CHAPTER 3

ARBUSCULAR MYCORRHIZAL INOCULUM POTENTIAL OF SOME BANANA PLANTATION SOILS IN UGANDA

3.1 Introduction

Uganda is a major producer and consumer of bananas in Africa (Karamura, 1993). Over 80% of the bananas grown are East African highland bananas (genotype AAA) (Karamura *et al.*, 1996) and approximately 8% of the total banana population consists of the recently introduced genotype *Pisang Awak* (genotype ABB). Unfortunately, serious yield declines attributable to complex interactions between soil fertility depletion and the accumulation of banana pests and diseases have been observed during the past two decades (Bekunda and Woomer, 1996). Current soil, pest and disease management practices on bananas are based on cultural control: clean planting material and crop sanitation (Bekunda and Woomer, 1996).

Developments emphasize the potential use of AMF (arbuscular mycorrhizal fungi) since they were shown to stimulate plant growth and provide protection against some soil-borne pathogens. Inoculation experiments with AMF have resulted in increased banana plant growth and P uptake (Knight, 1988; Rizzardi, 1990; Lin and Fox, 1992, Declerck *et al.*, 1994; Jaizme-Vega and Azcon, 1995;

Shashikala *et al.*, 1999; Yano-Melo *et al.*, 1999), and increased banana plant tolerance to nematodes (Umesh *et al.*, 1988; Pinochet and Fernandez, 1997; Jaizme-Vega *et al.*, 1997; Jaizme-Vega and Pinochet, 1998).

Assessment of mycorrhizal inoculum potential (MIP) defined as the capacity of mycorrhizal propagules in the soil to colonize plant roots provides the means to investigate the role that AMF symbiosis plays in ecosystem processes across different habitats or microsites (Asbjornsen and Montagnini, 1994). Measurement of the population level in soil is necessary for planning a strategy of maintenance, enhancement, or replacement with more desirable AMF.

The purpose of this investigation was to estimate and compare the mycorrhizal inoculum potential of AMF in soils collected from various banana plantations in Uganda. This is, to my knowledge, the first assessment of AMF in banana plantation soils in Uganda.

3.2 Materials and method

3.2.1 Sampling sites and methods

Sampling was carried out in February-March 1999 in Uganda in the districts of Luwero, Masaka, Kabale, and Ntungamo (Appendix C). The sites were used in a previous countrywide survey on biotic production constraints of *Musa* in Uganda (Gold *et al.*, 1994). At each site soil samples were taken from (1) three farms with good banana production and, (2) three farms with declining banana production. In a single farm, five soil samples from East African highland banana (*Musa acuminata*, genotype AAA) and a further five soil samples from *Pisang awak* (*Musa acuminata*, genotype ABB) were randomly collected. When the representatives of the two genotypes were not obtainable together from a single farm, the nearest farm with the required genotype was sampled. For each banana genotype, the five soil samples were pooled into a single paper bag.

The soil samples were collected from young suckers less than 50 cm in height. A standard-size excavation of 20x20x20 cm extending outward from the corm of the sucker was dug. A sub-sample of approximately 100 g soil was taken.

Farm description (geographical position, age, genotype distribution) was noted (Appendix A). The geographical position was obtained using a Global Positioning System (GPS) receiver (Ensign).

3.2.2 MIP assessment

A modified dilution-series technique (Moorman and Reeves, 1979) was developed to assay the total number of infective mycorrhizal propagules (spores, hyphae and root fragments) in the soil samples. Test soils were crushed to pass through a 2 mm sieve. A ten-fold dilution series was then prepared by thoroughly mixing the freshly sieved inoculum soil with a sterilized soil substrate by shaking for 30 s in an inflated sealed plastic bag. Dilutions were prepared (10^{-2}, 10^{-1} and undiluted) and the soil packed into 60 g plastic growing containers, with five replicate containers per dilution level. All soils were then watered to field capacity. Sudan grass (*Sorghum sudanense* (Piper) Staph.) was the test plant, and sterilized seeds were sown into each container and seedlings thinned to one per container after germination. The containers were kept in a glasshouse (12°C/25°C) and equally watered as needed with deionised water with no addition of nutrients. After 21 days, the seedlings from all dilution levels were harvested by carefully washing their roots free of soil. Whole root samples were fixed in 50% ethanol for a minimum of 24 hours prior to staining according to the method described by Koske and Gemma (1989). Estimation of the percentage root colonization by AMF and the intensity of colonization within the roots were performed using the subjective visual technique (Kormanik and McGraw, 1982). Using a dissecting microscope (Wild M5-101485, Switzerland) at a magnification of 400X, assessment of the percentage root colonization was made according to

a classification used at the Institute for Mycorrhizal Research and Development, USDA Forest Service, Athens, Georgia. The classification in broad classes of percentage colonization is as follows: Class 1 = 0-5%; Class 2 = 6-26%; Class 3 = 26-50%; Class 4 = 51-75%; and Class 5 = 76-100%. Intensity of colonization was evaluated in three categories according to Kormanik *et al.* (1980). An intensity of 1 was assigned to roots with small colonization sites widely scattered along the roots; an intensity of 2 represented larger colonization sites more uniformly distributed through the colonized roots, but rarely coalescing; and an intensity of 3 was given when feeder roots were almost solidly colonized with few easily identified, isolated patches of colonization. Average infection index (AII) was calculated by adding the replicates of percentage root colonization rating of a sample and its replicates of intensity rating and then diving by five. Verification of unidentifiable fungal structures in the roots was made using a compound microscope (Nikon 59533, Japan).

Analysis of variance (ANOVA) was performed to determine if the factors site, banana genotype, age and productivity status of a farm significantly influenced the mycorrhizal inoculum potential of Ugandan soils. Wherever a significant difference was obtained, the LSM (Least Square Mean) was used for pair-wise comparisons.

3.3 Results

Results of the mycorrhizal inoculum potential test are shown in Tables 1, 2 and 3. All the sites analysed had detectable infective propagules of AMF, however, the degree of root infection ranged widely among the sites. Arbuscules, vesicles, infection points and non-septate hyphal coils were observed (Plate 1). The highest root percent colonization ranged between 26-50% infection of test plant root system.

Statistical analysis of the data revealed significant differences in MIP among the sites (Table 1). Site BNT (second farm sampled in the Ntungamo district) had a notably higher mycorrhizal inoculum potential when compared to all other sites. Within the sites, statistically significant differences in MIP between farm soils growing the banana genotype *Musa* AAA and farm soils growing the banana genotype *Musa* ABB ($p < 0.05$) were observed. Farm soils containing the genotype *Musa* AAA had a higher mycorrhizal inoculum potential than farm soils containing the genotype *Musa* ABB (Table 2). This trend was evident at all the sites except for sites BNT and CNT.

Interestingly, the statistical interaction between site and genotype revealed that site had a significantly overwhelming influence on the mycorrhizal inoculum potential of Ugandan soils (Table 3). This interaction resulted in the sites being

split into two groups of significantly different MIP ($P<0.05$). None of the other factors considered could be found to correlate this difference.

Table 1: Effect of site on MIP of Ugandan banana farm soils

Site	LSM
BNT	2.50[a]
CMS	2.00[b]
CKB	1.77[b]
ANT	1.67[b]
AMS	1.63[b]
AKB	1.57[b]
CLW	1.50[b]
CNT	1.33[b]
BLW	1.33[b]

First letter of site, A, B, C = first, second and third farm sampled at a district respectively, next two letters= district: NT= Ntungamo, MS= Masaka, KB=Kabale, LW=Luwero. LSM = Least Square Mean. a significantly different to b at p<0 05.

Table 2: Effect of banana genotype on MIP of Ugandan banana farm soils

Genotype	LSM
Musa AAA	1.86[a]
Musa ABB	1.54[b]

a significantly different to b at p<0 05.

Table 3: Effect of site-genotype interaction on MIP of Ugandan banana plantation soils

		Site	BNT	BNT	CKB	AKB	CMS	CMS	AMS	ANT	ANT	BLW	CLW	CNT	AMS	CLW	CKB	CNT	BLW	AKB
		genotype	ABB	AAA	AAA	AAA	AAA	ABB	AAA	AAA	ABB	AAA	AAA	ABB	ABB	ABB	ABB	AAA	ABB	ABB
		LSM	2.67a	2.33a	2.33a	2.27a	2a	2a	1.8b	1.67b	1.67b	1.6b	1.57b	1.5b	1.47b	1.43b	1.2b	1.17b	1.07b	0.87b
Site	genotype	LSM																		
BNT	ABB	2.67a							0.0497	0.0237	0.0237	0.0159	0.0042	0.0024	0.0067	0.013	0.001	0.0001	0.0003	0.0001
BNT	AAA	2.33a											0.045	0.0294	0.0497	0.0188	0.0104	0.0024	0.0043	0.001
CKB	AAA	2.33a												0.045		0.0294	0.0159	0.0042	0.0067	0.0016
AKB	AAA	2.27a																0.0294	0.0346	0.0104
CMS	AAA	2a																0.0294	0.0346	0.0104
CMS	ABB	2a																		0.0346
AMS	AAA	1.8b	0.0497																	
ANT	AAA	1.67b	0.0237																	
ANT	ABB	1.67b	0.0237																	
BLW	AAA	1.6b	0.0159																	
CLW	AAA	1.57b	0.0042	0.045																
CNT	ABB	1.5b	0.0024	0.0294	0.045															
AMS	ABB	1.47b	0.0067	0.0497																
CLW	ABB	1.43b	0.0013	0.0188	0.0294															
CKB	ABB	1.2b	0.001	0.0104	0.0159															
CNT	AAA	1.17b	0.0001	0.0024	0.0042	0.0294	0.029													
BLW	ABB	1.07b	0.0003	0.0043	0.0067	0.0346	0.035													
AKB	ABB	0.87b	0.0001	0.001	0.0016	0.0104	0.01	0.0346												

3.4 Discussion

In order to determine the mycorrhizal inoculum potential of Ugandan soils it was necessary to establish the mycorrhizae on a suitable host in the glasshouse. *S. sudanense* was found to be a suitable host proving to be highly susceptible to AMF colonization and producing sufficient root system for infection within the 21-day test period. *S. sudanense* has the added benefit of being easily cleared and stained for observation of colonization.

Although all the soils tested in this experiment contained an active native AMF population capable of infecting *S. sudanense*, less than 10% of the samples supported colonization in the range 26-50% of the roots of bioassay plants. Sixty four percent of the samples gave rise to 0-5% colonization of bioassay plants. According to a report by Douds *et al.* (1994), soils under low-input agriculture produce high AMF colonization in the greenhouse assay. Expectations were that the MIP of Ugandan soils would be high at all the sites as the soils fall under low-input agriculture. However, the Ugandan banana rhizospheric soils tested in this experiment appear to be generally low in AMF. It is possible that the low MIP recorded at the majority of the sites may be due to the characteristic of the MIP method employed in this experiment. The MIP bioassay method was performed in a glasshouse upon soil samples removed from the field. The unavoidable problem of extractive bioassays is that removal of soil from the field disrupts

mycelia and can lower inoculum potential (Janos, 1996). Bioassays also have a limitation of estimating only those propagules which germinate, regrow, intercept a root and initiate an identifiable infection during the experiment (Tommerup, 1994). Furthermore, estimates are affected by all the variables that change plant or fungal growth. Estimates derived from plant colonization tests rarely detect all the propagules (Rajapakse and Miller, 1994).

Statistical analysis of the data showed differences between farm soils growing banana genotype *Musa* AAA and farm soils growing banana genotype *Musa* ABB, which might suggest that banana genotype *Musa* AAA is more receptive to AMF infection than banana genotype *Musa* ABB. Differences in levels of root colonization by AMF is probably attributable to differences in mycorrhizal dependency among varieties of banana (Yano-Melo *et al.*, 1999). It is possible that the banana genotype *Musa* AAA has a greater mycorrhizal dependency (MD) than the banana genotype *Musa* ABB. Mycorrhizal dependency has been defined by Gerdemann (1975) as "the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility". The magnitude of response is known to vary both between and within species and is mainly attributed to the ability of these species to absorb P from soils that are low in available P (Mosse *et al.*, 1973). Morphological root properties (root geometry, rate of root growth, density and length of root hair) as well as physiological root properties may influence P uptake of plants from soil and therefore influence mycorrhizal dependency. Baylis (1975) suggested that

the length and density of the root hairs could be indicative of the degree of Relative Mycorhizal Dependency (RMD) of a plant species or cultivar. Long and abundant root hairs allow for better uptake of P and other nutrients, thereby reducing the need for a strong mycorrhizal relationship (Declerck *et al.*, 1995).

Differences in AMF receptivity may also be under genetic control and may be affected by plant and fungal species as well as environmental conditions (Declerk *et al.*, 1995). This indicates that there is a need for screening of plant or cultivar receptivity in AMF research. Selection of efficient AMF/banana cultivar "couples" should be a consideration in the improvement of sustainability of banana cropping systems with the use of AMF.

Although the statistical interaction between genotype and site separated the 18 sites into two groups (Table 3), there were no similar characteristics distinguishing one group of sites from the other (Appendix A). Highly producing banana farms demonstrated high soil MIP, however a correlative relationship could not be proven statistically due to the experimental design. Too few samples prevented isolating variables to enable testing correlations. An extended set of sampling and variables such as detailed information regarding site-farming management practices as well as detailed soil characteristics and site climatology could explain the differences in MIP of the different sites.

Observations of site-farming management practices (Appendix A) made in this study are not supported by statistical evaluations (not enough data). However, in retrospect they could have been a significant influence on the findings since the results of this study show that site has an overwhelming influence on the MIP of Ugandan soils. During the survey, it was observed that most of the farmers visited practice tillage as a management practice. Disturbance of the top soil layers, which occurs during tillage, appears to reduce the establishment and efficacy of the mycorrhizal symbiosis and in some cases can render the fungi completely ineffective in providing any plant growth benefit (Stahl *et al.*, 1988). Disturbance effects largely appear to be due to destruction of the hyphal network (Jasper *et al.*, 1989), which may result in reduced levels of mycorrhizal infection in the host plants (Fairchild and Miller, 1988; Read, 1993) and also leads to a reduction in the volume of soil, which the hyphal network exploits (Evans and Miller 1990; McGonigle *et al.*, 1990a). Land clearing methods that remove mycorrhizae-rich surface soil exposing subsoil, or that result in the death of mycorrhizal fungi in the surface soil can be expected to lower MIP or to eliminate inocula entirely (Alexander, 1989; Sieverding, 1991; Michelsen, 1992).

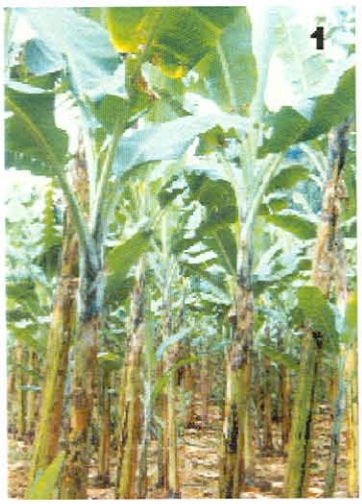
Baltruschat and Dehne (1989) reported that green manuring in the form of intercropping with rape and oil radish had a negative influence on AMF inoculum potential of soil from winter barley in a continuous monoculture. Similarly, banana production in Uganda is comprised of both continuous monoculture and intercropping, the majority of farms having some form of intercropping with

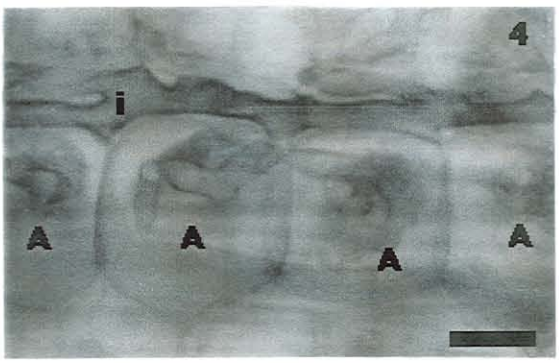
annual food crops. It is possible that some of the crops used for intercropping in Ugandan banana farms may have contributed to the low MIP recorded.

Observations were made that most of the farms with declining banana production in Uganda were overgrown with weeds. It is possible that the presence of these weed species may have contributed to the low MIP of these soils. The presence of non-mycorrhizal weed species at a location/site can reduce MIP (Baltruschat and Dehne, 1989; Janos, 1996). Non-host plants may lead to a reduction of AMF population in the soil with the consequence that subsequent crops are less infected with mycorrhizal fungi. A possible reason for the decrease of AMF populations in cultivation of non-host plants is that the germination of AMF spores takes place in the presence of roots of non-host plants and does not result in infection thus finally resulting in a reduction of AMF potential in the soil (Daniels and Trappe, 1980) due to this loss.

This study clearly confirmed the presence of viable AMF propagules in Ugandan banana rhizospheric soil and the potential of these soils as sources of AMF inoculum. Economic pressures dictate that maximum agricultural productivity be achieved at minimum economic and environmental cost. Agricultural systems, which enhance naturally occurring soil microbiota, such as AMF, should be considered as a possible means of achieving those goals in Uganda. Field and laboratory research have demonstrated that cultural practices have profound effects on AMF and could be effective tools for their management (Sieverding,

1991). Cultural practices should be developed or modified to exploit AMF symbiosis. Such practices include (1) minimum tillage; (2) intercropping with mycorrhiza-dependent plants that sustain a high inoculum potential of AMF. Intercrops such as millet (*Pennisetum americanum*); sudan grass (*Sorghum sudanense*) and milo (*Sorghum bicolor*) have been successfully used to increase population densities of AMF and improve growth of hardwood tree seedlings (Johnson and Pfleger, 1992); (3) managing weed communities to increase AMF inoculum potential; (4) breeding plants for either increased colonization or increased receptivity to AMF to improve water and nutrient uptake or reduce disease susceptibility.





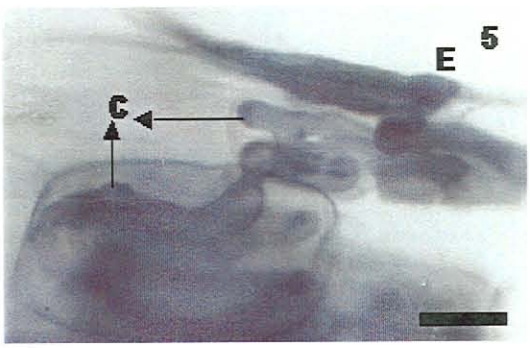


Plate 1

Figure 1: *Musa acuminata*, genotype AAA (East African highland banana).

Figure 2: *Musa acuminata*, genotype AAA showing two suckers less than 50 cm in height.

Figure 3: *Musa acuminata*, genotype ABB (*Pisang awak*).

Figure 4: *Arum* type root colonization of sorghum. The fungus spreads in the root cortex via intercellular hyphae (i). Short side branches penetrate the cortical cells and branch dichotomously to produce characteristic arbuscules (A). Bar = 9.5µm.

Figure 5: Penetration of sorghum root by AMF. The hypha entered the epidermis (E), penetrated a hypodermal cell, in which it formed a tightly packed coil (C), and then formed another coil in the adjacent cortical cell (C). This photograph represents typical *Paris* type AMF root colonization. Intracellular hyphae spread directly from cell to cell within the cortex. Bar = 9 5µm.