

## GENERAL INTRODUCTION

### 1 Banana and its importance

Bananas originated from south-east Asia and the western Pacific islands where several wild seed-bearing *Musa* spp. still exist in natural vegetation. The wild relatives were inedible, however a cross between two produced a sterile plant that has developed or been shaped into the varieties of edible banana and plantain growing today (Simmonds, 1962; 1966). All edible bananas are sterile and are propagated vegetatively. Of the very great number of recognized clones, some are derived from *Musa acuminata* Colla and others from natural hybridisations of *Musa acuminata* and *Musa balbisiana* Colla. Currently accepted nomenclature of clones indicates ploidy and genomic origin, with A for *acuminata* and B for *balbisiana* (Simmonds, 1966; Nestel, 1984; Gowen, et al. 2005).

Bananas, including the dessert bananas and the cooking types and plantains, are cultivated in more than 130 countries throughout the tropical and subtropical regions of the world (UNCTAD, 2008). While commercial production of bananas is oriented to the fresh export trade destined mainly for temperate-zone markets, plantains and even unripe bananas consumed boiled, fried, roasted, or even brewed are a major staple food throughout the tropics. Bananas are grown and harvested for fruit over an area of approximately 10 million hectares, with an annual production of around 90 million metric tons (FAO, 2008).

Bananas are the main fruit in international trade and play a key role in the economics of many developing countries. In terms of volume, it is the top exported fruit. According to the Food and Agriculture Organization of the United Nations (FAO) statistical estimations, total world exports of banana accounted for 15.9 million tonnes in 2004 (FAO, 2008).

In commercial terms, the most important dessert bananas are those of the Cavendish subgroup sterile, seedless triploids (AAA) of *M. acuminata*, of which the best known cultivars are ‘Grande Naine’ and ‘Dwarf Cavendish’. Others include AAA triploids (the best known is ‘Gros Michel’, absent from cultivation because of its high susceptibility to Panama disease, a fungal wilt of serious economic importance), AA diploids (such as ‘Pisang Mas’ in Southeast Asia and ‘Bocadillo’ in Latin America), various AB diploids (*acuminata balbisiana*), and AAB triploids such as ‘Silk Fig’ (also known as ‘Pome’ and ‘Manzano’), and the recently

obtained AAAB tetraploid 'Goldfinger' (Nestel, 1984; Stover and Simmonds, 1987; De Langhe 1995).

### **1.1 Botanical description**

Bananas and plantains are evergreen herbaceous tropical plants that can be considered giant herbs, as some varieties reach up to ten meters in height, although most commercial types grow to between two and five meters. The pseudostem formed by the concentric assembly of the leaf sheaths is crowned by a rosette of large, oblong to elliptic shaped leaves, conferring on the plant the aspect of a herbaceous tree. The true stem is a subterranean organ that extends upward at the core of the pseudostem until culminating in the inflorescence, which emerges from the top of the plant. It is responsible for producing all the other parts of the plant: roots, leaves, and shoots or suckers. Leaves are produced successively until the inflorescence is cast and in variable quantity depending on the specific variety of banana or plantain, climate, and cultural practices (Simmonds, 1966; Karamura and Karamura, 1995).

Although the plant dies after producing fruit, banana is considered perennial as suckers successively replace the senescent aerial parts without need for replanting. Several suckers emerge consecutively from buds located at the axil of leaves; under commercial cultivation, they are regularly eliminated, leaving either the most vigorous sucker (Simmonds, 1966), or the one with better position in relation with the other plants, to replace the mother plant.

The large and complex inflorescence is composed of double rows of flowers, called hands, and covered by bracts, usually red or reddish in colour, grouped helixoidally along the inflorescence axis, reproducing the pattern of the leaf system. All flowers are hermaphroditic, but only the female or so called "first" hands will give rise to the edible fruit, technically known as fingers (Simmonds, 1966). Depending mainly on climate, cultivation conditions, and varieties, the time lapse between emission of the inflorescence and harvesting of the bunch can be anywhere from three to ten months. Bananas are harvested year round, with normal commercial bunch weights of 15-45 kg.

## 2 Nematode pests of banana

Nematodes are an important pest of bananas in most producing areas. Average annual losses worldwide are believed to be in the order of 20% (Sasser and Freckman, 1987). However, the losses can be higher, and are influenced by many factors, such as soil texture, drainage and fertility, climatic conditions, nematode species and biotype (O'Bannon and Esser, 1985). Nematode parasitism in banana roots is characterized by simultaneous infestations by several species. The most widespread and damaging nematode species are *Radopholus similis* and species of *Pratylenchus*, *Meloidogyne* and *Helicotylenchus multicinctus* (Speijer and De Waele, 1997; Gowen et al., 2005). In addition to this major group, there are approximately 150 other species belonging to 43 genera of nematodes associated with *Musa* spp. throughout the world (Gowen and Quénehervé, 1990).

### 2.1 Burrowing nematode *Radopholus similis*

The burrowing nematode *R. similis* is a migratory endoparasite of the root system and the most important root pathogen attacking bananas. This nematode is considered to be the main nematode problem of intensive commercial bananas, especially Cavendish types, oriented towards export markets. Vegetative propagation using infested corms or suckers has disseminated this pest throughout the world (Gowen and Queneherve, 1990; Sarah et al., 1996).

The damage caused by *R. similis* in banana is known by different names, such as black head, black head toppling disease and toppling disease. The nematode destroys root and corm tissue, reducing the capacity of the plant to uptake water and nutrient. Secondary infections of damaged tissue by fungi and bacteria result in lengthening of the vegetative growth cycle, production of small bunches, shortened life of the production unit and toppling of the plants, particularly during windstorms and heavy rain periods (Stanton, 1994; Sarah, 2000; Viaene et al., 2003; Gowen et al., 2005). Inside the root, the nematode migrates inter- and intracellularly, feeding on the cytoplasm of cortex cells and forming cavities (Williams and Siddiqui, 1973; Esser et al., 1984). These cavities coalesce and are continuously enlarged by nematode feeding and tunnelling laterally towards the endodermis, producing the characteristic reddish brown lesions throughout the cortex (Loos, 1962; Williams and Siddiqui, 1973). Some weeks after infection, when extensive cavities have been formed, one or more deep cracks with raised margins appear on the root surface (Gowen and Quénehervé,

1990). Banana production losses, caused by *R. similis* in Central America (Costa Rica and Panama) and South America (Colombia) are between 12 to 18% (Sarah, 2000). In the Ivory Coast they are generally below 30%, but in poor and eroded soils losses can reach up to 75% (Sarah, 1989). 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 and Fogain, 1999; Fogain, 2000).

## 2.2 Threshold and control measures for nematodes in banana

To define an economic threshold level of a pest is essential in the implementation of control measures. At the same time, it is an important key in Integrated Pest Management (IPM) (Duncan, 1991). Nevertheless, economic thresholds for banana nematodes have not been established in subsistence banana farming (Gowen, 1995), where nematode problems are either not known, not recognized or control measures are too expensive (Sikora, 2002).

Population densities of *R. similis* estimated to produce yield loss on banana are very variable worldwide (Marin et al., 1998). In West Africa, 1000 *R. similis*/100 g roots are considered to cause serious yield loss, while 20000 *R. similis*/100 g roots are required to cause similar losses in Central America (Gowen, 1995). In Central America, 10000 *R. similis* /100 g of functional roots is the threshold for nematicide applications on commercial plantations (Chávez and Araya, 2001). However, Gowen and Quénéhervé (1990) and Quénéhervé (1989) consider that 2000 *R. similis*/100 g roots are a potential cause of yield loss in commercial cultivars.

In commercial banana plantations in Latin America, nematode control basically relies on the use of granular organophosphate and carbamate nematicides (Bunt, 1987; Moens et al., 2004), which can result in bunch weight increases between 15% and 41% (Araya and Chevez, 1997). Cultural practices, such as the use of organic amendments, crop rotations, fallows and clean planing material are also used, but with varying success (zum Felde et al., 2006). In many instances, the banana crop cannot be grown economically without the use of nematicides (Gowen et al., 2005). Repeated use of nematicides has led in some cases to a condition known as “enhanced biodegradation” where the active ingredient is rapidly metabolized by soil microflora (Smelt et al., 1987; Racke and Coats, 1988; Stirling et al., 1992; Anderson and Lafuerza, 1992; Anderson, 1998; Pattison et al., 2000; Moens et al., 2004; Gowen et al., 2005).

### 3 Biological approaches for sustainable nematode management in banana

The integration of habitat management with cultural control practices are important measures for current IPM approaches to nematode control. In this contest, the combination of more than one strategy to control nematodes has been considered more effective than any single control method alone (Barker and Koenning, 1998). A combination of practices based on cultural and biological control techniques can reduce dependence and improve the efficacy of the routinely used pesticides when they are included as tools in IPM. On the other hand, the use of antagonists for biological control against nematodes favours the development and restoration of the naturally occurring antagonistic potential in soils (Sikora 1992).

Although many studies have been conducted to identify potential biocontrol agents against *Radopholus similis*, such as: *Pseudomonas* spp. (Aalten et al., 1998), mycorrhizae (Umesh, 1988; Elsen et al., 2003; Jaizme-Vega et al., 2003), *Paecilomyces lilacinus* (Davide, 1987; Devrajan and Rajendran, 2002; Mendoza et al., 2004; Khan et al., 2006), endophytes including *Trichoderma atroviride*, *Fusarium* spp. and *Fusarium oxysporum* strain 162 (Niere et al., 1999; Pocasangre, 2000; Athman et al., 2006; Vu et al., 2006; zum Felde et al., 2004; 2006; Mendoza and Sikora, 2008), biological control is not widely used. Some biocontrol products, which contain bacteria, such as Deny<sup>®</sup> or Blue Circle<sup>®</sup> (*Burkholderia cepacia*), a fungus, such as Paecil<sup>™</sup> or BioAct<sup>®</sup> (*Paecilomyces lilacinus*), or the killed fermentation products of a fungus, such as DiTera<sup>®</sup> (*Myrothecium verrucaria*), are available for nematode management (zum Felde et al., 2006; Mendoza and Sikora, 2008), but banana producers do not generally use them because of a lack of long-term control.

The combination of biocontrol agents with different modes of action is considered an alternative to improve and increase options for biological management of *R. similis* in banana and increase yields (Esnard, et al., 1998; Kashaija et al., 1999; Pocasangre et al., 2000; Khan et al., 2006; zum Felde et al., 2006; Mendoza and Sikora, 2008). However, limited information is available.

The strategic application of biocontrol agents with different modes-of-action such as repellency induced resistance (*Fusarium oxysporum* strain 162), egg pathogenesis (*Paecilomyces lilacinus* strain 251) and direct toxicity (*Bacillus firmus*) before and at planting could increase biocontrol of nematodes during plant establishment and over time (Mendoza and Sikora 2008).

### 3.1 Mutualistic fungal endophytes

*Fusarium oxysporum* is one of the most common and diversity species among soil fungi in cultivated soil worldwide, where it survives as dormant propagules (chlamydospores) for long time periods and grows on organic matter in soil as well as in the rhizosphere and inside of roots of many plant species. This fungal species also includes many important plant pathogens that can induce necroses or wilts in crops of economic importance. However, strains of *F. oxysporum* also are commonly isolated from healthy roots. Strains isolated from healthy plants are termed non-pathogenic and are interesting, since some of them can induce resistance in host plants, which enhances the plant's ability to defend itself from pathogen attack (Stirling, 1991; Alabouvette et al., 1998; Olivain and Alabouvette, 1999; Larkin and Fravel, 1999; Pereira et al., 1999; Gnanamanickam et al., 2002; Trouvelot et al., 2002; Timper et al., 2005; Sikora et al., 2007).

Several studies demonstrated that tissue culture banana inoculated with non-pathogenic isolates of *F. oxysporum*, including *F. oxysporum* strain 162 growing endophytically were effective under glasshouse conditions in reducing densities of the root-knot nematode, *Meloidogyne incognita*, on tomato (Hallmann and Sikora 1994; Diedhiou, et al., 2003; Dababat and Sikora, 2007; Sikora et al., 2007), the burrowing nematode, *Radopholus similis* and Fusarium wilt, on banana (Amin, 1994; Niere et al., 1999; Pocasangre, 2000; Sikora and Pocasangre, 2004; Vu et al., 2006; Felde et al., 2006; Mendoza et al., 2006; Mendoza and Sikora, 2008) and banana weevil (*Cosmopolites sordidus*) under field conditions (Griesbach, 2000).

### 3.2 Opportunistic egg-pathogenic fungi

Several species of fungi are known to infect nematodes eggs. In addition, they also can parasitize females and sedentary nematodes and their reproductive structures. More than 150 species of fungi have been isolated from the cyst, females, or eggs of nematodes, but the parasitic status of fewer than 10% has been tested (Kerry, 1988). Some egg-parasitic fungi, such as *Pochonia* spp. (syn. *Verticillium* spp.) (Kerry and Crump, 1977; Kerry, 1980; Morgan-Jones et al., 1984; Siddiqui and Mahmood, 1996; Hirsch et al., 2001); *Paecilomyces lilacinus* (Jatala et al., 1979; Dube and Smart, 1987; Gaspard et al., 1990; Gomes Carneiro et al., 1991; Rao et al., 1998; Holland, et al., 1999; Kiewnick and Sikora, 2003; 2006; Mendoza et al., 2004; 2007; Khan et al., 2006; Mendoza and Sikora, 2008), *Fusarium* spp. (Nigh et al.,

1980; Jatala, 1986), *Cylindrocarpon destructans* (Crump, 1987), and *Dactylella oviparasitica* (Mankau, 1981; Olatinwo et al., 2006) have been studied. Apart from the opportunistic egg-parasitic fungi, there have been reports of some oomycetous fungi that are obligate parasites of the females of cyst nematodes that are difficult to manipulate and have not been grown on artificial media (Kerry, 2000).

Most of the fungi isolated from nematodes eggs have been readily grown on a range of artificial media, and two species in particular, *Pochonia chlamydosporia* (syn. *Verticillium chlamydosporium*) and *Paecilomyces lilacinus*, have been much studied and their potential as biological control agents assessed (Kerry, 2000). *P. lilacinus* strain 251 is currently commercialized as BioAct<sup>®</sup>WG or MeloCon<sup>®</sup>WG ([www.prophyta.de](http://www.prophyta.de)).

The interaction between many pathogenic fungi and their hosts follows a series of steps that starts with recognition of the host, adhesion to its surface, followed by the development of infective structures required for penetration into the host. Surface recognition is often highly specific and it is assumed that it represents an early event in the specificity of the host-parasitic infection. This process may involve a combination of chemical mediators and physical characteristics of the egg surface to be infected (Allen et al., 1991; Holland et al., 1999). Following recognition, adhesion is considered to be a necessary prerequisite for infection by the fungi. This adhesion involves an active process of secretion of adhesive materials by the fungus which may enhance the physical contact between fungal enzymes and the host, facilitating the breakdown of the host surface. After adhesion, a fungus forms an appressorium, which is a specialized infection structure adhering to the surface that achieves penetration (Dunn et al., 1982; Lopez-Llorca and Claugher, 1990). Through a combination of enzymes and mechanical pressure, the fungus penetrates the eggshell and can proceed to parasitize the developing juveniles (Segers et al., 1996; Holland et al., 1999).

### 3.3 Antagonistic bacteria

Bacteria are numerically the most abundant group of organisms in the soil associated with plant root systems. Extensive investigations have been conducted to assess their potential to control plant-parasitic nematodes (Table 1). Biological control of nematodes by bacteria involves different modes-of-action, such as: parasitism, production of toxins, enzymes and other metabolic products, interference with nematode-plant-host recognition, competition for

nutrients, promotion of plant growth and induction of systemic resistance (Kloepper et al., 1999; Siddiqui and Mahmood, 1999; Hallmann et al., 2004; Sikora et al., 2007).

### 3.3.1 Rhizobacteria

Rhizobacteria are a subset of the total soil bacteria which have the capacity to colonize the developing root system in the presence of competing soil microflora. Certain strains of rhizobacteria are able to suppress a variety of nematodes and diseases and stimulate plant growth, and are therefore called as plant growth-promoting rhizobacteria (PGPR) (Kloepper et al., 1980; 2004) or plant health-promoting rhizobacteria (PHPR) (Sikora, 1992). Aerobic endospore forming bacteria, mainly *Bacillus* and *Pseudomonas* spp. are among the dominant populations in the rhizosphere that are antagonistic to nematodes (Tian et al., 2007). Other rhizobacteria reported to show antagonistic effects against nematodes include members of the genera *Actinomyces*, *Agrobacterium*, *Arthrobacter*, *Alcaligenes*, *Aureobacterium*, *Azotobacter*, *Beijerinckia*, *Burkholderia*, *Chromobacterium*, *Clostridium* and *Rhizobium* (Racke and Sikora, 1992; Kloepper et al., 1999; Siddiqui & Haque, 2000; Hallmann et al., 2004; Tian et al., 2007).

### 3.3.2 Endophytic bacteria

Root colonization by bacteria may be viewed as a continuum from the rhizosphere to the rhizoplane to internal tissue of roots, and the latter bacteria are named endophytic bacteria. Endophytic bacteria have been defined by Hallmann et al. (1998) as bacteria that can be isolated from surface-disinfected plant tissues or extracted from within the plant, and additionally, do not visibly harm the plant. *Pseudomonas* spp. and *Rhizobium etli* are two of the most studied endophytic bacteria against nematodes. Other endophytic bacteria reported for their antagonistic effects against nematodes include: *Brevundimonas vesticularis*, *Burkholderia cepacia*, *Cedecea davisae*, *Phyllobacterium rubiacearum*, and *Pantoea agglomerans* (Hallmann et al., 2004).

Endophytic bacteria as biocontrol agents can be divided into two groups: (i) strains that extensively colonize the internal plant tissue and suppress invading pathogens by niche occupation, antibiosis, or both, and (ii) strains that primarily colonize the root cortex where they stimulate general plant defense/resistance mechanisms (Hallmann et al. 2001).