenfalvay, 1995); (ii) high rates of P and N fertiliser application, which both reduce the effectiveness and alter the population structure of mycorrhizal fungi (Sieverding, 1991; Scullion et al, 1998); (iii) the use of pesticides, which can adversely affect mycorrhizal associations and communities (Perrin and Plennchette, 1993); (iv) intensive tillage, which reduces the amount of mycelium and adversely affects AM spore density in agricultural soils (Schreiner and Bethlenfalvay, 1995; Kabir et al, 1997; Miller et al., 1995; Gavito and Miller, 1998, Menendez and Scervino, 2001); (v) continuous monocropping, even in the case of perennial crops, which can lead to significant changes in the composition of native mycorrhizal fungal species (Sieverding, 1991). The effects of high-input agricultural practices include: (i) reduction in the diversity and efficacy of native mycorrhizal populations (Schreiner and Bethlenfalvay, 1995); (ii) high rates of P and N fertilization, which both reduce the efficacy and alter the population structure of mycorrhizal fungi (Scullion et al, 1998); (iii) the use of pesticides, which can adversely affect mycorrhizal associations and communities (Perrin and Plennchette, 1993); (v) continuous monocropping, even in the case of perennial crops, which can lead to significant changes in the composition of native mycorrhizal fungal species (Sieverding, 1991). The effects of high-input agricultural practices include: (i) reduction in the diversity and efficacy of native mycorrhizal populations (Schreiner and Bethlenfalvay, 1995); (ii) high rates of P and N fertilization, which both reduce the efficacy and alter the population structure of mycorrhizal fungi (Scullion et al, 1998); (iii) the use of pesticides, which can adversely affect mycorrhizal associations and communities (Perrin and Plennchette, 1993); (iv) intensive tillage, which reduces the amount of mycelium and adversely affects AM spore density in agricultural soils (Schreiner and Bethlenfalvay, 1995; Kabir et al, 1997; Miller et al., 1995; Gavito and Miller, 1998, Menendez and Scervino, 2001); (v) continuous monocropping, even in the case of perennial crops, which can lead to significant changes in the composition of native mycorrhizal fungal species (Sieverding, 1991). As certain crops have a preferential selection for certain AM fungal species and strains, cropping sequence may also influence species composition (Sieverding, 1991).

Low-input systems often involve reduced tillage, increased crop diversity, maintenance of vegetation cover, reduced chemical inputs, and also tend to establish more undisturbed ecosystems in which the mycorrhizal symbiosis can provide some of the ecological functions mentioned above (Sieverding, 1991; Kabir et al., 1997; Hamel, 1996). Such systems also influence the composition of AM fungal communities by increasing diversity and altering the relative abundance of fungal species (Douds et al., 1993) and increasing sporulation (Kurle and Pfleger, 1994) by having a higher infectivity of AMF spores than the high input systems (Sieverding, 1991; Hamel, 1996: Scullion et al., 1998). However, the selection and management of non-host plants can delay or inhibit the infective potential of propagules (Thompson, 1987; Sieverding, 1991). If at least one of the plant components in an intercropping or agroforestry system is mycorrhizal or potentially mycorrhizal, adequate inoculum levels are likely to be

maintained (Sieverding, 1991; Michel-Rosales and Valdes, 1996).

The use of AM for the above benefits is not well advanced because inoculum cannot be readily produced in artificial (axenic) culture. The need for large quantities of inoculum may limit artificial inoculation of agricultural crops with AMF in the field (Sieverding, 1991). Therefore, production on roots and their environment in the field is of great importance for AMF. In the case of agroforestry, standing farm trees or tree inoculation at nursery level could help to overcome the need for large amounts of inoculum. However, in order to properly manage the mycorrhizal symbiosis on the farm, it is necessary to know the current status of the agroecosystem at farm, field and plot level.

In the traditional agroforestry land use systems of the Sidama, it is not known how farmers' practices in selecting and managing crop and/or tree components affect native mycorrhizal associations. In these systems, 87 tree species are reported (Zebene Asfaw 2003), of which Cordia africana and Millettia ferruginea are the second and fourth most abundant tree species, respectively. However, studies on the AM fungal hosting capacity of these two tree species in these traditional agroforestry systems are lacking. Research aimed at understanding the status of mycorrhizal fungi in a Sidama traditional agroforestry system in general and around these two tree species is valuable in determining appropriate management strategies as a background against which inoculation techniques will be developed. For this, it is necessary to understand the host capacity of these agricultural trees and the compatibility of the symbionts. Some AMF have a wide host range. Therefore, the roots of standing crop trees may harbor inocula that are also of value to crops intercropped with them. Therefore, it was hypothesised that Cordia africana and Millettia ferruginea trees in the traditional agroforestry land use system of Sidama could promote greater reservoirs of AM inoculum for agricultural crops, with particular reference to maize and Millettia /Ensete plants grown on the underlying soils. This study was designed to (i) determine the number of spores of AM fungi in the soil under Cordia and Millettia trees grown on enset and maize fields, including without trees in maize monoculture; and estimate the extent of colonisation of Cordia and Millettia trees and maize roots by AMF(ii) assess the potential of field soils from the differently managed plots, under Cordia and Millettia trees, to cause AMF colonisation of maize and Millettia.

2. Materials and Methods

2.1 Description of the study area

This study was carried out in the Awassa Zuria District of the Sidama Zone in southern Ethiopia. The present report is a part of other work carried out at three sites namely Entaye (Enta), Haranfama (Hara) and Murancho Kutela (Figure 1). For this particular study, two sites, namely Entaye and Murancho Kutela, were randomly selected to collect soil and root samples for both nursery experiments and routine laboratory activities at the Wondo Genet College of Forestry.

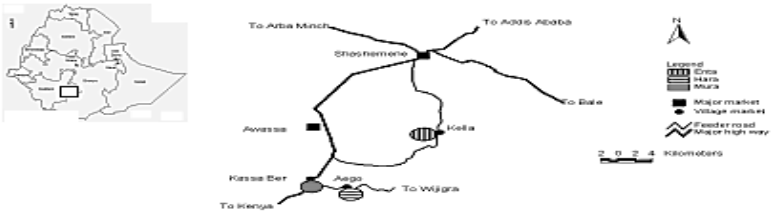

Fig.1. Map of the study sites showing the nearest markets and road network in Sidama, south Ethiopia.

2.2 Sampling plots within the fields

Sidama farms have about 10 different types of fields (Zebene Asfaw, 2003). For this study, we selected two predominant field types, namely enset and maize fields. A plot was defined as a relatively homogeneous area within a field, characterised by a single species or species mix and common management (Huxley, 1983). Soil samples for AMF spore counts (n=36) and root extractions (n=72) Table 2. For nursery level growth media, soil was collected from these plots. In the sample plots, the main weeds were first identified by the farmers themselves, then the botanical name was given based on the corresponding local names (Kelecha, 1980) or in the description by Edward et al. (1995).

Tree and enset-coffee:- These plots were characterised by multi-storey structure in which Cordia or Millettia are the upper storey. The age of the sample trees ranges from 24-32 and 17-25 year for Cordia and Millettia, respectively. Under both tree species at mid storey, a mixture of enset and coffee crop has been grown for the last five years. The ground cover, lower storey, includes Ethiopian kale, local cabbage "*Tunaye*", and pepper. The major weeds were *Snowdenia poly*stachya "merge", *Galinsoga parviflora* "Butissa", *Commelina benghalensis* "Laluntie" *Digitaria abyssinica* "Hiele", *Cyperus spp.* "Gicha", *Agrostis semiverticillata* "serdo". No external input was used. Hand hoeing was carried out twice a year. The topsoil properties are indicated in Table 1.

Tree and enset:- These plots are also located in enset fields but without coffee. The main under growth was Ethiopian Kale, "Tuneye" and pepper. At these plots enset has been grown for 5 and 7 years beneath Cordia and Millettia plots, respectively. No external input is applied. Hand hoeing is carried out twice a year. The recorded weeds were *Amaranthus s*pp., *Snowdenia polystachya*, *Cyperus spp.*, and *Galinsoga parviflora.* Soil properties are indicated in Table 1.

2.3 Spore and root sampling

Topsoil samples (0-20 cm) for spore counts and estimation of mycorrhizal colonisation and soil for the nursery experiment were collected at the beginning of the main rainy season. The physicochemical properties of all soil samples are shown in Table 1. To identify the specific plant roots in a composite soil sample is very difficult, but efforts were made to directly collect fine roots of standing plants to learn about specific characteristics, mainly their colour. Based on this fine root colour, we learnt that the maize root colour is white, while that of Millettia is cream and that of Cordia is very light brown. Using this common approach, we therefore made educated guesses based on these known root characteristics of these plants. For Cordia and Millettia grown in the top layer of enset mixed with coffee, soil samples were taken under the canopy at three distances in four different directions. Soils from each direction were pooled to obtain a composite sample. Finally, 400 g of the sample was taken separately for estimation of arbuscular mycorrhizal spores and root colonisation. Similarly, soil under Cordia and Millettia grown on enset coffee and scattered on enset and maize plots was also sampled.

For the nursery experiment, four lines, E-W and S-N, were established in both the enset and maize plots from the trunk to the canopy edge or open field. On each line from the trunk, three sample points were marked at 0.75 m, mid-canopy and canopy edge, and a fourth sample point was marked along the line in the open field. Soil samples of approximately 3 kg were then taken from a randomly selected line. During sampling, every effort was made to avoid disturbing the soil, such as repeatedly mixing by hand, as was done with the spore count samples. This precaution was taken because the process of repeated mixing can lead to loss of the hyphal network. Three replicates of soil samples from each plot were used to start the growth of maize and millet seedlings.

Table 1. Top soil characteristics from which spore density, tree root colonisation and on which seedling of maize and Millettia was raised

Plot Type	Sand %	Silt %	Clay %	pH[1)]	Na	K	Ca	Mg	CEC	T:N[2)]	O:C[3)]	P[4)]
					Meq/100 gm soil					%	%	ppm
Tree -enset-coffee												
Cordia	36	34	30	6.7	0.7	2.7	15.4	3.1	28.5	0.4	3.1	12.6
Millettia	36	34	30	6.3	0.9	1.7	16.7	3.0	29.9	0.4	3.1	9.5
Tree and enset												
Cordia	36	36	28	7.0	0.6	2.0	17.7	3.0	27.7	0.3	3.4	15.4
Millettia	36	35	29	6.6	0.8	1.4	14.0	2.1	27.3	0.4	2.7	9.0
Cordia-maize[5)]												
CT	33	35	32	6.5	0.5	2.2	18.1	3.2	29.0	0.3	3.5	9.4
MC	35	34	31	6.5	0.5	1.9	17.1	3.2	27.4	0.3	3.4	8.4
CE	35	33	32	6.4	0.4	1.9	16.7	2.9	26.4	0.3	3.1	7.4
OF	36	33	31	6.2	0.4	1.6	16.0	2.8	25.9	0.3	2.8	5.7
Millettia- maize												
CT	39	31	30	6.2	0.7	1.0	12.4	2.1	29.1	0.3	2.7	6.6
MC	39	35	26	6.1	0.8	1.2	12.6	2.2	29.0	0.3	2.9	6.4
CE	40	34	26	6.0	0.8	1.0	12.0	2.0	28.8	0.3	2.5	4.9
OF	38	34	28	6.0	0.7	1.3	10.7	1. 92	26.5	0.2	1.3	4.6

1) pH in H_20 1:2.5; [2)] T:N total nitrogen in %; [3)] O:C organic carbon [4)] Available phosphorus ppm
[5)]For maize-tree plots at laterally increasing distance from the tree trunks: CT= 0.75 m, MC= mid canopy, CE= canopy edge OF= open-maize field

2.4 AMF spore estimation

As one of the objectives of the present study was to estimate the number of AMF spores in the soil on different plot types, it was decided to keep the samples separate (Table 2). Spores were isolated from 50 g subsamples of soil. The methods described by Brundrett et al. (1996) were used for spore extraction. The spore suspension was stored in a refrigerator at -4 °C for preservation, as suggested by Dalpe (1993), until counting. Spores were counted by placing them in an 8 cm diameter nematode counting dish. Some spores were densely grouped in sporocarps and it was difficult to count the number of spores per sporocarp, so in these cases the sporocarp was referred to as one spore. A dissecting microscope was used to count the spores.

Table 2. Number of soil samples collected from different plots for estimation of number of AM spores (n=36) and root colonisation n=72).

Plot type	Tree species	No. of samples Spore	growth substrate maize	Millettia
Tree and enset-coffee	Cordia	3	3	3
	Millettia	3	3	3
Tree and enset	Cordia	3	3	3
	Millettia	3	3	3
Tree and maize	Cordia	12	12	12
	Millettia	12	12	12

2.5 Root colonization by AM fungi

Root sub-sampling and cleaning

Each 400 g soil sample was immersed in a bucket of water and gently swirled to mix and remove most of the soil. To obtain a cleaner sample of root fragments, the soil with roots was washed vigorously and poured off, the liquid passed

through 350-500 μm sieves and the roots washed free of soil. The suspended root fragments were rapidly decanted onto the 50-100 μm sieve and the decanting was repeated until all the root fragments were on the 50 μm sieve. The fine 50 μm sieve was placed in a pan of shallow water so that the floating roots and any debris could be picked up. Roots were sorted into two categories, living <1 and 1-2 mm, representing fine roots. Depending on the type of sample, up to 0.5-1.5 g of the <1 mm categories were taken and samples were stored in 50% ethanol until clarified and stained (Brundrett et al., 1996).

Root clearing and staining

The duration of autoclave sterilisation can vary depending on the type and density of the plant root. Autoclave sterilisation at 121°C is usually the same temperature for most materials. To standardise sterilisation we used a range of times and replicated three times. Based on this, the better time was found to be 20 minutes for Millettia and 25 minutes for Cordia. Young roots of maize and Millettia plants grown in the nursery were autoclaved for 5 minutes.

From the fresh field soil samples, the roots were gently washed with tap water to remove adhering soil particles. The roots were then cut into short segments (typically 1-2 cm) using a sharp scalpel. The roots were boiled in 10% KOH solution for 15-20 min at 90-0°C in a boiling water bath, rinsed several times with water and acidified with 1% HCI solution. Root samples were stained by adapting the techniques developed by Phillips and Hayman (1970) as modified by Koske and Gemma (1989). Roots were stained by boiling in 0.05% trypan blue at 90 0°C for 10 min, followed by distaining in acidic glycerol at room temperature. The stained root segments were mounted on slides in acidic glycerol, the coverslips placed over them and gently tapped with the blunt end of a pen. The stained roots were poured onto 50 cm sieves and destaining solution. The stained root segments were evenly distributed in a petri dish marked with grid lines (the method of intersecting grid lines on the bottom to form approximately 1.3 cm squares and root colonisation of individual species was estimated as described by Brundrett et al. (1996)). Cross-checks were made at a higher magnification of x200 (McGonigle et al., 1990). In most cases, Cordia roots were crushed when examined to confirm the presence of arbuscules, vesicles and/or In general, the intersections of the grid with the root were examined for the presence of AMF structures. The presence of colonisation in a root segment was recorded if hyphae (only), vesicles or arbuscules were found. Approximately 100-150 sections were examined for each sample. Total root colonisation was calculated as % colonisation = total number of positive segments/total number of segments examined x 100.

Nursery experiment

The aim was to assess the potential of field-collected soil to induce root colonisation of the two important plants in the study area. Thirty-six soil samples were used as growth medium in the nursery (Table 2). Maize and millet were used as host plants. Both species started germination after 5-8 days. A local variety of maize was purchased from the local market and sown. For Millettia, a locally collected seed source was used after four months of storage. Four seeds of each species were sown on 7 July 2000 in soil contained in a perforated plastic pot of approximately 3000 ml, with three replicates of each soil type and host seedling species. Plants were thinned to one per pot 15 days after sowing. All plants were harvested 45 days after sowing. Feeder roots were cut from the main root system, chopped into 1 cm lengths and mixed to represent fine roots < 2 mm. Then, 1.5 g of fine roots < 1 mm were collected for clearing and staining as described above. The percentage of root colonisation was calculated and the total fresh weight of the plant was determined.

2.6 Data analysis

To assess the relationships between spore counts in field soils and AM colonisation percentage n of maize and Millettia plants grown on these soils from the same sample plots, spore counts per sample were log-transformed. Data from colonised roots were also arcsine transformed to conform to the normal distribution (Sokal and Rohlf, 1981). Pearson's correlation was used to assess the relationships between the number of spores in field soils and the degree of root colonisation in the nursery experiment. The transformed values were also used for the ANOVA calculation. One-way ANOVA with STATISTICA (1999) was used to test for differences between the number of spores and the degree of root colonisation in each sampling plot and the data at the nursery level. Where significant F values were obtained ($P < 0.05$), the plot means were separated by the Duncan test. Results for spore counts and percent root colonisation are presented as back-transformed data.

3. Results

3.1. Number of AM spores and root colonization in the field

Spore number

Spore counts varied from 3 to 31 and 1 to 24 g -1 dry soil under Cordia and Millettia canopy, respectively, at enset-coffee, enset and open maize fields (Tables 3) at the different and grown at increasing distances from tree trunks of scatted trees (Table 4).. Soil samples beneath Cordia and Millettia had about the same number of spores. There were significancant differences ($n = 24$, $F = 3.807$, $p < 0.004$) in number of spores between soil samples from varying tree-crop combination plots. Significantly, ($p < 0.05$) lower numbers of spore were observed in soils under Cordia and Millettia trees grown on maize plots than under tree grown in enset-coffee and enset plots (Table 3). Numbers of spores were variable among soil samples collected at increasing distances from tree trunks (Table 4). However, the number of spores under the canopy did not significantly ($p < 0.05$) vary with increasing distances from tree trunks (Table 4). In the maize field outside the canopy the number of spores was about half the number under the canopy.

Percentage of AMF root colonization

Tables 3 shows mean spore count g -1 dry soil from under Cordia and Millettia canopy, enset-coffee, enset and open maize fields at the different sites. Spore counts varied from 3 to 31 and 1 to 24 g -1 dry soil under Cordia and Millettia canopy, respectively. Soil samples beneath Cordia and Millettia had about the same number of spores. There were significance differences ($n = 36$, $F = 3.807$, $p < 0.004$) in number of spores between soil samples from varying tree-crop combination plots. Significantly, ($P < 0.05$) lower numbers of spore were observed in soils under Cordia and Millettia trees grown on maize plots than under tree grow in enset-coffee and enset plots (Table 3). Numbers of spores were variable among soil samples collected at increasing distances from tree trunks (Table 4). However, the number of spores under the canopy did not significantly ($p < 0.05$) vary with increasing distances from tree trunks. In the maize field outside the canopy the number of spores was about half the number under the canopy (Table 4).

Table 3. Number of spores g^{-1} soil dry weight and percent length of colonised roots of *Cordia africana* and *Millettia ferruginea* grown at different sites. Mean ± std of three replications.

Plot type	species	Spore g^{-1} soil	Colonised root length in%
Tree-enset-coffee	Cordia	20a ± 11.0	44.49 ±11.0
	Millettia	15a ± 6.7	41.47±17.0
Tree-enset	Cordia	14a± 8.8	38.01±8.4
	Millettia	17a± 6.5	48.38±11.4
Tree-maize	Cordia	8bc± 4.8	30.31±12.2
	Millettia	6bc± 4.1	39.76±14.0

The same letter(s) following mean values indicate no significant ($P < 0.05$) difference between tree species

Table 4. Mean number of spore g^{-1} dry weight soil and percent length of colonised roots of *Cordia*

africana and *Millettia ferruginea* grown at increasing distances from tree trunks of scatted trees in maize fields. Mean ± std of three replications

Distances from	Spore g $^{-1}$soil		Colonised root length %	
	Cordia	Millettia	Cordia	Millettia
CT at =-0.75 m	9±3.4	7±5.2	31.54±17.1	44.06±14.3
Mid Canopy	6±6.6	6±4.2	30.30±10.3	39.58±17.4
Canopy edge	8±5.5	7±4.8	29.09±9.5	35.65±10.9
open-maize field	4±3.9	5±2.6		-

For Cordia and Millettia trees scattered on maize fields, there was a non-significant tendency for lower proportion of colonized roots with increasing distances from the tree trunks (Table 4). At all distances from tree trunk, higher root colonization was also noticed for Millettia than for Cordia trees.

3.2 ***Nursery grown plants***

Levels of AM colonization

Table 5 shows mean level of root colonization while the overall value ranged from 4 to 66 % for maize and 3 to 37 for Millettia. Colonization of roots by AMF was relatively more intensive in maize than in Millettia seedlings. There were significant differences (n = 24, F = 2.671, P < 0.030) in levels of root colonization between maize plants grown on soils samples from different tree crop combinations, with the lowest colonization in soil from maize fields. Although not significant, root colonization of nursery grown Millettia plants tended to be higher than values on soils from under Cordia trees at enset-coffee plots, followed by soil from under Millettia trees at enset plots. Lowest root colonization was noted in soils from maize fields. The percentages of AMF colonized roots of maize plants grown on soil from under Cordia trees at enset-coffee plots were 25 % greater than those grown on soil from under Cordia tree canopy at maize plot. For both maize and Millettia plants grown on soils collected at laterally increasing distances from tree trunks, no consistent pattern of colonization was observed (Table 6). The relationships between estimates of AMF colonisation of maize roots and Millettia seedlings grown in the nursery and the number of spore in the soil samples used as growth media was determined. Percentage of AM colonized roots of maize was significantly positively correlated with the number of spore counts from field soils (Table 7).

Table 5. Mean roportion of colonised roots of maize and Millettia seedlings grown in nursery soils taken from under the canopy of Cordia and Millettia trees grown in different fields. Mean ± std of three replications

Plot type	Colonised root of maize seedling (%)		Colonised root of Millettia seedling (%)	
	Cordia	Millettia	Cordia	Millettia
Tree-enset-coffee	51.9a±13.6	32.4a±8.4	27.4±10.2	19.5±10.6
Tree-enset	42.9ab±11.4	48.2ab±14.3	21.7±8.1	26.0±8.4
Tree-maize	24.9b±13.2	26.4b±9.8	15.2±8.2	12.4±7.1

The same letter(s) following mean values indicate no significant (P < 0.05) difference between tree specie

Table 6.Mean proportion of colonised roots of maize and Millettia seedlings grown in nursery soils taken at increasing distances from the trunk under Cordia and Millettia trees grown on maize fields. Mean ± std of three replications

Distances from trunk[1)]	Colonised roots of maize seedling (%)		Colonised roots of Millettia seedling (%)	
	Cordia	Millettia	Cordia	Millettia

CT	31.1±16.9	25.2±12.9	12.5±9.5	15.4±9.5
MC	25.6±6.72	26.8±10.5	11.1±3.1	11.2±8.8
CE	18.1±15.45	27.1±10.3	15.9±10.7	10.5±3.8
OF	24.0±13.42	23.1±14.9	19.8±13.0	11.4±6.1

[1)]CT= 0.75 m, MC= mid canopy, CE= canopy edge OF= open-maize field .

Table 7. Pearson correlation between log-transformed spore density and arcsin-transformed proportion of AM colonization of maize and Millettia seedling grown in soils from under Cordia and Millettia canopy of different fields and in soils from open maize fields

Species	r	p	n
Maize	0.340	0.043	36
Millettia	0.131	0.447	36

Fresh weight of plant

Soils used as growth media in nursery, from different plots beneath Cordia and Millettia trees had no significant effects on the fresh weight of either maize or Millettia plants 45 days after sowing. In all cases, maize plants attained higher fresh weight than Millettia (Fig. 2a, 2b, 3a and 3b). Fresh weights of plants grown in the soil collected from under Cordia and Millettia trees grown in a enset fields (Fig. 2a and 3a) were greater than from maize fields. Although not statistically significant ($P < 0.05$) a decrease in fresh weight was noticed for both maize (Fig. 2b) and Millettia (Fig. 3b) plants grown on soils from plots at laterally increasing distances from Cordia and Millettia tree trunks.

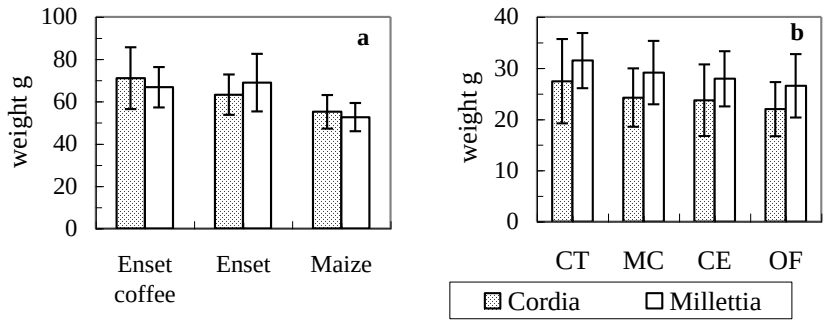

Fig. 2. Mean and standard error fresh weight of maize plants grown on soils from beneath Cordia and Millettia trees (a) grown at enset and maize fields and (b) at laterally increasing distances from tree trunks at maize fields. CT = distance at 0.75 m, MC = at mid-canopy CE = at canopy edge and OF = at open maize field

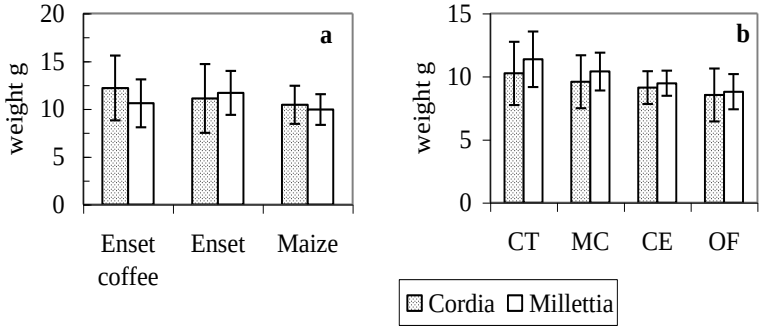

Fig. 3. Mean and standard error fresh weight of Millettia plants grown on soils from beneath Cordia and Millettia trees (a) grown at enset and maize fields and (b) at laterally increasing distances from tree trunks at maize fields. CT = distance at 0.75 m, MC = at mid-canopy, CE = at canopy edge and OF = at open maize field

4. Discussion

Studies on the effect of Cordia and Millettia trees on the number of AM fungi spores in field soils are lacking. It is therefore difficult to compare the results of this study with those of others, as most of the studies on spore numbers and colonisation levels have been carried out on other trees, crops and/or land-use systems under different ecological conditions. Spore counts and/or root colonisation levels are commonly used to study mycorrhizal associations (Dalpe, 1993; Brundrett et al., 1996). However, the number of spores and the percentage of mycorrhizal colonisation of root systems are not always good measures of the potential effectiveness of mycorrhization (Reich and Barnard, 1984; Merryweather and Fitter, 1998). Despite these limitations, both the number of spores and the percentage of infection in roots in the soil are used as a means of assessing mycorrhizal association.

Number of spores

Depending upon the seasons, AM fungi spores formation could be different at a given site (Allen et al., 1998). Since the sampling period of this work was 6 weeks after the onset of the main rainy season, the high number of spores could be related to seasonality effect, as suggested by other workers for other species (Brundrett et al., 1996; Janos, 1996).

The number of AM fungi spores ranged 1-31 per gram of dry soil, which is comparable with some of the previous findings from various land use types (Table 8). The number of spores was approximately the same under Cordia and Millettia canopies (maximum 31 and 25 g-1 soil, respectively). The occurrences of spores reflect many factors: such as the ability of native AM fungi to produce spores, the successful development of AM fungi and host tree/plants, or suitability of the environmental conditions. Soil physical, chemical and biological conditions are well-known to affect spore germination and mycelial dispersion of AM fungal species (Sieverding, 1991). Soil beneath Cordia and Millettia at maize fields had a significantly lower number of spores than in enset fields. Similarly, soils beneath trees at enset-coffee and at enset plots in enset fields contained four to five as many spores as in open-maize plots on maize fields.

The increased number of spores beneath Cordia and Millettia trees at enset-coffee and enset plots could probably be explained by four major factors or by their combined effects: (1) less soil disturbance/tillage(; Gavito and Miller,

1998, Menendez and Scervino, 2001), (2) higher number of plant species,(Schreiner and Bethlenfalvay, 1995) (3) more active biological conditions (Sieverding1991) and (4) higher cover/vegetation as compared with plots in open-maize fields (Sieverding;1991;Janos, 1996). Although those factors have not been critically investigated in this study, based on farmers' knowledge on frequency of tillage, one could speculate that tillage could influence mycorrhizal inoculum/spore. Farmers practice relatively higher frequency of tillage for maize than enset plots. Available reports for different species and land uses types indicated a lower AM fungi spore density in disturbed soils than in less disturbed (Miller et al., 1995; Gavito and Miller, 1998, Menendez and Scervino, 2001) or undisturbed soils (Lovera and Cuenca, 1996). There are also several reports of low AM fungi spore number in disturbed maize fields (Jasper et al., 1991; Douds et al., 1993; McGonigle and Miller 1996; Kabir et al., 1998; Boddington and Dodd, 2000). Variation in spore number could also be related to the differences between soil properties of studied plots.

Regarding biological conditions, the host genotype, vegetation cover and microbial activities could influence AM symbiosis (Janos, 1996; Smith and Read, 1997). The difference in number of spores between the Cordia and Millettia trees could be explained by the hosting ability for AM fungi of individual tree species. As having dominantly perennial plant species and high vegetation cover, the enset-coffee and enset plots have some characters of forest-dominated ecosystems. The numbers of spores recovered from tree-enset combination plots are higher than those reported from some tropical natural forests (Table 8). However, in the tropical moist forests the number of spores tend to be lower (Janos, 1980; Sieverding, 1991; Fischer et al., 1994) than in polyculture agricultural systems. In these systems, like the Sidama traditional agroforestry land use, the spore numbers are much greater than in intensive or high input systems (Sieverding, 1991). In general, the effect of vegetation and microorganisms on the amount of spore/inoculum is site specific (Sieverding, 1991; Fischer et al, 1994).

Table 8. Spore density g^{-1} soil of AMF recorded at different places under three major land uses

Land use	Country	Site/species/forest type	Spore no. g^{-1} dry soil	References
Tropical natural forest	Argentina	Semi-humid	5 – 23	Fontenla *et al.*, 1998
	Mexico	deciduous	2 – 28	Allen *et al.*, 1998
	Costa Rica	moist forest	120	Johnson and Wedin 1997
	Cameroon	deciduous	2 – 5	Musoko *et al*., 1994
	China	moist forest	6 – 19	Zhao *et al*., 2001
Woodland	UK	Oak	1 – 5	Merryweather and Fitter, 1998
	UK	sycamores	1 – 50	"
Agriculture	Colombia	Cassava	7 – 28	Sieverding 1991

	USA	Orchard	5 – 36	Reich and Barnard, 1984
Agroforestry				
	Senegal	plantation	1 – 8	Ingleby *et al*., 1997
	Indonesian	tree-maize	4 – 28	Boddington and Dodd, 2000
	Mexico	citrus/ dry tropics	1 – 4	Michel-Rosales and Valdes, 1996

The number of spores did not significantly vary with increasing distances from tree trunks (Table 4), which was observed only on maize fields with a generally low number of spores. At the closest distances from tree trunks, the number of spores was only slightly higher than further away from the crown cover. This is in agreement with Musoko et al. (1994) who reported that distance from *Terminalia superba* trees trunk has no influence on the number of spores, although more spores were observed close to the trunks. The open field samples were taken well outside the area less influenced by tree roots and the farmers are growing maize under the tree canopy with almost the same intensity of weeding and tillage practice as outside the canopy. The lower spore number outside the crown canopy in the maize field sample may thus indicate that the high spore number under the canopy is influenced by the root system of trees.

Root colonization in the field

The degree of AM fungi colonisation of Cordia and Millettia trees was variable, with fairly high standard deviation. This could be explained by differences in rapid root growth between trees and field plots during the wet period when the sampling was carried out. The seasonal influence on colonization of grass roots and common forbs and graminoids was reported by Allen (2001) and Michelsen et al., (1993), respectively. In this investigation, levels of root colonization were relatively high for both Cordia (25 to 57%) and Millettia (33 to 62%) reflecting their mycotropic nature. This is comparable with 27 to 57% root colonisation for lime grown in three agroecosystems reported by Michel-Rosales and Valdes (1996). Difference in AM colonization in the soil beneath Cordia and Millettia trees grown at enset-coffee and enset, and at maize plots examined in this study, probably resulted from interactions between tillage practices and species composition/cover. The relatively higher plant cover and richness at enset plots may explain differences in mycorrhizal infection between soil samples collected from various plots. At tree enset-coffee and tree enset plots, the infection of new roots probably occurred directly from the roots in a manner more rapid and efficient way than from germinating spores. Moreover, as being a perennially dominated and more diverse system, the tree enset-coffee and tree enset combination plots are probably suitable to host various AM fungi species. It is possible that due to low frequency of tillage practices for these tree-enset coffee and tree enset plots, plant roots suffer less damage and the hyphae network is less disturbed. This is in agreement with Michel-Rosales and Valdes (1996) who reported a higher percentage (57%) of root colonization for lime trees grown in gardens than those grown (27%) in plantations. Boddington and Dodd (2000) reported a greater length of extra-radical mycelium of AM fungi in soil under agroforestry systems than in a monoculture. McGonigle et al. (1999) also reported that reduced tillage increased the mycorrhizal association. However, different species of AM fungi that colonize host roots can respond to soil disturbance in a different ways (Merryweather and Fitter, 1998). Although not quantitatively and qualitatively assessed in this study, it is possible that tree species or maize might selectively favour only certain AM fungal species, which may initiate relatively low root

infection or are less compatible at this stage of development.

Slight spatial differences were detected in levels of mycorrhizal colonization. The lack of significant differences among plots at increasing lateral distances from tree trunks could be attributed to the fact that roots might be extended at least up to the edge of the crown with uniform distributions of AM fungal infection.

Root colonization in nursery

At nursery level, relatively more intensive root colonization was observed for maize plants than for Millettia plants. The literature does not appear to contain details of mycorrhizal dependency of Millettia. The high colonisation of maize could be due to its high mycorrhizal dependency, as it is well known to be a facultative host for AM fungi (Sieverding, 1991). However, soil under Cordia and Millettia trees at enset-coffee and at enset plots resulted in significantly higher levels of maize root colonisation than in soil from beneath tree-maize and open-maize plots.

The difference in number of spores that occurred as a result of differences in tree-crop plots and open-maize were reflected in the levels of root colonization of maize and Millettia grown in the nursery. Significantly positive correlation of spore numbers in field soil with the level of colonisation on maize roots (Table 7) indicates that the original set of AM fungi might play a role in the functional relationship between the roots and the fungi, as well as to support hyphae for nutrient uptake. These results agree with the conclusion that level of colonization was positively correlated with the number of AM fungi spores (Simpson and Daft, 1990; Frank and Morton, 1994; Frioni et al., 1999; Oliveira and Sanders, 1999). On the other hand, it is in disagreement with the lack of relationships between spore number and root colonization as suggested by Merryweather and Fitter (1998).

The relatively poor correlation of the spore number in the soil samples before planting and level of root colonization in Millettia plants could partially be explained by the low number of suitable spore of AM fungi species, lack of compatibility of AM fungi or most probably a too small Millettia plant not capable of acting as an effective host at this stage of development. The low proportion in the extent of AM colonization of maize and Millettia roots with increasing distances from the tree trunk was not significantly related to the number of spores in field soil.

Plant size

Both maize and Millettia plants with high level of root colonization, attained higher fresh weight when grown in soil under Cordia at enset-coffee, and Millettia at enset plots. The differences in fresh weight might also be related to the differences in the level of soil fertility, since soil fertility indicators under tree-enset combinations were relatively higher than in all other soil samples (Table 1). Apart from these, there was no readily apparent explanation of the difference in fresh weight between plants grown on soils from different plots.

Regarding AM association, we believe that this is the first report to deal with Cordia and Millettia trees in the Sidama traditional agroforestry land use systems. It could contribute the base line data necessary for future in-depth studies, on the relationship to environmental components of this complex system. For better understanding, of the factors that influence AM colonization, continuous sampling in needed, during both dry season and rainy seasons. Furthermore, detailed investigations are needed to understand the reasons for occurrences and species diversity of AM fungi as well as the predominant species.

5. Conclusions

In conclusion, tree-enset-coffee and tree-enset combinations can induce a higher number of spores and level of colonisation than tree-maize plots or the open maize field. Soil under Cordia and Millettia in maize fields had a significantly

lower number of spores than in enset fields. The number of spores did not vary significantly with increasing distance from the tree trunk. Although not statistically significant, differences were observed in the degree of root colonisation for both Cordia and Millettia trees grown in different plots. In general, the proportions of colonised roots were in the following order: tree enset coffee > tree enset > tree enset maize for Cordia trees and tree enset > tree enset coffee > tree enset maize for Millettia trees.

Soil under Cordia and Millettia trees in enset coffee and enset plots resulted in significantly higher levels of maize root colonisation than soil under tree maize and open maize plots. The high number of spores in the field soil had a positive, statistically significant effect on the colonisation of maize and Millettia plants in the nursery. Both maize and Millettia plants with high root colonisation achieved higher fresh weight when grown in soil under Cordia in enset coffee and Millettia in enset plots.

Acknowledgements

The financial and material support for this study was obtained from Swedish International Development Cooperation Agency and Wondo Genet College of Forestry. Assistance provided by National Soil Laboratory in Addis Ababa is acknowledgements. We thank Mr. Solomon Zewdie, head of Department of Basic Sciences at Wondo Genet College of Forestry, for providing pleasant working conditions during laboratory work.

References

Allen, E. B., Rincon, E., Allen, M. F, Perez-Jimenez, A. and Huante, P. 1998. Disturbance and seasonal dynamics of mycorrhizae in a tropical deciduous forest in Mexico. *Biotropica* 30(2): 261-274.

Allen, M. F. 2001. Modelling arbuscular mycorrhizal infection. Is % infection an appropriate variable? Mycorrhiza 10:255-258. Farmers, local knowledge and topsoil properties of agroforestry practices in Sidama, southern Ethiopia. Agroforest Systems 71:35-48

Boddington, C. L. and Dodd, J. C. 2000. The effect of agricultural practices on the development of indigenous arbuscular mycorrhizal fungi. I. Field studies in an Indonesian ultisol. Plant and Soil 218:137-144.

Brundrett, M., Bougher, N., Dell, B., Grove, T. and Malajczuk, N. 1996. Working with mycorrhizas in forestry and agriculture. Australian Centre for International Agriculture Research.

Dalpe, Y. 1993. Vesicular-arbuscular mycorrhiza. M. R. ed: Soil Sampling and methods of analysis. 287-301 Canadian society of soil science, Lewis Publishers.

den Biggelaar, C. 1996. Farmer experimentation and innovation: a case study of knowledge generation process in agroforestry systems in Rwanda. Nancy Hart (ed) Community Forestry Case Study Series 12, FAO, Rome.

Douds, D. D., Janke, R. R. and Peters, S. E. 1993. VAM fungus spore populations and colonization of roots of maize and soybean under conventional and low-input sustainable agriculture. Agriculture, Ecosystems and Environment 43 (3-4): 325-335.

Edwards, S., Sebsebe, D. and Hedberg, I. 1995. Flora of Ethiopia and Eritrea, part 2 (canellaceae to euphorbiaceae), Vol. 2. The National Herbarium (Addis Ababa, Ethiopia) and Department of Systematic Botany (Uppsala, Sweden).

Fischer, C. R., D. P. Janos, D. A. Perry, R. G. Linderman, and P. Sollins. 1994. Mycorrhiza inoculum potentials in tropical secondary forests. Biotropica 26: 369-377.

Franke, M. and Morton, J. 1994. Ontogenetic Comparisons of Arbuscular Mycorrhizal Fungi Scutellospora Heterogama and Scutellospora Pellucida - Revision of Taxonomic Character Concepts, Species Descriptions, and Phylogenetic Hypotheses. Canadian Journal of Botany 72:122-134.

Frioni, L. Minasian, H. and Volfovicz, R. 1999. Arbuscular mycorrhizae and ectomycorrhizae in native tree legumes in Uruguay: Forest Ecology and Management 115(1): 41-47.

Gavito M. E. and Miller M. H. 1998. Changes in mycorrhiza development in maize induced by crop management practices. Plant and Soil 198 (2): 185-192.

Hamel, C. 1996. Prospects and problems pertaining to the management of arbuscular mycorrhizae in agriculture. Agriculture, Ecosystems and Environment 60:197 – 210.

Huxley, P. A. 1983. Comment on agroforestry classifications: with special reference to plant aspects. In: Plant research and Agroforestry, ed, P.A. Huxley, 161-171, ICRAF, Nairobi, Kenya.

Ibijbijen, J., Urquaiga, S., Ismali, M., Alves, J. R. and Boddey, R. M. 1996. Effect of arbuscular mycorrhizal fungi on uptake of nitrogen by Brachara arrecta and Sorghum vulgare from soils labelled for several years with N15. New Phytologist. 133: 487-494.

Janos D P. 1980. Vesicular-arbuscular mycorrhizae affect low land tropical rainforest plant growth. Ecology 61:151-162.

Janos D. P. 1996. Mycorrhizas, succession, and the rehabilitation of deforested lands in the humid tropics. In: Fungi and environmental changes, eds. J.C Frank, N. Magan and G.M. Gadd, 129-162. British Mycological Society, Cambridge University press.

Jasper, D. A., Abbott, L. K. and Robson, A. D. 1991. The effect of soil disturbance on vesicular-arbuscular mycorrhizal fungi in soils from different vegetation types. New Phytol. 118:471-76.

Kabir, Z., O'Halloran, I. P., Widden, P. and Hamel, C. 1998. Short note: Vertical distribution of arbuscular mycorrhizal fungi under corn (Zea mays L.) in no-till and conventional tillage systems. Mycorrhiza 8: 53-55.

Kabir, Z., O'Halloran, I. P. Fyles, J. W. and Hamel, C. 1997. Seasonal changes of arbuscular mycorrhizal fungi as affected by tillage practices and fertilization: Hyphal density and mycorrhizal root colonization. Plant and Soil 192: 285-293.

Kelecha, W. M. 1980. A glossary of Ethiopian plant names. 3rd edition. Addis Ababa.

Koske, R. E. and Gemma, J. N. 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycotoxin Research 92: 486-505.

Kurle, J. E. and Pfleger, F. L. 1994. Arbuscular mycorrhizal fungus spore populations respond to conversions between low-input and conventional management practices in a corn-soybean rotation. Agronomy Journal 86:467-475.

Lovera, M. Y. and Cuenca, G. 1996. Arbuscular mycorrhizal infection in Cyperaceae and Gramineae from natural disturbed and restored savannahs in La Gran Sabana, Venezuela. Mycorrhiza. 6:111-118.

McGonigle, T. P. and Miller, M. H. 1996. Development of fungi below ground in association with plants growing in disturbed and undisturbed soils. Soil Biology and Biochemistry 28:263-269.

McGonigle, T. P., Miller, M. H., Evans, D. G. Fairchild, G. L, and Swan, J. A. 1990. A new method, which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist 115:495-501.

McGonigle, T. P., Miller, M. H. and Young, D. 1999. Mycorrhizae, crop growth, and crop phosphorus nutrition in maize-soybean rotations given various tillage treatments. Plant and soil 210:33-42.

Menendez, A. B. Scervino, J. M. and Godeas, A. M. 2001. Arbuscular mycorrhizal populations associated with natural and cultivated vegetation on a site of Buenos Aires province, Argentina. Biology and Fertility of Soils 33(5): 373-381.

Merryweather, J. W. and Fitter, A. H. 1998. Patterns of arbuscular mycorrhiza colonisation of the roots of Hyacinthoides non-scripta after disruption of soil mycelium, Mycorrhiza 8 (2): 87-91.

Michel-Rosales, A. and Valdes, M. 1996. Arbuscular mycorrhizal colonization of lime in different agroecosystems of the dry tropics. Mycorrhiza 6(2): 105-109.

Michelsen, A. 1993. Growth improvement of Ethiopian acacias by addition of vesicular-arbuscular mycorrhizal fungi or roots of native plants to non-sterile nursery soil. Forest Ecology and Management 59:193-206.

Miller, M..H., McGonigle, T.P. and Addy, H. D. 1995. Functional ecology of VA mycorrhizas as influenced by P fertilization and tillage in an agricultural ecosystem. Critical Reviews in Biotechnology 15: 241-255.

Musoko M; Last FT; Mason PA. 1994. Populations of spores of vesicular-arbuscular mycorrhizal fungi in undisturbed soils of secondary semi-deciduous moist tropical forest in Cameroon. Forest Ecology and Management 63(2-3): 359-377.

Negash L. 1995. Indigenous trees of Ethiopia: biology uses and propagation techniques. Printed by SLU Reprocentralen, Umeå, Sweden.

Oliveira, A. A. R. and Sanders, F. E. 1999. Effect of management practices on mycorrhizal infection, growth and dry matter partitioning in field-grown bean. Pesqui. Agropecu. Bras., 34: 1247-1254.

Perrin, R. and Plenchette, C. 1993. Effect of some fungicides applied as soil drenches on the mycorrhizal infectivity of two cultivated soils and their receptiveness to Glomus Intraradices. Crop Protection 12: 127-133.

Phillips, J. M. and Hayman, D. S. 1970. Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:158-161.

Reader, R.J. 1998. Relationship between species relative abundance and plant traits for infertile habitat. Plant Ecology, 134: 43-51.

Reich, L. and Barnard, J. 1984. Sampling strategies for mycorrhizal research. New Phytol. 98:475-479.

Schreiner, R. P. and Bethlenfalvay, G.J. (1995). Mycorrhizal interaction in sustainable agriculture. Critical Reviews in Biotechnology, 15: 271-285.

Scullion, J. Eason, W.R. and Scott, E.P. 1998. The effectivity of arbuscular mycorrhizal fungi from high input conventional and organic grassland and grass arable rotations. Plant and Soil 204: 243-254.

Sieverding, E. 1991. Vesicular arbuscular mycorrhiza management in tropical agrosystems. Technical cooperation. CTZ, Eschborn, Germany.

Simpson, D. and Daft, M. J. 1990. Interactions between water-stress and different mycorrhizal inocula on plant growth and mycorrhizal development in maize and sorghum. Plant and Soil 121: 179-186.

Smith, S.E. and Read, D. J. 1997. Mycorrhizal symbiosis. 2nd Edition. Academic Press, UK.

Sokal, R. R. and Rohlf, F. J. 1981. Biometry: principles and practice of statistics in biological research. 2nd edition. W.H. Freeman and company, San Francisco, USA. 417-429pp.

Thompson, J.P. 1987. Decline of vesicular-arbuscular mycorrhizae in the long fallow disorder of field crops and its expression in phosphorus deficiency of sunflower. Australian Journal of Agricultural research, 38: 847-867.

Zebne Asfaw (2003) Tree species diversity, topsoil conditions and arbuscular mycorrhizal association in the Sidama traditional agroforestry land use, Southern Ethiopia. Doctoral Thesis, Swedish University of Agricultural Sciences,Uppsala. Silvestria, 263