ARBUSCULAR MYCORRHIZAL FUNGI OF UGANDAN BANANA PLANTATION SOILS

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SUMMARY

ARBUSCULAR MYCORRHIZAL FUNGI OF UGANDAN BANANA PLANTATION SOILS

This first study of arbuscular mycorrhizal fungi in Uganda involved (1) the assessment of the mycorrhizal inoculum potential of banana farm soils, (2) isolation of AMF species and (3) determination of the potential of a selected AMF among the isolated indigenous species to colonize micropropagated banana plantlets.

A greenhouse bioassay was conducted to assess the arbuscular mycorrhizal (AM) fungi inoculum potential of 18 different banana farm soils. Sudan grass was used as a host plant. Root colonization by AMF occurred in all the 18 sites analyzed. The highest mycorrhizal inoculum potential was recorded at Ntugamo district. The mycorrhizal inoculum potential of Ugandan soils was found to be higher in soils containing the banana type *Musa* AAA than soils containing banana type *Musa* ABB. However, it was found that site was the main factor influencing the mycorrhizal inoculum potential of Ugandan soils.

Banana rhizospheric soils were retrieved for single morphotype pot culture production of some individual species of AMF. Pure cultures of *Glomus mosseae* (Nicolson & Gerdemann) Gerdemann and Trappe and *Glomus etunicatum*

Becker and Gerdemann were isolated under glasshouse conditions using sudan grass a host plant.

The ability of the indigenous AMF to colonize micropropagated banana plantlets was evaluated at the *in vitro* and weaning phase of growth. Surface sterilized *G. mosseae* spores were used as inoculum. Mycorrhization was demonstrable at 10 weeks post-inoculation for both *in vitro* phase and weaning phase banana plantlets under misting tunnel conditions. Root colonization levels ranged from 0-5% of the test plant for both stages of banana growth. Thirty percent of the *in vitro* plantlets were mycorrhizal and 50% of the weaning phase plantlets were mycorrhizal.

OPSOMMING

ARBUSKULÊRE MIKORISALE SWAMME IN UGANDESE PIESANGPLANTASIE GRONDE

Hierdie eerste studie van arbuskulêre mikorisale swamme (AMS) in Uganda het ingesluit: (1) die bepaling van die mikorisale inokulumpotensiaal van piesanggronde, (2) isolasie van AMS spesies en (3) bepaling van die potensiaal van 'n geselekteerde inheemse spesie om piesangplante in weefselkultuur te koloniseer.

'n Glashuisbiotoets is uitgevoer om die arbuskulêre mikorisale (AM) swaminokulum potensiaal van 18 verskillende piesangplaas-gronde te bepaal. Sudan gras is as gasheerplant gebruik. Wortelkolonisering deur AMS het in al 18 van die persele ontleed, plaasgevind. Die hoogste mikorisale inokulumpotensiaal is in die Ntugamo distrik opgeteken. Die mikorisale inokulumpotensiaal van Ugandese gronde is gevind om hoër te wees in gronde met die piesangtipe *Musa* AAA as gronde met die piesangtipe *Musa* ABB. Dit is nietemin gevind dat ligging die belangrikste invloed op die mikorisale inokulumpotensiaal van Ugandese gronde gehad het.

Piesang risosfeergronde is versamel vir enkel-morfotipe produksie in potte van sommige individuele spesies van AMS. Suiwer kulture van *Glomus mosseae*

(Nicolson & Gerdemann) Gerdemann & Trappe en *Glomus etunicatum* Becker & Gerdemann is onder glashuistoestande, met sudan gras as gasheerplant, geisoleer.

Die vermoë van die inheemse AMS om piesangplante in weefselkulture te koloniseer is in die *in vitro*- en speengroei fases bepaal. Oppervlak-ontsmette *G. mosseae* spore is as inokulum gebruik.

Mikorisering is 10 weke na inokulering vir beide die *in vitro*- en speengroei fases onder mistonneltoestande aangedui. Wortel koloniseringsvlakke het van 0-5% van die toetsplante in beide groeifases, gewissel. Dertig persent van die *in vitro* plante en 50% van die speengroeifase plante was mikorisaal.

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single large oil vacuole (C). The outer wall has completely sloughed off. Note thickening of laminate layer at point of hyphal attachment. Bar = 19.6 µm.

Figure 3: *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe spore showing funnel-shaped hypha (FH) and fragment of outer wall layer (SW). Spore contents consisting of lipid droplets of different sizes (C). Bar = 13 µm.

Figure 4: *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe spore showing funnel-shaped hypha. Bar =16 μm.

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Figure 1 and 2. *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe in roots of weaning phase banana plantlets showing darkly staining vesicles (V). Note the abundant root hairs (R) of Grainde Naine (*Musa acuminata* AAA). Bar = 21 µm.

Figure 3 and 4: *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe in roots of *in vitro* banana plantlets showing intraradical spores (S) and intratradical foraging hyphae (i) growing parallel to each other and the root axis. Vesicles are seen in figure 3. Bar =14.4 µm.

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ABBREVIATIONS

s	second
min	minute(s)
ml	millilitre
cm	centimeter
g	gram
mm	millimeter
µm	micrometer
AMF	Arbuscular mycorrhizal fungi
AF	Acid fuschin
SDH	Succinate dehydrogenase

CHAPTER 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 *Musa* AAA) (Karamura *et al.*, 1996) and approximately 8% of the total banana production consists of the recently introduced cultivar *Pisang Awak* (genotype *Musa* ABB). Banana growing is generally at the subsistence level with some farms raising surplus for family income. A few large-scale cooking banana farms exist in parts of the country.

Ugandan banana farming is under pressure from continuously declining production due to biotic and abiotic factors that vary between farmers and regions. The counter-productive biotic factors include plant parasitic nematodes, banana weevil, leaf diseases, *Fusarium* wilt and, Banana Streak Virus. The major abiotic constraints are from declining soil fertility as a result of intensive land use or conversely a reduction of farm inputs such as mulches. Thus small-scale Ugandan farmers are often confronted by a complex of agricultural constraints which are beyond their means for management. Furthermore, all inorganic fertilizers are imported in Uganda (FAO, 1991), making these applications financially restricted. The use of nitrogen bearing fertilizers dropped by 64% between 1980 and 1990. Similarly, the use of phosphorus fertilizers declined by

45% (Bekunda and Woomer, 1996) Pest management options available consist exclusively of cultural controls: clean planting material (i.e. paring and/or hot water treatment of banana suckers) and crop sanitation (i.e. removal of post-harvest residues). Crop sanitation requires continual labour from the farmer and is of uncertain value. However, clean planting material can significantly increase the performance of newly established plantations. These plants will have an increased tolerance to pests and diseases. Generally within a period of three to four years, these plants will get re-infestated.

Scientists have recognized the potential for manipulation of biological factors to create sustainable plant growth. The beneficial effects on growth and health of plants of economic importance and the increased concern for environmental quality have made biological factors important components for management practices in agricultural systems. Soil is a natural resource whose proper management is important for food security. A major component of soil management in agriculture involves maintenance, replacement and enhancement of the natural soil biota. Such a critical component includes AMF (arbuscular mycorrhizal fungi), which form mutualistic associations with the roots of most vascular plants.

Colonization of plant roots by AMF has been shown to have very beneficial effects for plant growth. Some of these effects include: increased uptake of phosphorus, zinc and other minerals (Ames *et al.*, 1983); reduction in the

incidence of disease (Dehne, 1982); increased transplant uniformity; reduction of both transplant morbidity and mortality; and improved drought tolerance (Auge *et al.*, 1986) by such means as decreasing leaf water potential, reducing stomatal and root hydraulic resistances, and increasing transpiration rates (Allen and Boosalis, 1983). The beneficial effects seen on micropropagated plants include: improved rooting; enhanced root function (absorption and translocation); and increased vitality (Varma and Schuepp, 1995).

It is my interest to investigate AMF occurring naturally in Uganda and experiment by introducing these fungi to micropropagated banana plants in attempts to form a successful association between the plant and the fungus. Micropropagated banana have been shown to have a high arbuscular mycorhizal dependency (Rizzardi, 1990; Jaizme-Vega and Azcon, 1995).

The aims of this study are to:

1 Determine the mycorrhizal inoculum potential of Ugandan banana farm soils and statistically examine some factors for correlations.

2 Identify some AMF from Ugandan banana farm soils.

3 Determine the ability of AMF, isolated from Ugandan banana farm soils, to colonize micropropagated banana plantlets.

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy of AMF: Spore-based taxonomy and identification

Morton (1990a) used all available data on selected morphological characters of somatic (hyphae, arbuscules, vesicles) and reproductive (resting spores) stages of known arbuscular species to hypothesize explicit genealogical trends. Cladistic analysis and a phylogenetic reconstruction were based on an evolutionary species definition: clonal populations which together maintained a distinct morphological identity from other clonal assemblages and which had the same evolutionary role and tendencies. A revised species concept to accommodate phenotypic variability as well as historical and contemporary evolutionary processes was elaborated by Morton (1990b). Currently, the classification of AMF has been revised by Morton and Benny (1990) to more accurately arrange groups of AMF according to patterns of common descent. It is based in part on results of Morton (1990a), with additional considerations of spore ontogeny and modes of spore germination. All the known AMF are currently classified in five families within the order Glomales(http //invam.caf.wvu.edu/Myc_info/Taxonomy).

Order: Glomales

Suborder: Glomineae

Family: Glomaceae

Genus: *Glomus* (~89 species)

Family: Paraglomaceae

Genus: *Paraglomus* (~2 species)

Family: Acaulosporaceae

Genus: *Acaulospora* (~34 species)

Genus: *Entrophosphora* (~4 species)

Family: Archaesporaceae

Genus: *Archaespora* (~3 species)

Suborder: Gigasporineae

Family: Gigasporaceae

Genus: *Gigaspora* (~8 species)

Genus: *Scutellospora* (~28 species)

Morphological characteristics of spores are the most important for the identification of AMF. According to Morton (1988), the following are the most important:

Spore development

Spore development is one of the main criteria used to define genera of Glomalean fungi. *Scutellospora* and *Gigaspora* species have spores which develop from bulbous subtending hyphae while those of *Glomus* species form on narrow or flaring hyphae. *Acaulospora* and *Entrophosphora* have spores which become sessile after detachment from a soporiferous saccule. Spores of many *Glomus* species can form within roots as well as in the soil, but the other four genera generally do not sporulate in living roots (Morton, 1988).

Spore arrangement

Spores of Glomalean fungi can be produced singly or in aggregations, which are called sporocarps. Sporocarps differ in size, colour, shape and surface texture between and within isolates of Glomalean fungi. Sporocarps may have a peridium (outer covering of hyphae) (Morton, 1988).

Spore shape

Spores of most Glomalean fungi are globose (spherical), but some species have spores which are oval, oblong, or occasionally other shapes. Subtending hyphae which remain attached to spores can be cylindrical, flared into conical shape, or swollen, and some species have multiple or branched subtending hyphae. Spore

attachments of mature spores can be occluded by wall layers or other material (Morton, 1988).

Spore size

Spore size is considered to be less useful than many other taxonomic criteria because of its variability, but substantial differences in spore sizes can help to distinguish species. Glomalean fungi have spores which fall within a range of sizes from very small (20-50 µm) to very large (200-1000 µm) (Morton, 1988).

Spore colour

Spore colour varies between and within isolates of Glomalean fungi and can be used to help identify them (Morton, 1988).

Spore ornamentation

Examples of spore surface features include pits, reticulations, spines and papillae, and these are most often found on *Scutellospora* and *Acaulospora* spores (Morton, 1988).

Spore wall layers and staining reactions

Walls of Glomalean fungus spores have one or more layers that may vary in their thickness, structure, appearance and staining reactions, and can be described using standardized terminology or diagrams (murographs) (Walker, 1983, Morton, 1988). *Acaulospora*, *Entrophosphora* and *Scutellospora* species typically have a complex wall structure consisting of a thicker outer wall and one or more thin inner wall layers. One or more wall layers may stain red or purple with Melzer's reagent. Melzer's staining reactions may occur in inner or outer wall layers of spores in all genera, but typical staining reactions may not occur in spores that are old, damaged or have been stored in preservatives. *Glomus* and *Gigaspora* spores are generally simpler in structure than those of other genera, but *Glomus* spores often have several wall layers. Immature *Glomus* spores may have a weak Melzer's staining reaction that is absent in older spores. Young *Glomus* spores often have a fragile outer wall layer which is lost as spores age (Morton, 1988).

Spore contents

Spores contain lipids and other contents which vary in colour and may be arranged in large or small droplets or granules. The size or arrangement of lipid droplets can help identify fungi, but will change when spores age (Morton, 1988).

Spore germination

Spore germination mechanisms can also be used to distinguish Glomalean fungi, especially *Scutellospora* species which have germination shields with complex infoldings on their inner walls. When *Scutellopsora* spores germinate, hyphae arise from compartments within these shields and then grow through the outer wall. There are also characteristic features of spore germination of *Acaulospora* (germination shields) and *Gigaspora* (warts inside the spore wall) spores (Morton, 1988).

Non-morphological characteristics

Careful study of biochemical, immunological and genetic properties have also provided valuable information in the classification of the Glomales (Morton, 1988; Walker, 1992).

Biochemical characteristics

Biochemical criteria to separate AMF have been limited so far to defining stained isozyme banding patterns from spores (Sen and Hepper, 1986) and mycorrhizal infection (Hepper *et al.*, 1986) after polyacrylamide gel electrophoresis. The most common enzyme assayed are esterase, glutamate oxaloacetate transaminase, hexokinase, malate dehydrogenase, peptidase,and phospho-glucomutase.

Based on results using spores of five *Glomus* species, malate dehydrogenase was most diagnostic for distinguishing between pairs of fungi (Sen and Hepper, 1986).

Immunological characteristics

Two approaches have been used thus far to identify specific AMF: the fluorescent antibody (FA) technique and the enzyme-linked immnuosorbent assay (ELISA). Immuno-localization procedures require the use of an antibody specific to either an external or internal antigen from the fungus. Though the sensitivity is excellent, immunological methods rarely overcome the problem of specificity beyond the subgeneric level (Wilson *et al.*, 1983). Antisera react most strongly with their homologous antigen, but some cross reactivity to heterologous AMF occurs (Wilson *et al.*, 1983). Monoclonal antibodies (MAbs), each of which is secreted by an immortal hybridoma culture derived from a single B-lymphocyte, are much more specific and therefore overcome problems associated with interspecific cross-reactions. Wright *et al.* (1987) demonstrated that MAbs against spores and hyphae of the target fungus, *G. occultum* Walker did not react against 15 other *Glomus* species or 14 AMF in other genera.

Genetic characteristics

Ribosomal genes are by far the most studied genes in glomalean fungi. In eukaryotes, the ribosomal genes code for a pre-RNA of 45S which, after processing, gives the small ribosomal subunit (18S), the 5.8S subunit and the large ribosomal subunit (28S) (Perry, 1976). The small, 5.8S and large ribosomal subunit coding regions (rDNA) are separated from each other by an internal transcribed spacer (ITS), and the ribosomal genes by an intergenic nontranscribed spacer (IGS). Sequence variations are not evenly distributed throughout the ribosomal genes and the three regions evolve at different rates. ITS and IGS are variable regions which mutate more frequently than the three conserved coding subunit regions (18S, 5.8S, 28S) (Mitchell *et al.*, 1995). The coding regions of the small and the large ribosomal subunit have been used to discriminate between AMF at the species/family level (Simon *et al.*, 1993; van Tuinen *et al.*, 1994). The variability observed in the ITS regions has been used to distinguish between different isolates of a species of AMF (Lloyd-MacGilp *et al.*, 1996).

2.2 Techniques for determining soil inoculum potentials of AMF

Practically all field studies involving AMF require an assessment of infection in the roots of plants studied/used in the investigation. This may be a simple check that infection has occurred or quantitative record of soil infectivity. Measurement

of the population level in soil is necessary for planning a strategy of maintenance, enhancement, or replacement with more desirable fungi (Janos, 1988; Plenchette *et al.*, 1989; Mason and Wilson, 1994). A number of techniques have been used to obtain indications of total arbuscular mycorrhizal fungal (AMF) propagules in soil. These are spore enumeration, determination of AMF mycelium biomass in soil, infection unit method, the 'Most Probable Number' technique (MPN) and the Mycorrhizal Inoculum Potential' technique (MIP) Each technique has both advantages and disadvantages. The most commonly used techniques are the MPN and MIP bioassays.

2.2.1 Spore enumeration

Direct enumeration of propagules suffer a number of problems but foremost is the difficulty of determining propagule viability. Spores of AMF can be extracted from soil, identified and counted relatively readily but because thick walls of dead spores are persistent, total spore counts can be poorly correlated with mycorrhiza formation/activity (Alexander *et al.*, 1992; Fischer *et al.*, 1994). Spores of some AMF are too small to be reliably extracted by wet sieving (Hall, 1977). Some AMF produce only a few infective spores, and others appear perfectly able to complete their life cycle without sporulating at all (Baylis, 1969). Spore density can only be related to inoculum potential if it is known whether the spores are alive, dead or dormant (Liu and Luo, 1994). Most often spore counts underestimate numbers of AMF since colonized roots and hyphae also serve as

propagules. Spore counts can also overestimate numbers of AMF if AMF in soil is dead and dead spores are persistent.

2.2.2 Determination of AMF mycelium biomass in soil

There is no completely satisfactory method to quantify external hyphae of AMF in soil. Three major problems have yet to be overcome: (1) there is no reliable method to distinguish AMF hyphae from the myriad of other fungal hyphae in soil, (2) assessment of the viability and activity of hyphae is problematic, and (3) meaningful quantification is very time-consuming (Sylvia, 1992; Jarstfer and Sylvia, 1996). Colometric methods to determine chitin in cell walls of AMF have been used to estimate hyphal biomass in soil (Hepper, 1977). The utility of these methods for natural soils is limited because chitin is ubiquitous in nature. It is found in the cell walls of many fungi and the exoskeletons of insects. Certain soils exhibit physical and chemical properties that interfere with the chitin analysis (Jarstfer and Miller, 1985).

2.2.3 Most Probable Number

The MPN was first used to estimate the propagule density of AMF in soil by Porter (1979). It provides a relative measure of the density of propagules capable of colonizing roots. Four main assumptions of the method are: (1) that the propagules are randomly distributed in the soil; (2) that propagules are single and

aggregrates; (3) that dilution is proportional to the number of propagules; (4) and that if one organism is present it will be detected by the assay method (Tommerup, 1994). The general procedure for the MPN assay is to dilute natural soil with disinfested soil. Equal portions of the dilution series are placed into small containers (5 to 10 replications of each dilution). A susceptible host plant is planted into in each container, and the plants are grown long enough (6-8 weeks) to obtain good root colonization. Plants are then washed free of soil, and roots are assessed for the presence or absence of colonization. Results are interpreted as a probability estimate of propagule numbers from statistical tables (Fisher and Yates, 1963). However, these tables restrict experimental design, thereby reducing the accuracy that can be obtained. A better approach is to program the equations into a computer and directly solve for the MPN value on the basis of optimal experimental design, i.e., increased replication and decreased dilution factor improve accuracy and reduce confidence limits. Five or ten fold dilutions are often used when inoculum is perceived to be highly infective but are not recommended because precision in quantification is lost (Sieverding, 1991). Two fold dilutions are optimal but require too much space and are too laborious. Numerous factors affect the outcome of an MPN assay (de Man, 1975; Wilson and Trinick, 1982; Morton, 1985, An *et al.*, 1990) therefore caution should be exercised when values from different experiments are compared. Nonetheless, this assay has been a useful tool for estimating propagule numbers in field soil, pot cultures, and various forms of inocula. The MPN assay has the advantage of providing a single number that can be compared directly with other

tests in the same assay and is relatively easy to conduct. However, some space and time (6-8 weeks) are required for this test (Morton, 1985).

2.2.4 Mycorrhizal inoculum potential

This assay measures the percentage mycorrhizal colonization in a host plant over time, after the host plant has been grown in a series of inoculum dilutions (Moorman and Reeves, 1979). The host plant is colonized by the AMF population to an extent that corresponds to the infection potential (infectivity) of the AMF population (Sieverding, 1991). The MIP is an indirect bioassay because there is not a 1:1 correspondence between number of infectious propagules and the assay result. The amount of mycorrhizal colonization includes a measure of both primary ingress (from propagules) and secondary spread (new infection units from those already established).

The MIP technique is less complex and time-consuming than the MPN assay (Jarstfer and Sylvia, 1996). The technique is simple and the test can be conducted with basic laboratory equipment However this technique is sensitive to the environment. Actual propagule numbers are not estimated; rather the assay provides a relative comparison of propagule density among various soils or treatments.

Other variations of measuring soil infectivity have been described. Plenchette *et al.* (1989) described a technique whereby soil infectivity can be estimated by a standard bioassay from a dose-response relationship. The technique described for measuring soil infectivity involved cultivation of a population of susceptible plantlets in controlled conditions on a range of concentrations of natural soil diluted with the same disinfected soil. Soil infectivity was expressed as arbuscular mycorrhizal soil infectivity (MSI) units/100g of soil. An MSI unit is the minimum dry weight (g) of soil required to infect 50% of a plant's population under the bioassay conditions (MSI_{50}). For each soil the percentage of mycorrhizal plants is plotted against the logarithm of unsterilized soil concentrations. Regression equations are calculated for each soil and the soil infectivity is determined by calculating the value at which 50% of the plants are mycorrhizal.

Typically, bioassays (MPN and MIP) are performed in a greenhouse upon soil samples removed from the field. The inevitable problem of extractive bioassays is that removal from the field may alter inoculum potential (Janos, 1996). The length of the bioassay is critical. If plants are grown for a short time, the full potential for colonization is not going to be realized. On the other hand, if plants are grown for too long a time, they may become uniformly colonized despite differences in AMF populations. Bioassays also have a limitation of estimating only those propagules which germinate, regrow, intercept a root and initiate an identifiable infection during the experiment. Estimates are affected by all the

variables that change plant or fungal growth. Estimates derived from plant colonization tests rarely detect all the propagules present (Tommerup, 1994).

2.2.5 Infection unit technique

An infection unit technique may also be used to quantify mycorrhizal propagules (Franson and Bethlenfalvay, 1989). The principle is that a count of discrete points of infection is a more reliable measure of the number of viable propagules than are other methods. However, this method is applicable only in short-term experiments because infection units are discernible only during the initial stages (1 to 3 weeks) of colonization (Jastfer and Sylvia, 1996).

2.3 Visualization techniques for measuring AMF infection in roots

The primary purpose of studying root colonization in many AMF experiments is to observe and confirm mycorrhiza within the root system. The study of root colonization is also necessary to observe and describe the morphology of specific mycorrhizal structures formed within the root. Root colonization is also measured to evaluate the extent of host specificity in different host-fungus combinations Furthermore, because the anatomical features of some of the fungal structures inside the root are diagnostic for certain species, it is sometimes possible to differentiate the AMF responsible for root colonization.

There is no standard method for quantification of root colonization in cleared and stained root samples. Researchers have used various assessment methods to meet their requirements. Most of the common assessment methods are described below.

2.3.1 Detecting the presence or absence of colonization

Whole stained root samples are scanned for the presence of any mycorrhizal structures, i.e. hyphae, arbuscules, vesicles or internal spores and rated positive (+) or negative (-) on a per sample or per plant basis. This is the most basic and rapid assessment method of those discussed here. This method is not quantitative, but adequate for some types of work such as checking host-fungus specificity and observing non-inoculated "control" plants for root colonization (Rajapakse and Miller, 1994).

2.3.2 Calculating the percentage of root segments colonized

Several studies have calculated colonization as the number of root segments with any colonization, divided by the total number of segments examined (Daft and Nicholson, 1972, Khan, 1974; Read *et al.*, 1976; Bierman and Linderman, 1981; Malibari *et al.*, 1988). This is the same in principle as calculating the number of microscope fields of view with any colonization divided by the total number of fields of view examined (Baylis, 1967; Sutton, 1973; Newman *et al.*,

1981; Plenchette *et al.*, 1982, Dodd and Jeffries, 1986). This method always overestimates percentage colonization, the degree of overestimation depending on the lengths of the segments, and on the lengths of the regions of colonization.

2.3.3 Grid-line intersect technique

The grid-line intersect technique (Giovannetti and Mosse, 1980) or various modifications of it, is a procedure whereby the presence or absence of colonization at each intersection of root and gridline is noted, after dispersing the roots above a grid of squares drawn on a Petri dish, and observing under a dissecting microscope at X40 magnification. In many cases colonization by AMF can be readily determined. However, even at X80 magnification it is not possible to ascertain if the roots are mycorrhizal at all intersections. This is because cortical cells or parts of the stele can become stained (Dodd and Jeffries, 1986), the roots may be crowded with hyphae, and because arbuscules can be difficult to detect when they are small. Structures formed by other fungi may also be confused with arbuscules at low magnification. Different researchers are unlikely to be consistent in the way they record these difficult intersections, and may arrive at different answers The gridline-intersect technique using the dissecting microscope can therefore be expected to give a relative measure of colonization.

To determine unequivocally whether arbuscules are present in all cases requires examination at X200 magnification. Ambler and Young (1977) described a grid-

line intersect technique involving the compound microscope, but this still has the difficulty that some intersections must be classified as colonized or not when hyphae but no arbuscules are seen. Since arbuscules are the only unique feature of AMF, decisions as to whether hyphae seen alone are mycorrhizal may vary from person to person. This technique is therefore vulnerable to bias and probably generates a relative measure of colonization.

McGonigle *et al.* (1990b) proposed use of a magnified-intersect method whereby roots are observed at a magnification of X200 and arbuscules are quantified separately from vesicles and hyphae. Nevertheless, another limitation of the gridline-intersect technique is that the intensity of colonization at each location is not estimated. To obtain an estimate of intensity, one can use a morphometric technique (Toth and Toth, 1982) whereby a grid of dots is placed over an image of squashed roots and colonized cortical cells are counted.

2.3.4 Visual estimation method

The subjective visual estimation technique is the most commonly used to evaluate the level of AMF infection (Mosse, 1973; Sanders *et al.*, 1977; Abbott and Robson, 1978; Buwalda and Goh, 1982; Hopkins, 1987), and has been modified to estimate the intensity of colonization within the roots (Schenck, 1982). While this technique is subjective it can give quite reliable results with only a few hours training (Giovannetti and Mosse, 1980). Giovannetti and Mosse

(1980) calculated a standard error of between 2 and 5% for subjective visual estimation. The subjective visual estimation is unquestionably the most time-efficient technique.

All of the above-mentioned methods use biological stains in the preparation of plant roots for quantification of the extent of AMF colonization. Gange *et al.* (1999) suggest that the extent of AMF root colonization recorded may depend on the stain used. They have reported having encountered many stained preparations in which arbuscules could not be seen. In such preparations, recording "arbuscular mycorrhiza" depends on a completely subjective decision, based on the presence of aseptate hyphae or vesicles. Neither of these characters, alone or together, is a reliable indicator of AMF colonization. Mycorrhizal hyphae cannot be easily distinguished from those of saprotrophic fungi or root pathogens (Smith and Read, 1997), while many other fungi can produce vesicles (Jennings and Lysek, 1996). Smith and Read (1997) comment that another problem with stains is their differential tissue penetration capacities. Clapp *et al.* (1996) state that acid fuchsin (AF) preparation quality varies according to the root tissue used.

As an alternative to biological stains, Ames *et al.* (1982) described a method that involves subjecting roots to ultraviolet illumination, under which arbuscules autofluoresce. While autofluorescence might be better for arbuscule recording in some plant species, this technique also has its drawbacks. For example, the fact

that other structures in a root, such as lignin-like compounds sometimes fluoresce (Stockwell and Hanchey, 1987), or yellow pigments might hamper the recognition of arbuscules (Klingner *et al.*, 1995). Ames *et al.* (1982) comment that both live and dead arbuscules fluoresce, but conventional stains cannot differentiate them either. Furthermore, if a plant is sampled when it is in the early or late stages of mycorrhizal colonization, arbuscules might not be present and hence the recording from autofluorescence would be zero. Other useful mycorrhizal material such as vesicles, hyphae, hyphal coils, and entry points present in the root material would not be recorded.

Merryweather and Fitter (1991) subjected roots stained with AF to epifluorescence and found that the quality of the preparation improved. By using a combination of autofluorescence and staining, an accurate arbuscule count could be obtained, while still allowing determination of the presence of other mycorrhizal structures, such as entry points, intercellular hyphae and vesicles. According to Gange *et al.* (1999) the combination of autofluorescence and staining requires less than two minutes to score the colonization.

For the quantification of AMF activity in planta, a combination of autofluorescence and non-vital staining (trypan blue, chlorazol black, acid fuchsin) is not sufficient because these procedures do not indicate which part of the fungal material is active or even alive (Lovato *et al.*, 1995). This has led to the development of staining procedures based on physiological activities of the fungi.

One of these is the use of the succinate dehydrogenase reaction (SDH) to evaluate the amount of living AMF in the root cortex (Kough *et al.*, 1987; Smith and Gianinazzi-Pearson 1990). This technique is useful to observe the evolution of the fungal viability in relation to plant growth and environmental factors. The evaluation of the infection using SDH staining will allow the precise determination of the effect on AMF of management procedures, in particular, whether one of these factors will lower the viability of the symbiotic fungus at an early stage of AMF development (Lovato *et al.*, 1995).

Researchers working on AMF do not use one method exclusively, as no method is unequivocally superior (Rajapakse and Miller, 1994). An investigator should be able to make an informed choice of the method of assessing AMF infection that is most suitable for their needs. Not only accuracy and reproducibility of assessment are important but also the laboriousness of the method. This is obviously increased by mounting roots on the slides but the possibility of making other, detailed observations on infection patterns, development of external mycelium and presence of other fungi may outweigh this disadvantage. Root colonization, measured quantitatively by using any of the methods, does not necessarily reflect the effectiveness of AMF in nutrient transfer capacity. It is not known that such functions are even related to the proportion of arbuscules present. Caution should be taken in interpreting the functional basis of differences in the level of root colonization between any two sets (Rajapakse and Miller, 1994).

2.4 Procedures for mycorrhization of microplants

Three main factors are of importance in the production of mycorrhizal micropropagated plants: time and form of mycorrhizal inoculation, substrate to be used and choice of species/strain of the mycorrhizal inoculum.

2.4.1 Time and form of inoculation

Three periods during micropropagated plant production can be identified for introducing mycorrhizal fungi: during the *in vitro* phase, during the weaning phase, and after the weaning phase. Micropropagated plants can be inoculated with AMF *in vitro*, but this requires an extension of the *in vitro* phase, so the economic impact of the procedure is questionable. Inoculation during the *in vitro* phase presents other difficulties, such as: (1) differences in the conductive media for root initiation and AMF formation and (2) loss of roots produced during the *in vitro* stages after outplanting (Vidal *et al.*, 1992). Plant growth increases due to the presence of mycorrhiza are obtained if microplants are inoculated at the post *vitro* phase (Ravolanirina *et al.*, 1989) or at the beginning of the weaning phase when they show only two root primordia (Branzati *et al.*, 1992). The trend is to couple the weaning or acclimatization period with a rooting phase, following induction of root formation during the *in vitro* phase (Lovato *et al.*, 1996). Mycorrhizal inoculation at the beginning of the rooting phase has been

successful in a number of plant species such as pear (Gianinazzi *et al.*, 1990), pineapple (Guillemin *et al.*, 1992) and apple (Branzati *et al.*, 1992).

The procedure for inoculation of micropropagated plants can be performed in a number of ways: (1) micropropagated plants can be grouped together in trays containing the mycorrhizal inoculum mixed into the weaning substratum, (2) microplants can also be dipped in water suspension that contains the mycorrhizal inoculum before transplanting into the weaning substratum, (3) or most commonly, 1-5 g of soil-based inocula is placed in the planting hole made in the soil substrate of the container. The seedling is planted into the hole with the inoculum base (Lovato *et al.*, 1996).

2.4.2 Substratum composition

Substratum composition is very important to obtain optimal mycorrhizal infection. Vidal *et al.*(1992) found that the symbiosis could be established in peat-sand mixes although soil-sand mixes were more conducive to AMF root colonization of micropropagated avocado plants. Estuan *et al.* (1999) working on micropropagated peach X almond hybrid rootstock GF677also found that soil-less growing media (peat and a commercial peat-bark compost mix) is less conducive to AMF colonization than soil-based (soil-sand mix) growing media however, compost-peat mixes result in higher AMF colonization percentages.

2.4.3 Choice of the inoculant

Inoculants of AMF are chosen according to the target plant since, for arbuscular mycorrhizas, there are differences among fungal strains in promoting growth of diverse plant species or varieties. Most frequently a mixture of isolates, each of which is adapted to a specific set of environmental factors is used, so that a wide range of hosts and environmental management conditions can be covered. However, it is possible that in a given situation a less efficient fungal strain present in the inoculant may be more competitive for infection of host roots. Optimal plant improvement can, therefore, only be obtained through mycorrhizal fungal inoculation of micropropagated plants if soil characteristics and other environmental features are carefully considered (Lovato *et al.*, 1996).

2.4.4 Sources of AMF inocula

2.4.4.1 "Infected roots" as inoculum source

Infected roots contain internal fungal mycelium as well as external mycelium, vesicles and in some species AMF spores, i.e., *Glomus intraradices* Schenck & Smith. Before being used as inoculum, roots are often chopped into small pieces. It is well known that the infectiveness of the infected roots as an inoculum source is higher than that of spores (Sieverding, 1991).

There are several problems in using infected roots as an inoculum source in the field and nurseries. Even if the root inoculum is free of pathogenic microorganisms, the organic material introduced to the soil is probably an attractive nutrient source for omnipresent microorganisms (saprophytic as well as parasitic bacteria and fungi). The transport and commercial distribution of "infected root" is limited by the relatively short survival time of AMF in roots at ambient conditions. This applies especially to AMF which do not sporulate within the roots (Sieverding, 1991).

2.4.4.2 "Soil based" AMF inoculum sources.

Soil inoculum contains all AMF structures. Soil inoculum is most frequently used in greenhouse and field experiments. However, Mosse and Thompson (1984) have indicated that soil inoculum is too bulky to handle particularly for use on a larger scale. It is also difficult to keep this inoculum from contamination of unwanted microorganisms (Lombaard, 1993).

2.4.4.3 Spores of AMF as inoculum source

Spores are important sources of inoculum for the establishment of clean cultures of AMF on host plants in previously sterilized substrates. This is because (1) small numbers of spores can be isolated relatively easily from soil substrates, (2) spores can be morphologically distinguished for the identification of the endophytes, and (3) spores can be satisfactorily disinfected on their surfaces

with certain chemicals (with the object of producing inocula free of other microorganisms)(Budi *et al.*, 1999). Spores are suitable sources of inoculum for experimentation and for special cases, i.e., in nurseries or in conditions where aseptic inoculation is required (Sieverding, 1991).

The germination of AMF spores depends on certain environmental conditions such as temperature, soil moisture and photoperiod. Koske (1987) showed that AMF could be directly influenced by temperature or indirectly by the influence of the temperature on the host. Reports indicate that in general spore germination is best at temperatures between 20 to 25°C (Hetrick, 1986; Bagyaraj, 1991). Bagyaraj (1991) indicated that spores of the same AMF species would germinate at different temperatures, depending on other environmental conditions. Land and Schonbeck (1991) showed that the germination of spores cannot occur under 5°C.

The germination of AMF spores also depends on the species of AMF itself. There is evidence that the germination of spores of some species of AMF is blocked by a dormancy factor for a certain period of time depending on the species of AMF (Tommerup, 1983; Bowen, 1987; Bagyaraj, 1991). Bowen (1987) found that this dormancy period is longer in dry soils than in wet soils. Tommerup (1983) reported a dormancy period of approximately 6 weeks for *Glomus caledonium* (Nicolson & Gerdemann) Trappe & Gerdemann and *Glomus monosporum* Gerdemann & Trappe in wet soil while, *Gigaspora calospora* (Nicolson &

Gerdemann) Gerdemann & Trappe was found to have a dormancy period of 12 weeks. This period was significantly reduced to 1 week for the *Glomus spp* and 6 weeks for *G. calospora* in dry soil. *Acaulospora laevis* Gerdemann & Trappe was found to have a dormancy period of 6 months under all conditions. Several studies showed that AMF spores will not germinate in sterile soil and that this dormancy factor should be broken before the spores will germinate. The presence of certain soil microflora has been shown to break this dormancy and subsequently stimulates the germination of spores (Hetrick, 1986; Bowen, 1987). According to Bagyaraj (1991) cold treatments or desiccation in the laboratory can also break this dormancy. Linderman (1991) indicated that the germination of AMF spores is influenced by several factors including self-inhibitors within the spores. Chemical and physical conditions of the soil may affect AMF spore germination (Lombaard, 1993).

Infected root segments, 'soil-based inoculum and spores isolated from open pot cultures of AMF-inoculated plants have been the usual source of AMF inoculum for research purposes (Ferguson and Woodhead, 1982). However, this type of inoculum is prone to contamination even with good phytosanitary care (Ames and Linderman, 1978). As opposed to the above-mentioned inoculum sources, axenically produced spores have lower levels of fungal contamination. Axenically-produced spores as AMF inoculum are more suitable for large-scale production as well as for biochemical and molecular investigations of the AMF symbiosis (Vimard *et al.*, 1999).

2.5 AMF and micropropagated banana

Available data on banana trials derive mostly from controlled conditions, however, efficient associations between AMF and banana plants are formed under greenhouse conditions (Declerck *et al.*, 1994). Inoculation experiments have resulted in increased banana plant growth due, in the great majority of cases, to their ability to deliver essential nutrients like phosphorus to the plant (Knight, 1988; Rizzardi, 1990; Jaizme-Vega *et al.*, 1991; Lin and Fox, 1992; Declerck *et al.*, 1994; Yano-Melo *et al.*, 1999), especially in soils of low fertility. Shashikala *et al.* (1999) have demonstrated that micropropagated banana plantlets raised in soil inoculated with *Glomus mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe and amended with 50% of recommended P fertilizer exhibit a significant increase in plant height, number of leaves, stem girth, root volume, total biomass and total P content over those grown with 100% recommended P fertilizer.

Pre-colonization of roots by AMF has been shown to reduce root damage in micropropagated banana plants due to soil-borne nematodes. Studies conducted by Umesh *et al.* (1988) resulted in a reduction in the nematode *Radopholus similis* (Cobb, 1893) Thorne 1949 by *Glomus fasciculatum* (Thaxter) Gerdemann & Trappe emend. Walker & Koske inoculated in banana plantlets. Furthermore, inoculated banana plants had a lower root lesion index as compared to plants

with nematodes alone. Jaizme-Vega *et al.* (1997) showed that *G. mosseae* suppressed root galling and nematode buildup in micropropagated banana roots Pinochet and Fernandez (1997) reported that the association between micropropagated banana plantlets and *G. intraradices* increased host tolerance to the nematode *Meloidogyne javanica* (Treub) Chitwood.

A number of mechanisms of interaction between AMF and nematode pathogens have been considered, and the evidence supporting each is reasonable. All the proposed mechanisms depend on AMF-mediated changes in host physiology. Changes in root exudation by AMF plants may change the attractiveness of roots to nematode pathogens. AMF may improve host plant vigor, and thus reduce yield losses caused by nematode infection, especially in low P soils and if AMF are established early in the growth cycle, before nematode infection (Linderman, 1994). Increased resistance to nematodes may also involve competition for food or space between AMF and the nematodes present together on the same roots. Physiological changes in AMF roots could also change resistance to nematodes by increased production of inhibitory substances or by changes in root exudation, which could alter mycorrhizosphere population and affect nematode populations and survival. It is important to note that the mechanisms of interaction between AMF and nematode pathogens depend on conditions of the test, the host plant, edaphic conditions, and the species of AMF involved (Linderman, 1994).

Declerck *et al.* (1995) reported that for a given banana cultivar, the extent of root colonization was influenced by the species of fungus used as inoculum: *Glomus macrocarpum* Tulasne & Tulasne colonized roots less than *G. mosseae*. Jaizme-Vega and Azcon (1995) also found differences in colonization of banana by AMF species. Declerck *et al.* (1995) points out that although *G. macrocarpum* colonized roots less than some other species, the fungus produced higher relative mycorrhizal dependency for each banana cultivar tested. Differences in levels of root colonization by AMF is probably attributable to differences in mycorrhizal dependency among varieties of banana and to abiotic factors (Yano-Melo *et al.*, 1999).

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 (i.e. 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).

AMF species vary in effectivity at promoting growth of micropropagated banana plantlets. Declerck *et al.* (1994) reported that *G. mosseae* was more effective than *Glomus geosporum* (Nicolson & Gerdemann) Walker at improving growth of micropropagated banana plantlets. Later, Declerck *et al.* (1995) observed that promotion of growth varied among banana cultivars and that plants inoculated with *G. macrocarpum* grew more than those inoculated with *G. mosseae*. Studies conducted by Jaizme-Vega and Azcon (1995) indicated that *G. fasciculatum* was more effective than *G. mosseae* at improving growth and nutrient uptake of micropropagated banana plants. Difference in growth promoting effects could arise from different rates of spread of infection, different amounts of external mycelium or different efficiency of the latter in uptake or translocation of phosphorus (Sanders *et al.*, 1977).

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).

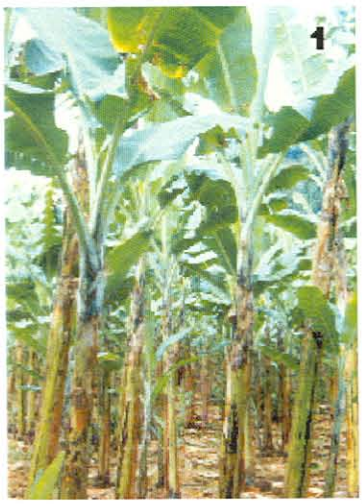
Baltruschat and Dehne (1989) reported that green manuring in the form of intercropping with rape and oil radish had a negative influence on AMF inoculum potential of soil from winter barley in a continuous monoculture. Similarly, banana production in Uganda is comprised of both continuous monoculture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

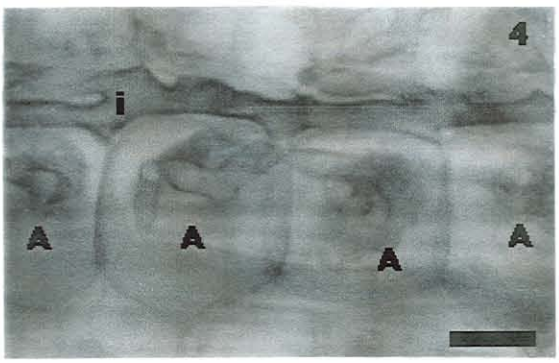
annual food crops. It is possible that some of the crops used for intercropping in Ugandan banana farms may have contributed to the low MIP recorded.

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.





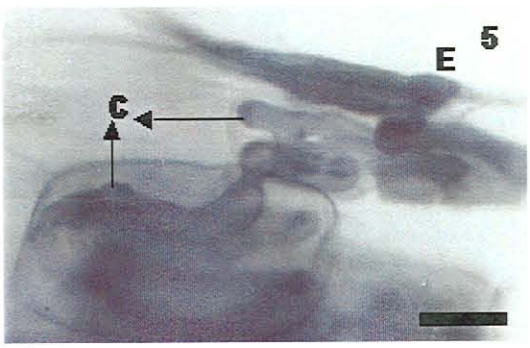


Plate 1

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.

CHAPTER 4

ISOLATION OF TWO AMF SPECIES FROM UGANDAN BANANA FARM SOILS

4.1 Introduction

The identification of AMF is based largely on the structure of their soil-borne resting spores (Brundrett *et al.*, 1996; Smith and Read, 1997). The task of identifying the fungi involved in the AMF symbiosis is a prerequisite to compare AMF research results obtained under different ecological conditions and has become increasingly important in recent years for the following reasons. Isolates of species differ in their life histories, growth-promoting activity, and tolerance to biotic and abiotic factors in the external environment. Isolates of species can be separated and cultured as single organisms only from spores grouped together by the same morphotype. Studies evaluating intra-and inter-specific differences among taxa at the biochemical, immunological, and molecular level require unambiguous definitions of species grouped by stable characters found at the morphological level. Relationships between form and function in biological, ecological and evolutionary processes begin with an understanding of the nature of morphological characters and the developmental relationships between them. From this, congruence (or conflict) between molecular, organismal and ecological levels of organization provide indications of where internal and external selection

pressures are affecting differential rates of evolution at each of these levels. Studies of species diversity, abundance, and geographic distribution require skills at identifying disparate organisms and grouping them accurately into a species (Morton, 1997).

There are approximately 163 species of AMF described worldwide. Over 43 records of AMF (Appendix B) have been reported from Africa and, to the best of my knowledge, none from Uganda.

Studies of AMF biota in Uganda involved evaluation of mycorrhizal inoculum potential of soils from banana plantations. Soils were retrieved for pot culture production of some individual species of AMF. *Glomus etunicatum* Becker & Gerdemann and *Glomus mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe were isolated under greenhouse conditions using sudan grass (*Sorghum sudanense* (Piper) Staph.) as a host plant. Descriptions of the species isolated are made with photographs of spore and spore wall structures.

4.2 Materials and methods

4.2.1 Trap cultures

Banana rhizospheric soil, obtained from Ugandan banana farms, containing roots chopped into small fragments was mixed with sterile soil. This 1:1 mixture was placed in 1-litre plastic pots (10 pots). Pots were seeded with *S. sudanense*, with 60-80 seeds/pot. Cultures were grown in a greenhouse (12°C/25°C) for 4 months after which they were left to dry undisturbed *in situ* (1-2 weeks). The shoots were removed from the pots and the remaining soil and root material was crushed to pass through a 2 mm sieve. Trap cultures were stored in brown paper bags in a fridge (4°C) until use.

4.2.2 Isolation of spores

Fifty grams (50 g) of soil was collected from the trap cultures and vigorously mixed in 1.5 litres of water, by blending for 1 min, to suspend the spores. The suspension was left to sediment for 20 s with the soil particles sedimenting more rapidly than the spores. The supernatant as well as the top (fine) layer of sediment was decanted through a 425 µm coarse soil-sieve (to remove large pieces of organic material) followed by a 45 µm fine soil-sieve (to retain total spore population). Spores and debris collected on the 45 µm sieve were transferred to a 100 ml centrifuge tube by washing with 50 ml distilled water. A

sucrose sedimentation gradient was established with one interface using 70% sucrose (40 ml) delivered slowly by pipette into the bottom of the centrifuge tube. A swinging bucket rotor centrifuge was used to spin down the suspension at 1800 rpm for 1.5 min. The spores were pipetted from the interface and water layer and washed on a 32 µm sieve. These were placed in water in Petri dishes and observed under a dissecting microscope (Nikon, Japan).

4.2.3 Spore sterilization

A modified technique of Budi *et al.* (1999) was used to surface sterilize the spores. Spores were immersed for 10 s in 96% ethanol and washed using a 32 µm sieve. Spores were then immersed for 10 min in a solution of 0.02% streptomycin, 2% chloramines T and a drop of Tween 20. Subsequent washing again took place on a 32 µm sieve. A final immersion of the spores took place in 6% bleach for 1 min and subsequent washing in sterile distilled water. Spores were stored in sterile distilled water in Petri dishes and glass vials in the fridge until use.

4.2.4 Establishment of monospecific cultures

Approximately 14 days before establishing a monospecific culture, *S. sudanense* plants were planted in a 500 ml pot using sterilized soil. Approximately 60-80 seeds were placed in the pot and spaced apart as evenly as possible to facilitate

seedling separation at harvest. When the plants were about 14 days old, the contents of the pot were gently removed (as intact as possible) and placed on a 425 µm sieve. The roots were gently placed under a stream of tap water to rinse off clinging soil medium and then gently teased apart by hand. Three seedlings were gently collected from the sieve. Spores (100) of similar morphology were selected from the collection of surface sterilized spores and placed along the length of the roots of the three seedlings. The seedlings were immediately transplanted into the center of a 1-litre pot containing sterilized soil seeded with 60 *S. sudanense* seeds. Ten pots containing different spore isolates were inoculated. When all inoculations were completed, sterile sand was added to all pots to within 1 cm of the pot lip. The medium was compacted with a glass rod until plants were not drooping over the edges. Then, all pots were watered gently and placed in a greenhouse (12°C/25°C). After 4 months, spores were isolated according to the method used in section 4.2.2 using a 425 µm and 75 µm sieves. Spores collected on the 75 µm sieve were examined.

4.3 Results

The isolated species showed quite typical spore morphology based on published descriptions (Schenck and Perez, 1990). All nomeclature for identifications follows the recommendations of Walker and Trappe (1993). Colour codes follow those of Brundrett *et al.* (1996).

4.3.1 *G. etunicatum* Becker & Gerdemann

Whole spores

Spores are borne terminally on a single undifferentiated hypha. They are orange brown (0-90-60)* to red brown (20-90-80)* in colour. Spores are of globose to subglobose shape. Spore size ranges from 60 µm-126 µm; mean = 75 µm; n = 100. Spore contents consist of a cytoplasm that is vacuolate, consisting of a continuous homogenous system with many lipid droplets of different sizes. Some spores consist of lipid droplets that are coalesced into a single large oil vacuole.

Spore wall

Spore wall consists of two detachable layers (**L1** and **L2**) 4.5 µm-9 µm thick (separate distinctly upon crushing of mature spore).

L1 appears as a single hyaline and variably mucilaginous layer. It is present in patches and appears to slough off with age.

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* Colour codes of Brundrett *et al.* (1996)

L2 consists of a smooth layer with thin adherent sublayers (laminae) yellow (0-60-0)* to brown (40-100-60)* in colour. In some specimens the innermost sublayer is separated slightly from the spore wall. This is rare and not considered stable enough to be taxonomically informative (Strumer and Morton, 1997).

Structure of subtending hypha

The subtending hypha is very slightly flared in shape at the point of attachment to the spore, measuring an average of 6 µm in thickness. Layers 1 and 2 of the spore wall continue into the hyphal attachment. Spore contents are separated from attached hypha by a bridging structure resembling a curved septum. Mature spores display spore contents separated from hypha by inner spore wall thickening.

4.3.2 *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe

Whole spores

Spores are borne terminally on a single undifferentiated hypha. They are yellow (0-60-0)* in colour. Spore shape varies from mostly globose to occasionally subglobose. Spore size ranges from 60 µm-240 µm; mean = 99 µm; n = 100. Spore contents consist of a cytoplasm that is vacuolate, consisting of a continuous homogenous system with many lipid droplets of different sizes.

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* Colour codes of Brundrett *et al.* (1996)

Some spores consist of lipid droplets that are coalesced into a single large oil vacuole.

Spore wall

Spore wall consists of two detachable wall layers (**L1** and **L2**) 4.5 µm-10.5 µm thick (separate distinctly upon crushing of mature spore).

L1 appears as a single hyaline, variably mucilaginous layer that appears to slough off with age.

L2 consists of yellow (0-60-0)* and brown (40-100-60)* tightly fused sublayers (laminae). The sublayers do not differ in texture.

Structure of subtending hypha

The subtending hypha is markedly funnel-shaped, measuring 4.5 µm-13.5 µm at the point of attachment to the spore. Occassionally, a short hypha extends from the funnel-shaped base and curves towards the spore. The hyphal wall consists of two wall layers. The funnel-shaped base of the hypha is separated from the spore contents by a septum.

* Colour codes of Brundrett *et al.* (1996)

4.4 Discussion

Two AMF species were isolated from Ugandan banana rhizospheric soil, namely *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe and *G. etunicatum* Becker & Gerdemann. The procedure of extracting spores from soil is very simple however, it is time consuming and labor intensive. The abundance of fungal propagules in soils and plant community are affected by local and seasonal environmental fluxes (Morton 1993). It is possible that only these two species were isolated due to the time of sampling. Sampling took place between February and March. If sampling had been done over a longer period of time, probably more species would have been isolated.

Morton (1985) isolated only two species (*Glomus diaphanum* Morton and Walker and *G. tenue* (Greenall) Hall from a reclaimed mine soil over a period of six years when rhizosphere samples were cultured on sudan grass (*Sorghum sudanense* (Piper) Staph.). Changes in pH of the medium resulted in the complete lost of *G. tenue* and spores of a previously undetected isolate of *G. etunicatum* Becker & Gerdemann appeared consistently. It is possible that the procedure used to culture spores (soil type, host species) from the Ugandan banana rhizospheric soil could have played a role as to which species would be isolated. The Ugandan rhizospheric samples were cultured on *S. sudanense* grown on a sterilized soil/sand mixture from local regions in Pretoria (South Africa), supporting local vegetation.

Certain agricultural practices may influence the species composition of AMF population (Linderman, 1994). Continuous monoculture of certain crops has been reported to alter AMF species composition (Johnson *et al.*, 1992). It is possible that only two species of AMF were isolated from Ugandan banana rhizospheric soil due to the fact that banana production in Uganda comprises of a continuous monoculture system. In an investigation of cropping systems with either continuous corn or continuous soybean, Johnson *et al.* (1991) observed that distinctly different mycorrhizal fungal communities developed over time according to the crop species in cultivation. The fact that only two species of AMF were isolated from banana rhizospheric soil of Uganda could also indicate that selective preference or specificity of AMF species with certain hosts occurs.

A hypothesis relating tillage with shifts in AMF species composition has been proposed by Kurle and Pfleger (1994). Where more intensive tillage systems are used, resulting in greater soil disturbance, it is likely that AMF species which sporulate more heavily would be favoured. Species which are more dependent on the mycelial network and on hyphal remnants in root fragments for carry-over would be disadvantaged because of disruption of the network and accelerated decomposition of roots. Seasonal pattern of AMF growth and sporulation would be important in determining the multiplication of a particular fungal species. Those species which sporulate most intensely early in the growing season would be favoured in many crops, since post harvest tillage would interfere with the

sporulation of species which colonize and multiply later in the growing season. Tillage is a common practice for the Ugandan banana farmers. It is possible that the species composition in Ugandan banana rhizospheric soil is influenced by this agricultural practice as described above.

In order to recognize spores of glomalean fungi from the Ugandan banana rhizospheric soil, trap cultures were established using *S. sudanense* as a host plant. This resulted in extraction of spores (1) consistent in appearance with most or all diagnostic features intact with hyphal attachments present, (2) of similar age and physiological condition, (3) abundant enough to produce replicate cultures from multi-spore inocula for use in inoculation of micropropagated banana plants (Chapter 5), (4) in a condition which permitted direct comparison of properties in spores from published referenced materials.

Taxonomic decisions at the species level required observations of broken spores with the light microscope, because many characters are subcellular (Morton and Benny, 1990). In this experiment it was found that structures within the spores were the most difficult to discern because they exhibit varying degrees of flexibility. All tend to wrinkle, fold, break in different places, overlap, clump, or become outstretched when pressure is applied to the spores. Some walls separated easily (Fig. 5, LL), others remained attached irrespective of manipulations (Fig. 1 and 3, LL).

Comparison of the Ugandan isolates with referenced published material is described below.

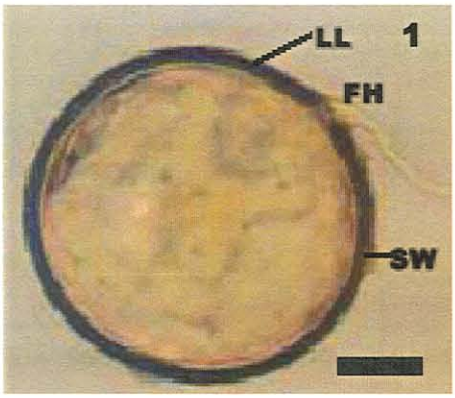
Becker and Gerdemann (1977) described *G. etunicatum* from a virgin sand prairie in central Illinois and agricultural fields in Illinois, Missouri and Florida. The Ugandan species record is smaller than this type description species. Spore size and spore wall thickness of the type description species range between 68 µm-144 µm and 4 µm -13µm respectively as opposed to 60 µm-126 µm and 4.5 µm - 9µm of the Ugandan species. The type description species has been recorded to have one or two hyphal attachments. The Ugandan species was never observed to have more than one hyphal attachment. Other morphological characteristics were similar. Both species display a spore wall composed of a hyaline outer wall and a persistent yellow to brown laminate inner wall. An intact outer wall layer is rarely present on mature spores. The spore contents are separated from the attached hypha by a thin curved septum. At maturity, the opening was sometimes occluded by inner wall thickening. Spores occur singly in soil.

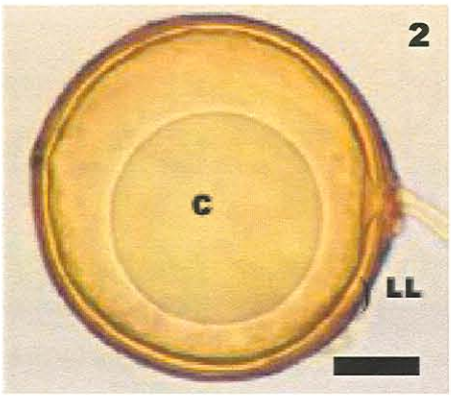
Nicolson and Gerdemann (1968) described *G. mosseae* spores extracted from cultivated soil in Scotland. The Ugandan species record is smaller than this type description species. Spore size of the type description species range between 60 µm –320 µm as opposed to 60 µm –240 µm of the Ugandan species. The type description species has been recorded to have one or two hyphal attachments. The Ugandan species was never observed to have more than one hyphal

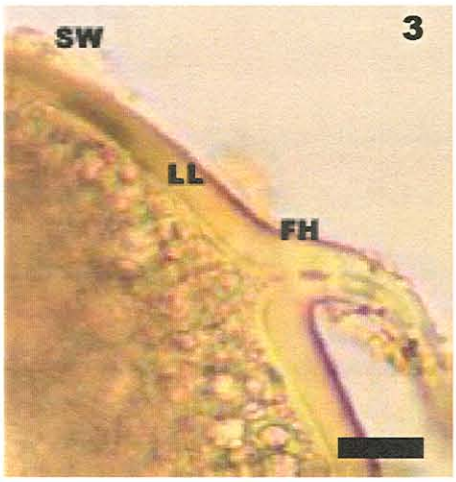
attachment. Sporocarps were not observed from the Ugandan species. Other morphological characteristics are similar. Both species display a distinctively funnel-shaped hyphal attachment. This funnel-shaped base is divided from the subtending hyphae by a curved septum. The spore wall is composed of a thin hyaline outer layer and a thick brownish-yellow laminate inner layer.

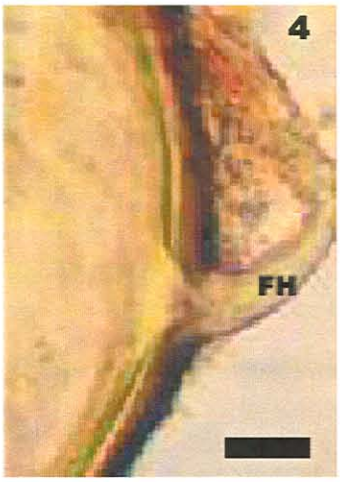
Five records of *G. etunicatum* Becker & Gerdemann and six records of *G. mosseae* have been reported on the African continent (Appendix B). Gaur *et al.* (1999) described *G. etunicatum* Becker & Gerdemann, from South Africa, with an average spore size of 100 μm. This falls within the size range described for the Ugandan isolate. El-Giahmi (1976) described *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe from Libyan soils. In comparison to the Ugandan isolate the Libyan spores are larger with an average diameter of 298 μm (220-353 μm). Unlike the Ugandan isolate, the Libyan isolate has been recorded to have 2 hyphal attachments. Comparisons with the other African isolates cannot be made as no descriptions were made.

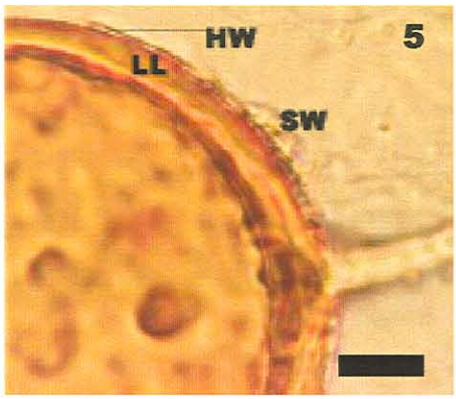
Both *G. etunicatum* Becker & Gerdemann and *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe are widely distributed on the African continent (Appendix B). To my knowledge, this is the first record of these species from Uganda.











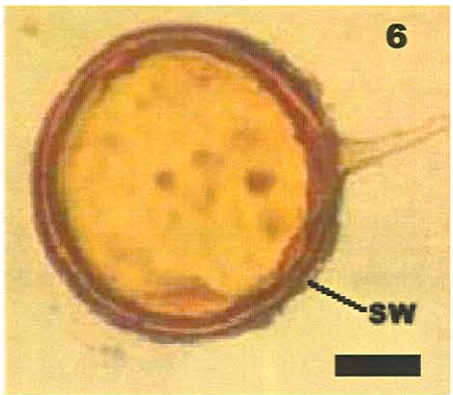


Plate 2

Figure 1: *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe spore showing funnel-shaped hypha (FH), tightly fused laminate wall layer (LL) and fragment of outer wall (SW). Bar = 23 µm.

Figure 2: *G. etunicatum* Becker & Gerdemann spore showing laminate wall layer (LL). Spore contents consist of lipid droplets that have coalesced into a single large oil vacuole (C). The outer wall has completely sloughed off. Note thickening of laminate layer at point of hyphal attachment. Bar = 19.6 µm.

Figure 3: *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe spore showing funnel-shaped hypha (FH) and fragment of outer wall layer (SW). Spore contents consisting of lipid droplets of different sizes (C). Bar = 13 µm.

Figure 4: *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe spore showing funnel-shaped hypha. Bar =16 µm.

Figure 5: *G. etunicatum* Becker & Gerdemann spore showing outer hyaline wall layer (HW) that is sloughing off (SW) and inner laminate wall layer (LL). Bar = 16.8 µm.

Figure 6: *G.etunicatum* Becker & Gerdemann spore with outer wall beginning to slough off. Bar =20 µm.

CHAPTER 5

MYCORRHIZATION OF MICROPROPAGATED GRAINDE NAINE (*Musa acuminata*, group AAA) WITH *GLOMUS MOSSEAE*

5.1 Introduction

Current developments emphasize the potential of plant tissue culture for the propagation, conservation and genetic improvement of bananas. A wide range of banana cultivars of all genomic constitutions has been found amenable to *in vitro* culture (Cronauer and Krikorian, 1984). However, micropropagated plants have problems concerning their survival and development, particularly in the acclimatization phase demonstrated by poor root, shoot and cuticle development (Varma and Schuepp, 1995). The sterile environment during the *in vitro* phase gives rise to pathogen free-plants, however, this technique presents the disadvantage of depriving plants of all soil microbiota which may include important rhizophere commensuals that maintain normal biological homeostasis as well as potentially crucial plant symbionts such as arbuscular mycorrhizal fungi (AMF) (Rizzardi, 1990).

The lack of exposure to rhizosphere organisms in micropropagated plants often continues in the nursery during the weaning phase and makes eventual outplanting stressful and/or unsuccessful.

The potting substrates are disinfected (either with steam or with general biocides) to eradicate or to prevent soil-borne plant pathogens and pests. General biocides are often applied to avoid weed problems in nursery soil substrates. It has been shown that general biocides greatly reduce the propagule density of indigenous AMF (Sieverding, 1991). Untreated potting material is derived from subsoils that have low natural content of soil-borne pathogens and these soils contain little in the way of AMF propagules. Composted organic materials and artificially produced media like vermiculite and perlite do not contain any AMF.

Non-mycorrhizal plants obtained from the micropropagation process eventually become mycorrhizal when they are planted in field soil. As demonstrated in chapter 3, AMF occur naturally in banana farm soils of Uganda. However, an early inoculation of these plants with selected AMF promises to improve plant survival and performance and allow lower chemical inputs (Varma and Schuepp, 1995) throughout the growth and productive phase of the plant.

Several workers have demonstrated that the acclimatization period of micropropagated plants can be shortened by the application of mycorrhizal technology (Lovato *et al.*, 1995). This reflects the potential gain in time and cost that is made possible through the use of mycorrhizal technology. Varma and Schuepp (1995) reported that mycorrhized apple plants were more uniform in size. From an industrial and economic point of view the uniformity of the plants is a desirable characteristic in any nursery, allowing for a homogenous

classification of the stock. Mycorrhization at the nursery level allows easy access to large numbers of plants, requires lower inoculum concentration and has an added advantage in that mycorrhiza and plant develop together.

This experiment was conducted to determine if a species of AMF, isolated from Ugandan banana farm soils, can colonize micropropagated banana plantlets (*Musa acuminata* cv. Grande Naine, group AAA).

5.2 Materials and methods

5.2.1 Experimental design

The following treatments were established:

(1) inoculated rooted banana plantlets from sterile modified banana growing medium (*in vitro* stage) (30 replicates),

(2) inoculated banana plantlets 5 cm in height from the weaning stage (30 replicates),

(3) control: micropropagated banana plantlets without inoculation for both stages of growth (10 replicates each) and,

(4) sterile sudan grass (*Sorghum sudanense* (Piper) Staph.) seedlings were inoculated to test viability of inoculum (10 pots) according to the method of Tommerup (1994).

5.2.2 Experimental plant material

Micropropagated banana plantlets (*Musa acuminata* cv. Grande Naine, group AAA) were supplied by African Biotechnologies, Tzaneen, South Africa. *In vitro* plantlets are derived from meristems tissue after multiple divisions on sterile modified banana growing medium. The weaning phase plantlets were allowed to grow to a 5 cm stage in plastic containers (60 ml) filled with peat moss in a

misting tunnel before inoculation. The experimental plants were washed free of the growing medium before inoculation.

5.2.3 Inoculum source

G. mosseae (Nicolson & Gerdemann) Gerdemann & Trappe spores collected from a monospecific culture as described in section 4.2.4 were surface sterilized according to the method used in section 4.2.3. The spores were then incubated in 0.02 % Streptomycin for 30 days in a fridge (4°C) to break any possible dormancy. Spores were washed several times with distilled water before inoculation.

5.2.4 Inoculation method and growth conditions

The experiment was carried out in May-July 1999 at African Biotechnologies, Tzaneen. Approximately 100 spores (per plant) were collected by pipette and placed directly unto the surface of the roots of the experimental plants prior to placing into growing substrate. The substrate consisted of a 1:1 sterilized soil/sand mixture obtained from the Agricultural Research Council-Plant Protection Research Institute (ARC-PPRI). The plants were maintained in a misting tunnel. Relative humidity was 60-70% and temperatures ranged between 25°C-27°C (day and night). The tunnel received a 12-hour day photoperiod and

had 40% shading. Plants were watered to field capacity once a day with no addition of nutrients.

5.2.5 Harvesting

At 3, 6 and 10 weeks after inoculation, ten plantlets were randomly harvested from treatments 1 and 2. At 3 and 6 weeks after inoculation, two plantlets were randomly harvested from treatment 3 and the remaining plantlets were harvested at 10 weeks. *S. sudanense* viability test plants were all harvested at 10 weeks.

5.2.6 Measurement of infection by AMF

Whole root systems of each plantlet were carefully washed under tap water, cut into 1 cm sections, cleared in 10% KOH and stained with trypan blue according to the method of Koske and Gemma (1989). The percentage root colonization by AMF was determined using the visual estimation technique (Kormanik and McGraw, 1982) under a dissecting microscope at a X400 magnification (Chapter 3; Section 3.2.2).

5.3 Results

Inoculum produced for mycorrhization of banana plantlets consisted of a pure culture of *G. mosseae*(Nicolson & Gerdemann) Gerdemann & Trappe. Photographs of wall structure and spore shape and colour are presented in chapter 4 (Plate 2). Inoculum produced was viability tested on *S. sudanense* in the greenhouse at the ARC-PPRI. Average root percentage colonization ranged between 6-26% of the test plant root system. This inoculum was subjectively considered to have a high infection potential.

Mycorrhization of both *in vitro* and weaning phase plantlets was demonstrated in the 10-week post-inoculation plantlets. All the other plantlets from the post-inoculation age groups were negative for the demonstration of infection by root staining using the methods of Koske and Gemma (1989). Examples of the infection are provided in Plate 3. Root percent colonization ranged between 0-5% infection of the test plant root system (Table 4). Thirty percent of the *in vitro* plants and fifty percent of the weaning phase plants harvested at 10 weeks were mycorrhizal.

Control plants consistently demonstrated no infection throughout the course of the experiment.

Table 4: Mycorrhizal colonization of micropropagated banana plantlets inoculated or not with *G. mosseae*

% colonization (Class)*			
	3 weeks	6 weeks	10 weeks
In vitro phase plantlets	0	0	1
Weaning phase plantlets	0	0	1
Non- inoculated control	0	0	0

*Class 0 = no infection.
*Class 1 = 0-5% infection.

5.4 Discussion

Micropropagated banana plantlets (both *in vitro* and weaning phase) exhibited AMF development 10 weeks after inoculation. It is possible that no infection occurred earlier in this experiment because the spores were under the influence of a dormancy period not broken in the conditions and time-span used. There is evidence that the germination of spores of some species of AMF is blocked by a dormancy factor for a certain period of time depending on the species of AMF (Tommerup, 1983; Bowen, 1987; Bagyaraj, 1991). Tommerup (1983) reported a dormancy period of approximately 6 weeks for *Glomus spp.* in wet soil and that no environmental condition will shorten this maturation period before germination.

In this experiment a low colonization level of the banana plantlets was recorded, ranging between 0-5% of the total root length. A high level of root colonization is in principle desirable, since it means that sites for the entrance of microorganisms into the roots are already occupied. This is the basis of potential resistance to pathogenic microorganisms (Gianinazzi *et al.*, 1990). Production of antimicrobial substances like phytoalexins increases with the level of arbuscular mycorrhizal infection (Morandi *et al.*, 1984). Considering the fact that Ugandan banana farming is under pressure from continuously declining production due to plant parasitic nematodes, banana weevil, leaf diseases, *Fusarium* wilt and, Banana Streak Virus, means that selection of efficient AMF requires a critical

evaluation of infection levels. Several studies have shown that plant growth enhancement by AMF generally is related to colonization level and to the extent of external mycelium, the latter being necessary for P uptake (Sanders *et al.*, 1977; Abbott and Robson, 1981; Tommerup, 1994)).

There is a range of factors that can affect the result of mycorrhizal inoculation in a micropropagation system. It has been shown that increased inoculum dosage results in increased colonization rate (Daft and Nicholson, 1969) and increased percentage root colonization (Johnson, 1977). It is possible that the amount of inoculum used in this experiment was low thereby resulting in the low percentage colonization observed. However, *S. sudanense* demonstrated high infection, which draws attention to possible host-dependent factors in receptivity to AMF infection.

Extent of root colonization of a fungal species varies with the host plant (Declerck *et al.* 1995). This may explain the difference in colonization levels between the banana plantlets and *S. sudanense*. It is possible that *S. sudanense* has a greater mycorrhizal dependency (MD) than Grainde Naine.

Both temperature and light have been shown to have a significant influence on colonization by AMF under greenhouse conditions. Higher temperatures generally result in greater root colonization (Hayman, 1974). According to Schenck and Schroder (1974), maximum arbuscule development occurs near

30°C, mycelial colonization of the root surface is greatest between 28°C and 34°C and vesicle development is greatest at 35°C. In this experiment, the banana plantlets were grown in a tunnel with temperatures ranging between 25°C-27°C (day/night). It is possible that the temperature settings of the tunnel were not ideal for increased AMF root colonization.

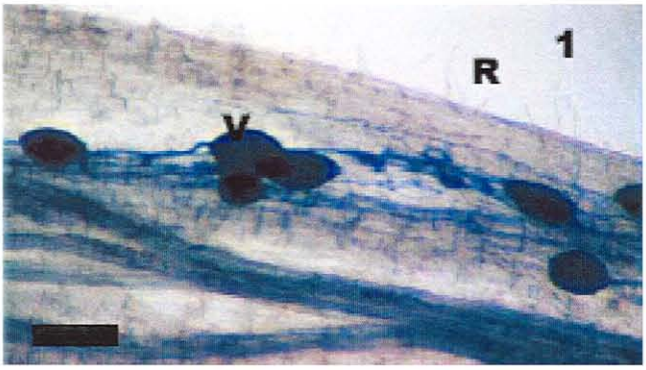
Low light intensity can significantly reduce root colonization (Hayman, 1974; Furlan and Fortin, 1977; Johnson *et al.*, 1982). In this experiment, the tunnel received a 16-hour photoperiod, however, it had 40% shading. It is therefore possible that the tunnel did not receive ideal light intensity for increased AMF root colonization. It is also possible that due to the diminished light intensity of the tunnel (40% shading), the banana plantlets carried out insufficient photosynthetic capacity to support high levels of colonization.

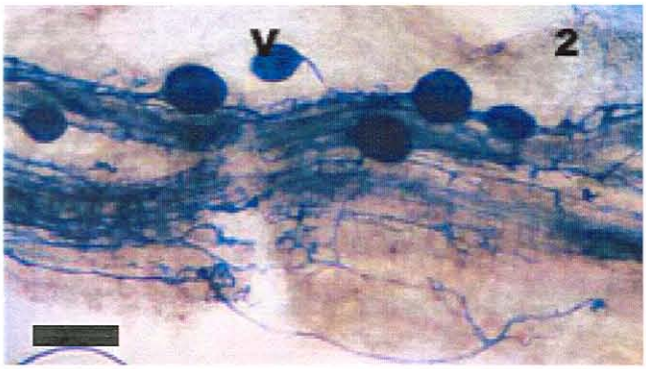
Gange *et al.* (1999) suggest that the level of mycorrhizal colonization recorded in any particular plant species at a particular time could be dependent on the stain employed. The differences in the ability of stains to reveal AMF is likely to be due to differential stain penetration of the roots of different plant species and the extent to which the stain is taken up by the fungus (Morton, 1985). The ability of a stain to penetrate a piece of plant tissue is affected by many factors including stain type, the vehicle in which it is dissolved, the concentration and pH of the staining solution, the temperature and duration of staining and the thickness of the plant material (Hayat, 1993). In addition, the nature and location of the target

material also has an effect, and it is possible that the depth at which arbuscules are located in the roots differs between plant species. This is because AMF hyphae grow through intercellular air spaces, and suberin in the cell walls regulate the growth direction of the fungus (Brundrett *et al.*, 1996), two factors which could vary between plant species. It would appear that trypan blue (used in this experiment) has different staining abilities in different plant species. It is possible that plants recorded as non-mycorrhizal or with low colonization are not so at all; the data simply reflect the inability of the stain used to detect the fungus.

The main hurdle in establishing strains of mycorrhizae on host plants is the starting point, the difficulty being two main points concerning the quality of spores: (1) ability of quick germination and (2) development of the mycelial network responsible for penetration of root cells (Rai, 2001). The results of this experiment suggest that *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe spores isolated from Uganda are of low quality with regards to the two points mentioned above. It is possible that the use of *G. etunicatum* Becker & Gerdemann or a combination of both species isolated from Uganda would have yielded different results. There is evidence that mixed inocula may interact with plants differently from single-species inocula (Daft, 1983). It is also possible that the use of whole inoculum (spores, roots, hyphae) as opposed to the use of spore-only inoculum for the inoculations would have also yielded different results.

It is conspicuous that *in vitro* inoculation of micropropagated banana plantlets at African Biotechnologies does not seem a feasible practice without changes to a number of factors such as maximizing greenhouse growth conditions conducive to AMF and root growth and screening for efficient mycorrhizal strains. Integrated technologies should be considered for future studies concerning the mycorrhization of micropropagated plants. Von (1998) emphasized the use of mycorrhizae helping bacteria (MHB) for enhancement of growth of plants. He reported that rhizosphere strains of *Bacillus mycoides* and *Pseudomonas fluorescens* promoted AMF formation in various crop plants by improving susceptibility of roots to AMF. The role of MHB was studied in detail by Garbaye *et al.* (1996). Evidently, MHB can also be inoculated into the roots for giving an opportunity to AMF for efficient, profuse and rapid colonization.





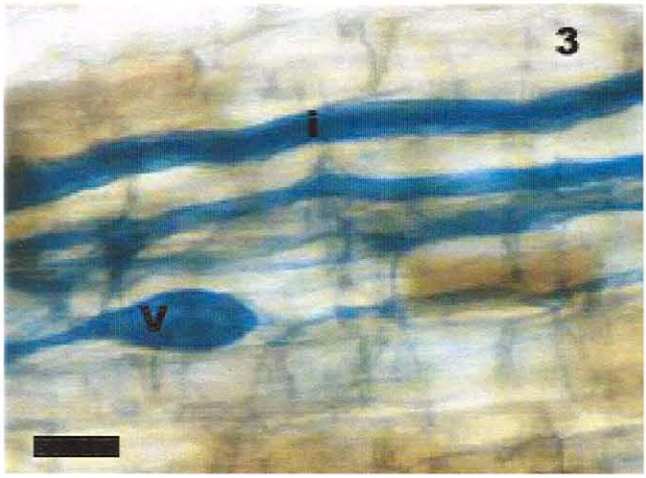


Plate 3

Figure 1 and 2. *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe in roots of weaning phase banana plantlets showing darkly staining vesicles (V). Note the abundant root hairs (R) of Grainde Naine (*Musa acuminata*, group AAA). Bar = 21 µm.

Figure 3 and 4: *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe in roots of *in vitro* banana plantlets showing intraradical spores (S) and intratradical foraging hyphae (i) growing parallel to each other and the root axis. Vesicles are seen in figure 3. Bar =14.4 µm.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

Consistent AMF colonization of *S. sudanense* trap plants by banana plantation soils from Uganda suggests that AMF are active biological components of this habitat. MIP assessment of the Ugandan banana farm soils showed that soils containing banana genotype *Musa* AAA had a higher mycorrhizal activity than soils containing banana genotype *Musa* ABB. This suggests that specific banana cultivars (*Musa* AAA) may support a more vigorous AMF population in the banana farm soils. Banana cultivars have been shown to differ in their receptivity to AMF infection (Declerk *et al.*, 1995). Therefore incorporation of the mycorrhizal technology in order to improve the sustainability of banana production in Uganda should look into selecting banana cultivars that are highly receptive to AMF.

The fact that *S. sudanense* plants became mycorrhizal from these soils implies that banana plants growing in these farm soils could also become mycorrhizal. Bananas are known to form facultative symbiosis with AMF in the field (Rizzardi, 1990). However, mycorrhization of these plants in the farm soils would be unplanned and uncontrolled. This would result in association with unknown indigenous AMF of unknown beneficial properties and quantity. Therefore screening for AMF effective at addressing specific problems faced by Ugandan

banana farmers should be a consideration for future research. One of the major factors contributing to declining banana production in Uganda is plant parasitic nematodes. Studies (Umesh *et al.*, 1988; Jaizme-Vega *et al.*, 1997; Pinochet and Fernandez, 1997) have shown that AMF may be effective at increasing plant resistance to nematodes. Aluminum toxicity, which is a common problem in Ugandan soils, has also been shown to be potentially alleviated by AMF (Rufyikiri *et al.*, 2000).

Successful inoculation of micropropagated banana plants with *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe isolated from Uganda has indicated the potential for using indigenous AMF species in inoculation programs. Selection of high-performance AMF species among isolated indigenous species should be a consideration for future research. Isolation of AMF species from soil is not difficult however it is time consuming. I was able to isolate only two AMF species in the time I had.

The majority of the farms in Uganda are of low-input agricultural practice. Suckers are used as planting material. Without having to incorporate a whole new technology of outplanting practice, perhaps investigations could be made into successfully mycorrhizing suckers to augment the habitat mycorrhiza. From my experience, it is not difficult to infect young plant material with AMF. However it is difficult to predict mycorrhizal species dominance and commensurate mycorrhizal plant benefit.

An efficient system can be developed to successfully mycorrhize highly receptive banana cultivars with a dominant and effective indigenous AMF such that outplanting provides a competitively dominant plant/symbiont combination for a specific habitat. Plant/symbiont couples of known effectivity for plant disease control, nutrient absorption, toxicity alleviation and stress conferance would be a realistic choice to provide the low-input farmers with sustainable and successful banana cropping systems. It is possible that the incorporation of the mycorrhizal technology can contribute to the improvement of sustainable and successful banana production by the low-input agricultural sector in Uganda.

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APPENDIX A

Survey sites used for MIP assessment of Ugandan banana farm soils

Sample*	Banana Genotype	District	Area	Farmer	Age of farm (yrs)	Latitude	Longitude	Elevation	Soil Temp °C	Observations
BLWD	AAA	Luwero	Kiteme	E. Kalibala	4	0 43 580	32 37 112	3500	20	Intercrop with coffee.
BLWD	ABB	Luwero	Kugu	C. Nanyombi	50	0 41 808	32 31 342	4000	20	Intercrop with coffee.
CLWG	AAA	Luwero	Kiteme	A. Sebyala	UN	0 32 867	32 47 025	4000	20	No intercrop.
CLWD	ABB	Luwero	Kalule	C. Semambo	12	0 38 002	32 31 931	4000	20	Intercrop with coffee.
AMSD	AAA	Masaka	Kyabakuza	Mr Chari	UN	0 19 681	31 42 066	3800	26	Intercrop with coco-yam, coffee, pineapple.
AMSD	ABB	Masaka	Selinya	B. Kwaso	26	0 17 236	31 39 716	4300	26	Intercrop with coco-yam, coffee.
CMSG	AAA	Masaka	Mwalu	F Nyombi	14	0 19 293	31 42 357	4400	22	Intercrop with coco-yam, cassava.
CMSG	ABB	Masaka	Mwalu	F Nyombi	14	0 19 293	31 42 357	4400	22	No inter crop.
AKBG	AAA	Kabale	Kitaburaza	P Barekye	75	1 13 34	30 07 031	6600	19	Intercrop with coco-yam.
AKBG	ABB	Kabale	Kitaburaza	P Barekye	75	1 13 34	30 07 031	6600	19	Intercrop with coco-yam.
CKBG	AAA	Kabale	Kayorero	F Kandali	150	1 12 998	30 07 682	5900	18	No inter crop.
CKBG	ABB	Kabale	Kayorero	F Kandali	150	1 12 998	30 07 682	5900	18	Intercrop with coco-yam, granadilla.
ANTG	AAA	Ntungamo	Karegeya	Kamugisha	10	0 52 758	30 14 182	4900	20	No inter crop.
ANTD	ABB	Ntungamo	Kabagenda	Bweseri	15	0 54 208	30 15 116	4600	19	No inter crop. Farm overgrown with weeds.
BNTG	AAA	Ntungamo	Ruguma	F Mujuni	9	0 52 764	30 13 998	4200	20	No inter crop.
BNTG	ABB	Ntungamo	Ruguma	F Mujuni	9	0 52 764	30 13 998	4200	20	No inter crop.
CNTD	AAA	Ntungamo	Ntungamo	Kashamba	12	0 52 600	30 15 836	4900	18	No inter crop. Farm overgrown with weeds.
CNTD	ABB	Ntungamo	Kabagenda	Kizungu	23	0 54 331	30 14 787	4900	19	No inter crop. Farm overgrown with weeds.

* See notation below Table 1. UN = Unknown.

APPENDIX B

Occurrence of AMF species in Africa

AMF species	Country(s) recorded	Author(s)
Acaulospora delicata *Walker, Pfeiffer & Bloss*	Sudan	Ba *et al.*, 1996
Acaulospora foveata *Trappe & Janos*	Ivory Coast	Wilson *et al.*, 1992
Acaulospora laevis Gerdemann & Trappe	Cameroon	Mason *et al.*, 1992
Acaulospora mellea Spain & Schenck	Cameroon Ivory Coast	Mason *et al.*, 1992; Wilson *et al.*, 1992
Acaulospora morrowiae Spain & Schenck	Namibia Cameroon	Stutz *et al.*, 2000; Mason *et al.*, 1992
Acaulospora scrobiculata Trappe	Cameroon Ivory Coast	Mason *et al.*, 1992; Wilson *et al.*, 1992
Acaulospora spinosa Walker & Trappe	Cameroon Ivory Coast	Mason *et al.*, 1992; Wilson *et al.*, 1992
Acaulospora trappei Ames & Linderman	Namibia	Stutz *et al.*, 2000
Acaulospora undulata Sieverding	Zaire	Sieverding, 1988
Gigaspora albida Schenck & Smith	South Africa Senegal	Gaur *et al.*, 1999; Dalpe *et al.*, 2000
Gigaspora calospora (Nicolson & Gerd.) Gerd.	Libya	El-Giahmi *et al.*, 1976
Gigaspora coralloidea Gerdemann & Trappe	Nigeria	Sani, 1976a
Gigaspora gigantea (Nicol. & Gerd.) Gerd. & Trappe	Nigeria Libya	Sani, 1976b El-Giahmi *et al.*, 1976
Gigaspora gilmorei Gerdemann & Trappe	Nigeria	Sani, 1976a
Gigaspora margarita Becker & Hall	South Africa Senegal Sudan	Gaur *et al.*, 1999; Dalpe *et al.*, 2000; Ba *et al.*, 1996
Glomus aggregatum Schenck & Smith	Senegal Sudan	Dalpe *et al.*, 2000; Ba *et al.*, 1996
Glomus avelingiae sp. nov. RC Sinclair	South Africa	Sinclair *et al.*, 2000
Glomus caledonium (Nicol. & Gerd.) Trappe & Gerd.	Senegal Libya	Dalpe *et al.*, 2000; El-Giahmi *et al.*, 1976
Glomus callosum Sieverding	Zaire, Rwanda	Sieverding, 1988

Glomus claroideum Schenck & Smith	Senegal	Diallo *et al.*, 1999
Glomus clavisporum (Trappe) Almeida & Schenck	Cameroon Ivory Coast	Mason *et al.*, 1992; Wilson *et al.*, 1992
Glomus constrictum Trappe	Senegal Ivory Coast	Diallo *et al.*, 1999; Wilson et al., 1992
Glomus eburneum sp. nov. Kennedy, Stutz & Morton	Namibia	Stutz *et al.*, 2000
Glomus etunicatum Becker & Gerdemann	South Africa Namibia Cameroon Ivory Coast Senegal	Gaur *et al.*, 1999; Stutz *et al.*, 2000; Mason *et al.*, 1992; Wilson *et al.*, 1992; Ginwal *et al.*, 1997
Glomus fasciculatum (Thaxter) Gerd. & Trappe emend. Walker & Koske	Nigeria Senegal Cameroon Egypt Libya	Sani 1976a Dalpe *et al.*, 2000; Mason *et al.*, 1992; Mankarios and Abdel-Fattah, 1994; El-Giahmi *et al.*, 1976
Glomus geosporum (Nicol. & Gerd.) Walker	Cameroon Sudan	Mason *et al.*, 1992; Ba *et al.*, 1996
Glomus intraradices Schenck & Smith	South Africa Namibia Senegal	Gaur *et al.*, 1999; Stutz *et al.*, 2000; Diallo *et al.*, 1999
Glomus lamellosum *Dalpe, Koske & Tews*	Sudan	Ba *et al.*, 1996
Glomus macrocarpum Tulasne & Tulasne	Cameroon Libya	Mason *et al.*, 1992; El-Giahmi *et al.*, 1976
Glomus manihotis Howeler, *Sieverding & Schenck*	Sudan	Ba *et al.*, 1996
Glomus microaggregatum Koske, Gemma & Olexia	Namibia	Stutz *et al.*, 2000
Glomus monosporum Gerdemann & Trappe	Ivory Coast	Wilson *et al.*, 1992
Glomus mosseae (Nicol. & Gerd.) Gerd. & Trappe	Nigeria Senegal Namibia Egypt Morocco Libya	Sani, 1976a Ginwal *et al.*, 1997; Stutz *et al.*, 2000; Mankarios and Abdel-Fattah, 1994; Meddich *et al.*, 2000 El-Giahmi *et al.*, 1976
Glomus occultum Walker	South Africa Namibia Cameroon Ivory Coast	Gaur *et al.*, 1999; Stutz *et al.*, 2000; Mason *et al.*, 1992; Wilson *et al.*, 1992

Glomus rubiforme (Gerd. & Trappe) Almeida & Schenck	Cameroon	Mason *et al.*, 1992
Glomus sinuosum (Gerd. & Bakshi) Almeida & Schenck	Ivory Coast	Wilson *et al.*, 1992
Glomus spurcum Pfeiffer, Walker & Bloss emend. Kennedy, Stutz & Morton	Namibia	Stutz *et al.*, 2000
Sclerocystis rubiformis Gerd. & Trappe	Senegal	Diallo *et al.*, 1999
Scutellospora coralloidea (Trappe, Gerd. & Ho) Walker & Sanders	Cameroon	Mason *et al.*, 1992
Scutellospora gregaria (Schenck & Nicol.) Walker & Sanders	Senegal Sudan	Diallo *et al.*, 1999; Ba *et al.*, 1996
Scutellospora nigra (Redhead) Walker & Sanders	Nigeria	Old *et al.*, 1973
Scutellospora pellucida (Nicol. & Schenck) Walker & Sanders	Cameroon Ivory Coast	Mason *et al.*, 1992; Wilson *et al.*, 1992
Scutellospora verrucosa (Koske & Walker) Walker & Sanders	Senegal	Diallo *et al.*, 1999

APPENDIX C

Figure 1: Map showing AMF survey sites enclosed in boxes.

Source: African Centre for the Constructive Resolution of Dispute