Arbuscular Mycorrhizal Fungi in the Sub-Saharan Savannas of Benin and their Association with Yam (*Dioscorea* spp.): Potential of Yam Growth Promotion and Reduction of Nematode Infestation

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Dedicace

To my late uncle, Mr Kankandana Ogagaraga Mouhamed, who brought me to school, paid my school fees and encouraged me to acquire knowledge in the field of agriculture. Thanks for your sacrifices.

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SUMMARY

The arbuscular mycorrhiza is the most widely occurring and important microbial symbiosis for agricultural crops and well known to facilitate plant mineral nutrient uptake, particularly under conditions of P-limitation - as it is common in tropical soils due to leaching or/and severe immobilization - and, moreover, it is understood to improve plant water relations and provide resistance against pests and pathogens. Yam (*Dioscorea* spp.) is the most important tuber crop in terms of coverage area in West Africa, particularly in Benin and Togo. Alarmingly, the annual yam production per hectare has recently decreased considerably due to a loss of soil fertility and pest and disease (especially nematode) damage. Under field conditions, yam and arbuscular mycorrhizal fungi (AMF) are naturally associated with each other. Currently, however, data on the ecology of AMF in West Africa are lacking with very limited information on the mycorrhizal status of yam. There may be potential to improve growth and to protect plants against nematodes by AMF but this is possibly dependent on specific AMF-nematode-host combinations. The present project aimed at studying AMF indigenous to West Africa, with respect to yam growth promotion and yam nematode suppression. The specific objectives focused on assessing the (1) diversity of AMF, including their distribution, abundance and relation to agronomic practices and ecological conditions; (2) the mycorrhizal status of yam; and (3) the specific associations between (a) yam-AMF, and (b) yam-AMF -nematode *in vivo* under greenhouse conditions.

In the first part of our study, we determined the influence of three ecological zones (from wettest to driest) and of land use intensity on the diversity of AMF in the yam growing area of Benin, West Africa. In each zone, four 'natural' and four 'cultivated' sites were selected. 'Natural' sites included three natural forest savannas (at least 25-30 years old) and a long-term fallow (6-7 years old). 'Cultivated' sites comprised yam fields established immediately following forest clearance, mixed cropping maize (*Zea mays*) and peanut (*Arachis hypogaea*) fields, peanut fields, and fields under cotton (*Gossypium hirsutum*), which was the most intensively managed crop. Soil samples were collected towards the end of the wet season in each zone. AMF spores were extracted and identified morphologically. A total of 60 AMF species was detected, with only seven species sporulating in AMF trap cultures that were set up with various AMF host plants. Higher species

richness was observed in the northern most, driest ecological zone Sudan Savanna (SU) than in the adjacent zones to the south with increasing humidity, namely the Northern Guinea Savanna (NG) and the Southern Guinea Savanna (SG), mainly due to a high proportion of species in the *Gigasporaceae*, *Acaulosporaceae* and *Glomeraceae*. Within each ecological zone, spore density and species richness were generally higher in the natural savannas and in association with yam than in the other cultivated sites. These parameters were lowest under the intensively managed cotton, and intermediate in the fallows, indicating that the high richness of the natural savannas is not necessarily restored during fallowing.

Assuming that yam is an arbuscular mycorrhizal crop, we addressed the question of which AMF species are associated with yam. Our aim was to propagate the AMF communities from three natural forests and three adjacent yam fields of the SG in Benin in trap cultures and to assess the AMF richness, identifying those associated with yam. Soil samples were collected in the dry season (February 2005) and used to identify AMF spores directly and also to establish AMF trap cultures on yam (tissue culture plantlets of *D. rotundata* and *D. cayenensis*) and, for comparison, on *Sorghum bicolor*. In the trap cultures, AMF root colonization was particularly high in yam (70-95%), compared with *S. bicolor* (11-20%). Based on spore morphotyping, 37 AMF species were detected in the 'trap' rhizosphere of *S. bicolor*, while 28 and 29 species were identified as fungal symbionts of *D. cayenensis* and *D. rotundata*, respectively. Following eight months cultivation in trap cultures, yam tuber dry weight was generally higher in mycorrhizal than in non-mycorrhizal control pots.

We also hypothesized that indigenous AMF species and strains isolated from yam plantlets in trap cultures may be more beneficial for yam plant growth compared to non-indigenous isolates. We screened indigenous AMF species and strains that have been isolated from the trap cultures and compared their effects on micro-propagated white yam plantlets (*D. rotundata*) (cv. TDr89-02461) against exotic AMF isolates in pot experiments over seven months. First, we tested several indigenous and non-indigenous (South America and Asia) *G. etunicatum* strains with regard to their effect on yam growth promotion and mineral accumulation in the tissues. Secondly, three isolates each of nine indigenous AMF species and three additional non-tropical AMF species were screened on the same yam cultivars. We found that most tropical AMF

isolates of *G. etunicatum* increased yam tuber dry weight, while the non-tropical AMF isolates had a lower or no effect, but instead increased tuber P concentrations, when compared to non-mycorrhizal controls. *Glomus mosseae*, *G. hoi*, *G. etunicatum*, *Acaulospora scrobiculata* and *A. spinosa* generally had a positive effect on tuber growth, while isolates of *G. sinuosum* and *Kuklospora kentinensis* generally did not.

Finally, we assessed the interaction between yam and AMF in the presence or absence of plant parasitic nematodes. Yam vplants cultivated in vitro were used, which were inoculated with commercial inocula of *G. mosseae* and *G. dussii* (Biorize, Dijon France). In the presence of nematodes *(Scutellonema bradys* and *Meloidogyne* spp.), inoculation of *G. mosseae* generally increased growth of micropropagated yam plantlets and yam tuber weight production, especially cultivars from *D. alata*. Tubers were, in general, less infected with *S. bradys,* but not necessarily with *Meloidogyne* spp. However, application of *G. mosseae* and *G. dussii* to micropropagated plantlets resulted in improved quality of yam tubers, when challenged with nematodes, compared to nematode inoculation without AMF, indicating a positive effect of AMF on yam productivity.

Our results indicate that the AMF richness is high in the 'yam belt' of Benin, but that it is strongly influenced by the ecological zone and by the intensity of land cultivation after forest clearance. Our results also indicate that in controlled pot studies, AMF can suppress nematode damage and additionally lead to improved quality and weight of yam tubers. The present results remain preliminary, however, while results from ongoing studies currently in the field will help to determine further their potential in the longer term. These results provide exciting prospects for African crop production, in addition to illuminating the wide and diverse species richness of West African AMF and their potential benefits.

CHAPTER 1: General introduction

1.1. Overall view

Yam (*Dioscorea* spp.) is a tuber crop belonging to the family of Dioscoreaceae. The genus *Dioscorea* includes more than 200 species, but only ten are important food yam species, from which water yam (*Dioscorea alata*, originating from Asia), yellow and white yam of the *Dioscorea* "*rotundata-cayenensis*" complex (indigenous to Africa) are the most frequently cultivated in West Africa (Coursey, 1967; Orkwor, 1998). Most of the other *Dioscorea* species are wild yam indicating the high biodiversity of wild yams. Yam is widely cultivated in West and Central Africa, in Asia and South American countries (Coursey, 1967; Orkwor, 1998; FAO, 2007). In West Africa, yam is the most important tuber crop in terms of area coverage and a key staple food, particularly in Nigeria, Ghana, Ivory Coast, Benin and Togo (Kalu and Erhabor 1992; Ile et al. 2006). More than 90% of the global world yam production (40 million tons fresh tubers/year) is produced in West Africa (FAOdata, 2007). Additionally, yam plays a vital role in traditional culture, rituals and religion, local commerce and is additionally referred to as a cultural symbol of fertility (Coursey, 1965). Yam is also an essential element of traditional marriages for instance in many West Africa cultures (Coursey, 1965; Coursey, 1967). In West Africa, yam consumption (especially in the cities) is increasing; consequently the area under yam cultivation is constantly increasing (IITA, 2006). Unfortunately, yam production is dramatically decreasing in productivity per area (IITA, 2006). Two major constraints are highlighted for their association with declining yam production: (1) soil fertility degradation, e.g. due to nutrient deficiency, leaching by erosion, high fixing of phosphorus (P) and low level of organic matter from most soils in West Africa (Schlecht et al., 2006); (2) damage by pests and diseases, especially plant parasitic nematodes and virus diseases (Odu et al., 2004; Egesi et al., 2007a b).

Originally, yam was planted only in the humid forest within intercropping based systems with banana, plantain (*Musa* spp.), sweet potatoes (*Ipomea batata*), vegetables and maize (*Zea Mays*) (Coursey, 1967; IITA, 1995). Over the past four decades, however, the yam production zone in West Africa has shifted from the humid forest zone towards the savannas in the North (Manyong

et al., 1996). In the savanna zones, farm sizes are larger and the number of intercrop components is fewer (Nweke et al., 1991; Manyong et al., 1996). The consequences from the reduction of intercropping practices are monoculture practices in rotation systems, where yam is planted as the first crop of the rotation following clearance of forests or long-term fallows (Carsky et al., 1999; O'Sullivan and Jenner, 2006). Each year, farmers have traditionally cleared forests for yam production and former yam production sites succeeded by other crops, such as maize, sorghum (*Sorghum* spp.), cassava (*Manihoti esculenta*) and cotton (*Gossipium hirsutum*) (IITA, 1995). Forest and fallow clearance are mainly characterized by the 'slash and burn' system of removing and burning grasses and trees (Orkwor and Asadu, 1998). Additionally, in West Africa, as in the rest of the continent, crop residues are often removed from the fields or burned, contributing to negative nutrient balances (Stoorvogel et al., 1993). These practices expose the soil to erosion, nutrient and organic matter leaching, natural resource degradations and decreasing soil microbial diversity including arbuscular mycorrhizal fungi (AMF) diversity (Cardoso and Kuyper, 2006), leading to soil degradation (Salako et al., 2007). With increasing demographic pressure, land use intensity and reducing savannah forest covering areas, suitable land for yam cultivation becomes gradually scarcer (Carsky et al., 2001). Furthermore, fallow periods in the savannas become shorter and most farmers increasingly cultivate yam without any fallow, leading to increased pathogen and pest attacks, including the harmful nematodes (Manyong et al., 1996; Carsky et al., 2001).

Nematode problems such as "dry rot disease" caused by the migratory endoparasitic nematode, *Scutellonema bradys,* and "galling" caused by the sedentary endoparasitic nematode *Meloidogyne* spp. cause important yield losses in West and Central Africa, while *Pratylenchus coffeae* is the most damaging in Asia (Bridge et al., 2005). *Scutellonema bradys* invade the roots tips or young tubers and feed intracellulary resulting in rupture of cell walls, loss of cell contents and the formation of cavities in yam tuber tissue (Bridge, 1973). *Scutellonema bradys,* causing "dry rot disease" (Bridge, 1973) is mainly confined to the subdermal, peridermal and underlying parenchymatus tissues in the outer 1-2 cm of the tuber, where the tissues become necrotic and die (Adessiyan, 1977). *Meloidogyne* spp. are sedentary endoparasitic nematodes of roots and tubers. The mobile second-stage juveniles (J2) emerge from the eggs, move towards the roots and penetrate the roots mostly at the root tip (Bridge, 1973). In the root, the J2 invade the

endodermis and, on entering, induce giant cells. Multiplication of the cortical cells is also induced, resulting in the formation of the characteristic galls (Vovlas and Ekanayake, 1985). The J2 feed on these giant cells and moult three times before becoming immobile adult females (Bridge, 1973). Both types of nematodes, *S. bradys* and *Meloidogyne* spp., produce a significant reduction in the quality, marketable value and edible portions of yam tubers in West Africa (Ayala and Acosta, 1971). The damage is more severe in yams following storage and often limited at harvest (Coyne et al., 2005; Baimey et al., 2006). Main nematode control methods, such as the application of chemicals can be used effectively and efficiently, particularly to treat planting material. However, one main problem is the limited and erratic availability of suitable pesticides, in addition to a lack of awareness of their beneficial effects, and understanding of the cause of the damage and losses. The identification of suitable sources of resistance, for the development of cultivars, resistant to the key nematode constraints would be highly desirable, but as yet remains to be realised (IITA, 2004).

Currently, research is underway to investigate the efficiency and economics of inorganic fertilizer application for yam. Available results on the effects and financial benefits of fertilizer application for yam production are often contradictory and not conclusive. Increased tuber weight is reported in Nigeria (Igwilo, 1989), Cameroun (Kayode, 1985) and in the savanna regions in Côte d'Ivoire (Dibby et al., 2004) for example, while no or limited effects are reported in other studies in the humid forest in Côte d'Ivoire (Dibby et al., 2004), in the savannas and coastal humid regions in Benin (Baimey et al., 2006) and in Hawaii and Ghana (Van der Zaag et al., 1980). The differences among results appear to be highly related to yam species or cultivars used and environmental conditions (Dibby et al., 2004). For example, the differences in yam responses to fertilizer application in savanna areas between studies of Baimey et al. (2006) and Dibby et al. (2004) were attributed to the yam cultivars (Baimey, 2006). Other factors are suggested to explain the non-response of yam to fertilizer application, such as Ferguson (1973), who attributed the lack of response of yam plants to fertilizer application to the fact that yam received P through a possible dependence on arbuscular mycorrhizal fungi (AMF), and for Vander-Zaag (1980), who speculated that yam requires only moderate or very low P, which can be readily delivered through mycorrhizal associations. It was suggested that imitating natural ecosystems, rather than planting monocultures or inorganic fertilizer application is the most

suitable agricultural strategy for the tropics e.g. agroforestry systems or mixed cultures of plant species(Cardoso and Kuyper, 2006). Use of adequate plant species diversity with perennial plants to maintain soil fertility and to protect ion of soils against erosion generally will lead to an increased diversity and abundance of AMF (Altieri, 2004; Cardoso and Kuyper, 2006).

AMF are an abundant and functionally important group of soil microorganisms, which can form symbiotic associations with more than 80% of terrestrial plant species (Smith and Read, 2008). They are supposed to be the most important microbial symbionts for the majority of plants. Many studies have shown an improvement of nutrient supply for crop plants (e.g. Caglar and Akgun, 2006; Schreiner, 2007) and suppressive effects on nematode infestations (e.g. Hol and Cook, 2005). Under phosphate-limited conditions, AMF can influence plant community development (van der Heijden et al., 1998), nutrient uptake, water relations and above-ground productivity (Clark and Zeto, 2000). They can also act against toxic stresses (Jeffries et al., 2003). Many studies have reported that native (=indigenous) AMF are often more effective mutualists than non-native AMF, presumably as a result of adaptation to edaphic factors, such as soil nutrient concentrations, or to environmental factors, such as drought (Lambert et al., 1980; Henkel et al., 1989; Caravaca et al., 2003; Oliveira et al., 2005; Querejeta et al., 2006). However, there are numerous reports where non-native AMF have outperformed native AMF (Trent et al., 1993; Sylvia and Burks, 1988; Calvente et al., 2004). The use of AMF, either by adding them into the field or by favoring systems and practices that facilitate their presence, may improve plant growth promotion or provide an interesting alternative or complement to manage soil fertility and nematodes in yam.

Until now, there is little information regarding the mycorrhizal status of yam (Uchendu, 2000; Ahulu et al., 2004; Dare et al., 2007; Oyetunji et al., 2007). To our knowledge no extensive study has been undertaken to isolate and identify AMF species associated with yam. Additionally, no information is available regarding the diversity of AMF species in yam-growing areas, specifically in yam fields or under natural conditions adjacent to yam fields in West African forest savannas. Furthermore, the interactions between AMF and yam and also between AMF and nematodes on yam have, to date, not been investigated to evaluate their potential to promote yam growth or nematode management. Thus, the present chapter 1 presents a literature review

providing a detailed background to the topic and is divided in four sections. The first section (1.2.) treats the importance of yam as food crop and the major constraints for its production. The second section (1.3.) is focused on *S. bradys* and *Meloidogyne* spp. as important pests of yam. The third section (1.4.) is concentrating on the biology of AMF and their importance in (agro)ecosystems and crop protection. The fourth section (1.5.) presents the general and specifics objectives of the current study.

1.2. *Dioscorea* spp. (yam)

1.2.1. Origin and distribution of yam

Yams are among the oldest food crops recorded and are defined as "an economically useful plant of the botanical genus *Dioscorea* for the tubers or rhizomes of these plants" (Coursey, 1967). Including cultivated and wild yam, the genus includes 194 species (http://www.aluka.org/: checked in May 2008), and about thirteen are important food yam species. They are listed in Table 1 with *D. rotundata* (white yam). *D. cayenensis* (yellow yam) and *D. alata* (water yam) as the economical most important species (Malaurie et al., 1998). Yam was believed to be indigenous to West Africa (Coursey, 1967; Nweke, 1981), but in reality, yam origins are variable according to a large species diversity of the genus *Dioscorea* (table 1). In general, food yam species originate in the tropical areas of three separate continents: Africa, South America and Southeast Asia. Details on yam origin and its evolution have been well discussed by Coursey (1967), Alexandre and Coursey (1969) and Orkwor et al. (1998).

The global distribution of yams was well documented by Coursey (1967), Ayensu (1972) and Orkwor et al. (1998). However, the majority of the *Dioscorea* species are distributed throughout the tropics and a few species of less economic importance are also found in the warmer regions of the temperate zones (Orkwor, 1998). The main area of production is in West Africa, which includes Nigeria, Benin, Togo, Ghana and Côte d'Ivoire, and Cameroon in Central Africa (Coursey, 1967 and Orkwor, 1998).

Table 1.1. Main edible species of yam (source: Malaurie et al., 1998),

Dioscorea spp.	Zone of origin	Zone of culture
D. rotundata Poir	West Africa	West & Central Africa, and Caribbean
D. cayenensis Lam	West Africa	West & Central Africa, and Caribbean
D. alata L.	South East Asia	South East Asia
D. esculenta (Lour.) Burk	South East Asia	Inter-tropical humid
D. dumetorum (Kunth) Pax.	West Africa	West Africa
D. bulbifera L.	South East Asia and Africa	Inter-tropical humid
D. trifida L	Guyana, Amazonian basin	Caribbean
D. opposita Kunth	China, Korea, Taiwan, Japan	China, Korea, Taiwan, Japan
D. japonica L.	China, Korea, Taiwan, Japan	China, Korea, Taiwan, Japan
D. nummularia Lamk	Indonesia, Oceania	Indonesia, Oceania and, Micronesia
D. transversa Br.	South Pacific	South Pacific
D. pentaphylla L.	Himalaya and Oceania	Himalaya and Oceania
D. hispida Dennst.	India, South-China, New Guinea	India, South-China, New Guinea

The yam domesticated earlier in West and Central Africa included *D. rotundata* and *D. cayenensis*, often summarized in the so-called *D. rotundata-cayenensis* complex (Malaurie et al., 1998) as well as *D. dumetorum*, while in Southeast Asia, *D.alata* was the first yam cultivated. Later, *D. alata* reached Africa, most likely on the East coast of Africa from Malaysia at about 1500 B.C. Today, the Asiatic yam, especially *D. alata,* is widely distributed in Africa, while the Africa *D. rotunda-cayenensis* complex is now widely grown in the Caribbean (Hahn et al. 1987). In Benin and in Togo, as well as in Nigeria, *D. alata* ranks second to the *D. rotunda-cayenensis* complex in production and consumption (Orkwor, 1998, MDR, 2000, IITA, 2005). In the West Indies, Papua New Guinea and New Caledonia, *D. alata* is the major food yam grown and consumed.

1.2.2. Taxonomy and morphology of yam

Yam was first described by Linnaeus (1737) as a monocotyledon plant in the Dioscoreales order. The family Dioscoreaceae has ten genera: *Dioscorea, Higinbothamia, Borderea, Epipetrum, Rajania, Tamus, Stenomeris, Trichopus, Avetra* and *Petermannia* (Knuth, 1924). The genus *Dioscorea* is the largest of the ten genera and consists mostly of tropical plants. Many edible yam species have a large number of cultivars that have yet to be systematically characterized and the distinctions between species are not always evident (Orkwor, 1998).

Morphologically, yam plants are composed of two parts: below-ground structures (the fibrous root system and the thick storage organs or tubers in which all starch is deposited) and the above aerial component, which comprises leaves and vines. Concerning the below-ground part, Onwueme (1978) showed that the fibrous root system is concentrated within the top 0.3 m of the soil with only few penetrating deeper than 1 m, while tubers can penetrate deeper than 2 m. Yam tuber shape, number and form depend on yam species and genotype (Martin and Sadik, 1977; Bai and Ekanayake, 1998). For example, *D. rotundata* tubers are generally large and cylindrical in shape, while *D. alata* has a variable shape, but the majority being cylindrical. Yam tubers are often referred to as stem tubers, because they are considered to be a modified stem structure; but in fact, they have no pre-formed buds or eyes, no scale leaves, and no equivalent of terminal bud at the distal end of the tubers (Hahn et al., 1987). Yam tubers originate from the hypocotyls, which is a small region of meristematic cells between the stem and the root (Lawton and Lawton, 1969). Orkwor et al. (1998) reported that the aerial part consists of vine-like stems on which leaves and inflorescence are formed. The vines can be several meters long and the leaves, which are borne on long petioles, are usually simple, cordate or acuminate, but are lobed or palmate in some species with pointed tips, alternate or opposite with a heart-shape (Bai and Ekanayake, 1998). The leaves may be smooth or hairy. In certain species, bulbils (aerial tubers) are formed in the leaf axils (Osagie, 1992). The yam plant is dioecious with white, green, or red flowers arranged in clusters or spikes. More extensive details about the origin, morphology and physiology of yam can be found in Orkwor et al. (1998).

1.2.3. Production, socio-economic importance, composition, consumption and use of yam

Yam production

The greatest proportion of global production is found in the area of West Africa known as the "yam belt", where *D. rotundata* and *D. cayenensis* are most widely grown and consumed. Approximately 96% of the world's annual yam production is produced in the 'yam belt' of West and Central Africa (FAOdata, 2007), which indicates a global production of yam of 42 million tonnes (Mt). In Africa, Nigeria is the largest producer with 34 Mt, followed by Ghana (3.8 Mt), Ivory Coast (3 Mt), Benin (2, 5 Mt) and Togo (600.000 t). In Benin, yam is one of the most important food crops cultivated in the Northern and Central part, while in Togo; yam is mainly cultivated in the Central and South-West part (MDR, 2000).

Socio-economic importance of yam

In many parts of the tropics where yam tubers are produced, the ethnocentric attachment to the crop is very strong (Ayensu and Coursey, 1972). In Africa, particularly in the "yam belt" yam plays a vital role in traditional culture, ritual and religion, as well as in local commerce. In all these respects, white yam (*D. rotundata*) is the most valued species (Hahn et al., 1993). Large tubers (5-10 kg) are used as gifts or for marriages. To appease the gods, special white yams are required. For example, at Maku in the east of Nigeria, an ancient white yam cultivar "Ukoli" is used by local priests for sacrifices to the gods (Akoroda and Hahn, 1995). No other type of yam can be used. In addition, wealthy people use white yam to set standards of social status to which the poor aspire, thereby creating competition and struggle for attainment (Ayensu, 1972; Hahn et al., 1993). In the Igbo district of Nigeria for instance, the yam is present in marriage ceremonies, birth and death rites, and other ceremonies (Ayensu, 1972). The same cultural practices with yam were observed with the Tem and Ani populations in Central Togo (MDR, 2000), where the population celebrates the "yam festival" each year before consuming newly harvested yam.

Composition of yam

The composition of yam tubers was well reviewed by Osagie (1992). Yam is composed mainly of starch, with a minor amount of proteins and lipids (1% dry matter) (Osagie, 1992; Omonigho, 1988) and all the vitamin C required by consumers (Bell, 1983). Yam is rich in minerals (Omonigho, 1988). The amount of starch depends on the cultivars and the age of the tubers

(Osagie, 1992). Yam tubers are important sources of carbohydrates comprising between 17 and 38% fresh weight (Orkwor, 1988). Some cultivars are sources of protein superior to cassava, comprising 1 to 3% fresh weight and a better balance of amino acids than many other root and tuber crops (Orkwor, 1988). In West Africa yam contributes approximately one-third of the calorific intake (FAOdata, 2007).

Consumption of edible yam

Yam is a valuable source of carbohydrate in the diet of West Africa, parts of South-East Asia, India, Islands of the South Pacific, the Caribbean and parts of Brazil (Osagie, 1992; FAOdata, 2007). The most common use of yam is as a boiled vegetable with some kind of sauce, but the skin may be removed before or after boiling, since it is normally not eaten. In West Africa, yam is often pounded into a thick paste after boiling and is eaten with soup (Orkwor et al., 1998). Yam is also processed into flour that is used in the preparation of another type of paste. It may also be baked, fried, roasted or mashed to suit regional tastes and customs. Other specific ways of preparing yam (puree, dry chips as basic ingredients for snacks…) can be found in other regions though (Okaka and Anajekwu, 1990; Okaka et al., 1991).

Other uses of yam

A number of *Dioscorea* species are also cultivated to provide a source of diosgenin, which is used in the manufacture of oral contraceptives and sex hormones (Coursey, 1967a; Kay, 1987; Ayensu, 1972). Also, some sapogenins, alkaloids, steroid derivatives and phenolic compounds are found in yam (for example *D. composita, D. floribunda* and *D. mexicana*) and are used in the pharmaceutical industry (Onwueme, 1978; Osagie, 1992; Degras, 1993).

1.2.4. Constraints of yam production

Several factors affect yam production. The main problems are the limited availability and high cost of planting material, pests and diseases and soil fertility issues. In addition, there is the problem of high cost of labor for operations such as land preparation, staking, weeding, harvesting.

Planting material

Yam planting material consistes of the whole tubers or pieces of tubers cut from a large tuber. Planting material accounts for about 50% of the cost of production in Nigeria (Nweke et al. 1991). To reduce the cost of planting material, numerous solutions are suggested for rapid and sustainable production of planting material, and especially for healthy pathogen-free material, such as the use of tissue culture technology, and also "minisett" technology (IITA, 2006), as described below.

The use of tissue culture has been well developed for disease elimination, and rapid multiplication, using meristem/shoot tip cultures for the former and meristem/shoot tip and nodal cultures for the latter (IITA, 2006). Using a combination of heat treatment and meristem culture, it was possible to eliminate yam mosaic virus from *D. rotundata* plantlets (IITA, 2006). At IITA, plantlets free of pathogens were also rapidly multiplied *in-vitro* using single node cuttings from *in vitro* plantlets previously obtained by the rapid multiplication methodology of yam. For this purpose, *in vitro* plantlets were maintained and multiplied under *in-vitro* conditions by sub-culturing nodal segments from established *in-vitro* plantlets under the laminar flow hood in culture test-tubes containing a specific yam multiplication medium (Ng, 1994). The yam plantlets were regenerated in the culture room with 12 hours photoperiod, 3000 lux light intensity, 27 ± 1°C of temperature and 70 ± 5% of relative humidity. The plantlets obtained in vitro are sterile, and consequently free of pathogens, but also of beneficial microorganisms, such as AMF. The plantlets are acclimatized for one month and later planted out, or used for dissemination of germplasm as certified disease-free material. After planting out, small tubers (minitubers) of 20-50 g are collected, which, if planted under sterile conditions, can also be used for germplasm distribution, as small minitubers, which are also less sensitive than vitroplants and in many ways, more suitable for transportation. The minitubers can be planted in the field for seed yam (100-250 g) production. Planting plantlets as well as minitubers in the field or in untreated soil exposes them to pathogen infection, of course.

An alternative type of planting material consists of whole yam tubers cut into sections of 25 g, 'minisetts'. Growing minisetts cut from mature tubers is a method used to relatively rapidly produce large numbers of seed tubers. It is important to cut the setts in such a way that each has a

reasonable amount of the peel of the tuber or periderm, from which the sprouting locus usually emanates. Prior to planting, the minisetts should be treated with a combination of insecticide/fungicide/bactericides/nematicide to prevent infection of the seed yam (IITA, 2004). The treated setts can be planted directly at the field site or pre-sprouted in beds in the nursery or in trays (boxes). Instead of using minisett pesticidal dust, application of AMF might prove useful in protecting minisetts against nematodes.

Yam yield lost by diseases

Yam is prone to infection by various diseases from the seedling stage to harvest (field diseases) and during storage (storage diseases) (Amusa et al., 2003). During the growth period, several pathogenic fungi have been found associated with yam, causing diseases such as anthracnose (caused by *Colletotrichum* spp. and *Glomerella* spp.) leaf spots and blight (caused by *Sclerotium rolfsii*), as well as rotting of yam tubers caused mainly by *Fusarium* spp. (IITA, 1975). Many viruses were also isolated from yam leaves that cause not only stress to yam, but also lead to yield losses (Osagie, 1992). Among the viruses that attack yam, Yam Mosaic Potyvirus (YMV) is the most frequent and economically important in Côte d'Ivoire, where the loss was estimated at between 4 to 10% (Osagie, 1992).

Concerning storage diseases, several fungi, viruses, and bacteria have been frequently associated with harvested tubers. The fungi most associated with rotted tubers are *Botryodiplodia theobromae* and *Fusarium* spp., while the bacteria most frequently isolated from wet rot tubers are *Erwinia* spp. (Adeniji, 1970; Osagie, 1992). In general, these pathogens occur in complexes causing pre-harvest and post-harvest losses. They are often a secondary consequence of tuber wounding, either as a result of mechanical damage during the harvest period and transportation, or due to pest damage to the cortex, permitting fungal and bacterial pathogens an entry point.

Yam yield lost by pests (nematodes excluded).

Several insect pests affects yam either in storage or in field depending on locality (Osagie, 1992; Bridge et al, 2005). These insects include larvae of three *Lepidoptera* spp. Viz**.** *Euzopherodes vapidella* (Sauphanor and Ratnadass, 1985) *Decadarchis minusculata* (Plumbley and Rees, 1983) and *Dasyses rugosella* (Dina, 1977); a Coleoptera *Araecerus fasciculatus* (Plumbley and

Rees, 1983), a Coccidae *Aspidiella* spp. and a Pseudococcidae *Phenacoccus* spp. (Nwankiti et al, 1988). Losses due to insect attacks can be estimated at 50% after several months of storage in Côte d'Ivoire (Osagie, 1992)

Nematode parasites of yam and their damage to yam

Among the nematodes associated with yam, three respective species groups are considered to be major constraints: the yam nematode (*Scutellonema bradys*), root-knot nematodes (*Meloidogyne* spp.) and lesion nematodes (*Pratylenchus coffeae* and *P. sudanensis*) (Bridge et al., 2005). In marketed yam tubers in West African yam markets, *S. bradys* and *Meloidogyne* spp. were the main nematodes with 2.84% of yam tubers infected by *S. bradys* and 2.94% infected by *Meloidogyne* spp. (Coyne et al., 2005). Similar observations were reported from Nigeria (Adesiyan and Odihirin, 1977). These two nematodes are described in more detail in the section 1.3.

The primary importance of *S. bradys* on yam is in the direct damage it causes to the tubers, resulting in dry rot disease (Bridge et al., 2005). The nematodes produce a significant reduction in the quality, marketable value and edible portions of tubers. These reductions are more severe in yam that has been stored (Coyne et al., 2005). Weight differences between healthy and diseased tubers harvested from the field have been estimated to be 20 to 30% in Côte d'Ivoire (Bridge, 1982) and 0 to 29% in Nigeria (Wood et al., 1980).
Meloidogyne spp. so far identified associated with yam are *M. incognita, M. javanica, M. arenaria* and *M. hapla,* of which *M. incognita* appears the most important (Bridge et al, 2005). Nwauzor and Fawole (1981) recorded losses of 25-75% due to *Meloidogyne* spp. infection on yam within a storage period of 16 weeks in Nigeria. The proportion of yam with galled tubers collected from yam barns and markets in Nigeria can be as high as 90% for *D. alata* and 70% for *D. rotundata* (Adesiyan and Odihirin, 1978), with an estimated value of between 39-52% in price reduction of galled tubers compared to healthy ones (Nwauzor and Fawole, 1981).

Pratylenchus coffeae lives endo-parasitically and is typically a root parasite, but it attacks also underground stems and tubers. It has been reported as a pest of yam in Puerto Rico, Jamaica, British Solomon Islands, Pacific and Central America (Thompson et al., 1973). *Pratylenchus*

coffeae is assumed to have a life cycle of 3-4 weeks on yam and causes dry rot of the peri-dermal and sub-dermal regions similar to that caused by *S. bradys* (Thompson et al., 1973). The nematode is concentrated in the apical portion within 6 mm up to 15 mm into yam tuber tissue. Dry rot can extend 1-2 cm into the outer tissues of *D. rotundata* tubers (Acosta, 1974), but has been estimated to extend up to 5 cm in *D. alata* tubers (Bridge and Page, 1984). *Scutellonema bradys* and *P. coffeae* can induce a synergistic effect on yam growth in pot experiments (Acosta and Ayala, 1976), but were together exclusively present in tubers harvested from fields in India (Castognone-Sereno and Kermarrec, 1988).

Pratylenchus sudanensis was recently observed on yam in Uganda (Coyne et al, 2003), where it was reported as dominant nematode on yam (Mudiope et al., 2001). *Pratylenchus sudanensis* is morphologically similar to *P. pseudopratensis* (Coyne et al., 2003) and associated with cracked tubers (Mudiope et al., 2004). Host range studies of *P. sudanensis* in Sudan showed that 20 plant species such as cotton, sorghum, and pigeonpea (*Cajanus cajan*) acted as favorable hosts while groundnut (*Arachis hypogea*)and wheat (*Triticum turgidum*) were considered as poor hosts (Saadabi, 1985). *Pratylenchus brachyurus* has been found in yam tubers, roots and yam soil in Nigeria (Caveness, 1967), Ivory Coast (Miège, 1957), Guatemala (Jenkins and Bird, 1962), Fiji and Tonga (Bridge, 1988), but appears to be of limited importance.

Radopholus cf. *similis* was associated with yam in Papua New Guinea (Bridge and Page, 1984), in Fiji (Butler and Vilsoni, 1975) and in the Solomon Islands (Bridge, 1988) causing dry rot disease, similar to that caused by *P. coffeae* and *S. bradys*. *Aphelenchoides besseyi* was also found associated with drying and blackening of the foliage, and cracking of the tuber of *D. trifida,* with internal decay in Guadeloupe (Kermarrec and Anais, 1973). *Paratrichodorus porosus* was reported associated with blackening, cracking and corkiness of the tuber tips of Chinese yam, *D. opposita* in Japan (Niashizawa, 1973).

Disease complexes on yam

It has been estimated that an average of over 25% of the yam yield is annually lost due to diseases and pests (Ezeh, 1998; FAO, 2007). Lesions caused by nematodes to yam tubers facilitate invasion by disease pathogens. The resulting of decay process often destroys the entire

tuber in the ground, but particularly during storage (Bridge, 1982). The more extensive internal decay of tubers known as wet rot or soft rot or watery rot is associated with fungal and/or bacterial pathogens (Adeniji, 1970). This general decay of tubers, which is a serious problem in stored yam, is increased when tubers are wounded or damaged by man, rodents, and insects or mainly by nematodes (Adeniji, 1970). Nematodes and fungi are often found in combination in the transitional stage between dry rot and wet rot although nematodes tend not to be found when yam tubers are completely decayed (Adeniji, 1970). Another complex associated with serious losses in yam production is the occurrence of nematodes especially in soils of poor fertility. According to Adeniji (1970), in soils of low fertility, yam plants are weak, nematode attacks increase and yam yields decrease.

Problems of soil fertility in West Africa

Many soils in the tropic and particularly in West Africa are fragile and prone to degradation (Schlecht et al., 2006). Major factors that constrain tropical soil fertility and sustainable agriculture are soil moisture stress (a dry season lasting longer than 3 months makes year-round crop production difficult), low nutrient contents, leaching and erosion risks, low pH with aluminium (Al) toxicity, high phosphorus (P) fixation, low levels of soil organic matter, and low soil biodiversity (Sanchez, 2002). However, savanna soils under natural conditions (forest or long fallow) have a diversity of species that tend to be productive, pest resistant, and maintain soil organic matter and soil biological activity at levels satisfactory for soil fertility (Ewel, 1999). As yam was thought to be a high nutrient demanding crop, farmers traditionally planted yam first in the rotation system following fallow or forest clearance (Carsky et al., 2001). Increasing human population pressure has decreased the availability of forest and arable land and it is becoming less feasible to use extended fallow periods to restore soil biodiversity and fertility (Manyong et al., 1996). It was estimated that over 70% of deforestation in West Africa is caused by farmers, who in their quest for arable land and food, have no incentive to ponder on long-term environmental consequences (FAOdata, 2007). Today, the fallow periods, which would have restored soil fertility and organic carbon, are reduced to lengths that cannot regenerate soil productivity leading to unsustainable farming systems (Schlecht et al., 2006). In addition, farmers in West Africa and other parts of Africa remove crop residues from field or burn them. This practice, coupled with a low rate of macronutrient application compared to their removal,

contributes to a strongly negative nutrient balance (Stoorvogel and Smaling, 1990). For nitrogen as an example, whereas 4.4 million tons are lost per year, only 0.8 million tons are applied in West African countries, such as Burkina Faso, Benin, Togo, and Mali, mainly for cotton growth (Bationo et al., 2004). The chemical characteristics of yam-growing soils showed that nutrient N, P, and K contents of such soils is low (Bationa et al., 1996; Bationo et al., 2004). Reversing the declining trend in agricultural productivity and preserving the environment for present and future generations in West Africa must begin with soil fertility restoration and maintenance (Bationa et al., 1996). Many solutions have been suggested for the restoration of soil fertility. These methods include mixed cropping of plant species, short fallows with cover crops, agroforestery, crop rotation mainly with mycorrhizal plants, which usually allow a larger diversity and abundance of AMF than monocultures (Altieri, 2004).

Labor

Yam cultivation requires large levels of energy for land preparation before planting, particularly since yam is planted on mounds, ridges or in beds (Toure and Ahoussou, 1982). Much time and labor is also required for weeding, staking, control of pests and diseases (during growth period), and also for tuber harvesting and storage (Toure and Ahoussou, 1982). The labor costs are estimated at > 40% of the annual income of the family (Nweke et al., 1991).

1.3. Plant parasitic nematodes studied in the current study

The main nematodes associated with yam cultivation in West Africa, the yam nematode (*S. bradys*) (Caveness, 1992) and root knot nematodes (*Meloidogyne* spp.) are among the major constraints to yam production and storage. These nematodes will therefore be the focus of further detail.

1.3.1. *Scutellonema bradys* "yam nematode"

Taxonomy and nomenclature

Scutellonema bradys was first described in 1933 from infected yam tuber from Jamaica in the genus *Hoplolaimus* (Steiner et al., 1933). According to Morgan (1971), *S. bradys* was redescribed from *Hoplolaimus* to *Rotylenchus* by (Goodey (1952) and then described to

Scutellonema by Andrassy (1958). The common name used is the "yam nematode" or "yam dry rot nematode".

The scheme of classification is according to Morgan (1971)

Kingdom: Animalia

Phylum: Nematoda Cobb,1919

Class: Secernentea Van Linstow, 1905

Oder: Tylenchida Thorne, 1949

Suborder: Tylenchoidea, Thorne, 1949

Superfamily: Tylenchoidea, Orley, 1880

Family: Hoplolaimidea, Filipjev, 1934

Genus: *Scutellonema* Andrassy, 1958

Species: *Scutellonema bradys*

(Steiner and LeHew, 1933) Andrassy, 1958.

Distribution and host range

Scutellonema bradys is widely distributed in the tropics, especially in yam growing areas. It has been reported from the West Africa countries of Nigeria, Côte d'Ivoire, Senegal, Gambia, Ghana, Benin and Togo, and from the central Africa country of Cameroon. It also occurs in Cuba, Jamaica, Guatemala, Puerto Rico, Guadeloupe, Haiti, and Martinique in the Caribbean and from Venezuela, Brazil and India (Bridge et al., 2005). All the *Dioscorea* spp. grown as food crops are susceptible to *S. bradys*. In addition, other yam species known to be affected by *S. bradys* are *D. bulbifera*, *D. trifida* and *D. transversa* (Ayala and Acosta, 1971). Two wild *Dioscorea* spp. growing in forests in Nigeria and Cameroon have been shown to be natural hosts (Bridge et al., 2005). A wide range of other crops and some weeds have been reported to support low root populations of *S. bradys* including yam bean (*Pachyrrhizus erosus*), pigeon pea (*Cajanus cajan*) okra (*Hibiscus esculentus*), tomato (*Lycopersicon esculentum*), sorghum (*Sorghum bicolour*), Loofah (*Luffa cylindrical*), and roselle (*Hibiscus sabdariffa*) (Adesiyan, 1976). It occurs also in cassava (Missah and Peters, 2001), taro (*Xanthosoma* sp., *Colocasia esculenta*) and sweet potato (*Ipomea batatas*) (Kermarrec et al., 1987). These alternative hosts permit the yams nematode to survive in the soil even in the absence of yams. However, only

cowpea (*Vigna unguiculata subsp. unguiculata)* water melon (*Citrullus* spp.) and sesame (*Sesamum indicum*) in addition to yam have been found to actually increase populations of *S. bradys* (Bridge, 1982).

Reproduction and ecology

The reproduction of *S. bradys* is amphimictic with separated sexes. Eggs are laid in the soil or in the roots and tubers. Juveniles develop into mature vermiform stages by subsequent moulting within 21 days while all active stages are infective (Kwoseh et al., 2001). Dense populations can build up in the tubers with a miximum of 62.000 nematodes/10g of tuber recorded in Nigeria (Bridge, 1972) but 100.000 nematodes were also reported to be found in the infested tubers in Nigeria (Bridge, 1982). The *S. bradys* populations are affected by storage conditions and increase at twice the rate in tubers stored at 22-32°C and relative humidity 40-85%, when compared to tubers stored at 16-18°C (Adesiyan, 1977).

Symptoms and diagnostics

Scutellonema bradys causes a characteristic disease of yam tubers known as “dry rot disease” (Bridge et al., 2005). The initial stage of rot consists of cream and light-yellow lesions below the outer skin of the tuber. There are no external symptoms at this stage. As the disease progresses, it spreads into the tuber, normally to a maximum depth of 2 cm, but sometimes deeper. In the later stages of dry rot, infected tissues first become light brown and then turn dark brown to black. External cracks appear in the skin of the tubers and parts can flake off exposing patches of dark brown, dry rot tissues. The most severe symptoms of dry rot are observed in mature tubers, especially during storage, when it is often associated with general decay of tubers. No foliar symptoms have been observed on yams growing in soil infested by *S. bradys* (Adesiyan and Adeniji, 1976; Bridge et al., 2005).

Assessment of the incidence and extent of dry rot disease in tubers can be undertaken by direct observation. In tubers without obvious external symptoms of damage, it is necessary to scrape away the surface layers, or cut tubers to determine the presence of dry rot. Nematodes in the soil and roots can be sampled during or at the end of the growing season. However, most nematodes

will be found in the outer cortex of tuber tissue. Sampling on the outer cortex is most appropriate for assessing populations and importance of *S. bradys* infestation (Adesiyan and Adeniji, 1976).

Fig.1.1. Healthy tubers (left photo) compared to tubers with dry rot disease (right photo) caused by *Scutellonema bradys* in outer part of yam (*Dioscorea rotundata*, cv. Kokoro)

1.3.2. *Meloidogyne* spp. or Root Knot Nematode

Taxonomy of Meloidogyne spp.

Phylum: Nemathelminthes

Class: Nematoda

Subclass: Secernentea

Order: Tylenchida

Superfamily: Tylenchoidea

Family: Heteroderideae

Genus: *Meloidogyne*

Specie: *Meloidogyne* spp.

Distribution and host range

Meloidogyne spp. has been found in yam in Africa (Ghana, Côte d'Ivoire, and Nigeria, Uganda, Ethiopia), the Caribbean (Jamaica, Martinique, Puerto Rico, Trinidad), Pacific (Fiji, Kiribati, Papua New Guinea, Western Samoa), Brazil, Guatemala and Japan (Bridge et al., 2005). They

are well distributed all over the world. The most widely spread species are *M. incognita* and *M. javanica* (Bridge et al., 2005). They can become abundant on yam above all in areas where *S. bradys* is not present. A general survey conducted by Coyne et al. (2005) in West Africa showed that the *Meloidogyne* spp is associated with yam across the whole region. In spite of their widespread occurrence and abundance, root-knot nematodes are considered of limited importance on yam in West Africa (IITA, 2005), since their infection does not cause lesion or an entry point for bacterial and fungal infestation. Especially *M. incognita* is highly polyphagous, with a very broad host range of more than 700 hosts, including most cultivated crops and ornamentals (Radewald, 1978).

Reproduction and ecology

Meloidogyne spp. reproduction is parthenogenetic. J2 moult to females under favorable conditions while a high percentage of males are produced under adverse conditions (Adesiyan and Odihirin, 1978). The eggs are laid within a gelatinous matrix to form an external egg mass. A single egg mass can contain several hundred eggs. The life cycle of *M. incognita* on yam tubers (*D. rotundata* or *D. alata*) is 35 days under controlled conditions at 28°C (Nwauzor and Fawole, 1981).

Symptoms and diagnostic

Root knot nematodes cause typical knotting or galling of yam roots. Yam tubers produce galls in the outer tissues, giving rise to abnormal, warty or knobbly tubers. Root proliferation from galls on tubers can occur (Nwauzor and Fawole, 1981).

Foliar symptoms, such as early yellowing, leaf fall and inhibited vine growth have been observed on yam infested with *Meloidogyne* spp. (Nwauzor and Fawole, 1981). Assessment of the incidence and extent of galles in tubers can be undertaken by direct observation. The tubers infected by *Meloidogyne* spp. show obvious external protuberance (galls) at the surface layers. The nematodes can be sampled in the soil and root at the end of the growing season. However, most nematodes will be found in the tuber tissues. Sampling on the infested tubers is the most appropriate means for assessing populations and importance of *Meloidogyne* spp., since the nematode has many host plants, as already mentioned above (Adesiyan and Odihirin, 1978).

Fig. 1.2. Yam tuber with galls caused by *Meloidogyne* spp. in the outer tissues of yam (*Dioscorea rotundata* cv. Kpana).

1.3.3 Yam nematode control

Although some nematodes can cause huge losses even when present in low numbers, most of them do not cause economically significant damage, except if their numbers are unusually high or the plant is highly stressed. Thus, the nematode population density should be kept under threshold levels. Host plants may either suppress (i.e. resistance) or allow (i.e. susceptibility) nematode development and reproduction. However, they may suffer only little injury (i.e. tolerance) even when heavily infected with nematodes (Bos and Parlevliet, 1995). In order to prevent and control nematode infestation, the management of *S. bradys* and *Meloidogyne* spp. can be achieved by one, or preferably, a combination of several measurements, which are presented in the following.

Phytosanitary and clean planting materials

Phytosanitary is the first method used for nematode control on yam. It is necessary to separate infested tubers before storage and planting in order to prevent establishment of nematode infection. Healthy planting materials can also be obtained by using tissue-cultured planting material (Dropkin, 1980; Speijer et al., 2000). In yam, use of nematode-free planting material is a practical and economic means of preventing damage by *S. bradys* and their dissemination. Aerial tubers of the yam *D. bulbifera* and some forms of *D. alata*, which are used for propagation should, however, be completely free of nematodes. A number of yam species, such as *D. alata*,

D. rotundata and *D. dumetorum*, can also be produced from vine cuttings (Coursey, 1967). Although these methods of propagation are not practical for producing yam tubers, they can be used to produce nematode-free seed tubers (IITA, 2005). The use of 'microsetts' or 'minisetts', cut from mature tubers (IITA, 2007) can be used to provide clean planting material if the mother seed yam tubers selected is free of nematodes.

Agronomic practices in the field

Agronomy practices such as fallow, crop rotation with non-host or cover crops are efficient for nematodes control. A fallow of 8 to 12 months as well as crop rotation with non-hosts, can reduce the nematode population in the soil (Adesiyan, 1976). However, high land pressure and the cultivation of perennials prevent the successful application of fallow. When it is practical, rotation of yam with non-hosts or poor hosts such as groundnut (*Arachis hypogea*), tabacco (*Nicotiana tabacum*) or cotton will limit damage by *S. bradys*. However, crop rotation to effectively reduce *S. bradys* populations seems to be non-realistic because of its absence or low density in the soil.

Using *Mucuna* sp. and *Crotalaria* sp. cropped as cover crops have been reported to reduce soil nematode populations, particularly *Meloidogyne* spp. and *S. bradys* (Claudius-Cole et al., 2004), Other cover crops such as *Tagetes* spp., *Stylosanthes* spp., *Centrosema* spp. and *Aspilia* spp. have been recommended to lower nematode populations and restore fertility for yam production in Nigeria (Atu and Ogbuji, 1983). Using cover crops in crop rotation is not efficient to control *Meloidogyne* spp. Since these nematodes have a wide host range; moreover, as these crops are not edible, farmers are deterred from using them as a rotation crop.

Organic and mineral fertilisers

It was reported that the application of mulch or organic matter might result in increased plant vigor and probably to some level, tolerance to nematodes (Kashaija, 1999). The use of cow dung, mixed in yam mound before planting at a rate of 1.5 kg per mound (1886.3kg/ha) was reported to increase yields of tubers and significantly decreased nematodes numbers (Adesiyan and Adeniji, 1976). The use of *Azadirachta indica* L. powder at a rate of 2.5 t/ha has also been reported to decrease nematode populations as well as to increase soil fertility and tuber yields

(Onalo et al., 2001). For inorganic fertilizer use, superphosphate fertilizers was reported to reduce *S. bradys* populations in tubers of *D. alata* to a low level, but did not increase yam yield (Baimey et al., 2006). In contrast, N (Nitrogen) alone was reported to increase both populations of *S. bradys* and the percentage of the infested tubers of *D. rotundata*, whereas P (Phosphorus) alone can decrease the percentage of infested tubers (Sobulo, 1972). However, the experimental conditions and the species of yam and cultivars used are likely to have a substantial influence on the results obtained. For example, *S. bradys* populations increased on *D. rotundata* but not *D. alata* or *D. cayenensis* following application of high rates of N combined with P (Obigbesan and Adesiyan, 1981). In addition, the traditional practice of using wood ash on yam tubers before planting is reported to decrease nematode numbers (Adesiyan and Adeniji, 1976). An efficient alternative practice to increase soil fertility and decrease nematode population in the soil, as well as on yam tubers could be to increase the levels of soil microorganisms and particularly AMF spore density and diversity or inoculation of selected AMF species or isolates (Cardoso and Kuyper, 2006; Smith and Read, 2008). But these practices are not necessarily effective and in some cases even reported to have no effect on nematode densities and damage, when compared to the controls (Claudius-Cole, 2005).

Physical control or hot water treatment

Hot water treatment consists of heating water at temperature of 50-55°C and submerging tubers for 45 min, which provides good control of *S. bradys* without damaging tubers (IITA, 2005). The hot water treatment is effective but far from practical for farmers, due to the need for temperature control, labor and fuel, and simply due to the huge volume of yam material needed to be treated. Other factors, such as yam species, cultivars, nematode densities, depth of infestation and age of tubers also conflict with efficiency (Bridge et al., 2005). However, it is feasible for small-scale operations and for establishing nematode-free planting material for research experiments.

Resistance

To date, no useful sources of resistance to the yam nematode *S. bradys* have been identified from cultivated yam (IITA, 2004). Sources of nematode resistance are likely to be found in non-cultivated yam, but breeding this into cultivated yam lines will be a lengthy process and not necessarily successful due to the complex nature of yam botany (Coursey, 1967; R. Asiedu, IITA

pers. comm.). However, resistance could prove to be the most practical and economic means of managing *S. bradys* for minimizing losses to such pests found in commercially acceptable cultivars (CABI International, 2001). Crop resistance is not as common against the migratory endoparasitic nematodes however, such as *S. bradys*, as it is against the sedentary endoparasites with specialized feeding sites, such as *Meloidogyne* spp., *Globodera* spp., *Heterodera* spp., *Rotylenchulus* spp. and *Tylenchulus* spp. One cultivar of yellow yam, *D. Esculenta,* and one of *D. dumetorum* have shown some resistance to *S. bradys* (Bridge, 1982; Kwoseh, 2000).

Nematicides

Nematicides (carbamates and organophosphates) can be used successfully against nematodes on yam, but these agents are not only very expensive but also toxic against non-target organisms, including the user. They are poorly biodegradable and, therefore present an important ecological risk (Gowen and Quénéhervé, 1990). However, application of chemical nematicides in the field has, at best, only produced moderate yield increases and control of *S. bradys* (Ayala and Acosta, 1971) and information on the economics of this means of control is lacking for large-scale use. Nematicide treatment of planting material however, to generate healthy seed yam free of nematodes can have a major effect and proved to be highly economical (IITA, 2006).

Biological control

Biological control is considered to be an alternative to nematicides, especially concerning the environmental and health risks associated with the use of these chemicals (Kerry, 2000; Viaenne, 2005). Integrated crop pest control may benefit from studying plant-parasitic nematodes and natural antagonistic interactions in natural systems, which have co-evolved for longer than crop-nematode-antagonist systems. Understanding how wild plants manage their association (plant-parasitic nematodes) may ultimately result in improving the sustainability of crop protection against plant-parasitic nematodes. Current research has focused mainly on predacious and parasitic micro-organisms. Nematophagous fungi such as *Pochonia chlamydosporium* and *Paecilomyces lilacinus* are nematode parasites (Gaspard et al., 1990). Bacteria such as *Pasteuria penetrans* also appear promising biological control agents of *Meloidogyne* spp. (Davies et al., 1991; Pembroke and Gowen, 1998). Recently, possible anti-nematode effects of the micro-organisms in the rhizosphere have been studied. Rhizobacteria such as *Rhizobium* spp. and

Pseudomonas spp. not only have a positive effect on plants by promoting their growth, but in addition they show a repellent effect towards nematodes (Aalten et al., 1998; Hallman et al., 2001).

Integrated pest management

A combination of several methods is the best way to control nematodes but is not usually applied by farmers for managing nematodes. For example, combinations of hot water treatment and phytosanitary measures as well as crop rotations were experimentally used in IITA-Ibadan, West Africa for successful yam nematode control (IITA, 2005).

1.4. Mycorrhiza association

1.4.1. General definition and its main types

Mycorrhiza (Greek words *mycos* = fungi, *rhiza* = root) can be defined as a close physical association between soil fungi and plant roots from which both fungi and plants appear to benefit. Harley and Smith (1983) preferred using the term symbiosis for describing this interdependent mutualistic relationship where the host plant receives mineral nutrients, while the fungus obtains photosynthesis derived carbon compounds from the plant. According to Smith and Read (2008), mycorrhizas were discovered during the late 19th century when several researchers noted the presence of fungi in plant roots without any apparent disease or necrosis. In general, the mycorrhiza was classified morphologically according to their association types (Peyronel et al., 1969).

Endomycorrhiza: The term endomycorrhiza refers to a symbiotic association between fungal mycelia and roots of certain plants, in which the fungal hyphae penetrates directly into cortical cells (endomycorrhizal fungi) with individual hyphae extending from the root surface outwards into the surrounding soil. Their hyphae penetrate the living cells of the cortex and they can form structures such as vesicles and/or arbuscules. The most important members belonging to the endomycorrhizal fungi are arbuscular mycorrhizal fungi (AMF), which include the vesicular-AMF and AMF that do not form vesicles in the roots. Over 200 fungal species belonging to the

Glomerales are known to form arbuscular mycorrhiza (Redecker et al., 2007; Smith and Read, 2008).

Ectomycorrhiza: This term refers to a symbiotic association of fungal mycelia and roots in which the fungal hyphae form a compact mantle on the surface of roots and extend into the surrounding soil and inwards between cortical cells, but not into these cells. The hyphae between cortical root cells produce a netlike structure, the Hartig net (Smith and Read, 2008). Many ectomycorrhizal fungi form a mantle of fungal tissue, completely covering the absorbing root. Ectomycorrhiza is found on woody plants, most belonging to the families Pinaceae, Fagaceae, Betulaceae and Myrtaceae (Smith and Read, 2008). Over 4000 fungal species, belonging primarily to the Basidiomycotina and fewer to the Ascomycotina, are known to form ectomycorrhizae (Schenk, 1991).

Ectoendomycorrhiza: This term refers to an ectomycorrhizal - endomycorrhizal intermediate type. In this association, the hyphae of involved fungi develop a mantle of fungal tissue and a Hartig net like the ectomycorrhiza association and their hyphae penetrate the living cells of their hosts as known for the endomycorrhiza association, but without forming special intracellular structures (Brundrett, 2004). The ectoendomycorrhizal fungi colonize only a few plant families from gymnosperms and angiosperms (Smith and Read, 2008).

Some additional mycorrhizas: Arbutoid mycorrhiza is a symbiotic association that involves Basidiomycete fungi and some specific host plants belonging to the Ericales. In this association the hyphae penetrate directly the cortex cells (endomycorrhiza) of the root but do not form arbuscules or vesicles (Read, 1998). Monotropoid mycorrhiza involves Basidiomycete fungi and plants from the Monotropaceae. They form intracellular colonization as well as a Hartig net but never arbuscules or vesicles (Harley and Smith, 1983). Ericoid mycorrhiza develops between Ascomycete fungi and plants belonging to the Ericales. The hyphae penetrate into the root cells, and form intracellular structures called coils, which are different from arbuscles or vesicles (Harley amd Smith, 1983). The Orchid mycorrhiza is developed between Basidiomycetes fungi and Orchidaceae plants. The hyphae penetrate the root cells and form intracellular coil structures (Smith and Read, 2008).

1.4.2. Taxonomy of AMF

AMF belong to the phylum Glomeromycota (Schüβler et al., 2001) and the class Glomeromycetes (Cavalier-Smith 1998), with four orders: Glomerales, Diversisporales, Archaeosporales and Paraglomerales, eight families and ten genera (Schüβler et al., 2001). Recently, four new AMF genera, *Ambispora*, *Kuklospora, Intraspora* and *Otospora*, and two new families, Ambisporaceae and Entrophosporaceae, have been described in the phylum *Glomeromycota* (Sieverding and Oehl, 2006; Palenzuela et al., 2008). In earlier classifications, the AMF were placed in the order Glomales within the division Zygomycota as they have non-septate hyphae, similar to the hyphae of most Zygomycota. However, AMF are distinguished from the Zygomycotan lineages due to some specific characteristics, e.g. mutualistic symbiotic nutritional habit and the lack of formation of characteristic zygospores (Smith and Read, 2008). Moreover, rDNA analysis has revealed a clear separation of AMF from other fungal groups and the AMF are now placed in a separate new phylum, Glomeromycota (Schüßler et al., 2001; Schwarzott et al., 2001).

Classical spore morphology and more recently PCR-based molecular approaches are generally used for identification of AMF species and communities, but there are problems with both these approaches. In the case of spore morphology, it is not always possible to identify all spores obtained directly by sieving field soil. There are variations in spore development and sometimes AMF colonizing the plant roots are not found as spores (Clapp et al., 1995; Clapp et al., 2002). The main problem with molecular approaches is that these are mostly based on rDNA sequences and AMF species have the peculiarity to contain polymorphic rDNA sequences often precluding a distinction of closely related species (Sanders, 2002; Redecker et al., 2003). It is normal to recover multiple rDNA sequences by PCR amplification from a single spore known to contain a thousand or more nuclei (Antoniolli et al., 2000; Pawlowska and Taylor, 2004). At present, there are no individual rDNA primers that permit identification of all major Glomalean lineages (Redecker, 2000; Vandenkoornhuyse et al., 2002; Redecker et al., 2003; Walker and Schüßler, 2004).

Table 1.2. Order, families and genera of AMF (Sieverding and Oehl, 2006; Palenzuela et al., 2008)

Orders	Families	Genera
Glomerales	Glomeraceae	*Glomus*
Diversisporales	Gigasporaceae	*Gigaspora, Scutellospora*
	Acaulosporaceae	*Acaulospora, Kuklospora*
	Entrophosporaceae	*Entrophospora*
	Pacisporaceae	*Pacispora*
	Diversisporaceae	*Diversispora, Otospora*
Paraglomerales	Geosiphonaceae	*Geosiphon*
	Paraglomeraceae	*Paraglomus*
Archaeosporales	Archaeosporaceae	*Archaeospora, Intraspora*
	Ambisporaceae	*Ambispora*

Thus the characterisation of AMF communities based on either spore morphology or molecular identification alone is insufficient to cover the whole spectrum within a community (Landis et al. 2004). In order to assess the total community present at a specific site, use of both methods is recommended because they complement each other (van der Heijden and Scheublin, 2007).

1.4.3. Morphology and biology of AMF

After colonization (=infection) of the host plant roots, AMF first develop a hyphal network of microscopic filaments in the soil. When these filaments come into contact with a young root, the extramatrical hyphae swell apically and increase in size to form an appressorium-like structure. Hyphae penetrate the roots in a number of ways: direct penetration of the root hair cell wall or of another epidermal cell or infection through crevices between cells in the outer layer of the root (Bonfante-Fasolo, 1984). After penetration, the infection develops an extramatrical phase which might consist of external vesicles and, above all, spores and a large mycelium in the surrounding soil, and an the intraradical phase with intercellular un-branched hyphae, the intracellular hyphae branching extensively to form arbuscules and, in some genera formed vesicles (Janse, 1897; Morton and Benny, 1990). Spores can be formed in the soil and within roots. The spores contain

a nutrient reserve and act as propagating organs. They are used for morphological species identification. The mycelium does not penetrate the meristematic regions and the endodermis, and is therefore absent from the central vascular cylinder. Active hyphae of AMF are non-septate, i.e. coenocytic (one cell with many nuclei).

Biologically, the close contact created between the plant and fungus through the intraradical filamentous network allows the exchange of nutrients between the two partners. The wide dispersal of the fungus in the soil through its large extraradical filament network gives the plant access to a much larger volume of soil than the root system itself. The fungal filaments act like conduits supplying the root with mineral salts to which it normally would not have access. In return, the fungus receives from the plant metabolized nutrients that it is unable to synthesize himself, such as sugars, amino acids and secondary metabolites (Smith and Read, 2008). AMF are recognized on the basis of their specific traits such as obligate biotrophy, asexual reproduction, large and multinucleate spores with layered walls, non-septate hyphae and arbuscule formation in plant roots. Though AMF are believed to be obligate symbionts and generally they need living plant roots to grow, some reports claimed that AMF species can grow up to the spore production phase *in vitro* in the absence of plant roots but in the presence of some selected strains of spore-associated bacteria (Hildebrandt et al., 2002; Hildebrandt et al., 2006). AMF reproduce asexually by spore (chlamydospore) production and there is no evidence that AMF can reproduce sexually (Kuhn et al., 2001). One study reports the formation of sexual zygospores by *Gigaspora* (Tommerup and Sivasithamparam, 1990), but this has not been confirmed so far. Only a low level of genetic recombination or non at all has been detected using molecular marker genes (Kuhn et al., 2001). Therefore, it is generally assumed that AMF are asexual. The spores are relatively large (40-800 µm) containing large amounts of lipids.

1.4.4. Role of AMF in agroecosystems

Contribution of AMF to improve soil structure

In tropical agriculture systems where most soils are fragile and of low fertility, a major beneficial effect of AMF is their role in maintenance and improvement of soil structure by their external hyphae and the production of a special protein excreted, the glomalin (Miller and Jastrow, 1990; 2000). The mechanisms involved are (1) the growth of external hyphae into the soil to create a

skeletal structure that holds soil particles together and (2) the formation of a 'sticky' string-bag of hyphae by the glomalin, which contributes to soil aggregate stabilization (Rillig et al., 2002).

Contribution of AMF to improve plant mineral uptake and plant growth.

The improvement of P nutrition of plants is the most recognized beneficial effect of AMF. These fungi can physically explore the soil with hyphae – significantly finer than roots - to access inorganic and organic P sources that are unavailable to non-mycorrhizal plants (Feng et al., 2003; Cardoso et al., 2006; Smith and Read, 2008). In particular, it is believed that plants with limited root hair development, such as cassava are frequently dependent on AMF for P nutrition under all soil conditions (Howeler and Sieverding, 1983). AMF may also enhance N acquisition by the plant (Mäder et al., 2000; Hogde et al., 2001). Uptake of other nutrients mediated by AMF hyphae, such as K, Mg, Ca, Zn and Cu has been demonstrated, but their translocation to plants has not been well established (Clark and Zeto, 2000).

Under P limited conditions, AMF have the potential to increase growth and yield e.g. as shown for watermelon (*Citullus lanatus*) inoculated with *G. clarum* (Kaya et al., 2003). Inoculation with *G. mosseae* increased shoot dry weight of several plum rootstock (*Prunus domestica*) cultivars (Camprubi et al., 1993), and dry weight of micropropagated banana (*Musa* spp.) plantlets (Declerck et al., 1994; Elsen el al., 2003). Other studies showed that inoculation of micropropagated plants of oil palm (*Elaeis guineensis*), pineapple (*Ananas comosus*) or kiwi (*Apteryx* spp.) with AMF also led to improved development (Schubert et al., 1990; Jaizme-Vega and Azcón, 1995). Caglar and Akgun (2006) found that Terebinth (*Pistacia terebinthus*) seedlings pre-inoculated with AMF had improved growth following transplanting. Stewart et al. (2005) showed that micropropagated plantlets of strawberry (*Fragaria X ananassa*) cultivars inoculated with *G. mosseae* and a mixture of *G. intraradices*, *G. mosseae* and *G. etunicatum* exhibited better growth at the acclimatization stage, than non inoculated plants when they were transplanted to P rich soil. It has been suggested that the extent of growth enhancement by AMF and the mycorrhizal dependency of the host varies with genera and species of AMF. For example, seedlings of narrowleaf plantain (*Plantago lanceolata*) inoculated with *Glomus* spp. grew faster than seedlings inoculated with *Archaeospora trappei* (Bennett and Bever, 2007).

In root and tuber crops, many studies have demonstrated the enhancement of growth and yield by AMF. For example, the AMF *G. manihotis* and *Entrophospora colombiana* both proved to be highly efficient for improving cassava (*Manihotis* spp.) growth in the greenhouse (Howeler and Sieverding, 1983). Potato (*Solanum tuberosum*) microplants inoculated with commercial AMF products (Vaminoc and Endorize IV) and with *G. intraradices* showed increased tuber yield and quality (Duffy and Cassells, 2000). Potato plantlets inoculated with *G. etunicatum* produced significantly greater shoot fresh weight, root dry weight and number of tubers per plant (Yao et al., 2002). Cultivars of potato, *S. aethiopicum* inoculated with *G. aggregatum* or with *G. mosseae* produced higher shoot dry weight than non-inoculated plants (Diop et al., 2003). Sweet potato (*Ipomoea batatas*) inoculated with 14 AMF species separately enhanced the biomass production and showed improved nutritional status with higher efficiency of *Glomus* spp. compared to *Acaulospora* spp. or *Scutellospora* spp.(Gai et al., 2006).

While numerous studies have shown the positive effects of AMF on growth and yield of plants, a few studies also indicated negative or neutral effects of AMF on plant growth and yield. For example, Duffy and Cassells (2000) reported that *Solanum* spp. plantlets inoculated with *G. intraradices* showed a reduction of growth, while no effect of *G. versiforme* inoculation on *S. aethiopicum* cultivars was recorded (Diop et al., 2003).

Role of AMF to enhance plant tolerance to pollution with toxic metals

The reports of AMF effects on plant tolerance to heavy metal or pollution have been contradictory (reviewed by Gadd, 1993). For example, in the case of nutrient toxicity, AMF can reduce metal translocation to the plants (Chen et al., 2005), reduce metal concentration build up in shoots and increase plant growth (Davies Jr et al., 2001). In addition, earlier studies by Bethlenfalvay and Franson (1989), found that the concentration of Mn in plants infected with *G. mosseae* was significantly lower but with no symptoms of Mn toxicity. However, other studies reported an increased accumulation of Cu, Ni, Pb and Zn in a grass (*Ehrartia calycina*) (Killham and Firestone, 1983) and maize (Liu et al., 2000), while *G. mosseae*, *G. intraradices* and *G. caledonium* inoculation to Chinese brake fern (*Pteris vittata*) in soil contaminated with U (Uranium) and As (Arsenic), increased U uptake, concentration in plant tissue and decreased plant growth were recorded (Shen et al., 2006).

Role of AMF to increase plant resistance to drought stress

In many arid and semiarid regions of the world, drought or low water availability limits crop productivity. Many studies reported that colonization of plant roots by AMF under drought stress resulted in improved productivity of numerous crop plants, such as wheat (*Triticum* spp.) (Al-Karaki et al., 2004), pepper (*Capsicum* spp.) (Davies et al., 2002), lettuce (Marulanda et al., 2003) and strawberries (Borkowska, 2002). Improved productivity of plants inoculated with AMF under drought stress was attributed to enhanced uptake of immobile nutrients such as P, Zn and Cu (Al-Karaki 1998). Other results have shown that AMF colonization increased the drought resistance of wheat (Allen and Boosalis, 1983; Ellis et al., 1985; Al-Karaki and Al-Raddad, 1997) and pepper (Davies et al., 1993). However, other studies established the negative effect of drought stress on AMF root colonization (Ryan and Ash, 1996).

1.4.5. Role of AMF in crop protection

Interaction between AMF and insects

A number of studies have investigated the interaction between AMF colonized plants and insects (Wardle e al., 2004), and have found both positive and negative effects on insect growth and survival (Bennett and Bever, 2007), dependind on plant host. For example, Gange et al. (2003) showed that AMF colonization reduced the level of narrowleaf plantain leaf damage by herbivorous insects at field sites, but had no such effect in ragwort (*Senecio jacobaea*). It was also showned that the effect of AMF colonized plants on insects depends on the type of insect (Gange and West, 1994; Borowicz, 1997; Goverd et al., 2004; Gang et al., 2005). The difference in feeding behaviour of herbivorous insects was explained by the assumption that AMF, which colonize roots, will alter plant physiology and chemistry (Smith and Read, 2008).

A positive effect of AMF inoculation on insects has also been reported. For example, Goverde et al. (2004) reported that survival and larval weight of third instars larvae of the common blue butterfly, *Polyommatus icarus* were greater when they were fed with small shoots of mycorrhizal plants than of non-mycorrhizal plants of bird's-foot trefoil (*Lotus corniculatus*).

By feeding on the plants leaves, the insects have been reported to have also negative effects on AMF infection. For example, Gange et al. (2003), performing a series of field and laboratory experiments reported that herbivorous insects reduced root colonization by AMF in narrowleaf plantain, which is a highly mycorrhizal, mycotrophic plant that sufferred continuously over a growing season, even after limited insect damage. On the other hand there was no such effect on *S. jacobaea* (weakly mycorrhizal, non-mycotrophic).

Interaction between AMF and pathogenic fungi

Various effects have been noted from AMF and pathogenic fungi interactions. AMF tend to decrease the harmful effects of fungal pathogens through a negative impact on pathogen development, leading to increased crop yields. For example, *Rhizoctonia solani* infected potato (*Solanum* spp.) plantlets, inoculated with *G. etunicatum*, produced greater tuber fresh weights than non-AMF plantlets (Borowicz, 2001). Similar observations were reported on *Verticillium dahliae* infected pepper plants (Garmendia et al., 2004), where AMF reduced the deleterious effect of *V. dahliae* on pepper growth and yield. *G. intraradices* has been also reported to suppress development of the potato dry rot (a post-harvest disease) caused by the fungus *Fusarium* spp. (Niemira et al., 1996). Mycorrhizal symbionts have been shown to improve resistance against pathogenic fungi such as *Fusarium oxysporum* f. sp. Cubense on banana (cv Maca) (Borges et al., 2007), *Phytophthora parasitica* on citrus (*Citrus* spp.) (Davis and Menge, 1981), *Sclerotium cepivorum* on onion (*Allium* spp.) (Torres-Barragan et al., 1996), *Fusarium* spp. on cotton (*G. hursitum*), tomato (*Lycorpersicum* spp.) and in cucumber (Zhipeng et al., 1991; Caron et al., 1996; Zhipeng et al., 2005), *F. oxysporum* f. sp. *Lycopersici* on tomato (Akköprü and Demir, 2005) and *Pythium ultimum* on white clover (*Trifolium repens*) (Carlsen et al. 2008).

In certain studies, reduced AMF colonization in pathogen-challenged plants was observed. For example, Garmendia et al. (2004) reported that prior inoculation of *Capsicum* sp. plants with *V. dahliae* negatively affected root colonization with *Glomus* sp. when the AMF were inoculated at the flowering stage. In addition, increased pathogen incidence was reported in mycorrhizal plants compared to the control non-mycorrhzal plants. For example, in mycorrhizal tobacco plants infested with the leaves pathogen *Botrytis cinerea,* the mycorrhizal treatments resulted in an

increase in disease symptom severity (necrotic lesions) when inoculated with *G. intraradices* compared to the non-mycorrhizal treatments (Shaul et al., 1999).

Interaction between AMF and pathogenic bacteria

Only recently has the interaction between AMF and pathogenic bacteria been assessed (Liu et al., 2007). The authors showed that in mycorrhizal medic (*M. truncatula*) plants infested with the bacteria *Xanthomonas campestris*, the symbiosis result in enhanced resistance to pathogenic bacteria compared to the nonmycorrhizal treatments.

Interaction between AMF and virus

A limited number of studies have assessed the interaction between AMF and viruses (Shaul et al., 1999). The authors reported that plants of tobacco colonized by *G. intraradices* and infested with tobacco mosaic virus showed a higher incidence of the virus disease than those of non-mycorrhizal plants.

Interaction between AMF and plant parasitic nematodes

In general, plant parasitic nematodes are detrimental to plant growth and yield, while AMF are beneficial. Plant parasitic nematodes and AMF share plant roots as a resource for food and space. The effects of both these organisms on plant growth and their interaction have been reviewed by numerous authors (Smith and Kaplan, 1988; Pinochet et al, 1996; Roncardori and Hussey, 1997; Borowicz, 2001; Hol and Cook, 2005; Borowicz, 2006). A general conclusion from these reviews suggests that AMF increase resistance to nematode infestation by slowing down nematode development. But it is now clear that the net effect of AMF on nematodes vary with environmental conditions, plant genotype, nematode species and fungal isolates. Here the interaction between AMF and the following four groups of nematodes is discussed: (1) sedentary endoparasitic nematodes, that induce feeding sites in the parenchyma cells of the roots; (2) migratory endoparasitic nematodes, which invade, multiply, feed and move within the root cortex of the host plant resulting in necrotic lesions and promoting fungal infections; (3) migratory ectoparasitic nematodes that feed on superficial cells, as well as in deeper cortical layers at the root tip, which is less colonized by AMF; (4) stem-and leaf nematodes.

The effect of AMF on sedentary nematodes have mostly been addressed in studies at assessing AMF-nematode interactions. These studies generally show a negative effect of AMF on nematode population densities. For example, Li et al. (2006) showed that inoculation of the grapevine (*Vitis amurensis*) with the AMF *G. versiforme* significantly increased resistance against the *M. incognita* possibly due to a transcriptional activation of the class III chitinase gene VCH3. A suppressive effect was also observed on *M. hapla,* when pyrethrum (*Anacyclus pyrethrum*) plants were inoculated with *G. etunicatum* (Waceke et al., 2001). Castillo et al. (2006) studied the effects of single and joint inoculation of olive (*Elaeagnus angustifolia*) planting stocks (cvs Arbequina and Picual) with the AMF *G. intraradices*, *G. mosseae* and *G. viscosum*, and the nematodes *M. incognita* and *M. javanica* under controlled conditions. They found that AMF in olive plants significantly reduced the severity of root galling up to 36.8% as well as reproduction of both *Meloidogyne* spp. up to 35.7%, indicating a protective effect against parasitism by *Meloidogyne* spp. They also reported that in plants free from AMF, infection by *Meloidogyne* spp. significantly reduced the plant main stem girth by 22.8-38.6%. A single inoculation of olive planting stocks with the AMF *G. intraradices*, *G. mosseae* and *G. viscosum* and the *M. incognita* and *M. javanica* under controlled conditions showed that the fungal symbiosis significantly increased growth of olive plants by 88.9% within a range of 11.9-214.0%, irrespective of olive cultivar and plant age (Castillo et al., 2006). In *Musa* spp., a number of studies have investigated the effect of AMF on *Meloidogyne* spp. population dynamics as well as on nematode damage on roots (reviewed by Pinochet et al., 1996; Jaime-Vega et al., 1997). In 2003, Elsen et al., reported that banana cultivars inoculated with *G. mosseae* reduced *M. javanica* populations as compared to the control. While however, root colonization by AMF was increased in plants free of nematodes as compared to plants infested with nematodes. In tomato for example, Diedhiou et al., (2003) reported that pre-inoculation of plants with *G. coronatum* stimulated plant growth and reduced *M. incognita* infestation. Among root and tuber crops, interactions between AMF and sedentary nematode were reported on potato, *Solanum tuberosum* and *S. melongena,* showing increased plant growth and reduced potato cyst population densities (Borah and Phukan, 2000; Jothi and Sundarababu, 2000; Jothi and Sundarababu, 2002; Rao et al., 2003).

While there are numerous studies indicating that AMF increase plant resistance to nematode infection by slowing down nematode development, there are also studies reporting no effect or synergistic effects on nematode populations. For example, Diedhiou et al., (2003) reported that a combined application of the AMF *G. coronatum* and a non-pathogenic *F. oxysporum* (Fo162) enhanced mycorrhization of *Lycopersicum* spp. roots, but did not increase overall nematode control. They also reported that a higher number of nematodes per gall was found for mycorrhizal than non-mycorrhizal plants. Ryan et al. (2003) reported that the population of potato cyst nematodes per plant was increased on potato plants inoculated with Vaminoc (commercial AMF product) compared to non-inoculated plants, by 200% for *Globodera rostochiensis* and by 57% for *Globodera pallida.* Other studies reported that a synergistic effect of soil pathogenic fungi and nematodes reduced bioprotection by AMF on the sand ryegrass (*Leymus arenarius*) (Greipsson and El-Mayas, 2002).

Concerning the AMF interaction with migratory endoparasitic nematodes, relatively few studies have been undertaken. Some notable successes in the management of migratory nematodes through application of AMF have been observed. This has been well documented for *G. intraradices* and *Radopholus similis* on rough lemon (*Citrus jambhiri*) seedlings (Smith and Kaplan, 1988). Camprubi et al. (1993) also reported that root weights of mycorrhizal plum (*Prunus domestica*) rootstock plants inoculated with *Pratylenchus vulnus* were higher than root weights of the same plum rootstock plants lacking mycorrhiza (*G. mosseae*). Some studies have shown increases in plant tolerance or resistance to *Pratylenchus* spp. as a consequence of inoculation with AMF, while others found no protective effect of AMF (Forge et al., 2001). For example, de la Peña et al., (2006) reported that AMF could out-compete migratory endoparasitic nematodes (*Pratylenchus penetrans*) when they occurred together in the same root compartment of pioneer dune grass (*Ammophila arenaria*). They also reported that root colonization by AMF was not affected by the nematode. In banana, Elsen et al., (2003; 2008) showed that *G. mosseae* or *G. intraradices* inoculation of plantlets increased plant tolerance or resistance to *Pratylenchus* spp. and *R. similis*. Similar observations were reported by Jaizme-Vega and Pinochet (1997), when they studied the interaction between *G. intraradices* and *P. goodeyi* on banana. In the case of root and tuber crops, few studies have been performed concerning the interaction of AMF with migratory endoparasitic nematodes. According to Kassab and Taha (1990b), sweet potato

(*Ipomoea batatas*) inoculated with *Glomus* sp., reduced *Tylenchorhynchus* spp. populations. In contrast, however, the same authors demonstrated that sweet potato inoculated with *Glomus* sp. led to increased *Criconemalla* spp. and *Rotylenchulus* spp. populations, but resulted in increased sweet potato tolerance (Kassab and Taha, 1990b). Other studies have shown that the associations of AMF with migratory endoparasitic nematodes can lead to increased nematode populations (Pinochet et al., 1996; Borowicz, 2001). Moreover, a few reports have dealt with the negative effects of migratory nematodes on AMF, as so-called fungivorous nematodes grazed on the mycorrhizal mycelium in soils (Bakhtiar et al., 2001). Mutual inhibitions were also detected between AMF and nematodes in banana (Francl, 1993; Elsen et al., 2003).
Concerning the migratory ectoparasitic nematodes, only limited studies have been performed, investigating mainly *Tylenchorhynchus* sp. It was clearly observed that the effect of ectoparasitic nematodes on AMF was more severe than the effect of *Meloidogyne* spp. (Hasan and Jain, 1987; Kassab and Taha, 1990a) with AMF plants of *Trifolium alexandrium* suffering more from ectoparasitic nematodes than non-AMF plants. A possible explanation might be that ectoparasitic nematodes damage the extra-radical hyphae growth and possibly diminish fungal entry into the roots (Hasan and Jain, 1987; Kassab and Taha, 1990a).

Furthermore, stem-and leaf nematodes infesting aerial plant parts should be included in the interaction studies. Unfortunately, up to now, only one report appears to have addressed the interaction between AMF and aerial nematodes. Sikora and Dehne (1979) reported that *Ditylenchus dipsaci* populations initially increased, then decreased on mycorrhizal bean (*Phaseolus vulgaris)* compared to non-mycorrhizal controls, while *Aphelenchoides* spp. populations decreased on mycorrhizal, compared to non-mycorrhizal tabacco (*Nicotiana* spp.).

Mechanisms involved in the AMF mediated bioprotection of plants against soil-borne pathogens
To our knowledge, there is no report elucidating the direct mechanisms of the interaction between AMF and pathogens e.g. through antagonism, antibiosis, and/ or mycoparasitism. Therefore, there are many hypotheses attempting to explain the mechanisms, which are likely to include indirect ones (Azcon-Aguilar and Barea, 1996; Harrier and Waston, 2004).

-Improved crop nutrition: AMF can enhance plant nutrient uptake, increasing plant vigor and consequently natural ability to resist/ tolerate pathogens, especially when AMF is established prior to pathogen infection (Smith and Kaplan, 1988; Smith and Read, 2008).

-Anatomical changes in the root system: It was reported that AMF infection increased root branching, leading to greater root ability to elude or avoid pathogen infections (Hooker et al., 1994) and, in addition, induced cell wall fortification by increased production of polysaccharides and an increased lignin, thus reducing pathogen penetration (Jalali and Jalali, 1991).

-Competition for infection and colonization sites: AMF and the soil-borne fungal and plant parasitic nematodes occupy similar root tissues, and therefore, they will compete for space especially if colonization occurs simultaneously (Smith, 1987). Biocontrol of the pathogen *Phytophtora parasitica* by the AMF *G. mosseae* was induced as a consequence of competition for infection sites (Cordier et al., 1996; Vigo et al., 2000).

-Competition for host photosynthates: Both AMF and pathogens, especially sedentary endoparasitic nematodes, depend on photosynthates produced by the host and compete for carbon reaching the root (Smith, 1987; Smith and Read, 2008). There is no solid evidence for the mechanism involved in the competition (Azcon-Aguilar and Barea, 1996).

-Soil microbial population changes: AMF inoculation reduced plant exudate production in maize plants (Marschner et al., 1997) and likely also in other plants, which could lead to stimulation of microorganisms such as antagonistic nematode and chitinase-producing actinomycetes (Burke et al., 2002).

-Pathogen damage compensation: Plants colonized by AMF can compensate for the loss of roots or root function caused by the pathogens by enhanced nutrient uptake and water absorption capacity of the mycorrhizal root system (Harrier and Waston, 2004).

-Activation of plant defense responses: Establishment of AMF symbiosis can predispose plants to respond more rapidly to pathogenic attacks (Dehne, 1982; Gianinazzi-Person, 1996), through

a pre-activation of plant defense responses (Slezack et al., 2000). Genes and corresponding proteins and other compounds involved in plant defense responses were extensively studied including phytoalexins, callose deposition, hydroxyproline-rich glycoproteins, phenolics, peroxidases, chitinases, β-1-3 glucanases and PR-pathogenesis related proteins (Cordier et al., 1996; Slezack et al., 2001).

1.5. Objectives of the current study

The general objective of the present thesis was to identify the diversity of AMF in the "yam belt" region of West Africa and to assess the potential of selected indigenous and non-indigenous AMF isolates for yam growth promotion and yam nematode suppression towards improved yam (*Dioscorea* spp.) production. The specific objectives divide this thesis in four related studies, which are presented accordingly, with an additional general discussion chapter. The studies have been prepared for publication in international scientific journals with one chapter recently published (Tchabi et al., 2008).

1.5.1. Arbuscular mycorrhizal fungal communities in sub-Saharan Savannas of Benin, West Africa, as affected by agricultural land use intensity and ecological zone (chapter 2).

The first specific objective was to explore the native AMF communities in three ecological zones of the 'yam belt' in Benin (from the more humid Southern Guinea savannah (SG) over the Northern Guinea savanna (NG) to the drier Sudan savanna (SU), and to assess the impact of farming practices on the diversity of AMF. We hypothesized that farming practices such as forest clearance by slash and burn, as well as crop rotation and cultivation of specific crops would lead to an erosion of AMF species diversity in West African soils. We further expected that AMF species composition would change with increasing dry season length from southern to northern succession of savannas (SG through NG to SU). For these, AMF spore density (= spore abundance) and species richness (= species numbers) were compared at various agricultural sites differing in land use intensity, from undisturbed natural forest savannas through yam fields and various low-input crops to relatively intensive cotton production in all three ecological zones.

1.5.2. Arbuscular mycorrhizal fungi associated with yam (*Dioscorea* spp.) in the Southern Guinea Savanna of West Africa (chapter 3).

The specific objective here was to assess the mycorrhizal status of yam by identifying root colonization by AMF among yam cultivars, and to identify AMF species associated with yam. It was assumed that yam is arbuscular mycorrhizal and dependent on AMF for its growth. The AMF communities present in soil samples from the field sites were propagated in trap cultures on cultivars of white and yellow yam in order to produce fresh living spores of different AMF species that could be used for the subsequent establishment of single spore derived AMF cultures (strains) necessary for the functional screening experiments for yam growth promotion and nematode suppression (chapters 3-5). The spore abundance and species richness of AMF was first determined in soil samples from three yam fields and three adjacent natural savanna forests. Therafter, the AMF trap cultures were etablished with yam and sorghum as host plants and soil inoculum from the field sites. While AMF present in the yam fields may not necessarily be associated with yam but possibly with the accompanying weed flora, AMF species detected in the yam trap cultures could unequivocally be assigned to yam. In these trap cultures, root colonization by AMF, spore density and species richness was determined, as well as yam shoot and tuber dry weight.

1.5.3. Increased growth of micro-propagated white yam (*Dioscorea rotundata*) following inoculation with indigenous arbuscular mycorrhizal fungal isolates (chapter 4).

This study assessed AMF isolates indigenous to the 'yam belt' for their potential to promote growth of micro-propagated white yam plantlets. For ecological reasons, the use of indigenous AMF instead of AMF of 'exotic' origin would certainly be recommendable. Furthermore, we hypothesized that indigenous AMF isolates might be more effective for plant growth promotion than non-indigenous isolates as being better adapted to the specific environment. Therefore, we first generated single spore derived (=monosporal) cultures of several AMF species indigenous to the Southern Guinea savanna of Benin. In a first growth experiment, we screened several indigenous and non-indigenous *G. etunicatum* isolates for AM root colonization as well as shoot, root and tuber growth of micro-propagated white yam. The non-indigenous *G. etunicatum* isolates were obtained from other tropical origins (Bolivia and India). In the second experiment, nine AMF species with three monosporal isolates per species were screened on the same yam

cultivar (TDr89/02461) for the same parameters. AMF isolates of non-tropical origin were included in both experiments.

1.5.4. Effect of arbuscular mycorrhizal fungal application on micropropagated yam plantlets and suppression of nematode damage caused by *Scutellonema bradys* (Tylenchideae) and *Meloidogyne* spp. (Meloidogyneae) (chapter 5).

Here, we assessed the potential of AMF species on in *vitro* yam plantlets for plant growth promotion and suppression of yam nematodes: migratory *S. bradys* and sedentary *Meloidogyne* spp. We hypothesized that AMF inoculation may be effective for plant growth promotion, but their effect might depend on AMF species and on yam genotype. In addition, we hypothesized that AMF inoculation could affect the nematode population dynamics and their consequent damage, but which probably depends on the type of nematode and especially on yam cultivar. We used two AMF species (*G. mosseae* and *G. dussii*) and four yam cultivars obtained from *in vitro*. Two experiments were established. Firstly, we challenged plants following AMF species inoculation with *S. bradys*, and secondly with *Meloidogyne* spp.

1.5.5. General discussion (chapter 6)

In the last chapter, we discussed the main results obtained from our overall study and presented some perspectives for further investigations in this exciting field of research.

1.6. References

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Chapter 2: Arbuscular mycorrhizal fungal communities in sub-Saharan Savannas of Benin, West Africa, as affected by agricultural land use intensity and ecological zone*

2.1. Abstract

The rapid decline of soil fertility of cultivated lands in the sub-Saharan savannas of West Africa is considered to be the main cause of the increasingly severe constraints of food production. The soils in this tropical area are highly fragile and crop yields are limited by characteristically low levels of available phosphorus. Under such preconditions, the multiple benefits of the arbuscular mycorrhizal (AM) symbiosis are likely to play a pivotal role for maintaining natural soil fertility, by enhancing plant nutrient use efficiency, plant health and stabilization of a favorable soil structure. Thus it is important to explore the impact of the commonly applied farming practices on the native AM fungal (AMF) community. In the present study, we determined the AMF species composition in three ecological zones differing by an increasingly prolonged dry season from South to North, from the Southern Guinea Savanna (SG), to the Northern Guinea Savanna (NG), to the Sudan Savanna (SU). In each zone, four 'natural' and four 'cultivated' sites were selected. 'Natural' sites were three natural forest savannas (at least 25-30 years old) and a long-term fallow (6-7 years old). 'Cultivated' sites comprised a field with yam (*Dioscorea* spp.) established during the first year following forest clearance, a field under mixed cropping with maize (*Zea mays*) and peanut (*Arachis hypogaea*), a field under peanut, and a field under cotton (*Gossypium hirsutum*) which was the most intensively managed crop. Soil samples were collected towards the end of the wet season in each zone. AMF spores were extracted and morphologically identified. Soil sub-samples were used to inoculate AMF trap cultures using *Stylosanthes guianensis* and *Brachiaria humidicola* as host plants to monitor AM root colonization and spore formation over 10 and 24 months, respectively. A total of 60 AMF species were detected, with only seven species sporulating in the trap cultures. Spore density and species richness were generally higher in the natural savannas and under yam than at the other cultivated sites, and lowest under the intensively managed cotton. In the fallows, species richness

was intermediate indicating that the high richness of the natural savannas was not restored. Surprisingly, higher species richness was observed in the SU than in the SG and NG, mainly due to a high proportion of species in the *Gigasporaceae*, *Acaulosporaceae* and *Glomeraceae*. We conclude that the West African savannas contain a high natural AMF species richness but that this natural richness is significantly affected by the common agricultural land use practices, and appears not to be quickly restored by fallow.

Key Word: agroecology; arbuscular mycorrhiza; biodiversity; *Dioscorea* spp.; ecological zones; farming practices; forest; yam; arid lands

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2.2. Introduction

Arbuscular mycorrhizal fungi (AMF) occur in most vegetation types and constitute an important component of the tropical soil microflora (Sieverding, 1991; Smith and Read, 2008; Cardoso and Kuyper, 2006). The symbiotic relationship between AMF and plants presents a particularly outstanding example of mutually beneficial interactions, where both organisms profit, primarily from the exchange of nutrients (Smith and Read, 2008). The benefit for the plants, however, extends to more than enhanced nutrient uptake, including pest and disease protection and favorable soil structure stabilization. Knowledge and understanding of the community structure and dynamics within AMF communities, however, is a necessary prerequisite to eventually identify the specific beneficial effects of individual AMF, especially in tropical low-input agro-ecosystems in which sustainable management of the generally low soil nutrient resources must consider the benefits of native microorganisms (Cardoso and Kuyper, 2006; Lovera and Cuenca, 2007).

During the infancy of AMF systematics (Gerdemann and Trappe, 1974; Morton and Benny, 1990), species diversity studies were few and generally limited (e.g. Gerdemann and Trappe, 1974; Nicolson and Schenck, 1979; Schenck et al., 1984) without consideration of climatic, vegetation or land use changes. Often the main purpose of these studies was the description of newly discovered species based on spore morphology. Further progress in this field (e.g. Schenck and Pérez, 1990) and particularly the rapid development of molecular tools for identification (e.g. Redecker, 2000), have steadily increased the number of AMF distribution and diversity studies and their quality in the recent past (e.g. Jansa et al., 2002; Wubet et al., 2003; Castillo et al., 2006; Gai et al., 2006; Bashan et al., 2007; Wu et al., 2007). Such studies have enabled progressive understanding of the connection between AMF communities and various parameters along broad gradients, such as land use intensity (Jansa et al., 2002; Oehl et al., 2003; 2004), soil type (Lekberg et al., 2007), soil depth (Oehl et al., 2005), host specificities (Bever et al., 2001; Eom et al., 2004), plant nutrient content (Egerton-Warburton and Allen, 2000; Landis et al., 2004), spatial, temporal (Lovelock et al., 2003; Lovelock and Ewel, 2005) and latitude (Koske et al., 1997) gradients. AMF identification and diversity studies from African ecosystems, however, are to date, relatively limited (e.g. Sieverding, 1988; Stutz and Morton, 1996; Wubet et al., 2004; 2006; Uhlmann et al. 2006; Lekberg et al., 2007; Mathimaran et al.,

2007) with a particular scarcity from tropical West Africa (Old et al., 1973; Ingleby et al., 1994; Sanginga et al., 1999; Dalpé et al., 2000; Friberg, 2001; Duponnois et al., 2001). AMF distribution and diversity in tropical ecosystems elsewhere, appears to be receiving increased attention (e.g. Sieverding, 1989; Maia and Trufem, 1990; Picone, 2000; Husband et al., 2002a; 2002b; Lovelock et al., 2003; Lovelock and Ewel, 2005).

In West Africa, particularly Benin, the impact of farming practices on composition and diversity of AMF remains largely unknown. The slash and burn technique of land clearance tends to prevail in many areas, leaving the land to fallow following a number of cultivation cycles. The Sudan and Guinea savannas of West Africa occur in a zone commonly referred to as the 'yam belt', where yam (*Dioscorea* spp.) is of particular importance and characteristically is used as the first crop cultivated after forest clearing (IITA, 2004). Depending on production levels, the same land is cultivated with other crops one or two years after yam, such as maize (*Zea mays*), sorghum (*Sorghum* spp.), peanut (*Arachis hypogaea*), cassava (*Manihot esculenta*), and later cotton (*Gossypium hirsutum*). The crops are generally cultivated in small-scale fields as mono-cropping or mixed cropping during the wet season, rotating the crops from one season to another. Except for cotton, which is cultivated as a cash crop, other crops are managed on low external input level and are intended to meet local demand (IITA, 2006). Use of agricultural machinery - even for soil cultivation - is not common and where present is applied to grow cotton. 'Ferralsol' soils are dominant in Benin and are characterized by low nutrient availability and high levels of soil degradation through physical loss and leaching of soil minerals, particularly available phosphorus, due to heavy rains, resulting in rapid yield decline (Defoer and Scoones, 2001; IITA, 2006). Soil infertility and subsequent yield decline is also partly related to the decreasing prevalence and loss of diversity of soil microflora and microfauna, such as beneficial AMF, following forest clearance (Johnson et al., 1992).

The present study investigated the impact of land use intensity on AMF communities in three ecological zones of Benin: the Sudan Savanna (SU) and the Northern and Southern Guinea Savannas (NG and SG, respectively). Based on similar studies elsewhere (e.g. Sieverding, 1989; Jansa et al., 2002; Oehl et al., 2003), we hypothesized that agricultural cultivation practices such as crop rotation and cultivation of specific crops would lead to an erosion of AMF species

diversity in West African soils. We further expected that AMF species composition change with increasing dry season length from south to north succession of savanna types (SG through NG to SU). In order to achieve an understanding on AMF dynamics in different ecological zones, AMF spore density (= spore abundance) and species richness (= species numbers) were compared at the various agricultural sites using a gradient of land use intensity, from undisturbed natural forest savannah sites through yam fields and various low-input crops to relatively intensive cotton production, in the three ecological zones.

2.3 Material and Methods

2.3.1. Study area

This study was undertaken in three ecological zones of Benin: in the SU the NG and SG (Table 2.1). The climate changes from SG through NG to SU, reflected by a decreasing annual rainfall and increasing length of dry season (Table 2.1). Moreover, the temperature differences between day and night are increasingly more pronounced with distance from south to north, especially during the dry season (Table 2.1). Remarkably, the SG has two wet seasons and two dry seasons per annum, while NG and SU have a single wet and dry season each per annum. The natural vegetation in the natural savannas consists of trees, shrubs and grasses with trees and shrubs becoming increasingly less prominent from south to north (see e.g. Adjakidje 1984; Adjanohoun 1989; Table 2.1). The soils are dominantly ferruginous Ferralsols (FAO, 2006: http://www.fao.org/AG/aGL/agll/landuse/docs/benin.doc).

Table 2.1. Some geographic characteristics of the sub-Saharan ecological zones under study for arbuscular mycorrhizal fungi

Parameters	Sudan Savanna	Northern Guinea Savanna	Southern Guinea Savanna,
Latitude	9-11 °N	8-9 °N	7-8 °N
Elevation	550m asl	400m asl	200m asl
Climate	One wet season: May-October 22-34°C One dry season: Oct-May 15-45°C	One wet season: April-October 22-34°C One dry season: Nov-Mar 20-40°C	Two wet seasons: March- July September –November 20-28°C Two dry seasons: Dec-Mar and Aug-Sep 24-30°C
Rainfall	600-700 mm	1000 -1200 mm	1200-1400 mm
Vegetation	*Zyziphus mauritania, Combretum* spp., *Balamiten* spp., *Acacia* spp., *Butyrospermum* spp., *Parkia biglobosa*, *Andropogon gayanus*, *Imperata cylindrica*	*Isoberlinia doka, Afzelia africana, Khaya senegalensis, Danielia oliveri, Anogeissus* spp., *Pterocarpus* spp., *Andropogon* spp.,	Combretaceae, Mimosaceae, Fabaceae, Poaceae

Sources: Adjakidje (1984); Adjanohoun (1989).

Table 2.2. Historical characteristics (cultural precedence, fallow duration, crop rotation, fertilizer and pesticide use) of sites in Benin sampled for arbuscular mycorrhizal fungi

Standing crops/vegetation at sampling date	Previous crops/vegetation	Estimated age of the forest or fallow (year)	Years of continuous cultivation after forest clearance	Historic of fertilizer use	Historic of pesticide use
Sudan Savanna (SU)					
Natural Forest1	-	25-30	-	-	-
Natural Forest2	-	25-30	-	-	-
Natural Forest3	-	25-30	-	-	-
yam	Natural forest	-	1	-	-
Mixed crops (maize-peanut)	yam	-	2	-	-
Peanut	Peanut and cassava	-	3	-	-
Cotton	Maize+peanut	-	4	Mineral fertilizer (N:P:K:S:B = 14:23:14:5:1; 150 kg ha^{-1}); Urea (50 kg ha^{-1})	Conquest Plus 388EC (Cypermetrine, Acetometride and Triasophos)
Fallow	-	6-7	-	-	-
Northern Guinea Savanna (NG)					
Natural Forest1	-	20-25	-	-	-
Natural Forest2	-	25-30	-	-	-
Natural Forest3	-	20-25	-	-	-
yam	Natural forest	-	1	-	-

Mixed crops (maize-peanut)	yam	-	2	-	-
Peanut	Cassava	-	3	-	-
Cotton	Maize+peanut	-	4	Mineral fertilizer (N:P:K:S:B = 14:23:14:5:1; 150 kg ha^{-1})	Conquest Plus 388EC (Cypermetrine, Acetometride and Triasophos)
Fallow	-	7	-	-	-
Southern Guinea Savanna (SG)					
Natural Forest1	-	25-30	-	-	-
Natural Forest2	-	25-30	-	-	-
Natural Forest3	-	20-25	-	-	-
yam	Natural forest	-	1	-	-
Mixed crops (maize-peanut)	yam	-	2	-	-
Peanut	Peanut and maize	-	3	-	-
Cotton	Maize+cassava	-	4	Mineral fertilizer (N:P:K:S:B = 14:23:14:5:1); 150 kg ha^{-1})	Conquest Plus 388EC (Cypermetrine, Acetometride and Triasophos)
Fallow	-	5-6	-	-	-

Table 2.3 Geographical position of study sites, and selected chemical soil parameters

Sampling sites	Geographical position	pH (H_20)	Organic C g kg^{-1}	Available P (Sodium acetate) mg kg^{-1}	Available P (Citrate) mg kg^{-1}
Ecological zone 1: Sudan Savanna (SU)					
Natural Forest 1	10°56.420N ; 001°32.003E	6.1	13.9	47.6	69.9
Natural Forest 2	10°17.060N ; 001°19.506E	6.3	10.4	11.8	17.5
Natural Forest 3	10°07.868N; 001°56.315E	6.5	23.8	3.9	8.7
Yam	10°07.868N; 001°51.104E	5.9	11.6	3.9	8.7
Mixed cropping	10°18.802N; 001°35.104E	6.2	6.4	7.4	13.1
Peanut	10°19.885N; 002°00.326E	6.2	12.8	6.5	13.1
Cotton	09°58.916N; 002°47.936E	5.9	13.9	47.6	69.8
Fallow	10°18.802N; 001°35.104E	5.9	6.4	3.9	4.4
Ecological zone 2: Northern Guinea Savanna (NG)					
Natural Forest 1	08°43.452N; 002°40.047E	6.6	9.3	8.7	8.7
Natural Forest 2	09°10.545N; 002°12.321E	6.5	28.4	13.1	21.8
Natural Forest 3	09°03.112N; 002°04.197E	6.7	36.0	46.3	65.5

Yam	08°54.966N;002°33.370E	5.8	11.0	7.0	8.7
Mixed cropping	09°57.436N; 001°51.797E	6.2	4.1	5.2	8.7
Peanut	08°58.619N; 002°28.714E	6.2	13.9	9.2	13.1
Cotton	09°21.962N; 001°34.121E	5.8	11.0	25.8	52.4
Fallow	08°54.966N; 002°33.370E	6.5	13.3	13.1	21.8
Ecological zone 3: Southern Guinea Savanna (SG)					
Natural Forest 1	07°45.739N; 002°27.519E	6.7	9.9	14.8	34.9
Natural Forest 2	07°57.217N; 002°26.935E	7.2	13.9	8.7	13.1
Natural Forest3	07°35.829N; 002°18.942E	6.4	13.9	28.4	43.6
Yam	07°55.111N; 002°10.507E	7.4	13.3	5.7	8.7
Mixed cropping	07°25.639N; 001°51.323E	6.2	9.9	14.8	34.9
Peanut	07°51.537N; 002°17.246E	6.6	7.5	13.1	17.5
Cotton	07°23.024N; 001°52.495E	6.2	16.2	43.7	61.1
Fallow	07°49.275N; 002°15155E	6.5	9.9	10.5	17.5

E, east of Greenwich; N, north of the Equator

2.3.2. Study sites and soil sampling

In each ecological zone, soils were sampled at eight sites (Table 2.2): three in natural forest savannas where soils and plant vegetation had been undisturbed for 25-30 years before sampling; one under long-term fallow of 6-7 years; one cultivated with yam, in the first season following forest clearance; two cultivated with peanut, either alone or in mixed cropping with maize; one cultivated with cotton under relatively intensive high-input conditions. The geographical position and selected chemical soil parameters were measured at each site (Table 2.3).

Sites were sampled towards the end of the wet season, in October 2004, when vegetation cover remained green and yam was approximately 5-6 months old. At each sampling site, four replicate quadrant plots (100 m^2) were selected and six soil cores randomly removed to a depth of 20 cm using 6-cm ∅ corers. The six soil-core samples per plot were combined as one composite sample constituting one replicate per site. Samples were stored in plastic bags and transported within 24-72 h to the International Institute of Tropical Agriculture station in Abomey-Calavi (IITA-Benin), air-dried on an open bench in the greenhouse for 72 h and then held at 4°C in a fridge for two weeks before transferal to the Botanical Institute of Basel (Switzerland).

2.3.3. Soil analyses

Each replicate air-dried soil sample from each quadrant plot was divided into three sub-sets: one for the determination of chemical soil parameters (pH, organic carbon and available phosphorus) in the "Laboratory Dr. Balzer", Wetter-Amönau, Germany, according to standard methods (Oehl et al. 2003); the second for direct isolation and identification of AMF spores (see below); the third for use as inoculum for AMF propagation and spore production in 'trap cultures' and subsequent AMF identification.

2.3.4. Trap cultures

Four trap culture pots (pots: 20 cm x 20 cm x 30 cm) were created for each site, one each per field plot replicate according to Oehl et al. (2003). For each pot, 4 kg of substrate was used, comprised of a sterilized 3:1 (wt/wt) mixture of Terragreen® (a calcined granular attapulgite clay mineral, American aluminium oxide, oil dry US special, type III R, >0.125mm; Lobbe Umwelttechnik, Iserlohn, Germany) and quartz sand (Alsace quartz sand, 5% of free silica,

Smurfit Company, France), respectively. The chemical composition of the substrate was 0.3 % organic matter, 10 mg kg^{-1} and 1480 mg kg^{-1} easily and heavily available phosphorus (P extracted with sodium acetate and citrate, respectively), and 191 mg kg^{-1} potassium (K extracted with sodium acetate), and pH 5.8. A 180 g sample, divided equally into three sub-portions of soil inoculum, was placed in each trap culture pot as three lines on the surface of 3 kg substrate, which was then covered with the remaining 1 kg of trap culture substrate. Five one-week old *Stylosanthes guianensis* plants and four one-week old *Brachiaria humidicola* plants were alternatively and equidistantly planted per pot along the three lines of the inoculum. A total of 100 pots were set up including four non-mycorrhizal control pots. Each control pot received 180 g sterilized soil and a non-mycorrhizal suspension of soil bacteria (1 mL per plantlet) obtained through fine filtration (LS 141/2; Schleicher and Schuell, Feldbach, Switzerland) of a soil suspension (final volume of 1 l bacterial soil suspension from 0.5 kg air-dried soil). The trap cultures were maintained in a greenhouse in Basel for 24 months under day: night regimes of 12h: 12h photoperiod and 25: 21°C temperature, with a mean relative humidity of 65 ± 5%. Trap cultures were irrigated using automated watering systems (Tropf-Blumat; Weninger GmbH, Telfs, Austria) (Oehl et al. 2003).

2.3.5. Sampling of trap cultures

At 4, 6, 8 10 and 24 months after planting, two soil core samples (volume 15 cm^3 per core) were removed from each pot for spore isolation and identification and to monitor mycorrhizal root colonization.

2.3.6. Isolation and morphological identification of AMF

AMF spores were isolated from 25 g air-dried field soil samples or from 30 cm^3 trap culture substrate that were suspended in water. Spores were extracted by wet sieving through nested sieves (1000µm, 125µm, 80µm and 32µm) followed by density gradient centrifugation. From the 1000 µm sieves no spores or sporocarps were obtained and therefore only the contents from the 125 µm, 80 µm and 35 µm sieves were poured into 50 ml vials and centrifuged in 70% sucrose solution gradient (Oehl et al. 2003). After centrifugation at 2000 rpm for 2 min, spores, spore clusters and sporocarps obtained from each pot were transferred into Petri dishes, and counted using a dissecting microscope (Olympus SZ12) at up to 90x magnification.

For microscopic identification, healthy spores were mounted on glass slides and stained with polyvinyl-lactic acid glycerol (PVLG) mixed with Melzer's reagent (1:1 vol/vol) (Brundrett et al. 1994). The spores were examined under a compound microscope (Zeiss; Axioplan) at up to 400x magnification. Identification was based on current species descriptions and identification manuals (Schenck and Pérez 1990; International Culture Collection of Arbuscular and Vesicular-Arbuscular Endomycorrhizal Fungi, INVAM: http://invam.caf.wvu.edu; Arbuscular Mycorrhizal Fungi (Glomeromycota), *Endogone* and *Complexipes* species deposited in the Department of Plant Pathology, University of Agriculture in Szczecin, Poland: http://www.agro.ar.szczecin.pl/~jblaszkowski/).

2.3.7. Statistical analysis

Spore density (= spore abundance) in a field sample was expressed as the number of AMF spores g^{-1} of soil. Spore density and species richness were analysed using the PROC ANOVA procedure of SAS version 9.1 packages (SAS, 2005). Significant differences between field sites were tested using Fisher's least significant difference (LSD) at $P < 0.05$. Prior to analysis, data on spore density were log(x+1) transformed to normalize the data.

2.4. Results

2.4.1. Soil characteristics

Soils were slightly acid across zones (Table 2.3). In general, natural forest savanna sites had relatively higher soil organic matter content and available P (phosphorus) than cultivated sites (except cotton fields). The C (carbon) and P soil contents also were dramatically reduced once under crop cultivation. Available P in the cotton fields, however, was similar to forest sites, probably as a result of fertilizer application. In the fallows, organic C and available P contents were similar to peanut or mixed cropping sites.

2.4.2. AMF spore density

AMF spore density was generally higher at the natural sites than at the cultivated sites. With a few exceptions forest soil spore density was consistently higher compared with peanut, mixed cropping and cotton sites (Fig.2.1). Spore density was similar in cultivated sites, but yam fields in SG had notably higher spore density than at all other sites, followed by forest and fallow soils. Spore density at fallow sites was variable, but generally comparable to forest sites. The lowest spore densities were observed under mixed cropping and in cotton fields in all three ecological zones (Fig. 2.1).

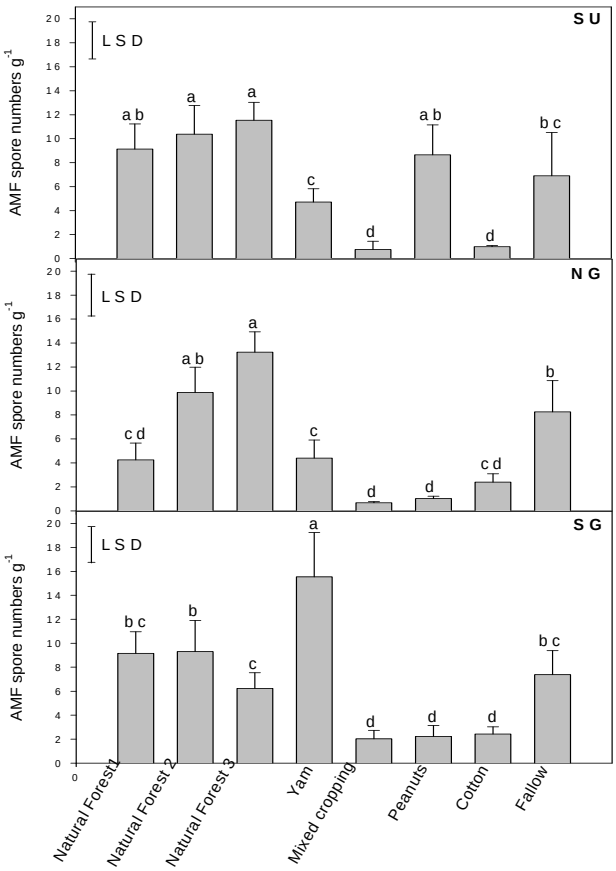

Fig. 2.1 Arbuscular mycorrhizal fungal spore density (in spore numbers g^{-1} of soil) at field sites in three agro-ecological zones: (SU) Sudan savanna, (NG) Northern Guinea savanna, and (SG) Southern Guinea savanna. Data are reported as averages and standard deviations for four replicate plots per field site. Non-significant differences between sites are shown by identical letters, determined using Fisher's Least Significant Difference (*LSD*) at the 5% level following one-way ANOVA.

Table 2.4. Arbuscular mycorrhizal fungal genera and species richness found at 24 sites in three ecological zones (SU: Sudan Savanna; NG: Northern Guinea Savanna and SG: Southern Guinea Savanna) of Benin

	SU	NG	SG	Sum of SU, NG, SG
Glomeraceae				
Glomus	23	18	19	30
Acaulosporaceae				
Acaulospora	10	10	8	12
Kuklospora	1	2	1	2
Gigasporaceae				
Gigaspora	2	1	0	2
Scutellospora	8	7	5	10
Entrophosporaceae				
Entrophospora	1	0	1	1
Ambisporaceae				
Ambispora	1	1	1	1
Paraglomeraceae				
Paraglomus	1	0	0	1
Total species richness	47	39	35	59

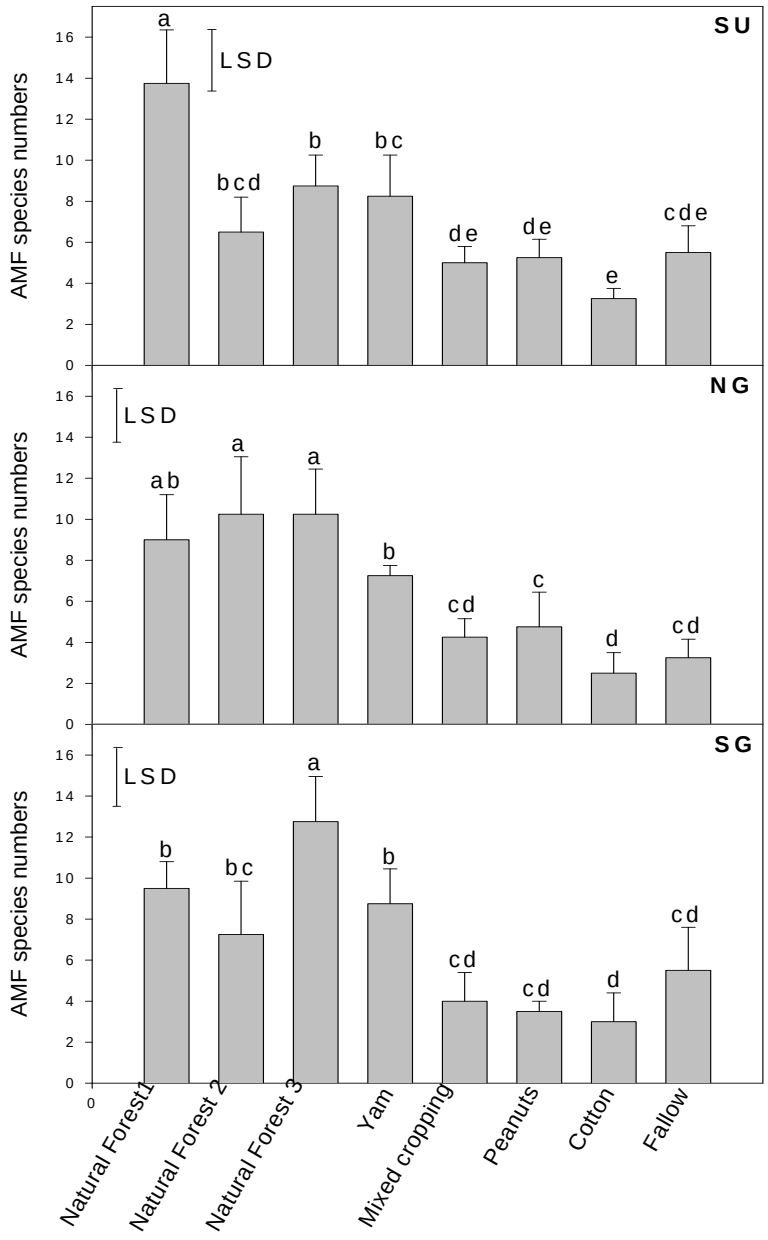

Fig. 2.2. Arbuscular mycorrhizal fungal species richness at field sites in three agro-ecological zones: (SU) Sudan savanna, (NG) Northern Guinea savanna, and (SG) Southern Guinea savanna. Data are reported as averages and standard deviations for four replicate plots per field site. Non-significant differences between sites are shown by identical letters, determined using Fisher's Least Significant Difference (*LSD*) at the 5% level following one-way ANOVA.

2.4.3. AMF species richness

A total of 59 AMF species were detected in soils sampled from the 24 sites in the study area (Table 2.4). Thirty species belonged to the genus *Glomus* in the family Glomeraceae, twelve species to *Acaulospora* and two species to *Kuklospora* in the Acaulosporaceae. There were ten *Scutellospora* and two *Gigaspora* species in the Gigasporaceae. One species each belonged to the families Entrophosporaceae, Archaeosporaceae, Ambisporaceae and Paraglomeraceae. The total number of AMF species detected was higher in the SU (47 species) than in the NG and SG (39 and 35, respectively; Table 2.4). A relatively greater proportion of particularly *Scutellospora* spp. and *Gigaspora* spp., but also of *Glomus* spp. and *Acaulospora* spp., was apparent in the SU than in the NG and SG.

2.4.4. AMF species richness and land use

AMF species richness was generally higher ($P<0.05$) in natural forest and yam field soils when compared to other field crop sites (Fig. 2.2). With one exception of yam fields in SU, species richness was higher than in other cultivated or fallow sites (Fig. 2.2). However, no difference in species richness was observed between any of the cotton, mixed cropping, peanut or fallow sites (Fig. 2.2).

Independent of the ecological zone, land cultivation negatively affected the species richness, particularly species of Gigasporaceae and sporocarp-forming *Glomus* species, such as *Glomus clavisporum*, *G. pachycaulis* and *G. taiwanense* (Tables 2.5, 2.6, 2.7 and 2.8). The number of species of Acaulosporaceae was also reduced, while a few *Glomus* species (e.g. *G. etunicatum*, *G. macrocarpum* and *G. intraradices*) and *Acaulospora scrobiculata* were less affected, with higher spore densities recorded under cotton production. These four species were recovered from most of the 24 sites under investigation.

Relationship between chemical soil parameters and spore density and species richness

Spore density, as well as species richness, were mostly positive correlated with soil organic carbon contents and soil pH ($P<0.05$). No significant correlation was observed, however, between available soil P and spore density or species richness (Table 2.9).

Table 2.5. Arbuscular mycorrhizal fungal species richness under various land use systems: natural forest savannas, cultivated fields, and fallows of three ecological zones (SU: Sudan Savanna; NG: Northern Guinea Savanna and SG: Southern Guinea Savanna) in Benin.

AMF family/genera and ecological zones	Natural Forest 1	Natural Forest 2	Natural Forest 3	Yam	Mixed Cropping	Peanut	Cotton	Fallow
Glomeraceae								
Glomus								
SU	13	10	10	8	3	8	5	7
NG	7	10	8	7	4	5	4	6
SG	9	9	8	10	5	3	4	6
Acaulosporaceae								
Acaulospora								
SU	4	6	2	7	2	4		2
NG	3	7	5	2	3	1	1	
SG	3	1	3	3	3	2	1	3
Kuklospora								
SU					1			
NG	1	2	1					
SG		1						
Gigasporaceae								
Gigaspora								
SU	1				1	1		
NG			1			1		
SG								
Scutellospora								
SU	6	1	4	2	3	1		1
NG	4	1	3	2		4		
SG	3	3	2	1				1
Entrophosporaceae								

Entrophospora						
SU		1		1		
NG						
SG			1			
Ambisporaceae						
Ambispora						
SU	1					
NG			1			
SG			1			1
Paraglomeraceae						
Paraglomus						
SU	1					
NG						
SG						

2.4.5. AMF in trap cultures

For unknown reasons, spore formation and mycorrhizal root colonization was low or even zero in most pots. After 10 months of trap culturing AMF propagation was low with only four AMF species having sporulated, and most pots remaining without mycorrhiza or spore formation, even after 24 months. Only spores of *Glomus etunicatum*, *G. claroideum*, *G. aggregatum*, a small-spored *Glomus* sp. and a few spores of *Gigaspora gigantea* were extracted during the first 10 months. After two years a further two species were recovered: *Paraglomus occultum* and *Acaulospora myriocarpa*. The latter species had not been detected in the field samples.

Table 2.6. Arbuscular mycorrhizal fungal species detected under various land use systems in the Sudan Savanna of Benin

AMF species	Natural Forest 1	Natural Forest 2	Natural Forest 3	Yam	Mixed cropping	Peanuts	Cotton	Fallow
Glomeraceae								
G. aggregatum						x		
Glomus etunicatum	x	x	x	x	x	x	x	x
G. macrocarpum	x	x	x	x		x	x	x
G. brohulti	x	x	x	x	x		x	x
G. claroideum	x		x		x			
G. aureum			x			x		
G. ambisporum	x		x			x		x
Glomus sp. WAG7[c]		x	x			x	x	
G. sinuosum	x			x		x		x
G. intraradices	x	x		x				x
G. mosseae								x
G. constrictum	x			x				
Glomus sp. WAG2[a]	x		x					
Glomus sp. WAG4[b]	x			x				
G. hyderabadense				x				
G. clarum		x						
G. taiwanense			x					
G. clavisporum	x							
G. geosporum	x	x						
Glomus sp. WAG5 [d]	x							
G. nanolumen		x						
Glomus sp. WAG3		x						
Glomus sp. WAG1[e]		x	x					
Acaulosporaceae								

Acaulospora scrobiculata	x	x	x	x	x	x		x
A. spinosa	x	x	x	x		x	x	x
A. elegans	x			x		x		
A. mellea		x			x			
A. excavata						x		
A. laevis				x				
A. longula		x		x				
Acaulospora sp. WAA1[f]	x	x		x				
Acaulospora sp. WAA2[g]				x				
A. morrowiae		x						
Kuklospora colombiana					x			
Gigasporaceae								
Scutellospora sp. WAS1	x	x		x	x			
Scutellospora verrucosa	x		x		x			
S. fulgida	x		x			x		
Scutellospora sp. WAS2					x			x
S. heterogama	x			x				
S. savannicola	x		x					
S. calospora	x							
S. nigra			x					
Gigaspora gigantea	x				x			
Gi. decipiens						x		
Entrophosporaceae								
Entrophospora infrequens		x		x				
Ambisporaceae								
Ambispora gerdemannii	x							
Paraglomaceae								
Paraglomus occultum	x							
	26	18	16	18	10	13	5	10
Total number of AMF	47							

species

[a] resembling *Glomus spinuliferum;* [b] resembling *Glomus coreomioides;* [c] resembling *Glomus microcarpum;* [d] resembling *Glomus tortuosum;* [e] resembling *Glomus rubiforme;* [f] resembling *Acaulospora scrobiculata;* [g] resembling *Acaulospora paulinae.*

Table 2.7 Arbuscular mycorrhizal fungal species detected under various land use systems in the Northern Guinea Savanna of Benin

AMF species	Natural Forest 1	Natural Forest 2	Natural Forest 3	Yam	Mixed cropping	Peanuts	Cotton	Fallow
Glomeraceae								
Glomus etunicatum	x	x	x	x	x	x	x	x
G. macrocarpum	x	x	x		x	x	x	x
G. brohulti	x	x	x	x		x	x	x
G. intraradices		x	x	x	x	x	x	
G. constrictum	x	x	x		x			x
G. claroideum			x					
G. sinuosum			x	x				
G. microaggregatum				x		x		x
Glomus sp. WAG3		x						x
G. versiforme				x				
Glomus sp. WAG7 [a]				x				
G. clavisporum	x	x						
G. taiwanense	x							
Glomus sp. WAG2 [b]	x							
G. aureum		x						
G. eburneum		x						
G. fasciculatum		x						
G. hoi			x					

Acaulosporaceae								
Acaulospora scrobiculata	x	x	x	x	x		x	
A. mellea		x	x			x		
A. laevis					x			
A. cavernata					x			
Acaulospora sp. WAA1[c]	x	x	x	x				
A. elegans			x					
A. spinosa	x	x	x					
A. excavata		x						
A. longula		x						
Acaulospora sp. WAA2		x						
Kuklospora kentinensis	x	x	x					
K. colombiana		x						
Gigasporaceae								
Scutellospora fulgida		x	x	x		x		
Scutellospora sp. WAS2	x			x		x		
S. savannicola						x		
S. heterogama						x		
Scutellospora sp. WAS1	x		x					
S. verrucosa	x		x					
S. pellucida	x							
Gigaspora decipiens			x			x		
Ambisporaceae								
Ambispora gerdemannii			x					
Number of AMF species	15	20	19	11	7	11	5	6
Total number of AMF species	39							

[a] resembling *Glomus microcarpum*; [b] resembling *Glomus spinuliferum*; [c] resembling *Acaulospora scrobiculata*

Table 2.8 Arbuscular mycorrhizal fungal species detected under various land use systems in the Southern Guinea Savanna of Benin

AMF species	Natural Forest 1	Natural Forest 2	Natural Forest 3	Yam	Mixed cropping	Peanuts	Cotton	Fallow
Glomeraceae								
G. etunicatum	x	x	x	x	x	x	x	x
G. macrocarpum	x	x	x	x	x	x	x	x
G. brohulti	x	x		x				x
G. intraradices	x	x	x	x	x			
G. fasciculatum	x			x				x
G. constrictum	x	x	x	x			x	
G. claroideum								x
G. geosporum						x		
G. aureum		x	x		x		x	
Glomus sp. WAG7 [a]					x			x
G. ambisporum	x							
G. sinuosum		x	x	x				
G. taiwanense			x	x				
G. clavisporum			x	x				
G. mosseae				x				
G. clarum	x							
G. pachycaulis		x						
Glomus sp. WAG2 [b]		x						
Glomus sp WAG6 [c]	x							
Acaulosporaceae								
Acaulospora scrobiculata	x		x	x	x	x	x	x
A. excavata			x	x	x			
A. spinosa	x		x					

A. mellea	x				x			
A. elegans				x				x
Acaulospora sp. WAA2 [e]						x		
A. laevis								x
Acaulospora sp. WAA1 [d]		x						
Kuklospora colombiana		x						
Gigasporaceae								
S. heterogama				x				x
Scutellospora sp. WAS1	x	x	x					
Scutellospora sp. WAS2	x	x						
S. fulgida		x	x					
S. verrucosa	x							
Entrophosporaceae								
E. infrequens			x					
Ambisporaceae								
Ambispora gerdemannii			x			x		
	15	14	15	14	8	6	5	10
Total number of AMF species	35							

[a] resembling *Glomus microcarpum*; [b] resembling *Glomus spinuliferum*; [c] resembling *Glomus arborense*; [d] resembling *Acaulospora scrobiculata*; [e] resembling *Acaulospora paulinae.*

Table 2.9 Correlation coefficients between soil chemical parameters and Arbuscular mycorrhizal fungal spore density and species richness from 24 sites sampled in four simultaneous replicates

Correlation factors	Correlation coefficient (r)	P value
P Acetic Acid x AMF spore density	0.13	0.18
P Acetic Acid x AMF species richness	0.17	0.09
P Citric Acid x AMF spore density	0.12	0.24
P Citric Acid x AMF species richness	0.19	0.05
Carbon(Humus) x AMF spore density	0.37	0.0002
Carbon(Humus) x AMF species richness	0.44	<0.0001
pH (H2O) x AMF spore density	0.54	<0.0001
pH (H2O) x AMF species richness	0.35	0.0005

2.5 Discussion

Our study amounts to the first comprehensive assessment of AMF species richness in the yam-growing region of West Africa, represented in this case by Benin. We detected a total of 60 AMF species (59 from field samples and *A. myriocarpa* additionally from trap cultures) belonging to eight AMF genera in soils from three separate ecological zones of Benin. This reflects results from studies in other tropical areas, such as India (Muthukumar and Udaiyan, 2000), Central America (Husband et al. 2002a; 2002b) and East Africa (Mathimaran et al., 2007), albeit with slightly higher species richness recorded in the present study. Data shown here may still represent an underestimation, however, as it is likely that not all AMF present will have sporulated at the time of sampling (see Bever et al., 1996; 2001), and especially since our trap culturing largely failed to support the field data. By using morphological tools for identification, AMF species richness may also be underestimated, because some species rarely, if ever sporulate, or the spores isolated from field are degraded and not suitable for identification purposes (Douds and Millner ,1999; Rodriguez et al., 2005). New data from ongoing studies further indicates that the timing of sampling in the current study, towards the end of the wet season, was not optimal, and at least partially explains why AMF propagation was largely unsuccessful (Tchabi et al. unpublished). However, the intensive sampling design used in the

current study will undoubtedly represent a high quality reflection of the occurrence and density of AMF species in the West African 'yam belt'.

Forty-seven AMF species were detected in the SU (~5 months wet season), while 39 were recovered from the NG (~7 months wet season) and 35 in the SG (~8 months wet season), respectively. The findings indicate that AMF species richness decreases with increasing length of wet season and towards the more humid tropics. However, 46 AMF species were detected from the SG + NG combined, similar to the number in the SU. Furthermore, species of the Gigasporaceae (mainly *Scutellospora,* but also *Gigaspora* spp.) became more numerous with increasing duration of annual dry season. Less obviously, *Acaulospora* and *Glomus* species numbers decreased with reducing duration of the annual dry season. Relatively high numbers of *Scutellospora* and *Acaulospora* spp. were also reported by Mathimaran et al., (2007) in a Kenyan savannah (with approximately 5 months annual rainfall) and also by Lekberg et al., (2007) in a Zimbabwean savannah (with approximately 5 months annual rainfall) in light textured soils. Indeed, most *Scutellospora* spp. has been described from warmer climates characterized by pronounced rainfall and a dry season (e.g. in Mediterranean and tropical and sub-tropical savannas, such as *S. savannicola*, *S. cerradensis*, *S. nigra*). In drier climates, with fewer rainfall months and lower total annual rainfall, *Gigasporaceae* tend to be represented by a small number of cosmopolitan species (Uhlmann et al., 2004; Bashan et al., 2007), if species of this family occur at all (Stutz and Morton, 1996). We conclude that SU has a high AMF species richness, and that a relatively long dry season (~7 months) does not negatively affect, but rather seems to stimulate species richness in this area of African savannas. It is also possible that species adapted to low water conditions, or species with a pronounced seasonal life cycle may be more competitive in SU, than in NG or SG (e.g. some *Scutellospora* and *Gigaspora* spp.). It would be interesting to further investigate the effect of rainfall and dry season length in the drier sub-Saharan savannas and deserts, where, under the driest conditions only a few *Glomus* and *Diversispora* spp., and rarely, an *Acaulospora* sp. can be expected (Friberg, 2001), to gain a better understanding of the adaptation of individual species along the climatic and vegetation gradient from tropical rain forest to the Sahelian desert.

Independent of the ecological zone, AMF species richness decreased with land use intensification, irrespective of duration of soil cultivation. The high species richness of the natural forest savannahs was not restored, however, by long-term fallows. Species of *Gigasporaceae* were most negatively affected following conversion from natural forest savannas into agro-ecosystems, and appeared most sensitive to recovery in the fallows, while several *Glomus* spp. and a few *Acaulospora* spp. were barely affected (Table 2.5) or reacted positively (e.g. *G. etunicatum* and *A. scrobiculata*). This observation concurs with the findings from temperate agro-ecosystems of Jansa et al., (2002; 2003) and Oehl et al., (2003; 2004; 2005), who observed negative correlations between land use intensification and soil disturbance, and the presence of *Scutellospora* spp., which is possibly related to the particularities in anastomosis processes of *Scutellospora* spp. (de la Providencia et al., 2005). Also, Menéndez et al. (2001) demonstrated that tillage and cereal monoculture negatively affected AMF species richness. As found by Oehl et al. (2003; 2004; 2005) for Central European regions, also in the sub-Saharan savannas the majority of sporocarpic *Glomus* spp. and *Entrophospora infrequens* were strongly affected by soil cultivation, becoming undetectable following the first year of arable land use, which, in the current study would correspond with yam cultivation (Table 2.5). It is possible that the persistence of such species as *G. etunicatum*, *G. macrocarpum, G. intraradices* and *A. scrobiculata* is related to their ability to rapidly colonize roots from spreading external hyphae (Kurle and Pfleger, 1994) or to the intensity and speed of spore formation (Oehl et al., 2003).

Spore density and species richness were not correlated to available P but were positively correlated with soil organic carbon and soil pH. Similar results were reported by Johnson et al., (1991) and Mohammad et al., (2003) who found that spore production increased with soil pH and organic carbon. Gryndler et al., (2006) also found that organic fertilizer application leads to increased external AMF mycelium development. No relationship between available P and spore density and species richness can be explained by the specific situation of our study, as a dramatic decrease in spore density and species richness was observed in the intensively managed cotton fields, compared to the natural forest savannas, despite the fact that soil P levels were similar at the time of sampling. However, it is well known that the response of AMF to available P is variable (Jasper et al., 1989) and, according to Neumann and George, (2004) and Subramanian et al., (2006), the application of P can influence spore production either positively or negatively.

Certainly, the period of time between forest clearance and cotton cultivation (4 years; Table 2.2), when other crops were cultivated without external fertilizer input, was already associated with species richness decline. In summary, the positive association between organic carbon contents and soil pH with spore density and species richness may reflect the change from natural ecosystems to crop production systems, particularly with application of acidifying fertilizers in the most intensive cropping system (Na Bhadalung et al., 2005). Neither parameter can be considered independently from each other for their impact on spore abundance and species richness (Coughlan et al., 2000).

Since in the mono-cropped fields several weed species were often present, AMF species recovered from these sites cannot unequivocally be identified as the AMF symbionts of the cultivated crop. To address such aspects, it would be necessary to apply molecular root analyses for single plant species. Also AMF dynamics within a single season cannot be deduced, but need to be interpreted in respect to long-term community dynamics in tropical agro- ecosystems. The natural forest savannas had a high AMF species and genus richness, but which remained relatively high for only one season, under yam, following forest clearance. Thereafter, the decline was precipitous and did not recover even after 7 years of natural fallow. We assume that at least some of the natural savannas - undisturbed for at least 25-30 years before sampling - had been used for agricultural purposes previously and thus, a relatively high AMF species richness had restored during this period. However, with the continuous erosion of fallow period length in West Africa and intensification of farming practices (IITA, 2006) it is likely that AMF communities will be subject to unrecoverable losses.

High levels of infective AMF propagules (Sieverding, 1991) and soil microbial activity are accepted as preconditions for sustainable low-input farming systems (Mäder et al., 2002), especially in the tropics (Oberson et al., 2004; Franchini et al., 2005). Present farming practices need to be studied with respect to the management of AMF, in order to better understand their importance, especially at the specific level. A common recommendation has often been the use of mixed culture or alley cropping systems with legumes to introduce nitrogen to the soil/plant system. However, our results indicated that mixed cropping of peanuts and maize had no effect on the AMF spore density and species richness when compared to peanuts alone. Moreover, the

decrease in AMF species richness after slash and burn and yam production was quite dramatic. Yam is usually grown in 70-120 cm high mounds heaped up following the high level of soil disturbance caused by the slash and burn of the forest, with further disturbance taking place at harvest. Such practices would certainly negatively affect both the AMF hyphal networks and the soil microbial biomass. With the change in cropping styles towards intensification, coupled with loss, or reduction of fallow periods as land becomes scarcer, there is need to assess the potentials of AMF and measures for maintaining AMF levels and communities for soil fertility and sustainable crop production. Therefore, besides yam being a highly important staple food crop in the study area, it is also particularly interesting in respect to AMF, as ongoing studies indicate it to be highly AMF dependent (personal observations). However, current soil and crop management strategies for yam would appear to have a strong negative impact on AMF communities, which will ultimately affect the AMF effectivity for the entire crop rotation. In order to improve the sustainability of yam production systems, it is suggested that future studies focus on the importance of AMF, and beneficial soil microorganisms in general, to yam production itself, in addition to other crops in the traditional rotational system.

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Chapter 3: Arbuscular mycorrhizal fungi associated with yam (*Dioscorea* spp.) in the Southern Guinea Savanna of West Africa

3.1. Abstract

In the southern Guinea Savanna of West Africa, yam (*Dioscorea* spp.) is a traditional staple food crop holding an important position in the prevailing farming systems. Traditionally, yam is cultivated as a first crop after forest clearing because, later in the crop rotation cycle, yam yields are declining substantially. The reason for this decline is still unknown as current knowledge on yam nutrient requirements and appropriate fertilizer management is limited and often contradictory. We hypothesized, therefore, that for nutrient acquisition yam may depend on an efficient symbiosis with arbuscular mycorrhizal fungi (AMF) left over from the preceding natural forest. We therefore investigated the AMF communities occurring in the natural forests and in the adjacent yam fields at several places in the southern Guinea Savanna of Benin. Soil samples were collected during the wet and subsequent dry seasons (October and February, respectively) and used for assessing the AMF community composition based on spore morphotyping. The same soil samples were used also as inocula for establishing trap cultures for AMF using *Sorghum bicolor* and yam (tissure culture plantlets of *D. rotundata* and *D. cayenensis*) as bait plants. AMF species richness, as determined directly in the soil samples from the field sites, was higher in the forests than in the neighboring yam fields (18-25 versus 11-16 spp, respectively). However, the species richness was found similar in both ecosystems if samples were taken from the trap cultures (29-36 spp). Trap cultures initiated with soil collected during the dry season exhibited a high arbuscular mycorrhiza (AM) root colonization, spore production and species richness (overall as much as 45 spp) whereas these parameters were all low in trap cultures initiated with samples from the wet season (2 spp). Using *S. bicolor* as bait plant, 37 AMF species were detected and with *D. cayenensis* and *D. rotundata* there were slightly less, namely 28 and 29 spp, respectively. AM root colonization, however, was much higher in yam than in sorghum (70-95% versus 11-20% of root length, respectively). After eight

months trap culturing, the tuber dry weights of the mycorrhizal yam was higher than that of the non-mycorrhizal controls. Our study demonstrates for the first time that yam is colonized with a multitude of indigenous AMF. It is likely, therefore, that AMF, probably inherited from the preceding natural forest, play an important role for yam nutrition, particularly in low-input traditional farming systems of West Africa.

Key words: biodiversity, arbuscular mycorrhiza, spore, sustainable agriculture, tropics, yam tubers.

3.2. Introduction

Yam (*Dioscorea* spp.) is a tuber crop widely cultivated in the humid and sub-humid lowland regions of West Africa, the Caribbean, the Atlantic coastal line of tropical South America and tropical Asia (Onwueme and Haverkort 1991; Sotomayor-Ramirez et al. 2003; Suja et al. 2003; Baimey et al. 2006; Egesi et al. 2007). In West Africa, yam is the most important tuber crop in terms of area coverage and a key staple food, particularly in Nigeria, Benin and Togo (Kalu and Erhabor 1990; Olasantan 1999; Ile et al. 2006; Baimey et al. 2006). More than 90% of the global production (40 million tons fresh tubers/year) is produced in West Africa (Ravi et al. 1996; FAOSTAT 2007). Additionally, yam plays an important cultural role in the traditions of West Africa (Coursey 1983, Orkwor 1998). Regionally, yam production is relatively static, while the area under production is steadily increasing (IITA, 2006), indicating a gradual decrease in productivity. Major constraints for yam production are presumed to be low soil fertility, e.g. due to macro-and micronutrient deficiency (O'Sullivan and Ernest 2007), nutrient leaching, or damage by plant parasitic nematodes and virus diseases (Odu et al. 2004; Baimey et al. 2005; Egesi et al. 2007). In terms of nutrient use, yam is a demanding crop and, consequently, it is planted traditionally at the beginning of the rotation cycle following forest clearing or long periods of fallow (Carsky et al. 2001; O'Sullivan and Jenner 2006). With increasing land use intensity, demographic pressure and reducing land availability, suitable land becomes gradually scarcer. Furthermore, cultivated lands are being continuously exposed to erosion, leading to soil degradation (Maduakor et al. 1984; Carsky et al. 2001; Salako et al. 2007). Studies conducted to explore the efficiency and economics of inorganic fertiliser application to yam are often

contradictory. For example, Ferguson (1973) found that *Dioscorea alata* did not respond to phosphorus (P) fertilizer application, particularly when seed yam setts of 100 g or more were used. Reasons for the lack of response were accorded to the assumption that the accumulated P in seed yam tubers of more than 100 g was sufficient to supply nutrition for the newly sprouting vines during early growth stages or that yam may depend on AMF for P aquisition. Similar, fertilizer application trials showed that yam yields, in the Southern Guinea savanna of Benin, are not increased by application of ammonium super phosphate (Baimey 2005). Sotomayor-Ramirez et al. (2003) found that the application of micronutrients was most important for increased yam (*D. alata* and *D. rotundata*) production, while a moderate application rate was adequate for macronutrients. Ahn (1993) attributed the moderate or limited need of P by yam to possible mycorrhizal benefits, as did Vander Zaag and Fox (1980), when observing that *D. esculenta* and *D. rotundata* did not respond to P fertilization in field experiments in Hawaii and Ghana. Valenzuela and deFrank (1995), in a review, also speculated that yam may depend on an effective mycorrhizal association to meet its P requirements.

Kowledge on the mycorrhizal status of yam has remained imprecise so far, with only limited information available (Ahulu et al. 2005). Micropropagated *D. rotundata* cv. TDr131 and cv. TDr179, however, were successfully inoculated with a mixture of AMF species when transferring from humidity chambers to field conditions (Uchendu 2000). Nevertheless, the extent of the association of yam with AMF, to date remains largely limited (Dare et al., 2007).

In a recent study, we investigated AMF diversity in the 'yam belt' of West Africa in three ecological zones differing by an increasingly prolonged dry season from South to North, from the Southern Guinea Savanna, to the Northern Guinea Savanna, to the Sudan Savanna, selecting in each zone a set of sites with 'natural' forest savanna, fallow and cultivated land differing in land use intensity (Tchabi et al. 2008). A high diversity of AMF was revealed at the 'natural' sites with a strong decrease of AMF species richness in the crops following yam in the crop rotation. In the present study, we focused on AMF associated with yam restricting the study area to the southern Guinea savanna (SGS) of Benin. Based on spore morphotyping, we determined the AMF species composition in three yam fields and, the adjacent natural savanna forest. Moreover, we set up trap cultures with soil inocula from the sites and using yam as bait plant for

specifically identifying yam AMF symbionts. For comparison, trap cultures were also established with a commonly used bait plant, *Sorghum bicolor*. In all these trap cultures, AM root rolonization, spore density and species richness were determined as well as yam shoot and tuber growth.

3.3. Materials and methods

3.3.1. Study area and study sites

The Southern Guinea savanna (SG) of Benin is situated between 7° and 9° N latitude in the sub-humid tropical savanna about 400 m asl. The soils are termed 'ferruginous' and generally classified as Ferralsols according to FAO (2007). The area is characterized by a wet season between April and October and a dry season between November and March, with a variant towards the southern latitude, where a short dry season intervenes in the wet season around August. The mean annual rainfall is 1000-1200 mm, with a vegetation composed mainly of Combretaceae, Mimosaceae, Fabaceae and grass layers of Poaceae (mainly *Andropogon gayanus*) (Adjakidje1984; Adjanohoun 1989).

Six sites were selected for this study: three natural forest sites (nf1-nf3), undisturbed for 25-30 years; and three fields cultivated with yam immediately following forest clearance (yf1-yf3). The sites were selected for the close proximity of the yam fields with the selected natural forests (Table 3.1).

Table 3.1 Geographic position of study sites.

Sampling sites	Geographic position
Natural Forest 1 (nf1)	07° 45. 739N; 002° 27. 519E
Natural Forest 2 (nf2)	07° 57. 217N; 002° 26. 935E
Natural Forest 3 (nf3)	08° 19. 661N; 001° 51 340 E
Yam field 1 (yf1)	07° 49. 114N; 002° 14. 519E
Yam field 2 (yf2)	07° 55. 111N; 002° 10.507E
Yam field 3 (yf3)	08° 19.730N; 001° 51. 332E

3.3.2. Soil sampling

Soils were sampled twice at each site: first towards the end of the wet season (September-October 2004), when yam plants in the field were approximately 5-6 months old and harvests were beginning (harvest period of yam is September to January in the study area and in West Africa in general, Ile et al. 2007). The second sampling occurred during the dry season (February 2005) following the yam harvest and when vegetation was dry. For each sampling occasion and site four replicate quadrant plots (100 m^2) were determined and six soil cores taken using a 6 cm diameter corer, to a depth of 20 cm. The six randomly located soil-core samples per quadrant were combined into one composite sample to constitute a replicate from per site for each occasion. Samples were air-dried on an open bench in the greenhouse for 72 hours at the International Institute of Tropical Agriculture (IITA) station in Abomey-Calavi, Benin, maintained in the refrigerator at 4° C for two weeks and transferred to the Institute of Botany in Basel, Switzerland.

Table 3.2 Selected chemical soil parameters at study sites.

Sampling sites	Available P (Sodium acetate) mg kg^{-1}		Available P (Citrate) mg kg^{-1}		Organic C g kg^{-1}		pH (H_20)	
	w.s.	d.s.	w.s.	d.s.	w.s.	d.s.	w.s.	d.s.
Natural Forest 1 (nf1)	52.8	74.2	83.0	144.1	24.9	26.1	6.7	6.8
Natural Forest 2 (nf2)	272.9	269.8	375.5	358.0	44.1	37.7	7.2	7.2
Natural Forest 3 (nf3)	28.8	21.8	34.9	30.6	20.3	23.8	6.5	6.9
Yam field 1 (yf1)	8.7	6.1	8.7	8.7	9.3	9.9	6.1	6.5
Yam field 2 (yf2)	10.9	8.7	13.1	13.1	16.8	15.1	6.7	6.7
Yam field 3 (yf3)	6.5	3.9	8.7	13.1	6.4	7.5	6.2	6.3

Study sites were natural forests (nf1-3) undisturbed since more than 25-30 years and yam fields (yf1-3) sampled during the first year after forest clearance. Soil samples were taken either in wet season (w.s.) or in dry season (d.s.).

3.3.3. Soil analyses

Each air-dried soil sample was divided equally into three sub-sets. Each separate sub-set per replicate, was used to: a) determine selected soil chemical parameters (pH, organic carbon and available phosphorus (see Table 3.2) in the "Laboratory Dr. Balzer", Wetter-Amönau, Germany), according to standard methods (Oehl et al., 2005); b) isolate and identify AMF spores (see below): c) to establish AMF trap cultures (see below). For three sites (nf1, nf2 and yf3), some spore data from the wet season field samples taken were already shown in a previous study (Tchabi et al., 2008).

3.3.4. Trap cultures

The first trap culture experiment was established using soil sub-sets from each replicate sample per site from end of the wet season. Four trap culture pots (pots: 20 cm x 20 cm x 30 cm) were created for each site according to Oehl et al., (2003), one each per field plot replicate which, including four non-inoculated control pots, totaled 28 trap culture pots. For each pot, 4 kg of

substrate was used, comprised of a sterilized 3:1 (wt/wt) mixture of Terragreen® (a calcined granular attapulgite clay mineral, American aluminium oxide, oil dry US special, type III R, >0.125mm; Lobbe Umwelttechnik, Iserlohn, Germany) and quartz sand (Alsace quartz sand, 5% of free silica, Smurfit Company, France), respectively. The chemical composition of the substrate was 0.3 % organic matter, 10 mg kg^{-1} and 1480 mg kg^{-1} easily and heavily available phosphorus (P extracted with sodium acetate and citrate, respectively), 191 mg kg^{-1} easily available potassium (K extracted with sodium acetate), and pH 5.8. A 180 g sample, divided equally into three sub-portions of soil inoculum, was placed in each trap culture pot as three lines on the surface of the 3 kg substrate, which was then covered with the remaining 1 kg of trap culture substrate. A 20-mm-thick drainage mat had been placed at the bottom of each pot (Enkadrain ST; Colbond Geosynthetics, Arnhem, the Netherland). Five one-week old *Stylosanthes guianensis* plants and four one-week old *Brachiaria humidicola* plants were alternatively and equidistantly planted per pot along the three lines of the inoculum. Automated watering systems (Tropf-Blumat; Weninger GmbH, Telfs, Austria) were used to irrigate all trap cultures, which were maintained in a greenhouse in Basel for 24 months under day:night regimes of 12h:12h photoperiod and 25:21°C temperature, with a mean relative humidity of 65 ± 5%. During the first year, trap plants were trimmed to about 4 cm above the substrate level at two months intervals and at 6-month intervals during the second year.

In order to specifically detect potential AMF symbionts associated with yam, a second trap culture experiment was established using the dry season soil samples. For this experiment, the yam cultivars *Dioscorea rotundata* cv TDr89/02461 and *Dioscorea cayenensis* cv TDc98-136 were used as host plants, and – for comparison – additionally *Sorghum bicolor*. The methodology was the same as above with a little modification. 1 L pots, 800 g of substrate and 50 g of inoculum were used. Pots were established and arranged as above, except that only one plant was used per pot, to unequivocally attribute the sporulating AMF species to its host. In order to compare the two trap culture experiments, four additional pots inoculated with wet season soil samples of field site YF2 were also included in this second experiment. Thus, including four non-mycorrhizal controls, 96 trap cultures were established in total. These trap cultures were maintained and irrigated for eight months as indicated above with the exception that trap plants were only cut once at the end of the experiment.

3.3.5. Sampling of the trap cultures

At 4, 6 and 8 (and – for first trap culture experiment – after 24) months, two separate soil cores of in total 30 cm^3 (core diameter of 1.5 cm and sampling depth 10 cm) were removed from each pot for the extraction of AMF spores and assessment of root colonization. The initial rate of root colonization and spore formation was determined after 4 months. In the trap cultures with the two yam cultivars as host plants, yam shoot and tuber growth was determined at the end of the experiment after 8 months.

3.3.6. Source and acclimatization of yam plantlets

In-vitro tissue culture plantlets of *D. rotundata* and *D. cayenensis* were obtained from IITA-Ibadan, Nigeria. They had been multiplied under *in-vitro* conditions by sub-culturing nodal segments from established *in-vitro* plantlets under laminar flow in culture test-tubes containing a specific yam multiplication medium (Ng, 1988; 1992; 1994). Plantlets were regenerated in a culture room with 12 hours photoperiod, 3000 lux light intensity, at 27 ± 1°C and 70 ± 5% relative humidity.

Plantlets were conveyed *in vitro* to Basel, Switzerland, de-flasked upon arrival and received three weeks post-flask acclimatization in a covered tray (50 cm x 30 cm) with 30 planting holes (each 16 cm^3 of volume). Plantlets were de-flasked into holes (one plantlet per hole). Each hole was filled with a substrate (Peat: vermiculite: Quartz sand: 2:1:1 w/w/w) mixture of sterilized peat, vermiculite (GERMEX, Vermica AG, Switzerland) and Quartz sand (Quartz d'Alsace, 5% of free silica; Smurfit Company, France). The chemical composition of this acclimatization substrate was 4.5 % organic matter, pH 5.0 (H_2O), 220 and 703 mg g^{-1} easily and heavily available P (P-natrium acetate and P-citrate, respectively and 717 mg g^{-1} easily available potassium (K-natrium acetate). The plantlets were then watered and the tray covered with a double white and transparent cover system (consisting of a thin plastic sheeting and a solid, plastic cover beneath) to maintain a consistently high relative humidity (70 ± 5 %), but which allowed air to circulate under the cover. The plantlets were maintained in the greenhouse with a day:night regime of 12h:12h photoperiod, 25:21°C temperature, and a mean relative humidity of

65 ± 5%. The plantlets were irrigated once to twice per day. At three days, the plastic cover was steadily removed for increasingly longer periods (6-12 hours) per day over the following week, before the cover system was completely removed at three weeks. The plantlets were transplanted one week later into the inoculated trap culture pots.

3.3.7. AMF spore isolation and identification from field and trap culture soil.

AMF spores from field samples or from trap cultures were extracted by wet sieving and sucrose density gradient centrifugation after Oehl et al., (2003). For this purpose, 25 g air-dried field soil samples or 250 ml trap culture substrate were suspended in 300 ml of water using a 500 ml beaker. The soil suspension was passed through 1000-, 500-, 125-, 80- and 32-µm sieves to discriminate particles. The 1000- and 500-µm µm sieves were checked for sporocarps, spore clusters and large spores adjacent to or inside roots. The contents of the 125-, 80- and 32-µm were layered onto a water-sucrose solution (70% ‹wt/vol›) gradient and centrifuged at 2000 tours/min for 2 minutes. After centrifugation, the supernatant was passed through the 32-µm sieve, washed with tap water, and transferred to Petri dishes. Spores, spore clusters, and sporocarps obtained from all sieves were transferred into Petri dishes, counted for each sample using a dissection microscope (Olympus SZ12) at up to x 90 magnification. The abundance of spores (= spore density) in a field sample was expressed as the number of AMF spores g^{-1} of soil (field samples) or mL^{-1} (of trap culture) substrate.

For species identification, healthy spores were mounted on glass microscope slides and stained with polyvinyl-acid-glycerol or polyvinyl-lactic acid-glycerol mixed 1:1 (vol/vol) with Melzer's reagent (Brundrett et al., 1994). The spores were examined under a compound microscope (Zeiss; Axioplan) at up to x 400 magnification. Identification was based on current species descriptions and identification manuals (Schenck and Pérez, 1990; International Culture Collection of Arbuscular and Vesicular-Arbuscular Endomycorrhizal Fungi, INVAM: http://invam.caf.wvu.edu). The relative abundance of each AMF species was recorded on a sacle: 0-3% (rare), only a few spores from a specific species were found; 3.1-20 % (frequent), spores from a specific species were found frequently; > 20% (abundant), the spores from a specific species were dominant among others.

3.3.8. AMF root colonization

The initial rate of root colonization by AMF was determined from trap culture samples taken at 4 months after planting. Therefore, roots obtained during wet sieving of spores were collected from the sieves, and the mycorrhizal structures were stained according to Brundrett et al., (1996) with trypan blue. The gridline-intersect technique (Giovannetti and Mosse, 1980) was then used to assess colonization.

3.3.9. Calculation and statistical analysis

For the field soil analyses, AMF spore density, as spore numbers g^{-1} of soil, and species richness (= species numbers) are recorded as mean total numbers per study site, per sampling occasion and - if useful – as sum of both dates. In the trap cultures, AMF spore density, as spore numbers per mL^{-1} substrate, and species richness are also recorded as mean total numbers per study site. The number of AMF species found to be symbiotic with both *Dioscorea* cultivars and with *S. bicolor* are presented. For the root length colonized by AMF, the mean percentage colonization is recorded per site. Prior to further analysis, in order to provide homogeneity of variance, data on spore numbers per gram (field spores) or per ml (trap culture spores) were log(x+1) transformed and mycorrhizal colonization were arcsin(x/100) transformed. Differences in spore density, species richness and mycorrhizal root colonization between field sites and sampling sites were separated using Fisher's least significant difference (LSD) at $P \leq 0.05$ after one-way ANOVA analysis (SAS program, version 9.1 package; SAS 2005).

3.4. Results

3.4.1. AMF spores and species at field sites

Between sites, AMF spore density varied from 2-13 spores g^{-1} of wet season soil (Fig.3.1 A). For dry season soil, mean spore density varied also between sites (3-17 spores g^{-1}; Fig. 3.1 B). Spore density, however, was less variable in soils from the forest (6-11 g^{-1} soil) than from yam fields (2-17 spores g^{-1} soil). No correlation was detected between spore density at field sites and any of the edaphic factors analyzed (pH, available phosphorus and organic carbon).

A total of 40 AMF species belonging to eight genera of six families were identified directly from the soil samples collected from field, without sitting trap culture (Table 3.3). Of the 40 species, 19 belonged to Glomeraceae and 9 each to Acaulosporaceae and Gigasporaceae. Entrophosporaceae, Paraglomeraceae and Ambisporaceae were each represented by one species (Table 3.3). Of the 40 species detected, seven could not be attributed to a described species and may represent new, undescribed species (*Glomus* spp. WAG1, WAG2, WAG3 and WAG4, *Acaulospora* spp.WA1 and *Scutellospora* spp. WAS1, *Scutellospora* spp. WAS2).

For the forest soils, the species richness was similar level for both seasons, while species richness in yam fields was relatively higher from soil sampled during the wet season than in the subsequent dry season (Fig. 3.2 and Table 3.3). The cumulative species numbers, including all species found either in the wet or in the dry season at the sites, also show a decline in AMF species richness following land-use change from natural forests into low-input yam production within one year after forest clearance (Fig. 3.2C). No correlation was found between species richness at field sites and any of the edaphic factors analyzed (pH, available P and organic C; data not shown). Twenty species were identified during the study, with 12 exclusive to the forests and 8 exclusive to the yam fields. In the yam field, *Glomus etunicatum* and *Acaulospora scrobiculata* dominated the spore populations, while sporocarpic *Glomus* spp. such as *G. sinuosum*, *G. clavisporum*, *G. taiwanense* and *G. pachycaulis* were more frequently found in the forests than in the yam fields. *Acaulospora* species were more frequently recorded in the forest samples than in the yam field samples.

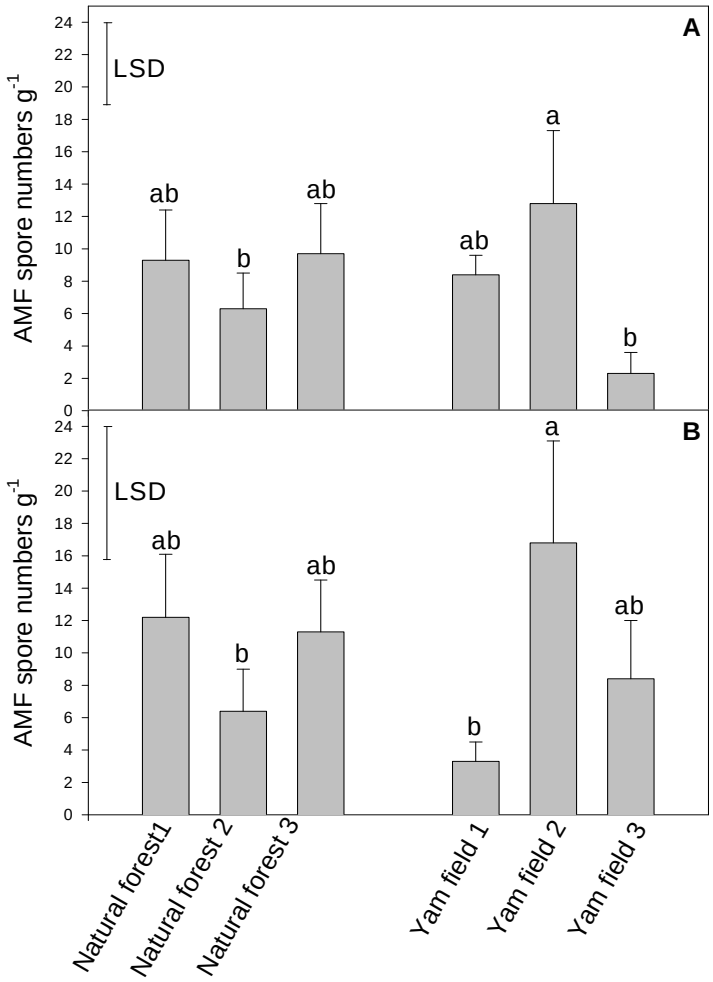

Fig. 3.1 AMF spore density presented as spore numbers g^{-1} in field soil samples collected from yam natural forests and adjacent yam fields in the southern Guinea savanna of Benin in the wet season (September-October 2004, A) and in the subsequent dry season (February 2005, B). Data are reported as averages and standard deviations for four replicate plots per field site. Non-significant differences between sites are shown by identical letters, determined using Fisher's Least Significant Difference (*LSD*) at the 5% level following one-way ANOVA.

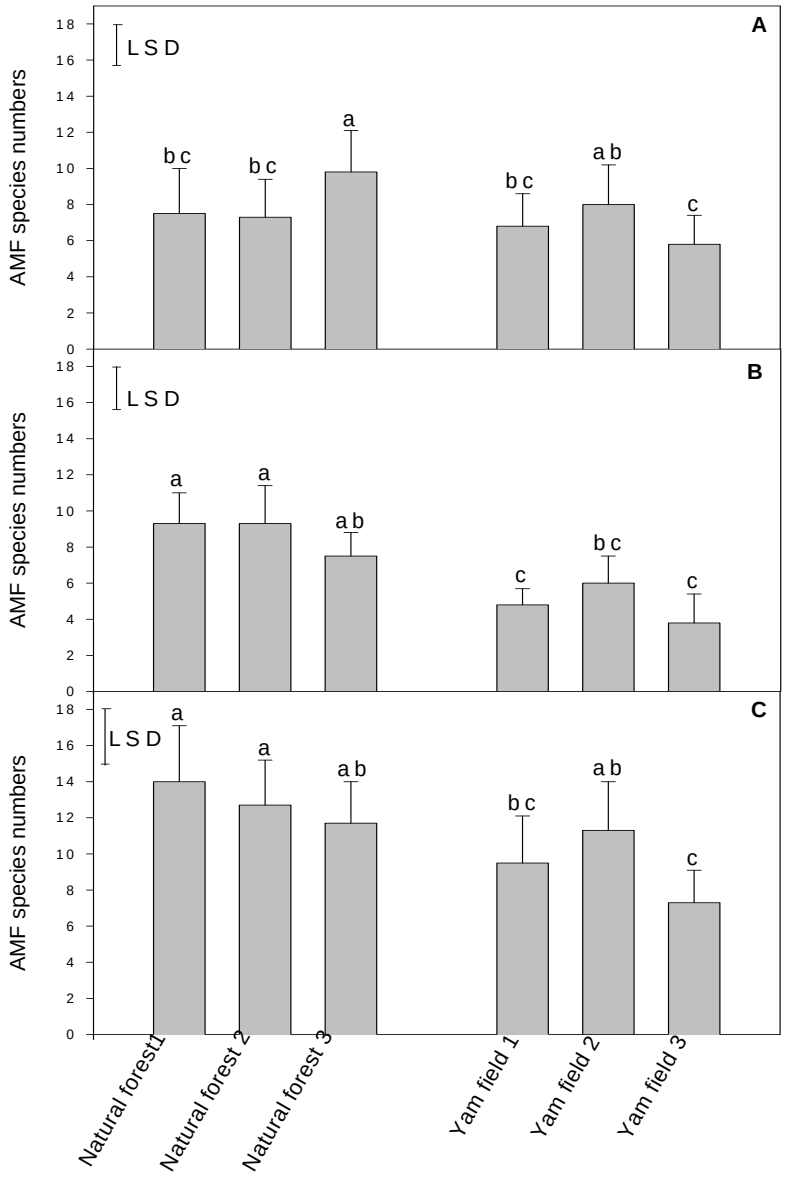

Fig. 3.2 AMF species richness in field soil samples collected from yam natural forests and adjacent yam fields in the southern Guinea savanna of Benin in the wet season (September-October 2004, A), in the subsequent dry season (February 2005, B), and cumulative from both seasons (C). Data are reported as averages and standard deviations for four replicate plots per field site. Non-significant differences between sites are shown by identical letters, determined using Fisher's Least Significant Difference (*LSD*) at the 5% level following one-way ANOVA.

Table 3.3 AMF species at field sites. Soils were taken from three natural forests (nf1-3) and three yam fields (yf1-3) in the wet season (w.s.) and in the subsequent dry season (d.s.)

AMF species	Natural forests						Yam fields					
	nf1		nf2		nf3		yf1		yf2		yf3	
	w.s	d.s.	w.s	d.s.	w.s	d.s.	w.s	d.s.	w.s	d.s.	w.s	d.s.
Glomeraceae												
Glomus etunicatum	●	●	●	●	●	●	⬤	⬤	⬤	⬤	⬤	⬤
G. macrocarpum	●	●	●	●	●	●	●	●	●	●	●	●
G. intraradices	●	●	●	●	●		●	●				
G. sinuosum	●	●			●				○			○
G. brohultii	●	●			●					●		
G. constrictum	●		●		●				○		○	
G. clavisporum			●	●	●				○			
G. taiwanense			●			●			○			
Glomus sp. WAG2 [a]	●										○	
Glomus sp. WAG7 [b]	●		●		○							
G.pachycaulis	●	●										
G. versiforme				○		○						
G. hoi				○		○						
G. mosseae				○		○						
Glomus sp. WAG3					○	○						
G. ambisporum											○	
G. fasciculatum										○		
G. claroideum											○	
Glomus sp. WAG1 [c]												○
Acaulosporaceae												
Acaulospora sp. WAA1 [d]	●	●		●			●		●	●		
Acaulospora scrobiculata		●	●		●		⬤	⬤	⬤	⬤	⬤	⬤
A. spinosa			●	●	●	●			●	●		
Kuklospora colombiana	●			●	●			●				
A. mellea						○	●		●	●		

A. laevis		○			○							
A. excavata			●	●								
A.elegans				●		●						
A. morrowiae				○								
Entrophosporaceae												
Entrophospora infrequens			○		○		○					
Gigasporaceae												
Scutellospora fulgida	●	●	●	●			●	●	●		●	
Scutellospora sp. WAS1	●	●	●		●		●	●	●	●	●	●
Scutellospora sp. WAS2	○	○				○	○	○				
Scutellospora verrucosa		○		○		○						
S. pellucida				○								
S. heterogama							○					
S. savannicola							○					
Gigaspora decipiens							○					
Gi. gigantea							○					
Paraglomaceae												
Paraglomus occultum		○				○		○				
Ambisporaceae												
Ambispora gerdemannii				○	○					○		
Total species numbers	14	14	13	17	16	13	14	9	12	10	9	6
Total species numbers per site	18		23		25		16		15		11	
Total species numbers per system	32						28					
Total species numbers in field samples	40											

[a] resembling *Glomus halonatum*; [b] resembling *Glomus aureum*; [c] resembling *Glomus rubiforme* (legend continue)

[d] resembling *Acaulospora rehmii.* The relative abundance of each AMF species was recorded following the scale: (rare): ○ = 0-3% or only a few specimens found; f (frequent): ● 3.1-20 %; (abundant): ⬤ > 20%.

Table 3.4 AMF species recovered on *Sorghum bicolor*, *Dioscorea cayenensis* and *Dioscorea rotundata* in trap cultures set up from field soils sampled in the dry season

AMF species	*Sorghum bicolor*		*Dioscorea cayenensis*		*Dioscorea rotundata*	
	Natural forests	Yam fields	Natural forests	Yam fields	Natural forests	Yam fields
Glomeraceae						
Glomus etunicatum	●●	●●	●●●	●●●	●●●	●●●
G. macrocarpum				●	●	
G. intraradices	●●●	●●●	●●	●●	●	●●
G. sinuosum	●●	●●		●		●
G. brohultii	●					
G. constrictum	●					●●
G. clavisporum	●		●			●●
G. taiwanense				●		
		●				
Glomus sp. WAG2 [a]						
Glomus sp. WAG7 [b]	●●		●	●●	●	●
G. pachycaulis						
G. versiforme	●		●			●●
G. hoi	●●	●●●	●	●	●	●
G. mosseae	●	●●●		●	●	●
Glomus sp. WAG3						
Glomus ambisporum		●				
G. fasciculatum		●	●	●●		
G. claroideum	●●●	●●●	●			
Glomus sp. WAG1 [c]						
G. tortuosum	●					
G. eburneum	●			●	●	●●●
Glomus sp. WAG4 [d]	●		●●	●●		

		●				
G. diaphanum	●					
G. aggregatum	●●●		●	●●	●	●
		●●●				
Glomus sp. WAG5 [e]						●
		●				
Glomus sp. WAG6 [f]	●			●		
Acaulosporaceae						
Acaulospora sp. WAA1 [g]	●●	●	●	●	●●	●
Acaulospora scrobiculata	●●●		●●●	●●●	●●●	●●
		●●				
A. spinosa	●	●	●●●			●
Kuklospora colombiana	●	●●	●			
A. mellea			●		●	●
A. laevis			●			
A. excavata						
A.elegans	●					
A. morrowiae					●	
		●				
A. rehmii				●	●●●	●●
A. dilatata		●				
A.caulospora sp. WAA3 [h]	● ●		●			
A. longula			●	●	●	
A. undulata				●		
Acaulospora sp. WAA2	●				●	
K. kentinensis		●●●		●	●	●
Kuklospora sp. WAK1		●				
Entrophosporaceae						
Entrophospora infrequens	●●		●●●		●●●	
Gigasporaceae						
Scutellospora fulgida	●			●		

Scutellospora sp. WAS1						
Scutellospora sp. WAS2						
S. verrucosa						
S. pellucida					●	
S. heterogama						
S. savannicola						
Gigaspora decipiens						
Gi. gigantea		●				●
Paraglomaceae						
Paraglomus occultum	●	●●	●	●	●●	●
Ambisporaceae						
Ambispora gerdemannii		●				
Archaeosporaceae						
Intraspora schenckii	●					●
Number of species per system and trap plant	29	23	19	21	20	22
Number of species per plant species	37		29		28	
Total number of species in trap cultures	45					

*Spores detected in the trap cultures after 4, 6, 8 months: first, second and third dot (●), respectively. [a] resembling *Glomus halonatum*; [b] resembling *Glomus aureum*; [c] resembling *Glomus rubiforme*; [d] resembling *Glomus tenue*; [e] resembling *Glomus tortuosum*; [f] resembling *Glomus arborense*; [g] resembling *Acaulospora rehmii*; [h] resembling *Acaulospora elegans.*

3.4.2. Trap cultures: root colonization, spore density and species richness

AMF root colonization was zero to insignificant in the wet season soil trap cultures (data not shown), but highly colonized in the trap cultures inoculated with dry season soil (Fig. 3.3). At four months after initiation, AMF root colonization was particularly high in both yam species (73-94 % in *D. cayenensis* and 78-95 % in *D. rotundata*), compared to *S. bicolor* (11-20 %). For

both yam cultivars, AMF root colonization was relatively higher in the trap cultures inoculated with forest soils than those with soil from the yam fields.

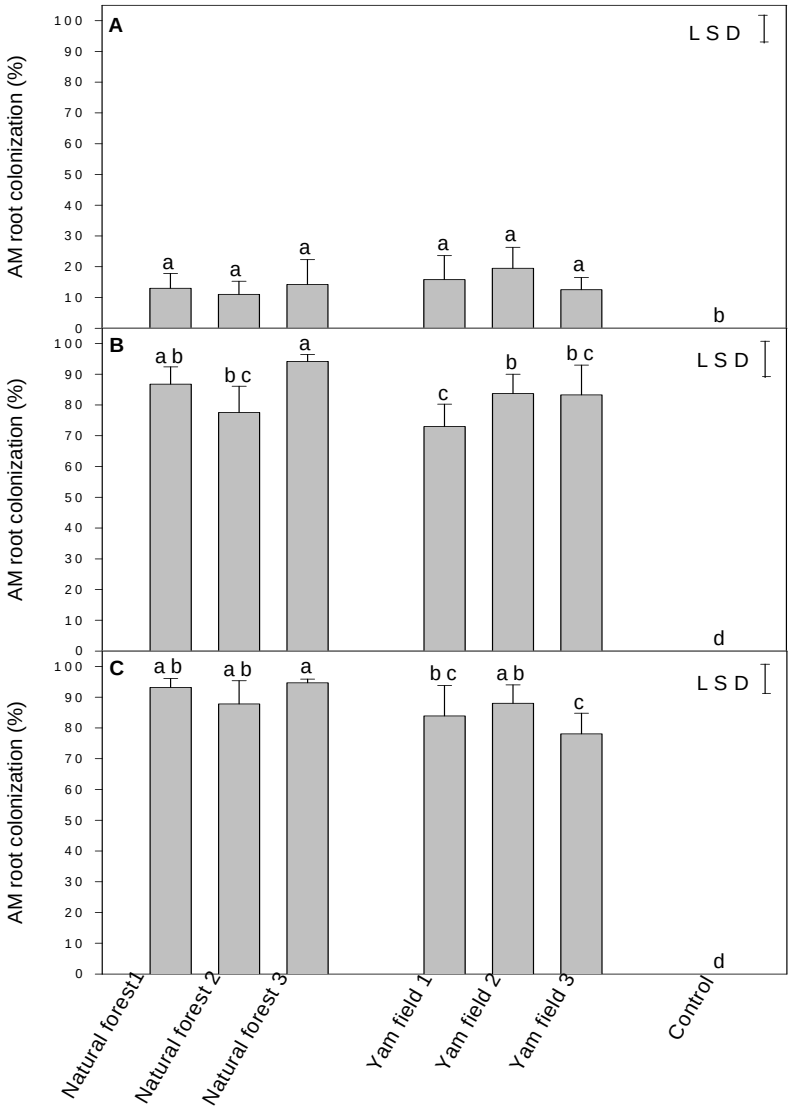

Fig. 3.3 AM root colonization (%) of *Sorghum bicolor* (A), *Dioscorea cayenensis* (B) and *D. rotundata* (C) following four months cultivation in trap cultures using dry season field soils from three forest and three yam field sites as inocula, and including a non-mycorrhizal control. Data are reported as averages and standard deviations for four replicate plots per field site. Non-significant differences between sites are shown by identical letters, determined using Fisher's Least Significant Difference (*LSD*) at the 5% level following one-way ANOVA.

Spores recovered from trap cultures inoculated with wet season soils were zero to insignificant (data not shown), while species abundantly sporulated in the trap cultures inoculated with dry season soils. Interestingly, despite the low mycorrhizal root colonization, spore numbers were higher with *S. bicolor* (12-94 ml^{-1} of substrate) than with *D. cayenensis* (0.1-17 ml^{-1} of substrate) or *D. rotundata* (1-34 ml^{-1} of substrate) as hosts (Fig. 3.4). The most frequently, most abundantly and first sporulating species were *G. etunicatum* and *A. scrobiculata* in the majority of the trap cultures. These two species had greater absolute and relative spore densities in the yam fields than in the forest sites (Table 3.3). High phosphorus availability in the forest soils had no obvious negative impact on root colonization or on spore production in the trap cultures (Table 3.1; Figs. 3.3 and 3.4).

In the trap cultures inoculated with wet season soils only *G. etunicatum* produced spores of any quantity over 8 months (sites NF1, YF1 and YF3). In one pot of YF3, a few spores of *Gigaspora gigantea* were also isolated, too. In the trap cultures inoculated with dry season soils, 45 AMF species, representing 9 genera and 7 families were recorded (Table 3.4). The greatest number of AMF species recovered were of the family Glomeraceae (22 species) followed by Acaulosporaceae (16), Gigasporaceae (3), Ambisporaceae, Archaeosporaceae, Entrophosporaceae and Paraglomeraceae (1 species each). Of the 45 species, 16 were not identified to species level in the corresponding field samples (Table 3.3). These were mainly species that form small and rapidly degrading spores such as *G. eburneum*, *A. undulata* and *Intraspora schenkii* or species such *G. aggregatum*, *Kuklospora kentinensis* and *K. colombiana*, which are usually difficult to distinguish from similar species (e.g. *G. intraradices*, *A. scrobiculata* and *A. dilatata*, respectively) in field samples.

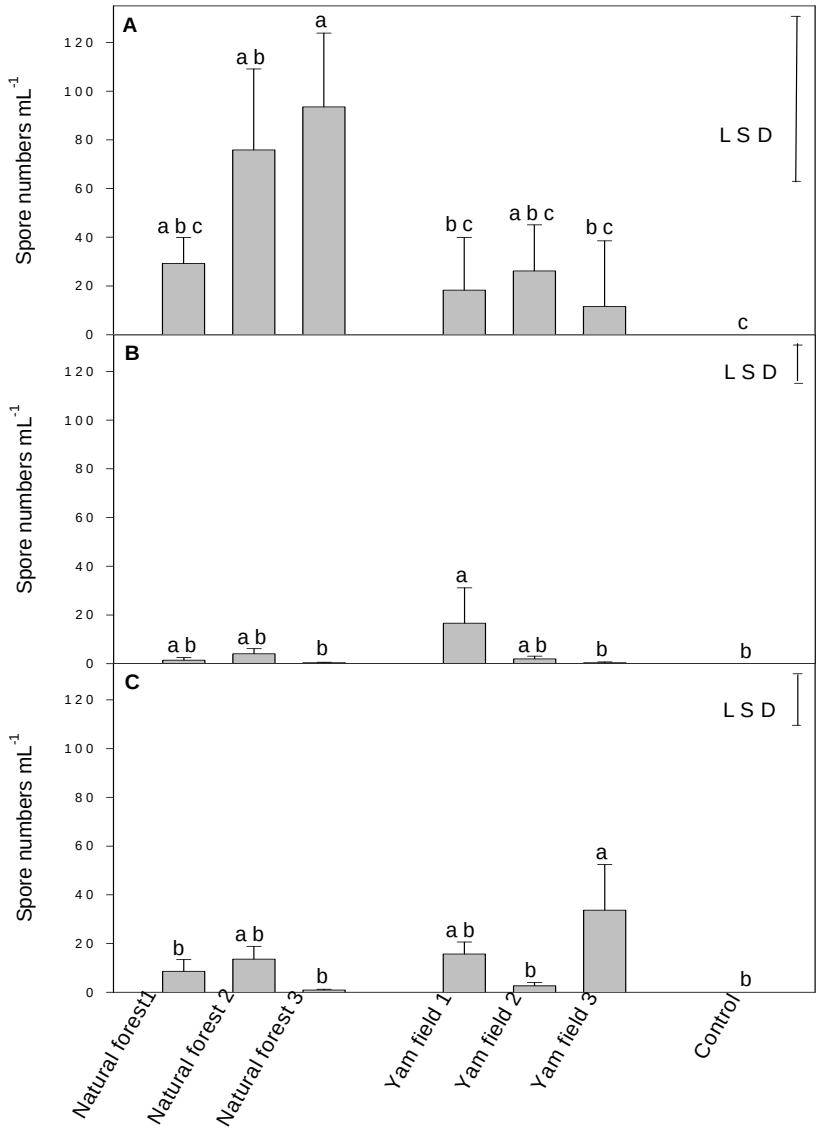

Fig. 3.4 AMF spore density (presented as spore numbers mL^{-1} of substrate) on *Sorghum bicolor* (A), *Dioscorea cayenensis* (B) and *D. rotundata* (C) following four months cultivation in trap cultures using dry season field soils from three forest and three yam field sites as inocula, and including a non-mycorrhizal control. Data are reported as averages and standard deviations for four replicate plots per field site. Non-significant differences between sites are shown by identical letters, determined using Fisher's Least Significant Difference (*LSD*) at the 5% level following one-way ANOVA.

Of the species detected in the field samples, 11 were not recovered from trap cultures. These were particularly sporocarpic *Glomus* spp. such as *G. pachycaulis* and all the above species of Gigasporaceae, such as *Scutellospora verrucosa*, *S. heterogama* and *Gigaspora decipiens*. Twenty-nine species were detected from trap cultures of both natural forests and yam fields, 8 species exclusive to the forests and 8 exclusive to the yam fields (Table 3.4).

3.4.3. Overall AMF species at study sites

Considering both field and trap cultures, a total of 56 species were recovered from across all study sites (Table 3.5), with similar numbers obtained from forest sites (29-36; in total 45) and from yam fields (29-35; in total 45). The mean number of AMF species per site was relatively higher in the forests (20-21) than in the yam fields (17-19). Interestingly, the high phosphorus availability at site NF2 did not negatively affect AMF species richness.

3.4.4. AMF species associated with yam cultivars

During the eight months of trap culturing a combined total of 37 species were associated with the two yam cultivars (Table 3.4). Of these, 29 and 28 AMF species were isolated from the rhizosphere of *D. cayenensis* and *D. rotundata*, respectively, of which 20 were common to both. Four species detected on *S. bicolor* were not recovered from yam. However, in mean numbers per site more AMF species were recovered in the trap cultures from *S. bicolor* (8-11), than *D. cayenensis* (4-7) or *D. rotundata* (3-8) (Fig. 3.5).

AMF species of six families were associated with yam. Approximately half (18) of the species were *Glomus* spp. of the Glomeraceae, followed by 13 Acaulosporaceae spp. (12 *Acaulospora* spp. and 1 *Kuklospora* sp.). Only three associated species were of Gigasporaceae (*Scutellospora fulgida*, *S. heterogama* and *Gigaspora gigantea*). *Entrophospora infrequens*, *Paraglomus occultum* and *Intraspora schenckii* were also found to be symbiotic on yam (Table 3.4).

Table 3.5 AMF species numbers at field sites found through soil and trap culture analyses

	Natural forests			Yam fields			
	nf1	nf2	nf3	yf1	yf2	yf3	*LSD*
Average numbers of AMF species [a]	20.5 a	20.3 a	20.0 a	19.3 a	17.3 a	16.8 a	3.9
Total numbers of AMF species per site (sum of four replicate plots)	29	36	33	29	35	30	-
Total numbers of AMF species per ecosystem	45			45			
Total numbers of AMF species in study area	56						

Field sites are three natural forests (nf1-nf3) and three yam fields (yf1-yf3). [a] Data are reported as averages for four replicate plots of field site. Nonsignificant differences between sites are shown by identical letters and were determined by Fisher's Least Significant Difference (*LSD*) at the 5% level after one-way ANOVA.

3.4.5. Impact of soil inoculum on yam growth

The different soil inocula did not affect the yam shoot dry weight when compared to the non-mycorrhizal control (Fig. 3.6). However, yam tuber growth was affected. Inoculum application from three origins (NF1, NF2 and YF3) positively affected tuber dry weight with an average growth increase of approximately 40% (*D. rotundata*), when compared to the control (Fig. 3.7). With *D. cayenensis*, tuber dry weight was 20% higher in the inoculated treatments than in the control, but this positive effect was significant ($P<0.05$) only for the NF1 site.

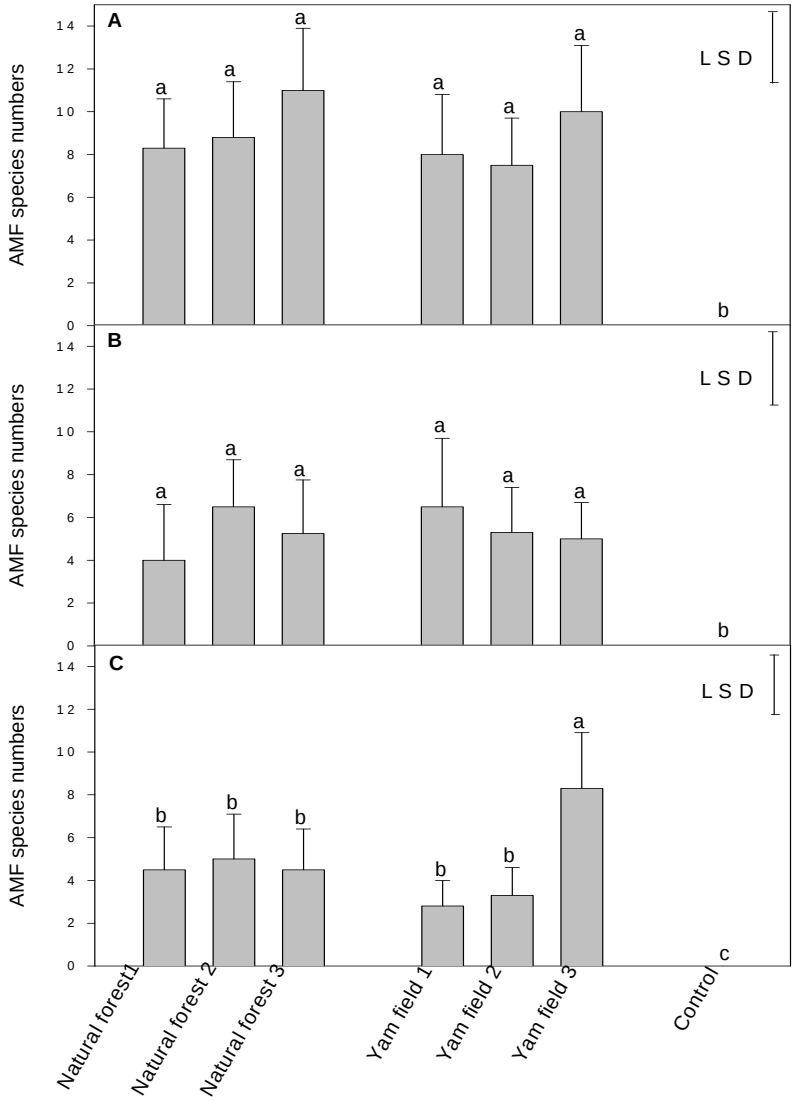

Fig. 3.5 AMF species richness on *Sorghum bicolor* (A), *Dioscorea cayenensis* (B) and *D. rotundata* (C) following four months cultivation in trap cultures using dry season field soils from three forest and three yam field sites as inocula, and including a non-mycorrhizal control. Data are reported as averages and standard deviations for four replicate plots per field site. Non-significant differences between sites are shown by identical letters, determined using Fisher's Least Significant Difference (*LSD*) at the 5% level following one-way ANOVA.

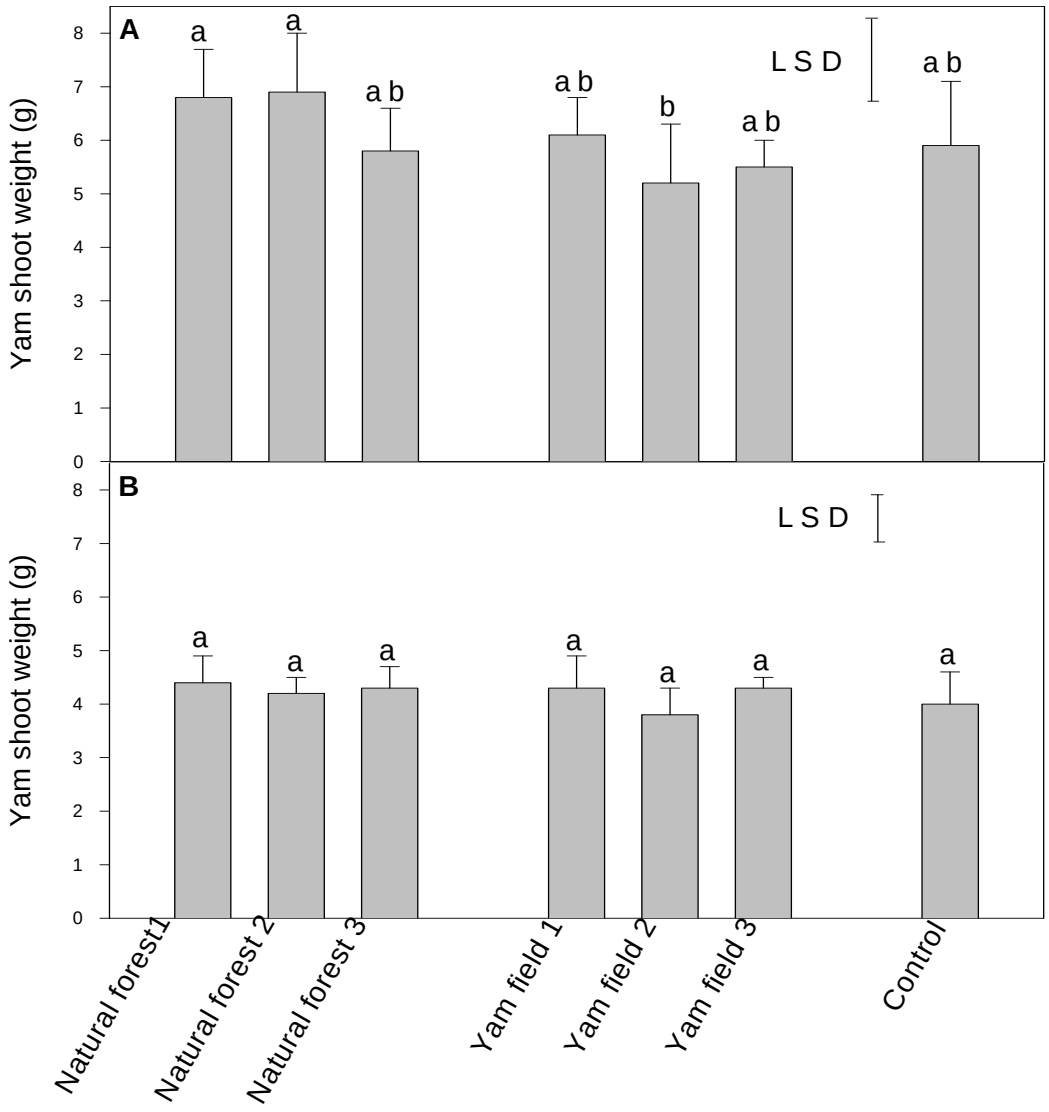

Fig. 3.6. Shoot dry weight of *Dioscorea cayenensis* (A) and *D. rotundata* (B) following eight months cultivation in trap cultures using dry season field soils from three forest and three yam field sites as inocula, and including a non-mycorrhizal control. Data are reported as averages and standard deviations for four replicate plots per field site. Columns with the same letter are not significant at P=0.05. LSD denotes the least significant difference between sites.

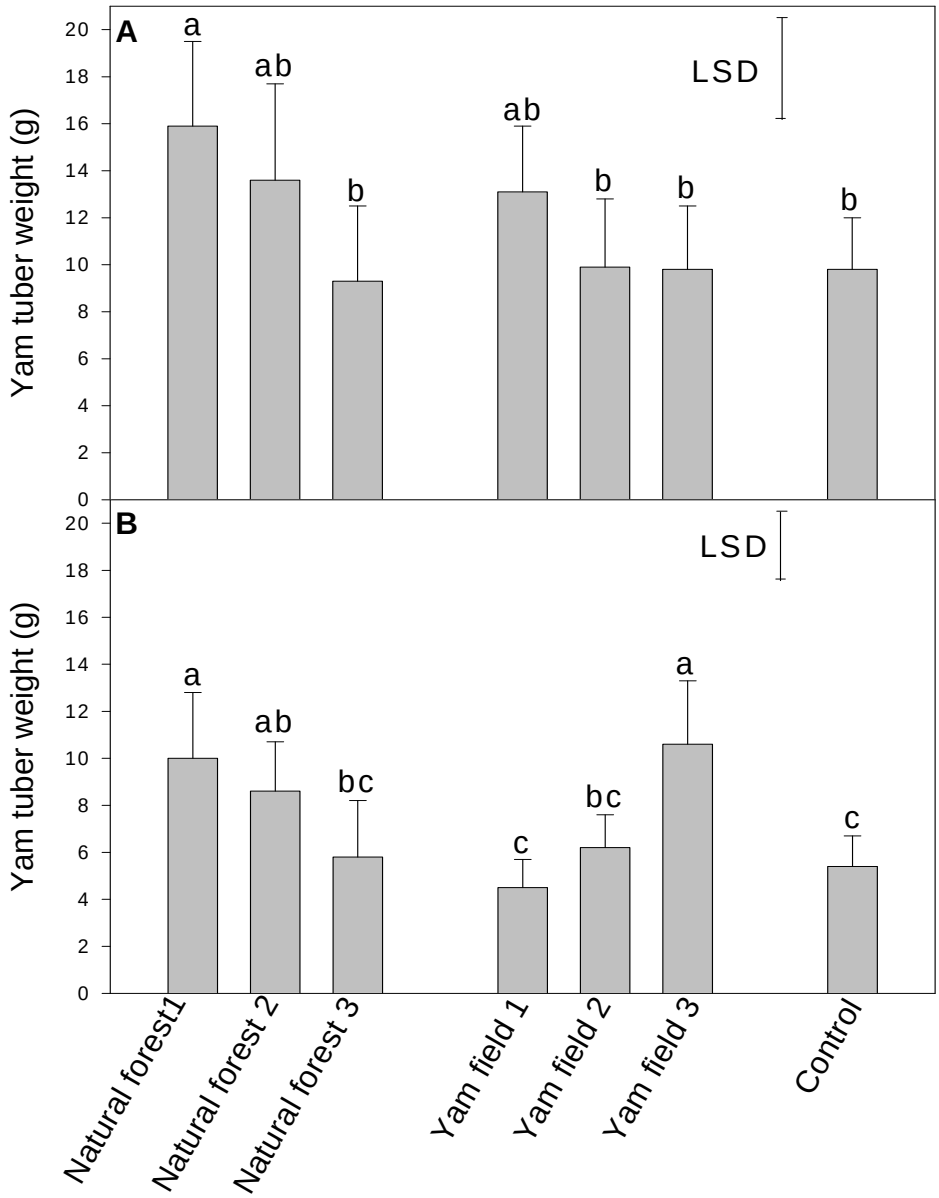

Fig. 3.7. Tuber dry weight of *Dioscorea cayenensis* (A) and *D. rotundata* (B) following eight months cultivation in trap cultures using dry season field soils from three forest and three yam field sites as inocula, and including a non-mycorrhizal control. Data are reported as averages and standard deviations for four replicate plots per field site. Non-significant differences between sites are shown by identical letters, determined using Fisher's Least Significant Difference (*LSD*) at the 5% level following one-way ANOVA.

3.5. Discussion

A total of 56 AMF species were recovered from three forest and three adjacent yam field sites, representing a high AMF diversity for the southern Guinea savanna of Benin, especially when compared to similar studies from tropical areas (e.g. Sanginga et al. 1999; Dalpé et al. 2000; review in Öpik et al. 2006; review in Gai et al. 2006; Lekberg et al. 2007; Mathimaran et al. 2007). By using molecular identification tools, Husband et al. (2002) recorded 30 AMF taxons from three sites in tropical forests in Panama. The high AMF species numbers in our study may, in part, be attributed to the extensive sampling during two different seasons, and also due to the combined recovery of AMF from fresh field samples and repeated analyses from trap cultures using three host plants over 8 months (longer than the ~7 months of wet season in the southern Guinea savanna). In a previous study which covered each eight sites under different levels of land use intensity in the southern and northern Guinea savanna in Benin, 35 and 39 AMF species were recovered from field soils sampled during the wet season, respectively (Tchabi et al., 2008). These results reflect the current findings from the field samples (40 species; Table 3.3).

The results from the trap cultures revealed that AMF communities recovered from the dry season samples enabled the recovery of more diverse communities than from soils sampled during the wet season. There may be several reasons for this result, such as the fact that AMF spores are more exposed to attack from soil microorganisms under wet, humid conditions (Klironomos et al. 1999; Bakhtiar et al. 2001; Tiunov and Scheu 2005). Furthermore, it is possible that many AMF species complete their life cycle only at the end of the wet season. Samples taken during the wet season therefore, would not enable germination in the trap cultures, even though dormancy aspects were considered. However, the results of the current study indicate that soil sampling for successful AMF propagation in the tropics should be performed primarily during the dry season. Nevertheless, despite intensive investigations, it is further assumed that additional species remain undetected, due to the lack of sporulation, both in the field soils and trap cultures.

The results of this study also demonstrate for the first time that a wide range of AMF species act as symbionts of yam, and that yam is highly mycorrhizal. The number of AMF species detected on *D. cayenensis* and *D. rotundata* was found to gradually increase over the course of the study,

with a single AMF species recovered at 2 months after inoculation (*G. etunicatum*), up 29 species at 8 months (Table 3.4). A similar observation was recorded by Oehl et al. (2003) and Chaurasia and Khare (2005) who found that the number of species sporulating in trap cultures increased from two to 20 months. Using molecular techniques, it is possible that future investigations will detect higher diversities of active AMF species, even during early yam root growth of trap cultures or field situations. This however, was not possible during the current study. Some species though consistently sporulated faster in the trap cultures than others, such as *G. etunicatum* and *A. scrobiculata,* which produced spores abundantly on both yam species as well as *S. bicolor* as early as four months (Table 4). Interestingly in this study, these species (*G. etunicatum* and *A. scrobiculata*) also tended to dominate the spore populations recovered from the field samples derived from the yam cultivated sites (Table 3.3).

Some AMF species that were recovered from *S. bicolor* did not associate with yam, while other AMF species were recovered only from yam plants. Even within yam species, some AMF species were recovered only from *D. rotundata* and from *D. cayenensis*, which suggests quite a high degree of specificity of some species of AMF, which is only recently being appreciated (e.g. Bever et al. 1996; Bever et al. 2001; Sýkorová et al. 2007). Lovelock et al. (2003) observed differences in the relative spore abundance of AMF communities according to host plants, and Vandenkoornhuyse et al. (2002) demonstrated that the AMF community colonizing *Trifolium repens* differed from that colonizing *Agrostis capillaris*. This specificity has obvious implications towards the efficient use of trap cultures for AMF species detection, especially for AMF ecosystem research (Oehl et al. 2003). However, information remains limited on host specificity and how individual or mixed communities affect interactions between specific AM fungal species or between AMF species and the host plants or host plant communities or even affect host growth promotion or improved plant health.

AM root colonization was also particularly high four months after inoculation for both yam cultivars, compared with *S. bicolor,* which had relatively low colonization. High mycorrhization of yam roots has also been observed in the field, although this varied with yam genotype (IITA 2005). The high yam root colonization by AMF may be explained by the relatively low density of yam roots, when compared to the extensive root system of *S. bicolor* (not quantified, own

observations during the concomitant spore analyses), although high root colonization has been attributed to the number and density of fine or secondary plant roots, which favor active mycorrhizal colonization (e.g. Mosse et al. 1976; Sanders et al. 1977). However, our results are highly indicative that yam is a highly AMF dependent plant, although higher levels of root colonization in the yam was accompanied by a lower spore reproduction than on *S. bicolor*. Similar observations were also made at the ecosystem level in the cold climate from Central Europe (Oehl et al. 2003) and in the semi-dry tropics in Bolivia (Pérez-Camacho and Oehl, unpublished): AMF communities from natural sites caused higher root colonization but had lower spore reproduction, compared with communities in adjacent cultivated soils.

Yam root colonization by AMF was higher using soil inoculum from the forests than adjacent yam fields. This was related with the lower AMF species richness during the year of yam cultivation following forest clearance: a lower number of species were also recovered from yam fields of the subsequent dry seasons than the natural forests (Fig. 3.2). The current study therefore, demonstrates the rapid decline in AMF infection potential (Fig. 3.3) and AMF species richness following land-use change from natural forests into traditional low-input farming, even during the first year after forest clearance. This observation is clearer in the current study than in the previous related study (Tchabi et al. 2008), which established a dramatic decrease in AMF species richness in relation to land-use intensity in the Sudan and Guinea savannas, but not necessarily during yam production in the first year after forest clearance. In the current study, AM root colonization was not negatively correlated with the available soil P contents, which has been observed elsewhere (e.g. Oehl et al., 2004; Wiseman and Wells, 2005).

Inoculation with field soil from the study sites had no affect on yam shoot growth in the trap cultures (Fig. 3.6), but increased yam tuber weight (Fig. 3.7). This result could be attributed to a number of factors, but highly likely to be related to the AMF given the outstanding high AMF root colonization of the yam roots. This further indicates that AMF might be particularly important components for yam nutrition and tuber production, and one possible reason why they perform better immediately following forest clearance, when AMF abundance is still increased when compared to subsequent years (Tchabi et al. 2008). Plant growth promotion in relation to AMF colonization is a well-established phenomenon across crops and climatic zones (e.g. Smith

and Read 1997; Chaurasia and Khare 2005; Caglar and Akgun 2006). Further studies on yam growth promotion by AMF should now focus on the specificity and inocula levels of AMF/yam interactions under controlled and field conditions with particular emphasis on the selection of indigenous AMF isolates respective species that promise to be most beneficial to yam.

Conclusion

The diversity of AMF species in the southern Guinea savanna (SG) was found to be higher after combined field and trap culture studies than after field studies solely. Thus, for AMF diversity studies in West Africa the use of trap cultures in addition to analyses of field samples is highly recommended, but sampling in the dry season, as opposed to the wet season, appears to be of much greater value for AMF propagation and subsequent AMF identification. Our results indicate that individual host plants favor different spore populations and thus AMF species compositions. However, in the current study it was clearly demonstrated that yam is an AM plant associated with at least 37 potential AMF species as fungal symbiotic partners in the SG. The AMF species most frequently found and most related to high and fast spore production during yam growth were *G. etunicatum* and *A. scrobiculata.* It will be challenging to determine in the future which of these, or whether rather slower sporulating species may be the more important symbionts for yam for improving crop productivity, especially when the soils become deficient in AMF with intensification of agricultural practices in West Africa.

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Chapter 4: Growth promotion in white yam (*Dioscorea rotundata*) following inoculation with West African arbuscular mycorrhizal fungal isolates

4.1. Abstract

Tuber yields of an important tropical staple crop, yam (*Dioscorea* spp.), per unit area, have declined in West Africa, mainly due to increasing land use pressure and decreasing soil fertility. Yam roots were recently found to be highly mycorrhizal and associated with a wide range of arbuscular mycorrhizal fungi (AMF). Thus, AMF inoculation of yam may present an option to increase tuber yields, above all under conditions of low soil nutrient availability. It is hypothesized that for plant growth promotion, application of indigenous AMF isolates might be more effective than non-indigenous exotic AMF isolates, and this would also sound more ecologically. We screened AMF isolates, indigenous to the West African 'yam belt', on micro-propagated plantlets of white yam (*Dioscorea rotundata*, cv.TDr89/02461) in pot experiments. Initially, single spore derived (=monosporal) cultures of nine AMF species (*Glomus etunicatum, G. claroideum*, *G. mosseae*, *G. sinuosum*, *G. hoi*, *Acaulospora scrobiculata*, an undescribed *Acaulospora* sp., *A. spinosa* and *Kuklospora kentinenses*) were generated on *Stylosanthes guianensis* and *Hieracium pilosella* following a first trap culturing on *Sorghum bicolor*. In the first experiment, several tropical indigenous and non-indigenous (South American and Asian) isolates of *G. etunicatum* were compared against three non-tropical AMF isolates from temperate Europe on yam. In the second experiment, three different isolates of each of the above nine AMF species derived from single spores and three strains from Biorize company® were screened on the same yam cultivar. The results indicated that, independent of origin in the tropics, most isolates of *G. etunicatum* increased yam tuber dry weight. However, the non-tropical AMF isolates had a lower or no effect on tuber growth, but instead increased tuber P concentrations as compared to non-mycorrhizal controls. The indigenous isolates of *G. mosseae*, *G. hoi*, *G. etunicatum*, *A. scrobiculata* and *A. spinosa* generally led to increased tuber growth, while isolates of *G. sinuosum* and *K. kentinensis* did not. In conclusion, our results indicate a high potential for an improvement of yam production by application of indigenous AMF. However, a

further screening for suitable isolates is necessary as different AMF species, and also different isolates are not equally beneficial.

Key words: beneficial microorganisms, indigenous AMF, Glomeromycota, growth, native, tuber crop, yam

4.2. Introduction

Yam (*Dioscorea* spp.) is a tuber crop widely cultivated in West and Central Africa, in Asia and in several tropical Central and South American countries (Ravi et al., 1996; Sotomayor-Ramirez et al., 2003; Suja et al., 2003; FAO data, 2007). Especially in Africa, yam production has been annually increasing, but yields realized per unit area have declined due to increased land pressure, declining soil fertility and an increase in pest and disease levels (Coyne et al., 2005; Baimey et al., 2006), above all of viruses and harmful nematodes (Egesi et al., 2006).

Arbuscular mycorrhizal fungi (AMF) are symbionts that colonize the roots of most terrestrial plants. The large majority of crop plants benefit from the AMF association e.g. by increasing plant nutrient uptake, plant growth and survival rates (Linderman, 1992; Smith and Read, 2008; Clark et al., 1999; Akhtar and Siddiqui, 2007). The AMF association may also increase host plant resistance/tolerance against pathogens (Jaizme-Vega et al., 1998; Declerck et al., 2002; Hol and Cook, 2005; Akhtar and Siddiqui, 2007) and abiotic stresses such as transplantation, salinity and drought (Gerdemann, 1968; Smith, 1987; Cartmil et al., 2007; Franco-Ramirez et al., 2007; Giri et al., 2007; Sudova et al., 2007).

Recently, it was shown that yam (*Dioscorea* spp.) is arbuscular mycorrhizal (Ahulu et al., 2005; Tchabi et al. 2008), with high levels of root colonization and a wide range of AMF species associated (Tchabi et al. 2008; Tchabi et al., chapter 3). Under field conditions, the association between AMF and cultivated yam may start immediately after tuber transplantation. Simultaneous AMF inoculation to the yam rhizosphere might therefore improve yam

development, especially under low soil nutrient conditions and in soils with low AMF infection potential.

The use of tissue culture as a technique for plant multiplication has become of broad use for commercial plant production in many cultivated crops (Nowak, 1998). *In vitro* propagated yam is increasingly used to produce yam planting material for research centers, but also increasingly for small and large scale-production systems in West Africa (IITA, 2007). However, such materials are not only free of viruses and nematodes, but also of beneficial micro-organisms including AMF. Thus, with transplantation, a simultaneous AMF inoculation might be especially useful for plant adaptation, improved yam development and ultimately increased tuber yields. Several studies have already reported a beneficial effect of AMF inoculation for micro-propagated tropical plants such as papaya, avocado, pineapple (Jaizme-Vega and Azcon, 1995) and bananas (Yano-Melo et al., 1999; Declerck et al., 2002). In tuber crops, AMF inoculation of micro-propagated potato improved viability during the transfer from *in vitro* conditions (Niemira et al., 1995; Vosatka and Gryndler, 2000), increased tuber yield and size (Duffy and Cassells, 2000; O'Herlihy et al., 2003) and in cassava shoot, root and tuber was increased by AMF inoculation (Azcón-Aguilar et al., 1997).

In a previous study, a high AMF species richness of the 'yam belt' (Benin, West Africa) was found for both sites with natural vegetation and sites with a broad range of agricultural uses in three ecological zones (Southern Guinea savanna, Northern Guinea, and Sudan savanna) (Tchabi et al., 2008). In a subsequent study, we identified the AMF associated with yellow yam (*D. cayanensis*) and white yam (*D. rotundata*) in trap cultures (Tchabi et al. chapter 3). In the present study, we testedthe AMF isolates from the West African 'yam belt' for their potential to promote growth of micro-propagated white yam plantlets. For ecological reasons, the use of indigenous AMF rather than AMF of 'exotic' origin would certainly be recommendable. We further hypothesized that indigenous AMF isolates might be more efficient for plant growth promotion than non-indigenous isolates because they are probably better adapted to the specific environment. Therefore, we generated single spore derived (=monosporal) cultures of several AMF species indigenous to the Southern Guinea savanna (SGS) of Benin. In a first growth experiment, we screened several indigenous and non-indigenous *G. etunicatum* isolates (from

tropical locations, in Bolivia and India, and from temperate region in central Europe) for AM root colonization as well as shoot, root, tuber growth and mineral accumulation in micro-propagated white yam(TDr89/02461). In a second experiment, nine AMF species with three monosporal isolates per species were screened on the same white yam cultivar for growth parameters. AMF isolates from Biorize Company® were included in this experiment.

4.3. Materials and methods

4.3.1. Establishment of single spore derived (=monosporal) AMF cultures

AMF communities indigenous to the SGS, derived from three natural forest and three adjacent yam fields, were previously propagated on white and yellow yam cultivars and on *Sorghum bicolor* as host plants (Tchabi et al., chapter 3). To generate indigenous single spore derived (=monosporal) isolates, spores of *Glomus etunicatum*, *G. claroideum*, *G. mosseae*, *G. sinuosum*, *G. hoi*, *G. ambisporum*, *Acaulospora scrobiculata*, *A.* sp. WAA1, *A. spinosa* and *Kuklospora kentinensis* were isolated from the trap cultures of *Sorghum bicolor* 6, 8 and 10 months after establishment of the trap cultures. Single spores of each species were used to generate pure cultures. In the case of *G. hoi,* single spore clusters with three to five spores formed on the same hypha were used as inocula instead of single spores; while in the case of *G. sinuosum* and *G. ambisporum* single sporocarp fragments of 5-10 adherent, connected spores were inoculated. The substrate was an autoclaved, fine-textured and water-saturated quartz sand:loess mixture (wt/wt 1:1; Alsace quartz, 5% of free silica, Smurfit Company, France). The substrate pH (KCl) was 7.0 and contained 5 mg g^{-1} organic matter, 5 mg g^{-1} available P and 50 mg g^{-1} available K, both extracted by sodium acetate. The substrate had been introduced to 1 mL pipette tip under dry conditions and watered from the bottom profiting from the good capillary conductivity of the selected substrate. The spores were placed in the top of ~4 mm of the substrate, with two to three *Stylosanthes guianensis* seeds sown at the same time and covered with an additional 1 mm of substrate. The pipette tip systems were watered every two days, as above. The prospective germinating AMF spore isolate and the host plant were grown in these tips for ~4 weeks until plant roots exhausted the space. After one month, the tips were then cut in half with sterilized scissors; the upper half was transplanted into 320 mL pots filled with an autoclaved substrate mixture (3:1 wt/wt) of Terragreen (calcined granulate Attapulgite clay, American aluminium

oxide, oil dry US special, type III R, >0.125 mm; Lobbe Umwelttechnik, Iserlohn, Germany) and quartz sand. The chemical composition of the substrate (pH 5.8) was 0.3 % organic matter, 10 mg kg^{-1} available P, 191 mg kg^{-1} available potassium (K), again extracted by sodium acetate. The AMF-plant systems were watered twice-weekly. No fertilizer was applied during the 5 months of culturing. The AMF-plant systems in the pipette tips and in the pots were grown in the greenhouse of the Institute of Botany in Basel with 25°/20°C day/night temperatures under ambient light conditions, and under additional artificial light when sun light was < 12 h per day, to assure 12 h light period every day.

The trap culture substrates were air-dried after 10 months of trap culturing and stored for 3 months in order to create an artificial dry season, more or less equivalent to the dry season in the SGS, in order to break possible dormancy of AMF spores (January to March 2006). Spores of all species were then isolated again from the trap culture substrate and used as monosporal inocula, as described above using *Hieracium pilosella*, a species typical of drier Central European grasslands, as a host plant. For these monosporal cultures, the 1 mL pipette tip system and the same substrate were used as described above. After four weeks, the upper tip half with the putative established AMF-*H. pilosella* symbiosis, was transplanted into 320 mL pots and cultured for five months as described above.

Six months after spore inoculation all monosporal pot cultures were assessed for new spore formation. After determination of the spore densities in the monosporal cultures, three isolates per species, with approximatively similar spore densities were selected for the subsequent functional screening experiments.

4.3.2. Experimental site details

Both experiments, following initial AMF inoculation, were conducted in the screenhouse of the International Institute of Tropical Agriculture (IITA), Cotonou, Benin. The IITA-Benin station is situated at the coastal region of Benin, 12 km Northwest of Cotonou, between 6°25.256N and 2°19.719E, 23 m asl with a sub-equatorial climate and a daily ambient temperature between 29-34°C at day and 24-27°C at night. Relative air humidity was between 70% and 85%.

4.3.3. Substrate used for the growth experiments

The substrate used for both experiments was a mixture (wt/wt 1/1) of soil derived from the SGS of Benin and sand collected from Cotonou beach. The soil was collected from a depth of 0-25 cm and passed through a 1 mm aperture sieve to remove roots and debris. The sea sand was thoroughly washed with tap water to remove salt. The substrate mixture was oven sterilized at 80 °C for 72 h. Substrate pH (H_2O) was 7.7, the organic carbon 20 g C kg^{-1}, and the total nitrogen (N) and available phosphorus (P-BrayI) were 3.4 g N kg^{-1} and 19 mg P kg^{-1}, respectively, which was analyzed at the Centre National Agro-Pedologique of Benin (CENAP, Cotonou).

4.3.4. Growth experiment I: Screening of *G. etunicatum* isolates of different geographical origin

The first experiment was established from May 2006 until November 2006. The experiment aimed at testing the effect of five indigenous and six non-indigenous monosporal *G. etunicatum* isolates on micropropagated yam plantlets (Table 4.1). The non-indigenous *G. etunicatum* isolates derived from other tropical origins (four from India and two from Bolivia) were obtained from Botanical Institute of Basel as pure culture generated from single spore culture. In the current experiment, non-indigenous isolates were propagated from the original monosporal inoculum simultaneously with the establishment of the indigenous isolates. Three monosporal isolates of non-tropical origin were also included in the experiment (each an isolate of *Glomus* sp. resembling *G. luteum*, *G. mosseae* and *G. constrictum*) generated on *Plantago lanceolata* from soils of the Upper Rhine valley close to Basel (Table 4.1). In total, the experiment comprised 14 monosporal isolates using 5 replicate pots per isolate and including both a control inoculated with yam field soil sourced from the SGS and a non-mycorrhizal control. The yam field was situated at 07° 50.121 N; 002° 12.445 E. Four-liter pots filled with 2 kg of the sterilized substrate (as described above), were used, arranged in a completely randomized design in the greenhouse. For each monosporal isolate 5 g of inocula (representing approximately 150 spores plus colonized roots and hyphal fragments) were placed in the planting hole before placing a single yam plantlet (*D. rotundata* cultivar TDr89/02461) in the hole. The non-mycorrhizal control pots received each 5 g of sterilized monosporal culture substrate. The second control received 5g of yam field soil. The plantlets were watered to 100% water capacity with tap water every other day and staked at five weeks after planting (one stake per plant). The stakes were

disinfected by submerging them in 70% alcohol. At harvest, 7 months after planting, AMF root colonization and yam shoot, root and tuber dry weights were determined. Shoot and tuber mineral concentrations (N, P, Mg and Ca) were determined in the Laboratory of Nutrition at IITA-Ibadan (Nigeria) according to standard methods (Anderson and Ingram, 1993).

Table 4.1 List of monosporal arbuscular mycorrhizal fungi (AMF) isolates[1] (included their ascension number, species name, short cut names, origin of samples from which they were isolated from and the Gene Bank where they were stored) used to assess the effect of AMF inoculation on yam (*D. rotundata*) cultivar 89/02461 micropropagated plantlets root colonization, growths parameters and mineral accumulation in the tissues.

Accession number	AMF species	Short cut	Origin	Gene Bank
BENTG102	*Glomus etunicatum*	WA-*G.etu1*	Benin yam field	IITA-Benin
BENTG103	*Glomus etunicatum*	WA-*G.etu2*	Benin natural forest	IITA-Benin
BENTG105	*Glomus etunicatum*	WA-*G.etu3*	Benin, natural forest	IITA-Benin
BENTG104	*Glomus etunicatum*	WA-*G.etu4*	Benin, cotton field	IITA-Benin
BENTG101	*Glomus etunicatum*	WA-*G.etu5*	Togo, sorghum field	IITA-Benin
FIND25	*Glomus etunicatum*	IN-*G.etu1*	Central India, cotton field	BIB-Basel
FIND62	*Glomus etunicatum*	IN-*G.etu2*	Southern India	BIB-Basel
FIND66	*Glomus etunicatum*	IN-*G.etu3*	Southern India	BIB-Basel
FIND73	*Glomus etunicatum*	IN-*G.etu4*	Southern India	BIB-Basel
FBOL28	*Glomus etunicatum*	SA-*G.etu1*	Bolivia, sorghum field	BIB-Basel
FBOL29	*Glomus etunicatum*	SA-*G.etu2*	Bolivia, sorghum field	BIB-Basel
FSWI39	*Glomus* sp resembling *G. luteum & G. claroideum*	EU-*G.'lut'*	Switzerland, Grass-clover field	BIB-Basel
FSWI22	*Glomus mosseae*	EU-*G.mos*	Switzerland, grassland	BIB-Basel
FGER45	*Glomus constrictum*	EU-*G.con*	Germany, grassland	BIB-Basel

[1]AMF isolates used for experiment1, WA = West Africa, IN = India, SA = South America, Eu = Europe, IITA = International Institute of Tropical Agriculture, BIB = Botanical Institute of Basel.

Table 4.2 List of monosporal arbuscular mycorrhizal fungi (AMF) isolates[2] indigenous to the Southern Guinea Savanna in Benin and non-indigenous isolate (included their ascension number, species name, short cut names, origin of samples from which they were isolated from) used to assess the effect of AMF inoculation on yam (*D. rotundata*) cultivar 89/02461 micropropagated plantlets root colonization and growths parameters

Accession code	AMF species	Short cut	Origin*
BENTG102	*Glomus etunicatum*	WA-*G.etu1*	Yam field yf2
BENTG103	*Glomus etunicatum*	WA-*G.etu2*	Natural forest nf1
BENTG105	*Glomus etunicatum*	WA-*G.etu3*	Natural forest nf1
BENTG141	*Glomus claroideum*	WA-*G.cla1*	Natural forest nf3
BENTG142	*Glomus claroideum*	WA-*G.cla2*	Natural forest nf3
BENTG143	*Glomus claroideum*	WA-*G.cla3*	Natural forest nf3
BENTG111	*Glomus mosseae*	WA-*G.mos1*	Yam field yf3
BENTG112	*Glomus mosseae*	WA-*G.mos2*	Yam field yf3
BENTG113	*Glomus mosseae*	WA-*G.mos3*	Yam field yf3
BENTG121	*Glomus sinuosum*	WA-*G.sin1*	Yam field yf3
BENTG122	*Glomus sinuosum*	WA-*G.sin2*	Yam field yf3
BENTG123	*Glomus sinuosum*	WA-*G.sin3*	Yam field yf3
BENTG131	*Glomus hoi*	WA-*G.hoi1*	Yam field yf3
BENTG132	*Glomus hoi*	WA-*G.hoi2*	Natural forest nf1
BENTG133	*Glomus hoi*	WA-*G.hoi3*	Natural forest nf1
BENTG201	*Acaulospora scrobiculata*	WA-*A.scr1*	Natural forest nf3
BENTG202	*Acaulospora scrobiculata*	WA-*A.scr2*	Natural forest nf3
BENTG203	*Acaulospora scrobiculata*	WA-*A.scr3*	Natural forest nf3
BENTG221	*Acaulospora* sp. WAA1	WA-*A.WA1*	Natural forest nf3
BENTG222	*Acaulospora* sp. WAA1	WA-*A.WA2*	Natural forest nf3
BENTG223	*Acaulospora* sp. WAA1	WA-*A.WA3*	Natural forest nf1
BENTG211	*Acaulospora spinosa*	WA-*A.spi1*	Natural forest nf1
BENTG212	*Acaulospora spinosa*	WA-*A.spi2*	Natural forest nf3

BENTG213	*Acaulospora spinosa*	WA-*A.spi3*	Natural forest nf3
BENTG301	*Kuklospora kentinensis*	WA-*K.ken1*	Yam field yf3
BENTG302	*Kuklospora kentinensis*	WA-*K.ken2*	Yam field yf3
BENTG303	*Kuklospora kentinensis*	WA-*K.ken3*	Yam field yf3
Biorize®	*G. mosseae*	*G.mosBEG*	Central Europe
Biorize®	*G. clarum*	*G.clrBEG*	Central Europe
Biorize®	*G. dussii* (= *G. coremioides*)	*G.dusBEG*	Côte d'Ivoire

[1]AMF isolates used for experiment II, WA = West Africa, [*]see (chapter3).

4.3.5. Growth experiment II: Screening of AMF isolates indigenous to the West African 'yam belt'

The second experiment screened all the AMF species previously isolated from the SGS in Benin study (Tchabi et al., 2008) using the same micropropagated yam cultivar as used in the first experiment, between May and November 2007. Three indigenous monosporal AMF isolates per AMF species were selected. Three indigenous *G. etunicatum* isolates which were previously screened in experiment I were included along with three non-indigenous species (*G. mosseae* (BEG12), *G. coremioides* (=*G. dussii*) and *G. clarum*), available as commercial products from Biorize (Dijon, France), and a non-mycorrhizal control (Table 4.2). Four pot replicates were established per treatment totalling 124 pots (30 isolates plus one control). Initially, 3 kg of the sterilized substrate was placed in 4 L pots, with 5 g of the corresponding AMF inoculum placed into the planting hole prior to transplanting the plantlets. The non-mycorrhizal control pots received 5 g of sterilized monosporal culture substrate. The experiment was arranged in a completely randomized design in the greenhouse. The experiment was maintained and harvested, recording the root colonization and growth parameters as in experiment I.

4.3.6. Source and acclimatization of yam plantlets

In-vitro tissue culture *D. rotundata* (cultivar TDr89/02461) plantlets, selected due to their availability, were supplied by the Biotechnology unit of IITA-Ibadan (Nigeria). The plantlets were multiplied under *in-vitro* conditions by sub-culturing nodal segments from established *in–*

vitro plantlets under a laminar flow hood in culture test tubes containing a yam specific multiplication medium (Ng, 1994). The plantlets were regenerated in the culture room with 12 h photoperiod, 3000 flux light intensity, 27 ± 1°C of temperature and 70 ± 5% of relative humidity.

Following removal from test tubes, before use in the trap cultures, the plantlets were acclimatized for three weeks. They were planted into peat pellets after first soaking them in water to swell. After removal from the test tube, their roots were gently rinsed in water and then transferred with a forceps into the peat pellets. They were then placed on a tray and covered with thin, transparent plastic sheet to maintain high relative humidity (70 ± 5%), but enabling air to circulate under the cover. The tray was kept under controlled conditions in the greenhouse (12 h of photoperiod; 25 °C in the day, 21 °C in the night; 65 ± 5% humidity). After one week, the plastic cover was steadily removed for increasingly longer periods (6-12 h) per day, before the plastic was completely removed after three weeks and the plants transplanted one week later into individual pots.

4.3.7. AM root colonization and spore formation

The AMF root colonization and spore density were assessed three months after AMF inoculation, according to Oehl et al. (2003), collecting two separate soil cores totalling 30 cm^3 (sampling depth 10 cm) from each pot. Roots were extracted by wet sieving and decantation, while the AMF spores were isolated by wet sieving and sucrose density gradient centrifugation (Oehl et al., 2003). AMF root colonization was determined according to Brundrett et al. (1996), using trypan blue to stain mycorrhizal structures. The gridline-intersect technique (Giovannetti and Mosse, 1980) was applied to analyze AM colonization using a Leica Wild M3C stereo microscope, at up to 90-fold magnification. AMF spores for each sample were counted with the same microscope.

4.3.8. Measurement of yam growth characteristics

The plants were harvested seven months after transplanting into pots. The shoots were cut to soil level and tubers were removed by hand by upending pots and gently breaking the soil away from around tubers and roots. The roots were removed with forceps and collected separately. Shoots, roots and tubers from each pot were rinsed gently under tap water, air dried and separately stored

in labeled paper bags. Dry weight of shoots, roots and tubers were recorded following oven drying in the well-ventilated Gallenkamp oven at 80 °C for 72 h.

4.3.9. Yam tissue nutrient concentration analysis

All shoots and tubers were sent to the Laboratory of Food Technology, IITA Ibadan, Nigeria for analyzing for total nitrogen (N), phosphorus (P), calcium (Ca) and magnesium (Mg) concentration. Shoot tissue was digested in a hydrogen peroxide-sulphuric acid digestion mixture according to the Kjeldahl procedure, followed by standard colorimetric assays (Anderson and Ingram, 1993) and nutrient contents determined calorimetrically according to Murphy and Riley (1962). From tubers, only P was analyzed.

4.3.10. Statistical treatment of data

The significance of differences between treatments for AMF root colonization, spore density, yam shoot, root and tuber dry weight and nutrient concentrations was assessed using Fisher's least significant difference (LSD) test at $P < 0.05$ after a one-way analysis of variance (ANOVA). Prior to analysis, data on spore density were $\log_{10}(x+1)$ transformed, while data on mycorrhizal colonization were arcsin (x/100) transformed.

4.4. Results

4.4.1. Establishment of single spore derived AMF isolates indigenous to the SGS

Monosporal isolates of nine AMF species, belonging to four AMF species groups were obtained: i) three species of *Glomus* group A sensu Schüßler et al. (2001): *G. mosseae*, *G. sinuosum* and *G. hoi*, ii) two species of *Glomus* group B sensu Schüßler et al. (2001): *G. etunicatum* and *G. claroideum*, iii) three species of *Acaulospora*: *A. scrobiculata*, *Acaulospora* sp. WAA1 and *A. spinosa*, and iv) *Kuklospora kentinensis*.

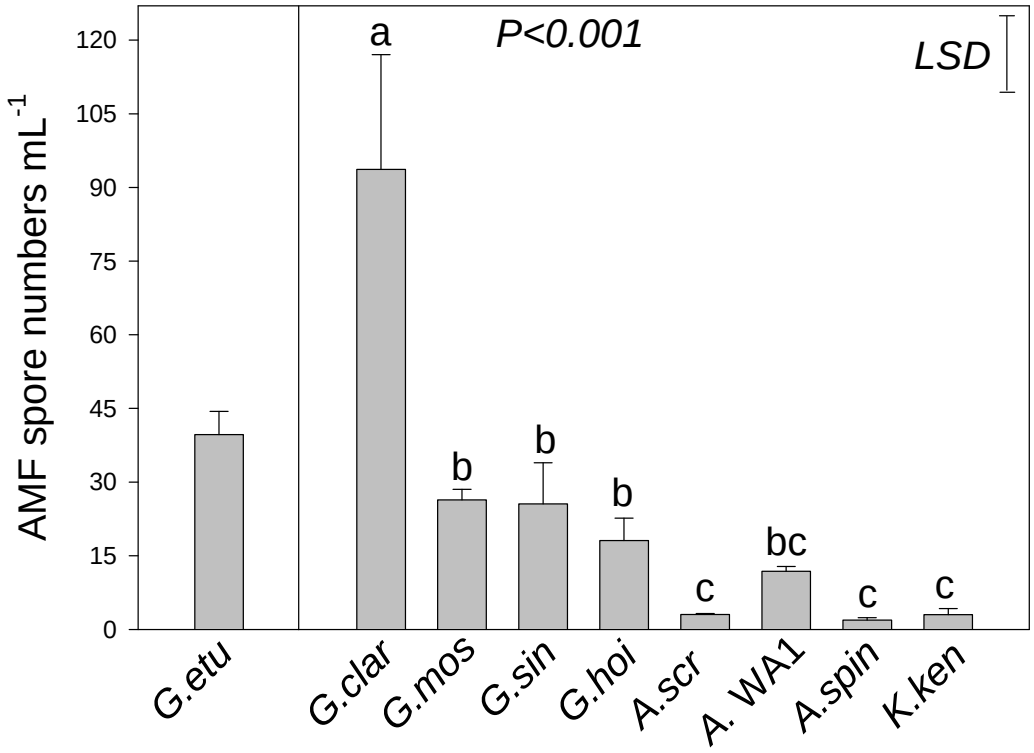

Fig. 4.1 Spore densities (= number of spores mL^{-1} substrate) after six months in single spore derived (= monosporal) AMF cultures, indigenous to the 'yam belt' of Benin. *G.etu* = *G. etunicatum*; *G.clar* = *G. claroideum*; *G.mos* = *G. mosseae*; *G.sin* = *G. sinuosum*; *G.hoi* = *G. hoi*; *A.scr* = *A. scrobiculata*; *A.WA1* = *A. sp. WAA1*; *A.spin* = *A. spinosa*; *K.ken* = *K. kentinensis*. The *G. etunicatum* isolates were established on *Stylosanthes guianensis*; the others on *Hieracium pilosella*. Data are reported as means and standard deviation of three isolates per AMF species. Non-significant differences between species propagated on *H. pilosella* are shown by identical letters, determined using Fisher's Least Significant Difference (LSD) at the 5% level following one-way ANOVA.

When monosporal cultures were established directly after spore isolation from growing trap cultures, an AMF symbiosis and new spores were formed only in 5% of the pots. Moreover, only monosporal cultures of *G. etunicatum* were successfully established, while from the other AMF species no cultures could be generated (data not show). When monosporal cultures were established from spores isolated from the same, but air-dried trap culture samples after 3-months storage in plastic bags, an AM symbiosis was established with new spore formation in 25% of the pots, and several isolates from nine species were successfully generated. After six months in the monosporal cultures, three isolates exhibiting typical species-specific and approximatively similar spore densities were selected from each of the nine species for subsequent screening experiments. The spore density (=number of spores g^{-1} soil) was generally higher for *Glomus* isolates than for *Acaulospora* and *Kuklospora* isolates (Fig. 4.1).

4.4.2. AM root colonization and spore formation

In both growth experiments, all AMF isolates of tropical and non-tropical origin had substantially colonized the micro-propagated yam plantlets after 3 months. No colonization occurred in non-mycorrhizal control plants (Figs. 4.2 and 4.3), while highest AMF colonization occurred on plantlets inoculated with the AMF yam field soil community (Fig. 4.2).

The degree of AMF colonization by the monosporal isolates were 25-60% in experiment I (Fig. 4.2) and 15-45 % in experiment II (Fig. 4.3). In experiment I, the *G. etunicatum* isolates WA-*G.etu4* and WA-*G.etu5* of West African origin had higher AM root colonization percentage than most other monosporal isolates (Fig. 4.2). The *G. constrictum* isolate from temperate Europe also highly colonized the roots when compared to the other isolates from all origins. In experiment II, isolates of *G. mosseae*, *G. hoi* and, less significantly *A.* sp. WAA1 and *K. kentinensis*, colonized roots the most, while *A. scrobiculata* and especially *G. sinuosum* and *A. spinosa* had lower colonization levels. Colonization levels of *G. etunicatum* and *G. claroideum* were intermediary and more variable, respectively. Percentage of AM root colonization was not correlated with the initial spore density of the inocula in which e.g. *K. kentinensis* had significantly lower spore densities than *G. mosseae* and *G. hoi,* while *G. claroideum* had highest spore densities (Fig. 4.1). Spore densities at 3 months after inoculation were between 3 and 23 per mL substrate, but it did not differ between the inoculated treatments. No spore production occurred in the non-mycorrhizal controls (data not shown).

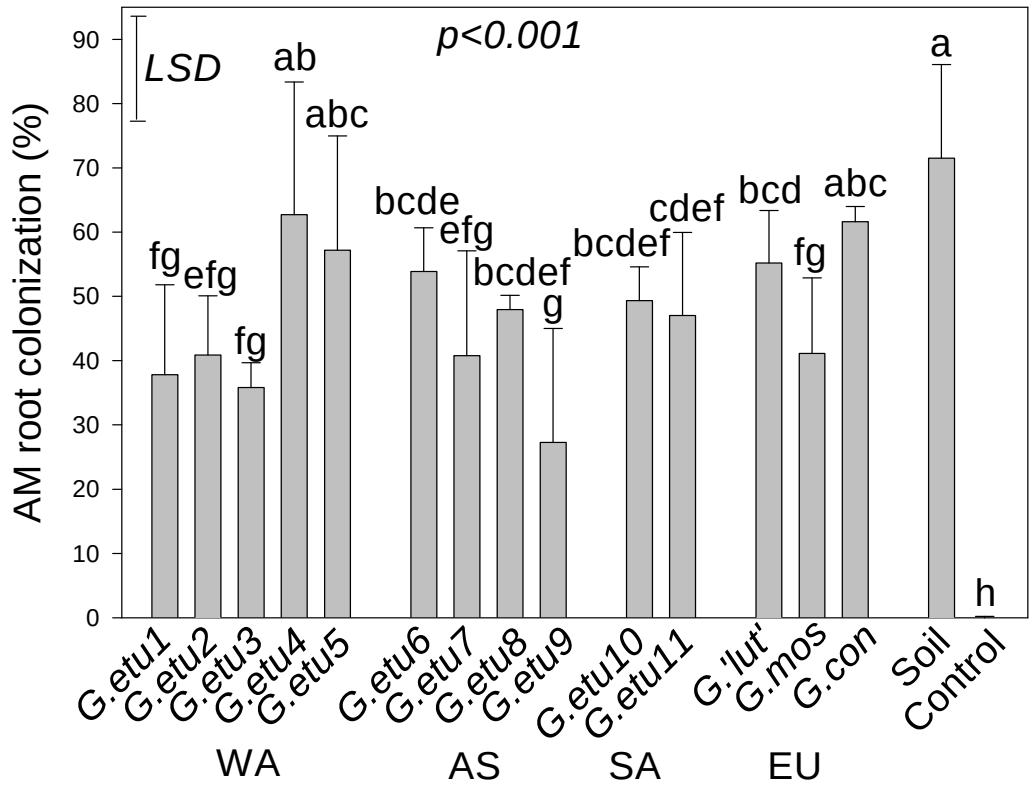

Fig. 4.2 Arbuscular mycorrhizal root colonization (%) in pot cultured plantlets of white yam (*D. rotundata*) 3 months after inoculation with 11 isolates of *G. etunicatum* from tropical West Africa (WA), Asia (AS) and South America (SA), compared with 3 non-tropical AMF isolates from temperate Europe (EU), one mycorrhizal control, inoculated with a West African yam field soil (Soil), and one non-mycorrhizal control. *G.etu* = *G. etunicatum*; *G.'lut'* = *Glomus* sp. resembling *G. luteum* and *G. claroideum*; *G.mos* = *G. mosseae*; *G.con* = *G. constrictum*. Data are reported as means and standard deviation of five replicates. Non-significant differences between treatments are shown by identical letters, determined using Fisher's Least Significant Difference (LSD) at the 5% level following one-way ANOVA.

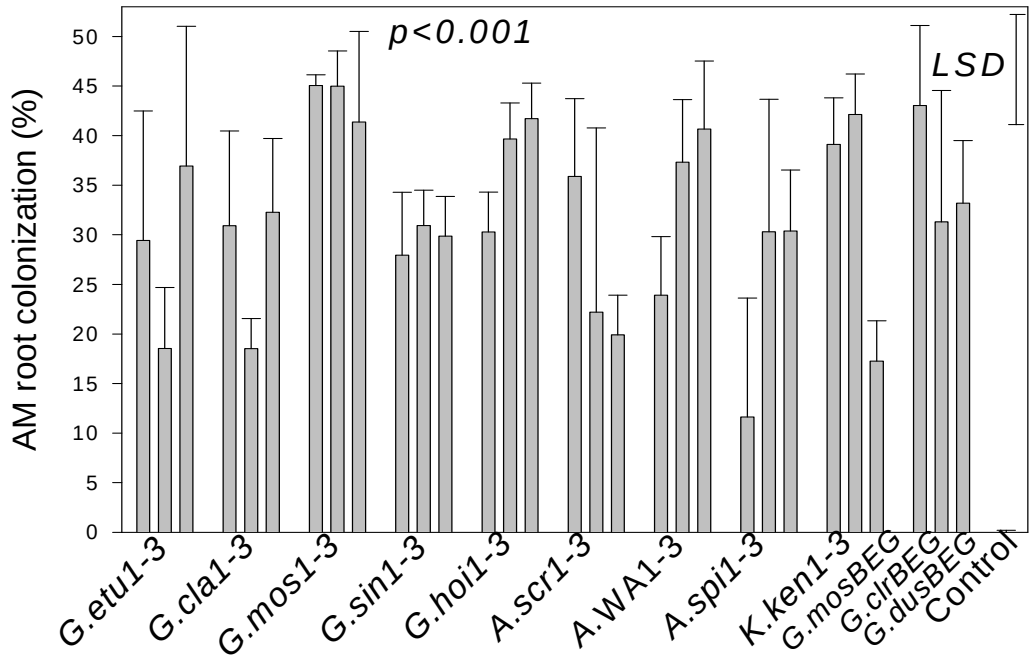

Fig. 4.3 Arbuscular mycorrhizal root colonization (%) in pot cultured plantlets of white yam (*D. rotundata*) 3 months after inoculation with 27 indigenous and 3 non-indigenous AMF isolates comprising 11 AMF species. *G.etu* = *G. etunicatum*; *G.cla* = *G. claroideum*; *G.mos* = *G. mosseae*; *G.sin* = *G. sinuosum*; *G.hoi* = *G. hoi*; *A.scr* = *A. scrobiculata*; *A.WA1* = *A. sp. WAA1*; *A.spin* = *A. spinosa*; *K.ken* = *K. kentinensis*; *G.clr* = *G. clarum*; *G.dus* = *G. coremioides*. A non-mycorrhizal control was included. Data are reported as means and standard deviation of four replicates. Non-significant differences between treatments are shown by identical letters, determined using Fisher's Least Significant Difference (LSD) at the 5% level following one-way ANOVA.

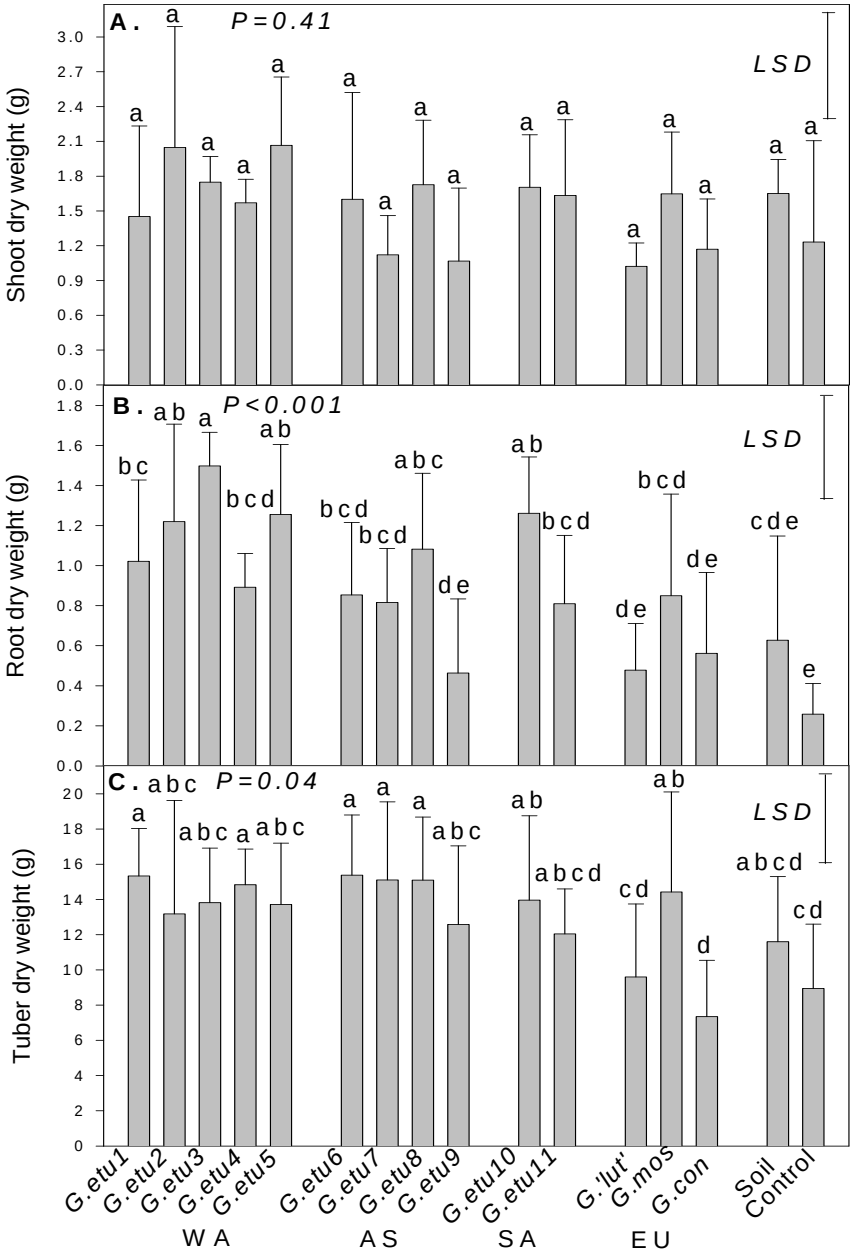

Fig. 4.4 Yam shoot (A), root (B) and tuber (C) dry weight of pot cultured plantlets of white yam (*D. rotundata*) 7 months after inoculation with 11 isolates of *G. etunicatum* from tropical West Africa (WA), Asia (AS) and South America (SA) compared with 3 non-tropical AMF isolates from temperate Europe (EU), one mycorrhizal control, inoculated with a West African yam field soil (SOIL), and one non-mycorrhizal control. *G.etu* = *G. etunicatum*; *G.'lut'* = *Glomus* sp. resembling *G. luteum* and *G. claroideum*; *G.mos* = *G. mosseae*; *G.con* = *G. constrictum*. Data are

reported as means and standard deviation of five replicates. Non-significant differences between treatments are shown by identical letters, determined using Fisher's Least Significant Difference (LSD) at the 5% level following one-way ANOVA.

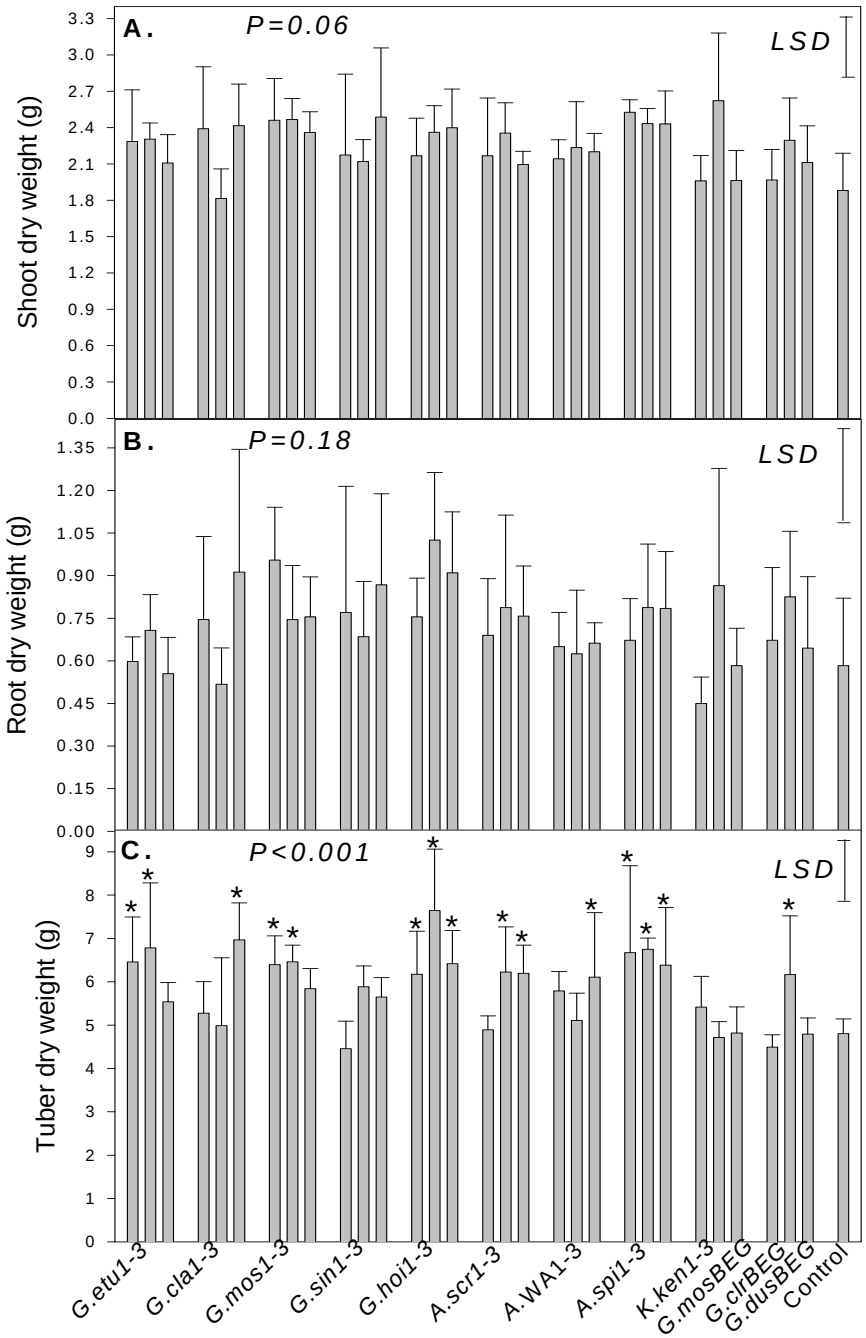

Fig. 4.5 Yam shoot (A), root (B) and tuber (C) dry weight of pot cultured plantlets of white yam (*D. rotundata*) 7 months after inoculation with 27 indigenous and 3 non-indigenous AMF isolates comprising 11 AMF species. *G.etu = G. etunicatum*; *G.cla = G. claroideum*; *G.mos = G. mosseae*; *G.sin = G. sinuosum*; *G.hoi = G. hoi*; (legend continue to next page); *A.scr = A. scrobiculata*; *A.WA1 = Acaulospora sp. WAA1*; *A.spin = A. spinosa*; *K.ken = K. kentinensis*; *G.clr = G. clarum*; *G.dus = G. coremioides*. A non-mycorrhizal control was included. Data are reported as means and standard deviation of four replicates. Non-significant differences between treatments are shown by identical letters, determined using Fisher's Least Significant Difference (LSD) at the 5% level following one-way ANOVA.

4.4.3. Shoot, root and tuber dry weight

For both growth experiments, inoculation with AMF had no effect on yam shoot dry weight at harvest after seven months of growth (Figs. 4.4A and 4.5A). In experiment I, yam root dry weight was higher following inoculation with several of the AMF isolates, while AS-*G.etu9* and two isolates of non-tropical origins had no effect, when compared to the control. The AMF isolates which led to increased yam root growth in the first experiment were WA-*G.etu3*, SA-*G.etu10*, WA-*G.etu5*, WA-*G.etu2* and AS-*G.etu8* from tropical West African, South American or Asian origin (Fig. 4.4B). In experiment II, no differences in root dry weight were observed between treatments (Fig. 4.5B). However, all *G. etunicatum* isolates increased yam tuber weight, when compared to the non-mycorrhizal control, and this was reflected for the majority of isolates (Fig. 4.4C). Of the AMF isolates from non-tropical origin only isolate EU-*G.mos* led to increased tuber weight. Inoculation with field soil did not affect shoot, root or tuber growth (Fig. 4.4).

4.4.4. Nutrient concentrations in the yam tissues

Yam tissue nutrient concentrations in experiment I showed that AMF inoculation had no significant ($P > 0.05$) effect on leaf N, Ca and Mg concentrations (Table 4.3). The effect of AMF inoculation increased leaf P concentration significantly only with *G. etunicatum* isolates (WA-*G.etu5*, AS-*G.etu6* and SA-*G.etu11*), when compared to the non-mycorrhizal control (Table 4.3). Tuber P concentrations, however were not affected by the tropical *G. etunicatum* isolates, while the concentrations were increased by the non-tropical AMF isolates EU-*G.'lut'* and EU-*G.con*

and the field soil inoculum (Fig. 4.6). Phosphorus concentrations in the tubers were negatively correlated with tuber dry weight ($P < 0.01$; linear regression $y = 0.260-0.008*x$).

In experiment II, isolates of *G. hoi* and *A. spinosa* had the greatest and most reproducible effect on yam tuber growth, followed by isolates of *G. etunicatum*, *G. mosseae* and *A. scrobiculata*. On the other hand, *G. sinuosum* and *K. kentinensis* had no effect, while isolates of *G. claroideum* and *A.* sp. WA1 had an intermediary or more variable effect (Fig. 4.5 C). Of the non-indigenous isolates, only *G. clarum* positively affected tuber dry weight (Fig. 4.5 C).

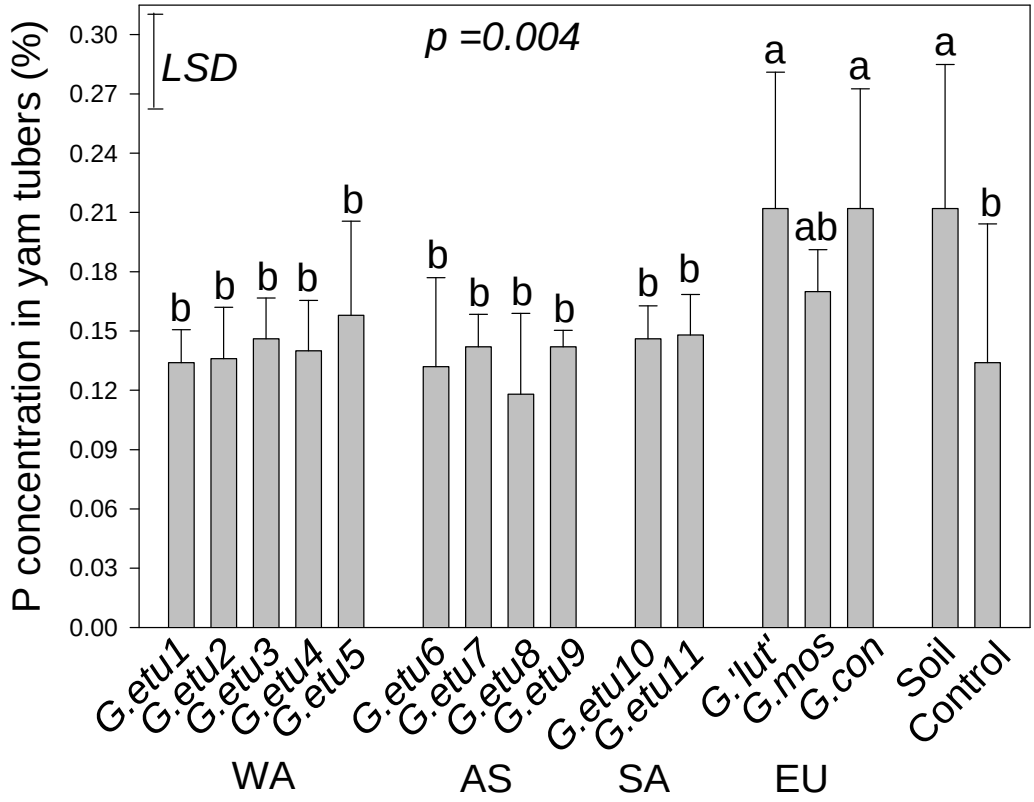

Fig. 4.6 Phosphorus concentration in tubers of pot cultured plantlets of white yam (*D. rotundata*) 7 months after inoculation with 11 isolates of *G. etunicatum* from tropical West Africa (WA), Asia (AS) and South America (SA) compared with 3 non-tropical AMF isolates from temperate Europe (EU), one mycorrhizal control, inoculated with a West African yam field soil (SOIL), and one non-mycorrhizal control. *G.etu* = *G. etunicatum*; *G.'lut'* = *Glomus* sp. resembling *G. luteum* and *G. claroideum*; *G.mos* = *G. mosseae*; *G.con* = *G. constrictum*. Data are reported as

means and standard deviation of five pot replicates per treatment. Non-significant differences between treatments are shown by identical letters, determined using Fisher's Least Significant Difference (LSD) at the 5% level following one-way ANOVA.

Table 4.3 Shoot nutrient concentrations of pot cultured white yam (*D. rotundata*) 7 months after inoculation with *G. etunicatum* from tropical West Africa (WA), Asia (AS) and South America (SA) compared against 3 non-tropical arbuscular mycorrhizal fungal isolates from temperate Europe (EU), one mycorrhizal control, inoculated with a West African yam field soil (SOIL), and one non-mycorrhizal control were included.

AMF isolates	Nutrient concentrations (%)			
	Nitrogen	Phosphorus	Calcium	Magnesium
WA-*G.etu1*	0.43±0.08a	0.12±0.01 bc	0.44±0.11a	0.38±0.25a
WA-*G.etu2*	0.42±0.17a	0.11±0.00 bc	0.44±0.25a	0.22±0.11a
WA-*G.etu3*	0.48±0.17a	0.11±0.01 bc	0.51±0.20a	0.31±0.14a
WA-*G.etu4*	0.43±0.08a	0.12±0.01 bc	0.48±0.16a	0.29±0.08a
WA-*G.etu5*	0.46±0.07a	0.13±0.01 bc	0.55±0.14a	0.33±0.14a
IN-*G.etu6*	0.48±0.19a	0.13±0.04 ab	0.46±0.15a	0.25±0.09a
IN-*G.etu7*	0.45±0.11a	0.12±0.02 ab	0.52±0.19a	0.34±0.18a
IN-*G.etu8*	0.46±0.09a	0.12±0.03 bc	0.53±0.18a	0.34±0.17a
IN-*G.etu9*	0.43±0.03a	0.12±0.02 bc	0.53±0.05a	0.34±0.04a
SA-*G.etu10*	0.42±0.11a	0.12±0.01 bc	0.56±0.09a	0.39±0.08a
SA-*G.etu11*	0.50±0.07a	0.15±0.02 bc	0.55±0.03a	0.36±0.04a
EU-*G.'lut'*	0.66±0.13a	0.11±0.04 a	0.56±0.08a	0.49±0.17a
EU-*G.mos*	0.51±0.05a	0.12±0.01 bc	0.51±0.16a	0.31±0.08a
EU-*G.con*	0.63±0.12a	0.13±0.03 bc	0.77±0.44a	0.64±0.31a
SOIL inoculum	0.43±0.03a	0.10±0.01 c	0.45±0.11a	0.40±0.23a
Control	0.39±0.03a	0.10±0.00 c	0.50±0.02a	0.36±0.07a
P value	0.08	0.02	0.95	0.19
LSD		0.024		

N = 5 per treatment. Non-significant differences between treatments are shown by identical letters, determined using Fisher's Least Significant Difference (LSD) at the 5% level following one-way ANOVA.

4.4.5. Correlation between AMF root colonization and yam dry weight and tuber P concentration

No relationship between AMF root colonization at three months and yam growth parameters at seven months were determined for either experiment (Table 4.4 and 4.5). In experiment I, yam tuber P concentration was positively correlated with AMF root colonization (P = 0.042; Table 4) but with a relatively small slope (0.0009; linear regression y = 0.112+0.0009*x).

Table 4.4 Linear regression between arbuscular mycorrhizal root colonization after 3 months and yam growth parameters and P concentrations in the yam tubers 7 months after inoculation with 11 *G. etunicatum* isolates from tropical West Africa (WA), Asia (AS) and South America (SA), 3 non-tropical AMF isolates from temperate Europe (EU), one mycorrhizal control, inoculated with a West African yam field soil (SOIL), and one non-mycorrhizal control*.

	R	P value
AM root colonization and shoot dry weight	0.141	0.601
AM root colonization and root dry weight	0.215	0.424
AM root colonization and tuber dry weight	0.092	0.736
AM root colonization and tuber P concentration	0.516	0.041

* refer to table1

Table 4.5 Linear regression between arbuscular mycorrhizal root colonization after 3 months and yam growth parameters 7 months after arbuscular mycorrhizal fungi (AMF) inoculation with 27 indigenous and 3 non-indigenous AMF isolates comprising 11 AMF species*.

	R	P value
AM root colonization and shoot dry weight	0.349	0.055
AM root colonization and root dry weight	0.347	0.056
AM root colonization and tuber dry weight	0.082	0.663

* refer to table2.

4.4.6. Correlation between AMF spore density in the monosporal inocula and parameters analyzed in experiment II

No correlation was observed between monosporal AMF inocula, spore density after 3-months storage of the spores in the dry trap culture substrate (comprising 8 species; Fig. 4.1) and AMF root colonization (Table 4.6). There was also no significant correlation between spore density of the monosporal AMF inocula and yam growth parameters (Table 4.6). Correlation analysis was not calculated for the experiment I as the different monosporal inocula derived from different propagation cycles (the West African isolates from first cycle, the Indian and Bolivian isolates from second cycle, and the European from third cycle).

Table 4.6 Linear regressions between spore densities of the 27 indigenous arbuscular mycorrhizal fungi (AMF) inocula and AM root colonization after 3 months and yam growth parameters after 7 months. Inocula comprised 9 AMF species obtained after propagation on *Hieracium pilosella*

Linear regression between	R	P value
Inoculum spore density and AM root colonization	0.091	0.653
Inoculum spore density and yam shoot dry weight	0.125	0.536
Inoculum spore density and yam root dry weight	0.015	0.942
Inoculum spore density and yam tuber dry weight	0.044	0.829

4.5. Discussion

The present study, involving 29 AMF isolates of 9 species and several species groups, is amongt the largest investigating the effect of AMF inocula from a single geographical origin on growth promotion of a single plant species. Furthermore, the study presents the first assessment of AMF indigenous to the SGS of West Africa, where information on AMF species is virtually known. Previously, studies have tended to be based on non-indigenous, exotic isolates (e.g. Frey et al., 1985; Copretta et al., 2006) or, alternatively, were restricted solely to the screening of *Glomus* sp. isolates (Munkvold et al. 2004; Jansa et al. 2005) or, were focused on relatively few isolates (e.g. Yano-Melo et al., 1999; Jansa et al. 2005; Cavallazzi et al., 2007). Moreover, the large

majority of such studies were not based on monosporal, but on multiple spore isolates (e.g. Sieverding, 1991; Fagbola et al., 1998; Duffy and Cassells, 2000; O'Herlihy et al., 2003; Oyekanmi et al., 2007), thus, increased of genetic variation and a risk of contamination by morphologically similar species (e.g. *G. intraradices* and *G. fasciculatum*). Also noteworthy for our study is that this is the first successful establishment of single spore, respectively single sporocarp-fragment derived cultures for several species, such as *A. scrobiculata*, *A. spinosa*, *K. kentinensis* and sporocarpic *G. sinuosum*.

When compared with the non-mycorrhizal control, the majority of tropical *G. etunicatum* isolates (6 of 11) positively influenced ($P \leq 0.05$) yam tuber weight, but not negatively, whereby their geographical origin appeared to be of minor importance. Remarkably, the non-tropical AMF isolates (EU-*G.'lut'* and EU-*G.con*) generally did not affect yam tuber dry weight (EU-*G.mos*) (Fig. 4.4 C) despite of their high colonization level. However, these results are likely, at least, partly dependent on the species or the isolate since the experiment II revealed that different AMF species and isolates affected yam growth differently, but the majority of indigenous AMF isolates (14 out of 27) led to increased yam tuber weights. Indigenous isolates of *G. mosseae*, *G. etunicatum*, and *A. scrobiculata*, and especially *G. hoi* and *A. spinosa* had a greater effect on yam tuber weight than indigenous *G. sinuosum* and *K. kentinensis*. Various other studies have also shown the variable effect of different AMF species on plant growth (e.g. Frey et al., 1985; Sieverding 1991), which can be related to specific compatibility between host plants and AMF species (Dodd et al., 2000; Klironomos, 2003). Variable compatibility between AMF species and host plants has been reported in a number of crops, such as potatoes (Yao et al., 2002; Diop et al., 2003), onion (Yao, 1996), maize (Khalill et al., 1994) and sweet potatoes (Gai et al., 2006). Recent studies also strongly suggest that such effects may vary intraspecifically at a high level (e.g. Munkvold et al. 2004; Koch et al., 2004; Koch et al., 2006). However, it has to be considered that in such studies the inocula applied were sometimes prepared in different laboratories, propagated on different host plants and substrates, and under different propagation conditions (time, pot sizes etc.).Thus, intraspecific variation of host growth promotion was probably not only a factor of genetic variation but also of the quality of the inocula. In the current study, we tried to minimize this 'background disturbance', by propagating the isolates as

close as possible under the same conditions, on the same plant species and substrate, and for the same duration.

None of the isolates affected the shoot dry weight, which might be explained by yam physiology, which is characterised by dry matter translocation from shoots to tubers during the growth period, especially towards tuber maturity when shoots senesce (Sobulo, 1971). Root dry weight was affected by the AMF isolate only in the first experiment, but with a rather high variability within treatments: The indigenous *G. etunicatum* isolates slightly affected root dry weight positively compared with non-indigenous isolates. A larger root system, though, may prove decisive for such crops, which are prone to production constraints under low nutrient and water availability, especially during extended dry periods within the yam-growing season.

In the first growth experiment, the non-tropical isolates of *G. etunicatum* were the only monosporal treatments that led to increased P concentrations in tubers, although they did not affect yam tuber dry weight. Such results could therefore lead to the assumption that these non-tropical isolates were less useful inocula for yam tuber production since they extracted more phosphorus from the soil when compared to the non-mycorrhizal control without leading to simultaneous improved tuber growth. Nutrient concentrations were not determined in the yam tubers in the second experiment. This would have been interesting to elucidate which indigenous isolates/species affected nutrient assimilation with or without concomitant tuber yield increase, to judge the benefits of the various isolates and identify those most suitable for yam tuber production without exaggerated nutrient export from the soils. However, the increase in tuber P concentration of the mycorrhizal control soil, inoculated with a yam field soil AMF community, indicates that yam association with some indigenous AMF isolates can also lead to increased P uptake without having simultaneous effects on tuber growth. Sporocarpic *G. sinuosum* and *K. kentinensis* might be two of such species. Interestingly, sporocarpic *Glomus* spp. were found to be amongt the first species to disappear from fields with intensification of agronomic practices (Oehl et al., 2003; Tchabi et al. 2008). On the other hand, they may be a decisive factor during fallow periods, leading to increased available and total P contents towards a rapid restoration of soil fertility. However, the current study focused on the assessment of AMF isolates on yam plantlets, shortly after weaning, which may prove not to be the most suitable period to assess the

effect of AMF species on yam tuber, given that only a small tuber is produced. The most informative period of the effects of AMF on yam tuber development may be during subsequent growth periods. Information on the effects of AMF on other tuber crop vitro-plantlets like potato inoculated at post-flask have however, shown higher number of minitubers, weight per minituber and total weight of minitubers per plant (Vosátka and Gryndler, 2000). In addition, *Glomus deserticola* inoculation to cassava micropropagated plantlets at acclimatization phase improved the percentage of plantlets survival but the growth parameters were similar when compared to plantlets inoculated with AMF after acclimatization phase (Azcón-Aguilar et al., 1997). However, the timing of AMF inoculation significantly improved cassava growth parameters compared to control (Azcón-Aguilar et al., 1997). In contrary, Monticelli et al., (2000) reported an increased development of shoots in terms of height and weight of fruit tree rootstocks cv GF677 and cv. Citation in double inoculation (at acclimatization and at transplanting) with *Scutellospora calospora* compared to a single inoculation. The general observation from these results was that micropropagated yam response positively to AMF inoculation but the level of the responses depend on AMF species even strains and on yam cultivar. We suggest that yam micropropagated plantlets inoculation after weaning phage might be successful regarding plant growth and yield; however, to increase the potential of the inoculation in practical production of yam minitubers, it is necessary to consider the differences of various yam cultivars and to select appropriate AMF strains.

Although none of the isolates reached the high colonization levels found for the indigenous AMF field soil community, all AMF isolates significantly colonized the yam roots independently of their geographical origin. Even the three isolates from Central Europe colonized the yam roots to a relatively high level (40-60%), confirming observations that the infectivity of any AMF is likely more dependent on its intrinsic ability to infect and spread in a specific host than its origin (Monzon and Azcon, 1996; van der Heijden and Kuyper, 2001; Klironomos, 2003). On the other hand, our results confirm that yam can be colonized by a wide and broad range of AMF species (Tchabi et al., chapter 3), and suggest that there was rather low host specificity for the 13 AMF species and 41 isolates tested. However, there were differences in root colonization levels between AMF species and isolates: isolates of *G. mosseae*, *G. hoi* and *K. kentinensis* had higher AM root colonization levels than isolates of *A. scrobiculata,* and especially *G. sinuosum* and *A.*

spinosa, which could not be necessarily explained by inoculum spore density. Similar results were obtained in previous studies where it was postulated that AM fungal infection and colonization is not necessarily host specific, but that the level of colonization and function can depend on the AM fungal ability to colonize a specific host and even on the genotype of the host plant (Buwalda et al., 1984; Gai et al., 2006). Several recent studies have revealed that there is at least a clear host preference in the AM symbiosis (e.g. Bever et al., 2001; Sýkorová et al., 2007), even though the reasons for this are yet to be established. The lower colonization levels in the second than in the first experiment might, however, be related to the higher amount of substrate used in the second (3 kg) than in the first experiment (2 kg).

Our attempts to achieve a large set of monosporal cultures of various AMF species using *S. guianensis* and *H. pilosella* yielded a series of isolates of nine AMF species. This represents approximately 25% of the species (37) that sporulated in the preceeding trap cultures on *S. bicolor* (Tchabi et al., chapter 3), but moreover represents 90% of the species selected for the single spore inoculation in the pipette tip system. In our trap cultures, spores e.g. of *Gigaspora*, *Scutellospora*, *Diversispora* and *Ambispora* species were scarce, creating difficulties to establish such species in pure cultures. Other species could also not be readily identified under the dissecting microscope, leading to their avoidance for establishing monosporal cultures, for example *Kuklospora colombiana* or *Paraglomus occultum*. We attribute our success in achieving several species in monosporal culture to our attempt to imitate the length of the wet and dry season in the SGS during culturing and subsequent storage in the air-dried substrate, both during trap culture and subsequent single spore propagation. This strategy was chosen to reflect as closely as possible the conditions during the life cycles of the AMF at their natural sites, breaking their putative (spore) dormancy during dry storage. This strategy was derived from our previous experience with AMF trap cultures from the SGS (Tchabi et al. 2008). There, even following a 3-month storage of air-dried field soil samples, trap culturing largely failed with field samples taken during the wet season, but with field samples from dry season, a total of 44 species were propagated (Tchabi et al. chapter 3). Remarkably, *H. pilosella*, a typical plant for Central Europe, was found to be an excellent host for the propagation of AMF species from tropical West Africa.

In conclusion, the results of the current study indicate a high potential for indigenous AMF to provide plant growth benefits for micro-propagated yam when applied at the stage of transplantation. Several species or isolates promoted tuber growth of white yam, although AMF species and isolates were not equally beneficial. The data indicate that *G. hoi*, *A. spinosa*, *G. mosseae* and *G. etunicatum* isolates offer particular promise, although future greenhouse and field studies on different yam cultivars and species are necessary to confirm these benefits. Interestingly, some non-indigenous isolates were also effective in promoting yam tuber growth, but since these species also occur in West Africa, there may be also indigenous isolates that may be more adapted to prevailing field conditions than the non-indigenous isolates. Although the presence of AMF led to increased tuber P concentrations in some cases, the results were variable and compounded by the fact that there was a general negative correlation between tuber growth and tuber P concentration. The physiology behind this different functionality of AMF species or isolates is not yet understood, it may be crucial to determine this in order to better judge the usefulness and applicability of AMF for various eco- and agricultural systems. For the 'yam belt' in West Africa, it might be especially interesting to identify AMF species that promote growth of high quality yam tubers, with concomitant efficient and sustainable use of soil nutrient resources. Together with the development of improved yam cultivars, this aspect may additionally help to provide a more stable and improved yam production.

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CHAPTER 5: Effect of arbuscular mycorrhizal fungal application on micropropagated yam plantlets and suppression of nematode damage caused by *Scutellonema bradys* (Tylenchideae) and *Meloidogyne* spp. (Meloidogyneae)

5.1. Abstract

Using two commercially available arbuscular mycorrhizal fungal (AMF) products, one based on *Glomus mosseae* and the other based on *Glomus dussii*, two experiments were conducted to assess their effect on yam growth and ability to suppress nematode damage in pots. Four cultivars (cvs) in total (*Dioscorea alata* cvs TDa98-01183 and TDa98-165, and *Dioscorea rotundata* cvs. TDr97-00551 and TDr 745) were used. In the first experiment, micropropagated yam plantlets were inoculated either with *G. mosseae* or with *G. dussii* at the stage of transplanting into 2L pots and - one month later - with 500 vermiform *Scutellonema bradys*. In the second experiment, AMF inoculated micropropagated plantlets were challenged with 500 infective juveniles of *Meloidogyne* spp. The plantlets were grown for further six months in the greenhouse at IITA-Ibadan. Although the results showed a wide variance, the presence of AMF tended to lead to improved growth of yam, especially *D. alata* cvs, as compared with the non-arbuscular mycorrhizal control plants. When challenged with the yam nematode *S. bradys*, plantlets of the two *D. alata* cultivars pre-inoculated with *G. mosseae* and cv TDr97-00551 pre-inoculated with *G. dussii* yielded significantly higher tuber weights compared to non-AMF control plantlets, indicating a relatively high degree of functional specificity of the AMF species. In addition, when challenged with *Meloidogyne* spp., cv TDa98-01183 plantlets pre-inoculated with either AMF species yielded heavier tubers than the non-mycorrhizal control plantlets. *Scutellonema bradys* densities on yam plantlets pre-inoculated with AMF were generally suppressed, although no differences were observed in visible damage scores, which remained low or absent across treatments. However, *Meloidogyne* spp. densities and galling damage tended to be similar with or without AMF. Thus, with regard to nematode suppression, AMF appears to counter *S. bradys* attack more than an attack by *Meloidogyne* spp., but obviously, this

finding requires further substantiation, particularly under field conditions. In conclusion, our pot experiments, using the delicate micropropagated plantlets, showed that yam cvs are highly mycorrhizal, though variably response to AMF species and thus, there is a potential to use AMF as inocula to sustainably improve yam productivity.

Keywords: Bio control, bio fertilizer, *Scutellonema bradys*, *Meloidogyne* spp., yam growth, yield

5.2. Introduction

Yam (*Dioscorea* spp.) is a tuber crop widely cultivated in West and Central Africa and is a particularly important staple source of carbohydrates and vitamins in the local diet (Coursey, 1967; IITA, 2006). It is also cultivated in Asia and in Central and South America (Malaurie et al., 1998). Yam cultivation is adversely affected by an array of biotic and abiotic factors, among which are the plant parasitic nematodes *Scutellonema bradys* (Andrassy, 1958) and *Meloidogyne* spp. (Bridge et al., 2005).

Scutellonema bradys (Andrassy, 1958) and *Meloidogyne* spp. are the economically most important nematodes affecting yam (Bridge et al., 2005). *Scutellonema bradys* is a migratory endo-parasite of roots and tubers, confined usually to the outer 1-2 cm of the tuber, feeding intracellular in yam tuber tissues. This results in ruptured cell walls, loss of cell contents, the formation of cavities (Goodey, 1935; Bridge, 1973), tuber dry rot (Bridge et al., 2005), tuber decay and higher rates of desiccation (Nwauzor and Fawole, 1981). *Meloidogyne* species so far identified associated with yam are *M. incognita, M. javanica, M. arenaria* and *M. hapla,* of which *M. incognita* is the most important (Bridge et al, 2005). *Meloidogyne* spp. are sedentary endo-parasites. Mobile second-stage juveniles (J_2) emerge from the eggs, move towards the roots and penetrate the roots, where they feed on specific cells and induce the formation of giant cells. The adult females of *Meloidogyne* spp. are immobile and enlarge rapidly (Bridge et al., 2005). The proportion of galled tubers collected from yam barns and markets in Nigeria can be as high as 90% for *D. alata* and 70% for *D. rotundata* (Adesiyan and Odihirin, 1978), although in general the proportion of affected yams is much lower (Coyne et al., 2005). The value of galled tubers is estimated to be between 39-52% lower than healthy ones (Nwauzor and Fawole, 1981).

Pesticides can be used for nematode control, but they are expensive, unavailable or highly toxic for both the user and the environment, constituting serious health hazards (FAO, 2007). Other nematode management practices, such as hot water treatment of tubers (Speijer, 1996; IITA, 2006), use of cover crops (Claudius-Cole et al., 2005), trap crops (El-Nagdi and Youssef, 2004), chemical fertilizers (Baimey, 2005) or organic fertilizers (Adesiyan and Adeniji, 1976; Youssef et al., 2005) have been explored for yam. Recent progress in biotechnology has also shown that tissue culture of yam will provide disease and pest-free planting material, using aseptic *in vitro* meristem/shoot tip culture techniques (IITA, 2006). However, the vitroplants obtained are delicate and fragile, and not only free of pathogens, but also free of all natural beneficial microorganisms, such as arbuscular mycorrhizal fungi (AMF).

AMF are important elements of the soil microflora in agroecosystems, which form a mutualistic symbiosis with most plant species, including almost all plants currently micropropagated (Smith and Read, 2008). AMF are active in increasing the availability and uptake of soil phosphorus and trace elements, thereby enhancing host plant growth (Hamel, 1996; Dodd, 2000, van der Heijden et al., 2006). They can alleviate biotic and abiotic stresses (Fred et al., 2001; Alarcon et al., 2007; Arriagada et al., 2007a; Aroca et al., 2007; Audet and Charest, 2007). They are also particularly important in sustainable agriculture systems where non-biological inputs are low (Brussard et al., 2007). Root colonization by AMF, in general, favors plant development by increasing nutrient uptake, hormonal activity, growth rate and consequently yield (Arriagada et al., 2007b; Smith and Read, 2008), but is also associated with pathogen suppression (Ryan et al., 2003; Vestberg et al., 2004; Hol and Cook, 2005). Some AMF species have recently become available as commercial products for promoting crop productivity (Duffy et al., 1999; Deliopoulos et al., 2007). In tuber crops such as potato (*Solanum* spp.) and sweet potato (*Ipomea batata*), results following the application of commercial AMF products have shown that individual species of AMF formulation, and even combinations of a number of AMF species in a single formulation, differ in their ability to promote plant growth, which depends on the specific compatibility between plant and fungal species (Carpio et al., 2005; Farmer et al., 2006). The existence of the interspecific variation in interactions between AMF and host plants increases the need for efficient screening of AMF for host-plant species compatibility and especially for broad-spectrum associations. On yam, no studies have yet been reported on the efficiency of

commercial AMF isolates or species for promoting yam growth and yield, or management of nematodes. The present study aimed at evaluating the effect of two commercial AMF products, based separately on *Glomus mosseae* and *G. dussii,* on yam growth parameters and on plant parasitic nematode management using *in vitro* cultures of various yam cultivars.

5.3. Materials and methods

5.3.1. Experimental details

Two separate experiments were conducted at the International Institute of Tropical Agriculture (IITA), Ibadan (Nigeria), in the Southern Guinea savanna zone of Nigeria (7°30'N, 3°05'E). The experiments were established under controlled conditions in the greenhouse with mean daily temperatures ranging between 25°C and 32°C and humidity between 70% and 85%. The soil used for both experiments was collected from a depth of 0 – 15 cm at IITA Ibadan. Soil was passed through a 1 mm sieve to remove roots, sterilized by autoclaving in the oven at 80°C for 3 days and then air-dried. The soil was characterized as a sandy loam soil with a pH 6.0 and total nitrogen and available phosphorus were at 0.7 g N kg^{-1} and 2.96 mg P kg^{-1}, respectively (Oyekanmi et al., 2006).

5.3.2. Source and acclimatization of yam plantlets

For both experiments, *in vitro* tissue culture plantlets of *D. alata* (TDa 98-165, TDa 98-01183) and *D. rotundata* (TDr 745, TDr 87-00551), selected due to their availability, were supplied by the Biotechnology unit of IITA-Ibadan (Nigeria). The plantlets were multiplied under *in vitro* conditions by sub-culturing nodal segments from established *in vitro* plantlets under a laminar flow hood in culture test tubes containing a yam specific multiplication medium (Ng, 1994). The plantlets were regenerated in the culture room with 12 h photoperiod, 3000 flux light intensity, 27 ± 1°C of temperature and 70 ± 5% of relative humidity.

Following removal from test tubes, before use in the trap cultures, the plantlets were acclimatized for three weeks. They were planted into peat pellets after first soaking them in water to swell. After removal from the test tube, their roots were gently rinsed in water and then

transferred with a forceps into the peat pellets. They were then placed on a tray and covered with thin, transparent plastic sheet to maintain high relative humidity (70 ± 5%), but enabling air to circulate under the cover. The tray was kept under controlled conditions in the greenhouse (12 h of photoperiod; 25 °C in the day, 21 °C in the night; 65 ± 5% humidity). After one week, the plastic cover was steadily removed for increasingly longer periods (6-12 h) per day, before the plastic was completely removed after three weeks and the plants transplanted one week later into individual pots.

5.3.3. Source of AMF inoculum and inoculation procedure

Glomus mosseae and *G. dussii* were obtained from BIORIZE Company© (Dijon, France). The *G. mosseae* strain originated from Western Europe, while *G. dussii* originated from Côte d'Ivoire, West Africa. These commercial inocula consisted of substrates, spores, hyphae and chopped infested fine roots, 2 g of which (representing approximately 300 spores) were inoculated to each plantlet at transplanting. A hole ~8 cm deep was made in the substrate at the middle of each 2-l pot using a pencil. The inoculum was then placed in the hole before planting a single plantlet above the inoculum. Each AMF product was applied separately, as individual treatment. Non-inoculated pots were included as controls. All control pots received 2 g of sterilized soil and 30 ml of filtrated suspensions from 20 g of both AMF products.

5.3.4. *Scutellonema bradys* inoculum preparation and procedure of inoculation

Scutellonema bradys were collected from infected peels obtained from heavily infected tubers of *D. rotundata* cv TDr 131 from IITA-Ibadan. To determine the nematode density for the required inoculum of *S. bradys*, the infected tubers were manually peeled using a kitchen peeler, and chopped in ~0.5 cm x 0.3 cm pieces (Baimey et al., 2005). Nematodes were extracted from the peels for 48 h using a modified Baermann pie pan method (Coyne et al., 2007). Prior to inoculation, the density of the *S. bradys* suspension, which had been adjusted to 100 ml with tap water, was estimated from 3 x 10 ml aliquots, after manually shaking without allowing the nematodes to settle down. Fornematodes counting, a Leica Wild M3C stereomicroscope was used. Two months after planting, each plant was inoculated with 8 ml suspension of 500 vermiform *S. bradys*. A water control of 8 ml was added to the control plants. Plants were subsequently watered with 300 ml tap water per each plant from the base.

5.3.5. *Meloidogyne* spp. inoculum preparation and inoculation procedure

Meloidogyne spp. were originally extracted from an infested yam tuber (*D. rotundata*, cv Amula) collected from the market in Ibadan. The yam tuber was peeled, chopped finely and nematodes were extracted using a modified Baermann method (Coyne et al., 2007). The nematodes were maintained in the greenhouse at IITA-Ibadan on tomato (cv Pello) plants. For inoculum, *Meloidogyn*e spp. J_2 and eggs were obtained by finely chopping infected tomato roots, and macerating in 1.0 % NaOCl for 4 min to aid their release from the eggmass matrix. Eggs were caught on a 20 µm aperture sieve after passing through nested sieves and rinsed in five changes of tap water, before maintaining in tap water for 10 days at room temperature. Hatched J_2 were inoculated into soil aside seedlings in a shallow trench made ~5 cm radius around each plant (~5-10 cm deep) that exposed some of the roots, at a rate of 500 J2/plant in 10 ml of water, at 2 months after planting. Control plants received 10 ml of water. All plants were subsequently watered with 300 ml tap water per plant from the base. Prior to inoculation, the density of the *Meloidogyne* spp. J_2 suspension, which had been adjusted to 100 ml with tap water, was estimated from 3 x 10 ml aliquots, after manually shaking without allowing the nematodes to settle before removing and counting using a Leica Wild M3C stereomicroscope.

5.3.6. Experimental design

In both experiments single plants of *D. alata* (TDa) and *D. rotundata* (TDr) were planted in 2-l pots with 2 kg sterilized soil and maintained in the greenhouse over 6 months. All yam plantlets were aged one month at initiation of the experiment. Plants were inoculated with two AMF species (*G. mosseae* and *G. dussii*) that were applied separately. In all experiments, pots were randomly arranged, using ten and six plants per treatment (respectively for experiment I and II). Plants were staked at two months in all cases, received no fertilizer during the experiment and were watered regularly as required. During the growing period, temperature ranged between 25°C and 35°C and relative humidity between 75% and 90% in the greenhouse.

The first experiment involved three factors: AMF (three levels: *G. mosseae, G. dussii* and non-inoculated control), yam cultivar (four levels: TDa 98-165, TDa98-01183, TDr745, TDr87/00551) and *S. bradys* inoculation (two levels: 0 and 500). Sixty pots per yam cultivar

were used, totalling 240 pots. The experiment was established in June 2006 and harvested in December 2006.

The second experiment involved three factors: AMF (three levels: *G. mosseae, G. dussii* and non-inoculated control) yam cultivar (four levels: TDa 98-165, TDa98-01183, TDr745, TDr87/00551) and *Meloidogyne* spp. inoculum (two levels: 0 and 500 J2). The experiment was established in June 2007 and harvested in December 2007. Thirty-six pots per yam cultivar were used, totalling 144 pots.

5.3.7. Assessment of AMF spore density and root colonization

In both experiments, soil core samples were removed one day before harvest of yam tubers, according to Oehl et al. (2003), which consisted of collecting two separate soil cores of a combined total of 30 cm^3 (sampling depth 10 cm) from each pot. Roots were extracted by wet sieving and decantation, while the AMF spores were isolated by wet sieving and sucrose density gradient centrifugation (Oehl et al., 2003). The root colonization by AMF was determined according to Brundrett et al. (1996), using trypan blue to stain the mycorrhizal structures. The gridline-intersect technique (Giovannetti and Mosse, 1980) was used to analyse AMF colonization under a dissecting stereo microscope (Leica Wild M3C) at up to 90x magnification.

5.3.8. Assessment of yam growth parameters at harvest

The plants were harvested six months after transplanting into individual pots. The shoots were cut to soil level and then tubers were removed by hand, and gently removed the soil away from tubers and roots. The roots were removed with forceps and collected separately. Shoots, roots and tubers from each pot were rinsed gently under tap water, air dried and separately stored in labelled paper bags. Dry weight of shoots and roots were recorded following oven-drying in a well-ventilated Gallenkamp oven at 80°C for 72 h. Only fresh tuber weight was recorded as they were used for planting in the subsequent season at field sites. Total dry root weights were calculated after taking into account material removed to determine mycorrhizal colonization and nematode density.

5.3.9. Assessment of nematode density and tuber damage symptoms

For both experiments, all tubers harvested were scored for both cracking and dry rot (experiment 1) or galling (experiment II) severity. Galling of tubers is the visible symptom of *Meloidogyne* spp. infestation, while cracking and dry rot are the main symptoms of *S. bradys* infestation. The tuber cracking, galling and dry rot severity were assessed on a scale of 1-5 (Claudius-Cole et al., 2005): where 1 = clean tuber; 2 = 1-25% tuber skin showing cracking, galling or dry rot symptoms (low level of damage); 3 = 25-50% of tuber skin symptoms (low to moderate level of damage); 4 = 51-75% tuber skin symptoms (moderate to severe level of damage); 5 = 76-100% tuber skin symptoms (high level of damage). All tubers per pot were scored and mean scores calculated per pot when more than one tuber per pot was present. Nematodes were not extracted from tubers, which were destructive, preventing planting through to the next season, which was undertaken for longer-term assessment on the effects of AMF on yam production.

Nematodes were extracted from the soil of each pot by mixing all the contents of each pot, removing 3 x 50 g sub-samples and extracting them using a modified Bearmann Pie Pan method (Coyne et al., 2007). Two methods were used to extract nematodes from roots: *Meloidogyne* spp. were extracted using a modified Baermann technique following root maceration in 1.0 % NaOCl for 4 min, while *S. bradys* were extracted directly using the modified Baermann technique as described in above sections. For each method, roots collected from each pot were chopped into small pieces of 0.1 to 0.2 cm and 2 x 5 g sub-samples were removed for nematode extraction. Nematodes were counted with a stereomicroscope at 400x magnification.

5.3.10. Data analyses

All data were analyzed using STATGRAPHICS, version 9.1 in Windows 2007. Two-Way ANOVA was used to compare yam growth parameters (shoot, root and tuber weight) between treatments. Data on nematodes and on mycorrhization were analyzed by one-way ANOVA. Prior to analysis, AMF spore density and nematode population density data were $\log_{10}$ (x+1) transformed, while data on mycorrhizal colonization were arcsin (x/100) transformed for homogeneous variances. The differences among treatment means were compared with Fischer's Least Significant Difference (LSD) Test. Pearson's correlation coefficient was used to assess the association between root colonization and various growth parameters.

5.4. Results

5.4.1. Effect of AMF and *S. bradys* inoculation on yam root colonization and plant growth parameters.

Mycorrhizal root colonization was moderately high (between 17% and 44%) with a significant interaction occurring between AMF inoculation and nematode inoculation (Df = 2, F = 4.3, p = 0.02; Table 5.1). Higher colonization by AMF was recorded from all cultivars inoculated with *G. mosseae,* compared to *G. dussii* in the treatment without *S. bradys,* while in the treatments with *S. bradys*, higher colonization by AMF was recorded from all yam cultivars except for cv. TDa98-165 inoculated with *G. dussii* (Table 5.2). Spore production was affected by both yam cultivars and *S. bradys* inoculation (Df = 1, F = 48.5, p = <0.001 Table 5.1). A higher spore density was recorded from cv TDr745 inoculated with *G. dussii* without *S. bradys* inoculation compared to cv. TDr745 plantlets inoculated with *G. dussii* and *S. bradys* (Table 5.2).

Without *S. bradys* inoculation, fresh weight and number of tubers were significantly affected by AMF inoculation (Df = 2, F = 39.44, p <0.001; Df = 2, F = 4.07, p = 0.01 respectively) and yam cultivar (DF = 3, F =11.83, p <0.001; Df = 3, F = 3.35, p = 0.02) (Table 5.1). AMF inoculation did not affect shoot or root weight. *S. bradys* inoculation significantly affected tuber fresh weight, shoot and root dry weight (Df = 1, F = 28.19, p <0.001 for tuber fresh weight; Df = 1, F = 4.98, p = 0.02 for shoot dry weight; Df = 1, F = 5.80, p = 0.01 for root dry weight) (Table 5.1). Without *S. bradys* inoculation, application of each AMF species led to a significantly higher fresh weight and number of tubers than the control for TDa98-01183 (Table 5.3). In addition, *G. dussii* application induced higher shoot and root dry weights of TDr97-000551 than the controls (Table 5.3). With *S. bradys* inoculation, only plantlets from cvs TDa98-165 and TDa98-01183 inoculated with *G. mosseae* yielded higher (p <0.01) than controls, while a higher number of tubers was recorded from cv TDr97-00551 when plantlets were inoculated with *G. mosseae* compared to controls (Table 5.3).

Table 5.1: Analysis of variance table for yam cultivar, inoculation of arbuscular mycorrhizal fungi[1] and *Scutellonema bradys*[2] factor effects on micropropagated yam plantlet growth (tuber fresh weight, shoot dry weight, root dry weight, tuber number), AMF development (root colonization, spore production) and nematode parameters (cracking, dry rot, population densities in soil and root) from a pot study conducted under greenhouse conditions at IITA-Ibadan, Nigeria, West Africa.

Variable	Factors						
	Cultivar (A)	AMF (B)	*S. bradys* (C)	AxB	AxC	BxC	AxBxC
Colonization							
Df	3	2	1	6	3	2	6
F	2.27	131.5	1.16	1.00	2.12	4.3	1.56
p	0.08	<0.001	0.18	0.42	0.09	0.02	0.16
Spore densities[3]							
Df	3	2	1	6	3	2	6
F	207.3	586.6	48.5	70.7	9.4	12.3	25.5
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
TFW							
Df	3	2	1	6	3	2	6
F	11.83	39.44	28.19	0.64	1.29	2.07	0.93
p	<0.001	<0.001	<0.001	0.69	0.27	0.12	0.47
SDW							
Df	3	2	1	6	3	2	6
F	2.42	2.13	4.98	3.51	2.72	2.02	2.62
p	0.06	0.12	0.02	0.002	0.04	0.13	0.018
RDW							
Df	3	2	1	6	3	2	6
F	0.70	0.13	5.80	1.57	2.10	0.53	1.60
p	0.55	0.87	0.01	0.15	0.10	0.58	0.14

Tuber No.							
Df	3	2	1	6	3	2	6
F	3.64	4.07	0.75	1.81	1.81	0.94	0.90
P	0.01	0.01	0.38	0.09	0.14	0.39	0.49
Tuber cracking							
Df	3	2	1	6	3	2	6
F	3.35	1.8	12.30	3.10	5.83	1.10	3.05
p	0.02	0.16	<0.001	0.006	<0.001	0.33	0.006
Tuber dry rot							
Df	3	2	1	6	3	2	6
F	2.20	0.01	12.31	1.91	4.74	0.54	2.63
p	0.08	0.98	<0.001	0.08	0.003	0.58	0.01
S. bradys root density							
Df	3	2	1	6	3	2	6
F	1.33	76.74	488.5	1.98	1.33	76.7	1.98
p	0.26	<0.001	<0.001	0.06	0.26	<0.001	0.06
S. bradys soil density							
Df	3	2	1	6	3	2	6
F	0.73	26.6	363.2	6.58	0.73	26.7	6.9
p	0.53	<0.001	<0.001	<0.001	0.53	<0.001	<0.001

Tuber cracking, and dry rot severity were assessed using an arbitrary scale from 1 to 5 (Claudius-Cole et al., 2005), where 1 = clean tuber; 2 = 1-25% tuber skin showing cracking or galling or dry rot symptoms (low level of damage); 3 = 25-50% of tuber skin showing cracking or galling or dry rot symptoms (low to moderate level of damage); 4 = 51-75% tuber skin showing cracking or galling or dry rot symptoms (moderate to severe level of damage); 5 = 76-100% tuber skin showing cracking or galling or dry rot symptoms (high level of damage). [1]AMF inoculated at rate of 300 spores per pot at yam plantlet transplanting into individual pot. [2] *S. bradys* inoculated at rate of 500 vermiform two months after AMF inoculation. *S. bradys* density g^{-1} root or 50 g^{-1} soil. [3]spore densities were per 30 cm^3 of substrate. Data on spore densities and nematode population densities were $\log_{10}$ (x+1) transformed while data on mycorrhizal colonization were arcsin (x/100) transformed prior to analysis.TFW = tuber fresh weight, SDW = shoot dry weight, RDW = root dry weight, Tuber No. = tuber number.

Table 5.2: Effect of arbuscular mycorrhizal fungal (*Glomus mosseae* and *G. dussii*) and *Scutellonema bradys* inoculation on root colonization and spore production of two cultivars of *Dioscorea rotundata* (TDr745, TDr87-00551) and two *D. alata* (TDa98-165, TDa98-01183) in pots under greenhouse conditions at IITA-Ibadan, Nigeria, West Africa.

Treatments	AMF Root colonization (%)		AMF spore density (30 cm^{-3} of soil)	
	Non-inoc	Inoc	Non-inoc	Inoc
TDa98-165				
Control	-	-	-	-
G. mosseae	35.9±6.5a	30.9±3.3a	19.3±2.1a	16.2±1.5a
G. dussii	24.4±3.6b	23.4±2.7b	21.8±1.5a	14.2±0.8a
P value	0.03	0.023	0.21	0.32
TDa98-01183				
Control	-	-	-	-
G. mosseae	44.04±4.6a	22.6±3.1b	19.8±1.7a	19.01±0.8a
G. dussii	21.5±4.8b	29.1±4.3a	13.5±0.8a	11.2±0.8a
P value	<0.01	0.04	0.16	0.09
TDr745				
Control	-	-	-	-
G. mosseae	31.1±4.3a	17.1±3.3b	28.3±2.6b	25.6±1.4a
G. dussii	19.8±3.7b	24.4±5.3a	52.5±3.1a	16.6±1.8b
P value	0.04	0.02	<0.01	<0.01
TDr97-00551				
Control	-	-	-	-
G. mosseae	22.9±3.6a	29.7±5.4a	79.3±4.2a	52.6±5.6a
G. dussii	22.02±3.7a	27.1±9.6a	39.9±2.1a	48.6±2.6a
P value	0.23	0.08	0.07	0.13

Non-inoculated AMF treatments were free of colonization and spore production. - = data collected were zero and were omitted from statistical analysis. Values = mean (± SE) of ten replicates (non transformed data) at harvest seven and five months after AMF and *S. bradys* inoculation respectively; Inoc = inoculated with *S. bradys*; Non-inoc = non-inoculated with *S. bradys*; Means followed by the same letter within a column for each cultivar were not significantly different ($p > 0.05$) according to the Protected Least Significant Difference test (LSD).

Table 5.3: Effect of arbuscular mycorrhizal fungal (*Glomus mosseae* and *G. dussii*) and *Scutellonema bradys* inoculation on micropropagated yam plantlet growth (tuber fresh weight (TFW), shoot dry weight (SDW), root dry weight (RDW), tuber number (Tuber No.) of two cultivars of *Dioscorea rotundata* (TDr745, TDr87/00551) and two cultivars of *D. alata* (TDa98-165; TDa98-01183) in pots study under greenhouse conditions at IITA-Ibadan, Nigeria, West Africa.

Treatments	TFW (g)		SDW (g)		RDW (g)		Tuber No.	
	Non-inoc	Inoc	Non-inoc	Inoc	Non-inoc	Inoc	Non-inoc	Inoc
TDa98-165								
Control	9.8±2.2a	3.6±1.2b	1.4±0.4b	1.9±0.4b	2.8±1.1a	1.3±0.2a	1.1±0.3a	1.3±0.2b
G. mosseae	12.5±2.3a	15.5±4.5a	3.5±0.6a	4.1±1.2a	3.2±0.9a	1.6±0.6a	1.7±0.4a	2.6±0.6a
G. dussii	3.1±1.4b	6.2±2.1b	3.7±0.9a	0.6±0.1c	1.8±0.6a	0.6±0.1a	1.4±0.6a	1.1±0.3b
P value	0.029	0.04	0.05	0.01	0.07	0.35	0.36	0.03
TDa98-01183								
Control	9.3±1.3b	11.9±1.5b	3.6±0.7a	1.3±0.4a	3.3±0.7a	2.0±0.9a	1.1±0.4a	1.3±0.3a
G. mosseae	16.7±3.8a	23.3±3.6a	1.9±0.4a	3.8±0.8a	2.4±0.8a	2.0±0.7a	1.0±0.1a	1.6±0.4a
G. dussii	13.3±3.4a	11.6±1.1b	2.9±0.6a	3.4±0.9a	2.5±0.7a	1.6±0.5a	1.3±0.5a	1.4±0.6a
P value	0.005	0.0013	0.14	0.08	0.31	0.66	0.33	0.48
TDr745								
Control	7.3±1.5a	4.9±18.8a	3.4±1.3a	3.4±0.8a	1.6±0.5a	1.1±0.2a	1.2±0.1a	1.1±0.2a
G. mosseae	6.8±0.6a	6.9±1.7a	3.7±0.9a	2.7±0.9a	1.8±0.5a	1.4±0.4a	1.3±0.1a	1.0±0.0a
G. dussii	8.11±2.2a	5.2±1.4a	2.7±0.6a	1.2±0.4a	3.1±0.8a	1.5±0.8a	1.3±0.1a	1.3±0.2a
P value	0.06	0.07	0.07	0.16	0.19	0.86	0.56	0.71
TDr97-00551								
Control	9.8±2.3a	2.7±0.6b	3.8±0.6b	3.6±1.2	2.3±0.8b	2.1±0.7a	1.1±0.2a	1.0±0.1a
G. mosseae	13.8±3.1a	2.7±0.6b	3.8±0.6b	3.6±1.2	2.3±0.8b	2.1±0.7a	1.3±0.2a	1.1±0.1a
G. dussii	3.1±1.1b	10.8±1.8a	4.1±0.7a	3.3±1.1	3.9±0.5a	1.3±0.5a	1.0±0.0a	1.3±0.2a
P value	<0.001	0.01	<0.001	0.8	0.002	0.6	0.53	0.39

Values = mean (± SE) of ten replicates at harvest seven and five months after AMF and *S. bradys* inoculation respectively; Means followed by the same letter within a column for each cultivar were not significantly different ($P > 0.05$) according to the Protected Least Significant Difference test (LSD). Inoc = inoculated *S. bradys;* Non-inoc = non-inoculated with *S. bradys*.

Across all cultivars, a negative correlation was observed between root colonization and tuber fresh weight (p = 0.0002) (Table 5.4). Within each cultivar, a negative correlation was observed between root colonization and tuber fresh weight for three of the four assessed cultivars: TDa98-165, TDa98-01183 and TDr745 (p = 0.0038, 0.0142 and 0.0147 respectively) (Table 5.4).

Table 5.4: Pearson correlation analysis between arbuscular mycorrhizal fungal root colonization and yam plant growth parameters (tuber number, tuber fresh weight) or nematode damage scores (cracking, dry rot)

Cultivars		Tuber No.	TFW	Cracking	Dry rot
All cultivars	*Correlation*	0.00019	-0.24101	-0.0144	0.03015
	P value	0.99	< 0.001	0.82	0.6421
TDa98-165	*Correlation*	0.12556	-0.36800	-0.2746	-0.0746
	P value	0.33	< 0.01	0.03	0.5709
TDa98-01183	*Correlation*	0.21152	-0.31521	.10099	0.16321
	P value	0.10	0.01	0.44	0.2128
TDr 745	*Correlation*	0.12436	-0.31367	-0.0878	-0.0154
	P value	0.35	0.01	0.50	0.90
TDr87-00551	*Correlation*	0.11005	-0.17042	0.07170	0.06486
	P value	0.40	0.19	0.5862	0.62

Tuber No. = tuber number, TFW = tuber fresh weight.

5.4.2. Effect of AMF inoculation on yam tuber quality and *S. bradys* density

The AMF inoculation apparently did not reduce the severity of *S. bradys* damage recorded as cracking and dry rot (Table 5.1). Across the experiment, symptoms were very low in general (Table 5.5). Tuber cracking was significantly lower though, where AMF species were applied compared with controls for cvs TDa98-165 and TDr745, while tuber dry rot was significantly lower for cv TDr745 (Table 5.5). Interestingly, AMF inoculation significantly suppressed *S. bradys* densities in roots (Df = 3, F = 2.20, p <0.01) and soil (Df = 1, F = 363.2, p <0.001) (Tables 5.1 and 5.5) on plantlets where both AMF + *S. bradys* were inoculated, compared to *S. bradys* inoculation only.

Table 5.5: *Scutellonema bradys* population densities and tuber damage at harvest seven and five months after arbuscular mycorrhizal fungal (*Glomus mosseae* and *G dussii*) and *S. bradys* inoculation, respectively, to micropropagated yam plantlets of two cultivars of *Dioscorea rotundata* (TDr745, TDr87/00551) and two cultivars of *D. alata* (TDa98-165, TDa98-01183) under greenhouse conditions at IITA-Ibadan, Nigeria, West Africa.

Treatments	*S. bradys* density (root)		*S. bradys* density (soil)		Tuber cracking		Tuber rot	
	Non-inoc	Inoc	Non-inoc	Inoc	Non-inoc	Inoc	Non-inoc	Inoc
TDa98-165								
Control	-	48.6±6.8a	-	5.2±1.01	1.1±0.1	2.4±0.4a	1.0±0.0	1.6±0.1
G. mosseae	-	23.8±2.2b	-	6.3±1.5	1.0±0.0	1.4±0.3b	1.0±0.2	1.6±0.2
G. dussii	-	20.3±3.2b	-	2.9±0.67	1.1±0.2	1.5±0.3b	1.1±0.1	1.4±0.2
p value		<0.001		0.104	0.23	0.04	0.6	0.08
TDa98-01183								
Control	-	69.0±4.5a	-	11.4±1.2	1.0±0.0	1.1±0.1	1.0±0.0	1.2±0.1
G. mosseae	-	21.8±6.5b	-	2.5±0.6	1.0±0.0	1.02±0.2	1.1±0.1	1.1±0.2
G. dussii	-	20.4±3.2b	-	3.8±0.8	1.0±0.0	1.2±0.1	1.0±0.0	1.4±0.2
p value		<0.001		<0.001	0.5	0.26	0.13	0.23
TDr745								
Control	-	73.2±4.6a	-	6.3±1.2	1.2±0.1	1.7±0.4a	1.1±0.1	1.6±0.3a
G. mosseae	-	20.3±2.3b	-	6.0±0.8	1.2±0.1	0.8±0.1b	1.2±0.1	0.7±0.1b
G. dussii	-	17.9±2.0b	-	3.3±0.5	1.3±0.1	0.7±0.1b	1.2±0.1	0.8±0.1b
p value		<0.001		0.055	0.061	0.016	0.07	0.04
TDr97-00551								
Control	-	70.5±8.4a	-	9.3±0.8a	1.0±0.0	1.1±0.1b	1.1±0.1	1.2±0.2
G. mosseae	-	18.4±9.8b	-	2.0±0.4b	1.0±0.0	1.5±0.3ab	1.0±0.0	1.6±0.3
G. dussii	-	32.2±7.5b	-	3.9±1.06 b	1.1±0.1	2.0±0.4a	1.0±0.0	1.8±0.3
p value		<0.001		<0.001	0.12	0.08	0.9	0.23

Roots and soil from non-inoculated treatments were free of nematode. - = data collected were zero and were not used for statistic analysis. Analysis and means separation of nematode densities were undertaken on $\log_{10}(x+1)$ transformed data. [1]Nematode densities were 5 g^{-1} root. [2]Nematode densities 50 g^{-1} soil. Values are means (± SE) (non transformed data) of ten replicates. For each yam cultivar treatment means were compared by columns, and means followed by same (or without) letters were not significantly different ($p > 0.05$) according to the Least Significant Difference test (LSD). Inoc = inoculated with *S. bradys*; Non-inoc = non-inoculated with *S. bradys*.

5.4.3. Effect of AMF and *Meloidogyne* spp. inoculation on yam root colonization and plant growth parameters

Root colonization was in general low (0.3% to 6%) but significantly influenced by both AMF species (Df = 2, F = 4.02, p = 0.02) and by *Meloidogyne* spp. inoculation (Df =1, F = 100.21, p = 0.028) and appears as a function of AMF species and yam cultivars compatibility (Table 5.6). *Glomus mosseae* application resulted in higher root colonization for cv TDa98-01183 than *G. dussii* application (p = 0.03). Both AMF species produced a high density of spores (10 to 136 spores/30 cm^{-3} of soil), but depending on AMF species (Df = 2, F = 8.7, p < 0.001) (Tables 5.6, 5.7). Without *Meloidogyne* spp. inoculation, *G. mosseae* produced higher density of spores on cv TDa98-01183 (p = 0.008) and fewer on cv TDr97-00551 (p = 0.02), than *G. dussii* (Table 5.7). With *Meloidogyne* spp. inoculation to cv. TDr97-00551, *G. dussii* produced more spores than *G. mosseae* (p = 0.03).

Table 5.6: Analysis of variance table for yam cultivar, inoculation of arbuscular mycorrhizal fungi[1] and *Meloidogyne* spp.[2] factor effects on micropropagated yam plantlet growth (tuber fresh weight, shoot dry weight, root dry weight, tuber number), AMF development (root colonization, spore production) and nematode parameters (cracking, rot, population densities) in pots under greenhouse conditions at IITA-Ibadan, Nigeria, West Africa.

Variable	Factors						
	Cultivar (A)	AMF (B)	*S. bradys* (C)	AxB	AxC	BxC	AxBxC
Colonization							
Df	3	2	1	6	3	2	6
F	0.25	4.02	100.21	5.88	34.8	42.36	18.41
p	0.86	0.02	0.028	0.94	0.69	0.130	0.496
Spore densities[3]							
Df	3	2	1	6	3	2	6
F	1.08	8.7	0.12	1.04	0.3	0.8	0.6
p	0.36	<0.001	0.7	0.4	0.8	0.4	0.7
TDW							

Df	3	2	1	6	3	2	6
F	2.28	6.87	4.29	2.18	1.06	0.08	0.68
p	0.086	0.001	0.041	0.054	0.36	0.92	0.66
SDW							
Df	3	2	1	6	3	2	6
F	3.21	0.71	2.03	1.93	4.06	1.21	0.19
p	0.027	0.49	0.15	0.086	0.0097	0.30	0.97
RDW							
Df	3	2	1	6	3	2	6
F	1.63	1.09	5.87	0.57	0.11	0.01	0.42
p	0.18	0.34	0.018	0.75	0.95	0.99	0.86
Tuber No.							
Df	3	2	1	6	3	2	6
F	0.80	0.87	0.02	0.40	1.58	1.004	0.21
p	0.49	0.42	0.89	0.87	0.20	0.35	0.97
Tub galling							
Df 3	2	1	6	3	2	6	
F	3.28	3.20	259.6	0.97	1.21	4.15	0.96
p	0.025	0.046	0.0001	0.45	0.309	0.019	0.460
No. Meloid. in root							
Df	3	2	1	6	3	2	6
F	1.38	1.00	12.62	1.10	1.38	1.00	1.10
p	0.25	0.37	0.001	0.36	0.25	0.37	0.36
No. Meloid. in soil							
Df	3	2	1	6	3	2	6
F	0.83	1.36	18.15	0.95	0.83	1.36	0.95
p	0.21	0.25	0.001	0.46	0.48	0.25	0.46

Tuber galling severity were assessed on an arbitrary scale from 1 to 5 (Claudius-Cole et al., 2005) where 1 = clean tuber; 2 = 1-25 % tuber skin showing cracking or galling or dry rot symptoms (low level of damage); 3 = 25-50% of tuber skin showing cracking or galling or dry rot symptoms (low to moderate level of damage); 4 = 51-75% tuber skin showing cracking or galling or dry rot symptoms (moderate to severe level of damage); 5 = 76-100% tuber skin showing cracking or galling or dry rot symptoms (high level of damage). [1]AMF inoculated at rate of 300 spores per pot at yam plantlet transplanting into individual pot. [2]*Meloidogyne* spp. inoculated at rate of 500 vermiform two months after AMF inoculation. [3]Spore densities were per 30 cm^3 of substrate. TFW = tuber fresh weight, SDW = shoot dry weight, RDW = root dry weight, Tuber No = tuber numbers, No. Meloid. = Number of *Meloidogyne* spp. per 5 g of root or 50 g of soil.

Table 5.7: Effect of arbuscular mycorrhizal fungal (*Glomus mosseae* and *G. dussii*) and *Meloidogyne* spp. inoculation on tuber number, yam root colonization and spore production of two cultivars of *Dioscorea rotundata* (TDr745, TDr87-00551) and two *D. alata* (TDa98-165, TDa98-01183) in pots under greenhouse conditions at IITA-Ibadan, Nigeria, West Africa.

Treatments	Tuber No.		Root colonization (%)		Spore density (g^{-1} of soil)	
	Non-inoc	Inoc	Non-inoc	Inoc	Non-inoc	Inoc
TDa98-165						
Control	1.7±1.7	1.8±1.3	-	-	-	-
G. mosseae	2.0±1.3	1.7±1.5	4.1±2.5	1.9±1.5	56.8±37.2	33.7±22.9
G. dussii	1.0±0.6	1.2±0.5	4.4±3.4	1.2±0.6	21.8±8.2	33.1±14.8
p value	0.36	0.76	0.41	0.46	0.23	0.25
TDa98-01183						
Control	1.4±0.9	1.4±0.6	-	-	-	-
G. mosseae	2.4±1.7	1.3±0.6	6.3±4.5	0.3±0.1b	136.3±52.9a	66.1±45.1
G. dussii	3.2±1.8	1.5±0.6	3.3±1.5	4.9±1.2a	17.6±4.0b	179.3±110.7
p value	0.42	0.10	0.13	0.03	0.008	0.26
TDr745						
Control	1.5±0.6	2.1±1.2	-	-	-	-
G. mosseae	2.0±0.8	2.3±1.5	2.5±1.2	0.9±0.5	43.5±38.2	14.8±7.4
G. dussii	2.4±1.7	1.7±0.9	4.6±2.9	4.6±2.4	11.8±5.4	35.2±24.3
p value	0.5	0.8	0.25	0.09	0.38	0.26
TDr97-00551						
Control	1.0±0.1	1.7±1.1	-	-	-	-
G. mosseae	1.3±0.6	2.1±1.6	1.5±0.9	0.3±0.2	10.5±3.8	23.3±11.8
G. dussii	1.3±1.2	2.0±0.9	0.2±0.5	4.9±3.2	43.7±17.1	49.8±10.6
p value	0.9	0.93	0.39	0.37	0.02	0.03

Non-inoculated AMF treatments were free of colonization and spores production. - = data collected were zero and were not used for statistic analysis. Values were means (± SE) of six replicates at harvest, seven and five months after AMF and *S. bradys* inoculation, respectively. For each yam cultivar treatment means were compared by column, and means followed by same letters (or without) were not significantly different ($p > 0.05$) according to the Protected Least Significant Difference test (LSD). Inoc = inoculated; Non-inoc = non-inoculated with *Meloidogyne* spp.

Table 5.8: Effect of arbuscular mycorrhizal fungal (*Glomus mosseae* and *G. dussii*) and *Meloidogyne* spp. inoculation on micropropagated yam plantlet growth (tuber fresh weight, shoot dry weight (SDW), root dry weight (RDW) of two cultivars of *Dioscorea rotundata* (TDr745, TDr87-00551) and two *D. alata* (TDa98-165, TDa98-01183) in pots under greenhouse conditions at IITA-Ibadan, Nigeria, West Africa.

Treatments	TFW (g)		SDW (g)		RDW (g)	
	Non-inoc	Inoc	Non-inoc	Inoc	Non-inoc	Inoc
TDa98-165						
Control	19.4±14.8b	34.8±14.6	17.6±15.9	26.5±8.1	7.7±6.0	4.5±2.7
G. mos.	75.8±14.6a	66.3±12.9	29.8±9.6	53.2±12.9	15.6±4.0	14.6±10.7
G. dussii	31.8±16.9ab	21.7±12.3	21.0±14.1	39.1±13.6	10.05±10.4	3.8±1.8
p value	0.039	0.25	0.79	0.3	0.60	0.27
TDa98-01183						
Control	8.0±2.1b	3.5±1.2b	23.2±5.4ab	3.4±0.4b	4.0±1.5	0.5±0.1
G. mos.	99.5±11.1a	54.3±9.8a	10.5±4.9b	3.6±1.1b	12.6±7.2	4.7±2.6
G. dussii	77.5±21.3a	42.0±6.5a	30.2±8.5a	8.6±1.9a	13.8±6.1	8.2±3.1
p value	0.050	0.04	0.051	0.003	0.4	0.56
TDr745						
Control	81.7±23.5	68.4±15.2	48.4±14.7	12.9±5.2	8.3±3.6	2.0±1.1
G. mos.	74.2±7.9	74.1±22.6	19.5±4.7	16.2±8.1	14.4±4.2	2.6±1.1
G. dussii	49.2±14.3	43.4±13.2	23.2±6.6	3.6±1.1	6.5±2.2	2.8±2.7
p value	0.46	0.56	0.11	0.32	0.25	0.94
TDr97-00551						
Control	5.1±0.12	10.7±3.4	5.0±2.3	1.1±0.8	9.0±3.6	1.0±0.5
G. mos.	144.3±34.5	80.2±22.5	26.6±13.2	26.3±4.3	4.1±2.3	1.5±0.3
G. dussii	66.3±23.6	38.7±11.8	42.3±31.8	23.2±10.1	4.3±2.6	3.2±1.9
p value	0.2	0.09	0.7	0.13	0.6	0.46

Values were means (± SE) of six replicates at harvest, seven and five months after AMF and *Meloidogyne* spp inoculation, respectively. For each yam cultivar treatments means were compared by columns, and means followed by same (or without) letters were not significantly different ($p > 0.05$) according to the Protected Least Significant Difference test (LSD). Inoc = inoculated and Non-inoc = non-inoculated with *Meloidogyne* spp. *G. moss.* = *G. mosseae*, TFW = tuber fresh weight, SDW = shoot dry weight, RDW = root dry weight.

Taking into account all cultivars, without *Meloidogyne* spp., AMF inoculated plantlets yielded greater than non-AMF inoculation plantlets (Df = 2, F = 6.87, p = 0.001) (Table 5.6). With *Meloidogyne* spp. inoculation, AMF inoculated plantlets also yielded greater than non-AMF inoculated plantlets (Df = 1, F = 4.29, p = 0.041) (Table 5.6). Data analysis for each yam cultivar showed that, with (P = 0.05) or without (P = 0.04) *Meloidogyne* spp. inoculation, only cv TDa98-01183 yielded greater with either of the AMF species application than the control (Table5.8). In addition, shoot dry weight was significantly higher for cv TDa98-01183 when inoculated with *G. dussii* and *Meloidogyne* spp. compared to control (p = 0.003) (Table 5.8). Taking into account all yam cultivars, AMF root colonization was positively correlated with tuber weight (p = 0.0006) and also with *Meloidogyne* spp. soil densities (p = 0.017) (Table 5.9). The correlation varied by cultivar however. Positive correlation was observed between root colonization and tuber weight for cv TDa98-01183 (p = 0.04) and TDr745 (p = 0.028) (Table 5.9).

Table 5.9: Results of Pearson Correlation analysis between arbuscular mycorrhizal fungal spore density and yam plant growth parameters and nematode damage symptoms.

Pearson Correlation Coefficients	r value	P value
Mycorrhization/tuber dry weight	0.32024	0.0006
Spore density/soil *Meloidogyne* spp. density	0.17768	0.0173
TDa 98-01183		
Mycorrization/tuber dry weight	0.39071	0.0484
TDa 98-165		
Spore density/root *Meloidogyne* spp.	-0.30402	0.0714
TDr745		
Mycorrization/root dry weight	0.42968	0.0285

5.4.4. Effect of AMF inoculation on yam tuber quality and *Meloidogyne* spp. density

At harvest tuber galling was significantly lower on plantlets inoculated with both AMF species and *Meloidogyne* spp. compared to *Meloidogyne* spp. inoculation alone (Df = 2, F = 3.20, p = 0.04; Table 5.6). Plants inoculated with *Meloidogyne* spp. had no roots at harvest and highly galled tubers, compared to tubers harvested from plantlets inoculated with AMF species and *Meloidogyne* spp. (Figure 5.1). AMF inoculation suppressed the densities of *Meloidogyne* spp. both in yam roots (Df = 2, F = 1, p = 0.37) and in soil (Df = 2, F = 1.36, p = 0.25) (Table 5.6). For individual yam cultivars, only *G. dussii* significantly suppressed *Meloidogyne* spp. soil density on TDa98-01183 (Table 5.10). However, application of either AMF species improved tuber quality of plants inoculated additionally with *Meloidogyne* spp. compared to nematode inoculation alone (Figure 5.1).

Figure 5.1: Quality of yam tubers and roots at harvest six months after planting following arbuscular mycorrhizal fungi inoculation at planting and *Meloidogyne* spp. inoculation two months after planting.

Table 5.10: Tuber galling and *Meloidogyne* spp. population density at harvest seven and five months after AMF (*G. mosseae* and *G. dussii*) and *Meloidogyne* spp. inoculation respectively to micropropagated yam plantlets of two cultivars of *Dioscorea rotundata* (TDr745, TDr87/00551) and two cultivars of *D. alata* (TDa98-165, TDa98-01183) under greenhouse growth conditions at IITA-Ibadan, Nigeria, West Africa.

Treatments	Tubers galling		*Meloidogyne* spp. density[1] (root)		*Meloidogyne* spp. density[2] (soil)	
	Non-inoc	Inoc	Non-inoc	Inoc	Non-inoc	Inoc
TDa98-165						
Control	0.8±0.2	5±0.3	-	2977.6±1615.3	-	734.2±356.9
G. mosseae	1±0.0	3±0.7	-	1687.3±716.7	-	148.3±55.9
G. dussii	0.8±0.4	3.2±0.7	-	236.4±167.5	-	202.5±138.1
pvalue	0.67	0.2		0.20		0.15
TDa98-01183						
Control	1.0±0.0	4.1±0.2	-	560.8±324.9a	-	727.3±655.8
G. mosseae	1.0±0.0	4.7±0.5	-	457.5±354.1a	-	1055.6±724.5
G. dussii	1.0±0.1	4.2±1.5	-	135.2±85.5b	-	354.3±117.9
p value	0.9	0.23		0.01		0.12
TDr745						
Control	1.0±0.1	4.7±0.7	-	1308.3±530.5	-	1048.3±581.8
G. mosseae	1.0±0.0	4.6±0.2	-	178.3±110.3	-	85.8±44.6
G. dussii	1.0±0.2	3.7±0.5	-	110.2±39.6	-	155.5±82.3
pvalue	0.81	0.09		0.3		0.26
TDr97-00551						
Control	1.0±0.0	3.8±0.3	-	53.3±13.2	-	225.6±142.4
G. mosseae	1.0±0.1	2.3±0.6	-	2720.3±1516.3	-	125.3±72.7
G. dussii	0.7±0.3	3.5±0.3	-	2053.3±1309.5	-	460.8±170.8
p value	0.6	0.06		0.51		0.23

Roots and soil from non-inoculated treatments were free of nematode. - = data collected were zero and were not used for statistical analysis. Analysis and means separation of nematode densities were undertaken on $\log_{10}(x+1)$ transformed data. [1]Nematode density $5g^{-1}$ of root; [2]Nematode 50 g^{-1} soil. Values were mean (± SE) of six replicates non transformed data. For each yam cultivar treatment means were compared by columns, and means followed by same (or without) letters were not significantly different ($p > 0.05$) according to the Protected Least Significant Difference test (LSD). Inoc = inoculated; Non-inoc = non-inoculated with *Meloidogne* spp.

5.5. Discussion

The results of our study clearly show the positive potential of AMF application on yam plantlets, both in terms of improved production under non-pest challenged conditions, and also under nematode pest challenged conditions. Although results were erratic to some degree following nematode inoculation, there is a strong indication that AMF will provide good protection against nematode infection. However, this is less convincing for *Meloidogyne* spp. than it is for *S. bradys*. On the other hand, while the use of *in vitro* plantlets proved useful in the current study, it must also be recognised that their use has its limitations, in respect to the fragile nature of the plantlets. To date no data exist on the interaction and protective potential of AMF against nematodes on yam. *In vitro* plantlets were used as an initial starting point to assess the potential of AMF on yam, with and without challenge from nematodes. It must be respected, however, that at this stage, most plantlets would not be challenged or come in contact with nematodes, but the study serves as an initial indicator. Understandably, the most effective stage to inoculate plantlets yet remains to be properly identified. It was suggested that the growth stage at which AMF is inoculated to tissue-cultured plantlets is important but varies according to plant genotype (Smith and Read, 2008). For example, Monticelli et al., (2000) reported that micropropagated tree fruit rootstock (*Prunus* spp.) growth was significantly affected depending on the growth stage at which AMF were inoculated (early acclimatization phase, at transplantation after acclimatization, or in both phases) with significantly greater plant growth in early acclimatization. In addition, the authors reported that the results were also influenced by the inocula strains (*G. mosseae* or *Scutellospora calospora*) and the plant genotype (cv M51, cv GF677 and cv Citation). A similar response was also reported for banana (*Musa* spp.) (Elsen et al., 2003) and cassava (*Manihot* spp.) (Azcón-Aguilar et al., 1997). In some cases a better plant response is produced when the inoculation is carried out at the transplant phase after acclimatization of vitroplants e.g. avocado (*Persea americana*) (Vidal et al., 1992), pineapple (*Ananas comosus*) (Guillemin et al., 1992) and apple rootstocks (*Malus* spp.) (Cavallazi et al., 2007). Further to the current study, a separate study has been undertaken to establish the nature of the relationship of AMF on yam planting setts, which are cut from tubers and prepared more traditionally by farmers, for both their effect on growth and protection against nematode attack during the sprouting phase (the results are not included in the present thesis).

In the current study, yam plants inoculated with AMF species yielded heavier tubers (microtubers) compared to non-AMF plantlets in the presence or absence of nematode pests (*S. bradys* or *Meloidogyne* spp.). Without nematode inoculation, the general observation from our results is that, AMF inoculation significantly increased number and fresh weight of tubers, but that the degree of effectiveness depends on yam cultivar and on AMF species. The latter confirms our previous results carried out with 13 AMF species and 41 AMF isolates (chapter 4). Tuber Plant growth promotion in relation to AMF colonization is a well-established phenomenon across crops and climatic zones (Elsen et al., 2003; Chaurasia and Khare, 2005; Caglar and Akgun, 2006). The effect of AMF inoculation was not constant across our experiments or yam cultivar, however. The lack of consistency of effectiveness may be attributed to a number of factors, including the slight variation in experimental set up, but more possibly as a result of the different feeding styles of the two nematodes assessed. It was proposed by Johnson et al. (1997) that mycorrhizal association could be considered as symbioses, but the functional range along a continuum of parasitism to mutualism according to environmental conditions (climate, temperature, abundance of soil nutrients, presence or absence of pathogens, etc.) and the host plants genotype (Klironomos, 2003).

In the current study, perhaps the most interesting result was that yam cultivars from *D. alata* species responded more efficiently to AMF inoculation compared to *D. rotundata* cultivars. One possible explanation could be related to the morphology and physiology of the two yam species. *D. alata* cultivars have larger leaves, intercepting more light for photosynthesis than *D. rotundata* species (Orkwor and Ekanayake, 1998), and probably transfer more carbohydrate to AMF, which in return uptake and transfer nutrients to the plant leading to greater tuber production. Furthermore, a possible difference in the change of phytohormone balance following the association between some yam cultivars and AMF may further explain differences in yam cultivar response to AMF inoculation (Allen et al., 1980 and 1982; Dannenberg et al., 1992). Such differences in response to AMF inoculation among plant cultivars are reported, indicating the variable response in root colonization and relation to yield for different cultivars in the field (Dare et al., 2007) and on other tuber crops, such as micropropagated potato plantlets (Yao et al., 2002) and sweet potato (Gai et al., 2006). The beneficial effect of AMF infection on plants has generally been attributed to improved uptake of nutrients, especially P (Smith et al., 1992; Smith

et al., 2003; Zhu et al., 2004) and other elements, such as Ca, Mg and micro-elements (Pinochet et al., 1997; Ryan and Angus, 2003). Further studies are warranted to verify this hypothesis, which is also likely to vary by situation, crop and cultivar. Alternatively, inoculation with two fungal taxa in dual combination might improve the overall synergistic interaction between plants and fungi and may reflect the possible different roles of AMF within a fungal community (van der Heijden and Kuyper, 2001), towards resolving the selection problem for the most specific fungal partner.

With nematode (*S. bradys* or *Meloidogyne* spp.) inoculation, yam plantlets inoculated with *G. mosseae,* followed by *S. bradys,* produced heavier tubers compared to *S. bradys* alone for TDa98-165, TDa98-01183 and TDr97-00551. Interestingly, a stimulatory effect on tuber weight was observed for plantlets inoculated with both *G. mosseae* and *S. bradys* compared to single inoculation of *G. mosseae* or *G. dussii* using cv TDa98-01183. In the second experiment, the tuber weight was also significantly increased for treatments with either of the AMF species followed by *Meloidogyne* spp. inoculation, compared to *Meloidogyne* spp. alone using cv TDa98-01183. These results indicate that AMF can lead to suppression of nematode damage through the phenomenon of compensation (Smith and Read, 2008). This means that plants colonized by AMF can compensate for the loss of root or root function caused by the pathogen infection by enhanced root growth and development (see Figure 1), nutrient uptake and water absorption capacity of the root system (Harrier and Watson, 2004).

Our observation was similar to those undertaken by Brown and Kerry (1987), Hao et al., (2005) and Zum-Felde et al., (2006), who also observed that a low nematode population in dual combination with endophytic microbes could stimulate host plant growth and yield. Also, considering that tuber formation in yam is hormonally mediated (Okwor and Ekanayake, 1998), it may be hypothesized that *G. mosseae,* in combination with *S. bradys* challenge, affected the hormone balance in yam plantlets (e.g. increased synthesis of growth regulators in response to nematode infection), leading to increased production of yam tubers. McKenry et al., (2001) reported that grapevine (*Vitis* spp.) (cvs VR 039-16, Schwarzmann, and Freedom rootstocks) had grown larger in the presence of *Xiphinema americanum* than in its absence. The mechanisms by which AMF reduces nematode damage has not been fully determined. Hypotheses range from

depression of nematode development by competition for nutrient and space (Smith et al., 1986; Elsen et al., 2003), microbial changes in the mycorrhizosphere that disturb nematode chemotaxis (Linderman, 1988) to induced resistance through a pre-activation of gene and corresponding proteins responsible for plant defence against pathogen attacks (Slezack et al., 2000). For an efficient option for using AMF to protect against nematode damage, AMF essentially need to be established in the roots before nematode attack in order to provide biological control (review Borowicz, 2001; Diedhiou et al., 2003). For this reason, the 'impregnation' of AMF at weaning of *in vitro* plantlets would appear suitable and appropriate, before planting out in the field, where nematode (and other pest and disease) challenge would occur.

Concerning the migratory endo-parasitic nematode *S. bradys*, our results clearly indicated for the first time that yam plants inoculated with AMF species can produce healthier tubers, compared to non-AMF plants, in the presence of nematodes, while additionally suppressing *S. bradys* densities. Tubers harvested from AMF inoculated plantlets followed by nematode inoculation had lower cracking and rotting symptoms caused by *S. bradys*. Similar studies assessing the impact of AMF on nematode pests on banana (Elsen, 2003) and potato (Yao et al., 2002) have also recorded reduced nematode symptoms. In the case of yam tubers, the severity of surface cracking and dry rot is well correlated with tuber nematode density (Bridge et al., 2005), although tubers showing cracking symptoms typical of *S. bradys* infestation have been observed which are not related to the nematode but to other unknown factors (Baimey et al., 2005; Coyne et al., 2006). Furthermore, we observed that *S. bradys* inoculation negatively affect AMF spore production while no effect was recorded on AMF root colonization. However, the present results conflict with those recorded in a study on bananas where it has been reported that AM root colonization was negatively affected by nematodes (Elsen et al., 2003; 2008).

Concerning the effect of AMF inoculation on sedentary endo-parasitic nematodes, the results of our study have shown for the first time that AMF plantlets can suppress galling damage symptoms caused by *Meloidogyne* spp., although there was less impact on nematode densities. Results from the current study reflect to some degree, those of Ryan et al. (2003), who reported that the population of potato cyst nematodes (sedentary nematode) was higher by 200% for *Globodera rostochiensis* and 57% for *Globodera pallida* on potato plants inoculated with

Vaminoc© (commercial product with combination of three *Glomus* spp.), compared to non-inoculated plants. Diedhiou et al., (2003), also reported that a combined application of the AMF *G. coronatum* and a non-pathogenic *Fusarium oxysporum* strain Fo162 led to a higher number of nematodes per gall on mycorrhizal than non-mycorrhizal plants. However, the present results differ from those of many studies, where AMF suppression of *Meloidogyne* spp. density and damage has previously been reported (review Azcon-Aguilar and Barea, 1997; review Hol and Cook, 2006; Li et al., 2006).

The present study shows that commercial *G. mosseae* and *G. dussii* could easily associate with yam plantlets and produce high quantities of spores. No single yam cultivar, within the study, appeared to be a more suitable host than the others, across both experiments. The cv. TDa98-01183 was successfully associated with both AMF species, with high root colonization and spore numbers produced. The high root colonization and high spore production had earlier been observed on yam plantlets, indicating the high mycorrhizal association with yam of a few cultivars with *G. etunicatum*, *G. hoi* and *Acaulospora scrobiculata* (Tchabi et al., chapters 3 and 4). In contrast to the high spore production, there was relatively low root colonization in the second experiment. Differences in root colonization was also observed among AMF species, with higher levels recorded for *G. mosseae,* compared to *G. dussii,* which may be explained by the difference in inoculum infectivity (Abbott and Robson, 1981; Cavallazzi et al., 2007), or the difference in compatibility between both AMF species and the yam cultivars used in the present study (Smith and Read, 2008). Alternatively, the specificity of yam species and cultivars may account for colonization differences, with yam being more compatible with *G. mosseae* than *G. dussii* in general. Recent work has demonstrated quite a high level of variability in yam cultivar specificity with AMF, recorded as differences in root colonization among cultivars (Uchendu, 2000; Dare et al., 2007), while *D. rotundata* cv TDr89-02461 and *D. cayenensis* cv TDc98-136 were shown to be highly colonized by bulk AMF from soil samples at up to 90% (Tchabi et al., submitted; chapter 3). However, it is interesting to note that the *G. dussii* inoculum used in the current study was originally sourced from West Africa, while *G. mosseae* is derived originally from Europe (Biorize, pers. comm.), indicating that geographical or ecological origin is not entirely an indicator of their potential to form associations with certain crops, to improve crop growth or as biocontrol agents.

Conclusion

Our results have shown for the first time that a commercial AMF can stimulate yam growth parameters. However, AMF species influence on plant growth appears highly dependent on the plant genotype with which they are associated. The fact that tuber cracking, dry rot, and galling symptoms in mycorrhizal yam were lower, compared to non-mycorrhizal plants, even though yam tuber weight was lower in some cases, indicates that mycorrhizal plants probably activate defence genes or improve compensation of root losses due to nematode attack, which leads to suppression of nematode density and injury. Taking into consideration tuber weight, number of tubers, AMF attributes, and nematode management, *G. mosseae*, originating from Europe, was a more effective AMF symbiont for association with yam plantlets under the conditions of the study (in pots on vitroplants) than *G. dussii* originating from West Africa.

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CHAPTER 6: General discussion

Beneficial plant-microbe interactions in the rhizosphere play a crucial role for plant health and soil fertility (Cardoso and Kuyper, 2006). The arbuscular mycorrhiza (AM) is the most widely occurring and important microbial symbiosis for agricultural crops (Smith and Read, 2008). In recent years, a wealth of evidence has shown that arbuscular mycorrhizal fungi (AMF) provide increased efficiency of use of soil nutrients and water, and enhance plant growth, particularly under sub-optimal soil conditions such as P-limitation, which is common in tropical soils due to leaching or/and severe immobilization (Clark and Zeto, 2000; Schlecht et al., 2006). Additionally, the use of AMF for suppression of plant root diseases and nematodes has been recognised and is gaining increasing attention recently (Hol and Cook, 2005).

Yam (*Dioscorea* spp.) is the most important tuber crop in terms of area of production in West Africa, particularly in Benin and Togo (IITA, 2006). It also provides cash income for a wide range of smallholders, including many women as producers, processors and traders (Orkwor and Ekenayeke, 1998). Yam is also particularly symbolic in the cultural and traditional history of the region and is integrally woven within the fabric of society (Coursey, 1967). Currently, the situation regarding yam production in West Africa has been summarised as, “the annual demand for yam consumption is increasing, the surface used for yam cultivation is increasing but the annual production per hectare or the yield is decreasing considerably”. Two main problems were defined for yam yield decrease: soil fertility (IITA, 2006; Schlecht et al., 2006) and disease damage (Odu et al., 2004; Egesi et al., 2007a; b), especially nematode damage due to S*cutellonema bradys* and *Meloidogyne* spp. (Coyne et al., 2005).

Nematodes can successfully be managed by nematicides (carbamates and organophosphates), but these are not commonly used due to a number of factors, one being their perceived high cost. Instead they use cheaper (sometimes obsolete or banned), poor quality (following extended storage and often dubious repackaging) or unconventional products such as pounded disused batteries, which leads to well-documented pollution and health problems (MDR, 2000; IITA, 2006). Recent studies at the International Institute of Tropical agriculture (IITA) show that hot

water therapy effectively reduces yam nematode infection of tubers and can reduce losses in the field, which can result in a production increase of up to 60% (IITA, 2006). However, although feasible, producing clean (healthy) planting material through the use of pesticides or heat treatment can be costly and often impractical. Management of nematode pests of yam should effectively involve an integrated approach, using a number of suitable options. Specifically compatible microbial agents, such as AMF, which provide resistance/tolerance against pests or diseases and increase yam establishment under poor soil conditions, may contribute to a sustainable practice for improving yam production.

The current study was therefore undertaken to address the knowledge gaps on the occurrence of AMF in sub-Saharan West Africa and on the AM status of yam on the one hand, but also the effectiveness of different AMF species to colonize yam roots and promote yam growth and disease control, especially nematodes. Several aspects of AMF ecology such as AMF richness in relation to their presence in various ecological zones and following increased land use intensity were assessed. The interaction between AMF and yam and the interaction between AMF, yam and nematodes were also examined.

Concerning the occurrence of AMF in sub-Saharan West Africa, soil samples were initially collected in the yam growing areas of Benin, in the so called “yam belt”, to assess the impact of agricultural practices and of dry season length in respective ecological zones on AMF species richness (Chapter 2). The soil sample sites included forests and their adjacent yam fields, peanut fields, mixed culture fields, cotton fields and 6-7 year old fallowed lands. Results showed that the savannas of Benin contain a high natural AMF species richness. This natural AMF species richness is significantly affected by the length of dry season among the three ecological zones with an apparently increasing AMF richness from the wetter Southern Guinea savanna through the Northern Guinea savanna to the drier zone of the Sudan savanna. The underlying mechanisms for the AMF community shifts among ecological zones remain unclear, but may be due to a reduced activity of spore grazing soil biota and lower fungal respiration in the driest zone (Baktiar et al., 2001). Within each ecological zone spore densities, as well as species richness, were affected by forest clearance, i.e. following “slash and burn” for yam production in the first year and the subsequent years of land cultivation with various other crops. Especially

under intensive cotton production, AMF richness was negatively influenced and appeared not to be readily restored by fallow thereafter. In tropical regions several studies have shown the impact of agricultural practices on AMF species richness and abundance, e.g. in Kenya (Mathimaran et al., 2007), Ethiopia (Mulata et al., 2007), South America (Dodd et al., 1990), India (Harinikumar and Bagyaraj, 1988), China (Wang and Vestberg, 2008) and in West African countries such as Burkina Faso and Niger (Bagayoko et al., 2000), Togo and Burkina Faso (Marschner et al., 2004). Although the current study was relatively intense across a range of agro-ecological zones in West Africa, given the geographic scale of the region, its climatic diversity, agricultural breadth - considering the size of the continent as a whole - our study has hardly touched upon the situation of AMF in Africa. However, our "small" study established a wide range of species indicating the potential magnitude of what possibly exists in terms of species diversity and extent of undescribed species yet to be discovered. The present results also indicate, to a certain extent, the potential biodiversity losses if actions are not taken to explore, preserve and utilise AMF before it is too late. We suggest mycorrhizal management through agroforestry - including the use of legume plants - and reduced soil disturbance, to improve mycorrhizal persistence.

In order to screen AMF species for their potential to improve yam growth and nematode control, it is necessary to understand which AMF species can easily associate with yam (Chapter 3). Within the current study, soil samples collected from three different forests and their adjacent yam fields were used as inoculum. By using sterile micropropagated plantlets of two yam species, *D. cayenensis* and *D. rotundata* (cv. TDc98-136, cv. TDr89-02461) as trap plants and inoculation with the soil inocula, 28 and 29 AMF species were identified as symbionts of *D. cayenensis* and *D. rotundata*, respectively. AMF species identified as symbionts of yam belong to six families, mainly 18 species from Glomeraceae and 13 species from Acaulosporaceae, but also some species from Gigasporaceae, Entrophosporaceae, Archaeosporaceae and Paraglomeraceae. The current study thus provides one of the most detailed investigations to date on AMF status of yam (Ahulu et al., 2005; Dare et al., 2007; Oyetunji and Afolayan, 2007). Currently, information is still limited on how mixed communities of AMF species compete for root colonization and how they affect host plant growth, health and, consequently crop yield (Jansa et al., 2008). Therefore, the current study definitively indicates the need for more detailed and comprehensive investigations of AMF communities and their value to agriculture in West

Africa (and beyond). We suggest the use of molecular techniques to identify the AMF species which actually colonize the roots at the various growth stages of yam, even during early yam root growth in trap cultures or under field situations.

In the present study the interaction between yam and indigenous as well as exotic AMF species was assessed (Chapter 4). Firstly, *D. rotundata* cv. TDr89-02461 plantlets were inoculated with *G. etunicatum* strains from Africa, India, Bolivia and Central Europe and compared against three AMF isolates from temperate Europe. Despite the relative similarity of AMF infectivity (27-61%), plant response to AMF colonization showed that tuber weight was significantly increased when infected with African strains and certain strains from other tropical regions, while the phosphorus (P) concentration in yam tubers was significantly increased with Central European strains, compared to non-mycorrhizal plants. These results suggest that similar species of AMF (e.g. *G. etunicatum* and *Glomus* sp. resembling *G. luteum*) and even same species confer different physiological benefits to the same plant species. Intraspecific variation in function of AMF was also reported on cucumber (*Cucumis sativus*) with *G. mosseae*, *G. claroideum* and *G. geosporum* isolates originating from different geographical zones (Munkvold et al., 2004). In addition, there was a negative correlation between yam tuber weight and tuber P concentration, at least for the cv. TDr89-02461 and the AMF species and isolates used in the present interaction study. The results clearly indicate that the hypothesized functional complementarity of AMF (Read, 1998; Koide, 2000) or incompatibility of function between AMF (Fitter et al., 2005) could exist, not only between, but also within AMF species. The physiology behind this different functionality of AMF species or isolates has yet to be determined. But, for the 'yam belt' in West Africa, it will be interesting to identify AMF species and isolates that promote growth of high quality tubers, confering efficient and sustainable use of soil P and – of particular interest - supporting yam production for more than one year after forest clearance on the same land, or in crop rotation systems without fallows. Furthermore, the same yam cultivar was inoculated with 9 AMF species (including 3 strains for each species, i.e. a total of 27 AMF strains) from Africa and compared to an inoculation with 3 AMF species from Biorize Company©. The results from pots showed that *G. mosseae*, *G. hoi*, *G. etunicatum*, *A. scrobiculata* and *A. spinosa* generally increased tuber weight while strains of *G. sinuosum* and *Kuklospora kentinensis* generally did not. Our results clearly indicated that the effect of AMF isolates on yam cv. TDr89-02461 yield

depends on AMF species, which can be related to the genetic difference between AMF species (e.g. Bâ et al., 2000; Munkvold et al., 2004). In this study, the interspecific variability among AMF species in promoting yam yield was high while the intraspecific variability in some AMF species was rather low. The low level of intraspecific differences of AMF isolates on promoting yam growth recorded in the present study (only from 3 AMF species: *G. claroideum, A. scrobiculata* and *G. etunicatum*) might be attributed to the few isolates (3) of each AMF species (Munkvold et al., 2004). In contrary, some studies have reported intraspecific differences in mycorrhizal plant growth response, by including only two isolates of each of *G. mosseae* and *G. caledonium* (Carling and Brown, 1980) or three isolates of *G. mosseae* (Stahl et al., 1990). Taking into account the ability of AMF isolates or species to promote growth of micropropagated plantlets of yam cv. TDr89-02461, we hypothesize that the performance of a single host yam cultivar depends on the particular AMF isolates associated, and therefore it is not easily possible to generalize the performance of a single AMF isolate against the entire range of possible isolates within a particular AMF species, or to the entire range of cultivars of a host plant species.

The results from the interactions between two AMF species (*G. mosseae*, *G. dussii*) and four yam cultivars (TDr745, TDr97-00551, TDa98-01183 and TDa98-165) (chapter 5) showed the potential of AMF species inoculation to improve yam growth. However, the beneficial effects provided by different AMF species to different yam cultivars differed and our results highlight the importance of the AMF species and the yam genotype on the response of yam to AMF inoculation. Our results showed that AMF inoculation significantly increased tuber weight of *D. alata* cvs (TDa98-01183 and TDa98-165). Despite being significantly colonized, cv. TDr745 did not physically respond to any of the AMF species, while cv. TDr97-00551 had lower yield following *G. dussii* colonization compared to the control. These results indicate that *D. alata* cultivars respond positively to AMF colonization through improved tuber yield, while *D. rotundata* cultivars did not respond in the same way, at least for the parameters analyzed in the present investigation and with the AMF species used. The difference between the two *Dioscorea* species in response to AMF colonization can partly be related to the crop morphological properties, particularly the hairyness of the root systems of yam species, with coarse and short root hairs on *D. alata* and fine, long hairs on *D. rotundata* (Orkwor and Ekanayeke, 1998). It was

suggested that few and short root hairs are indicative for plants responding to AM colonization (Baylis, 1975; Bâ et al., 2000). Alternatively, Orkwor and Ekanayeke (1998) reported that *D. alata* generally produces larger tubers, compared to *D. rotundata*, indicating that *D. alata* requires higher nutrient levels for growth, a requirement that can be partly fulfilled by AM colonization. Additionally, Asiedu et al. (1998) and Dare et al. (2007) commented that the genotype and the genotype X environment interactions can affect yield and/or yam response to AM colonization under field conditions.

With regard to control options of nematodes (*S. bradys* or *Meloidogyne* spp.) infesting yam, the inoculation of two AMF species (*G. mosseae*, *G. dussii*) was assessed for their effect on yam growth and yield, nematode densities and nematode damage to yam tubers (Chapter 5). This experiment was undertaken in consideration of intensification of yam production by biocontrol measures as opposed to using fertilizers or nematicides for nematode management (Coyne et al., 2004; Bridge et al., 2005). Using micropropagated yam plantlets there appears to be a better ability of AMF to suppress *S. bradys* attack than *Meloidogyne* spp., but this requires further substantiation, particularly under field conditions. *Scutellonema bradys* densities were in general suppressed following AMF inoculation prior to nematode inoculation, but *Meloidogyne* spp. densities were not necessarily suppressed. Most studies on AMF-nematode interactions have reported on the suppression of sedentary endoparasitic nematodes (e.g. *Meloidogyne* spp.) compared to migratory endoparasitic nematodes (e.g. *S. bradys*) (Hol and Cook, 2005). However, AMF suppression of migratory endoparasitic nematodes has been reported in banana (Elsen et al., 2003, 2008) and on quite a range of other crops (Borwicz, 2001; Hol and Cook, 2005). Similar studies assessing the impact of AMF on nematode pests in potato (Yao, 2002) have also reported reduced nematode symptoms and population densities. The current study further indicated that AMF application reduced galling damage symptoms of *Meloidogyne* spp. compared to non-AMF controls even though *Meloidogyne* spp. population densities were not suppressed. The suppression of *Meloidogyne* spp. damage by AMF inoculation has previously been reported in a number of studies (review of Azcón-Aguilar and Barea, 1997; review of Hol and Cook, 2006; Li et al., 2006), indicating their general potential as a management tool.

While not conclusive, the present results on nematode control provide basic and useful information to warrant their further investigation as an alternative, ecofriendly method of yam nematode management. Taking into account our experimental findings, we can speculate that for an efficient use of AMF to protect against nematode damage, AMF should be established in roots prior to nematode attack, in order to provide biological control (review Borowicz, 2001; Diedhiou et al., 2003). For this reason, we suggest that the 'impregnation' with AMF at weaning of *in vitro* plantlets would be an idealsolution, before planting out in the field, where nematode and other pest and disease challenges will occur.

Conclusion and perspectives:

The present work constitutes the first attempt to evaluate the diversity of AMF in Sub-Saharan savannas and agro-(eco)systems in Benin, West Africa - and also the first attempt to study AMF-nematode interactions in *Dioscorea* spp. Three principal conclusions can be drawn from the present work:

a-) West African savannas contain a high natural AMF species richness, which is significantly affected by the common agricultural land use practices, and the length of dry season. The soil inocula are highly infective, but for AMF-trapping and propagation studies, soil sampling should be conducted in the dry season and not in the wet season as the AMF propagation success with soil inocula from the wet season was poor.

b-) Yam is highly mycorrhizal with yam roots hosting numerous AMF species as indicated by the finding of > 30 AMF species in pots containing yam micropropagated plantlets that were used as trap plants.

c-) The functional study with several AMF species indicated a high potential of indigenous AMF to promote yam tuber growth and to provide bioprotection against nematodes. However, different AMF species and isolates are not equally beneficial to yam and, vice versa, different yam cultivars do not equally benefit from individual AMF species or strains. Our results indicate that the performance of a single specific host can depend on the AMF strain and similarly, the performance of a single AMF strain can depend on the specific host plant species or cultivar.

Due to the high level of response by certain yam cultivars to AMF inoculation, we suggest the inoculation of micropropagated *D. alata* cvs. TDa98-01183 and TDa98-165 or additionally *D. rotundata* cv. TDr89-02461 as model plant species in future AMF studies on yam. Furthermore, *G. mosseae*, *G. hoi*, *G. etunicatum*, *A. scrobiculata* and *A. spinosa* appear most adequate as AMF model species for future investigations on the interaction between yam, AMF and pathogens (nematodes, fungi, bacteria) for improving yam production. We believe that this pioneering work provides a solid platform of information upon which to build further towards understanding the ecology of AMF in West Africa, and furthermore represents a basis for future studies on the function of AMF for sustainable crop production in West African agroecosystems.

For further AMF studies on yam, we recommend to focus on the following two objectives:
Firstly, in order to evaluate the AMF species associated with yam, molecular tools should be applied to identify the AMF species colonizing the roots. Roots can be collected from field sites (e.g. from different ecological zones) or in trap cultures using soil inoculum. The sampling can be undertaken at two months intervals to enable interpretation of AMF population dynamics inside the roots. The results can be compared with morphological identifications of spores present in the yam rhizosphere.
Secondly, we suggest that greenhouse screening is continued for functional compatibility between yam cultivars and AMF species/strains subjected to a challenge with nematodes in order to select the AMF most promising as bio-protectants. All the isolated native AMF strains present in the Gene Bank should be tested, singly or in combinations, for selecting the strains most efficient in mediating yam yield improvement and nematode suppression. Another possibility is the selection of two or more AMF species or strains performing in concert one or both functions. Once the most appropriate AMF strains - or combinations of strains - are identified by the greenhouse pot experiments, field experiments have to be conducted for validation.

Finally it remains the task to convince the biotechnologists about the importance to establish the AM symbiosis already in the sterile micropropagated plants that are still free of pathogens. Plant pathologists and microbiologists engaged in mycorrhizal research may be unaware of the problems of micropropagation. We suggest that biotechnologists and plant breeders integrate

AMF knowledge and combine the technology with micropropagation techniques for stronger, more robust and healthier plants, which have 'in-built' protection against pests and pathogens.

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Annex

ANNEX

CURRICULUM VITAE

1-Personal

Name: TCHABI

Surname: Atti

Current address I: Botanical Institute, University of Basel
Hebelstrasse 1, 4056 Basel, Switzerland
Tel: +41 798566416

Current address II: IITA-Benin, 08BP0932 Tri Postal, Cotonou-Benin
Tel: +229 21 35 01 88

Permanent address I: Rijkeklarenstraat 57, 9050 Gent-Ledeberg, Belgium

Permanent address II: S/C Prof. Gumedzoe Mawuna, University of Lomé, Ecole Supérieure d'Agronomie, BP 1515 Lomé-Togo

Email: attitchabi@yahoo.fr; atti.tchabi@unibas.ch

2-Education

B.S. "Ingenieur Agronome" (1989-1995): Faculty of Agronomy, University of Lomé-Togo. Supervisor: Professor Gumedzoe Mawena.

Master of Science in Nematology (2001-2002): Faculty of Biology, University of Gent. Supervisor: Prof. Dirk de Weale.

PhD thesis: (August 2004-Sept. 2008): Botanical Institute, University of Basel. Supervisor: Prof. Andres Wiemken and Dr Fritz Oehl.

3- Specialist area

Plant protection especially Nematology
Stored products pests management
Arbuscular mycorrhizal fungi

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4-Professional experience

Present job description: Since September 2004, I am dealing with Abuscular mycorrhizal fungi identification and their use for improved yam production especially the interaction between AMF, yam (D*ioscorea* sp) and nematode.

Jan. 2003-August 2004**:** Employer**:** IITA- Benin**,** Plant Health Management Division, and University of Lomé.
I was working for IITA as consultant on nematology and as Research Assistant at University of Lomé (Togo) under Prof GUMEDZOE

Sept. 1998 – August 2001**:** Employer**:** IITA- Benin**,** Plant Health Management Division
I was involved in "Nematology project" where I was dealt with yam nematode, *Scutellonema bradys*, assessment in Benin under Dr. Danny COYNE

Jan 1997- August 1998: Employer: IITA-Benin, Plant Health Management Division.
I was involved in"Stored Products Pests Management Project" where I was dealt with *Callosobruchus maculatis* isolates collection from West Africa and their screening for cowpea resistance.

May 1995- Dec 1996: Employer: IITA-Benin, Plant Health Management Division
I was involved in Larger Grain Borer Project as trainee where I was studying *Prostephanus truncates* biology and ecology.

5- **Computer skills**

Windows 2008, Statview, Super ANOVA SAS, Delta graph, Excel, GIS courses.

6-**Language skills**

French: Native,
English: spoken and written

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7-Publications

Tchabi A, Coyne D, Hountondji F, Lawouin L, Wiemken A, Oehl F (2008). Arbuscular mycorrhizal fungal communities in sub-Saharan Savannas of Benin, West Africa, as affected by agricultural land use intensity and ecological zone. Mycorrhiza 18: 181-195.

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