

## Chapter 1

### GENERAL INTRODUCTION

#### 1. Bananas and Plantains

Bananas (*Musa* spp.) thrives in over 120 countries, mainly in lowland tropical and subtropical regions, where rainfall is in excess of 1250 mm per year and mean minimum temperatures are above 15 °C (Gowen & Quénéhervé, 1990). The banana came originally from Southeast Asia and still grows widely in the Philippines, Papua New Guinea and Indonesia. It migrated to the Indian peninsula, eastern and western Africa, central and southern America and the islands of the Pacific (INBAP, 2000).

Banana is a massive herb – the largest herbaceous plant in the world and can grow up to 15 m, consisting of a branched, underground stem, called rhizome or corm, roots, vegetative buds and an erect pseudostem composed of tightly packed leaf bases (Jones, 2000). The root system is shallow, not penetrating much below 0.6 m and horizontal spread can be as far as 5 m, but more commonly 1 - 2 m. Banana roots are sensitive to adverse conditions, such as water logging, desiccation and compacted soil structure (Robinson, 1996). In the beginning in Asia, there were two types of bananas with seeds, *Musa acuminata* and *Musa balbisiana*. A mixture of natural selection, crossings and breeding by humans gave rise to several varieties of sterile bananas whereby the female flower started to produce seedless fruit (INIBAP, 2000). From planting to harvest takes 9 to 12 months and the bunch of fruits will weigh 20 to 25 kg on average.

Bananas can be divided into two main categories: dessert bananas and cooking bananas. Annually, 55 million tonnes of dessert banana are produced. This is the second highest harvest of all fruits in the world, just behind the orange. However, less than one quarter of the production is traded on the international market. Consumers in the West tend to consume bananas mainly as a dessert, but in many countries they are eaten as a vegetable. Cooking bananas are common in the tropical regions, where they are eaten in many ways: fried, grilled, boiled or stewed. They can also be kept for a long time, usually in a dried form. The heart of the stem is a delicacy in India and Ethiopia. In the Far East the buds are eaten in salads. The

leaves are used to wrap food for cooking and the pseudostem is used to make dams, or feed livestock.

Banana is one of the most important economic crops produced in the tropics. Commercial production provides sustenance for more than 700 million people today and is an important source of nutrition for more than 400 million people in tropical countries (INIBAP, 2000). Banana is fourth on the list of the developing world's most important food crops after rice, wheat and maize. World production of banana was estimated at 85 million tonnes, of which 30 million tonnes are plantains. Production is centered in Latin American, the Caribbean, Africa and Asian-Pacific regions (Jones, 2000) where almost 90 % of total production is consumed locally. Developing countries account for 98 % of the production of bananas and for 100 % of plantain production (INIBAP, 2000).

## **2. Nematode parasites on banana**

### **2.1. Plant parasitic nematodes associated with bananas**

A complex of pests and diseases threatens banana production such as insects, fungi, bacteria, viruses and nematodes. Nematode parasites are among the most important pests on banana and are involved in the destruction of primary roots that disrupts the anchorage system resulting in toppling or uprooting of the plants. The most widespread and important are the burrowing nematode *Radopholus similis*, some species of root lesion nematode *Pratylenchus coffeae* and *P. goodeyi*; species of root-knot nematodes *Meloidogyne incognita*, *M. javanica*; the spiral nematode *Helicotylenchus multicinctus*, *H. mucronatus* and the semi-endoparasitic nematode *Rotylenchulus reniformis* (Gowen & Quénehervé, 1990; Stanton, 1994). In addition to these five major nematode parasites of banana, there are approximately 150 other species belonging to 43 genera of nematodes associated with *Musa* spp. throughout the world (Gowen & Quénehervé, 1990). Banana crop losses depend on several factors including: nematode species, banana cultivars, climate conditions and soil factors (Sarah, 2000) as well as fungal, virus and bacteria pathogens. Worldwide banana production losses of approximately 20 % are caused by nematodes annually (Sasser & Freckman, 1987).

### **2.2. Burrowing nematode *Radopholus similis***

The burrowing nematode *R. similis* is a migratory endoparasite of the root system and the most important nematode pathogen on banana. *R. similis* is able to attack almost all banana

cultivars as well as other *Musa* species (Gowen & Quénéhervé, 1990). The disease of banana caused by *R. similis* is known throughout the world by the common names “black head toppling disease” and “toppling disease”. The most obvious symptom of attack on banana is the toppling over of plants especially those bearing fruit (Gowen & Quénéhervé, 1990). The burrowing nematode destroys root and corm tissue, which reduces water and mineral uptake. This results in a reduction of plant growth and development and may lead to severe reduction of bunch weight and an increase in the time period to harvest (Stanton, 1994; Sarah, 2000). Furthermore, root destruction results in a tendency for plants to uproot or topple particularly during windstorms and heavy rain periods. On entering the root, the nematodes occupy an intercellular position in the cortical parenchyma where they feed on the cytoplasm of nearby cells, so destroying them and causing cavities to develop. These cavities coalesce and are continuously enlarged by the nematodes feeding and tunnelling laterally towards the endodermis, producing the characteristic reddish brown lesions throughout the cortex but never in the stele (Williams & Siddiqi, 1973). Some weeks after infection, when extensive cavities have formed, one or more deep cracks with raised margins appear on the root surface. *R. similis* migrates inside the roots and from the root to the rhizome cortex where it causes diffuse black lesions surrounding embedded roots (Gowen & Quénéhervé, 1990).

*R. similis* is also associated with another important pathogen on banana: Root rot disease caused by *Fusarium oxysporum* f.sp. *cubense*. *F. oxysporum* f.sp. *cubense* invades intact banana roots and colonizes cortical parenchyma cells especially when the roots are wounded mechanically or by the nematode (Williams & Siddiqi, 1973). In plants inoculated with both pathogens *F. oxysporum* and *R. similis*, the fungus was able to grow through the endodermis, causing necrosis of the stele and eventual atrophy of the whole root distal to the point of stellar invasion (Blake, 1966).

The burrowing nematode *R. similis* has spread worldwide. It infests commonly plantain and cooking banana in the lowlands of central and eastern Africa and Puerto Rico in the Caribbean (Sikora et al., 1989; Speijer et al., 1999; Sarah, 2000). *R. similis* also causes toppling disease on banana in South East Asia such as the Philippines, Indonesia, and Papua New Guinea as well as in Australia and the South Pacific (Stanton, 1994; INIBAP, 2000). Banana production losses, caused by *R. similis* depend on population density in the root systems. Banana crop losses due to *R. similis* in the Ivory Coast were reported generally below 30 % but can reach 75 % in poor and eroded soil (Sarah, 1989). In Central America

(Costa Rica and Panama) and South America (Columbia), production loses are between 12 to 18 % (Sarah, 2000). The losses of banana crop caused by *R. similis* in Uganda were estimated at approximately 30 % (Speijer et al., 1999) and can reach up to 50 % in the highlands of East Africa (Sikora et al., 1989; Speijer & Fogain, 1999; Fogain, 2000).



**Figure 1:** Toppling disease of banana caused by burrowing nematode *Radopholus similis* (Photo by Niere, IITA)



**A)**

**B)**

**Figure 2:** Symptoms of burrowing nematode *Radopholus similis* on banana roots. A) Necrotic roots caused by *R. similis* (Photo by Niere, IITA). B) *R. similis* inside root tissue of banana (Photo by zum Felde, 2004).

### 3. Specific antagonists of plant parasitic nematodes

Chemical control of plant parasitic nematodes is the most often used approach to nematode management. However, due to high toxicity, microbial degradation and negative impact of nematicides on the environment use is often restricted. Breeding for nematode resistance is a favourable alternative and has been used in the past (Gros Michel) and present (FHIA) (De Waele & Speijer, 1999), but nematode resistance is not yet available for cultivars used in commercial production. Therefore, the development of biological control agents as an additional nematode control component is necessary (Stirling, 1988; Sikora, 1992; Sikora 1997).

Bacteria and fungi are the most abundant organisms in the soil and some of them have shown great potential as biological control agents for plant parasitic nematodes (Stirling, 1991; Sikora 1992; Becker, 1993). Antagonist is an umbrella term for parasites, predators, pathogens, competitors, and or other organisms that repel, inhibit or kill plant parasitic nematodes. Antagonists most likely to be receptive to management for the biological control of nematodes are: plant-health promoting rhizobacteria, obligate bacterial parasites, and fungal egg pathogens/parasites, predacious or trapping fungi, mutualistic endoparasitic fungi, fungal pathogens/parasites of females and endomycorrhizal fungi (Sikora, 1992). The biological control of plant parasitic nematode diseases by antagonists in general, using rhizobacteria, endophytic bacteria and fungal endophytes has been well documented (Hallmann & Sikora, 1994; Hasky-Günther et al., 1998; Niere et al., 1998; Hoffmann-Hergarten et al., 1999; Niere et al., 1999; Pocasangre, 2000).

#### 3.1. Mycorrhizal fungi

Arbuscular mycorrhizal fungi are obligate symbionts, that biotrophically colonizes the root cortex of host plants and develops an extra-radical mycelium in the rhizosphere. The presence of arbuscular mycorrhizal fungi in the plant root system is well known to improve plant health and growth (Hussay & Roncadori, 1982; Sikora, 1992). A plant with a well established symbiosis showed increased resistance to fungal pathogens and nematode diseases (Hussay & Roncadori, 1982). Some species of the arbuscular mycorrhizal fungus *Glomus* were documented to reduce root gall index caused by root-knot nematode *Meloidogyne* spp. on tomato and pyrethrum (Waceke et al., 2001; Diedhiou et al, 2003; Molina, 2004).

Furthermore, biological control activity of species of *Glomus* decreased galls of *M. incognita* as well as lesion nematode such as *R. similis* and *P. coffeae* on banana (Jaizme-Vega et al., 1997; Elsen et al., 2003). Limits on large scale production of these fungi however limit their practical use in banana production.

### 3.2. Facultative fungal parasites

Fungi infecting the eggs or females of plant parasitic nematodes have been classified as facultative pathogens or parasites or facultative opportunistic fungi (Sikora, 1992). Nematophagous fungi may increase to densities that eventually control specific nematodes including root-knot and cyst nematodes (Sikora et al., 1990; Kerry & Evans, 1996). The egg pathogen *Pochonia chlamydosporia* (syn. *Verticillium chlamydosporium*) diminished nematode density of species of the root-knot nematode and cyst nematodes (Kerry & Bourne, 1996; Viaene & Abawi, 2000; Ismail et al., 2001). Similar results were observed with the facultative fungal parasite *Paecilomyces lilacinus* against *Meloidogyne* spp. (Siddiqui & Mahmood, 1993; Khan et al., 1997; Viaene & Abawi, 2000; Kiewnick & Sikora, 2003; Kiewnick & Sikora, 2004). These fungi do not depend on nematodes for their nutrition but they proliferate in the rhizosphere on root exudates and organic material and colonise the egg masses of root-knot nematodes or female nematodes as they enlarge and rupture the root cortex and are exposed on the root surface. During the infection process, *P. chlamydosporia* produces the enzyme subtilisins, which breaks down the outer membrane of the eggshell, and expose the chitin layer of eggs to infection (Kerry & Bourne, 1996). Recently, the control activity of *P. lilacinus* against *R. similis* on banana was documented (Mendoza et al., 2004). These types of fungi could be important in short cycle production systems and when applied to the soil before replanting of banana plantations, but not in long term production systems.

A combination between facultative fungi with other organism to enhance their ability to control nematodes was demonstrated (Zaki & Maqbool, 1992; Siddiqui & Mahmood, 1993; Khan et al., 1997; Hojat Jalali et al., 1998).

### 3.3. Fungal endophytes

All plants are colonized by endophytic fungi that in most case have unknown functions in the plants. Non-pathogenic endophytic fungi, which colonize the inner tissue of plants, are considered potential biological control agents. Some of these non-pathogenic fungi appear to

be mutualistic to the plant and are often numerous in root tissue. They have been shown to influence the host-parasitic relationship of both sedentary and migratory endoparasitic nematodes reducing infection significantly (Sikora, 1992; Schuster et al., 1995).

Non-pathogenic endophytic *F. oxysporum* isolates were demonstrated to have activity against the root-knot nematode *M. incognita* on tomato (Hallmann & Sikora, 1994; Diedhiou et al., 2003; Sikora et al., 2003). Some non-pathogenic *F. oxysporum* isolates were also documented for their ability in biocontrol of migratory endoparasitic nematodes such as *P. goodeyi* and *Radopholus similis* on banana (Speijer et al., 1993; Schuster et al., 1995; Niere et al., 1998; Niere et al., 1999; Pocasangre, 2000; zum Felde et al., 2004). These fungi are now considered good candidates for biocontrol in the field because of ease in production and application.

#### **4. Non-pathogenic endophytic *Fusarium oxysporum* as biological control agents**

Fusarium wilt diseases caused by formae speciales of *Fusarium oxysporum*, and also Fusarium root rots, caused by more or less specific strains of *F. oxysporum* are among the most severe diseases in the world. Fusarium wilts affect many plant species belonging to all the botanical families (Alabouvette et al., 1998). *F. oxysporum* is one of the most common species among soil fungi in cultivated soil all over the world. It includes a large diversity of strains: saprophytic, parasitic and pathogenic. The wilt inducing *F. oxysporum* strains represent a high degree of host specificity, which results in the concept of forma speciales that are strictly adapted to a plant species and races that are adapted to cultivars of the host plant (Alabouvette et al., 1998). Plant pathologists have described more than one hundred formae speciales.

Many non-pathogenic endophytic *F. oxysporum* strains can be isolated from asymptomatic roots where they grow without causing damage. Such endophytic strains were isolated from many crops that are not affected by *Fusarium* wilt such as rice, maize, tomato, banana (Hallmann & Sikora, 1994; Amin, 1994; Schuster et al., 1995; Sikora et al., 2003). Since 1986, a method characterizing non-pathogenic versus pathogenic strains of *F. oxysporum* based on vegetative compatibility groups (VCG test) was developed (Correll et al., 1986).

In the 1970s, the first evidence of a possible role of high densities of non-pathogenic endophytic *Fusarium* spp. in roots of plants growing in suppressive soils to Fusarium wilts

was reported (Smith & Snyder, 1971; Toussoun, 1975). Since then, many studies clearly point to a role of non-pathogenic *Fusarium* spp. in the suppression of Fusarium wilts in suppressive soils from different areas in the world (Chang S.P., et al., 1988; Biles C.L. & Martyn R.D., 1989; Kroon B.A.M. et al., 1991; Minuto A. et al., 1995; Leeman M. et al., 1996; Fuchs J.P. et al., 1997). The mechanisms of action of non-pathogenic *F. oxysporum* strains responsible for control of Fusarium wilts were also studied. Induced resistance has been considered as a mechanism, which could be responsible for disease control caused by non-pathogenic endophytic *F. oxysporum* strains (Biles & Martyn, 1989; Kroon et al., 1991; Fuchs et al., 1997).

Beginning in 1990, non-pathogenic endophytic *F. oxysporum* were studied as biocontrol agents against plant parasitic nematodes (Sikora, 1992; Sikora et al., 2003). Non-pathogenic endophytic *F. oxysporum* considerably decreased nematode penetration as well as root galls caused by the root-knot nematode *M. incognita* on tomato (Hallmann & Sikora, 1994; Diedhiou et al., 2003). Non-pathogenic endophytic *F. oxysporum* strains also were documented to reduce penetration and population development of burrowing nematode *Radopholus similis* on banana (Amin, 1994; Reißinger, 1995; Schuster et al., 1995; Niere et al., 1999; Pocasangre, 2000). They have now been reported as the main antagonist in suppressive soils to *R. similis* (zum Felde et al., 2004).

Additionally, non-pathogenic endophytic *F. oxysporum* isolates were demonstrated to produce nematicidal or nematostatic compounds (Amin, 1994; Hallmann & Sikora, 1996). Secondary metabolites of non-pathogenic endophytic *F. oxysporum* strains were shown to have effects on the mobility and survival of nematodes such as *R. similis* and *M. incognita* *in vitro*. The relationship between fungal secondary metabolites of endophytic *F. oxysporum* and inactivity of plant parasitic nematodes has not been elucidated.

Non-pathogenic endophytic *F. oxysporum* have also been reported for their biological promotion of banana growth; however this promotion only affected banana growth in the absence of burrowing nematode *R. similis* (Niere et al., 1999; Pocasangre, 2000).

Even though activity of non-pathogenic endophytic *F. oxysporum* as well as their potential for biocontrol of plant parasitic nematodes has been studied, little is known about the mechanism of action. Future prospects for using them as a biocontrol agent against plant parasitic