"Effect of plant parasitic nematodes and arbuscular mycorrhizal fungi on banana (Musa spp.) in the East African Highland cropping systems"

Gaidashova, Svetlana

Abstract
Banana (Musa spp.) is an important crop in the East African Highlands (EAH). Although root health and interaction with soil microorganisms are amongst its major yield determinants, they remain poorly investigated in this region. Understanding these relationships may help to improve yield. In this PhD thesis, we investigated the effect of plant parasitic nematodes, in particular, Pratylenchus goodeyi, and arbuscular mycorrhizal (AM) fungi on the EAH banana. We demonstrated that P. goodeyi was the main species contributing to root necrosis. However, its abundance was positively correlated with high plant density and mulch. Pratylenchus goodeyi had limited impact on yields in flatter fields (crest and valley bottom) where soil conditions were more optimal for root growth, whereas on steep slopes root death was increased and yield was reduced even under moderate pressure from P. goodeyi. A field experiment showed that P. goodeyi had low impact on yield of highland banana (AAA-EA). Hence...

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Effect of plant parasitic nematodes and arbuscular mycorrhizal fungi on banana (*Musa* spp.) in the East African Highland cropping systems

Thèse présentée par Svetlana Gaidashova
en vue de l’obtention du grade de Docteur en Sciences agronomiques et ingénierie biologique

President du Jury : Prof. J. Mahillon (UCL)

Promoteurs : Prof. S. Declerck (UCL)
Prof. D. De Waele (K.U.Leuven)

Membres du Jury : Prof. B. Delvaux (UCL)
Dr. A. Elsen (BDB)
Dr. P. Van Asten (IITA)

Louvain-la-Neuve, Octobre 2009
A mes maîtres
qui ont contribué à ma formation
intellectuelle, émotionnelle et spirituelle
depuis mon très jeune âge jusqu'à ce jour
“Learn as if you were to live for ever.”

Mahatma Gandhi
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Svetlana Gaidashova
Louvain-la-Neuve, October 12, 2009
Summary

Banana (Musa spp.) is an important crop in the East African Highlands (EAH). Although root health and interaction with soil microorganisms are amongst its major yield determinants, they remain poorly investigated in this region. Understanding these relationships may help to improve yield. In this PhD thesis, we report on the effect of plant parasitic nematodes, in particular, Pratylenchus goodeyi, and arbuscular mycorrhizal (AM) fungi on banana. Our studies at eco-regional level showed that P. goodeyi was the dominant and the only species significantly (P < 0.001) contributing to root necrosis. However, a possible negative impact of P. goodeyi on banana yields was masked by the fact that nematode populations were positively correlated with high plant density and mulching practices, both associated with relatively high plant vigour of the most infected plants.

A study at watershed level within one eco-region (Lake Kivu border) revealed that the spread of P. goodeyi had generally limited impact on banana yields in flatter fields (crest and valley bottom) where soil conditions were more optimal for root growth, whereas on the middle slope root death was increased and bunch weight was reduced even under moderate pressure (25482 nematodes 100g\(^{-1}\) fresh roots) from P. goodeyi.

In a field experiment, P. goodeyi had low impact on yield of highland banana (AAA-EA). Low to medium levels (\(\leq 50\%\)) of root necrosis were associated with improved plant growth while higher root necrosis (\(> 50\%\)) had no effect. Hence, the three studies at country, regional, and field level all suggest that root necrosis caused by P. goodeyi has very little to no negative impact on banana.
Summary

plant vigour. These results challenge general perceptions and previous findings on the negative impact of root lesion nematodes on banana crop performance. Our studies revealed that AM fungi were widespread, but root colonization, AM fungi population density and diversity highly varied according to edapho-climatic conditions (i.e. rainfall, soil texture and P content) and soil management practices (tillage). The AM fungi colonized banana roots up to 120 cm depth, while poor vigour plants were colonized significantly higher (P < 0.05) and may have been, therefore, more dependent on their associated AM fungal partners compared to plants having good vigor. Root colonization by AM fungi decreased with soil depth and with reduction in soil water content, and higher colonization lead to more vigorous root systems.

Studies of mycorrhizal status of different banana genotypes revealed that all studied genotypes had similar root colonization. However, its extent is largely determined by environmental conditions: rainfall and soil chemical properties (especially soil P). This study highlighted strong effect of environment on the AM fungi-banana genotype interaction.

The effect of banana plant inoculation with indigenous AM fungi was dependent on soil type and on banana genotype. Acrisol, having low soil fertility, coarser texture and low natural AM fungi populations, favored greatest plant growth increase as compared to Ferralsol and Nitisol having low P, higher natural AM fungi populations and better soil fertility. Poorer root development of the banana variety Musakala coincided with its highest response to the AM fungi inoculation compared to FHIA 17 and Sukali Ndizi, which suggested higher dependency of Musakala on the AM fungi inoculation.

This research opens new perspectives for development of appropriate environment friendly technologies for low-input production systems in the EAH.
Further research should focus on development and promotion of appropriate crop and soil management practices at farm and watershed scale. These practices should be not only crop- and soil-friendly, but also AM fungi-friendly. Functional groups of the AM fungi populating disturbed environments (with low soil fertility, high erosion-prone steep slopes, and intensive tillage) in the EAH should be identified and characterized, while these areas should constitute priority sites for research and promotion of integrated soil and crop management in banana cropping systems.
List of abbreviations

ANOVA: Analysis of variance

AAA-EA: *Musa* spp. belonging to East African Highland banana (sub-group Lujugira-Mutika)

AM fungi: Arbuscular mycorrhizal fungi

CEC: Cation exchange capacity

E: East (longitude)

EAH: East African Highlands

IITA: International Institute of Tropical Agriculture

INERA: Institut National pour l’Etude et la Recherche Agronomique (DR Congo)

IRAZ: Institut de Recherche Agronomique et Zootechnique

ISABU: Institut des Sciences Agronomiques du Burundi

ISAR: Institut des Sciences Agronomiques du Rwanda

KARI: Kenya Agricultural Research Institute

LSD: Least significant difference

masl: meters above sea level

MPN: Most Probable Number

NARO: National Agricultural Research Organization (Uganda)

OM: Soil organic matter content
**List of abbreviations**

PP nematodes: Plant parasitic nematodes

S: South (latitude)

°C: Degrees Celcius

% DR: Percentage of dead roots

% F: Frequency of mycorrhizal colonization in root length, comprising frequency of arbuscules, hyphae and vesicles all together

% FA: Frequency of mycorrhizal colonization in root length, comprising frequency of arbuscules

% FV: Frequency of mycorrhizal colonization in root length, comprising frequency of vesicles

% I: Intensity of mycorrhizal colonization in root length, comprising intensity of arbuscules, hyphae and vesicles all together

% IA: Intensity of mycorrhizal colonization in root length, comprising intensity of arbuscules

% IV: Intensity of mycorrhizal colonization in root length, comprising frequency of vesicles

% RN: Percentage of root necrosis
Foreword

The thesis is presented in form of articles, corresponding to chapters, which are either published or prepared for submission to peer review journals. I apologize for any redundancy among the chapters related to this form of presentation and inconveniences for the reader.
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General introduction

Banana is one of the most important crops in the East African Highlands (EAH) providing food and income to more than 30 million people living in densely populated areas (Karamura et al., 1999). Bananas are grown in small plots and production is subsistence oriented. These systems are characterized by low input and declining crop yields (Karamura et al., 1999; Gold et al., 1999). In recent years, high population pressure on land intensified soil exploitation and losses, and led to the reduction of pastures and forests. This is particularly noticeable in marginal steeper landscapes. At the same time, growing urban demand increased marketing of agricultural produce, enhancing nutrient exports from smallhold farms (Schoenbrun, 1993; Davies, 1995; Van Asten et al., 2004). The nutrients exported are not replaced through external nutrient inputs; the EAH have one of the lowest fertilizer use in the world (Van Asten et al., 2004). The sustainability of the EAH agriculture is thus under pressure through nutrient mining.

Crops differ in their response to soil fertility. This response is determined by abiotic environmental factors and soil microbial communities that impact root growth and efficiency. In the EAH, soil micro-organisms of banana rhizosphere remain poorly investigated. Understanding their role and relationship with plants may help to find ways to optimize plant growth and improve sustainable crop production.

Plant parasitic nematodes are known to reduce crop yields by damaging plant roots, while arbuscular mycorrhizal (AM) fungi have been shown to improve nutrients uptake (e.g. P, N).
General introduction

While the effect of plant parasitic nematodes (*Radopholus similis*) has been studied in lower parts of the EAH region (Speijer & Kayumba, 2000), the effect of nematodes in higher areas dominated by *Pratylenchus goodeyi* was seldom evaluated (Gowen, 1995). Identically, there had been limited research about the presence and role of the AM fungi, and their effect on banana in the EAH.

The thesis research presented here will focus on the banana rhizosphere, considering both, plant parasitic nematodes and AM fungi, and their effect, and relative contribution in determining banana performance in the EAH cropping systems. The first part of the thesis will include assessment of the distribution of plant parasitic nematodes in banana cropping systems of Rwanda as related to species, population densities, root damage and crop yield. We will also investigate the possible factors affecting nematode damage. The second part of the thesis will focus on AM fungi by characterizing their distribution, abundance and incidence on banana cropping systems in the EAH as well as the relation between AM fungi and plant performance.
Chapter 1. Banana rhizosphere with emphasis on plant parasitic nematodes and arbuscular mycorrhizal fungi in the East African Highland cropping systems

This chapter is in preparation for submission to “Agriculture, Ecosystems and Environment” as Gaidashova S.V., Van Asten P.J.A., De Waele D., Declerck S. and Delvaux B. Banana rhizosphere with emphasis on plant parasitic nematodes and arbuscular mycorrhizal fungi in the East African Highland cropping systems.
1.1 Introduction

The German scientist Hiltner (1904) introduced the term “rhizosphere” and defined it as a soil space influenced by the root (Hartmann, 2005). The major components of the rhizosphere thus include plant roots, surrounding soil environment and soil micro-organisms. The rhizosphere zone extends a few millimetres from the root surface and accommodates multiple interactions between the soil (i.e. solid, gaseous and liquid components), plant roots and a wide range of soil micro-organisms including plant symbionts and pathogens (Cardon & Whitbeck, 2007).

A well developed root system contributes to sustainable productivity (Gauggel et al., 2005). Thus, there is, an increasing need to understand the factors contributing to optimal development and function of the rhizosphere. Multiple and complex interactions in banana rhizosphere remain poorly understood (Delvaux et al., 2005). The knowledge is especially limited in the East African Highland (EAH) cropping systems, where banana is a traditional staple since many centuries (Karamura et al., 1999).

Soil ecosystems in the EAH are threatened by anthropogenic pressure on land through reduction of fallows, land fragmentation, nutrient mining, soil loss from erosion and run-off, and sub-optimal cropping practices leading to inadequate nutrient replenishment, exposure of bare soil, use of inappropriate crop density and soil loss on steep slopes. Environmental degradation in the EAH was studied at different scales, ranging from pedological (Bekunda et al., 2002) and ecological and agronomical (Clay & Lewis, 1990), to socio-economical (Kangasniemi, 1998) points of view. Degradation of the soil quality makes research oriented towards the sustainable improvement of crop productivity particularly relevant. Research on belowground microbial
communities and their effect on crops appear relevant in this context. The present chapter provides a review of research on the banana rhizosphere with particular emphasis on EAH cropping systems.

1.2 East African Highland banana ecology

The East African Highlands where EAH bananas are grown comprise a system of mountain plateaus characterized by hills, valleys and lakes. The area is located between 2°N and 10°S, and extends into six countries – Burundi, Rwanda, Kenya, Tanzania, Uganda and Eastern DR Congo (Figure 1.1). The altitude ranges from 600 to 5895 masl (Mount Kilimanjaro). In this region, banana is mostly cultivated between 900 and 1800 masl (Davies, 1995).

The climate in the EAH is strongly influenced by altitude and the proximity of large lakes, which affect temperature and rainfall. The bi-modal rainfall distribution across the region (800–2000 mm yr⁻¹) generally fulfills minimum requirements for banana crop (1000 mm yr⁻¹) (Davies, 1995). The EAH region is characterized by a high diversity of soils (Eswaran et al., 1989), ranging from old, acidic, strongly weathered ferrallitic soils to young and rich volcanic ash soils (Anonymous, 2001). The Rift Valley fault system is the principal geological feature of the highlands (Davies, 1995) where the most common soil types are Acrisols, Ferralsols and Nitisols (UNESCO, 1977). Low soil K, N and P levels were frequently reported in this region (Lassoudière, 1989; Rubaihayo et al., 1994; Bosch et al., 1996; Gaidashova et al., 2007).
The EAH region represents an important centre of banana diversity where several hundreds of cultivars co-exist (Figure 1.2). While South East Asia is known to be a center of origin of *Musa*, the wild ancestor of the EAH bananas is unknown, and these cultivars have specific traits that are not found elsewhere in the world (Karamura, 1998). Recent archaeological findings related banana cultivation in East Africa to the fourth millennium before Christ (Lejju *et al.*, 2006). Linguistic evidence confirms banana presence in the African Great Lakes region around 800-1500 years after Christ (Schoenbrun, 1993). Over this time,
two major types of EAH banana have evolved, which have been named by their end-use: cooking and brewing (Sebasigari, 1990).

Cooking bananas, used for cooking when mature but green, prevail in the lower Eastern part of the region: Kenya, Tanzania, Uganda, and Eastern Rwanda. They constitute the main staple food in this area. Brewing cultivars dominate in higher altitudes in the west of the region: Central and Eastern Rwanda, Burundi, and much of East DR Congo (Figure 1.3), where production, distribution and sale of banana ‘beer’ are essential for social and economic transactions within the community (Davies, 1995).
Chapter 1. Banana rhizosphere in the EAH

Figure 1.3 Highland banana growing on the Nyakinama plain (Andosol), North Rwanda.

The banana cropping systems in the EAH are dominated by smallholder (<2 ha) and subsistence-oriented farms with low external input use (Karamura et al., 1999). Principal management practices include fertilization with organic waste or cow manure, mulching with grasses or banana and bean residues, de-leafing, de-budding, de-suckering, and stalking of heavier bunches to prevent toppling or snapping (Bananuka & Rubaihayo, 1994; Okech et al., 2002). Manure applications are proportional to the livestock held by the household, and may be more intensive when banana is intercropped with beans, main protein source in local food basket (Okech et al., 2005). De-suckering and fertilization are done before intercropping and mulch is applied after legume harvest, normally before the dry season (Okech et al., 2002). Banana plantations are rain-fed and are often established on lower slopes, valleys and/or between-hill depressions where soil moisture is higher during dry periods (Jones & Edli, 1984; Rockström,
2000). However, in Rwanda, Burundi, and Eastern DRC, bananas can also frequently be found on hilltops and plateaus wherever the soil is deep enough to sustain banana production.

High rates of human population growth and introduction of banana cultivation in areas where it was not a traditional crop have increased banana production and consumption in the EAH (Kangasniemi, 1998). Urban growth increased market demand for banana which, in turn, has stimulated greater commercial production (Davies, 1995; Ferris et al., 2002) as well as nutrient flow from the production areas (Van Asten et al., 2004). Population growth has negatively affected farm size, which drastically reduced over the last years (Jayne et al., 2003). Reduction of farm size contributed to increase in poverty, reduced livestock and minimized investments in soil conservation (Jayne et al., 2003). Higher dependence on brewing banana of the smallest households (Kangasniemi, 1998) may be an evidence of higher margins and/or market size of the ‘beer’ banana sub-sector (Kangasniemi, 1998; Gaidashova et al., 2005).

Because of low external input, the productivity of the EAH banana cropping systems is also constrained by diseases, pests, inadequate management or crop cultivation in marginal zones (Karamura et al., 1999). Major banana diseases are of bacterial, fungal and viral origin, while banana weevil and root parasiting nematodes are main pests. Fusarium wilt, leaf spot diseases, nematodes (*Radopholus similis*, *Pratylenchus goodeyi* and *Helicotylenchus* spp.), banana bunchy top and banana streak virus have spread in many areas of the EAH (Tushemereirwe & Bagabe, 1999). Banana *Xanthomonas* wilt appeared recently and spread at pandemic scale in the region (Mwangi & Nakato, 2007; Karamura et al., 2007).
Gold et al. (1999) observed that some of the banana fields may remain highly productive for a long time (>100 years) without replanting. However, the cases of reduction of plantation longevity to 5-10 years become more and more common (Okech et al., 2002). A shift of production zones from the nutrient depleted Victoria Lake border region to more fertile South-West of Uganda has happened in the last century to meet increasing demand for banana by the urban centers (Rubaihayo & Gold, 1993; Gold et al., 1999). Banana cultivation appears more environment-friendly on steep slopes than cultivation of annual crops. Erosion was reduced by three folds under banana compared to the erosion rate of soil under annual crops (Rishirumuhirwa, 1997), which may be one of the most important considerations for driving long term strategies of soil conservation in steep slopes where banana cropping predominates.

In addition to the stresses imposed by agricultural use, the EAH are exposed to global climate change as most of the other regions of the world. Climate change in the East African region may result in a raise of the aire and soil temperature, and greater variability and lesser predictability of rainfall (Hulme et al., 2001; WWF, 2006). In recent years, the average temperatures were distinctly warmer than 100 years ago, while the amplitude of annual rainfall patterns increased (Hulme et al., 2001). Forest reduction in the EAH, non-optimal use of wetlands and lakes along with agricultural expansion due to demographic increase also contributed to regional climate change. Therefore, in the near future, the EAH cropping systems may experience higher pressure at environmental (climate), biological (higher pressure from humans and crop diseases) and economic scale (higher production required from the same acreage, higher proportion of produce marketed to cities to feed more people).
1.3 Rhizosphere

1.3.1 Major processes in the banana rhizosphere

The major processes in banana rhizosphere are: (i) nutrient and water uptake from surrounding soil by roots and beneficial microbes such as mycorrhizal fungi, (ii) demineralization of nutrients from solid soil fraction surrounding rhizosphere and alteration of pH in soil solution; (iii) carbon release resulting from root respiration, secretion of root exudates and carbon flow from roots to various root symbionts (mycorrhizal symbiosis), (iv) modification of soil structure by roots through the exploration of new spaces.

The uptake of water and nutrients, which is the major function of roots, results in either accumulation or depletion of all the ions contained in the soil solution in the rhizosphere (Hinsinger, 1998). Calcium uptake in banana roots is directed by simple convection, or mass flow, while Mg uptake by convection happens only at low water uptake rate (Delvaux et al., 2005). Uptake rates of $K^+$, $NO_3^-$, $NH_4^+$, P and Mn uptake clearly depend on active mechanisms. For Fe, Mo, B, exclusion mechanisms were suspected when supply is greater than demand (Delvaux et al., 2005). Large uptake of cations ($K^+$ and $NH_4^+$) over anions induced strong release of acidity by banana roots, which subsequently promoted the dissolution of aluminosilicate minerals present in the surrounding rhizosphere (Rufyikiri et al., 2004). The uptake of the latter by banana roots significantly decreased plant transpiration rate, uptake of Ca and particularly Mg (Rufyikiri et al., 2000a; 2002; Delvaux et al., 2005). The efficiency of nutrient uptake from the soil to the plant is affected by soil K. Low K supply not only reduces banana yields but also root system effectiveness in absorbing N, P, Ca, Mn, Na and Zn (Turner & Barkus, 1981) but not K that is available from soil (Turner, 2005).
Apart from plant roots, AM fungi are actively involved in nutrient transfer within the rhizosphere. Uptake and transfer of P, \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) as well as other non-essential elements (U and Cs) by the AM fungi to plant roots were demonstrated experimentally (Rufyikiri et al., 2005). Such uptake may be substantial in the agro-ecosystems, however, its magnitude has not yet been quantified.

Water uptake by the roots is determined by plant requirements for water and quantity of water available for plants in a given soil (Hinsinger, 1998). Banana is an exigent crop for water, and it accumulates up to 80% of water in its biomass (Robinson, 1996), while about 100 mm rainfall per month are considered as optimal. Water uptake by the plant is dependent on leaf surface and water evaporation from soil (Draye, 2002a) as well as from temperature (Rufyikiri et al., 2001) and its diurnal fluctuations (Milbum et al., 1990). Lower temperatures decreased water uptake by banana roots (Rufyikiri et al., 2001). At high evaporation rates from soil, about 60% of water taken up by the banana plant from soil was extracted from upper 30 cm soil depth where most of the roots are concentrated (Kashaija et al., 2004; FAO, 2009). Reduction of water uptake particularly leads to the decrease of uptake of those nutrients which are absorbed by mass flow (Ca) (Rufyikiri et al., 2000). Genotype-related differences in water and nutrient uptake were observed for banana where the cultivars with lower water uptake (AAB, plantain) were more tolerant to Al toxicity compared to cultivars with higher water uptake (AAA, Cavendish) (Rufyikiri et al., 2000). These physiological differences may play an important role for plant adaptability and survival in the EAH region where the dry season can last up to 3 to 4 months (Davies, 1995).
Root respiration may contribute up to 50% of the total soil respiration. Growing root tips are the most intensively respiring parts of the banana rhizosphere (Aguilar et al., 1998), while root hairs consume up to 60% of the oxygen available at root surface (Aguilar et al., 2003).

The release of organic compounds by roots (Sen, 2005) is at the origin of the stimulation of soil microflora in the rhizosphere (Hinsinger, 1998). Up to 20-30% of the total C assimilated by higher plants is released in the rhizosphere as diversified exudates, including respired CO$_2$ (Helal & Sauerberg, 1989; Nguyen, 2003). Being a huge herbaceous plant producing up to 900 cord roots per plant (Sebuwufu et al., 2004), banana root systems secrete important quantity of root rhizodeposits (also termed exudates) during its life time. Rhizodeposits may account up to 40% of total dry biomass matter (Lynch & Whypps, 1990). Root exudates serve as a major food source for rhizosphere microorganisms (Hinsinger et al., 2005). Rhizodeposits are involved in mineral nutrient mobilization and acquisition either directly by the action of enzymes and organic acids or indirectly by stimulating symbiotic organisms (rhizobia and mycorrhizae) and facilitating their establishment in roots (Dakora & Phillips, 2002). Plant root exudates may stimulate AM fungi spore germination and mycelium growth (Jones et al., 2004) and attract plant parasitic nematodes (Wuyts et al., 2006). Little research has been conducted on rhizodeposits of banana. Their role in the EAH cropping systems is unknown.

Another process in the rhizosphere concerns the physical effect of roots on soil structure. Continuous root growth and senescence modify the soil structure through the exploration of new spaces. The empty corridors left in soil after root death serve further as secondary niches for the roots themselves and other microorganisms. Maintenance of the soil porosity contributes to its aeration and water
retention and reduces plant expenses for root growth through reduction of soil physical resistance during root penetration.

1.3.2 Banana roots

The capacity of banana roots to absorb nutrients and water may be affected by the following characteristics of the root system: (i) root surface determined by the number, branching and length of roots of all orders produced by the plants as well as their lifetime; (ii) proportion of laterals to adventitious roots; (iii) root hair growth and properties; (iv) root growth rate (Draye et al., 2005). The banana root system is composed of a group of primary root axes initiated from the rhizome and branching to lateral roots of subsequent orders (Draye, 2002a). In the EAH, banana roots may reach 5-6 m length and up to 70 cm depth, with 70 to 90% of the root biomass concentrated in the upper 30 cm soil (Godefroy et al., 1992; Kashaija et al., 2004). Lateral roots and root hairs account for more than 98% of the total root length (Swennen et al., 1986) and increase root absorption surface. However, they received very little research attention (Draye et al., 1999).

Root system size (Swennen et al., 1986), number (Stoffelen, 2000) and the proportion of primary laterals bearing the secondary roots vary among banana cultivars (Swennen et al., 1986; Rufyikiri et al., 2000a). However, it is not clear, which of these traits are genotype-dependent, and how much of their variability can be attributed to environmental factors (Draye, 2005). Studies of the root system of the EAH bananas are rare and fragmentary. The cultivar diversity of this sub-group in terms of root system variability remains practically unknown. Considering very tight interdependence of root and shoot development in many EAH banana genotypes (Blomme, 2000) and large variability from dwarf to
long-height cultivars, it is possible that large differences exist. Sebuwufu et al. (2004) observed large variations in root system size and extent in ten EAH cultivars. In this study, cultivars Kibuzi, Nakinyika and Butobe had as much root axes as other best *Musa* performers (e.g. Yangambi km 5), while other cultivars of the same group (Musakala, Imbululu, Namadhi and Katalibwambuzi) had significantly (30-40%) lower root axes number. Depth of soil exploration by banana roots is a cultivar/genotype dependent trait (Sebuwufu et al., 2004), thus indicating that different cultivars are able to explore soil to a various extent.

Root branching and proportions of laterals to adventitious roots, which contribute to the surface of nutrient uptake within the rhizosphere, are almost blank areas of banana root research. The EAH bananas, as well as other *M. acuminata* genotypes, have well developed root branching, resulting in higher proportions of tertiary roots compared to AAB and ABB cultivars (Swennen et al., 1986; Stoffelen, 2000; Elsen et al., 2003).

Banana roots develop a dense cover of large root hairs often exceeding 2 mm in length. These appear immediately behind the root elongation zone and reach full size at 8-12 cm proximal to their origin and persist up to 60 cm behind the root tips of actively growing roots (Draye, 2002a). The maintenance of a root hair zone depends on a continuous root growth (Robinson, 1996), while nutrient and water uptake take place in fine roots covered by root hairs whose life doesn’t exceed a few days or weeks (Draye, 2002a).

Primary root axes represent routes of water and nutrient transport to the shoot and assure anchorage of the important above ground plant biomass (Price, 1995a), while lateral roots and root hairs provide absorptive surface to the plant, thus controlling its capacity to exploit soil resources (Draye, 2002b). They also are the entry points for many soil organisms including AM fungi and parasitic
nematodes (Bilgrami, 1994; Bilgrami & Gaugler, 2004; Smith & Read, 2008). The length of root axes depends primarily on their elongation rate and the time over which they persist (Draye, 2002a). The two are apparently strongly controlled by environmental factors, while the effect of genotype is suspected but so far remains underinvestigated (Draye et al., 1999). In Musa, the elongation rate follows the diurnal and seasonal variations of the leaf emergence rate and is adversely affected by mechanical impedance, low pH, elevated water tables, suboptimal soil temperatures and oxygen deficiency (Draye, 2002a). It also depends on the physical and chemical properties of the soil, the age of the roots, and the root type (primary roots grow fast, secondary roots grow slower) (Lecompte et al., 2003).

1.3.3 Impact of edapho-climatic and management factors on roots

Among the factors contributing to root deterioration (Gauggel et al., 2005), the most relevant in the EAH are the following: limited effective depth of soil, very high sand, gravel or clay content, the degradation of soil physical and chemical conditions, deterioration of soil biological activity, and poor crop management practices (disease control, nematodes and weeds).

The exchange surface between the plant and the soil may be affected by the volume of soil influenced by root activity and the depth of soil exploration. The output of this interaction is highly dependent on type, depth and texture of soil (Delvaux & Guyot, 1989; Delvaux, 1995). Some soils with appropriate physical properties allow deep root development even under conditions of poor nutrient content (Godefroy, 1969). In compact soil, root development is often limited to the upper 30-35 cm horizons (Delvaux 1995). Root density in soil was inversely correlated to soil bulk density (Delvaux & Guyot, 1989). Highly diverse soil
conditions result in large variations of the banana growth and yield (Godefroy et al., 1991a, b). In the EAH, banana is usually cultivated on soils having good physical properties (Bekunda et al., 2003), but limited in nutrients (e.g., K and P) (Lassoulière, 1989).

The studies of management practices showed beneficial effect of mulch (Bananuka et al., 2000; McIntyre et al., 2000; Zake et al., 2000; Talwana et al., 2003a) and other soil organic amendments (Akhtar & Malik, 2000) on strengthening of the banana root systems. Mulch increased root density via greater branching (Blomme et al., 2003), decreased soil bulk density (McIntyre et al., 2000), increased organic matter in soil (Blomme et al., 2003), improved soil humidity (Bananuka et al., 2000), improved soil drainage (McIntyre et al., 2000) and mitigated soil temperatures (McIntyre et al., 2000; Talwana et al., 2003a). Mulch practice is recommended for the improvement of the soil microclimate (Stigter, 1984). Organic matter added to the soil (e.g. in the form of mulch) increases the soil’s cation exchange capacity and releases nutrients upon decomposition. Mulch suppresses weed growth (Bananuka et al., 2000) and increases soil biological activity (Zake et al., 2000; Bananuka et al., 2000; Akhtar & Malik, 2000). Since root growth rate is sensitive to temperature variations, mulch promotes rapid root extension (Turner, 1995). Higher soil temperatures of bare fields accelerated root decay (Speijer & Fogain, 1999), while in mulched banana fields root deterioration was substantially reduced (Robinson, 1996).

1.4 Plant parasitic nematodes

Studies on plant parasitic nematodes in banana rhizosphere in the EAH region were mostly limited to identification and impact assessment of the
migratory endoparasitic species (e.g., *R. similis*) (Bridge, 2000). These reports concern mainly endoparasitic species, which cause extensive visual damage to the roots. The impact of ectoparasitic species has been less investigated. Root extraction studies have reported lower numbers of plant parasitic (PP) nematode species diversity (4-7 species from 4-6 genera; Speijer & De Waele, 2001; Seshu Reddy *et al.*, 2007) as compared to soil extraction surveys (10-22 species from 7-12 genera; Bridge, 1988; Gaidashova *et al.*, 2004). According to the ecological classification of Yeates *et al.* (1993), using feeding habits for grouping, the banana nematodes include (i) ecto-parasites (*Helicotylenchus multicinctus*); (ii) semi-endoparasites (*Hoplolaimus pararobustus*); (iii) sedentary endoparasites (*Meloidogyne* spp.); (iv) migratory endoparasites (*Radopholus similis* and *Pratylenchus goodeyi*) (Speijer & De Waele, 1997).

The abundance of two predominant endo-parasitic species (*R. similis* and *P. goodeyi*) in the EAH banana cropping systems is related to altitude. *Radopholus similis* prevails on warmer lower altitudes (<1300m) (Gowen, 1995), while *P. goodeyi* predominates at cooler higher altitude (>1400 masl) (Kashaija *et al.*, 1994; Speijer & Fogain, 1999; Gaidashova *et al.*, 2004). These differences, resulting from different temperature optimums for reproduction (Pinochet *et al.*, 1995), assure separation of for the ecological niches of these two species within the EAH region.

Vertical and horizontal distributions of banana nematodes in soil are related to (i) root growth and (ii) nematode migrations in soil. Because of their small size (<1 mm), the absolute distances the nematodes can move are small and do not exceed a few dozens of centimeters per year (Boag & Yeates, 2004). Plant parasitic nematodes use water to move in soil. Because of adhering to the substrate, they stop moving when the water film in the soil evaporates (Warton,
2004). Among banana root nematode species, little difference was observed in horizontal migration, while vertical distribution was species-related. *Radopholus similis* was present exclusively in the upper 30 cm soil, while *H. multicinctus* prevailed in the upper 30 cm layers and *P. goodeyi* was more homogenously spread across soil depths but was concentrated in immediate soil/root margins (Kashaija et al., 2004).

The life cycle of the banana affecting nematodes generally lasts 1 month when the temperature is optimal (Gowen & Quénéhévé, 1990). After egg hatching, all nematodes species affecting banana moult four times into subsequent juvenile stages with the first moult usually occurring in the egg (Lewis & Perez, 2004) except for *H. multicinctus* (Karakas, 2007). The length of the life cycle of the nematodes varies and is strongly controlled by environmental factors (Barbercheck & Duncan, 2004). The most aggressive species, *R. similis*, is also the one developing the most rapidly as compared to *P. goodeyi* and *H. multicinctus*: 20-25; 25-30 and 39 days (from egg to egg), for *R. similis*, *P. goodeyi* and *H. multicinctus*, respectively (Loos, 1962; Prasad et al., 1999; Karakaş, 2007). Food quality apparently affects nematode nutrition and growth speed as there is an indication that the length of the life cycle may be host dependent. *Pratylenchus goodeyi* was able to complete its life cycle in 25 days when feeding on cv. Nakietengu (AAA-EA), but did so in 30 days on Sukali Ndiizi (AAB) (Prasad et al., 1999). In reality, this period may be longer if the temperature or moisture conditions are not favorable (Gowen & Quénéhévé, 1990). Thus, within an average 3 months rainy season in the EAH, all banana root parasitic nematodes develop several generations within the rhizosphere.

Although roots can be considered as a protecting (buffering) area for endoparasitic nematode species, edaphic factors (soil texture and organic matter
content) and soil climate (water content and temperature) may influence their dynamics. These factors have mostly been studied in lower altitude (below 700 m asl) banana production areas of the world where most of the Cavendish dessert bananas is grown. Higher soil moisture conditions favor root growth and nematode movement in soil. Nematode population densities generally reach a maximum following the rainfall peak (Hugon et al., 1984; Mateille et al., 1988; Quénéhévé, 1989a, b, c). Soil texture and humidity were associated with significant variations in nematode dynamics (Fargette & Quénéhévé, 1989; Quénéhévé, 1988) which is probably related to the presence of water film around soil particles which nematodes use to move in soil (Barbercheck & Duncan, 2004). In banana, the highest necrosis was observed in soils with coarse texture and lowest in fine-textured soils (Delvaux & Guyot, 1989). Excess in soil water and drought both reduce nematode population growth and affect their survival (Hugon et al., 1984; Mateille et al., 1988; Quénéhévé, 1988, 1989a, b, c). There is a little knowledge how and to which extent variation of soil conditions in the EAH affects banana root growth and nematode damage.

Banana root dynamics and plant phenological stage also affect nematode populations. Population dynamics of PP nematodes on banana roots follows logistics functions (Tixier et al., 2006), which are characterized by growth in parallel with banana plant, stabilization during inflorescence development and decreasing after bunch harvest (Quénéhévé, 1993). Since no new roots are formed after flower initiation (Price, 1995a), root biomass of the flowered plant decreases. By harvest time, the nematodes are forced to move to the emerging suckers for invasion (Speijer & De Waele, 1997).

Among the management practices affecting PP nematodes, mulch and intercrops effects are all well documented (Objefuna, 1990, 1991; Speijer et al.,
1999; Talwana et al., 2003a). Mulch is altering soil microclimate by cooling the environment conditions (Stigter, 1984). Shifting temperature may affect the reproduction of various PP nematode species. Intercrops may host the same PP nematode species as the banana roots and therefore, they may positively contribute to PP nematode population build-up depending on their host status (Namaganda et al., 2000). It is, however, unclear, how the availability of the intercrop root population as a food source for nematodes does affect their damage to the banana crop.

Banana plant-nematode relationships have particularly focused on R. similis. The population densities of this species, which is present sometimes in lower numbers as compared to other PP nematode species, were strongly correlated to root necrosis in highland banana (AAA-EA) (Kashaija et al., 1994; Barekye et al., 2000). Radopholus similis is considered as the most important species causing significant economic damage to banana (Fogain, 2000; Speijer & Kajumba, 2000). In the EAH, R. similis- induced yield losses up to 50% are documented (Speijer & Kayumba, 2000). In highland banana, this species also causes delay in flower production, poorer plant growth (Speijer et al., 1999) and reduction of root system size (Mukasa et al., 2006). Occurrence of strains varying in pathogenicity (Sarah et al., 1993) and reproductive fitness (Fallas et al., 1995) was confirmed for R. similis. Information on pathogenicity differences between P. goodeyi populations from different locations is not available. Although R. similis is believed to be a more damaging species than P. goodeyi (Elsen et al., 2000), information on yield losses due to P. goodeyi in the EAH banana cropping systems is virtually lacking.

Many of PP nematode species, feeding on banana roots, developed polyphagous behavior, e.g. feeding on multiple host species. Radopholus similis
may feed on plant species including weeds, crops and trees. Its host list exceeds 350 plant species (Price, 1994; Marin et al., 1998; Araya & De Waele, 2005). *Pratylenchus Goodeyi*, which is considered as an endemic species for the EAH (Price, 1995b), has multiple hosts but possibly of much narrower range compared to *R. similis* (Namaganda et al., 2000). The host range of *H. multicinctus* is believed to be very wide but it remains poorly studied (Gowen et al., 2005). Presence of the PP nematodes in fallows at below-detectable levels (Prasad et al., 2000) and in non-hosts at very low levels (Namaganda et al., 2000) suggests that possibly all banana PP nematode species have eggs capable to survive prolonged stress periods.

Feeding places within the banana root may be different for various PP nematode species. *Radopholus similis*, a migratory endoparasitic species, feeds mostly on cortical parenchyma cells (Araya & De Waele, 2004) through periodical intracellular migration within the root (Bilgrami & Gaugler, 2004), while *Pratylenchus* spp. and *Helicotylenchus* spp. prevail on epidermal root cells (Araya & De Waele, 2004). Although the information on preferred feeding sites within the root is missing for *P. goodeyi* in the EAH, this species together with *R. similis* was associated with extensive root necrosis (Speijer & De Waele, 2001; Talwana et al., 2003a). Hugon & Picard (1988) showed that in case of *R. similis* attack, the most infected zones within a root are not those showing the most visible necrotic symptoms but rather their neighboring zones that still appear healthy. However, for *P. goodeyi*, nearly equally large populations were observed in alive and dead roots (De Waele et al., 1997).

Management practices may affect PP nematode populations and their damage on banana in different ways. Intercrops may harbor the same PP nematode species as the banana crop. This may decrease PP nematode pressure
on banana crop, but higher root population in soil would encourage greater nematode reproduction. Mulch may create soil conditions which alter PP nematodes populations. Some agricultural research showed no effect of intercropping and mulch practices on the population densities of *R. similis* and *H. multicinctus* (Speijer *et al.*, 1999). However, root damage, caused by these species, was the major factor contributing to reduced yield (Ssango *et al.*, 2004). Other experiment at the same site showed reduction of banana plant biomass and yield due to the same nematode species in mulched plots (McIntyre *et al.*, 2000, 2001). Source of nitrogen (ammonium versus nitrate) was shown to moderate nematode (*R. similis*) population densities in highland banana (Bwamiki, 2004), while in field conditions under *R. similis* infection, root damage was associated with decreased K levels in banana leaves (Talwana *et al.*, 2003b). Manure moderated nematode populations and their impact on plants (Akhtar & Malik, 2000). Other management practices may also have an effect on the PP nematodes. For example, higher plant density and weeding alter root population size in soil. There exists limited information about the effects of these crop management practices on PP nematodes in the EAH.

### 1.5 Arbuscular mycorrhizal fungi

Banana is a highly mycotrophic plant (Declerck *et al.*, 1995) which forms symbiotic association with many AM fungal species (Adriano-Anaya *et al.*, 2006). The major functions of AM fungi in the rhizosphere are: (i) nutrient transfer from soil to plant via the extraradical mycelium, (ii) soil structure improvement via aggregation, and (iii) protection of plants from drought and abiotic stresses and soil root pathogens (Dodd, 2000).
Banana is known as a highly mycotrophic plant (Declerck et al., 1995). However, studies on AM fungi in banana cropping systems have been rare (Adriano-Anaya et al. 2006). Very little research has been conducted to document the presence of the AM fungi in the EAH region (Jefwa et al., 2008).

The process of nutrient exchange in the rhizosphere between AM fungi and plant depends on (i) the abundance of arbuscules, which is supposed to be the preferential structure for nutrient and carbohydrates exchanges between the plant and the fungus; and (ii) the extent of hyphae inside the roots and in the surrounding soil (Smith & Read, 2008). The last significantly increases the surface of soil exploration (Rillig & Mummey, 2006). The part of the rhizosphere that is under direct influence of the AM fungi is frequently renamed the mycorrhizosphere.

Mycorrhizal development is a dynamic process paralleled with root growth in the finer branches of the root system (Wilcox, 2002). Major structures of AM fungi are arbuscules, vesicles, auxiliary cells, hyphae and spores. Spores, vesicles and hyphae participate in fungal re-growth and may serve as sources of inoculum (Smith & Read, 2008). This is also suggested for auxiliary cells (de Souza & Declerck, 2003). The AM fungi enter the root via an appresorium. The proportion of an individual root axis which ultimately becomes colonized by the fungus depends on interrelationships between the rate of growth of the root and the rate of fungal growth within the root (Wilcox, 1996). Extent of colonization depends not only on rate of root and fungus growth, but also on root age as related to root tissue maturation, branching level (Hepper, 1985), AM fungal and plant host species (Hepper, 1985), root density and AM fungi inoculum density in soil as well as differences in soil P levels (Wilcox, 1996).
The AM fungi are classified in the phylum *Glomeromycota* (Schüßler *et al.*, 2001), in which six genera, classified on spore morphology, include *Glomus* and *Sclerocystis* of the family *Glomacea*; *Acaulospora* and *Entrophospora* of the family *Acaulosporacea*; *Gigaspora* and *Scutellospora* of the family *Gigasporacea* (Morton & Benny, 1990). Recent molecular studies revealed huge diversity of the AM fungi (Daniell *et al.*, 2001; Vanderkooi *et al.*, 2002), which underlined the limits of morpho-taxonomy. However, the concept of ‘species’ is still not clearly defined for the AM fungi, because these organisms lack sexual reproduction (Smith & Read, 2008). Until new taxonomy is available, we will further use the traditional AM fungi systematics.

The occurrence of *Glomus* spp., *Sclerocystis* sp. and *Acaulospora* sp. has been documented in mature banana fields of Martinique and Central America regions (Declerck, 1996; Adriano-Anaya *et al.*, 2006). Information is lacking for the EAH banana cropping systems. The abundance of AM fungi in the soil was studied in banana cropping systems in Martinique (Declerck *et al.*, 1999). Large differences in AM fungal population densities were reported and related to variations in soil and agro-ecological conditions. Recent volcanic soils that were used for banana cultivation contained only 1.5 propagules (p) per 100 g soil while Vertisols had 163 p. per 100g (Declerck *et al.*, 1999). Adriano-Anaya *et al.* (2006) used spore density assessment from soil under banana and reported a range between 40 and 277 spores per 2 kg of rhizosphere soil under cv. Grande Naine plantation.

Edapho-climatic conditions determine growth patterns of roots and fungal hyphae in the rhizosphere. Higher root colonization was observed in poorer soils (Chapin, 1988), however, the samplings were often limited to upper soil layers. Mechanical soil disturbance breaks AM mycelium network in soil and thus, strongly reduces absorptive surface of the rhizosphere (Jansa et al., 2006). In banana cropping systems, the soil population density of the AM fungi was affected by soil texture and P, agro-chemical use and soil wetness. Sandy soils were associated with lower and clay soils with higher AM fungi soil populations in intensive banana cultivation systems of Martinique (Declerck et al., 1999). Low P was associated with higher AM fungal populations in soil and roots (Declerck et al., 1999). Higher agrochemical inputs negatively affected AM fungi spore densities and root colonization in banana while spore numbers decreased during the dry season and at the end of rainy season, possibly, because of too dry or too wet conditions of soil rhizosphere (Adriano-Anaya et al., 2006).

Effect of AM fungi on banana plant growth was studied almost entirely in controlled conditions and on young plants (mostly Cavendish cultivars) derived from tissue culture (Yano-Melo et al., 1999; Declerck et al., 2002a; Thaker & Jasrai, 2002; Jaizme-Vega et al., 2002). Banana plants inoculated with AM fungi had increased growth rate and/or foliar nutrient concentrations. Inoculation with AM fungi decreased the impact of Al toxicity (Rufyikiri et al., 2000b).

Inoculations of banana plantlets during the hardening phase (i.e. using micropropagated plantlets) increased root and shoot biomass (Jaizme-Vega & Azcon, 1995; Reyes et al., 1995; Yano-Melo et al., 1999; Thaker & Jasrai, 2002; Jaizme-Vega et al., 2002; 2003), photosynthesis and respiration (Yano-Melo et al., 1999). In many cases, banana plants responded better in terms of biomass
Chapter 1. Banana rhizosphere in the EAH

increase to the inoculum consisting of Glomus species as compared to other genera (Acaulospora scrobiculata (Yano-Melo et al., 1999) and Scutellospora heterogama (Jaizme-Vega & Azcon, 1995). Identically, within the Glomus genus, differences were noted among species (Declerck et al., 1995; Reyes et al., 1995; Jaizme-Vega et al., 2002; 2003). Under controlled pot culture conditions, bananas inoculated with G. macrocarpum had the highest dry plant biomass increase as compared to those of plants inoculated with G. mosseae (Declerck et al., 1995).

The effect of the AM fungi on foliar nutrient concentration increase in banana was not always observed. Jaizme-Vega & Azcon (1995) reported increase of foliar N, P and K in banana plants due to inoculation, while Jaizme-Vega et al. (2003) observed only increase of foliar P.

Since the increase of foliar nutrient concentrations in banana is not always achieved, the magnitude of the AM fungi inoculation effect is measured by biomass increase of the inoculated plant. The relative mycorrhizal dependency (RMD) of a given plant is determined as the difference between the dry weight mycorrhizal plant and the dry weight of non-mycorrhizal plant as a percentage of the dry weight of the mycorrhizal plant (Plenchette et al., 1983; Declerck et al., 1995). The RMD in banana was affected by root morphology (Declerck et al., 1995; Elsen et al., 2003a), development stage of the plant (Jaizme-Vega et al., 2002), plant genotype (Declerck et al., 1995; Jaizme-Vega et al., 2002; Elsen et al., 2003 a, b) and the composition of the AM fungal inoculum (Declerck et al., 1995). Genotypes with the least developed root systems (cv. Williams, AAA-Cavendish), lower root hair length and density (cv. Intokatoke, AAA-EA) had the highest RMD as compared to those with well developed roots and high hair density (cv. Poyo, AAA-Cavendish) (Declerck et al., 1995).
Fungus species affected RMD as well. Inoculation with *G. macrocarpum* resulted in higher RMD than that with *G. mosseae*, irrespective of plant genotype (Deckerck *et al.*, 1995). In another study, cultivars Igitsiri and Mbwazirume (both AAA-EA) had higher RMD compared to Grande Naine (AAA, Cavendish) after inoculation with *G. mosseae* (Elsen *et al.*, 2003b). Jaizme-Vega *et al.* (2002) observed that Grande Naine (AAA-Cavendish) decreased its RMD in field conditions by 9 months after re-planting while cv. Gruesa (AAA-Cavendish) remained mycorrhizal dependent. Thus, further research should study the factors determining RMD in mature plantations and plant response to the AM fungi colonization under field conditions.

### 1.6 Biotic interactions in banana rhizosphere

Rhizosphere microcosm is a crossroad accommodating multiple interactions between nematodes, fungi, bacteria and macrofauna (earthworms, mites, collembola, insects etc). In banana cropping systems, only a limited number of this multitude of interactions has been studied. We will shortly point only the interactions related to plant parasitic nematodes and AM fungi.

#### 1.6.1 Nematode - nematode interactions

In banana rhizosphere, interspecific competition for feeding resources among nematodes species leads to exclusion or reduction of the concurrent population through deterioration of the quality of the resource (root tissues). Migratory endoparasites are generally more invasive and capable to exclude the populations of other root feeders (Eisenback, 1993). Interspecific competition among *R. similis*, *P. coffeea*, *H. multicinctus* and *H. pararobustus* has lead to the dominance of *R. similis* while suppressing other species (Quénéhervé, 1989).
Suppression may be mutual as demonstrated by Moens et al. (2005) who reported that, in experimental conditions, *P. coffeae*, *H. multicinctus* and *M. incognita* reduced *R. similis* populations, while out of the three species, only *P. coffeae* population was affected by the presence of *R. similis*. However, in poorly managed bananas, the combination of of two endoparasitic species – *P. goodeyi* and *R. similis* – still resulted in lower banana growth and yield than that of single species (Talwana et al., 2003a).

1.6.2 AM fungi and nematode interactions

In-root habitat of the AM fungi overlaps with those of plant parasitic nematodes. As in case of nematode infection, colonization by the AM fungi takes place in the epidermis and cortical parenchyma of roots (Smith & Read, 2008). Interactions between AM fungi (*Glomus* spp.) and nematodes were first investigated experimentally on dessert AAA banana infected by *R. similis* and *Meloidogyne* spp. (Umesh et al., 1988; Jaizme-Vega et al., 1997; Pinochet et al., 1997). The presence of the AM fungi reduced nematode infection and damage to the root systems in most cases. This was confirmed on wider range of banana genotypes (AA, AAA, AAB) and nematode species (*P. coffeae*) (Elsen et al., 2003a, b). The effect of nematodes and AM fungi on banana root system was opposite, as they decreased and increased root branching, respectively, while the magnitude of interactions was also genotype-dependent (Elsen et al., 2003a, Elsen et al., 2003b).
1.7 Research needs

Rhizosphere interactions between banana roots, the PP nematodes, the AM fungi and other micro-organisms have important implications on plant performance and the ability of the plant to cope with abiotic and biotic stressess in the various agro-ecologies. Information about the impact of PP nematodes on banana, as well as the presence, spread and abundance of the AM fungi in the EAH banana cropping systems is lacking. Understanding the ecological significance of the PP nematodes and the AM fungi in the EAH banana cropping systems, their relationships and effect on plant growth and nutrition, soil fertility and crop management practices could provide an insight on the rhizosphere processes contributing to sustainable productivity of the EAH banana cropping systems. This will further help to develop long-term and environmentally sound improved production technologies.
Research objectives, hypotheses and thesis outline
Research objectives

The main objectives of the thesis were the following:
I. to investigate the abundance, incidence and impact of *Pratylenchus goodeyi* on banana crops in different soil types in the EAH;
II. to characterize the presence, abundance and incidence of AM fungi as a function of soil type, soil depth, and banana genotype in the EAH;
III. to assess the impact of the indigenous AM fungi on plant growth in different soil types and banana genotypes.

Research questions and hypotheses

In relation to the research objectives, the following research questions were proposed:
1. How are the plant parasitic nematode distribution and abundance related to banana plant vigor, soil, climate, and management practices in the EAH at eco-regional scale?
2. How do nematode abundance and root damage affect banana performance at watershed scale?
3. Do the nematodes cause yield reduction in EAH banana and to which extent?
4. How are the abundance, composition and distribution of AM fungi related to soil, climate, and management practices in the EAH banana cropping systems at eco-regional scale?
5. How does root colonization by AM fungi vary with soil depth?
6. Is the root colonization by AM fungi affected by banana genotype?
7. How is the impact of AM fungi inoculation on plant growth affected by soil type and banana genotype?
Research objectives, hypotheses and thesis outline

To help to respond to these questions, the following hypotheses were proposed:
I. The incidence and impact of the plant parasitic nematodes on banana crop are affected by the edapho-climatic conditions but are low;

II. The AM fungi play an important role in the EAH and have impact on banana plant performance.

Thesis outline

Chapter 1 provides a state of the art review of the knowledge on banana rhizosphere and interactions between banana roots, plant parasitic nematodes and arbuscular mycorrhizal fungi with emphasis on the EAH.

The first part of the study (Chapters 2, 3 and 4) includes the assessment of the impact of *Pratylenchus goodeyi* population densities, root damage and yield of banana, and the possible factors affecting nematode damage in the EAH banana cropping systems. Chapter 2 describes the abundance and damage caused by *P. goodeyi* in banana cropping systems as well as their relationship with environmental factors (climate, soil type) and agricultural practices at farm level. Chapter 3 investigates the effect of topography and soil properties on nematodes and subsequent impact on root damage and banana yields at a watershed level. Chapter 4 assesses the impact of *P. goodeyi* on banana yield under two plantation management types.

The second part of the study (Chapters 5, 6, 7 and 8) investigates the presence, distribution, population densities, and impact of AM fungi on and in
Research objectives, hypotheses and thesis outline

banana plants in the EAH. **Chapter 5** investigates the AM fungi colonization as related to soil type, soil properties and crop management practices. **Chapter 6** describes the pattern of root colonization by AM fungi versus soil depths. **Chapter 7** studies how root colonization varies with banana genotype and site. **Chapter 8** explores the effect of indigenous AM fungi populations on banana plant growth in different soil types.

Thesis outline in form of chapters is presented in Figure 1.4.
Figure 1.4 Thesis research outline in form of chapters showing the major relationships studied.

This figure illustrates the relationship between chapters of this study. On the left, it is illustrated that chapters 2 to 4 focus nematode x banana interactions from eco-regional to field scale. On the right, chapters 5 to 8 focus on AMF x banana interactions from eco-regional to plant scale. At the bottom of the figure, we indicate that chapter 1 provides an overview of nematodes, AMF, and banana interactions to which we refer back in the concluding remarks in chapter 9.
Chapter 2. Relationship between soil property, crop management, plant growth and vigor, nematode occurrence and root damage in East African Highland banana cropping systems

This chapter was adapted from Gaidashova S.V., Van Asten P.J.A., De Waele D. and Delvaux B., 2009. Relationship between soil property, crop management, plant growth and vigour, nematode occurrence and root damage in East African Highland banana cropping systems. *Nematology* (11), *in press.*
Chapter 2. Relationships soil-crop management-plant vigour-nematodes

2.1 Abstract

Parasitic nematodes are widespread in *Musa* cropping systems in the African lowlands where they are known to limit crop production. Their spread is, however, very poorly known in large parts of the East African Highland banana ecology. We carried out a survey in 188 fields in Rwanda to assess and understand nematode occurrence and damage under a wide range of agro-ecological conditions. Altitude varied from 900-1800 masl and soil types were distinctly different in the five eco-regions sampled and deriving from diverse parent materials; i.e. Ruhengeri (Andosol), Gitarama - Butare (Acrisol), Kibungo (Nitisol), Gashonga (Ferralsol) and Bugarama (Fluvisol and Vertisol). Crop management practices, root health parameters and nematode infection in roots were recorded for a single East African highland banana cultivar (Intuntu, AAA-EA). Five nematode parasitic species were identified: *Pratylenchus goodeyi*, *Helicotylenchus multicinctus*, *Meloidogyne spp.*, *Radopholus similis* and *Hoplolaimus pararobustus*. *P. goodeyi* was the dominant species in all eco-regions except in Bugarama (lowland). Only *P. goodeyi* significantly (P < 0.001) correlated (r=0.61) with root necroses. Altitude was strongly correlated with root densities of *P. goodeyi* (r = +0.91, P < 0.001) and *R. similis* (r= -0.72, P < 0.001). A possible negative impact by *P. goodeyi* on banana yields was masked by the fact that nematode populations were positively correlated with high plant density and/or mulching practices, which was associated with relatively high plant vigour irrespective of soil type. Therefore, controlled field experiments will be needed to assess whether root necrosis caused by *P. goodeyi* at high altitude (>1400m) actually has a detrimental impact on banana yields, similar to what has been observed for root lesion nematodes at lower altitudes.
Chapter 2. Relationships soil-crop management-plant vigour-nematodes

2.2 Introduction

Banana (Musa spp.) is an important food and cash crop in Africa, a leading producer and consumer of banana and plantain (Gowen, 2005). In many regions of Africa, Musa yields are declining (Macharia et al., 2008; Karamura et al., 1999) due to a combination of biotic and abiotic constraints (Karamura, 1993). Suboptimal soil fertility in combination with pest and disease complexes are the major factors contributing to overall yield decline in the East African highlands (EAH) (Van Asten et al., 2004).

Plant parasitic nematodes are considered as important pests of bananas and plantains worldwide (Gowen et al., 1995). This is also the case in Africa where they may cause up to 50% yield reduction in highland bananas (Speijer & Kajumba, 2000). Yield losses vary depending on nematode species (Talwana et al., 2003a) and level of pathogenicity of a specific nematode population (Hahn et al., 1996). Yield reduction is mainly caused by the burrowing nematode (Radopholus similis) (Fogain, 2000; Speijer & Kajumba, 2000). The effect of nematodes on crop yield has mainly been studied in lowland bananas and plantains cultivated at altitudes below 1400 meters above sea level (masl) where Radopholus similis largely dominates the plant parasitic nematode communities (Gowen, 1995). In contrast, the effect of nematodes on bananas grown in highlands, such as the EAH where Pratylenchus goodeyi dominates the plant parasitic nematode communities, has poorly been studied despite the major importance of banana as a staple food in these areas (Davies, 1995).

In Rwanda, the banana-based cropping systems are characterized by subsistence small-farm production (Karamura et al., 1999) where the predominant nematode species is P. goodeyi (Kashaija et al., 1994; Speijer & Fogain, 1999; Gaidashova et al., 2004). These systems are situated between 900
Chapter 2. Relationships soil-crop management-plant vigour-nematodes

and 1800 masl and highly diverse as well in terms of soils, ranging from old and strongly weathered ferrallitic soils to young fertile volcanic ash soils, as in climate (Anonymous, 2001).

There is little understanding how differences in environment affect nematodes and the damage they cause to banana growth and yield. Existing data concern mainly the effects of mulching and intercropping on nematode communities dominated by *Radopholus similis* and *Helicotylenchus multicinctus* (Speijer et al., 1999; McIntyre et al., 2000; McIntyre et al., 2001), nutrient-nematode (*R. similis*) interactions (Bwamiki, 2004; Talwana et al., 2003b) and root damage caused by *P. goodeyi* on plants grown under poor management conditions (Talwana et al., 2003a).

In this study, the occurrence of plant parasitic nematodes in general and *P. goodeyi* in particular were examined in EAH banana cropping systems in five different eco-regions varying in altitude (900-1800 masl), soil, and climate. The aim is to assess and understand the impact of nematodes, in particular, migratory endoparasitic species, on banana performance and its relationship with the ecological parameters that are determined by nature (geology, altitude and climate) and influenced by man (soil and crop management).

2.3 Material and methods

2.3.1 Study sites

A diagnostic survey was conducted in banana fields in Rwanda during March-April 2006 (eco-regions 1-3 as explained below) and April-May 2007 (eco-regions 4-5). The methodology was adapted from Delvaux et al. (1986) and Perrier & Delvaux (1991). Five contrasting ecological regions were selected on the basis of climate and soil conditions: (1) Butare – Gitarama axis (South
Chapter 2. Relationships soil-crop management-plant vigour-nematodes

Rwanda); (2) Kibungo (East Rwanda); (3) Nyakinama plain of Ruhengeri (North Rwanda); (4) Gashonga (Southwest Rwanda); and (5) Bugarama (Southwest Rwanda) (Fig. 2.1).

Each eco-region corresponded to a major soil type (Table 2.1). The term ‘eco-region’ was used to distinguish the regions on the basis of their environmental conditions. The eco-regions differed in climate (altitude and rainfall) and soil type. Each eco-region corresponded to a distinct soil type. At each farm, data on altitude and geographic position of the homestead were recorded using a GPS.

Figure 2.1 Map of Rwanda showing the study sites.
Stars show the location of the sites.
Table 2.1 Major ecological characteristics of the surveyed eco-regions.

<table>
<thead>
<tr>
<th>Region</th>
<th>Altitude, masl ¹</th>
<th>Temp, °C</th>
<th>Rainfall, mm/year</th>
<th>Soil type ²</th>
<th>Soil parent rock</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bugarama</td>
<td>965 - 992</td>
<td>24.0</td>
<td>1079</td>
<td>Fluvisol/Vertisol</td>
<td>Alluvium</td>
<td>Old banana growing area, currently banana are being replaced by rice</td>
</tr>
<tr>
<td>Kibungo</td>
<td>1498-1623</td>
<td>20.6</td>
<td>900</td>
<td>Nitisol</td>
<td>Shale</td>
<td>Ancient banana growing area, medium to high production</td>
</tr>
<tr>
<td>Ruhengeri</td>
<td>1659-1692</td>
<td>19.6</td>
<td>1307</td>
<td>Andosol</td>
<td>Volcanic ash</td>
<td>Old banana growing area, high production</td>
</tr>
<tr>
<td>Gashonga</td>
<td>1607-1732</td>
<td>19.4</td>
<td>1200</td>
<td>Ferralsol</td>
<td>Basalt</td>
<td>Relatively old banana growing area, medium production</td>
</tr>
<tr>
<td>Butare-Gitarama</td>
<td>1675-1763</td>
<td>19.2</td>
<td>1170</td>
<td>Acrisol</td>
<td>Granite</td>
<td>Old banana growing area, production in decline</td>
</tr>
</tbody>
</table>

¹ - masl – meters above sea level; ² - mean annual air temperature, degrees Celsius; ³ - IUSS-WRB (FAO), 2006.
2.3.2 Farm, plot and cultivar selection

The term ‘farm’ was used to designate banana fields belonging to one farmer. At farm level, plot types differing in plant vigour were identified, and two different extremes were selected: one plot with the best performing plants and another plot where the plants looked relatively poor. These plots are referred to as ‘good or poor plant vigour plot types’. The decision on differentiating plant vigour as “good” or “poor” was based on 1) visual appraisal of each plant in the field and 2) measurement of plant growth and yield components (height, girth, number of functional leaves, number of fruits per bunch), while an attempt was made to select the plants showing the biggest difference in growth (=vigour) at each single farm level. Farm selection was made according to the following criteria: 1) belong to the required soil type (Table 2.1) according to the soil map (Anonymous 2001); 2) have plot types differing in plant vigour; and 3) have at least five flowering plants at the stage of fully opened hands (Martin-Prével, 1980) of the selected cultivar per plot.

In each eco-region (Fig. 2.1), twenty farms were selected. Usually two plot types were taken at each farm. In the Butare-Gitarama axis, where farm sizes were particularly small, the two contrasting plots could sometimes belong to neighbouring farmers, but for the data analysis they would be classified as one. Five flowering plants were sampled at each plant vigour plot type.

To minimize the effect of genotype on the data collected, the survey was performed on a single cultivar Intuntu. This was selected as the most common cultivar in Rwanda belonging to East African highland banana sub-group (AAA-EA, Lajugira-Mutika) (Nsabimana et al., 2008).
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2.3.3 Soil chemical properties

With each sampled plant, a composite soil sample was taken from three sub-samples at 0-25 cm depth at a horizontal distance of 50-70 cm from the plant. The five composite samples per plot were again thoroughly mixed to prepare one composite soil sample per plot.

The soil’s cation exchange capacity (CEC) and the soil exchangeable contents of Ca$^{2+}$, K$^+$, Mg$^{2+}$ and Na$^+$ were determined by atomic absorption spectrometry (VARIAN 300), after extraction with the normalized ammonium acetate extract at pH 7. In addition, KCl-extractable Al, N, P, pH $H_2O$ and pH KCl were determined. Soil C and N contents were determined using Walkley-Black and Kjeldhal methods, respectively. P was determined by colorimetry (SHIMADZU UV-1205).

2.3.4 Crop management

For each plot type, field management practices were recorded. Banana plant density, mulch, weeding and intercrops were assessed. Density was deduced from measuring the distances between each selected mat and four surrounding mats. Mulch thickness was recorded from five random measurements around each selected banana plant. The surface of weeds and intercrops in banana field was estimated in the radius of 2 m from the selected banana plant by visual appraisal using the following ranks: 1) none (< 2%); 2) very little (3-5%); 3) little (6-10%); 4) medium (11-25%); 5) high (26-50%) and 6) very high (>50%). Mean values of each rank were used to calculate the percentages.
Chapter 2. Relationship soil-crop management-plant vigor-nematodes

2.3.5 Plant growth

Plant growth measurements included plant height and girth at 1 m height, number of functional leaves, length and width of the 3rd leaf, number of hands and fruit number per bunch. An estimator of bunch weight based on plant vigour parameters was used to quantify the crop yield as reported recently by Gaidashova et al. (2007):

\[
EBW = 0.173 \times \text{Girth} + 0.995 \times \text{NFL} + 0.123 \times \text{Fruit number/bunch} - 16.1 \quad (r^2 = 0.56)
\]

where EBW is the estimated bunch weight, NFL is the number of functional leaves at flowering.

2.3.6 Nematode population densities

Nematode population density per farm and per plant vigour plot (composite root sample from 5 plants) were assessed from 20x20x20cm soil volume excavated in proximity of each selected banana plant at flowering stage (Speijer & De Waele, 1997). Nematodes were extracted from fresh banana roots using modified Baerman funnel as described by Speijer et al. (1998) and Carlier et al. (2002), identified and counted from 30 ml aliquots in Crop Protection Laboratory, ISAR Rubona Research Station. The nematode population density was calculated using one composite root sample from 5 plants per plot type (two nematode extractions per farm).

2.3.7 Root damage

Percentages of root necroses and dead roots per farm and per plant vigour plot (composite root sample from 5 plants) were assessed following the same sampling procedure as described for nematode population densities. Percentages
Chapter 2. Relationship soil-crop management-plant vigor-nematodes

of root necroses were assessed using five randomly selected roots from each plant as described in Speijer et al. (1998). The numbers of functional and dead roots were counted per 20x20x20 cm soil volume and percentage of dead roots was determined.

2.3.8 Data analysis

Some variables were transformed prior to the analysis. Type of transformation was selected to meet at best the assumption of linearity (Quinn & Keogh, 2006): square root for % dead roots and % root necroses; $\log_{10}(x+1)$ for nematode numbers and $\text{Al}^{3+}$, $\log_{10}$ for soil K, Mg, Na, CEC, C, N, P and pH. Management practices were ranked. Analysis of variance under General Linear Model was used to compare differences in plant vigour, nematode population densities and soil chemical characteristics between different eco-regions. Duncan multiple range test was used to separate means in case of significant differences. Two-sample t-test was used to compare nematode population densities and root variables in zones of good and poor plant vigor. Bivariate correlation analysis was performed to study the relationships between nematode population densities, root damage, soil chemical characteristics and management practices. Bonferoni adjustment was used to correct levels of significance. Pearson correlation was used for parametric values and Spearman correlation.
Table 2.2 Average values of plant vigour indicators in different farm plot types and eco-regions.

<table>
<thead>
<tr>
<th>Plant vigour Eco-region</th>
<th>Girth, cm</th>
<th>Height, cm</th>
<th>Leaf length, cm</th>
<th>Fruit number/bunch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (±SD)</td>
<td>Good</td>
<td>Poor</td>
<td>Mean (±SD)</td>
</tr>
<tr>
<td>Bugarama</td>
<td>Good: 64.6a (±7.7)</td>
<td>59.2B</td>
<td>418a</td>
<td>393B (±49.2)</td>
</tr>
<tr>
<td></td>
<td>Poor: 52.2b</td>
<td>52.2b</td>
<td>360b</td>
<td>360b (±49.2)</td>
</tr>
<tr>
<td>Kibungo</td>
<td>Good: 59.4a (±8.0)</td>
<td>54.7C</td>
<td>388a</td>
<td>368C (±39.3)</td>
</tr>
<tr>
<td></td>
<td>Poor: 49.6b</td>
<td>49.6b</td>
<td>345b</td>
<td>345b (±39.3)</td>
</tr>
<tr>
<td>Ruhengeri</td>
<td>Good: 68.7a (±5.9)</td>
<td>64.3A</td>
<td>438a</td>
<td>432A (±29.4)</td>
</tr>
<tr>
<td></td>
<td>Poor: 59.9b</td>
<td>59.9b</td>
<td>404b</td>
<td>404b (±29.4)</td>
</tr>
<tr>
<td>Gashonga</td>
<td>Good: 64.4a (±10.0)</td>
<td>55.6BC</td>
<td>445a</td>
<td>396B (±65.0)</td>
</tr>
<tr>
<td></td>
<td>Poor: 46.8b</td>
<td>46.8b</td>
<td>348b</td>
<td>348b (±65.0)</td>
</tr>
<tr>
<td>Butare-Git.</td>
<td>Good: 55.0a (±10.0)</td>
<td>47.9D</td>
<td>378a</td>
<td>340D (±63.1)</td>
</tr>
<tr>
<td></td>
<td>Poor: 40.9b</td>
<td>40.9b</td>
<td>302b</td>
<td>302b (±63.1)</td>
</tr>
</tbody>
</table>

P<0.0001, P<0.0001, P<0.0001, P<0.0001  

A, B, C and D – vertical and a, b – horizontal comparisons, values followed by the same letter are not significant at 0.05 level. SD - standard deviation. ‘Butare-Git.’ is Butare-Gitarama eco-region.
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was used for ranks (ex. management practices). SAS 9.1 Enterprise Guide 4 was the software package used for the above.

2.4 Results

2.4.1 Plant vigour

Highly significant differences (P < 0.001) in plant height, girth, leaf length and fruit number per bunch between different eco-regions have been observed (Table 2.2). Plants had the poorest growth in Butare-Gitarama and the best in Ruhengeri and Bugarama. Plant growth in Kibungo (Nitisols) was very similar to that observed in Ruhengeri (Andosols) and Bugarama (Fluvisols and Vertisols) (Table 2.2). The results demonstrate that the separation between plots was sufficient (Table 2.2). This was observed in all eco-regions with the largest differences between poor and good plant vigour plots in Gashonga.

2.4.2 Soil properties

Soil analysis revealed that the sites that were selected are typical/representative for the eco-regions (Godefroy et al., 1991; Nizeyimana, 1997). As such, the soil analysis results presented confirm the wide range of ecological conditions of the banana-based cropping systems. What new of this study is that it explores if and how these soil characteristics impact the spread and the damage of plant parasitic nematodes. Soils of eco-regions differed significantly in all measured characteristics (Table 2.3). Weathered soil on granite (Butare-Gitarama axis) had the lowest contents of exchangeable cations (Ca, Mg, K) and CEC, the lowest C and N contents, low pH values, moderate contents of KCl-
extractable Al\(^{3+}\). Nitisol (Kibungo) had high pH values, highest levels of exchangeable cations, CEC and high C, but moderately low P. Young volcanic ash soil of Nyakinama (Ruhengeri) had high contents of exchangeable cations and CEC, high pH, P and C contents. Ferralsol of Gashonga had very low in P but high in C; they were also low in exchangeable cations, and they contained substantial quantity of KCl-extractable Al. The Bugarama volcanic alluvial soil had a high pH, but moderate contents of exchangeable cations and CEC, low C and N contents.

Plots with poor plant vigour had lower quantities of all soil nutrients in most eco-regions/soils, however, this was significantly lower (P < 0.05) in Butare - Gitarama and Gashonga for K, pH and Na. Soil in Bugarama also had lower (P < 0.05) pH and Na in plots with poor plant vigour (Table 2.3). In Butare - Gitarama, Gashonga and Bugarama, pH and soil nutrient contents significantly differed between plots with, respectively, poor and good vigour, while Kibungo and Rugengeri were more uniform in soil characteristics at farm level (Table 2.3).

2.4.3 Management practices

Plant densities, mulching, intercropping and weeding varied distinctly (P < 0.05) between the eco-regions (Table 2.4).

The least distance between banana plants and therefore the highest plant density (3496 plants/ha) was recorded in Ruhengeri. The lowest plant density was in Bugarama (1096 plants/ha), followed by Kibungo (1600 plants/ha). In Kibungo and Ruhengeri, farmers adopted a lower density of banana plants in
Table 2.3 Average values of major soil chemical properties in different farm plot types and eco-regions.

<table>
<thead>
<tr>
<th>Plant vigour</th>
<th>pH–KCl</th>
<th>C (%)</th>
<th>N (%)</th>
<th>P (mg kg⁻¹)</th>
<th>KCl-extractable Al³⁺ (cmol kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eco-region</td>
<td>Good</td>
<td>Poor</td>
<td>Mean</td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Bugarama</td>
<td>6.75a</td>
<td>6.59b</td>
<td>6.68A</td>
<td>2.19a</td>
<td>2.03a</td>
</tr>
<tr>
<td>Kibungo</td>
<td>6.50a</td>
<td>6.23a</td>
<td>6.37B</td>
<td>3.64a</td>
<td>3.48a</td>
</tr>
<tr>
<td>Ruhengeri</td>
<td>6.21a</td>
<td>6.10a</td>
<td>6.11C</td>
<td>3.40a</td>
<td>3.57A</td>
</tr>
<tr>
<td>Gashonga</td>
<td>5.34a</td>
<td>4.92b</td>
<td>5.13D</td>
<td>3.60a</td>
<td>3.72a</td>
</tr>
<tr>
<td>Butare-Git.</td>
<td>5.51a</td>
<td>5.02b</td>
<td>5.26D</td>
<td>1.54a</td>
<td>1.34a</td>
</tr>
</tbody>
</table>

a, b – horizontal comparisons between plots with good and poor vigour within each eco-region, values followed by the same letter are not significant at 0.05 level; A, B, C, D, E – vertical comparisons between the eco-regions, values followed by the same letter are not significant at 0.05 level. ‘Butare-Git.’ is Butare-Gitarama eco-region.
good vigour plots than in poor ones (Table 2.4), while in Butare-Gitarama, the inverse trend was observed. Plant density had strong log-normal relationships with estimated bunch weight per unit area (Fig. 2.2). Distance between the plants positively and strongly correlated with estimated bunch weight ($r=0.73$, $P < 0.001$).

Mulch thickness was the highest in Kibungo (3.2cm), followed by Ruhengeri (1.5cm) and the other eco-regions (<1.3cm). Plots with poor plant vigour generally were mulched thinner, but differences within the eco-regions were not significant with the exception of Gashonga.

![Scatterplot of EBW/area vs Distance](image)

Figure 2.2 Relationship between the productivity per unit area (EBW/area: estimated bunch weight per unit area) and the average distance between banana plants in five eco-regions of Rwanda (1 – Butare-Gitarama, 2 – Kibungo, 3 – Ruhengeri, 4 – Gashonga, 5 – Bugarama).
<table>
<thead>
<tr>
<th>Plant vigour Eco-region</th>
<th>Distance between banana plants (cm)</th>
<th>Mulch thickness (cm)</th>
<th>Surface of cropping in banana fields (%)</th>
<th>Surface of weeds (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>Poor</td>
<td>Mean</td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Bugarama</td>
<td>301a</td>
<td>305a</td>
<td>302A</td>
<td>0.7a</td>
</tr>
<tr>
<td>Kibungo</td>
<td>264a</td>
<td>234b</td>
<td>250B</td>
<td>3.3a</td>
</tr>
<tr>
<td>Ruhengeri</td>
<td>178a</td>
<td>160b</td>
<td>169E</td>
<td>1.8a</td>
</tr>
<tr>
<td>Gashonga</td>
<td>211b</td>
<td>233a</td>
<td>222C</td>
<td>1.7a</td>
</tr>
<tr>
<td>Butare-Git.</td>
<td>181a</td>
<td>209a</td>
<td>195D</td>
<td>1.6a</td>
</tr>
</tbody>
</table>

a, b – horizontal comparisons between plots with good and poor plant vigour within each eco-region, values followed by the same letter are not significant at 0.05 level; A, B, C, D, E – vertical comparisons between the eco-regions; values followed by the same letter are not significant at 0.05 level. ‘Butare-Git.’ is Butare-Gitarama eco-region.
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Mulch thickness positively but weakly correlated with banana productivity, namely, the estimated bunch weight (EBW) \( (r=0.32, P < 0.001) \).

Intercropping of banana plantations consisted mostly of beans and cocoyam and much less of other crops (soya, peas, sorghum, maize, sweet potato, cassava). Intercrops had higher \( (P < 0.05) \) proportions in Butare-Gitarama, Gashonga and Bugarama. In Butare-Gitarama, Kibungo and Gashonga, intercrops were generally more frequently practiced, with significant difference in the last eco-region (Table 2.4).

Surface proportion covered by weeds was the lowest in Kibungo and the highest in Ruhengeri \( (P < 0.05) \) with 2% and 28% weed cover, respectively (Table 2.4). It generally tended to be higher in plots with poor plant vigour, but was only significantly higher \( (P < 0.05) \) in poor vigour plots of Ruhengeri compared to good vigour plots. Plant performance parameters did not significantly differ between plots that had no intercrops or weeds and those that had.

2.4.4 Nematode population densities and root damage

Four nematode parasitic species were identified (Table 2.5) from banana roots: \( P. goodeyi \) was present in 83% of fields, followed by \( H. multicinctus \) (36.7%), \( R. similis \) (13.3%) and \( H. pararobustus \) (1.6%), while the genus \( Meloidogyne \) (27.1%) was also present. \( P. goodeyi \) was the most abundant, frequent and dominant species in all eco-regions except in Bugarama (lowland, 900 masl) (Tables 2.5 and 2.6). The difference in \( P. goodeyi \) densities between highland eco-regions (>1450masl) and the lowland one (900 masl) was highly significant \( (P < 0.001) \). In Bugarama, \( H. multicinctus \) and \( R. similis \) dominated in banana roots. \( H. multicinctus \) was present in all eco-regions except one
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(Ruhengeri). However, it was the most frequent and its densities were significantly higher (P < 0.001) in Bugarama (Tables 2.5 and 2.6). *R. similis* was only found in lowlands of Bugarama where it was present in 62.5% of fields but

Table 2.5 Frequency of occurrence of plant parasitic nematodes in five eco-regions.

<table>
<thead>
<tr>
<th>Nematode</th>
<th>Pratylenchus goodeyi</th>
<th>Helicotylenchus multicinctus</th>
<th>Radopholus similis</th>
<th>Meloidogyne spp.</th>
<th>Hoplolaimus pararobustus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bugarama</td>
<td>20.5</td>
<td>100</td>
<td>61.5</td>
<td>48.5</td>
<td>5.1</td>
</tr>
<tr>
<td>Kibungo</td>
<td>100</td>
<td>10.5</td>
<td>0</td>
<td>15.8</td>
<td>0</td>
</tr>
<tr>
<td>Ruhengeri</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td>2.5</td>
</tr>
<tr>
<td>Gashonga</td>
<td>100</td>
<td>22.5</td>
<td>0</td>
<td>17.5</td>
<td>0</td>
</tr>
<tr>
<td>Butare-Git.</td>
<td>100</td>
<td>53.3</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>83</td>
<td>36.7</td>
<td>13.3</td>
<td>27.1</td>
<td>1.6</td>
</tr>
</tbody>
</table>

generally in low densities (Tables 2.5 and 2.6). *Meloidogyne spp.* was the least abundant species and *Hoplolaimus pararobustus* was only present in trace numbers in Ruhengeri and Bugarama (Table 2.5).

Highly significant correlations were found between altitude and population densities of *P. goodeyi, R. similis, H. multicinctus* (r=0.91, P < 0.001; r=-0.72, P < 0.001; r=-0.75, p<0.001, respectively) and a low, negative correlation (r=0.25, P < 0.001) for *Meloidogyne* spp. Correlations were higher in good vigour plots (r=0.93, P < 0.001; r=-0.66, P < 0.001; r=-0.77, P < 0.001; r=-0.34, P < 0.001) compared to poor vigor plots (r=0.76, P < 0.001; r=-0.58, P < 0.001; r=0.11 (not
Table 2.6 Population densities (100 g⁻¹ roots) of parasitic nematodes in different eco-regions and farm plot types.

<table>
<thead>
<tr>
<th>Plant vigour</th>
<th>Eco-region</th>
<th>P. goodeyi (±SD)</th>
<th>H. multicinctus (±SD)</th>
<th>R. similis (±SD)</th>
<th>Meloidogyne spp. (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
<td>Poor</td>
<td>Good</td>
<td>Poor</td>
<td>Good</td>
</tr>
<tr>
<td>Bugarama</td>
<td>186a</td>
<td>141a</td>
<td>167B** (±518)</td>
<td>11350a</td>
<td>8 743a</td>
</tr>
<tr>
<td>Kibungo</td>
<td>2667a</td>
<td>26691a</td>
<td>26489A (±18851)</td>
<td>90a</td>
<td>22a</td>
</tr>
<tr>
<td>Ruhengeri</td>
<td>46747a</td>
<td>38207a</td>
<td>41342A (±31418)</td>
<td>0a</td>
<td>0a</td>
</tr>
<tr>
<td>Gashonga</td>
<td>54658a</td>
<td>41425a</td>
<td>46895A (±35755)</td>
<td>77a</td>
<td>115a</td>
</tr>
<tr>
<td>Butare-Git.</td>
<td>59047a*</td>
<td>30347b</td>
<td>44697A (±36646)</td>
<td>1102a</td>
<td>1 031a</td>
</tr>
</tbody>
</table>

P<0.001   P<0.001   P<0.001   NS

a, b – horizontal comparisons between plots with good and poor plant vigour within each eco-region; A, B, C, D, E – vertical comparisons between the eco-regions; values followed by the same letter are not significant at 0.05 level. SD – standard deviation. ‘Butare-Git.’ is Butare-Gitarama eco-region.
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significant) and r=0.0 (not significant) for *P. goodeyi, R. similis, H. multicinctus* and *Meloidogyne* spp., respectively.

Number of roots (both: functional and total) was lower (P < 0.05) in Butare - Gitarama and Gashonga. However, the percentage of dead roots did not differ between the eco-regions (Table 2.7). Average root necrosis was higher in Butare - Gitarama (43.7%) and Kibungo (47.9%) than in other eco-regions, with Bugarama having the lowest value (16.8%).

Higher (P < 0.05) number of functional roots was observed in plots of good vigour at each of the eco-regions. Total root number did not differ significantly in Butare-Gitarama, Ruhengeri and Gashonga but it was higher in good vigour plots in Kibungo and Bugarama (Table 2.7). Plants in poor vigour plots had higher proportion of dead roots which was significant (P < 0.05) in all eco-regions except Butare - Gitarama where all plots had similar proportion of dead roots. No significant difference in root necroses was observed between the plots with good and poor plant vigour in any of the eco-regions.

*P. goodeyi* population densities correlated positively with root necrosis (r=0.61, P < 0.001 and r=0.50, P < 0.001) for good and poor vigour plots, respectively. Other nematode species did not have significant correlations with root necrosis.
Table 2.7 Root number and damage in different eco-regions and plot types.

<table>
<thead>
<tr>
<th>Plant vigour</th>
<th>Eco-region</th>
<th>Good (±SD)***</th>
<th>Poor (±SD)</th>
<th>Mean (±SD)</th>
<th>Good (±SD)***</th>
<th>Poor (±SD)</th>
<th>Mean (±SD)</th>
<th>Good (±SD)***</th>
<th>Poor (±SD)</th>
<th>Mean (±SD)</th>
<th>Good (±SD)***</th>
<th>Poor (±SD)</th>
<th>Mean (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bugarama</td>
<td>19.0a (±2.6)</td>
<td>15.6b (±2.6)</td>
<td>17.5A (±2.6)</td>
<td>22.7a (±2.2)</td>
<td>20.0b (±2.2)</td>
<td>20.5a (±2.2)</td>
<td>16.0b (±6.7)</td>
<td>21.9a (±6.7)</td>
<td>18.6A (±6.7)</td>
<td>15.0a (±6.7)</td>
<td>19.2a (±6.7)</td>
<td>16.8C (±6.7)</td>
</tr>
<tr>
<td></td>
<td>Kibungo</td>
<td>18.4a (±1.9)</td>
<td>15.5b (±1.9)</td>
<td>17.0A (±1.9)</td>
<td>21.7a (±1.7)</td>
<td>19.5b (±1.7)</td>
<td>20.7A (±1.7)</td>
<td>15.2b (±1.7)</td>
<td>20.6a (±1.7)</td>
<td>17.7A (±1.7)</td>
<td>45.4a (±1.7)</td>
<td>50.7a (±1.7)</td>
<td>47.9A (±1.7)</td>
</tr>
<tr>
<td></td>
<td>Ruhengeri</td>
<td>17.8a (±2.4)</td>
<td>16.2b (±2.4)</td>
<td>17.0A (±2.4)</td>
<td>20.8a (±2.0)</td>
<td>19.9a (±2.0)</td>
<td>20.4A (±2.0)</td>
<td>14.7b (±5.9)</td>
<td>18.7a (±5.9)</td>
<td>16.7A (±5.9)</td>
<td>38.0a (±5.9)</td>
<td>34.1a (±5.9)</td>
<td>36.1B (±5.9)</td>
</tr>
<tr>
<td></td>
<td>Gashonga</td>
<td>16.8a (±3.0)</td>
<td>14.7b (±3.0)</td>
<td>15.8B (±3.0)</td>
<td>19.9a (±3.9)</td>
<td>18.2a (±3.9)</td>
<td>19.1B (±3.9)</td>
<td>15.4b (±7.1)</td>
<td>22.7a (±7.1)</td>
<td>19.0A (±7.1)</td>
<td>22.7a (±7.1)</td>
<td>23.6a (±7.1)</td>
<td>23.1C (±7.1)</td>
</tr>
<tr>
<td></td>
<td>Butare-Git.</td>
<td>15.2a* (±2.1)</td>
<td>13.7b (±2.1)</td>
<td>14.5C** (±2.1)</td>
<td>18.4a (±2.4)</td>
<td>17.1a (±2.4)</td>
<td>17.7C (±2.4)</td>
<td>17.3a (±5.7)</td>
<td>19.2a (±5.7)</td>
<td>18.3A (±5.7)</td>
<td>45.9a (±5.7)</td>
<td>41.5a (±5.7)</td>
<td>43.7A (±5.7)</td>
</tr>
</tbody>
</table>

P<0.001   P<0.001   NS   <0.001 a, b – horizontal comparisons between plots with good and poor plant vigour within each eco-region; A, B, C, D, E – vertical comparisons between the eco-regions; values followed by the same letter are not significant at 0.05 level; SD – standard deviation.* - as described in Speijer et al. (1998). ‘Butare-Git.’ is Butare-Gitarama eco-region.
2.4.5 Nematodes, root damage and soil properties

Root necrosis correlated positively with soil C (r=0.63, P < 0.001), N (r=0.51, P < 0.01), CEC (r=0.62, P < 0.001), Mg (r=0.58, P < 0.001) and K (r=0.52, P < 0.01) in Butare - Gitarama, positively with CEC in Gashonga (r=0.43, P < 0.01) with and negatively with soil P in Kibungo (r=-0.47, P < 0.01). No significant correlations were found between soil characteristics and root necrosis in other eco-regions. *P. goodeyi* population densities correlated positively with soil K (r=0.50, P < 0.001) in Butare - Gitarama, positively with soil pH (r=0.34, P < 0.05) and negatively with KCl-extractable Al (r=-0.31, P < 0.05) in Gashonga. No significant correlation was observed in other eco-regions.

2.4.6 Nematodes and management practices

Root necrosis positively correlated with mulch thickness in Butare - Gitarama (r=0.42, P < 0.05) and plant density in Kibungo (r=0.41, P < 0.05). *P. goodeyi* population densities correlated positively with plant density in Butare - Gitarama, Kibungo and Gashonga (r=0.53, P < 0.01, r=0.31, P < 0.05 and r=0.31, P < 0.05, respectively) and mulch thickness in the first eco-region only (r=0.34, P < 0.05).

2.5 Discussion

Banana plants exhibited the best growth and, therefore, crop yields in Ruhengeri and Bugarama where climate conditions (temperature, rainfall), topography (lack of steep slopes) and soil fertility (nutrient contents, soil depth and physical properties) were combined optimally. Banana plants in Kibungo were almost as vigorous and had equivalent yields as those for Ruhengeri and
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Bugarama. Plant growth and vigour was less optimal in Butare - Gitarama and Gashonga, where low pH, low P, and particularly low levels of exchangeable K and Mg seem all to contribute to the poor fertility of these soils thereby limiting banana plant vigour.

The effect of management practices on plant vigour seems to be remarkable in Kibungo having more uniform mulching, vigorous weeding, and lower plant density, resulting from rigorous sucker control. High management standards in this area had also positive impacts on maintaining soil fertility levels. The significant higher mulch thickness in Kibungo area likely levelled the soil fertility parameters (Okech et al., 2002), thereby reducing the difference in plant growth.

Increased plant density and mulch thickness seem to exert a positive impact on the productivity of the banana cropping systems. Farmers double their banana productivity per unit area by reducing plant spacing from 3.5x3.5m (816 plants/ha) to 1.7x1.7m (3460 plants/ha) (Fig. 2.2). The latter density was observed in Ruhengeri on young volcanic ash soils.

For the first time, this study attempted to describe host-nematode relationships existing in real field situations in contrasted ecologies of the East African highland cropping systems with the case of banana (*Musa* AAA-EA) and *P. goodeyi*.

*P. goodeyi* was the most predominant plant parasitic nematode species affecting banana roots in different Rwandan soils/climates. Our results confirm the previous reports about *P. goodeyi* occurrence and abundance in major banana producing regions in Rwanda (Gaidashova et al., 2004). This species was reported also as major banana root pest in other East African countries – Uganda (Kashaija et al., 1994), Tanzania (Bridge, 1988; Speijer & Bosch,
Other nematode species (*H. multicintus, R. similis, Meloidogyne* spp. and *H. pararobustus*) were spread across the Rwandan environments in very low numbers and much lower occurrence compared to Uganda. This may be related to higher average altitudes of most Rwandan soils. Higher altitude eco-regions are cooler that optimises the reproduction of *P. goodeyi* (Pinochet *et al.*, 1995). Our study confirms that temperature may be the major factor affecting the spread of all major plant parasitic nematode species in the EAH banana cropping systems.

Root deterioration is caused by climatic, edaphic and biological factors (Gauggel *et al.*, 2002). Impact of endo-parasitic nematodes on plants ranges from localized forms of damage caused during invasion and feeding to overall systemic effects such as retarded plant growth (Back *et al.*, 2002). *P. goodeyi* was the only nematode species correlated with root necrosis according to the factors explained below and therefore the impact of only this species on banana cropping systems will be discussed further.

In the present study, although plant growth and root development were closely related to soil properties at inter-regional and intra-regional scale, root damage and nematode population densities were not linked directly to soil fertility patterns. The population densities of *P. goodeyi* tended to be higher in good vigour plots, where soil contained more exchangeable cations, higher C, N and P content and had higher pH value, which all together contributed to better plant growth and vigour. The latter confirms the observations of Declerck *et al.* (1998).

Gowen (1995) recommended mulch as a mean to mitigate the effect of nematodes on plant root system. However, some studies revealed that the effect
mulch has on plant parasitic nematode populations and resulting damage is inconsistent (e.g., Obiefuna, 1990; Pattison & Stanton, 1999). The first author observed reduction of nematode populations and the second team could not see any effect of mulch on nematodes. Talwana et al. (2003a) reported that \textit{P. goodeyi} population densities were higher in mulched plots having better plant growth. Therefore, management practices and soil properties may be involved in regulating \textit{P. goodeyi} populations.

Population densities of plant parasitic nematodes might be related to microclimate or soil conditions (Speijer & De Waele, 2001), however, information on this issue is limited and fragmentary. Few existing reports, however, put an evidence that such relationships exist for ecto- as well as for endo-parasitic nematode species in wide range of plant hosts. Negative relationships were observed between nematode population densities and soil pH (Sarah et al. (1991) on \textit{Pratylenchus brachiurus} in pineapple; manganese (Cadet et al., 2004) on \textit{Helicotylenchus dihystera} and \textit{Xiphinema elongatum} in sugarcane; positive relationships were mentioned between nematode abundance and soil exchangeable bases (Mg and K) for root knot nematodes in tobacco (Kincaid et al., 1970) and tomato (Dabire et al., 2007), \textit{Scutellonema} spp. in maize/beans, \textit{Crotalaria}, \textit{Tephrosia} and \textit{Sesbania} spp. cropping systems (Kandji et al., 2001).

This last trend was also observed for \textit{P. goodeyi} population densities which significantly and positively correlated with soil K in Butare – Gitarama, the eco-region having the lowest K levels in soil. In other eco-regions, where K levels were not as much limiting, no such relationships were observed. Similarly, \textit{P. goodeyi} significantly correlated with CEC in eco-regions with lower CEC values (Butare-Gitarama and Gashonga). Nematode populations might have larger impact on banana root systems in terms of root necroses and population
Chapter 2. Relationship soil-crop management-plant vigor-nematodes

densities when other limiting factors are present. It is possible that plant parasitic nematode populations and, therefore, root damage due to nematodes may be affected to some extent by critically low levels of soil nutrients. However, the mechanism of such relationships is unknown, complicated and challenging. Only pot experiments in controlled conditions can yield answer to questions regarding the impact of individual factors.

Crop management practices may modify soil environment, and thereby influence root development in soil (Watt et al., 2006). Changes may involve soil temperature, soil moisture and biological activity in terms of biodiversity including plant parasitic nematodes. Soil temperature is lower in mulched soil compared to bare soil (Talwana et al., 2003; Speijer et al., 1999). The similar effect may be expected when high plant density is used as bananas have good canopy cover and therefore more dense plantations may be cooler and probably more humid compared to fields with low plant density and lesser canopy (Stigter, 1984). Since both, mulch and plant density, significantly and positively correlated with root necroses and *P. goodeyi* densities, substantiates this assumption. Higher temperature affects negatively reproduction of *P. goodeyi* (Pinochet et al., 1995), it may explain why this species was more abundant in good vigour plots (which were heavier mulched). Sarah & Hugon (1991) reported about a decrease in plant parasitic nematode densities during dry seasons following reduced soil moisture. Uniform mulch may also explain higher root necroses in Kibungo in both plot types.

2.6 Conclusions

Results from this study indicate that the plant parasitic nematode population densities in the selected banana cropping systems of Rwanda were low except
numbers of *P. goodeyi* which seemed to be high. *P. goodeyi* was the only species correlated with root necrosis. The wide spread of this species was related to the cooler climate at higher altitudes (>1400m).

Although *P. goodeyi* was present in high population densities and apparently associated with root damage, it is not clear from our study how it really affects plant performance. In fact, the highest root population densities of *P. goodeyi* were recorded in the best performing banana plots that were characterized by high nutrient and labour inputs. A possible negative impact by *P. goodeyi* on banana yields was masked by the fact that nematode populations were positively correlated with high plant density and/or mulching practices, which was associated with relatively high plant vigour irrespective of soil type. A host reaction expressed in compensation of the root systems in response to plant parasitic nematode invasion may also be possible. There is, therefore, a need to further evaluate its impact on productivity in controlled conditions to make a final conclusion on the importance of this species. To minimize the interference between the nematodes and other factors of the environment, such experiment should be conducted in a site with low limitations for water and nutrient supply.
Chapter 3. Highland banana (*Musa* spp. AAA-EA, cv. Intuntu) x nematode interactions are influenced by topographic position in Western Rwanda

This work was submitted in *Scientia Horticulturae* as Gaidashova S.V., Van Asten P.J.A., Delvaux B. and De Waele D. Highland banana (*Musa* spp. AAA-EA, cv. Intuntu) x nematode interactions are influenced by topographic position in Western Rwanda.
3.1 Abstract
Soil properties are known to vary according to topography. They affect water uptake and root exploration in the soil. Consequently, they may also influence the spread of plant-parasitic nematodes. This study reports on the effect of toposequence related variations in soil on banana yields, foliar nutrient status, as well as nematode impact. Significantly better plant growth was observed in valley bottoms, where banana bunch weight was 1.7 to 3.4 kg higher than at upper toposequence positions. Lowest bunch yield (12.8 kg) and highest root death percentages were observed in middle slope. Heaviest bunches (16.2 kg) were observed in valley bottoms in contrast to the highest N and K foliar deficiencies in this location. Plants in valley bottoms had higher foliar Ca and Mg (P < 0.001 for both), and K (P < 0.05) compared to those in crest. Plants in middle slope had greater (P < 0.01) percentage of dead roots (19.1%), compared to the plants in valley bottoms (12.3%) and crest (14.2%). Soils in valley bottoms were deeper (P < 0.001), sandier (P < 0.001), with lower (P < 0.05) organic matter, lower N (P < 0.01), and K (P < 0.001) compared to the soils at higher topo-sequence. The spread of *P. goodeyi* had generally limited impact on banana yields in flatter fields (crest and valley bottom) where soil conditions were more optimal for root growth. However, in the presence of increased run-off on steeper middle slopes, root death was increased and bunch weight was reduced even under moderate pressure from *P. goodeyi*.

3.2 Introduction
Banana has been an important traditional staple and cash crop in the East African highlands (EAH) for at least a few centuries (Karamura *et al.*, 1999). Human expansion in this region was associated with a change of natural
vegetation to pastures along with the expansion of cattle herds, and thereafter or in parallel, with progressive switch from pastures to agricultural cultivation (Schoenbrun, 1993b, 1994) where banana expansion took place (Schoenbrun, 1993a). Cropping history and land use left their imprints on natural soil fertility patterns that were related to topography and geology. In Rwanda, population growth led to an intensification of agricultural land use (Kangasniemi, 1998), with banana cultivation spreading to more and more marginal zones in terms of altitude, soil fertility and topography. Consequently, bananas are grown on a wide range of soils (Andosols, Acrisols, Ferralsols, Nitisols) from 900 to 2100 m altitude.

In the EAH region, research was mostly confined to screenhouse and plot-scale studies, with little focus on the agro-ecosystem and watershed level. Soil quality and micro-climate can vary strongly as a function of topography. Farmers of the EAH region use this variation to preferably plant bananas at concave slope parts, foot-slopes and land depressions when fertile flat fields are not available (Jones & Edli, 1984; Rockström, 2000). However, land pressure has increasingly forced them to grow bananas on hill tops and steep slopes.

Among the pests and diseases, limiting banana production in Rwanda, plant parasitic nematodes (Pratylenchus goodeyi) were identified as important pests (Okech et al., 2002). They were associated with high root damage (Gaidashova et al., 2004), which negatively affected yields in other countries of the East African region (Speijer et al., 1999; Speijer & Kajumba, 2000; Talwana et al., 2003).

Inadequate understanding exists how toposequence location of field influences plant performance by differences in soil quality, and to what extent this is affected by the spread and impact of nematodes on banana yields. This
study has been conducted with the objective to investigate the effect of
toposequence related variations in soil on banana yields, foliar nutrient status, as
well as nematode impact on banana roots and crop yields in the EAH cropping
systems of Western Rwanda.

3.3 Material and methods

3.3.1 Site description

The study site was located near Kibuye (29°21’00”E, 02°03’20”S) at the
border of Kivu Lake. Kibuye is located at 1470 meters at sea level (masl) of
altitude, it has 21.5°C of mean annual temperature and receives 1185 mm rain
annually (Farrow et al., 2007). Rainfall is bimodal with the minor rainy season
between October and December and the major rainy season between March and
June. The plains of the Kivu Lake shore are of alluvial nature and have deep
soils with good soil physical structure. Soils on hills surrounding the plains are
lying on shale sometimes alternating with quartzite (Anonymous, 2001). They
have generally less optimal soil fertility and depth for banana growth.

The study area was located between 2°04’ to 2°06’ South and 29°21’ to 29
°25’ East. The altitude of the studied plots varied between 1477 and 1780 masl.

3.3.2 Topo-sequence location, plot, cultivar and plant phenological
stage

Within the toposequence, surveyed plots were classified according to location
on the hill: 1) valley bottom, 2) middle slope and 3) crest (Fig. 3.1). The
topography at the valley bottom was completely flat, whereas the middle slope had beyond 15% slope and the 'crest' had 0 to 5% slope. At each topo-sequence position, 10 farms, 2 plots per farm and 5 plants with a recent, within 14 days, flower emergence (Speijer et al., 1998) per farm were selected. At each farm, a range of banana plants with the poorest and best growth were identified by visual appraisal. This was done to achieve maximum plant performance variability to better identify the factors driving plant performance differences. The study only focused on the cultivar Intuntu (AAA-EA), since it was the dominant cultivar in this area.

Figure 3.1 The studied topo-sequence locations. Arrows show soil depth.
3.3.3 Sampling methodology

Topsoil samples (0-30 cm) were taken at 1 m distance from the selected banana plants to make composite soil samples at plot level. Leaf samples were taken from the inner lamina of the 3rd leaf of the flowered banana plant (Martin-Prével, 1980). Root pieces were taken from a 20x20x20 cm upper soil volume excavated 20 to 30 cm from the plant pseudostem base as described by Speijer & De Waele (1997). Soil depth was measured with an auger.

3.3.4 Crop management

The following crop management practices were recorded in proximity (3 m radius) near each banana plant: banana plant density, thickness of mulch, intercrop and weeding intensities. Each practice was ranked. Banana density was estimated as 1) low (800 plants /ha or less, equal to 3.5 x 3.5 m or more), 2) medium (800 to 1200 plants /ha, equal to spacing 2.5 to 3.0 x 3.5 m) and 3) high (more than 1200 plants /ha or spaced less than 2.5 x 3 m). Mulch thickness was ranked after measurements as the following: (1) none, (2) low (< 2 cm), (3) medium (2 – 5 cm), (4) high (≥ 5 cm). Intercrop intensity was ranked as (1) none, (2) less than 20%, (3) 20 to 60% and (4) more than 60% surface area under intercrops. Weeding intensity was ranked as (1) none, (2) more than 60%, (3) 20 to 60% and (4) less than 20% surface area under weeds.

3.3.5 Plant growth

Plant measurements comprised plant height, girth at 50 cm from soil level, number of functional leaves and total number of plants per mat, whereby a mat
was defined as a family of plants with interconnected corms. Bunch weight was measured at each farm whenever it was possible to find a mature bunch.

3.3.6 Root damage and nematode population densities

Percentage root necrosis, and number and percentage of functional and dead roots were assessed using five randomly selected roots from each plant as described in Speijer et al. (1998). Nematodes were extracted from fresh banana roots using the modified Baerman funnel method as described by Speijer et al. (1998), thereafter they were identified and counted from 30 ml aliquots. The nematode population density was calculated using one composite sample from five plants per plot, resulting in two nematode extractions per farm.

3.3.7 Soil and leaf analyses

Soil samples were oven dried at 40°C for 24 hours, ground and sieved to pass through a 2 mm mesh. Soil pH was measured in 1/2.5 sediment/water suspension as described in Okalebo et al. (2003). Total N was measured using a spectrophotometer in a sulphuric acid and selenium acid extract as described in Okalebo et al. (2003). Available P was extracted using Mehlic-3 solution (Mehlich, 1984) prior to colorimetric determination. Exchangeable cations (Ca, Mg, K and Zn) were extracted using 1 M ammonium acetate, and determined using an Atomic Absorption Spectrophotometer (AAS). Foliar sub-samples were oven dried at 72°C for 48-96 hours, ground to pass through a 2mm mesh and then digested in a sulphuric and selenium acid mixture. N and P were determined by colorimetry while K, Ca, Mg and Zn were determined using the AAS.
3.3.8 Compositional nutrient diagnosis

Since nutrient deficiencies are not only a function of absolute plant nutrient concentrations but of the concentration ratios between different nutrients, a compositional nutrient diagnosis (CND) approach was adopted. CND is based on row-centered log ratios where each nutrient is adjusted to the geometric mean of all nutrients and to a filling value ($R_d$) (Parent & Dafir, 1992). CND was done to identify major nutrient deficiencies using foliar nutrient contents data from our study and norms for East African highland banana developed by Wairegi & Van Asten (in press), using optimum partitioning into low and high yield subpopulations using the procedure described by Khiari et al. (2001). The negative value of the indices of nutrient deficiency (ind) of K (indn), P (indp), K (indk), Mg (indmg) and Ca (indca) reflects the magnitude of deficiency.

3.3.9 Data analysis

All variables were checked for normality of data distribution using Kolmogorov-Smirnov test. The following transformations were done to improve normality: $\log_{10}$ for soil P, Ca and Mg; $\log_{10}(x+1)$ for nematode numbers; square root for % clay, % dead roots, % root necrosis, leaf N, P and K; power transformation for soil K, K-ratio and % silt. Analysis of variance under General Linear Model was used for parametric variables, and non-parametric one-way ANOVA was performed for ranks. Linear regression analysis was applied to investigate the variability in root necrosis as related to field management practices, nematode population densities and leaf nutrient concentrations and/or deficiencies. SAS 9.1 Enterprise Guide was performed at these steps. Boundary line (Schnug et al. 1996) analysis was used to derive functional relationships.
between yield (bunch weight) and environmental variables/factors (soil properties, field management practices, foliar nutrient concentrations and deficiencies, nematode damage and population densities). In the first step, variables correlating significantly with yield ($r > 0.3$, $p < 0.05$) were selected. In the second step, these variables (x-axis) were plotted against yield (y-axis) and subjected to boundary function to delineate the upper points of the cloud (Schnug et al., 1996) The boundary lines express the maximum attainable yield as a function of the x-variable. For each plot, the actual attainable yield was then considered to be the minimum of the predicted maximum attainable yields following Liebig’s law of the minimum. For each factor, the number of plots where it was ranked as most limiting was counted. This allowed a ranking of the importance of yield limiting factors. The same approach to identify and rank yield constraints was previously applied for other tropical crops in Cassonova et al. (1999), Van Asten et al. (2003) and Fermont et al. (2009).

3.4 Results

3.4.1 Soil properties

Soils in valley bottoms were much deeper ($P < 0.001$), with significantly lower organic matter content ($P < 0.05$), lower N ($P < 0.01$), and K ($P < 0.001$) compared to soils in middle slope and crest (Table 3.1, Fig. 3.1). Valley soils contained higher ($P < 0.001$) sand and lower clay and silt ($P < 0.001$) compared to soils from middle slope and crest.
Table 3.1 Mean soil properties in the toposequence at Kibuye, Western Rwanda.

<table>
<thead>
<tr>
<th>Soil properties</th>
<th>Valley bottom</th>
<th>Mid-slope</th>
<th>Crest</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil depth, cm</td>
<td>78.4A</td>
<td>42.3B</td>
<td>46.3B</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pH–KCl</td>
<td>6.37A</td>
<td>6.45A</td>
<td>5.97B</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OM (%)</td>
<td>2.8B</td>
<td>3.7A</td>
<td>3.5A</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>N (%)</td>
<td>0.12B</td>
<td>0.14A</td>
<td>0.13A</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>P (ppm)</td>
<td>30.2</td>
<td>84.8</td>
<td>58.6</td>
<td>NS</td>
</tr>
<tr>
<td>Ca (cmol, kg⁻¹)</td>
<td>7.44AB</td>
<td>9.41A</td>
<td>6.61B</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mg (cmol, kg⁻¹)</td>
<td>2.56A</td>
<td>3.01A</td>
<td>2.40A</td>
<td>0.053</td>
</tr>
<tr>
<td>K (cmol, kg⁻¹)</td>
<td>0.37C</td>
<td>1.08A</td>
<td>0.66B</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clay+silt (%)</td>
<td>24.4B</td>
<td>35.0A</td>
<td>37.1A</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>75.6A</td>
<td>65.0B</td>
<td>62.9B</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

A, B, C = significant differences at 0.05 level. OM = Organic matter.

3.4.2 Leaf nutrient concentrations and deficiencies

Plants grown in valley bottoms had significantly (P < 0.001 for both) higher leaf concentrations of Ca and Mg compared to higher toposequence positions, and significantly (P < 0.05) higher leaf K concentration compared to crest position (Table 3.2). Plants grown in crest had lowest concentrations of leaf P (P < 0.01), K (P < 0.05), Ca (P < 0.001), Mg (P < 0.001). The CND analysis of foliar nutrient concentrations showed that the valley bottom plants had the highest N and K deficiency and excess of Ca. They also tended to have P deficiency (P = 0.056) (Table 3.2). Crest and middle slope plants had significantly lower N and K deficiencies, no P deficiency, but big Ca deficiency. Plants in all locations had excess Mg, which was less pronounced in lower toposequence locations.
3.4.3 Plant growth and root characteristics

Plants grown in valley bottoms were tallest ($P < 0.05$ for height) and thickest ($P < 0.01$ for girth), but with the least number of functional leaves ($P < 0.001$). Similarly, bunch weight in valley bottom was 1.7-3.4 kg higher than in upper toposequence locations, although the effect was not significant (Table 3.3). Plants from middle slope were with the thinnest girth, while the plants grown on the upper slope were shortest and produced the highest number of functional leaves. Mean root number per sample varied between 14.9 and 20.6 for total roots and from 12.2 to 16.6 for functional roots. Average total root number (15.3) and number of functional roots (13.7) were much lower ($P < 0.001$) in

<table>
<thead>
<tr>
<th>Nutrient deficiency index</th>
<th>Valley bottom</th>
<th>Mid-slope</th>
<th>Crest</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ind N</td>
<td>-0.0022B</td>
<td>-0.0011A</td>
<td>-0.0002A</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ind P</td>
<td>-0.169</td>
<td>0.827</td>
<td>0.122</td>
<td>0.056</td>
</tr>
<tr>
<td>Ind K</td>
<td>-0.816B</td>
<td>-0.194A</td>
<td>0.269A</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ind Ca</td>
<td>0.872A</td>
<td>-1.086B</td>
<td>-1.532B</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ind Mg</td>
<td>1.428A</td>
<td>1.417A</td>
<td>0.794B</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

A, B, C – significant differences at 0.05 level.
valley bottom than in middle slope (21 and 17% lower, respectively) or on the crest (19% and 16% lower) (Table 3.3). Percentage of dead roots varied from 8.3 to 20.7% and root necrosis from 23.6 to 38.4%. Plants on the middle slope had significantly (P < 0.01) higher percentage of dead roots (19.1%), compared to the plants in valley bottom (12.3%) and crest (14.2%). Root necrosis tended to increase slightly from crest to valley bottom but the trend was not significant (Table 3.3).

Table 3.3 Mean values of banana plant growth and root damage in the toposequence at Kibuye, Western Rwanda.

<table>
<thead>
<tr>
<th></th>
<th>Valley bottom</th>
<th>Mid-slope</th>
<th>Crest</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height, cm</td>
<td>380A</td>
<td>363AB</td>
<td>355B</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Girth, cm</td>
<td>69.6A</td>
<td>62.9B</td>
<td>65.7AB</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NFL</td>
<td>7.9B</td>
<td>9.5A</td>
<td>9.9A</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bunch weight, kg</td>
<td>16.2</td>
<td>12.8</td>
<td>14.5</td>
<td>NS</td>
</tr>
<tr>
<td>NFR</td>
<td>13.7B</td>
<td>16.5A</td>
<td>16.4A</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% Dead roots</td>
<td>12.3B</td>
<td>19.1A</td>
<td>14.2AB</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>% Root necrosis</td>
<td>32.1A</td>
<td>31.0A</td>
<td>28.2A</td>
<td>NS</td>
</tr>
</tbody>
</table>

A, B – significant differences at 0.05 level. NFL = number of functional leaves; NFR = number of functional roots

3.4.4 Management practices

Management practices were generally homogenous within farm and across toposequence locations, except intercropping which was lower in valley bottom (Table 3.4). Intercrops varied from farm to farm, but were hardly dominated by
beans (85%) with some cocoyam and/or arrow root (10%), soybean and sorghum (5% or less).

### Table 3.4 Average ranks of management practices at the toposquence at Kibuye, Western Rwanda.

<table>
<thead>
<tr>
<th>Management practices (ranks)</th>
<th>Valley bottom</th>
<th>Mid-slope</th>
<th>Crest</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana plant density</td>
<td>2.2</td>
<td>1.9</td>
<td>2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Mulch</td>
<td>1.8</td>
<td>1.4</td>
<td>1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Weeding</td>
<td>2.6</td>
<td>2.4</td>
<td>3.2</td>
<td>NS</td>
</tr>
<tr>
<td>Intercropping intensity</td>
<td>2.1B</td>
<td>3.4A</td>
<td>3.1A</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

NS – not significant at P = 0.05; A, B – significant differences at 0.05 level.

Management practices (ranks): Plant density - 1- low, 2 – medium, 3- high; other practices – 1- none, 2 – low, 3 – medium, 4 – high.

### 3.4.5 Nematode species occurrence and abundance

The following nematode species were recovered from banana roots: *P. goodeyi*, *Hoplolaimus pararobustus*, *Meloidogyne* sp. and a mix of *Helicotylenchus* spp. (*H. dihystera*, *H. egyptiensis* and *Helicotylenchus* sp.). *Pratylenchus goodeyi* was predominant and present in 100% of fields in all toposquence locations. It was followed by *Meloidogyne* sp. (70-85%) and *Helicotylenchus* spp. (55-80%). *Hoplolaimus pararobustus* was the least frequent and abundant species (Table 3.5). All nematode species seemed to have a similar frequency of occurrence in all soil toposquences, except *H. pararobustus* which was more frequent in crest where it occurred in 35% of the plots examined.
Pratylenchus goodeyi largely dominated other nematode species in banana roots (Table 3.5) with population densities varying between 22000 and 44000 vermiforms per 100 g fresh root weight. Other nematode species were present in much lower numbers (Table 3.5). None of the nematode species showed the significant increase or decrease along within soil toposequence, although P. goodeyi tended be slightly more abundant in valley bottoms (Table 3.5).

Table 3.5 Frequency of occurrence and population densities of parasitic nematodes in banana roots in the toposequence at Kibuye, Western Rwanda.

<table>
<thead>
<tr>
<th></th>
<th>Frequency (%)</th>
<th>Mean population density (Number./ 100g roots)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Valley bottom</td>
<td>Middle slope</td>
<td>Crest</td>
</tr>
<tr>
<td>P. goodeyi</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Meloidogyne sp.</td>
<td>75</td>
<td>85</td>
<td>70</td>
</tr>
<tr>
<td>Helicotylenchus spp.</td>
<td>55</td>
<td>80</td>
<td>70</td>
</tr>
<tr>
<td>H. pararobustus</td>
<td>15</td>
<td>10</td>
<td>35</td>
</tr>
</tbody>
</table>

Frequency of occurrence (F) was estimated as % fields where species was observed; NS – non-significant differences at 0.05 level. P – probability.

3.4.6 Banana yield determinants on the toposequence

Boundary line analysis revealed that intercropping intensity, foliar nitrogen concentrations and percentage of dead roots were the major factors limiting bunch weight in 68.2% of banana plots (Table 3.6). Other factors (mulch, foliar Ca, weeding, K deficiency and plant density) appeared less important (Table 3.6). Intercrop intensity, percentage of dead roots and foliar N were the main
limiting factors to bunch weight in the crest, middle slope and valley bottom, respectively (Table 3.6).

Table 3.6 Boundary line analysis summary list of the factors limiting banana yields at soil toposequence at Kibuye, Western Rwanda, (2005).

<table>
<thead>
<tr>
<th>Most limiting yield factor</th>
<th>Number of banana fields</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Valley bottom</td>
</tr>
<tr>
<td>Intercrop intensity</td>
<td>4</td>
</tr>
<tr>
<td>Foliar N content (%)</td>
<td>5</td>
</tr>
<tr>
<td>Dead roots (%)</td>
<td>3</td>
</tr>
<tr>
<td>Mulch</td>
<td>4</td>
</tr>
<tr>
<td>Foliar Ca content (%)</td>
<td>-</td>
</tr>
<tr>
<td>Weeding</td>
<td>2</td>
</tr>
<tr>
<td>K foliar deficiency (Ind K)</td>
<td>-</td>
</tr>
<tr>
<td>Banana plant density</td>
<td>2</td>
</tr>
</tbody>
</table>

3.4.7 Factors associated with root damage on the toposequence

Among the plant parasitic species recovered from banana roots, *P. goodeyi* was the only species significantly contributing to root necrosis ($r^2 = 14.42\%$, $P < 0.01$, model not shown). Relationships between root necrosis, percentage of dead roots and *P. goodeyi* population densities were poor and not significant in crest and significant strong in middle slope (Table 3.7). In the latter location, *P. goodeyi* population density highly positively correlated with root necrosis ($r = 0.70$, $P = 0.001$) and root necrosis highly positively correlated with % dead roots ($r = 0.61$, $P < 0.01$). In the valley bottom, *P. goodeyi* population densities were
positively associated with root necrosis ($r = 0.44$, $P = 0.05$) but not with % dead roots (Table 3.7).

Table 3.7 Pearson correlations between percentage of dead root, root necrosis and \textit{P. goodeyi} population densities in toposequence at Kibuye, Western Rwanda, (2005).

<table>
<thead>
<tr>
<th></th>
<th>Valley bottom</th>
<th>Mid-slope</th>
<th>Crest</th>
</tr>
</thead>
<tbody>
<tr>
<td>% root necrosis - % dead roots</td>
<td>-0.29</td>
<td>0.61**</td>
<td>-0.03</td>
</tr>
<tr>
<td>% root necrosis – \textit{P. goodeyi} population density</td>
<td>0.44*</td>
<td>0.70***</td>
<td>0.13</td>
</tr>
<tr>
<td>% dead roots - \textit{P. goodeyi} population density</td>
<td>-0.29</td>
<td>0.44*</td>
<td>0.33</td>
</tr>
</tbody>
</table>

* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

### 3.5 Discussion

In our previous reports (Gaidashova \textit{et al.}, 2007, 2009), a low contribution by nematodes via root damage in determination of banana yields was observed for Rwandan cropping systems. However, these studies have focused on soil types of contrasted geology and fertility, while the topographic aspect was not taken in account.

Banana bunch yields in our study (12.8 to 16.2 kg /bunch) confirmed yield range (10.4 to 16 kg /bunch) from our earlier reports for Rwanda (Gaidashova \textit{et al.}, 2007) but were lower than those reported by Talwana \textit{et al.} (2003) (14 to 20 kg /bunch) and Bekunda & Woomer (1996). Major banana bunch yield determinants in the present study were similar to those from our earlier reports.
for Rwanda: percentage of dead roots and foliar N content (Gaidashova \textit{et al.}, 2007).

Although banana planting in concave slope parts, foot-slopes, and land depressions is common in the EAH (Jones & Edli, 1984; Rockström, 2000), reports on variation of banana yields on soil topo-sequence are lacking. Higher bunch weights in valley bottom observed in our study may possibly be related to deeper soils, better soil texture and lower proportion of dead roots, which all together overweights the highest N and K foliar deficiencies at this location.

Optimal plant growth and yield result from healthy roots capable to exploring soil efficiently (Fitter, 1996; Rosales & Ramiro, 2004; Sen, 2005). Root system performance is a function of (i) root number, length and growth (Draye \textit{et al.}, 2005), (ii) root health (Barea \textit{et al.}, 2005), (iii) soil volume that can be explored (Rosales & Ramiro, 2004), – proxied by soil depth, (iv) soil fertility status and (v) water availability (Delvaux, 1995). Deeper and moister soils of valley bottoms favoured optimal root development which resulted in higher bunch weights. Lower root densities (total root number and functional root number) at valley bottom may have been compensated by higher soil depth and volume available for root exploration. Possibly individual roots are longer and total root biomass is the same or even higher in valley bottom soils as compared to higher topo-sequence locations with thinner soil layers. Increased root death in middle slope was associated with much lower yields.

Sub-optimal plant nutrition was apparently widespread in our study area. This is confirmed by (i) lower foliar concentrations of N and K for all toposquence locations than those reported for the EAH region (Wairegi & Van Asten, \textit{in press}; Gaidashova \textit{et al.}, 2009; Wortmann \textit{et al.}, 1993); and (ii) boundary line analysis results of limiting factors for bunch weight from our
study. Particular decrease of Ca and K foliar concentrations in our study seems to be related to higher toposequence locations, where they become factors limiting banana yields in 15-25% of banana fields. In valley bottom, nutrient imbalances are related to excessive Mg and deficient K, while in the crest, Ca deficiency becomes major. Increasing negative values for N, P and K foliar deficiencies from crest to valley bottom suggest that plant nutrition for these nutrients was better in middle slope and crest. Nonetheless, there were no significant yield differences between toposequence locations, because better soil fertility at the upper locations may have been partially offset reduced soil depth and reduced water availability.

Impact of nematodes on root health markedly differed along the toposequence. On the one hand, there was a gradual, although non-significant increase in root necrosis and *P. goodeyi* population densities from crest to valley bottom; from the other hand, proportion of dead roots was much high in the steeper fields at middle slope. This suggests a possible contribution of run-off in passive accumulation of nematodes from upper fields to foot slopes (Chabrier *et al.*, 2009) and that the poorest conditions for root growth and health were in the middle slope due to increased run-off and reduced water availability. Moister soil conditions of valley bottom may have favoured not only plant and root growth, but also nematode population build up (Sarah *et al.*, 1991). Thus, the differences in soil humidity and water availability may be the driving factors between topogsequence-related differences in nematode impact on banana plants.

Soil fertility seems to be better at higher toposequence locations, although this contrasts with plant growth and foliar nutrient concentrations, both being
greater in valley bottom fields. Deeper and wetter soils in lower toposequence may explain these controversial contrasts.

Variation of soil conditions in our case study was related to soil depth, increasing down slope, soil fertility gradient and water availability. Soil water availability largely decreases with the increase of slope, while the highest runoff and nutrient loss happen at the steeper sloping hillsides (Briggs & Twomlow, 2002). Progressive accumulation of run-off soil in valley bottoms explains the formation of hydromorphic soil with their greater depth, sandier texture and higher humidity (Van Wambeke, 1992). Consequently, higher soil fertility would be expected in lower toposequence locations. It is possible that lower values of % soil organic matter and nutrients would be a consequence of past cropping, which continuously relied on valley bottoms (Clay & Lewis, 1990) and only recently was shifted to hill slopes (Clay, 1993; Byiringiro & Reardon, 1996).

Seldom authors reported about the effect of topo-sequence variations on plant parasitic nematode occurrence and abundance and related them to soil characteristics (Nyhan et al., 1972) and water regime (Prot & Mattias, 1995). Our report is the first work which investigated how nematode impact on plant varies as related to toposequence variations and how it does affect crop yields.

The nearly equal occurrence and abundance of *P. goodeyi* in this study confirmed its heavy dominance in banana roots and its association with root necrosis at all toposequence locations. However, its contribution to root health and crop yield was important only at the middle slope fields. We hypothesize that the association of nematode factor with reduced water availability of the middle slope soil due to thinner soil layer and higher slope may have all contributed to bunch yield reduction at this location. Earlier research in the EAH
region suggested that *P. goodeyi* impact in combination of abiotic stresses may be important for banana yield reduction (Talwana *et al.*, 2003a).

### 3.6 Conclusion

Our study highlighted the important effect of the topography-related variations on the relationship between plant parasitic nematodes (*P. goodeyi*) and banana plant growth, yield and root health. We conclude that *P. goodeyi* spread alone has a limited impact on banana yields when soil conditions are optimal for root growth and plant development. However, yield of crop grown in less favorable conditions, as in the presence of abiotic stress in steepy slope fields, may be substantially limited due to *P. goodeyi*-related damage. The trend of increase of plant parasitic nematode pressure and their damage to roots downstream on topo-sequence remains to be confirmed by further studies. Role of nutrient imbalances in addition to nematode damage may also be important.
Chapter 4. Impact of plant parasitic nematode
*Pratylenchus goodeyi* and mulch on East African
Highland banana performance in Kibuye,
Western Rwanda

This work was accepted for publication in *Nematology* as Gaidashova S.V., Van Asten P.J.A., Dochez C., Delvaux B. & De Waele D. Impact of plant parasitic nematode *Pratylenchus goodeyi* and mulch on East African Highland banana performance in Kibuye, Western Rwanda.
4.1 Abstract
The effect of nematode root injuries on banana crop yield is very poorly known in higher parts of the East African highlands. This study assessed the impact of *P. goodeyi* root lesion nematode on growth and yield of three banana cultivars (*Musa* spp. AAA-EA) in a field experiment involving nematicide and mulch applications at conditions of high altitude (about 1500 m). Plant growth, yield, root damage and nematode population densities were observed over three production cycles. Low to medium levels (≤ 50%) of root necroses were associated with improved plant growth while higher root necrosis (> 50%) had no effect. No significant reduction in bunch weight was associated with high root necrosis in any cycle and any of the three cultivars. Mulch significantly (P < 0.05) reduced root necrosis and *P. goodeyi* population densities. Bunch weight significantly increased in all mulched plots irrespective of root necrosis intensity. These results are in line with those of earlier surveys in Rwanda that suggested little negative impact of *P. goodeyi* on banana yields. However, they challenge general perceptions and previous findings on the negative impact of root lesion nematodes on banana crop performance.

4.2 Introduction
Bananas (*Musa* spp.) are considered as important food and cash crops in East Africa (Karamura *et al.*, 1999). In Rwanda, banana is grown on 23% of the cultivated land and provides nearly 50% of the country’s raw food production (INSR, 2008). In this country, banana is grown by small-scale farmers in low-input systems with low yields.

Decline in banana productivity has been reported by many authors for the East African region (Karamura *et al.*, 1999; Van Asten *et al.*, 2004) and Rwanda
Chapter 4. Impact of Pratylenchus goodeyi on banana yield

(Musabyimana et al., 1989; Okech et al., 2005). Pests and diseases were identified as the major constraints to banana productivity in Rwanda (Okech et al., 2002).

In recent years, plant parasitic nematodes were reported to cause substantial yield reduction in bananas grown in the East African highlands (Speijer & Fogain, 1999; Speijer & Kajumba, 2000). Radopholus similis and Pratylenchus goodeyi are considered as the most important nematode species. The first species is associated with high yield losses on banana worldwide, while the impact of P. goodeyi is less studied (Bridge, 2000). Pratylenchus goodeyi usually occurs at higher altitude and cooler environments (Speijer & Fogain, 1999).

Amplitude of the damaging effect to plant roots may depend on (i) species biology, i.e. species reproduction rate and feeding habits, length of the life cycle, host specificity and range, environmental optimum as temperature, moisture (Yeates et al., 1993; Pinochet et al., 1995; Gowen, 1995; Duyck et al., 2009); (ii) host-related factors, i.e. host identity, root growth rate, size of the root system (Brinkman et al., 2008; Stoffelen, 2000; Tixier et al., 2006); (iii) suitable habitat characteristics, i.e. food (root) availability, adequate soil moisture, soil texture and organic matter content, crop management practices (Hugon et al., 1984; Speijer et al., 1999; Barbercheck & Duncan, 2004; Gaidashova et al., 2009) and (iv) interactions with other plant parasitic nematode species and other soil and root inhabitants (Eisenback, 1993; Elsen et al., 2003; Brinkman et al., 2008).

Many authors reported high population densities of P. goodeyi on banana in existing fields (Kashaija et al., 1994; Fogain, 2001; Gaidashova et al., 2004). However, very limited information is available about the impact of this nematode species on plant growth and crop yield (Gowen, 1995). This study
Chapter 4. Impact of Pratylenchus goodeyi on banana yield

4.3 Material and methods

4.3.1 Site description and experimental lay out

The experiment was established on a farm at 29°22'30''E longitude and 2°04'38''S latitude, at Kibuye, Western Rwanda. The field was located in a plain near the Kivu lake border at 1470 m altitude with 1185 mm mean annual rainfall and 21.5°C mean air temperature (means of 30 year period). The plains of Kivu Lake have alluvial soils, exhibiting physical and chemical properties, which are highly suitable for banana production (Delvaux, 1995; Anonymous, 2001). In this area, high root damage and P. goodeyi population densities were reported on banana (Okech et al., 2002). Besides, no other important species were present (Gaidashova et al., 2004).

A complete randomized block design with four treatments in full factorial combination of (i) nematicide-applied (Nemacur®, 15 g a.i. plant⁻¹, twice year⁻¹) and nematicide-free, and (ii) mulched and mulch-free plots, was used. Three commonly grown highland banana cultivars (Musa sp. AAA-EA) were used: Intuntu (brewing), Injagi and Mbwaziruma (cooking). Six plants per cultivar were planted in three replications and four treatments. Total number of plants was 216, with 72 plants per block (replication). Plants were arranged in 2 x 3 m spacing. A single border line of banana plants was planted at the external side of the experimental field, whose surface was 280 m² including border line plants.
4.3.2 Selection of the experimental field with high natural infestation by *P. goodeyi*

The field for the experiment was selected based on the following criteria: (i) to be naturally infected with *P. goodeyi* at high population densities on grown *P. goodeyi* host crops; (ii) to present high root damage (more than 40% root necrosis) on the roots of the old crops; (iii) to have low populations of other plant parasitic nematode species in soil and roots. The experimental field selected for the experiment had been previously cropped with banana (*Musa* spp., AAA-EA cultivars), taro (*Colocasia esculenta*) and beans (*Phaseolus vulgaris*) during more than 20 years.

Roots from five randomly selected plants of the old crops grown at this field and soil surrounding roots were sampled from 20 x 20 x 20 cm upper soil volume in near (15-20 cm) proximity of plant pseudostem base (Carlier *et al.*, 2002) and assessed prior to the establishment of the experiment. Examination revealed high root necrosis of banana roots (41.5%) in contrast to the lack of apparent necrosis on bean and taro roots, and the presence of two plant parasitic nematode species, *P. goodeyi* and *Meloidogyne* sp. Nematode population densities in banana roots averaged 44084 and 390 verminforms 100 g⁻¹ of fresh roots for *P. goodeyi* and *Meloidogyne* sp., respectively, while nematode extractions of soil samples yielded very low number of both species (below 50 verminforms per 100 ml of soil).
4.3.3 Establishment and management of the experimental field

The old crop was uprooted, and necrotic banana roots of the freshly uprooted banana plants were spread in the field and in planting holes to serve as natural inoculum. Pared and solar dried during 3 days 6-month-old suckers originated from local neighboring field (cv. Intuntu) and ISAR Rubona research station (cvs. Injagi and Mbwaziruma) were used as planting material. Well decomposed cow manure originated from a nearby local farm was applied at planting at the rate of 15 kg per plant. The field was regularly weeded, de-suckered to maintain a maximum of three plants per mat, and mulched at planting, then once a year with banana residues (5-8 cm width of mulch layer), which originated from neighboring banana fields.

4.3.4 Data collection

Plant growth, root damage and nematode population densities were collected for each plant at flowering, more precisely, at flower emergence from pseudostem and at harvest during three crop cycles, from the beginning of July 2004 to the end of January 2007. Height, girth at 50 cm height, date of bunch emergence, harvest date and bunch weight were recorded. Harvest intervals were estimated by calculation of number of days between planting and harvest (crop cycle 1) and between two successive harvests (crop cycles 2 and 3). Root data included number of functional roots, percentages of root necrosis, and dead roots (Carlier et al., 2002) that were taken from mother plants at flowering (within 14 days from bunch emergence) and from sword suckers (from 10 to 100 cm height) at harvest. Numbers of functional and of dead roots were counted and necroses evaluated from 20 x 20 x 20 cm upper soil volume in close proximity
Chapter 4. Impact of Pratylenchus goodeyi on banana yield

from the selected banana plant within the mat as described in (Carlier et al., 2002). Nematodes were extracted from 5 randomly selected banana roots, which were assessed for root necroses, using a modified Baerman funnel method as described in Speijer et al. (1998), thereafter they were identified and counted from 30 ml aliquots. Root sampling and extractions, as well as plant measurements were done for each plant progressively when a required phenological stage was attained.

4.3.5 Data analysis

To achieve normal distribution of the data, the bunch weight, harvest interval, height, girth, % root necrosis, and % dead roots were square root transformed, while nematode counts were log_{10}(x+1)-transformed. The relationships between nematode populations, root necrosis, and % dead roots were assessed using Pearson correlation. Boundary line (Schnug et al., 1996) and linear regression analysis were used to investigate the functional relationships between root necrosis and number of functional roots. Analysis of variance under General Linear Model was applied to test the effect of treatments on root necrosis. The impact of root necrosis on plant growth and yield was examined using both regression analysis and ANOVA. All models were checked for goodness of fit and violations of assumptions based on residual diagnostics and adjusted for cycle, mulch and cultivar. LSD test was applied to separate means. SAS 9.1.3 and/or Enterprise Guide 4.1 were used at all steps.
4.4 Results

4.4.1 Nematode populations and root damage relationships

Population densities of *P. goodeyi* at flowering were strongly (P < 0.001) and positively correlated with percentages of root necrosis at flowering and harvest (r = 0.63 and r = 0.33, respectively) and dead roots at flowering and harvest (r = 0.15 and r = 0.17, respectively) (Table 4.1). Since the relationship was stronger for root necrosis at flowering, the latter was used as a variable to investigate the impact of *P. goodeyi* on plants.

Table 4.1 Correlations between root damage and nematode (*P. goodeyi*) population densities in a field trial, Kibuye, Western Rwanda (2003-2007).

<table>
<thead>
<tr>
<th></th>
<th>% RN at harvest</th>
<th>% RN at flowering</th>
<th>% DR at harvest</th>
<th>% DR at flowering</th>
<th>P. goodeyi at harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. goodeyi</em> at flowering</td>
<td>0.327***</td>
<td>0.626***</td>
<td>0.174***</td>
<td>0.150***</td>
<td>0.317***</td>
</tr>
<tr>
<td><em>P. goodeyi</em> at harvest</td>
<td>0.629***</td>
<td>0.223***</td>
<td>0.049</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>% DR at flowering</td>
<td>0.152***</td>
<td>0.295***</td>
<td>0.222***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% DR at harvest</td>
<td>0.159***</td>
<td>0.305***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% RN at flowering</td>
<td>0.272***</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

“***” means P < 0.001. RN means root necrosis, DR means dead roots

4.4.2 Effect of treatments on root necrosis

Nematicide application did not have any impact on root necrosis (P = 0.9785) (Table 4.2). The three cultivars included in the experiment were equally
susceptible to root necrosis. Mulch application significantly reduced root necrosis (27.4% vs 31.8%; P < 0.05). Root necrosis increased significantly (P < 0.001) from an average of 15.5% in crop cycle 1 to 40.4% in crop cycle 2 and only decreasing slightly to 34.2% in crop cycle 3 (data not shown).

Table 4.2 ANOVA, General linear model, showing the significance of the effect of treatments (nematicide, mulch, cultivar, cycle) on root necrosis in banana field trial, Kibuye, Western Rwanda, 2003-2007.

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>Partial SS</th>
<th>MS</th>
<th>F</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>19</td>
<td>1020.6</td>
<td>53.7</td>
<td>12.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nematicide</td>
<td>1</td>
<td>0.003</td>
<td>0.003</td>
<td>0</td>
<td>0.9785</td>
</tr>
<tr>
<td>Cycle</td>
<td>2</td>
<td>862.9</td>
<td>431.5</td>
<td>99.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cultivar</td>
<td>1</td>
<td>14.7</td>
<td>23.5</td>
<td>5.4</td>
<td>0.1844</td>
</tr>
<tr>
<td>Mulch</td>
<td>2</td>
<td>23.5</td>
<td>7.4</td>
<td>1.7</td>
<td>0.0204</td>
</tr>
<tr>
<td>Nematicide x cycle</td>
<td>2</td>
<td>16.6</td>
<td>8.3</td>
<td>1.9</td>
<td>0.1491</td>
</tr>
<tr>
<td>Nematicide x mulch</td>
<td>1</td>
<td>20.7</td>
<td>20.7</td>
<td>4.8</td>
<td>0.0293</td>
</tr>
<tr>
<td>Nematicide x cultivar</td>
<td>2</td>
<td>15.4</td>
<td>7.7</td>
<td>1.8</td>
<td>0.1711</td>
</tr>
<tr>
<td>Mulch x cycle</td>
<td>2</td>
<td>15.1</td>
<td>7.6</td>
<td>1.7</td>
<td>0.176</td>
</tr>
<tr>
<td>Cycle x cultivar</td>
<td>4</td>
<td>10.7</td>
<td>2.7</td>
<td>0.6</td>
<td>0.6522</td>
</tr>
<tr>
<td>Mulch x cultivar</td>
<td>2</td>
<td>25.9</td>
<td>13.0</td>
<td>3.0</td>
<td>0.0513</td>
</tr>
<tr>
<td>Residual</td>
<td>552</td>
<td>2395.8</td>
<td>4.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>571</td>
<td>3416.3</td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Df is degrees of freedom; partial SS is partial sum of squares, MS is mean square.

4.4.3 Ranking root necrosis based on its relationships with number of functional roots

Relationships between root necrosis percentage and number of functional roots at flowering (Fig. 4.1) were significantly positive (linear regression slope = 0.49; P < 0.001, Fig. 4.1) till root necrosis reached 50%. From the 50% level
Chapter 4. Impact of Pratylenchus goodeyi on banana yield

upwards, there was a significant negative relationship (linear regression slope = -1.37; P < 0.01, Fig. 4.1). Based on the above, the percentage of root necrosis was categorized into three groups: (1) 0-24.9%; (2) 25-49.9%, and (3) ≥ 50% for further analysis of the impact of root necrosis on plant performance through ANOVA and LSD.

4.4.4 Impact of root necrosis on plant growth and yield

Root necrosis rank significantly affected number of functional roots (P < 0.01), height (P < 0.001) and girth (P < 0.05) and did not affect bunch weight and harvest interval at P = 0.05 (Table 4.3). Root necrosis rank interacted with
crop cycle on the number of functional roots and height (P < 0.001 for both); and with cultivar on height, girth and harvest interval (P < 0.05 for all).

Root necrosis below 50% was significantly positively associated with the number of functional roots (NFR), plant height (H), girth (G) \((r^2 = 18.4\% ; P < 0.01; r^2 = 79.3\% , P < 0.001; r^2 = 64.7\% , P < 0.001\) for NFR, H and G, respectively) regardless of mulch and crop cycle. There was no significant relationship between root necrosis higher than 50% on NFR, H and G. Root necrosis was negatively but not significantly associated with number of functional leaves (NFL) irrespective of whether it was below or above 50%.

Table 4.3 Summary of ANOVA General linear model, showing the significance of the impact of root necrosis rank and other factors on banana performance in a field trial, Kibuye, Western Rwanda, 2003-2007.

<table>
<thead>
<tr>
<th>Model factors</th>
<th>NFR</th>
<th>Height</th>
<th>Girth</th>
<th>NFL</th>
<th>BW</th>
<th>HI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root necrosis rank (RNR) *</td>
<td>**</td>
<td>***</td>
<td>*</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mulch</td>
<td>-</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>***</td>
<td>-</td>
</tr>
<tr>
<td>Cultivar</td>
<td>-</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>***</td>
<td>-</td>
</tr>
<tr>
<td>Cycle</td>
<td>-</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>RNR x mulch</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RNR x cultivar</td>
<td>-</td>
<td>*</td>
<td>*</td>
<td>-</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>RNR x cycle</td>
<td>***</td>
<td>***</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mulch x cultivar</td>
<td>-</td>
<td>-</td>
<td>**</td>
<td>-</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>Mulch x cycle</td>
<td>-</td>
<td>-</td>
<td>***</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cultivar x cycle</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>Model significance</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

RNR is root necrosis rank; NFR is number of functional roots; NFL is number of functional leaves; BW is bunch weight; HI is harvest interval; ns is not significant at P < 0.05.
Using regression models, the effect of root necrosis below and above 50% adjusted for crop cycle, mulch, and cultivar on bunch weight was not significant (P > 0.05) (data not shown). Comparisons of means with LSD test did not show significant effect of root necrosis on mean bunch weight for cultivars Injagi and Mbwaziruma, while cultivar Intuntu in mulched plots showed significant increase in bunch weight with increase of root necrosis (Table 4.4).

Higher root necrosis rank increased harvest interval in crop cycle 1, but this effect was no longer significant in subsequent cycles (data not shown). This trend was common for all cultivars, being significant (P < 0.05) in mulched plots of Intuntu and Mbwaziruma, and in non-mulched plots of Injagi (Table 4.4).

Rate of plant toppling in the experimental field remained below 1% plants, irrespective of cultivar, crop cycle and mulch.

4.4.5 Effect of mulch on banana yield, root necrosis and nematode populations

Mulch significantly (P < 0.01) affected bunch weight but not harvest interval, the bunch weights of Intuntu and Injagi significantly (P < 0.01) increased in mulched plots irrespective of root necrosis intensity (Table 4.4).

Plants in mulched plots had significantly (P < 0.05) lower root necroses during the 1st and 2nd cycles (Table 4.5). Population densities of *P. goodeyi* were significantly higher (P < 0.05) in non-mulched plots throughout the 2nd and 3rd crop cycles (Table 4.5), with the exception of flowering plants in the 3rd crop cycle. In general, a lower percentage of mulched plants had > 50% root necrosis compared to non-mulched plants (18% versus 23%), although an overall 79% of the plants had necrosis below 50%.
Table 4.4 Bunch weight, period from planting to harvest and yield at different root necrosis ranks (means from three production cycles) in mulched and non-mulched banana plots in a field trial, Kibuye, Western Rwanda, 2003-2007.

<table>
<thead>
<tr>
<th>Root necrosis rank</th>
<th>Intuntu</th>
<th>Injagi</th>
<th>Mbwaziruma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Mulch +</td>
<td>Mulch -</td>
<td>P</td>
</tr>
<tr>
<td>Mean bunch weight, kg</td>
<td>22.3b</td>
<td>18.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Planting to harvest, days</td>
<td>24.5b</td>
<td>19.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Estimated yield, kg per plant yr⁻¹</td>
<td>27.0a</td>
<td>20.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.001</td>
<td>ns</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intuntu</th>
<th>Injagi</th>
<th>Mbwaziruma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>Mulch +</td>
<td>Mulch -</td>
</tr>
<tr>
<td>Mean bunch weight, kg</td>
<td>24.5</td>
<td>19.8</td>
</tr>
<tr>
<td>Planting to harvest, days</td>
<td>29.1</td>
<td>20.5</td>
</tr>
<tr>
<td>Estimated yield, kg per plant yr⁻¹</td>
<td>24.7</td>
<td>19.3</td>
</tr>
<tr>
<td>P</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mbwaziruma</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
</tr>
<tr>
<td>Mean bunch weight, kg</td>
</tr>
<tr>
<td>Planting to harvest, days</td>
</tr>
<tr>
<td>Estimated yield, kg per plant yr⁻¹</td>
</tr>
<tr>
<td>P</td>
</tr>
</tbody>
</table>

a, b – vertical comparisons significant at 0.05 level; ns – not significant at P < 0.05; ne – non-estimated.
Chapter 4. Impact of Pratylenchus goodeyi on banana yield

Table 4.5 Evolution of root necrosis and P. goodeyi population densities along three production cycles in mulched and non-mulched banana plots, Kibuye, Western Rwanda, 2003-2007.

<table>
<thead>
<tr>
<th>Plant stage</th>
<th>Flowering (mother)</th>
<th>Harvest (daughter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle</td>
<td>Mulch +</td>
<td>Mulch -</td>
</tr>
<tr>
<td>1</td>
<td>13.5b</td>
<td>18.3c</td>
</tr>
<tr>
<td>Root necrosis (%)</td>
<td>2</td>
<td>38.3a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>36.4a</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1</td>
<td>40957b</td>
<td>39231c</td>
</tr>
<tr>
<td>P. goodeyi number/100g fresh roots</td>
<td>2</td>
<td>78751a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>51398a</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

a, b, c = vertical comparisons significant at 0.05 level; ns = not significant at 0.05 level.

4.5 Discussion

Strong relationships between nematode population densities and root necrosis observed in this experiment confirm previous reports (Speijer et al., 1999; Moens et al., 2001; Speijer & De Waele, 2001; Coyne et al., 2005). However, what contrasts with these reports is the quasi-total lack of suppressive
effect of root damage on plant growth and yield. Pinouchet (1998) reported an estimated 16% yield loss for agro-climatic conditions of Canary Islands. Although this figure is much lower than the yield losses reported for *R. similis* (Speijer & Kajumba, 2000; Coyne *et al.*, 2005), our study challenges both findings by reporting zero yield losses from *P. goodeyi*. Elsen *et al.* (2000) concluded that EAH banana were susceptible to this nematode, as their bunch weight significantly negatively correlated with *P. goodeyi* population densities. Talwana *et al.* (2003) reported higher nematode damage on roots and plant growth reduction in case of combined effect of *P. goodeyi* and *R. similis* compared to *P. goodeyi* alone. This study, however, lacked a nematode free control to quantify the magnitude of both effects. Besides, the higher plant toppling rates observed in this study in case of *P. goodeyi* infestation as compared to ours may possibly be linked to higher pathogenicity of the Ugandan race of *P. goodeyi*, and/or poorer soil conditions at this site. Our study reported about the impact of *P. goodeyi* on growth and yield of EAH banana under field conditions where the presence of other nematode species was low and in soils with suitable physical properties (Delvaux, 1995; Anonymous, 2001). The latter might have facilitated intensive root growth to compensate root losses due to nematodes, thus obscuring the response of crop yield to nematode damage.

Field observations confirmed association of high *P. goodeyi* population densities and damage with better performing plants (Gaidashova *et al.*, 2009). Our results showed the positive impact of *P. goodeyi* on banana plants, expressed in better growth. One of the possible causes of this ‘improved’ growth may be the contribution of plant parasitic nematodes to nutrient mineralization from organic matter of the root system (Tu *et al.*, 2003). When pest pressure on root systems is moderate, host plants may ‘benefit’ from its root nematode
populations as they may contribute as much as 2 to 5% directly and much more indirectly through the soil microbial food chains of the overall nitrogen mineralization in soil (Tu et al., 2003; Verschoor, 2002). This may generate growth response from host plant, as observed in our study increase in height and girth of banana plants. This proposition is supported by the reports of increased root biomass at low pressure levels from plant parasitic nematodes (Bardgett et al., 2001). Lack of negative response from plant to P. goodeyi infection may be related to possible polyphagous behaviour of this species which is able to survive in dead roots (De Waele et al., 1997). Records about the effect of mulch on nematodes are numerous, although some of them are controversial in terms of reducing (Obiefuna, 1991), increasing (Talwana et al., 2003) or having no impact (Speijer et al., 1999) on the number of root nematodes. In this experiment, mulch was effective in reducing root necrosis and to a lesser extent root populations of P. goodeyi. Some authors reported the crucial role of mulch for plant survival and yield increase in case of nematode infestation (Coyne et al., 2005). However, in our study, impact of nematodes on plants remained low, but mulch application still increased bunch weights. This suggests that mulch may have helped to reduce water and nutrient stresses. Mulch may mitigate the effect of nematodes where soil conditions are less optimal for plant growth (Talwana et al., 2003). It may therefore be applied to improve crop yields and root health.

Recommendations on nematicide control of P. goodeyi are lacking. Practically, no research was conducted on this issue as this species is absent at lowland commercial banana plantations worldwide (Bridge, 2000) where most of research on nematicides was generated. In contrast, P. goodeyi attacks banana in highland cropping systems (Gowen, 1995) which are mostly subsistence
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oriented (Karamura et al., 1999). Nemacur®, successfully used for R. similis control in commercial banana production (Jackson et al., 2003), was not efficient for P. goodeyi in this study. This suggests that P. goodeyi may be resistant to this nematicide, or that the doses and application modes used were not appropriate.

4.6 Conclusion

Low to medium levels of root necrosis were associated with improved plant growth irrespective of cultivar. Pratylenchus goodeyi was a strong contributor to root necrosis. However, nematode population densities in roots did not reduce banana yields in this experiment even at high levels of root necrosis. These results suggest that P. goodeyi root lesions are not an important yield constraint at this study site. They challenge most other nematode studies in bananas, especially those focused on R. similis impact. It would be interesting to understand how the feeding behaviour of these two PP nematode species differs and how that could explain the observed differences in plant response.

Mulch was efficient to improve root health and increase crop yields. We have previously reported that soil fertility appears to be amongst the most important constraints to banana productivity. Mineral fertilizer use in the EAH is often limited by low input-purchase capacity of the small scale farmers. Potential low-external input technologies (e.g. use of arbuscular mycorrhizal fungi) that may efficiently contribute to improved banana plant nutrition and yield should be further explored to achieve yield improvements for better food and income security in these highland cropping systems.
Chapter 5. Arbuscular mycorrhizal fungi in East African Highland banana cropping systems as related to edapho-climatic conditions and crop management practices

This chapter was accepted for publication in Fungal Ecology as Gaidashova S.V., Van Asten P.J.A., Jefwa J., Delvaux B. and De clerck S. Arbuscular mycorrhizal fungi in East African Highland banana cropping systems as related to edapho-climatic conditions and crop management practices.
5.1 Abstract

Root colonization, soil population density and diversity of arbuscular mycorrhizal (AM) fungi were assessed in 188 banana fields in contrasted soil types across five eco-regions in Rwanda (Butare-Gitarama, Gashonga, Bugarama, Kibungo, Ruhengeri). Root colonization was observed in all banana plants, whatever the soil type, field site and farm. The population density was higher on the wetter (1300-1500 mm yr\(^{-1}\)) volcanic soils (Gashonga 59.8 and Ruhengeri 48.5 propagules 100g\(^{-1}\) soil, respectively) as compared to the dryer (900-1200 mm yr\(^{-1}\)) soils derived from schist and granite (Butare-Gitarama 2.0, Kibungo 8.5, Bugarama 14.7 propagules 100g\(^{-1}\) soil). The diversity was highest in Kibungo and lowest in Butare-Gitarama (10 and 2 spore morphotypes, respectively). These results suggested that AM fungi were widespread in banana cropping systems in Rwanda, but that root colonization, population density and diversity highly varied according to edapho-climatic conditions (i.e. rainfall, soil texture and P content) and soil management practices (tillage).

5.2 Introduction

Arbuscular mycorrhizal (AM) fungi are ubiquitous soil micro-organisms present in most of terrestrial eco-systems (Smith & Read, 2008) where they form symbiosis with the roots of nearly 80% of plant species (Brundrett, 2002). Their major functions are nutrient transfer from soil to plant, soil structure improvement through aggregation, and plant protection from drought stress and root pathogens. The combination of these attributes participates to the long term stability of the eco-systems (Dodds, 2000).

The AM fungi have been studied in a wide range of natural and agro-ecosystems. Their presence, diversity and abundance are a function of climatic
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conditions (Öpik et al., 2006), plant community composition (Johnson et al., 2003) and diversity (Burrows & Pfleger, 2002), and soil disturbance (Jansa et al., 2006). The diversity of ‘indigenous’ AM fungal species existing in different environments remains largely under-explored (Öpik et al., 2006). This is particularly true in tropical agro-ecosystems of Africa. Despite the great diversity of African plant species, few have been described in relation to their mycorrhizal association (Quilambo, 2003).

East African highland (EAH) banana cropping systems provide food and income to over 30 million people living in high land pressure areas (Karamura et al., 1999) that can no longer increase production by expanding area under cultivation. Because of low use of external inputs (e.g. fertilizers) and increased nutrient removal through banana exports to urban markets, the productivity of these systems is declining (van Asten et al., 2004). Consequently, novel approaches should be developed to improve crop yields.

The most diverse and highly productive terrestrial eco-systems in the world support high abundance and diversity of AM fungi (Öpik et al., 2006; Smith & Read, 2008). As compared to systems with annual crops, banana cropping systems show a lower soil disturbance both in terms of soil work and erosion levels (Rishirumuhirwa, 1997). From these two statements, we hypothesize that the abundance and diversity of the AM fungi in EAH banana cropping systems may be high.

The EAH banana cropping systems deserve a special interest for studying AM fungi for several reasons: i) highly productive fields do exist up to date where banana is grown without replanting for more than 100 years (Gold et al., 1999); ii) banana cultivation was practiced in this region for more than a thousand years (Schoenbrun, 1993); iii) these systems strongly differ from each
other in soil and climate conditions (Eswaran et al., 1989). Besides, the East African region represents an important center of banana diversity where several hundreds of cultivars co-exist (Karamura, 1998).

The objectives of the present study was to assess (i) the AM fungi root colonization of bananas and the population density in soils of contrasted origin and fertility in a selection of highland banana cropping systems; (ii) the relationship between AM colonization in roots and banana plant performance, soil chemical properties, parasitic nematode damage, and field management practices; (iii) the morphotype composition of AM fungi in highland banana cropping systems in Rwanda.

5.3 Material and methods

5.3.1 Study sites characteristics

Five ecological regions (eco-regions) were selected for this study on the basis of contrasting climatic, geologic, and soil fertility conditions: (1) Butare – Gitarama (South Rwanda), (2) Gashonga (Southwest Rwanda), (3) Bugarama (Southwest Rwanda), (4) Kibungo (East Rwanda) and (5) Nyakinama plain of Ruhengeri (North Rwanda) (Fig. 5.1). Butare-Gitarama soils belong to Acrisol on granite, of sandy clay texture, with 1170 mm annual rainfall (ar), +19.2°C mean annual temperature (mat), 1675-1763 meters of altitude at sea level (masl). This eco-region is an old banana production area with low banana yields. Gashonga soil is a fine clayey Ferralsol derived from basalt, of highly clayish texture and receiving 1480 mm ar with +19.4°C mat at 1607-1732 m asl. This eco-region is a relatively old banana production area with medium yields. Bugarama plain is located on Fluvisol and Vertisol, of loam to sandy loam texture. It receives 1079 mm ar with +24.0°C mat at 965-992 masl. This eco-
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region is an old banana growing area where banana was mostly replaced by rice in the plain area. Kibungo has fertile Nitisol on shale, of clay texture, receiving 900 mm ar, +20.6°C mat, 1498-1623 m asl. It is an ancient banana growing area with medium to high yields. Nyakinama plain in Ruhengeri is a region with fertile volcanic Andosol, of loam to sandy loam texture, 1307 mm ar, +19.6°C mat and a range of altitudes between 1659 to 1692 m asl, representing an old banana growing area with high yields.

Figure 5.1 Map of Rwanda showing the five eco-regions studied: 1 – Butare - Gitarama; 2 - Gashonga; 3 – Bugarama; 4 - Kibungo; 5 – Ruhengeri.
5.3.2 Farm, plot and cultivar selection

Sixteen to twenty farms were sampled per eco-region with two plot types per farm. The term ‘farm’ was used for banana fields belonging to a single farmer. Farm selection was made to meet the following criteria: 1) belong to the required soil type according to the soil map (Anonymous, 2001); 2) have plot types differing in plant vigour; and 3) have at least five flowering plants of the selected variety per plot. Plot types differing in plant vigour were identified by visual appraisal. At each farm, one ‘good’ plot with well performing plants (i.e. having taller and thicker stems, higher number of leaves), and one ‘poor’ plot with plants having poorer performance as compared to the above, were selected. In total, 188 fields were sampled. Plant vigour was measured by recording plant growth parameters as described below (2.5.1) on five flowering plants. To minimize the effect of plant genotype on AM fungi, the survey was conducted on a single cultivar “Intuntu” which belongs to the EAH banana sub-group (AAA-EA) most widely cultivated in Rwanda (Nsabimana et al., 2008).

5.3.3 Root, leaf and soil sampling

For evaluation of root colonization by the AM fungi, fine lateral roots were collected in the upper 25 cm rhizosphere of each selected banana plant at a distance of 50 – 70 cm from the corm. One composite sample of roots was made per plot type. Numbers of dead and alive roots were counted from 20x20x20 cm soil volume, excavated at proximity of each selected banana plant at flowering stage (Speijer & De Waele, 1997). Leaf samples were taken from the internal lamina of the third leaf (Martin-Prével, 1980) at each selected plant at flower emergence stage.
Soil samples were taken at 0-25 cm depth at a horizontal distance of 50-70 cm from the plant from three single sub-samples per plant. Five composite samples per plot were again thoroughly mixed to prepare one composite soil sample per plot.

5.3.4 Plant growth

Banana plant growth, including plant height and girth at 1 m from the soil surface, number of functional (i.e. having more than 50 % green leaf area) leaves, length and width of the 3rd leaf, number of hands and number of fruits per bunch, was recorded for each selected plant. An indicator of bunch weight based on plant growth parameters was used to quantify the crop yield as reported recently by Gaidashova et al. (2007):

\[ EBW = 0.173 \times \text{Girth} + 0.995 \times \text{NFL} + 0.123 \times \text{FN} - 16.1 \ (r^2 = 0.56) \]

where EBW is the estimated bunch weight, NFL is the number of functional leaves at flowering and FN is the finger number per bunch.

5.3.5 Field management practices

At each plot type, management practices were observed by visual appraisal of the field in 2-3 m radius from each selected banana plant. Management practices were: banana plant density, mulch thickness, surface coverage of weeds and intercrops. Plant density was deduced from measuring the distances between each selected mat and four surrounding mats. The surface covered by weeds and intercrops in the radius of 2 m from the selected banana plants was assessed by visual observation using the following ranks: 1) less than 2 %; 2) 3-5 %; 3) 6-10 %; 4) 11-25 %; 5) 26-50 % and 6) >50 % (modified from Okech et al., 2002). Mulch thickness was recorded from five random measurements within 50 to 100
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cm circle around each selected banana plant. Tillage intensity was estimated through visual appraisal of the soil work at the moment when the survey was done using the following ranks: 1 = none; 2 = occasional; 3 = moderate; 4 = intensive and 5 = very intensive.

5.3.6 Assessment of banana AMF root colonization

Roots were oven-dried at 60°C for 48 hours. Dried root samples were placed in 10% KOH solution for 1 hour at 80°C. Roots were subsequently bleached during 30 min in 3% H₂O₂ solution freshly alkalinized by NH₄OH (8 ml of 25% NH₄OH for 100 ml 3% H₂O₂) as described by Koske & Gemma (1989). After bleaching, roots were stained in acidified ink solution (20 ml of permanent ink “Parker” in 1l of 1% HCl) for overnight (Vierheilig et al., 1998). Stained root samples were de-stained in water for 1 hour and observed under compound microscope (Olympus BH2 RFCA) at 200 X magnification. Frequency and intensity of root colonization was evaluated from fifty root fragments (1-cm length) for each sample. Frequency was estimated as the percentage of root fragments containing AM fungi structures. Intensity, which is the abundance of hyphae, vesicles and arbuscules in roots, was assessed as described in Plenchette & Morel (1996).

5.3.7 Assessment of AM fungi population density in soil

One composite soil sample per eco-region of approximately 3 kg was prepared by taking 80-100 g of soil from all composite samples taken at plot level. The samples were sieved (<2 mm) and 2.5 kg per sample was sterilized by γ-radiation (25 kGy) while the remaining 0.5 kg was left untreated. AM fungi
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population density was determined by the most probable number method (MPN) as described in Declerck et al. (1999). A two-fold dilution series was made of each soil by mixing the non-sterilized soil with γ-sterilized samples of the same soil. Twelve successive dilutions were prepared with three replicates per dilution. Plastic multi-pots were filled with 70 g of each soil dilution. Each multi-pot contained the dilution series for one soil. Two-week old leek plantlets grown on sterilized calcined clay (Terragreen®) were transplanted in each pot. Pots were subsequently placed in a greenhouse with temperature set at 24/18°C day/night and 16 h of daylight. Plants were watered every 2 days with de-ionized sterilized water. No nutrient solution or fertilizer was added during the experiment. After 9 weeks, the entire root system of each plant was collected and stained as above. Each root system was mounted on microscope slides and monitored for the presence or absence of AM fungal colonization. A single entry point was enough to confirm the presence of AM fungi in root.

5.3.8 AM fungi spore extraction from soil

In four of the five eco-regions, namely, Butare-Gitarama, Gashonga, Kibungo and Ruhengeri, additional soil samples were collected from the upper 25 cm using the same procedure as described above for soil samples. One single farm was considered per eco-region. One composite soil sample per farm was made from 10 points near randomly selected banana plants. Spores were extracted from 50 g of soil. Spore extraction was done using the sucrose (concentration of 47% w/v) centrifugation method (Walker et al. 1982) after washing and sieving through 710 μ, 250 μ and 37 μ sieves. The fraction of 710 μ was checked under dissecting microscope (Leica) at 32 X magnification for collection of
sporocarps. The other fractions were placed in centrifuge tubes containing 50 ml of sucrose solution. The tubes were centrifuged at 1800 rpm for 5 minutes. Spores were isolated using automatic pipette under dissecting microscope, washed from sucrose on 5 µm sieve and dried from excess water. Spores were subsequently mounted on slides in polyvinyl-alcohol and/or Melzer’s iodine reagent and observed under compound microscope Leica DME under X 400 magnification. Identification up to genera level was done at the National Museums of Kenya, Nairobi.

5.3.9 Root damage and nematode population densities

Percentages of root necroses were assessed using five randomly selected roots per plant as described in Speijer et al. (1998). The numbers of functional (i.e. alive) and dead roots were counted from 20x20x20 cm soil volume and percentage of dead roots was determined.

Nematodes were extracted from fresh banana roots using blender, sieves and tissue paper as described by Carlier et al. (2002), identified and counted under compound microscope Leica DME under X 200 magnification from 30 ml aliquots in Crop Protection Laboratory, ISAR Rubona Research Station. Nematode population density was calculated using one composite root sample from 5 plants per plot type (two nematode extractions per farm). The nematode population densities and root damage used in this study were those reported earlier by Gaidashova et al. (2009).
5.3.10 Soil and leaf analysis

Relative proportion of coarse fragments (>2 mm) was determined by weighting of soil stony fraction after sieving through 2 mm mesh. The soil exchangeable contents of Ca$^{2+}$, K$^+$, Mg$^{2+}$, Na$^+$ and cation exchange capacity (CEC) were determined by atomic absorption spectrometry (VARIAN 300), after extraction with the normalized ammonium acetate extract at pH7. In addition, KCl-extractable Al, N, available P (Olsen method), pH H$_2$O and pH KCl were determined (Page et al., 1982). Soil C and N contents were determined using Walkley-Black and Kjeldhal methods, respectively. Phosphorus was determined by colorimetry (SHIMADZU UV-1205). Leaf nutrient concentrations were determined by atomic absorption spectrometry (K, Ca, Mg, Cu, Zn and Mn) and colorimetry (N, P) after acid wet digestion.

5.3.11 Statistical analysis

The following transformations were used to improve linearity: square root for percentages of AM fungi frequency and intensity of colonization, percentage of dead roots and root necroses; $\log_{10}(x+1)$ for nematode population densities and Al$^{3+}$; $\log_{10}$ for soil K, Mg, Na, CEC, C, N, P and pH. Analysis of variance under General Linear Model was used to test the effect of eco-region and LSD test was used to separate homogenous groups. Correlation analysis was performed to study the relationships between AM fungi colonization, plant growth, soil chemical characteristics, management practices, root damage and nematode population densities. Bonferoni adjustment was used to correct levels of significance. Pearson correlation was used for parametric values and Spearman correlation was used for ranks (rainfall and management practices). SAS 9.1
Enterprise Guide 4 statistical software was used at all above-mentioned analytical steps. The population densities of AM fungi were calculated using table VIII2 of Ficher & Yates (1948). The 95% confidence limits were calculated as described in Cochran (1950) to separate homogenous groups at 0.05 level.

5.4 Results

5.4.1 Soil properties

Soil physico-chemical parameters differed between the eco-regions (Table 5.1). The Acrisol on granite parent rock (Butare-Gitarama) contained the lowest levels of exchangeable cations (Ca, Mg, K), CEC, C, N and highest coarse fraction compared to soils of other eco-regions. The first had low pH and moderate levels of KCl-extractive Al. The old Ferralsol on basalt parent rock of Gashonga contained the lowest coarse fraction, very low P but high C. This soil had low exchangeable cations and contained substantial quantity of KCl-extractable Al. The relatively young alluvial soil of Bugarama had a high pH, but moderate contents of exchangeable cations and CEC, low C and N contents. The Nitisol (Kibungo) on schist parent material was rich in exchangeable cations, CEC and C, with high pH, but moderately low P. The Andosol (Ruhengeri) on recent volcanic deposits had high levels of exchangeable cations and CEC, high pH, P and C contents. Plots with poor plant vigour had lower quantities of all soil elements in most soils (Table 5.1).
5.4.2 Management practices

The percentage of intercropping, percentage of weed surface, mulching and plant densities significantly differed (P < 0.05) between the eco-regions (Table 5.2). Main intercrop cultures were beans and cocoyam. Intercrops were more intensive (P < 0.05) in Butare-Gitarama, Gashonga and Bugarama. Intercropping was more frequently practiced in poor vigour plots (Table 5.2). Surface proportion covered by weeds was the lowest in Kibungo (2 %) and the highest in Ruhengeri (28 %) (P < 0.05) (Table 5.2). Mulch thickness was the highest in Kibungo (3.2 cm) compared to all other eco-regions (<1.5cm). Plots with poor plant vigour generally had less mulch. Highest plant densities were recorded in Ruhengeri (3496 plants ha\(^{-1}\)) and the lowest in Bugarama (1096 plants ha\(^{-1}\)) and Kibungo (1600 plants ha\(^{-1}\)). Tillage intensity ranks were the highest in Butare-Gitarama and the lowest in Kibungo and Ruhengeri. Good and poor vigour plots had similar tillage intensity in all eco-regions except Gashonga where higher (P < 0.05) tillage intensity was observed in poor vigour plots (Table 5.2).
<table>
<thead>
<tr>
<th>Eco-region</th>
<th>Butare - Gitarama</th>
<th>Gashonga</th>
<th>Bugarama</th>
<th>Kibungo</th>
<th>Ruhengeri</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plant vigour</strong></td>
<td><strong>Good</strong></td>
<td><strong>Poor</strong></td>
<td><strong>Mean</strong></td>
<td><strong>Good</strong></td>
<td><strong>Poor</strong></td>
</tr>
<tr>
<td>pH-KCl</td>
<td>5.51a</td>
<td>5.02b</td>
<td>5.26d</td>
<td>5.34a</td>
<td>4.92b</td>
</tr>
<tr>
<td>C (%)</td>
<td>1.54a</td>
<td>1.34a</td>
<td>1.44c</td>
<td>3.60a</td>
<td>3.72a</td>
</tr>
<tr>
<td>N (%)</td>
<td>0.17a</td>
<td>0.14a</td>
<td>0.16c</td>
<td>0.29a</td>
<td>0.30a</td>
</tr>
<tr>
<td>P (mg kg⁻¹)</td>
<td>80.1a</td>
<td>50.9a</td>
<td>65.5AB</td>
<td>10.7a</td>
<td>6.9a</td>
</tr>
<tr>
<td>Ca⁺²</td>
<td>6.15a</td>
<td>5.50a</td>
<td>5.82D</td>
<td>11.18a</td>
<td>9.48a</td>
</tr>
<tr>
<td>Mg⁺²</td>
<td>2.34a</td>
<td>1.93a</td>
<td>2.13E</td>
<td>3.04a</td>
<td>2.66a</td>
</tr>
<tr>
<td>K⁺</td>
<td>2.14a</td>
<td>1.33b</td>
<td>1.74B</td>
<td>2.21b</td>
<td>1.12a</td>
</tr>
<tr>
<td>CEC</td>
<td>12.4a</td>
<td>11.7a</td>
<td>12.1D</td>
<td>24.0a</td>
<td>22.5a</td>
</tr>
<tr>
<td>Al³⁺</td>
<td>0.03a</td>
<td>0.07a</td>
<td>0.05b</td>
<td>0.04a</td>
<td>0.20a</td>
</tr>
<tr>
<td>Gravel, %</td>
<td>14.4a</td>
<td>18.9a</td>
<td>16.6A</td>
<td>5.4a</td>
<td>2.6b</td>
</tr>
</tbody>
</table>

Units of exchangeable cations (Ca, Mg, K), CEC and KCl-extractable Al³⁺ are in cmol, kg⁻¹. a, b = horizontal comparisons between plots with good and poor plant vigour. A, B, C, D, E = horizontal comparisons of means between the eco-regions. Values followed by the same letter are not different at p = 0.05.
### Table 5.2 Management practices in banana fields in five eco-regions of Rwanda, 2006-2007.

<table>
<thead>
<tr>
<th>Plant vigour Practice</th>
<th>Butare - Gitarama</th>
<th>Gashonga</th>
<th>Bugarama</th>
<th>Kibungo</th>
<th>Ruhengeri</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
<td>Poor</td>
<td>Mean</td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Distance between plants, cm</td>
<td>181a*</td>
<td>209a</td>
<td>195D**</td>
<td>211b</td>
<td>233a</td>
</tr>
<tr>
<td>Mulch thickness, cm</td>
<td>1.6a</td>
<td>1.1a</td>
<td>1.3BC</td>
<td>1.7a</td>
<td>0.7b</td>
</tr>
<tr>
<td>Intercropping, %</td>
<td>8.8a</td>
<td>12.1a</td>
<td>10.4A</td>
<td>11.0b</td>
<td>29.3a</td>
</tr>
<tr>
<td>Weeds, %</td>
<td>18.1a</td>
<td>17.2a</td>
<td>17.6B</td>
<td>12.7a</td>
<td>14.6a</td>
</tr>
<tr>
<td>Tillage (rank)</td>
<td>4.1a</td>
<td>4.2a</td>
<td>4.2A</td>
<td>3.0b</td>
<td>4.0a</td>
</tr>
</tbody>
</table>

a, b = horizontal comparisons between plots with good and poor plant vigour. A, B, C, D, E = horizontal comparisons of means between the eco-regions.

Values followed by the same letter are not different at p = 0.05. Tillage rank: 1= none, 2= low; 3= moderate; 4 = high; 5 = very high.
5.4.3 Plant growth and vigor

Banana plants were significantly (P < 0.001) more vigorous (i.e. girth and finger number) on the young soils in Ruhengeri and Bugarama (Table 5.3). Plants had the poorest growth (i.e. lowest girth, height, leaf length and number of fruits) at Butare – Gitarama.

At farm level, plants in plots of poor plant vigour had lower (P < 0.05) girth and fruit number per bunch as compared to the plants in the plots with good vigour in all eco-regions (Table 5.3).

5.4.4 In situ AM fungi banana root colonization

AM fungal root colonization varied significantly (P < 0.001) between the eco-regions (Fig. 5.2). The frequency and intensity of root colonization were the lowest in Butare-Gitarama (17.2 % and 16.5 %, respectively) and the highest in Gashonga (62.6 % and 35.4 % respectively). Butare-Gitarama eco-region had the most heterogenous farms. In some fields frequency and intensity of root colonization were low, while in other fields high colonization levels were recorded, with the highest coefficient of variation (CV): 129 % for frequency and 63.8 % for intensity. The roots, sampled in the bananas from this eco-region, rarely contained vesicles and had few arbuscules. Roots from Gashonga contained numerous vesicles and arbuscules. This eco-region had more uniform levels of root colonization (i.e. CV =27.6 % and CV = 36.8 % for frequency and intensity, respectively). The three other eco-regions (i.e. Kibungo, Ruhengeri and Bugarama) had intermediate values of frequency and intensity of AM fungi root colonization that were significantly (P < 0.001) higher than those found in Butare-Gitarama but significantly (P < 0.001) lower than those found in Gashonga (Fig. 5.2).
Table 5.3 Banana plant growth parameters and root colonization by AM fungi in plots varying in plant vigour in five eco-regions of Rwanda, 2006-2007.

<table>
<thead>
<tr>
<th>Eco-region</th>
<th>Plant girth, cm</th>
<th>Fruit number per bunch</th>
<th>AM fungi frequency, (%)</th>
<th>AM fungi intensity, (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plant vigour</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>Poor</td>
<td>P</td>
<td>Good</td>
</tr>
<tr>
<td>Butare-Gitarama</td>
<td>55.0a</td>
<td>40.9b</td>
<td>&lt;0.001</td>
<td>99a</td>
</tr>
<tr>
<td>Gashonga</td>
<td>64.4a</td>
<td>46.8b</td>
<td>&lt;0.001</td>
<td>115a</td>
</tr>
<tr>
<td>Bugarama</td>
<td>64.6a</td>
<td>52.2b</td>
<td>&lt;0.001</td>
<td>137a</td>
</tr>
<tr>
<td>Kibungo</td>
<td>59.4a</td>
<td>49.6b</td>
<td>&lt;0.001</td>
<td>130a</td>
</tr>
<tr>
<td>Ruhengeri</td>
<td>68.7a</td>
<td>59.9b</td>
<td>&lt;0.001</td>
<td>126a</td>
</tr>
</tbody>
</table>

a, b = horizontal comparisons between good and poor vigour plots. Values followed by the same letter are not significantly different at 0.05 level. NS – not significant at p<0.05.
Figure 5.2 Mean colonization (frequency and intensity, %) of banana roots by the AM fungi in five eco-regions of Rwanda, 2006-2007.

(A, B, C – significant differences at p = 0.05 for intensity; a,b,c – significant differences at p = 0.05 for frequency. Error bars indicate standard error)

Within each eco-region, no significant difference in frequency and intensity of root colonization was observed between plots with poor and good plant vigour (Table 5.3).
5.4.5 Relationships between root colonization and other parameters

**Root colonization by AM fungi and edapho-climatic conditions:** Increased rainfall was positively correlated to intensity (r = 0.16, P < 0.05) and to less extent to frequency of root colonization, although the latter was not significant (r = 0.13, P = 0.0777). Frequency and intensity of root colonization were negatively correlated with coarse fraction of soil (r = -0.36 for frequency and r = -0.28 for intensity, P < 0.001 for both). Frequency and intensity of root colonization were negatively correlated with soil P (r = -0.34, P < 0.001 and r = -0.22, P < 0.01). Separate correlation analysis of soil P and root colonization per region revealed similar negative relationships, which were significant only for Kibungo (r = -0.42, P < 0.01; r = -0.32, P < 0.05 for frequency and intensity, respectively). Frequency of root colonization positively correlated with C/N ratio of soil (r = 0.48, P < 0.001), soil C (r = 0.39, P < 0.001), N (r = 0.25; P < 0.001) and CEC (r = 0.30, P < 0.001). Similarly, intensity of colonization was positively correlated with soil C (r = 0.36, P < 0.001), CEC (r = 0.27, P < 0.01) and N (r = 0.27, P < 0.001).

**Root colonization by AM fungi and field management practices:** Tillage intensity rank was negatively correlated to frequency (r = -0.30, P < 0.001) and intensity of root colonization (r = -0.20, P < 0.01). No significant correlations were observed between root colonization and other management practices.

**Root colonization by AM fungi and plant performance:** Frequency of root colonization was positively correlated with plant height (r = 0.19, P < 0.01), girth (r = 0.14, P < 0.05), pseudostem volume (r = 0.15, P < 0.05), number of functional roots (r = 0.19, P < 0.01) and total root number (r = 0.22, P < 0.01). Higher root colonization was associated with higher Cu foliar concentrations (r =...
Chapter 5. Arbuscular mycorrhizal fungi in the EAH

= 0.31 and r = 0.26, P < 0.001 for both, frequency and intensity, respectively) and lower Zn foliar concentrations (r = -0.25, P < 0.001 and r = -0.15, P < 0.05, for frequency and intensity, respectively). Foliar concentration of Fe were positively correlated with frequency of root colonization (r = 0.18, P < 0.05). Running separate correlations per eco-region did not provide consistent trend in the relationships between root colonization and foliar nutrient concentrations.

Root colonization by AM fungi, root damage and nematode population densities: Frequency of colonization was negatively correlated with root necroses (r = -0.24, P < 0.01) and with P. goodeyi population densities (r = -0.13, P < 0.10).

5.4.6 AM fungi population density in soil

Our results (Table 5.4) showed that AM fungal population densityies in Gashonga and Ruhengeri (59.8 and 48.5 propagules (p.) per 100g of soil) were significantly higher (P < 0.05) than in Butare-Gitarama and Kibungo (2.0 and 8.5 p. per 100g of soil), with Bugarama values in-between (14.7 p. per 100g of soil). This density was higher (P < 0.05) compared to Butare-Gitarama but did not differ significantly (P > 0.05) from Gashonga and Ruhengeri (Table 5.4). The soil propagule density of the AM fungi was highly (P < 0.001) correlated to frequency and intensity of root colonization (r = 0.41 and r = 0.39, respectively).
Table 5.4 Infective propagule population density of the AM fungi in soils from banana rhizosphere in five eco-regions of Rwanda, (2006-2007).

<table>
<thead>
<tr>
<th>Eco-region</th>
<th>Number of infective propagules/100g soil</th>
<th>95% confidence intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butare-Gitarama</td>
<td>2.0 a</td>
<td>0.9 – 4.5</td>
</tr>
<tr>
<td>Gashonga</td>
<td>59.8 c</td>
<td>26.8 – 133.4</td>
</tr>
<tr>
<td>Bugarama</td>
<td>14.7 bc</td>
<td>6.6 – 32.8</td>
</tr>
<tr>
<td>Kibungo</td>
<td>8.5 ab</td>
<td>3.8 – 19.0</td>
</tr>
<tr>
<td>Ruhengeri</td>
<td>48.5 c</td>
<td>21.8 – 108.2</td>
</tr>
</tbody>
</table>

Mean values followed by the same letter do not differ significantly (P<0.05) according to 95% confidence intervals.

5.4.7 AM fungi morphotypes in soils

Four genera of AM fungi were identified based on soil spore identification, namely *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora*. The highest number of morphotypes (10) was observed in Kibungo and the lowest (2) in Butare-Gitarama. The other eco-regions had intermediate numbers (Table 5.5).
Table 5.5 Number of the AM fungi spore morphotypes per genus observed in banana rhizosphere in four eco-regions of Rwanda, 2006-2007.

<table>
<thead>
<tr>
<th>Eco-region</th>
<th>Genus observed</th>
<th>Number of morphotypes</th>
<th>Number of morphotypes per eco-region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butare - Gitarama</td>
<td><em>Glomus</em></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Gashonga</td>
<td><em>Glomus</em></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Acaulospora</em></td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><em>Gigaspora</em></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Kibungo</td>
<td><em>Glomus</em></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Acaulospora</em></td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>*Scutellasp.</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Gigaspora</em></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Ruhengeri</td>
<td><em>Glomus</em></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*Scutellasp.</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><em>Acaulospora</em></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

5.5 Discussion

In this study, we reported for the first time the root colonization, population density and composition of AM fungi in a selection of highland banana cropping systems of Rwanda. The AM fungi were present in all the study sites, both in banana roots and in soils, but population densities and colonization levels varied with edapho-climatic conditions and management practices.

Bananas are mycotrophic plants (Jaizme-Vega & Azcón, 1995), which dependency versus AM fungi has been shown to markedly differ between cultivars (Declerck et al., 1995). Intuntu cultivar, used in this study, also known as “Igitsiri” in Burundi (Sebasigari 1990), had values of root colonization varying from 18 to 62% for frequency and 17 to 33% for intensity that
corroborates earlier findings by Elsen et al. (2003a) on AMF dependency in ex-situ conditions.

The AM fungi population density estimated in soil of the five eco-regions of Rwanda were in a similar range (2.0 to 59.8 propagules (p.) 100 g\(^{-1}\) of soil) as those observed for banana cropping systems in Martinique (Declerck et al., 1999) (1.5 and 163 p. 100 g\(^{-1}\) soil). In our study, the highest population density of AM fungi propagules was in Ruhengeri and Gashonga, the two eco-regions with highest rainfall and it was the lowest in Butare-Gitarama and Kibungo where the rainfall patterns were less abundant. Rainfall in Bugarama and Butare-Gitarama were of similar range, however, soils in Bugarama were more humid because of the position in flat plain, and due to finer physical structure and better water retention. These conditions may have favoured fine root growth and therefore better survival of the AM fungi in soil.

In the five eco-regions, banana root colonization levels showed similar trends to the AM fungi propagule densities in soil, while correlations between the two were positive and significant. The highest root colonization was observed in Gashonga and the lowest in Butare-Gitarama, however, frequency of root colonization was more variable compared to intensity.

Since, the AM fungi population density in soil was well related to root colonization levels, both may be affected by environmental factors in a similar way. Among these factors, the edapho-climatic conditions (Mathimaran et al., 2005; Plenchette, 2000; Land & Schönbeck, 1991) and management practices (Entry et al., 2002; Oehl et al., 2003; Jansa et al., 2006) have been reported.

Higher rainfall in Gashonga and Ruhengeri contributed to maintain better moisture of soil promoting fine root growth in these eco-regions. Thus, higher root densities were in soil for colonization by the AM fungi. In moist soil, root
growth was less interrupted by drought, which possibly assured continuous
growth of the AM fungi which were able to colonize roots with higher intensity.
Soil of Butare-Gitarama contained high coarse and sand fractions which
provided sub-optimal conditions for water retention and root growth. It is
important to note that soils with lowest coarse fraction (Gashonga and Kibungo)
were rich in clay and had important root colonization levels by the AM fungi.
Although no other study to our knowledge tried to link root colonization of
banana by the AM fungi to soil properties, studies of soil populations of the AM
fungi pointed similar findings. Lower AM fungi populations were observed in
sandy soils (Declerck et al., 1999), while higher AM fungi populations were
associated with clay soils (Plenchette, 2000).

Many studies reported high AM fungi colonization in roots and high
population densities in low P soil, although significant correlations were rarely
mentioned (Declerck et al., 1999). Identically, in our study, higher root
colonization was associated with low P content in soil. Lack of significant
correlations in Gashonga may be possibly linked to very low P values and their
narrow variation at this eco-region. In Kibungo, where soil P was higher and
much more variable compared to Gashonga, these relationships were very
strong.

Relationships between root colonization by the AM fungi and other soil
characteristics (soil C, CEC, N, C/N ratio) were observed, however, their
significance is less understood (Entry et al., 2002).

High soil disturbance observed in Butare-Gitarama, which consisted in very
depth, up to 50 cm, soil labour before sowing intercrops in banana plantations,
may have contributed to the low root colonization by the AM fungi in this eco-
region as compared to the other field situations under study. In the other eco-
regions, tillage was less marked. Detrimental effect of tillage on AM fungi was well documented (Douds et al., 1995; Anken et al., 2004; Kabir, 2004; Borie et al., 2006). For example, in Kibungo, most of the farmers, in visited farms, left soil intact when intercropping banana with beans. Manual sowing was used inside of mulch cover, while very delicate and localized soil work was done by a mini-hoe, which left the soil and roots intact. Highest levels of frequency and intensity of root colonization in Gashonga may be triggered by low soil P, as soil work was still quite intensive (high intercropping), although less intensive than in Butare-Gitarama.

Lack of consistent differences within the eco-regions in the relationship between root colonization by the AM fungi and foliar nutrient concentrations could possibly be explained by a lower within-eco-region variability of foliar nutrients in plants growth on the same soil type. If the AM fungi contribute to micro-nutrient content in banana leaves, only controlled experiments could confirm such effect.

The suppressive effect of AM fungal species on banana parasitic nematode populations in roots has been widely documented in experiments with young tissue culture banana plants, infested with *Meloidogyne incognita* (Jaizme-Vega et al., 1997), *Radopholus similis*/*Pratylenchus coffeae* (Elsen et al., 2001; 2003a; 2003b). In our study, similar negative relationships, although of much lower magnitude, were observed between *P. goodeyi* and root necrosis in mature banana plantations *in situ*.

Our study illustrated the diversity of AM fungi in the EAH banana cropping systems. Several spore morphotypes belonging to four major AM fungal genera (i.e. *Glomus, Acaulospora, Gigaspora* and *Scutellospora*) were detected. It has been shown that significant variability exists in agro-ecosystems in terms of AM
species diversity (Dodds & Millner, 1999) and banana cropping systems still remain *terra incognita* in this regard. Agronomic practices (e.g. tillage) may affect community structure of the AM fungi species (Dodds & Millner, 1999) through altering sporulation patterns (Jansa et al., 2002). Lowest number of spore morphotypes observed in Butare-Gitarama and highest number of morphotypes in Kibungo may possibly be affected by contrasted tillage in these two eco-regions as it was discussed above.

### 5.6 Conclusion

Our study provided the first general picture of root colonization, soil population densities and morphotype diversity of the AM fungi in a selection of the existing banana cropping systems in the EAH region. Results suggested that the AM fungi in banana cropping systems in Rwanda were widespread but highly variable in different edapho-climatic conditions. These variations are most probably linked to rainfall, management practices (tillage) and soil properties (texture and P content). Soils cultivated for bananas contained substantial and diverse populations of AM fungi capable to colonize roots in highly contrasted edapho-climatic conditions. These strains should be further isolated, identified and characterized for their beneficial effects on bananas under contrasted edapho-climatic and management practices in EAH banana cropping systems.
Chapter 6. Effect of soil depth and plant vigour on banana root colonization by arbuscular mycorrhizal fungi in the East African Highland cropping systems

This work is in preparation for submission to Plant and Soil as Gaidashova S.V., Van Asten P.J.A., Delvaux B. and Deelerck S. Effect of soil depth and plant vigour on banana root colonization by arbuscular mycorrhizal fungi in the East African Highland cropping systems.
Chapter 6. Effect of soil depth on root colonization by AM fungi

6.1 Abstract
East African Highland bananas are intimately associated with arbuscular mycorrhizal (AM) fungi which have been shown to improve water and nutrient uptake. However, root colonization as well as the distribution of AMF within roots is still poorly understood. In this study we investigated the AM fungi root colonization as a function of banana root characteristics. Three soil depths were considered for vigorous and weak plants in farmer fields at five sites in Rwanda and Burundi. AM fungi colonized the banana roots at all soil depths. However, frequency and intensity of root colonisation decreased with depth. Identically, root colonization was significantly higher (P < 0.05) in plants with poor vigour and growth in all sites except Kibungo, where high crop management standards reduced the contrast between good vigour backyard plots and poor vigour distant plots. Frequency and intensity of arbuscules in roots were positively correlated (P < 0.01) with the number of thick roots (r=0.41 and r=0.36, respectively), the number of medium-size roots (r=0.40 and r=0.33), and the total number of primary roots (r=0.38 and r=0.32). Root colonization frequency and intensity were positively correlated (P < 0.001) with soil organic matter (r=0.30 for %F and %I), but negatively correlated (P < 0.05) with soil water content (r=-0.22 and r=-0.21). Our results suggested that (i) poor vigour plants seem more dependent on their associated AM fungal partner; (ii) that AM fungi colonization decreases with depth and with reduction in soil water content, and (iii) that AMF colonization leads to more vigorous root systems.

6.2 Introduction
Banana (Musa spp.) is an important food and cash crop in Africa (Gowen et al., 2005) which occupies large parcels (10 to 30%) of cultivated land in the East
Chapter 6. Effect of soil depth on root colonization by AM fungi

African highlands (EAH) to sustain about 30 million people (Karamura et al., 1999). Within Musa, highland bananas (genome group AAA-EA) are the dominant (>80%) cultivars in the East African Great Lakes region (Bagamba et al., 1999).

Banana yields within this region tend to decrease (Karamura et al., 1999) and are reverse to population growth (Baijukya & de Steenhuijsen, 1998). Soil depletion resulting from erosion and nutrient removal, drought stress, and pest and disease outbreaks are the main causes of this yield decline (Gold et al., 1999; Okech et al., 2000; Bekunda et al., 2002; Murekezi & Van Asten, 2008; Van Asten et al., 2008). Nutrient removal increased over the past decades as traditional small scale farmers export their production to urban markets, without increasing external nutrient inputs (Van Asten et al., 2004). Banana is a high demanding crop (Delvaux, 1999), but its cultivation in the East Africa is commonly practiced on soils having low inherent fertility (Sanchez et al., 1989; Bekunda & Woomer, 1996).

In recent years, there have been increasing efforts in high input agriculture systems to develop technologies oriented to enhance the ability of the crop to take up nutrient and water, or to overcome pest and diseases. The utilization of root symbionts such as endophytes and arbuscular mycorrhizal (AM) fungi is amongst the options proposed (Dubois et al., 2006; Marschner, 2007; Rivera et al., 2007). Banana is a highly mycotrophic plant (Jaizme-Vega & Azcón, 1995; Declerck et al., 1995). Positive effects of AM fungi on banana growth and protection versus abiotic and biotic stresses were reported under controlled pot culture conditions (Reyes et al., 1995; Yano-Melo et al., 1999; Jaizme-Vega et al., 2002; 2003). However, within-field studies and data in the EAH banana cropping systems are
scarce (Jefwa et al., 2008). Recently, we reported the widespread presence of AM fungi in existing banana systems in Rwanda (Gaidashova et al., accepted). This study revealed differences in AM fungal colonization between different agro-ecological zones, with the highest colonization found in the areas with higher rainfall and younger soils. However, this study did not consider within-site variation in AM fungal colonization. Large variations in plant vigour often exist within farms, but how they relate to horizontal and vertical variations in soil fertility, root system characteristics, and AM fungi colonization is still unknown.

Banana roots may occupy a volume of soil up to 5.2 m in radius from the plant and develop until 1 m depth (Draye, 2002; Draye et al., 2005) depending on physical soil properties (Delvaux & Guyot, 1989). Although the majority of the roots are concentrated in the upper 30 cm top soil, the role of the deeper roots in nutrient uptake is poorly understood. The presence of AM fungi associated to roots at various depths may help unravelling their role in nutrient uptake.

To better understand the role of AM fungi in banana cropping systems in terms of (i) the variables that influence AM fungi distribution, and (ii) the impact of AM fungi on banana root systems, the selected farms previously characterized by Gaidashova et al. (accepted) were revisited. Roots and AM fungi were assessed at different depths from plants that exhibited relative poor and good plant performance within the same farm. The objectives of this study were to better understand what factors impact AM fungi colonization along the root system from surface to deeper soil and how this knowledge could help to exploit the beneficial effects of AM fungi on root system performance.
6.3 Material and methods

6.3.1 Study sites and period

Five sites, with distinct soil types in terms of fertility and parent material, were selected: (1) Butare (Acrisols on granite); (2) Gashonga (Ferralsol on basalt); (3) Kibungo (Nitisol on shale), (4) Ruhengeri (Andosol on volcanic ash) and (5) Giheta (Nitisol on shale). One single farm per site was selected in Rwanda (sites 1-4) and Burundi (site 5) based on previous physico-chemical soil characterisation (Delstanche et al., 2008; Gaidashova et al., 2009). The selected sites represented major soil types from the EAH banana cropping systems. These soils ranged from poor weathered Acrisol to rich volcanic Andosol, with Ferralsol and Nitisol having high clay and low P content. The study was conducted at the end of the rainy season (May-June 2008).

6.3.2 Sample size

At farm level, two banana plots with contrasting plant performance (good and poor) were selected by visual appraisal. ‘Good’ plots had well developed plants (i.e. having taller and thicker pseudostems, higher number of leaves), and ‘poor’ plots had plants with poorer developed plants (i.e. with slimmer pseudostems and fewer leaves). These were referred as good and poor plant vigour plots. All plots with good plant growth (i.e. vigour) were in the immediate proximity (5 to 30 m) from farm houses, while plots with poor plant growth were located more distantly (30 to 60 m) from farm houses. Five flowering plants of Intuntu (Rwandan name) or Igitsiri (Burundian synonym of Intuntu, Sebasigari 1990) cultivar (genome AAA-EA) were identified in each plot. For each plant, pits of 1.2 m length 1 m width and 1.5 m depth were prepared in the near proximity (10
to 20 cm) of the pseudostem of the selected flowered plant. Three depths (0-20 cm; 50-70 cm and 100-120 cm) were selected for root, AM fungi and soil characterization.

6.3.3 Soil and root sampling procedure and handling

One soil sample was taken at each plant and at the three depths for physico-chemical analysis. In addition, a soil volume of 1 dm$^3$ was excavated at each plant at the centre of each depth class for root characterization. Root samples were stored at + 4°C to prevent decay. Fine lateral roots were also collected at each plant and soil depth class (from 0 to 50 g weight per sample depending on root presence at a given depth class) to evaluate root colonization by the AM fungi. These root samples were carefully washed and placed in 50% alcohol for storage.

6.3.4 Soil analysis

Soil samples were oven dried at 60°C for 72 hours, ground and sieved to pass through a 2 mm mesh. Gravimetric water content was determined by weighting soil aggregates before and after drying. Soil organic matter (OM) was determined by a modified Walkley-Black method (Walkley & Black 1934). Available P was extracted using Mehlich-3 solution (Mehlich 1984) prior to colorimetric determination. Soil pH H$_2$O was determined as described in Okalebo et al. (2003).
6.3.5 Root measurements

For each sample of 1 dm³, the following data were evaluated: average diameter of primary roots, cumulative length of primary and secondary roots. In addition, measurements were made on the whole pit profile (1.2 m length) at the 3 depths as described above (6.3.2): numbers of thin (<3 mm), medium (3-6 mm) and thick (>6 mm) primary roots and total number of primary roots. To facilitate the count of primary roots in the field, a metallic grid with 10 cm mesh was used. Additionally, the depth of the deepest root was measured at each pit. Depth of the pit was 1.5 m, which was 0.5 m deeper than literature reports on maximum banana root depth (Draye et al., 2005).

6.3.6 AM fungi root colonization

After storage in 50% alcohol, fine lateral root (<1.5 mm diameter) samples were washed, cleared and stained to assess root colonization by the AM fungi. For clearing, roots were placed in 10% KOH solution for 1 hour at 80 °C. Roots were subsequently bleached during 30 min in 3% H₂O₂ solution freshly alkalinized by NH₄OH (8 ml of 25% NH₄OH for 100 ml 3% H₂O₂) as described by Koske & Gemma (1989). After bleaching, roots were stained in acidified ink solution (20 ml of permanent ink “Parker” in 1l of 1% HCl) overnight (Vierheilig et al., 1998). Stained root samples were de-stained in water for 1 hour and observed under compound microscope (Leica DME) at 200 X magnification. Fifty 1-cm root fragments were assessed for frequency and intensity of (i) arbuscules, (ii) vesicles and (iii) overall root colonization, including arbuscules, vesicles and hyphae combined. The parameters i – iii were evaluated separately. Frequency of i - iii was estimated as percentage of root...
Chapter 6. Effect of soil depth on root colonization by AM fungi

fragments containing respective AM fungal structures. Intensity, which is the abundance of these respective structures (hyphae, vesicles, arbuscules separately or all together) in roots, was assessed by estimation of the proportion of the root surface containing the respective AM fungal structures related to the total surface of the root fragment (Plenchette & Morel, 1996).

6.3.7 Statistical analysis

The variables were transformed to achieve normality (Quinn & Keough, 2006): (i) log for % organic matter; (ii) power for soil P and water content, frequency of vesicles and arbuscules, intensity of arbuscules and overall colonization, primary and secondary root length, primary root numbers; and (iii) arcsin for frequency of overall colonization and intensity of vesicles. Different ways of variable transformation were used as with a single way (eg. arcsin) normality was not always achieved. Analysis of variance under General Linear Model was used. Scheffe’s multiple comparison test was used to separate homogenous groups. Pearson correlation analysis was performed to study the relationships between AM fungi colonization, root, and soil chemical and physical characteristics. SAS 9.1 Enterprise Guide 4 software was used at all analytical steps.

6.4 Results

6.4.1 Soil properties

Effect of plant vigour: Within the same site, soil pH did not vary significantly between good and poor vigour plots except in Butare. In this site, the pH was lower (P < 0.05) in poor vigour plots (Table 6.1). The OM content
Chapter 6. Effect of soil depth on root colonization by AM fungi

did not differ in good and poor vigour plots in Butare and Gashonga, while in Giheta and Ruhengeri, the values of OM were lower (P < 0.001) in the poor vigour plots. In Kibungo, higher OM (P < 0.001) were measured in poor vigour plots as compared to good vigour plots (Table 6.1). Soil P did not vary between vigour plots except in Giheta, where it was lower (P < 0.001) and Kibungo where it was higher (P < 0.001) in poor vigour plots than good vigour plots. Water content was identical in poor and good vigour plots except in Giheta where water content was higher (P < 0.05) in poor vigour plots and in Ruhengeri (P < 0.001) (Table 6.1) where the reserve was observed.

Effect of soil depth: Soil pH, OM and P significantly decreased (P < 0.001) with depth except for pH in Giheta, Kibungo and Ruhengeri (P > 0.05 for all), and P in Gashonga and Kibungo (P > 0.05) where it had very low values at all depths (Table 6.1). Water content did not vary with depth except in Giheta and Ruhengeri, where it increased (P < 0.01) with soil depth (Table 6.1).

6.4.2 Root development

Effect of plant vigour: Average root diameter, and lengths of primary and secondary roots were similar irrespective of plant vigour in all sites except Giheta. In this site, reduced (P < 0.01) primary and secondary root lengths were observed in poor vigour plots as compared to good vigour plots (Table 6.2). Root penetration in depth did not vary between good and poor vigour plots except in Giheta and Kibungo where depth of the deepest root was reduced (P < 0.001) in poor vigour plots (Table 6.2). Good vigour plots had higher (P < 0.001) number of thin roots in all sites except Kibungo and Ruhengeri (Table 6.3). This was also the case in Giheta for the number of medium roots (P < 0.01). Number of thick roots did not vary with plant vigour in any site. Total
root number was higher ($P < 0.05$) in good compared to poor vigour plots except in Kibungo and Ruhengeri (Table 6.3) were no differences were observed.

**Effect of soil depth**: Average root diameter, length of primary and secondary roots, and number of thick roots decreased ($P < 0.05$) with depth in all sites, except Kibungo (Table 6.2). Medium-sized, thin and total root numbers decreased with soil depth in all sites. It was also the case for thin roots in all sites except Kibungo ($P < 0.01$ for Gashonga, $P < 0.001$ for Giheta, Butare and Ruhengeri); medium roots in all sites ($P < 0.01$ for Butare, $P < 0.001$ for other sites); thick roots and total root number in all sites ($P < 0.001$ for all) (Table 6.3).
Table 6.1 Soil properties at three soil depths and plots with good and poor plant vigour in the studied sites, Rwanda (1-4) and Burundi (5), African Great Lakes region, May-June 2008.

<table>
<thead>
<tr>
<th>Site/soil type</th>
<th>Soil depth class</th>
<th>(1) Butare /Acrisol</th>
<th>(2) Gashonga/Ferralsol</th>
<th>(3) Giheta /Nitisol</th>
<th>(4) Kibungo /Nitisol</th>
<th>(5) Ruhengeri /Andosol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>P</td>
<td>1</td>
<td>2</td>
</tr>
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<td>pH</td>
<td>Good</td>
<td>6.9a</td>
<td>6.3b</td>
<td>6.1b ***</td>
<td>5.8a</td>
<td>5.1b</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>6.4a</td>
<td>6.2b</td>
<td>5.9b ***</td>
<td>6.0a</td>
<td>5.2b</td>
</tr>
<tr>
<td>P</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>OM (%)</td>
<td>Good</td>
<td>5.2a</td>
<td>2.6b</td>
<td>1.4c ***</td>
<td>5.5a</td>
<td>4.0b</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>4.2a</td>
<td>3.1b</td>
<td>1.9c ***</td>
<td>6.1a</td>
<td>2.3b</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P (mg/kg)</td>
<td>Good</td>
<td>40.9a</td>
<td>22.2a</td>
<td>3.2b ***</td>
<td>1.8</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>39.3a</td>
<td>28.6a</td>
<td>8.0b ***</td>
<td>2.0</td>
<td>1.2</td>
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<td>Water (%)</td>
<td>Good</td>
<td>14.7a</td>
<td>11.0a</td>
<td>12.5a NS</td>
<td>33.8a</td>
<td>36.5a</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>12.1a</td>
<td>10.9a</td>
<td>15.0a NS</td>
<td>31.1a</td>
<td>33.2a</td>
</tr>
</tbody>
</table>

Soil depth class: 1 = 0-20 cm; 2 = 50-70 cm; 3 = 100-120 cm. * 'Good' and 'Poor' are plant vigour plots; NS = non significant; NE = non-estimated; a, b = horizontal comparisons significant at P = 0.05. Soil depth classes: 1 = 0-20cm, 2 = 50-70cm, 3 = 100-120cm.
Table 6.2 Selected root characteristics (I) at three soil depths and in two plant vigour plots, Rwanda (sites 1-4) and Burundi (site 5), African Great Lakes region, May-June 2008.

<table>
<thead>
<tr>
<th>Site /soil type</th>
<th>Butare /Acrisol</th>
<th>Gashonga/Ferralsol</th>
<th>Giheta /Nitisol</th>
<th>Kibungo /Nitisol</th>
<th>Ruhengeri /Andosol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soil depth class</td>
<td>1 2 3 P</td>
<td>1 2 3 P</td>
<td>1 2 3 P</td>
<td>1 2 3 P</td>
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<tr>
<td></td>
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<td>1 2 3 P</td>
<td>1 2 3 P</td>
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<td>1 2 3 P</td>
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<td>1 2 3 P</td>
<td>1 2 3 P</td>
<td>1 2 3 P</td>
<td>1 2 3 P</td>
</tr>
<tr>
<td>PRL, mm**</td>
<td>Good*</td>
<td>722 239ab 131b *</td>
<td>389a 311ab 134b **</td>
<td>840a 201b 256b ***</td>
<td>484a 193a 398a NS</td>
</tr>
<tr>
<td></td>
<td>Poor*</td>
<td>323b 579a 269b *</td>
<td>538a 161b 141b **</td>
<td>452a 129b 0c ***</td>
<td>436a 286a - NS</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>NS NS NS</td>
<td>NS NS NE</td>
<td>NS NS NE</td>
<td>NS NS NE</td>
</tr>
<tr>
<td>SRL, mm**</td>
<td>Good*</td>
<td>6072a 2996a 1156b **</td>
<td>2453a 441b ***</td>
<td>11290a 1005b 743c **</td>
<td>2166a 1519a 1007a NS</td>
</tr>
<tr>
<td></td>
<td>Poor*</td>
<td>4051a 1207a b 614b **</td>
<td>3039a 290b ***</td>
<td>4759a 417b 0c ***</td>
<td>2336a 2816a 0c ***</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>NS NS NS</td>
<td>NS NS ***</td>
<td>NS NS ***</td>
<td>NS NS ***</td>
</tr>
<tr>
<td>DDR, cm</td>
<td>Good</td>
<td>119.8 121 110.6 131.4 130.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>111.0 121.6 61.2 56.9 128.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>NS NS ***</td>
<td>NS NS ***</td>
<td>NS NS ***</td>
<td>NS</td>
</tr>
</tbody>
</table>

ARD = average primary root diameter; PRL = primary root length; SRL = secondary root length; DDR = depth of the deepest root. * ‘Good’ and ‘Poor’ are plant vigour plots; ** Values for PRL and SRL are given per cubic decimeter of soil volume. Values followed by the letters ‘a’, ‘b’ and ‘c’ are significantly different at $P = 0.05$. Soil depth classes: 1 = 0-20cm, 2 = 50-70cm, 3 = 100-120cm.
Table 6.3 Selected root characteristics (II) at three soil depths and in two plant vigour plots, Rwanda (sites 1-4) and Burundi (site 5), African Great Lakes region, May-June 2008.

<table>
<thead>
<tr>
<th>Site /soil type</th>
<th>Butare /Acrisol</th>
<th>Gashonga/Ferralsol</th>
<th>Giheta /Nitisol</th>
<th>Kibungo /Nitisol</th>
<th>Ruhengeri /Andosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thin PRN</td>
<td>Good*</td>
<td>19.4a 6.4b 2.8c ***</td>
<td>22.4a 13.4a 3.6b **</td>
<td>11.2a 4.6b 2.6b ***</td>
<td>1.6a 7.6a 2.6a NS</td>
</tr>
<tr>
<td></td>
<td>Poor*</td>
<td>6.8a 5.0b 0.6c ***</td>
<td>5.0a 4.6a 3.2b **</td>
<td>3.6a 0.8b 0.0b ***</td>
<td>7.6a 0.0a - NS</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>*** *** *** *** ***</td>
<td>*** *** *** ***</td>
<td>NS NS NE NS NS NS NE</td>
<td></td>
</tr>
<tr>
<td>Medium PRN</td>
<td>Good</td>
<td>13.0a 1.8b 1.8b **</td>
<td>18.6a 6.2b 1.4c ***</td>
<td>13.0a 2.2b 0.4b ***</td>
<td>15.0a 7.2b 3.0b ***</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>1.6ab 4.2a 0.6b **</td>
<td>21.0a 5.2b 0.8c ***</td>
<td>7.4a 0.2b 0.0b ***</td>
<td>13.8a 4.5b - ***</td>
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<tr>
<td></td>
<td>*</td>
<td>NS NS NS NS NS ** ** **</td>
<td>NS NS NE NS NS NS NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thick PRN</td>
<td>Good</td>
<td>15.8a 2.0b 0.0c ***</td>
<td>11.0a 5.8b 0.0c ***</td>
<td>11.2a 0.0b 0.0b ***</td>
<td>16.0a 4.6b 1.0b ***</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>3.6a 2.2b 0.2c ***</td>
<td>14.0a 1.6b 0.0c ***</td>
<td>13.0a 0.0b 0.0b ***</td>
<td>11.2a 2.0b - ***</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>NS NS NS NS NS ** ** **</td>
<td>NS NS NE NS NS NS NS</td>
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<tr>
<td>Total PRN</td>
<td>Good</td>
<td>47.8a 10.2b 4.6c ***</td>
<td>52.0a 25.4b 5.0c ***</td>
<td>35.4a 6.8b 3.0c ***</td>
<td>32.6a 19.4ab 6.6b ***</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>12.0a 11.4a 1.8b ***</td>
<td>40.0a 11.4b 4.0c ***</td>
<td>24.0a 1.0b 0.0c ***</td>
<td>32.6a 6.5b - ***</td>
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<tr>
<td></td>
<td>***</td>
<td>NS NS *** * * *** ***</td>
<td></td>
<td>NS NS NE NS NS NS NS</td>
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</tbody>
</table>

* ‘Good’ and ‘Poor’ are plant vigour plots; PRN = primary root number; soil depth classes: 1 = 0-20cm, 2 = 50-70cm, 3 = 100-120cm.
Table 6.4 Root colonization at three depths and in two plant vigour plots, Rwanda (1-4) and Burundi (5), May-June 2008.

<table>
<thead>
<tr>
<th>Site/soil type</th>
<th>Butare/Acrisol</th>
<th>Gashonga/Ferralsol</th>
<th>Gilheta/Nitisol</th>
<th>Kibungo/Nitisol</th>
<th>Ruhengeri/Andosol</th>
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<tr>
<td>Soil depth class</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>FA (%)</td>
<td>8.8a</td>
<td>10.0a</td>
<td>11.6a</td>
<td>ns</td>
<td>61.2a</td>
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<tr>
<td>'Good'***</td>
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<td>'Poor'***</td>
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<td>'Good'***</td>
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<td>'Poor'***</td>
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<td>'Good'***</td>
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<td>'Poor'***</td>
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<td>'Good'***</td>
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<tr>
<td>'Poor'***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>ne</td>
<td>ne</td>
<td>ne</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>'Good'***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Poor'***</td>
<td></td>
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<tr>
<td>P</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

* 'Good' and 'Poor' are plant vigour plots; FA = frequency of arbuscules; IA = intensity of arbuscules; FV = frequency of vesicles; IV = Intensity of vesicles; F = overall frequency; I = overall intensity.
6.4.3 AM fungi root colonization of banana plants

Effect of plant vigour: Plant vigour significantly (P < 0.05) affected most of root colonization parameters irrespective of site. Frequency of arbuscules was higher (P < 0.05) in poor compared to good vigour plots in all sites except Kibungo (Table 6.4). Similarly, poor vigour plots were more intensively (P < 0.01) colonized as compared to good vigour plots in all sites except in Giheta and Kibungo (Table 6.4).

Frequency of vesicles was higher in poor vigour plots as compared to good vigour plots in all sites and all depth classes; however, it was significantly higher (P < 0.05) in Giheta, Butare and Ruhengeri where it averaged 25%, 19.2% and 7.1%, respectively (Table 6.4). Similarly, intensity of vesicles was higher (P < 0.05) in poor as compared to good vigour plots in all sites and all depth classes except Kibungo and the medium depth class (50-70cm) of Gashonga (Table 6.4).

Overall frequency (%F) and intensity (%I) of colonization were higher (P < 0.05) in poor than in good vigour plots in all depth classes and all sites except Kibungo (Table 6.4).

Effect of soil depth: Frequency (%FA) and intensity of arbuscules (%IA) decreased (P < 0.05) with depth in both poor and good vigour plots in all sites except Butare and Ruhengeri (P > 0.05 for both) (Table 6.4). Lack of soil and/or roots at these sites at the depth 100-120cm in poor vigour plots has constraint evaluation. Reduction of frequency of vesicles with soil depth was observed in fewer sites: Ruhengeri (P < 0.001) and Giheta (P < 0.05). Intensity of vesicles did not vary with soil depth at any of sites except in good vigour plots of Giheta (P < 0.001) (Table 6.4). Overall frequency of colonization (= %F of hyphae, vesicles and arbuscules all together) was significantly (P < 0.01) reduced with
soil depth in Gashonga, Giheta and Ruhengeri. Overall intensity (=%I) was reduced (P < 0.05) with depth in Ruhengeri and good vigour plots of Giheta (Table 6.4).

6.4.4 Relationships between the AM fungi and soil properties

Organic matter content positively correlated with %FA and %IA (r=0.30, P < 0.001 for %FA and %IA) and general frequency (%F) (r=0.23, P < 0.01) and intensity (%I) (r=0.18, P < 0.05) of root colonization. Reduced water in soil was associated with higher frequency (%FV) (r=-0.22, P < 0.01) and intensity (%IV) of vesicles (r=-0.21, P < 0.05), and %F (r=-0.19, P < 0.05). Correlations between various colonization parameters and soil P were negative but low and not significant at P < 0.05.

6.4.5 Relationships between the AM fungi colonization and root characteristics

Average primary root diameter positively correlated with %FA and %FI (r=0.43 and r=0.33, P < 0.001 for both), overall %F and %I (r=0.36, P < 0.001 and r=0.27, P < 0.01). Length of primary roots was positively correlated with %FA, %IA and overall %F (r=0.23, P < 0.01, r=0.18, P < 0.05 and r=0.21, P < 0.05, respectively). Similarly, length of secondary roots positively correlated with %FA, %IA and overall %F (r=0.28, P < 0.01, r=0.16, P < 0.05 and r=0.23, P < 0.01, respectively). Number of thin primary roots negatively correlated with %IV (r=-0.22, P < 0.01) and number of medium and thick roots positively correlated with %FA, %IA, overall %F and %I (r=0.41, P < 0.001 for %FA, r=0.36, P < 0.001 for %IA, r=0.34, P < 0.001 for %F and r=0.26, P < 0.01 for
%I and medium roots; and \( r=0.40 \) for %FA, \( r=0.33 \) for %IA, \( r=0.37 \) for %F and \( r=0.28 \) for %I, and thick roots, \( P < 0.001 \) for all). Similarly, total number of primary roots was positively correlated with %FA and %IA (\( r=0.38 \) and \( r=0.32 \), respectively, \( P < 0.001 \) for both), and general %F and %I (\( r=0.27 \), \( P < 0.01 \) and \( r=0.20 \), \( P < 0.05 \), respectively). Most of these relationships were also observed at significant (\( P < 0.05 \)) level and with varying strength within each site.

### 6.5 Discussion

Plots with poor plant vigour had generally lesser quantities of nutrients available from soil at all depth classes and all sites (Gaidashova et al., 2009). Farm-level management practices are different in backyard and distant plots (Rishirumuhirwa, 1993, 1997). Backyard plots regularly benefit from home waste and manure, and bananas are grown as dense monocrops, while distant plots are heavily intercropped, only accidentally manured and lower banana density is used (Lassoudière, 1989; Rishirumuhirwa, 1997). Our results indicate that management differences result in less developed root systems in distant plots, i.e. smaller root (both primary and secondary) length and number at all sites and all depths.

The difference in banana plant growth in plots of good and poor plant vigour, imposed by natural soil fertility patterns and resuming from the existing farm management practices, resulted in highly significant differences in crop yield (Gaidashova et al., 2007), as well as root system size (this study). We observed consistently higher root colonization in poor vigour plants. This may be related to lower soil fertility status in poor vigour plot. Chapin (1988) reported that higher root colonization by AM fungi was observed as soil fertility declined. We
hypothesize that the plants are more actively developing AM symbiosis in conditions where less nutrients are available.

In a recent report, Gaidashova et al. (accepted), AM fungi root colonization were higher on the wetter and younger soils at superficial layers (0-25 cm depth). In the present study, root colonization was observed from top soil till 120 cm depth, even though roots were rare at this depth. Average root colonization frequency from the three soil depths was generally within the range of 17 to 80% for colonization frequency, similarly to the values reported for the upper soil depths (Gaidashova et al., accepted).

The fact that root colonization decreases with soil depth was known from earlier studies. In annual crops in temperate agro-ecosystems, a sharp decrease in proportion of roots colonized by AM fungi was observed (Jakobsen & Nielsen, 1983; Rillig & Field, 2003). Since banana has shallow root system compared to trees (Draye et al., 2002), while most of its roots are concentrated in the upper 30 cm soil layer (Kashaija et al., 2004), it may be expected that AM fungi colonization takes place mostly in the top soil where most of fine lateral roots are located. However, in our study, root colonization was observed in the deepest banana roots (> 120 cm), although to a lesser extent than roots from the upper soil layers. This may be an indication of effective investment of the crop resource (roots) and the fungal resource (whole biomass) across the soil space limited largely to the place where highest food resource (soil nutrients) is the most available. Fine lateral roots are the most abundant in top-soil layers, while deeper sub-soil contains more large roots assuring plant anchorage in soil as compared to fine lateral roots (Draye et al., 2005).

It was shown recently that banana plants with deeper root systems have better growth and vigour, and could better sustain drought, because they are able to
Chapter 6. Effect of soil depth on root colonization by AM fungi

pump water from more humid deeper soil compared to the plants having more shallow root systems (Araya, 2005). In support of this observation, controlled irrigation experiments indicated that banana roots tended to extract water from deeper soil layers with increasing soil water depletion (Robinson & Alberts, 1989). In our study, in the deepest soil layer (100-120 cm), banana roots colonized by the AM fungi maintained high frequency (some 30 to 60%) and intensity (11 to 58%) of arbuscules, a typical intra-cellular AM fungi structures, where exchange of nutrients between the fungi and plant cell takes place. This may be an indication that AM fungi are actively involved in nutrient transfer from soil to roots at this depth. This role may be of critical importance during dry periods when most of fine superficial soil roots die off (Draye, 2002). Thus, we hypothesize that the biological role of the AM fungi colonizing the deepest banana roots may be linked to water and nutrient transfer from more humid deeper sub-soil during drought periods when most of the top-soil roots die off. In support to this hypothesis, Diop et al. (1994) observed that in Sudano-Guinean region of Senegal, *Acacia* roots were colonized up to 4.5 m soil depth during dry season, although frequency of colonization was not reported.

The fact, that positive relationships between various parameters of AM fungi root colonization and quantitative roots characteristics were observed in mature plantations, suggests that the association of the AM fungi with banana roots in the EAH has positive implications for root growth. Previous studies demonstrated that in pot experiments, inoculation with AM fungi increased banana root branching (Elsen et al., 2003).

We have earlier reported (Gaidashova et al., accepted) about root colonization as related to edapho-climatic factors in four of the five sites studied in this paper. The present study confirmed the lower root colonization in coarse-
Chapter 6. Effect of soil depth on root colonization by AM fungi

rich soil (Butare/Acrisol) and positive relationships between root colonization and soil organic matter. Although the role of AM fungi in P uptake from low P soils was well documented (Smith & Read, 2008), lack of correlations between root colonization and soil P (as in our study) was often mentioned (Declerck et al., 1999). Positive contribution of the AM fungi colonization to water uptake and, in particularly, of the abundance of arbuscules in plant roots in stress conditions of dehydration was shown in pot experiments (Estrada-Luna & Davies, 2003) and in field (Adriano-Anaya et al., 2006). The latter authors observed higher root colonization in banana roots during dry season. However, in our study which was conducted from mid- to end of wet season, no relationships were observed. If these relationships are present, the repeated root sampling at various soil depths should be done after peaks of rain and drought.

6.6 Conclusion

Our study reported for the first time on root colonization by AM fungi at different soil depths in the EAH banana cropping systems. Results suggested that AM fungi colonized banana roots from the top soil till the deepest soil layers which were explored by roots, although colonization was lower in deep compared to top soil. Thus, the AM fungi are actively involved in association with banana roots at all depths that roots may reach. We hypothesize that biological role of the AM fungi colonizing the deepest banana roots may be linked to water and nutrient transfer from more humid deeper sub-soil during drought periods when most of the top-soil roots die off.

Root colonization frequency and intensity were highly related to plant vigour with higher colonization in poor vigour plants grown on less fertile soil. However, plants with higher root colonization had better developed root
characteristics across the three soil depths investigated. Our study, limited to a single cultivar, could not reflect possible variability of relationships and potential for development of the AM symbiosis in different Musa genotypes. Further research should investigate diverse banana germplasm of the EAH region.
Chapter 7. Mycorrhizal status of major banana genotypes sampled in six field germplasm collections in East Africa

This work is in preparation for submission to *Fungal Ecology* as Gaidashova S.V., Nsabimana A., Karamura D. and Declerck S. Mycorrhizal status of major banana genotypes sampled in six field germplasm collections in East Africa.
Chapter 7. Mycorrhizal status of banana genotypes in East Africa

7.1 Abstract
In the East African highlands (EAH), bananas are a major staple and cash crop. However, yields are low, amongst others due to soil fertility problems. Utilization of root symbionts, such as arbuscular mycorrhizal (AM) fungi may be a reasonable option to boost production for smallholders. Even though the association of banana with AM fungi has been investigated and positive effects demonstrated, the extent to which mycorrhizal colonization is controlled by banana genotype and environment is largely unknown. In the present study we assessed the banana AM fungal root colonization in six banana germplasm collections across East Africa. Eighteen genotypes were sampled among which 5 were present in the six locations and 6 were found in five locations. Location, genotype, and interaction between both had significant (P < 0.001) effects on AM fungal colonization. The highest colonization level was reported in the germplasm collection of Burundi where soil had an extremely low P content and a high acidity. Frequency (%F) of root colonization was 90.7% and intensity (%I) was 63.0%. The lowest %F was observed in the germplasm collection of Uganda and Rwanda with values of 46.1% and 50.3%, respectively, while the lowest %I was observed in the germplasm collection of Uganda with a value of 22.5%. Differences in root colonization were observed between the genotypes. Even though the root colonization varied among the genotypes from one location to another, the variation in both %F and % was remarkably lower for Kayinja having coefficient of variation (CV) of 10.9% and 19.9%, for %F and %I, respectively compared to FHIA tetraploids, Intuntu and Ingenge having CV of 50.0-70.8% for %F and 63.1-84.6% for %I, respectively. Whatever the genotype, the %F and %I were positively correlated with altitude ($r^2=0.17$, P < 0.01 and $r^2=0.30$, P < 0.001, respectively) and 3-last-month rainfall ($r^2=0.46$ and
Chapter 7. Mycorrhizal status of banana genotypes in East Africa

\[ r^2 = 0.27, \ P < 0.001 \] for both). Soil P, C, CEC and pH, 3-last month rainfall and number of functional roots explained 38.4% of variation in %F and 56.1% in %I. Soil P, C and pH were negatively and CEC, 3-last month rainfall and number of functional roots were positively associated with increased %F and %I of root colonization. This study highlighted strong effect of environment on the AM fungi – banana genotype interaction.

7.2 Introduction

Banana is amongst the most important staple and cash crops in the East African highlands (EAH) (Frison & Sharrock, 1999). In this region, production systems are mostly small-scale and oriented to local marketing and consumption (Karamura et al., 1999). In the recent years, the productivity of these systems has declined due to the low use of external inputs (e.g. fertilizers), the increased nutrient removal via banana exports and the emergence of diseases (Tushemereirwe & Bagabe, 1998; Van Asten et al., 2004; Mwangi & Nakato, 2007). Therefore, new agricultural practices adapted to the local edapho-cilmatic and socio-economic conditions are highly demanded.

Exploiting the genetic diversity in bananas in combination with beneficial soil micro-organisms such as arbuscular mycorrhizal (AM) fungi appears promising but has not been tested yet in the field. The AM fungi are obligate root symbionts present in nearly 80% of vascular plants. They have been demonstrated to improve plant nutrition and to provide protection against various abiotic stresses, pests and diseases (Smith & Read, 2008).

Banana is a highly mycotrophic plant (Declerck et al., 1995; Jaizme-Vega & Azcón, 1995). Results to date reported improved biomass production (Reyes et al., 1995) and resistance against nematodes (Elsen et al., 2003a, b) and fungi
Chapter 7. Mycorrhizal status of banana genotypes in East Africa

(Jaizme-Vega et al., 1998; Declerck et al., 2002b). The efficiency of the association is dependent on the host plant (Plenchette et al., 1983), the AM fungi (Yano-Melo et al., 1999) and the environment (Öpik et al., 2006) and may be influenced by management-practices (Jansa et al., 2006; Adriano-Anaya et al., 2006). The banana germplasm diversity exceeds 1100 genotypes worldwide (INIBAP, 2005) and more than 300 in the EAH region (Karamura, 1999). Only for a very narrow range of cultivars, some studies were conducted on mycorrhizal symbiosis (Declerck et al., 1995, 2002; Yano-Melo et al., 1999; Elsen et al., 2003a, b).

In the EAH, climatic and edaphic conditions vary substantially (Eswaran et al., 1989). Soils are highly diverse and range from poor and old weathered acidic soils to highly fertile young volcanic soils (UNESCO, 1977). Bananas are grown on a wide range of soils within this region (Bekunda et al., 2003).

It is well known that AM fungi colonize plant roots to various extend depending on soil fertility and AM fungal population (Plenchette et al., 1989). Plant benefits (nutrient uptake and growth increase) from mycorrhizal symbiosis also depend on soil fertility (Plenchette et al., 1983). Conversely, it is poorly known under field conditions whether and to which extent banana root colonization is controlled by host genotype and environment.

In the present study, we assessed (i) root colonization of different banana genotypes grown in six sites of the EAH region, represented by field collections of Musa; and (ii) investigated the relationships between banana genotype and environmental characteristics.
Chapter 7. Mycorrhizal status of banana genotypes in East Africa

7.3 Material & methods

7.3.1 Characteristics of the collection sites

The sites of study were fields of banana germplasm collections belonging to the national agricultural research organizations from the following six East African countries: Rwanda (ISAR-Rubona); Burundi (IRAZ-Gitega), DR Congo (INERA-Mulungu); Uganda (NARO-Mbarara); Tanzania (ARI-Maruku) and Kenya (KARI-Kisii) (Fig. 7.1).

Figure 7.1 Map of the banana germplasm collection locations in East Africa (Stars represent collection locations).
Table 7.1 Site characteristics of the six East African banana germplasm collections.

<table>
<thead>
<tr>
<th>Country</th>
<th>Location (Institute)</th>
<th>Geographic coordinates</th>
<th>Rain, mm yr⁻¹</th>
<th>Altitude, m asl</th>
<th>Parent material</th>
<th>Soil characteristics</th>
<th>Soil type*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rwanda</td>
<td>Rubona (ISAR)</td>
<td>S 02° 29'15&quot; E 29° 46'21&quot;</td>
<td>1104</td>
<td>1724</td>
<td>Granite</td>
<td>Well drained, sandy clay loam, moderately deep</td>
<td>Acrisol</td>
</tr>
<tr>
<td>Burundi</td>
<td>Mashitsi (IRAZ)</td>
<td>S 03° 21'48&quot; E 29° 54'09&quot;</td>
<td>1189</td>
<td>1650</td>
<td>Shale</td>
<td>Poor acidic soil, P deficiency</td>
<td>Nitisol</td>
</tr>
<tr>
<td>DR Congo</td>
<td>Mulungu (INERA)</td>
<td>S 02° 12'07&quot; E 28° 28'33&quot;</td>
<td>1420</td>
<td>1650</td>
<td>Volcanic ash</td>
<td>Well drained, very deep, fertile</td>
<td>Andosol</td>
</tr>
<tr>
<td>Uganda</td>
<td>Mbarara (NARO)</td>
<td>S 0° 39'00&quot; E 30° 41'00&quot;</td>
<td>1426</td>
<td>1500</td>
<td>Shale</td>
<td>Medium fertility, compacted soils</td>
<td>Lixisol</td>
</tr>
<tr>
<td>Tanzania</td>
<td>Maruku (ARI)</td>
<td>S 01° 14'37&quot; E 31° 28'02&quot;</td>
<td>1809</td>
<td>1200</td>
<td>Shale, sandstone</td>
<td>Well drained, deep, clay loam with loamy top-soil</td>
<td>Acrisol</td>
</tr>
<tr>
<td>Kenya</td>
<td>Kisii (KARI)</td>
<td>S 0° 00' 41&quot; E 0° 34' 37&quot;</td>
<td>1965</td>
<td>1800</td>
<td>Basalt</td>
<td>Well drained, deep, of clay texture, P deficient</td>
<td>Nitisol</td>
</tr>
</tbody>
</table>

* IUSS- WRB (FAO), 2006.
The collection sites highly differed in rainfall and soil type (Table 7.1). Rainfall was the highest in Kisii (1965 mm year⁻¹) and the lowest in Rubona (1104 mm year⁻¹). Altitude ranged from 1200 m asl in Maruku to 1800 m sal in Kisii. The sites were represented by Acrisol (Rwanda, Tanzania), Ferralsol (Burundi), Lixisol (Uganda), Nitisol (Kenya) and Andosol (DR Congo).

### 7.3.2 Genotype identification and selection

At each collection site, 10 to 18 genotypes were selected. Preference in selection was given to genotypes known to be present in the other sites under synonym name(s). An attempt was made to select the same genotypes in all or majority of the sites. Genotypes were identified after comparison of morphological traits of the genotype selected with those from a ‘reference’ collection (ISAR Rubona). The list of genotypes and their local synonyms are presented in Table 7.2. In total, 18 genotypes were sampled. Five out of the 18 genotypes were present in all sites: Kamaramasenge (AAB, Ndiizi); Incakara (AAA-EA, Musakala); Gisukari (AAA, Green Red); Yangambi km 5 (AAA, Ibota) and Gros Michel (AAA, Gros Michel) (Table 7.2).
Table 7.2 List of cultivar names and their synonyms, sampled from the six banana germplasm collections of East African region, February-March 2007. Grey color is for the common genotypes.

<table>
<thead>
<tr>
<th>N</th>
<th>Genotype(s/group)/clone set</th>
<th>Rwanda</th>
<th>Burundi (region)</th>
<th>DR Congo (region)</th>
<th>Tanzania (region)</th>
<th>Uganda (region)</th>
<th>Kenya (region)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AA M. acum. burmannoides</td>
<td>Calcutta 4</td>
<td>Calcutta 4</td>
<td></td>
<td></td>
<td>Calcutta 4</td>
<td>Muraru</td>
</tr>
<tr>
<td>2</td>
<td>AA Mshale</td>
<td></td>
<td>Burhale</td>
<td></td>
<td>Mshale</td>
<td>Mshale</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>AAA-EA-Beer</td>
<td>Intuntu</td>
<td>Igtiri</td>
<td>Ndundu</td>
<td>Entundu</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>AAA-EA-Nakabululu</td>
<td>Injagi</td>
<td>Incakara</td>
<td></td>
<td></td>
<td>Nakabululu</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>AAA-EA-Musakala</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Musakala</td>
<td>Nshakara</td>
</tr>
<tr>
<td>6</td>
<td>AAA-EA-Nakitembe</td>
<td>Mwaziruma</td>
<td>Mbwaziruma</td>
<td></td>
<td></td>
<td>Mbwazirume</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>AAA-EA-Nfuuka</td>
<td>Mutsinawuburo</td>
<td>Inzirabushera</td>
<td></td>
<td></td>
<td>Enzirabushera</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>AAA-Gros Michel</td>
<td>Gros Michel</td>
<td>Gros Michel</td>
<td></td>
<td></td>
<td>Kgjoge</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>AAA-Cavendish</td>
<td>Poyo</td>
<td>Poyo</td>
<td></td>
<td></td>
<td>Bogoya</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>AAA-Bota</td>
<td>Yangambi km5</td>
<td>Yangambi km5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>AAA-Green Red</td>
<td>Gisukari Red</td>
<td>Cisukari Red</td>
<td></td>
<td></td>
<td>Green Red</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>AAB-Apple banana</td>
<td>Kamaramasenge</td>
<td>Kamaramasenge</td>
<td></td>
<td></td>
<td>Kamaramasenge</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>ABB-Pisang Awak</td>
<td>Gisubi</td>
<td>Kayinja</td>
<td></td>
<td></td>
<td>Kayinja</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>AAAA</td>
<td>FHIA 17</td>
<td>FHIA 17</td>
<td>FHIA 17</td>
<td>FHIA 17</td>
<td>FHIA 17</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>AAAB</td>
<td>FHIA 21</td>
<td>FHIA 21</td>
<td>FHIA 21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>AABB</td>
<td>FHIA 03</td>
<td>FHIA 03</td>
<td>FHIA 03</td>
<td>FHIA 03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>AAA</td>
<td>FHIA 25</td>
<td>FHIA 25</td>
<td>FHIA 25</td>
<td>FHIA 25</td>
<td>FHIA 25</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>BB</td>
<td>M. balbisiana</td>
<td>M. balbisiana</td>
<td></td>
<td></td>
<td>M. balbisiana</td>
<td></td>
</tr>
</tbody>
</table>
7.3.3 Sampling: roots and soil

Roots of the selected cultivars were sampled from flowering plants (i.e. within 1-2 weeks after or before bunch emergence). Three plants (replicates) per cultivar were sampled. For each plant, two sub-samples of fine lateral roots were collected in the upper 25 cm rhizosphere at a distance of 50 cm from the corm. These two sub-samples were taken from two opposite sides from the pseudostem of the sampled plant to make one composite root sample per plant. Numbers of dead and alive roots were counted from 20x20x20 cm soil volume, excavated in proximity (20 cm) of the pseudostem of the selected banana plant (Speijer & De Waele, 1997).

Soil sub-samples were taken at 0-25 cm depth at a horizontal distance of 50 cm from each plant sampled. These sub-samples were mixed up to form one composite sample per site, all genotypes and plant replicates mixed. The composite sample was then split into three replicates per site.

7.3.4 Study period and geo-data record

Sampling was done from mid-February to mid-April 2007 which corresponded to the period from the beginning to the middle of large rainy season in East Africa. Altitude and geographic position of the collection field were measured using a GPS.

7.3.5 Data collection

Data were collected on root colonization frequency and intensity by the AM fungi, soil pH, CEC and nutrients (N, C, Ca, Mg, Na and P), and rainfall (average rainfall for the past 2 years and for the last 3 months before the sampling date).
7.3.6 Soil analysis

The soil exchangeable contents of Ca$^{2+}$, K$^+$, Mg$^{2+}$, Na$^+$ and cation exchange capacity (CEC) were determined by atomic absorption spectrometry (VARIAN 300), after extraction with the normalized ammonium acetate extract at pH=7. In addition, KCl-extractable Al, C (Walkley-Black method), N (Kjeldhal method), available P (Olsen method), pH H$_2$O and pH KCl were determined (Page et al., 1982). Phosphorus was determined by colorimetry (SHIMADZU UV-1205).

7.3.7 Assessment of banana AMF root colonization

Fresh roots were stored in 50% alcohol before evaluation. Root samples were subsequently placed in 10 % KOH solution for 1 hour at 80$^\circ$C. Roots were then bleached during 30 min in 3 % H$_2$O$_2$ solution freshly alkalinized by NH$_4$OH (8 ml of 25 % NH$_4$OH for 100 ml 3 % H$_2$O$_2$) as described by Koske & Gemma (1989). After bleaching, roots were stained in acidified ink solution (20 ml of permanent ink “Parker” in 1l of 1 % HCl) overnight (Vierheilig et al., 1998). Stained root samples were de-stained in water for 1 hour and observed under compound microscope (Olympus BH2 RFCA) at 200X magnification. Frequency and intensity of the AM fungi root colonization was evaluated from fifty root fragments (1-cm length) for each sample. Frequency was estimated as the percentage of root fragments containing AM fungi structures. Intensity, which is the abundance of hyphae, vesicles and arbuscules in roots, was assessed as described in Plenchette & Morel (1996).
7.3.8 Statistical analysis

To achieve normal distribution of data, percentage values were square root transformed (Quinn & Keogh, 2006). Analysis of variance under General Linear model was used. Where significant differences were obtained, Scheffe’s test was used for homogenous group separation. Pearson correlation analysis was used to investigate bi-variate relationships between climate and root colonization variables. Relationships between root colonization frequency and intensity, soil and climate characteristics were investigated with step-wise multiple regressions. SAS 9.1 Enterprise Guide 4 was used on all analytical steps.

7.4 Results

7.4.1 Soil characteristics of the study sites

Soil physico-chemical characteristics differed among the sites (Table 7.3). Soil P was significantly lower in all sites as compared to that in Mulungu (P < 0.01). Soil C and N were the highest in Mulungu, medium in Maruku, and the lowest in the other sites (P < 0.001 for both). Soil cations (Ca, Mg, K and Na) were significantly higher at Mulungu as compared to the other sites in most of the cases (P < 0.01 for Ca, K and Na; P < 0.001 for Mg). Soil pH was the highest in Mulungu and the lowest in Mashitsi and Maruku.
Table 7.3 Soil characteristics of the fields of the six East African banana germplasm collections, February-March 2007.

<table>
<thead>
<tr>
<th>Location</th>
<th>P  mg/kg</th>
<th>C  %</th>
<th>N  %</th>
<th>Ca  %</th>
<th>K  %</th>
<th>Mg cmol/kg</th>
<th>Na  %</th>
<th>CEC cmol/kg</th>
<th>pH H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mashitsi/ Bu</td>
<td>0.04b</td>
<td>2.3c</td>
<td>0.16c</td>
<td>2.1b</td>
<td>0.2b</td>
<td>1.8b</td>
<td>0.04ab</td>
<td>4.1b</td>
<td>5.5b</td>
</tr>
<tr>
<td>Kisi/ Ke</td>
<td>0.65b</td>
<td>3.3c</td>
<td>0.31bc</td>
<td>9.1b</td>
<td>2.2ab</td>
<td>2.8b</td>
<td>0.06ab</td>
<td>14.2b</td>
<td>6.0ab</td>
</tr>
<tr>
<td>Maruku/ Tz</td>
<td>1.35b</td>
<td>5.6b</td>
<td>0.49b</td>
<td>1.8b</td>
<td>0.9ab</td>
<td>0.8b</td>
<td>0.06ab</td>
<td>3.5b</td>
<td>5.4b</td>
</tr>
<tr>
<td>Mbarara/ Ug</td>
<td>9.23b</td>
<td>1.3c</td>
<td>0.11c</td>
<td>3.2b</td>
<td>0.6b</td>
<td>1.2b</td>
<td>0.03b</td>
<td>5.1b</td>
<td>5.8ab</td>
</tr>
<tr>
<td>Rubona/ Rw</td>
<td>23.9ab</td>
<td>1.7c</td>
<td>0.14c</td>
<td>3.1b</td>
<td>0.6b</td>
<td>1.2b</td>
<td>0.03b</td>
<td>5.0b</td>
<td>5.5ab</td>
</tr>
<tr>
<td>Mulungu/ DRC</td>
<td>118.50a</td>
<td>8.7a</td>
<td>0.79a</td>
<td>38.5a</td>
<td>6.8a</td>
<td>9.7a</td>
<td>0.12a</td>
<td>55.1a</td>
<td>6.5a</td>
</tr>
</tbody>
</table>

P <0.01 <0.001 <0.001 <0.01 <0.01 <0.001 <0.01 <0.001 <0.05

Bu = Burundi; Ke = Kenya; Tz = Tanzania; Ug = Uganda; Rw = Rwanda; DRC = DR Congo. Different letters behind numbers represent significant differences in the same column (P < 0.05).

7.4.2 Root colonization by the AM fungi: effect of environment (location)

Averaged over the five genotypes present in the six locations (Table 7.2), the root colonization significantly (P < 0.001) differed between the locations. The highest root colonization was in Mashitsi, with values of 91.9% and 66.4% for %F and %I, respectively (Fig. 7.2). The lowest %F were recorded in Mbarara and Rubona, with values of 54.6% and 45.5%, respectively, while the lowest %I was observed in Mbarara with values of 25.8%. In the other locations, intermediate values for %F and %I were recorded (Fig. 7.2).
7.4.3 Root colonization by the AM fungi: effect of banana genotype

Within each location, the effect of banana genotype on frequency of root colonization (% F) was highly significant (P < 0.001 for Kisii, Mbarara, Rubona and Mulungu) or significant (P < 0.05 for Mashitsi and Maruku (Table 7.4)). In Mashitsi, the highest (P < 0.05) %F values were observed in FHIA 21 and Mshale genotypes (99.3% and 98.7%, respectively) and the lowest values were in Poyo (72%). In Kisii, significantly (P < 0.001) higher %F values were observed in Kamaramasenge, Poyo and Kayinja (96.7%, 94% and 80.7%, respectively) as compared to Yangambi km 5 (32%). In Maruku, Kayinja had significantly higher (P < 0.05) %F value (94%) as compared to Mshale (56.7%),
Chapter 7. Mycorrhizal status of banana genotypes in East Africa

Gros Michel (65.3%), FHIA 17 (68%) and FHIA 03 (67%). In Mbarara, higher (P < 0.001) %F values were observed in Yangambi km 5 (88%) as compared to Ingenge (6.7%). In Rubona, higher (P < 0.001) %F value was observed in Kayinja (90.7%) as compared to FHIA 17 (5.3%). In Mulungu, Kamaramasenge had higher (P < 0.01) %F (96%) as compared to Intuntu (26.7%). Other clones had intermediate values for %F in each site, respectively.

The effect of banana genotype on %I was significant for all locations (Table 7.5). In Mashitsi, FHIA 21 and FHIA 25 had higher (P < 0.05) %I (79.3% and 74.6%, respectively) as compared to M. balbisiana and Poyo (42.5% and 46.8%, respectively). In Kisii, higher (P < 0.01) %I were recorded in Kamaramasenge and Poyo (51.9% and 52.6%, respectively) as compared to Yangambi km 5 and FHIA 25 (25% and 23.9%, respectively). In Maruku, higher (P < 0.001) %I values was in Kayinja (51.4%) as compared to Intuntu, FHIA 17 and Mshale (13.8%, 18.0% and 20.0%, respectively). In Mbarara, Yangambi km 5 had higher (P < 0.001) %I (39.1%) as compared to Ingenge (10.0%). In Rubona, Kayinja had highest %I (P < 0.01) (52.5%) as compared to FHIA 17 (8.0%). In Mulungu, higher (P < 0.01) %I value was recorded in Kamaramasenge (50.9%) as compared to Intuntu (15.3%). Other clones had intermediate position for %I at each location, respectively. The interaction between environment (location) and banana genotype was highly significant (P < 0.001) for %F and %I.

Variation in both frequency (%F) and intensity (%I) of colonization was lower for Kayinja (10.9% and 19.9%, respectively) compared to FHIA tetraploids, Intuntu and Ingenge (50.0 to 70.8% for %F and 63.1 to 84.6% for %I, respectively). Coefficient of variation of other genotypes was in the range of 27.2-44.7% for %F and 39.0-52.5% for %I, respectively (Fig. 7.3).
Table 7.4 Frequency of the root colonization by the AM fungi in 18 selected genotypes from the six banana germplasm collections of East Africa, February-March 2007: effect of genotype within each location.

<table>
<thead>
<tr>
<th>Cultivar/common name</th>
<th>Genotype/ sub-group</th>
<th>Burundi</th>
<th>Kenya</th>
<th>Tanzania</th>
<th>Uganda</th>
<th>Rwanda</th>
<th>DR Congo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcutta 4</td>
<td>AA M. acum. burm.</td>
<td>96.0ab</td>
<td>66.0ab</td>
<td>56.7d</td>
<td>46.7ab</td>
<td>26.7c</td>
<td></td>
</tr>
<tr>
<td>Mshale</td>
<td>AA Mshale</td>
<td>98.7a</td>
<td>79.0abc</td>
<td>67.0ab</td>
<td>46.7ab</td>
<td>26.7c</td>
<td></td>
</tr>
<tr>
<td>Intuntu</td>
<td>AAA-EA-Beer</td>
<td>96.7ab</td>
<td>65.3abc</td>
<td>89.0ab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ingege</td>
<td>AAA-EA-Nakabululu</td>
<td>86.7abcd</td>
<td></td>
<td>68.0ab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musakala</td>
<td>AAA-EA-Musakala</td>
<td>76.7bcd</td>
<td>87.3abc</td>
<td>54.7ab</td>
<td>38.0ab</td>
<td>65.3abc</td>
<td></td>
</tr>
<tr>
<td>Mbwaziruma</td>
<td>AAA-EA-Nakitembe</td>
<td>95.3ab</td>
<td>82.0abc</td>
<td>54.7ab</td>
<td>44.0ab</td>
<td>88.7ab</td>
<td></td>
</tr>
<tr>
<td>Mutsimawuburo</td>
<td>AAA-EA-Nfuuka</td>
<td>88.7abcd</td>
<td></td>
<td>62.0ab</td>
<td>66.0abc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gros Michel</td>
<td>AAA-Gros Michel</td>
<td>96.0ab</td>
<td>84.7a</td>
<td>65.3dc</td>
<td>41.3ab</td>
<td>35.3bc</td>
<td></td>
</tr>
<tr>
<td>Poyo</td>
<td>AAA-Cavendish</td>
<td>72.0d</td>
<td>94.0a</td>
<td>70.7ab</td>
<td>76.0ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yangambi km 5</td>
<td>AAA-Ibota</td>
<td>97.3ab</td>
<td>80.7abc</td>
<td>88.0a</td>
<td>52.7ab</td>
<td>80.0ab</td>
<td></td>
</tr>
<tr>
<td>Gisukari</td>
<td>AAA-Green Red</td>
<td>97.3ab</td>
<td>89.3ab</td>
<td>29.3ab</td>
<td>38.0ab</td>
<td>80.0ab</td>
<td></td>
</tr>
<tr>
<td>Kamaramasenge</td>
<td>AAB-Ney Poovan</td>
<td>92.0abc</td>
<td>79.0abc</td>
<td>21.0ab</td>
<td>57.3ab</td>
<td>96.0a</td>
<td></td>
</tr>
<tr>
<td>Kayinja</td>
<td>ABB-Pisang Awak</td>
<td>88.0abcd</td>
<td>94.0a</td>
<td>90.7a</td>
<td>88.0ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FHIA 17</td>
<td>AAAA</td>
<td>85.3abcd</td>
<td>68.0bcd</td>
<td>31.3ab</td>
<td>5.3b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FHIA21</td>
<td>AAAB</td>
<td>99.3a</td>
<td></td>
<td>43.3ab</td>
<td>26.7ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FHIA03</td>
<td>AABB</td>
<td>95.3ab</td>
<td>67.0bcd</td>
<td>11.3ab</td>
<td>40.0ab</td>
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<td></td>
</tr>
<tr>
<td>FHIA25</td>
<td>AAA</td>
<td>97.3ab</td>
<td>75.3abcd</td>
<td>58.0ab</td>
<td>29.3ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. balbisiana</td>
<td>BB M. balbisiana</td>
<td>73.3dc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Different letters behind numbers represent significant differences in the same column.
<table>
<thead>
<tr>
<th>Cultivar/common name</th>
<th>Genotype/ sub-group</th>
<th>Burundi</th>
<th>Kenya</th>
<th>Tanzania</th>
<th>Uganda</th>
<th>Rwanda</th>
<th>DR Congo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcutta 4</td>
<td>AA <em>M. acum. burm.</em></td>
<td>61.9abcd</td>
<td>21.6bcde</td>
<td>30.2abc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mshale</td>
<td>AA M. acetabile</td>
<td>66.5ab</td>
<td>38.5abc</td>
<td>20.0b</td>
<td>31.3ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intuntu</td>
<td>AAA-EA-Beer</td>
<td>61.3abcd</td>
<td>13.8b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ingege</td>
<td>AAA-EA-Nakabululu</td>
<td>55.8abcd</td>
<td>10.0e</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musakala</td>
<td>AAA-EA-Musakala</td>
<td>58.7abcd</td>
<td>34.9abc</td>
<td>35.0ab</td>
<td>28.8abc</td>
<td>26.4bc</td>
<td>27.2ab</td>
</tr>
<tr>
<td>Mbwaziruma</td>
<td>AAA-EA-Nakitembe</td>
<td>58.0abcd</td>
<td>33.5ab</td>
<td>21.0bcde</td>
<td>23.8bc</td>
<td>42.1ab</td>
<td></td>
</tr>
<tr>
<td>Mutsimawuburo</td>
<td>AAA-EA-Nfuuka</td>
<td>50.9bcd</td>
<td>22.8bcd</td>
<td>33.3abc</td>
<td>38.5ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gros Michel</td>
<td>AAA-Gros Michel</td>
<td>67.3ab</td>
<td>38.4abc</td>
<td>27.9ab</td>
<td>31.8ab</td>
<td>16.3dc</td>
<td>26.8ab</td>
</tr>
<tr>
<td>Poyo</td>
<td>AAA-Cavendish</td>
<td>46.8dc</td>
<td>52.6a</td>
<td></td>
<td>23.5bcd</td>
<td>40.9ab</td>
<td>50.9a</td>
</tr>
<tr>
<td>Yangambi km 5</td>
<td>AAA-Ibota</td>
<td>68.7ab</td>
<td>25.0c</td>
<td>33.1ab</td>
<td>39.1a</td>
<td>40.2abc</td>
<td>40.2ab</td>
</tr>
<tr>
<td>Gisukari</td>
<td>AAA-Green Red</td>
<td>72.9ab</td>
<td>36.6abc</td>
<td>32.9ab</td>
<td>12.2ed</td>
<td>34.3abc</td>
<td>40.2ab</td>
</tr>
<tr>
<td>Kamaramasenge</td>
<td>AAB-Ney Poovan</td>
<td>64.5abc</td>
<td>51.9a</td>
<td>38.9ab</td>
<td>17.1cde</td>
<td>36.4abc</td>
<td>46.9ab</td>
</tr>
<tr>
<td>Kayinja</td>
<td>ABB-Pisang Awak</td>
<td>64.9abc</td>
<td>42.4ab</td>
<td>51.4a</td>
<td>52.5a</td>
<td>46.4ab</td>
<td></td>
</tr>
<tr>
<td>FHIA 17</td>
<td>AAAA</td>
<td>68.0ab</td>
<td>30.1bc</td>
<td>18.0b</td>
<td>12.7ed</td>
<td>8.0d</td>
<td></td>
</tr>
<tr>
<td>FHIA21</td>
<td>AABAB</td>
<td>79.3a</td>
<td>22.7bcd</td>
<td>21.4bcd</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FHIA03</td>
<td>AABBB</td>
<td>72.0ab</td>
<td>21.8ab</td>
<td>11.1e</td>
<td>20.3bc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FHIA25</td>
<td>AAA</td>
<td>74.6a</td>
<td>23.9c</td>
<td>31.7ab</td>
<td>34.4ab</td>
<td>16.4dc</td>
<td></td>
</tr>
<tr>
<td><em>M. balbisiana</em></td>
<td>BB <em>M. balbisiana</em></td>
<td>42.5d</td>
<td>19.2bcd</td>
<td>55.9a</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Different letters behind numbers represent significant differences in the same column.
Figure 7.3 Variability of root colonization in the 18 selected genotypes from the six banana germplasm collections of East Africa, February-March 2007.

### 7.4.4 Relationship between root colonization and other variables

Whatever the location, the %F was positively correlated with mean annual rainfall for the last 2 years ($r^2=0.24$, $P < 0.001$) and 3-last-month rainfall ($r^2=0.46$, $P < 0.001$), while %I was only significantly correlated with the latter ($r^2=0.27$, $P < 0.001$). Soil P, C, CEC and pH, 3-last month rainfall and number of functional roots explained 38.4% of variation in %F and 56.1% of variation in %I. Soil P, C and pH were negatively and CEC, 3-last month rainfall and number of functional roots were positively associated with increased %F and %I (Table 7.6).
Table 7.6 Regression analysis of variables associated with frequency and intensity of root colonization by the AM fungi in six banana germplasm collections of East African region, February-March 2007.

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Parameter estimate</th>
<th>Partial R²</th>
<th>Model R²</th>
<th>Pr &gt; F</th>
<th>Explanatory variable</th>
<th>Parameter estimate</th>
<th>Partial R²</th>
<th>Model R²</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil P, mg/kg</td>
<td>-0.82</td>
<td>0.22%</td>
<td>0.22%</td>
<td>0.0022</td>
<td>Soil P, mg/kg</td>
<td>-0.45</td>
<td>0.73%</td>
<td>0.73%</td>
<td>0.0040</td>
</tr>
<tr>
<td>3 month rain, mm</td>
<td>0.14</td>
<td>20.87%</td>
<td>21.09%</td>
<td>&lt;0.0001</td>
<td>3-month rain, mm</td>
<td>0.16</td>
<td>5.42%</td>
<td>6.15%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Soil C, %</td>
<td>-13.88</td>
<td>1.85%</td>
<td>22.94%</td>
<td>&lt;0.0001</td>
<td>Soil C, %</td>
<td>-22.13</td>
<td>14.15%</td>
<td>20.30%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>pH</td>
<td>-47.87</td>
<td>1.15%</td>
<td>24.09%</td>
<td>&lt;0.0001</td>
<td>pH</td>
<td>-52.48</td>
<td>3.97%</td>
<td>24.27%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CEC, cmol/kg</td>
<td>4.86</td>
<td>13.66%</td>
<td>37.75%</td>
<td>&lt;0.0001</td>
<td>CEC, cmol/kg</td>
<td>5.08</td>
<td>31.13%</td>
<td>55.40%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NFR</td>
<td>0.44</td>
<td>0.62%</td>
<td>38.37%</td>
<td>0.1170</td>
<td>NFR</td>
<td>0.32</td>
<td>0.65%</td>
<td>56.05%</td>
<td>0.0577</td>
</tr>
</tbody>
</table>

CEC = Cation exchange capacity, cmol/kg soil; NFR = number of functional roots
7.5 Discussions

Our study reported large differences in mycorrhizal status between the genotypes within and between locations. Root colonization and plant mycorrhizal dependency may be genotype- and environment-dependent traits (Fitter & Merryweather, 1992; Smith & Read, 2008). Earlier workers reported about genotype related differences in both these parameters in greenhouse experiments (Declerck et al., 1995; Elsen et al., 2003a). In our study, frequency and intensity of root colonization significantly differed between the genotypes grown under the same soil conditions (i.e. within the same location). However, within the five genotypes common to all sites, the differences in root colonization were not statistically significant; meaning that, for the five genotypes present in all locations, level of root colonization is probably more dependent on the environmental conditions than on genotype.

Another factor which could have affected root colonization is the size of the AM fungal population in soil. Although we did not estimate this parameter in this study, our earlier report showed significant differences in AM fungal population density between different soil types (Gaidashova et al., accepted). The same authors reported significant correlations existing between the size of infectious AM fungal population and frequency of root colonization. Larger sample size, repeated seasonal examination of roots and soil for the size of infectious AM fungal population presence and dynamics may be required to understand better the mechanisms by which environment conditions and AM fungal population densities control the extent of root colonization.

In our study, Kayinja was the only genotype with markedly lower variation and consistently high values of %F and %I as compared to all other genotypes studied. It has low contribution of tertiary roots to total root length (Stoffelen,
and lower concentrations of nutrients (e.g. foliar N) (Van Asten & Gaidashova, unpublished). Earlier investigations in greenhouse pot experiments reported large and significant differences in root colonization between different Musa genotypes at early growth stages (Declerck et al., 1995; Elsen et al., 2003a) and under identical controlled conditions. Root colonization varied with the characteristics of the root system (Declerck et al., 1995), which are genotype and environment dependent traits in banana (Delvaux & Guyot, 1989; Stoffelen, 2000; Draye, 2002).

Characteristics of the root system contributed to plant mycorrhizal dependency (Declerck et al., 1995). For example, lower density of root and root hairs and shorter root length were characteristics typical for genotypes with high mycorrhizal dependency (Declerck et al., 1995). In our study, however, the genotypes with strong root systems which were known for this reason for their resistance to plant parasitic nematodes (Stoffelen, 2000), such as Gros Michel and Yangambi km 5, were colonized as much as Musakala. This genotype has less extended root system compared to Gros Michel and Yangambi km 5 (Sebuwufu et al., 2004), and it is known in the EAH for its poor tolerance of intercropping (Gaidashova et al., 2005). This may suggest that the size of the root system has possibly a limited effect on root colonization.

The observed large differences in root colonization of the cultivars present in all locations confirm the strong impact of environment. Because climatic conditions and soil properties of the selected locations markedly differed, root system performance may have been affected to large extent, which has in turn determined root-AM fungi interaction. Since both – AM fungi and root are subjected to environment, any factor that influences the rate of root growth and
plant nutrition, will inevitably influence percent of root colonization (Smith & Read, 2008).

The fact, that highly significant positive correlations were observed in our study between %F and %I of root colonization and 2-year mean of annual rainfall; and more stronger correlations between %F and %I, and 3-last-month rain, suggests that root growth dynamics was certainly affected by quantity of water available in soil. The observations of Adriano-Anaya et al. (2006) about the highest root colonization during the dry season and in the beginning of rainy season are possibly also related to root growth dynamics.

The fact that different banana genotypes exhibited high variation in %F and %I under a range of ecologies (6 locations in the EAH) may be a sign of genotype-related plasticity in banana genotype adaptability to different ecological conditions.

Environmental factors explained higher part of variation in %I (56.1%) compared to %F (38.4%). Possibly, the latter is more dependent on ‘internal’ conditions of the rhizosphere: chance of infection to take place in root, root growth pattern, extent of hyphal network in soil etc. The positive association of root colonization %F and %I with soil acidity and the negative association with available soil P soil was mentioned in previous studies (Declerck et al., 1999).

7.6 Conclusion

Our study provided the first report on root colonization by the AM fungi in a wide range of banana clones in EAH ecologies. The results suggested that (i) high receptivity but low banana genotype specificity to the AM fungi exist among the five genotypes common in all sites; (ii) high variability in root colonization exists within the site among a wide range of *Musa* genotypes: from
Chapter 7. Mycorrhizal status of banana genotypes in East Africa

AA, AAA to AAB, ABB and tetraploids. The latter suggests about strong genotype-environmental interactions that affect banana root system and development of the AM symbiosis. Further research should investigate these interactions to envision appropriate selection strategies for banana genotypes and AM fungi. Some banana genotypes may be the most adequate whatever the soil conditions and may thus be of interest. Conversely, some other genotypes may be more interesting in some regions and others in other regions. The strategy would be to use different genotypes in different regions.
Chapter 8. Effect of indigenous arbuscular mycorrhizal fungi on growth and roots of banana (Musa spp.) grown in three different soils
8.1 Abstract

Arbuscular mycorrhizal (AM) fungi are known to improve plant growth during the early stages of development. This effect may vary depending on soil type. Here we reported the effect of inoculation with indigenous AM fungi isolated from a Nitisol in Kibungo (Rwanda) on the growth and root characteristics of three banana genotypes: FHIA 17 (AAAA), Musakala (AAA-EA) and Sukali Ndiizi (AAB), grown in pasteurized and non-pasteurized Acrisol, Ferralsol and Nitisol. Root characteristics differed significantly between soil types (P < 0.001) and banana genotype (P < 0.05). The poorest root development was observed on Acrisol and the largest on Nitisol whatever the genotypes. Musakala had a smaller root system compared to FHIA 17 and Sukali Ndiizi. Inoculation resulted in highly significant (P < 0.001) differences in frequency of arbuscules (FA) between soil types in all genotypes and treatments. The highest FA was observed in Nitisol and the lowest in Acrisol. Inoculation increased plant growth and dry biomass (P < 0.05) but its effect was less marked in non-pasteurized treatments as compared to pasteurized treatments in all soils and genotypes. The exception was Ferralsol where pasteurization did not result in significant increase of plant growth. Inoculation increased plant growth most in poor Acrisol. This was observed in all genotypes and treatments. This was most probably linked to greater limitations in root growth in Acrisol. Poorer root development of Musakala coincided with its highest response to the AM fungi inoculation compared to other genotypes, which suggested higher AMF dependency.
8.2 Introduction

The important role of East African Highland bananas (Musa spp., AAA-EA) as a staple crop, cash crop and essential element in landscape and soil conservation in the East African Highlands (EAH) was stressed by different authors (Davies, 1995; Karamura et al., 1999; Rishirumuhirwa, 1997; Kangasniemi, 1998). However, declining yields throughout the region compromise production (Okech et al., 2004; 2005; Baijukya et al., 2005; Macharia et al., 2008). Among the major constraints causing yield decline are the low inherent soil fertility (Sanchez et al., 1997), inadequate soil management (Bekunda et al., 2002), nutrient mining (Van Asten et al., 2004, 2006) and pests and diseases pressure (Tushemereirwe & Bagabe, 1998; Okech et al., 2002; Mwangi & Nakato, 2007).

To overcome some of these production constraints, the application of soil beneficial microorganisms such as arbuscular mycorrhizal (AM) fungi has received increasing attention (Jaizme-Vega & Azcón, 1995; Declerck et al., 2002a; Jefwa et al., 2008). The potential role of AM fungi to address plant nutrient constraints in the EAH cropping systems may be particularly high (Jefwa et al., 2008), since these systems do normally not receive chemical input and are, therefore, more AM fungi friendly (Jansa et al., 2006; Adriano-Anaya et al., 2006).

In the EAH region, a high range of soil diversity is found (Eswaran et al., 1989), and bananas are grown under wide range of agro-ecological conditions (Davies, 1995).

A positive effect of AM fungi on banana growth was shown in controlled conditions (Jaizme-Vega & Azcon, 1995; Yano-Melo et al., 1999; Thaker & Jasrai, 2002). The magnitude of the effect of the AM fungi on growth of annual
crops was dependent on soil type (Plenchette, 1989, 2000). However, it is still unknown how the response of banana on AM fungal inoculation is modulated in different soil types.

In the present study, we investigated the effects of soil inoculation with AM fungi on banana growth parameters in three soils differing in physico-chemical properties. Soils were either pasteurized or non-pasteurized. We evaluated the impact of (1) natural microflora (non-pasteurized soil non inoculated; NP-NI) alone or (2) in combination with introduced inoculum (non-pasteurized soil inoculated; NP-I), (3) inoculated AM fungi alone (pasteurized soil inoculated; P-I) and (4) absence of inoculum (pasteurized soil non inoculated; P-NI).

8.3 Material & methods

8.3.1 Experimental lay out

The experiment consisted of four treatments with the three soils (Acrisol (A), Ferralsol (F) and Nitisol (N)), pasteurized (P) or non-pasteurized (NP) and the plants inoculated (I) or non inoculated (NI) with a population of AM fungi. Ten replicates were used per treatment and the plants were arranged in a complete randomized design.

8.3.2 Plant material

Tissue-culture plantlets were supplied by the Agro-Genetic Technologies laboratory in Uganda. Plantlets belonged to three Musa spp. genotypes: FHIA 17 (AAAA), Musakala (AAA-EA) and Sukali Ndiizi (AAB). The first genotype (FHIA 17) is an improved banana variety recently introduced in the EAH, while
Musakala and Sukali Ndiizi are traditionally (i.e. > 50 years) grown cultivars in this region.

8.3.3 Soils

The three different soils used in this experiment originated from Rwanda: i.e. an Acrisol from Butare, a Ferralsol from Gashonga and a Nitisol from Kibungo. These soils were characterized earlier for fertility, nematode infestation by *P. goodeyi* and AM fungi populations in soil and colonization in banana roots (Chapters 2 and 5).

8.3.4 Soil analysis

Three soil samples of 200 g were taken per treatment and soil type. They were analyzed for major nutrients (N, P, K) to observe the physico-chemical changes caused by soil pasteurization (100°C for 1 h). After air drying during 3 days, soil samples were oven dried at 40°C for 24 hours, ground and sieved on a 2 mm mesh. Soil pH was measured in 1/2.5 ratio of sediment/water suspension as described in Okalebo *et al.* (2003). Total N was measured using a spectrophotometer in a sulphuric acid and selenium acid extract as described in Okalebo *et al.* (2003). Available P was extracted using Mehlich-3 solution (Mehlich 1984) prior to colorimetric determination. Exchangeable cations (Ca, Mg, K and Zn) were extracted using 1 M ammonium acetate and then determined using an Atomic Absorption Spectrophotometer (AAS).
Chapter 8. Effect of AM fungi on banana growth in three soils

8.3.5 Experiment set up and inoculation

The experiment was conducted at ISAR-Rubona Research station in Rwanda. Prior to planting, rooted plantlets were removed from flasks and the rooting medium was carefully washed off. Plantlets were transplanted in pots (15 cm diameter, 15 cm height) filled with 800 g of pasteurized or non pasteurized soil under greenhouse conditions (22/18°C average day/night temperature and 12 hours daylight). During the first four weeks, plants were covered with plastic cups and sprayed with sterilized water. From the fifth week, cups were removed and plants were watered (50 ml per plant) regularly (each 1-3 days, depending on soil desiccation). Half of the plants were subsequently inoculated with a mix of the native AM fungi species isolated from a farm at Kibungo (Rwanda) and multiplied on leek, as trap plant, at UCL, Belgium.

Plants were inoculated after removal of medium when they were first planted in soil substrate. Inoculum consisted of a population of species belonging to Glomus, Acaulospora, Scutellospora and Gigaspora genera (Chapter 5). Leek roots were colonized by the AM fungi population with 89% frequency and 72.5% intensity. The inoculated plants received 1 g fresh leek root pieces and 30 g sand containing spores (number not estimated) and root fragments from leek culture. The non-inoculated plants also received 1 g of boiled leek roots and 30 g of pasteurized sand.

8.3.6 Data collection at harvest

Plants were harvested after 20 weeks from planting date. Data were recorded at harvest on plant growth (plant height, number of functional leaves, leaf length of the 3\textsuperscript{rd} opened leaf), biomass (fresh and dry shoot weight and fresh root weight) and root characteristics (number and length of primary roots).
Fine root samples were placed in 10 % KOH solution for 1 hour at 80 °C. Roots were subsequently bleached during 30 min in 3 % H₂O₂ solution freshly alkalinized by NH₄OH (8 ml of 25 % NH₄OH for 100 ml 3 % H₂O₂) as described by Koske & Gemma (1989). After bleaching, roots were stained in acidified ink solution (20 ml of permanent ink “Parker” in 1 l of 1 % HCl) overnight (Vierheilig et al. 1998). Stained root samples were de-stained in water for 1 hour and observed under compound microscope (Leica DME) at 200 X magnification. Frequency and intensity of arbuscules, vesicles and overall root colonization (i.e. arbuscules, vesicles and hyphae combined), was evaluated from fifty root fragments (1-cm length) for each sample. Frequency was estimated as the percentage of root fragments containing the respective AM fungi structures. Intensity, which is the abundance of (1) hyphae, (2) vesicles, (3) arbuscules or all (1-3) in roots, was assessed as described in Plenchette & Morel (1996).

8.3.7 Statistical data analysis

The variables were transformed to achieve normality: (i) arcsin transformation for percentages and (ii) power transformations for growth variables. Analysis of variance under General Linear Model and Scheffe’s multiple comparison test was used to test significance of the differences observed and to separate homogenous groups. SAS 9.1 Enterprise Guide 4 statistical software was used for the above-mentioned analytical steps.
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8.4 Results

8.4.1 Soil fertility in pasteurized and non-pasteurized soils

Soil pasteurization did not affect soil nutrient content significantly except organic matter in Nitisol which was slightly but significantly (P < 0.05) reduced in pasteurized soil (Table 8.1).

Table 8.1 Soil fertility characteristics in pasteurized and non-pasteurized soils.

<table>
<thead>
<tr>
<th>Soil treatment</th>
<th>pH</th>
<th>OM (%)</th>
<th>N (cmol⁺/kg)</th>
<th>Ca (cmol⁺/kg)</th>
<th>Mg (cmol⁺/kg)</th>
<th>K (cmol⁺/kg)</th>
<th>p (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasteurised Acrisol</td>
<td>6.53</td>
<td>4.65</td>
<td>0.24</td>
<td>3.23</td>
<td>1.45</td>
<td>0.37</td>
<td>48.4</td>
</tr>
<tr>
<td>Non-pasteurised Acrisol</td>
<td>6.50</td>
<td>4.45</td>
<td>0.23</td>
<td>2.91</td>
<td>1.36</td>
<td>0.33</td>
<td>30.0</td>
</tr>
<tr>
<td>Pasteurised Ferralsol</td>
<td>5.73</td>
<td>3.36</td>
<td>0.19</td>
<td>1.76</td>
<td>1.14</td>
<td>0.66</td>
<td>12.9</td>
</tr>
<tr>
<td>Non-pasteurised Ferralsol</td>
<td>5.70</td>
<td>3.32</td>
<td>0.19</td>
<td>1.74</td>
<td>1.12</td>
<td>0.63</td>
<td>12.7</td>
</tr>
<tr>
<td>Pasteurised Nitisol</td>
<td>6.57</td>
<td>6.32</td>
<td>0.32</td>
<td>6.71</td>
<td>2.36</td>
<td>0.58</td>
<td>12.9</td>
</tr>
<tr>
<td>Non-pasteurised Nitisol</td>
<td>6.53</td>
<td>6.73</td>
<td>0.31</td>
<td>6.61</td>
<td>2.28</td>
<td>0.52</td>
<td>2.82</td>
</tr>
</tbody>
</table>

OM = organic matter content; NS = Not significant at P = 0.05.

8.4.2 AM fungal population density in the three selected soils

Infectious propagule density of the AM fungi in the three selected soils was determined using the MPN method (see Chapter 5 for details). Ferralsol from Gashonga contained significantly (P < 0.05) higher soil densities of AM fungi propagules as compared to Acrisol and Ferrisol (Table 8.2).
Chapter 8. Effect of AM fungi on banana growth in three soils

Table 8.2 AM fungal population density in three selected soils of Rwanda.

<table>
<thead>
<tr>
<th>Eco-region</th>
<th>Number of infective propagules/100 g soil</th>
<th>95% confidence intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrisol (Butare)</td>
<td>2.0 c</td>
<td>0.9 – 4.5</td>
</tr>
<tr>
<td>Ferralsol (Gashonga)</td>
<td>59.8 a</td>
<td>26.8 – 133.4</td>
</tr>
<tr>
<td>Nitisol (Kibungo)</td>
<td>8.5 bc</td>
<td>3.8 – 19.0</td>
</tr>
</tbody>
</table>

The letters a, b, c mean significant differences (P < 0.05).

8.4.3 Root development

Root characteristics differed significantly between soil types (P < 0.001) and banana genotype (P < 0.05). Whatever the genotype, the poorest root development was observed on Acrisol and the largest on Nitisol (Table 8.3). Musakala had smaller root system compared to FHIA 17 and Sukali Ndiizi: i.e. the number of primary roots was significantly lower on the Ferralsol (P < 0.001) and Nitisol (P < 0.05). Identically, fresh root weights were significantly (P < 0.05) lower on these two soils (Table 8.3).
8.4.4 Root colonization by the AM fungi in different soils

Highly significant (P < 0.001) differences in root colonization between the soils were observed in all the P-I, NP-I, NP-NI treatments. Irrespective of the genotype, the frequency of arbuscules (FA) was the highest (more 70%) in plants grown on Nitisol (Table 8.4). Similarly, plants grown on Acrisol exhibited significantly (P < 0.001) lower FA in all the I-P, I-NP, NI-NP treatments as compared to the plants, grown on Nitisol.
Chapter 8. Effect of AM fungi on banana growth in three soils

Table 8.4 Frequency of arbuscules (FA) in banana roots grown in different soils at 20 weeks after inoculation.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>Acrisol</th>
<th>Ferralsol</th>
<th>Nitisol</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I-P</td>
<td>31.2B</td>
<td>16.6B</td>
<td>92.2A</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FHIA 17</td>
<td>I-NP</td>
<td>33.3C</td>
<td>65.0B</td>
<td>84.0A</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(AAAA)</td>
<td>NI-P</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>NE</td>
</tr>
<tr>
<td></td>
<td>NI-NP</td>
<td>3.6B</td>
<td>80.0A</td>
<td>69.2A</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>I-P</td>
<td>5.0B</td>
<td>4.8B</td>
<td>79.2A</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Musakala</td>
<td>I-NP</td>
<td>23.9B</td>
<td>62.4A</td>
<td>70.2A</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>(AAA-EA)</td>
<td>NI-P</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>NE</td>
</tr>
<tr>
<td></td>
<td>NI-NP</td>
<td>3.3B</td>
<td>73.8A</td>
<td>82.8A</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>I-P</td>
<td>30.2B</td>
<td>19.6B</td>
<td>96.5A</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Sukali</td>
<td>I-NP</td>
<td>11.7C</td>
<td>76.6B</td>
<td>97.2A</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>(AAB)</td>
<td>NI-P</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>NE</td>
</tr>
<tr>
<td></td>
<td>NI-NP</td>
<td>0.0C</td>
<td>61.3B</td>
<td>90.5A</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

NE = non-estimated. A, B, C = significant differences in rows at P < 0.05.

Plants grown on Ferralsol had consistently (what means consistently – does it mean significantly. If yes use this term. If no, then there is not difference) higher FA (61.3 – 80%) in NP-I and NP-NI treatments and lower FA (4.8 – 19.6%) in P-I treatments irrespective of genotype (Table 8.4, Fig. 8.1).

On Acrisol, plants of FHIA 17 and S. Ndiizi in P-I and NP-I treatments were significantly (P < 0.001) better colonized by the AM fungi (=higher FA) as compared to those in NP-NI treatments, while Musakala plants only had significantly (P < 0.001) higher FA in NP-I as compared to other treatments (Fig. 8.1).
On the Ferralsol, inoculation with the AM fungi in pasteurized (P-I and P-NI) treatments significantly increased FA in roots of FHIA 17 and S. Ndiizi, but inoculation had no effect in Musakala (Fig. 8.1).

On Nitisol, FHIA 17 plants had significantly ($P < 0.001$) higher FA in all inoculated treatments while FA in these treatments was of similar range in Musakala and S. Ndiizi as that of NP-NI treatments (Fig. 8.1).
Figure 8.1 Root colonization in different treatments at 20 weeks after inoculation. Letters above bars mean significant differences (P < 0.05) between treatments on the same soil.
Table 8.5 Growth characteristics and biomass of banana plants after 20 weeks of pot growth on Acrisol.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>Height, cm</th>
<th>1st leaf length, cm</th>
<th>Primary root</th>
<th>Shoot</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Number</td>
<td>Length, cm</td>
<td>FW, g</td>
</tr>
<tr>
<td>FHIA 17 (AAAA)</td>
<td>I-P</td>
<td>13.9A</td>
<td>19.0A</td>
<td>12.8A</td>
<td>107.0A</td>
<td>24.3A</td>
</tr>
<tr>
<td></td>
<td>I-NP</td>
<td>5.7B</td>
<td>10.0B</td>
<td>12.0A</td>
<td>68.7AB</td>
<td>6.8B</td>
</tr>
<tr>
<td></td>
<td>NI-P</td>
<td>11.6A</td>
<td>17.4A</td>
<td>8.1B</td>
<td>59.9AB</td>
<td>17.3A</td>
</tr>
<tr>
<td></td>
<td>NI-NP</td>
<td>5.9B</td>
<td>11.1B</td>
<td>7.3B</td>
<td>36.2B</td>
<td>5.2B</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.0001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

| Musakala (AAA-EA)       | I-P       | 19.2A      | 24.5A               | 14.9A        | 94.4A     | 28.4A | 4.0A  | 6.7A  |
|                         | I-NP      | 6.8B       | 9.7B                | 9.4B         | 42.9B     | 3.6B  | 0.6B  | 0.9B  |
|                         | NI-P      | 5.6B       | 14.5B               | 5.0C         | 59.4AB    | 1.7B  | 0.5B  | 1.2B  |
|                         | NI-NP     | 7.2B       | 12.3B               | 6.0C         | 22.0C     | 2.9B  | 0.3B  | 0.5B  |
| P                       | < 0.0001  | <0.0001    | <0.0001             | <0.0001      | <0.0001   | <0.0001| <0.001|

| Sukali Ndiizi (AAB)     | I-P       | 17.7A      | 22.8                | 14.9A        | 82.5A     | 22.1A | 3.8A  | 4.1A  |
|                         | I-NP      | 6.8B       | 10.2                | 7.8B         | 30.4B     | 3.3C  | 0.6C  | 0.7B  |
|                         | NI-P      | 14.6A      | 19.2                | 10.3B        | 73.2A     | 13.3B | 2.3B  | 3.7A  |
|                         | NI-NP     | 6.0B       | 8.6                 | 6.3B         | 18.7B     | 2.0C  | 0.8C  | 0.4B  |
| P                       | < 0.0001  | NS         | <0.01               | <0.01        | <0.0001   | <0.0001| <0.0001|

NFL = number of functional leaves; 1st leaf L = length of the 1st opened leaf; FW = fresh weight; DW = dry weight. The letters A, B, C show significant (P < 0.05) differences in columns.
Table 8.6 Growth characteristics and biomass of banana plants after 20 weeks of pot growth on Ferralsol.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>Height, cm</th>
<th>NFL</th>
<th>1st leaf L, cm</th>
<th>NPR</th>
<th>LPR, cm</th>
<th>FSB, g</th>
<th>DSB, g</th>
<th>FRB, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHIA 17 (AAAA)</td>
<td>I-P</td>
<td>13.3B</td>
<td>5.4A</td>
<td>21.0A</td>
<td>15.0A</td>
<td>203.2A</td>
<td>39.6A</td>
<td>2.9A</td>
<td>25.8A</td>
</tr>
<tr>
<td></td>
<td>I-NP</td>
<td>10.0C</td>
<td>5.0A</td>
<td>15.9B</td>
<td>10.4B</td>
<td>138.6B</td>
<td>17.1B</td>
<td>1.1B</td>
<td>8.6B</td>
</tr>
<tr>
<td></td>
<td>NI-P</td>
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<td>4.0B</td>
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<td>2.8A</td>
<td>26.3A</td>
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<td>3.9B</td>
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<td>4.0</td>
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<td>4.0</td>
<td>24.5A</td>
<td>16.1A</td>
<td>236.4A</td>
<td>54.3A</td>
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</table>

NFL = number of functional leaves; 1st leaf L = length of the 1st opened leaf; NPR = number of primary roots; LPR = length of primary roots; FSB = fresh shoot biomass; DSB = dry shoot biomass; FRB = fresh root biomass. The letters A, B, C show significant (P < 0.05) differences in columns.
Table 8.7 Growth characteristics and biomass of banana plants after 20 weeks of pot growth on Nitisol.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>Height, cm</th>
<th>NFL</th>
<th>1st leaf L, cm</th>
<th>NPR</th>
<th>LPR, cm</th>
<th>FSB, g</th>
<th>DSB, g</th>
<th>FRB, g</th>
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<tr>
<td>FHIA 17 (AAAA)</td>
<td>I-P</td>
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<td>6.2</td>
<td>23.8A</td>
<td>14.2A</td>
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</tr>
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<td>2.5A</td>
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</tr>
<tr>
<td></td>
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<td>10.7BC</td>
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<tr>
<td>Musakala (AAA-EA)</td>
<td>I-P</td>
<td>18.7A</td>
<td>6.6A</td>
<td>25.8A</td>
<td>12.7A</td>
<td>275.9A</td>
<td>41.7A</td>
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<td>11.0C</td>
<td>6.2A</td>
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<tr>
<td>Sukali Ndiizi (AAB)</td>
<td>I-P</td>
<td>21.6A</td>
<td>5.4</td>
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</table>

NFL = number of functional leaves; 1st leaf L = length of the 1st opened leaf; NPR = number of primary roots; LPR = length of primary roots; FSB = fresh shoot biomass; DSB = dry shoot biomass; FRB = fresh root biomass. The letters A, B, C show significant (P < 0.05) differences in columns.
Chapter 8. Effect of AM fungi on banana growth in three soils

8.4.5 Effect of inoculation on banana growth and biomass

In the Acrisol, inoculation with the AM fungi in the pasteurized (P-I and P-NI) substrate significantly increased plant height, length of the 1st leaf, number and length of primary roots, fresh weight of roots and shoots, and dry aboveground biomass for all genotypes as compared to other treatments (Table 8.5). In NP-I treatments, FHIA 17 and Musakala produced significantly more primary roots than in P-NI and NP-NI treatments, but they had similar growth characteristics as the non-inoculated (P-NI and NP-NI) plants (Table 8.5). Inoculation increased root length in Musakala irrespective of pasteurization (Table 8.5).

In the Ferralsol, inoculation did not produce significant increase of height, leaf length, number and length of primary roots, fresh root and shoot biomass and dry shoot biomass. This was irrespective of substrate pasteurization in all genotypes. The only exception was the number of functional leaves in FHIA 17 and Musakala, which was greater in inoculated (P-I and NP-I) than non-inoculated (P-NI and NP-NI) treatments (Table 8.6).

In the Nitisol, inoculation increased height of FHIA 17 and Musakala but not Sukali Ndiizi (P < 0.001 for all) (Table 8.7). Inoculation of FHIA 17 resulted in longer leaves and greater fresh shoot biomass (P < 0.001) (Table 8.7). Inoculation of Musakala increased number of leaves in P-I treatments (P < 0.05), number of primary roots in NP-I treatments (P < 0.001), root length irrespective of pasteurization (P < 0.001), dry shoot and fresh root biomass in NP-I treatments (P < 0.001 for both) (Table 8.7). Effect of inoculation in S. Ndiizi plants resumed in greater number and length of primary roots in P-I treatments (P < 0.01), while that in NP-I treatments resulted in greater fresh shoot biomass (P < 0.001) (Table 8.7).
Relative mycorrhizal dependency (RMD) varied with pasteurization, soil type and genotype. In pasteurized treatments, the highest RMD was observed in Acrisol (30 to 50%), followed by Nitisol (-10 to 20%) and the lowest was in Ferralsol (-35 to 5%). In non-pasteurized treatments, RMD values were variable with a larger magnitude as compared to pasteurized treatments: from -45 to 45% in Acrisol, -93.8 to 15% in Ferralsol, and -7 to 50% in Nitisol (Fig. 8.2).

In Acrisol, inoculation increased the most the RMD of Musakala: by 52.1% and 48.5% in P-I and NP-I treatments compared to other genotypes with half or lesser RMD values (Fig. 8.2). In Ferralsol, inoculation resulted mostly in negative RMD values for all three genotypes in both P-I and NP-I treatments, while Musakala had the lowest RMD value (-93.8%) in NP-I treatment (Fig. 8.2). In Nitisol, amongst the three genotypes, the highest RMD value (52.4%) was in Musakala in NP-I treatments, with lower values for other genotypes (21.9-26.4% for S. Ndiizi and -7 – 15.2% for FHIA 17).
Chapter 8. Effect of AM fungi on banana growth in three soils

Figure 8.2 Relative mycorrhizal dependency (expressed in %) of three banana genotypes grown on three different soils (Acrisol, Ferralsol and Nitisol) in pasteurized and non-pasteurized treatments.
8.5 Discussion

The lower level of root colonization in inoculated treatments on Acrisol may be related to the physical and biological properties of this soil. The first may concerns coarser texture of Acrisol, which limits water holding capacity of this soil and therefore root growth as suggested by our results. The second regards the size and the activity of soil microbial communities present in soil, including natural AM fungal populations. Although limited research was conducted to investigate the effect of environmental factors on the AM colonization in roots (Smith & Read, 2008), the studies of infectious AM fungi population densities in soil showed lower AM fungi populations in sandy soils (Declerck et al., 1999), and higher AM fungi populations in clay soils (Plenchette, 2000). Our results are in line with this trend, since plants grown on the more clayey Ferralsol and Nitisol had higher root colonization compared to the sandier Acrisol. The fact that these clayey soils were also low in P has probably favoured the establishment of the AM association (Smith & Read, 2008).

Variations in trends of root colonization observed between pasteurized and non-pasteurized treatments suggested that the native AM fungi present in non-pasteurized soil have possibly affected root colonization. In Acrisol, the generally similar positive effect of inoculation on root colonization by the AM fungi (FA) irrespective of pasteurization suggested that the size of the native population of the AM fungi was low and its effect on inoculum was minimal. This was very different in the Ferralsol, where the frequency of arbuscules was very low in sterile and very high in non-sterile treatments. This suggested that the native AMF population was important and its effect surpassed the effect of the introduced inoculum. A high infective propagule density in the soil, as observed in our Ferralsol (Chapter 5), increased the percentage of root
colonization (Smith & Smith, 1981; Smith & Read, 2008). Alternatively, a low infective propagule density as observed in Acrisol (Chapter 5), led to poor root colonization. On the Nitisol, lack of significant differences in FA between inoculated pasteurized and non-pasteurized treatments may signify the highest ‘compatibility’ between the introduced and native inoculum. This would be logical, since the introduced inoculum was isolated from the soil sample originated from a banana grown on Nitisol (Rwanda, Kibungo), a location close to where Nitisol soil substrate was collected for this experiment.

Various shoot and root growth characteristics showed a strong positive response to AM fungi inoculation, which is in line with existing literature (e.g., Declerck et al., 1995; Jaizme-Vega & Azcon, 1995; Yano-Melo et al., 1999; Thaker & Jasrai, 2002). The magnitude of this increase was largest on poor soil (Acrisol), where even modest colonization levels were associated with the highest plant response in growth.

Genotype seems also to affect the magnitude of the plant response to the AM fungi, both in terms of plant growth and AM fungi colonization in roots. For example, Musakala seemed particularly responsive to AMF than FHIA-17 and Sukali Ndiizi. Declerck et al. (1995) already demonstrated that relative mycorrhizal dependency was cultivar dependent in banana, and that it was related to cultivar-specific characteristics in root hair development. Increased root branching may also increase the chances of encounters between roots and infective hyphae (Koske & Gemma, 1992). In our study, slightly higher colonization was observed in FHIA 17 and S. Ndiizi possibly since both genotypes had greater root systems compared to Musakala.

The introduced inoculum competed with the AM fungi populations present in non-pasteurized soil. However, the observed colonization levels were not
directly associated with better plant growth. For example, in the Ferralsol, higher root colonization in non-pasteurized treatments did not result in height, root and biomass increase. Antagonistic relationships and competition for root resources between soil microorganisms, including the introduced and native AM inoculum (Declerck et al., 2002a), may have contributed to the lack of plant growth increase in non-pasteurized treatments. This effect was more pronounced on the Ferralsol than on the Nitisol from where the introduced inoculum originated from. We suggest that in this situation an effect of ‘compatibility’ of a specific microbial community in a given soil to the introduced and ex-situ multiplied AM fungal inoculum may also have place.

### 8.6 Conclusion

The positive effect of inoculation by the AM fungi on plant growth was consistently larger on a poor, more sandy, Acrisol, than on a clayey Ferralsol or Nitisol. This was most probably linked to greater physical limitations to root growth and nutritional limitations to plant growth in Acrisol. Antagonistic relationships and competition for root resources between soil microorganisms, including the introduced and native AM inoculum, seem to have limited plant response to inoculation in non-pasteurized treatments. This effect was minimized when the inoculum originated from a site close to where the soil substrate originates. Practical implications of this study may be (i) use of the higher potential of AM fungi application in genotypes that have a relatively poor root system; (ii) use of indigenous inoculum, especially in sites where high AM fungi populations are present.
Chapter 8. Effect of AM fungi on banana growth in three soils

Further studies should focus on isolation of the native AM fungi species/strains and characterization of their efficiency in single and multi-species combinations to improve plant growth and performance.
Chapter 9. General conclusions and perspectives
9.1 General conclusions and perspectives

As outlined in Chapter 1, East African Highland (EAH) banana cropping systems feed more than 30 million people living in the relatively humid (rainfall >900 mm yr$^{-1}$) East African highlands (Karamura et al., 1999). Crops are most often produced in small plots, and production systems are subsistence oriented, with low input and low or declining yields. Major abiotic constraints contributing to low banana yields are drought stress, low soil fertility, inappropriate crop and soil management. These contraints are further aggravated by the presence of pests and diseases. Population growth, particularly in urban centres, encourages export of bananas from the farm to the markets. However, related nutrient losses are not sufficiently compensated by external farm inputs. The resulting decreasing soil fertility is probably the main cause of low and declining yields in the region. The sustainability of this important production system is at risk.

As for all crops, banana productivity is a function of the size and the efficiency of the root system. Root performance is affected by soil microbial communities. Understanding their role in determining plant productivity may help to improve plant growth and optimize the use, efficiency, and profitability of external chemical inputs (i.e. pesticides, fertilizers). This thesis has focused on two groups of soil biota - plant parasitic nematodes and beneficial root symbionts (i.e. arbuscular mycorrhizal fungi). Our general objective was to investigate the role and impact of both groups of microorganisms on banana crop in the EAH cropping systems. More precisely, our objectives were to:

(i) assess the abundance and incidence of *P. goodeyi* in major soil types represented in the EAH, and to assess its impact on banana yields;
(ii) evaluate the presence and abundance of AM fungi in the EAH banana cropping systems;
(iii) assess the impact of the indigenous AM fungi on banana plant growth in experimental conditions ex-situ.

At the beginning of the research work, the following hypotheses were proposed: (i) Incidence and impact of the plant parasitic nematodes on banana are affected by local edapho-climatic conditions but are low in the EAH; and (ii) AM fungi are important in the EAH in terms of distribution, abundance and impact on banana growth. To test the first hypothesis, the following research questions were formulated:

1. How are the plant parasitic nematode distribution and abundance related to banana plant vigor, soil, climate, and management practices in the EAH at eco-regional scale?
2. How do nematode abundance and root damage affect banana performance at watershed scale?
3. Do the nematodes cause yield reduction in EAH banana and to which extent?

In a first step (Chapter 2), an extensive field diagnostic survey was conducted in 188 banana plots in Rwanda to characterize plant parasitic nematode presence in five distinct soils of contrasting origin and fertility. This study revealed that *P. goodeyi* was the most abundant nematode in all the soils. It was also the only species which abundance correlated with root necroses. *P. goodeyi* population densities in banana roots were positively correlated with high plant density and/or mulching practices. The fact that the highest root population densities of *P. goodeyi* were recorded in the best performing banana plots that were characterized by high nutrient and labour inputs supported the evidence that this nematode species was not directly associated with banana yield decline in the studied farm plots.

Because of the increasing land pressure and the hilly nature of most of the EAH banana production regions, a study was undertaken to assess how
General conclusions and perspectives

topography was related to variations in soil quality, plant performance, root health, and nematode infestation (P. goodeyi) (Chapter 3). This study showed that the spread of P. goodeyi had generally limited impact on banana yields in flatter fields (crest and valley bottom) where soil conditions were more optimal for root growth. However, in the presence of increased run-off on steeper middle slopes, root death was increased and bunch weight was reduced even under moderate P. goodeyi pressure. Most of the traditional banana producing fields are located in flatter fields and only new, more recently established banana plots are planted on steep slopes as a result of increased land pressure. Thus, we conclude that P. goodeyi spread alone has limited to no impact on banana yields in flatter fields. However, on steep slopes where run-off is high, moderate P. goodeyi levels may have some negative impact on plant performance.

Exact information about the impact of P. goodeyi on banana yield in controlled environments was lacking. Therefore, we assessed how infestation with P. goodeyi nematode affects banana yields in a researcher-managed yield loss trial (Chapter 4). After three crop cycles, our results revealed that P. goodeyi was a strong contributor to root necrosis, similar to trends observed in on-farm diagnostic studies (see Chapters 2 and 3). However, in this experiment, low to medium levels of root necroses were associated with improved plant growth irrespective of cultivar. Moreover, nematode population densities in roots did not reduce banana yields even at high levels of root necrosis. If we combine the results of chapters 2, 3, and 4, we can conclude that there is no much evidence to suggest that P. goodeyi is an important yield constraint for the EAH bananas. These results challenge most other nematode studies on EAH bananas, especially those focused on R. similis impact in the somewhat lower areas of the EAH (<1300 masl) and other banana production regions worldwide.
Mulch was efficient to improve root health and increase crop yields. We reported previously that soil fertility was amongst the most important constraints to banana productivity (Gaidashova et al., 2007). Mineral fertilizer use in the EAH is often limited by poor availability and low purchase capacity of small-scale farmers. Potential low-external input technologies (e.g. use of arbuscular mycorrhizal fungi) that may efficiently contribute to improved banana plant nutrition and yield were further explored. In the EAH, practically no research was conducted on AM fungi in banana cropping systems. Our work therefore focused on isolation, characterization, inoculum production and testing of the indigenous AM fungi in pot experiments. We hypothesized that AM fungi are important in the EAH in terms of geographical distribution, abundance, and impact on banana growth. To test this second hypothesis, the following research questions were formulated:

4. How are the abundance, composition and distribution of AM fungi related to soil, climate, and management practices in the EAH banana cropping systems at eco-regional scale?

5. How does root colonization vary with soil depth?

6. Is the root colonization by the AM fungi affected by banana genotype?

7. How is the impact of AM fungi inoculation on plant growth affected by soil type and banana genotype?

In a second step, an extensive field diagnostic work was conducted in 188 banana plots in Rwanda to characterize AM fungi presence in five agro-ecological regions (Chapter 5). This study revealed that the AM fungi were widespread but highly variable in terms of propagule density, extent of root colonization, and morphotype diversity in different edapho-climatic conditions. These variations are most probably linked to rainfall pattern,
management practices (tillage), and soil properties (texture and P content). Soils cultivated for bananas contained substantial and diverse populations of AM fungi able to colonize roots in highly contrasting edapho-climatic conditions. Another study investigated in depth AM fungi colonization of banana roots at farm level in the selected agro-ecological study sites (Chapter 6). The results suggested that AM fungi colonized banana roots from the top soil until the most deep soil layers which were explored by roots, and this study confirmed in general the root colonization levels observed previously for a selected eco-region (Chapter 5). Although colonization was lower in sub-soil compared to top soil layers, the presence of arbuscules in the deepest roots showed evidence that the AM fungi are actively involved in symbiosis with banana plants. Trend of higher colonization of roots by AM fungi, which was observed in plants with poor vigor at eco-region level (Chapter 5) was clearly confirmed at farm level (Chapter 6). This may be an indication of higher dependency of plants on AM fungi on less fertile soil. Based on the findings from this thesis about ultimate association of the AM fungi to banana roots at all depths, possible biological role of the AM fungi colonizing the deepest banana roots may be linked to water and nutrient transfer from more humid deeper sub-soil during drought periods when most of the top-soil roots die off.

Banana is a good host for AM fungi, and the five genotypes (Gisukari (AAA-Green Red), Gros Michel (AAA-Gros Michel), Kamaramasenge (AAB), Musakala (AAA-EA) and Yangambi (AAA-Ibota), which were grown in six different EAH agro-ecologies, were equally receptive to AM fungi (Chapter 7) but were strongly affected by specific edapho-climatic conditions of the site. This information underlines the importance that should be attributed to the development of appropriate management options to
conserve natural AM fungi population in soil, and favor their establishment in banana roots.

The last study (Chapter 8) attempted to answer the question about the impact of AM fungi inoculation on plant growth under various soil type with different banana genotypes. The results suggested that, in poor Acrisol, the effect of inoculation by the AM fungi on plant growth increase was the greatest and the most consistent across the genotypes and in both pasteurized and non-pasteurized substrate. This was most probably linked to greater limitations of root growth in Acrisol. Antagonistic relationships and competition for root resources between soil microorganisms, including the introduced and native AM inoculum, seem to have limited the plant response to inoculation in the non-pasteurized treatments. This effect was minimized when the inoculum originated from closely related location and from the same soil type. Poorer root development of Musakala coincided with its highest response to the AM fungi inoculation.

Our research attempted to characterize the role of \textit{P. goodeyi} nematode and AM fungi in the EAH banana cropping systems. The first part of our study has demonstrated the low to none impact of \textit{P. goodeyi} nematode on banana yields (hypothesis i), while the second unveiled the important effect of the AM fungi on banana growth and root development in the experimental conditions, as well as in the existing mature plantations (hypothesis ii). The highlights of the thesis finding are summarized in Figure 9.1.
Figure 9.1 Highlight of the thesis findings per chapter.

Ch. 2: Nematodes-banana-agro-ecology: higher nematode incidence in intensive use cropping systems (high plant density & mulch)

Ch. 3: Nematodes-banana-topography: yield reduction on steep slopes, but no effect on flat fields

Ch. 4: Nematodes-banana: no yield reduction

Ch. 5: AM fungi-banana-agro-ecology: AM fungi widespread but root colonization is higher in more humid and low P soils

Ch. 6: AM fungi-banana-soil depth:
- presence of active AM fungi (arbuscules) up to 120cm soil depth;
- higher dependence of weak plants on the AM symbiosis

Ch. 7: AM fungi-banana genotype-agro-ecology: 5 common genotypes are equally receptive to AM fungi, and colonization is highly affected by agro-ecology (ref. Ch. 5)

Ch. 8: AM fungi-banana: highest growth increase in poorest soil (Acrisol) & in cultivars with smaller root systems
General conclusions and perspectives

This research opens new perspectives for the development of appropriate and environment friendly technologies for low-input production systems in the EAH. The results of this work showed that *P. goodeyi* has very little effect on banana yields, although its impact may be important when another limiting factor is present (e.g. low soil fertility, high soil and nutrient leaching on steep slopes). Thus, further research should focus on development and promotion of appropriate crop and soil management options at farm and watershed scale to assure yields sustainably. These options should be not only crop- and soil-friendly, but also AM fungi-friendly. Some of the management practices observed in different agro-ecological regions appear to meet these requirements. For example, minimum tillage with miniature hoe when sowing annual crops in mulched banana fields in Kibungo leaves the soil almost undisturbed. This practice in combination with mulch conserves extra-radical mycelium of AM fungi in soil and thus accelerates the establishment of root colonization. It allows multiple cropping, a common practice in the EAH, while providing an advantage of optimal soil conservation. Such practice should be tested and adapted in other agro-ecological regions.

Recent studies have shown the existence of functional groups of AM fungi adapted to the systems of varied land use intensity (Oehl et al., 2003). This study reported the existence of species ‘generalists’ and ‘specialists’, present widely or restricted to specific land use systems. These species constitute distinct functional groups and have different survival strategies (Oehl et al., 2003). Identification of these functional groups in the EAH cropping systems and understanding their survival strategies would further contribute to the development of environmentally sound crop and field management options. The functional groups of AM fungal species that colonize disturbed environments
General conclusions and perspectives

(with low soil fertility, high erosion prone steep slopes, and intensive tillage) in the EAH would be identified and characterized, while such disturbed environments should constitute priority sites for research and promotion of integrated soil and crop management in banana cropping systems.
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Overview of the scientific achievements
Overview of scientific achievements

I. Scientific publications

A. Thesis research papers in journals

Accepted

Gaidashova SV, Van Asten PJA, De Waele D and Delvaux B. Relationship between soil properties, crop management, plant growth and vigor, nematode occurrence and root damage in East African Highland banana cropping systems. *Nematology*, *in press*.


Submitted

Overview of scientific achievements

B. Thesis research papers presented on conferences

Published in proceedings


II. Other publications

A. Published papers

Overview of scientific achievements


Overview of scientific achievements


B. Conference presentations


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