# Chapter 1

# Review of the role of endophytes in biological control of plant-parasitic nematodes with special reference to the banana nematode, *Radopholus similis* (Cobb) Thorne

## 1. Introduction

Plant-parasitic nematodes cause significant damage and losses to most agricultural crops in the tropics and sub-tropics (Luc *et al*., 2005). The need to control and manage nematode populations to acceptable levels and reduce losses remains a big concern for nematologists. Increased scientific interest in biological control of plant parasitic nematodes is mainly a response to growing public concerns over the use of agrochemicals. The need to reduce dependence on chemical control using nematicides and the increased pressure to use pest control measures that do not pollute or degrade the environment has provided the impetus for more research geared towards the search for and exploitation of potential biological control agents of plant parasitic nematodes (Cook, 1988; Gerhardson, 2002). Stirling (1991) defined biological control as 'the reduction of nematode populations through the action of living organisms other than the nematode-resistant host plant, and which occurs naturally, or through the manipulation of the environment or the introduction of antagonists'.

Nematodes have long been known to have numerous antagonists (Kerry, 1987). Several organisms have been described and exploited for the management of plant parasitic nematodes in agricultural crops. A large number of organisms including fungi, bacteria, viruses, predatory nematodes, insects and mites have been found to parasitize on vermiform stages of nematodes or females and eggs of root-knot nematodes and cyst nematodes (Stirling, 1991). To date, most research on biological control of plant-parasitic nematodes has concentrated on nematophagous egg parasitic fungi and the nematode predatory fungi and antagonistic bacteria. In this respect, several reviews have been published (Jatala, 1986; Stirling 1991; Sikora, 1992; Siddiqui and Mahmood, 1995; 1996; Akhtar and Malik, 2000; Kerry, 2000).

More recently the use of endophytic micro-organisms resident within plant tissues for protection of plants against pests and diseases has been exploited. The most studied is the grass-endophyte associations in which endophytic fungi associated with grasses have been shown to protect grasses against pests and diseases. Most grass endophytes are members of the Ascomycete family Clavicipitaceae (Clay, 1991). Biological control with endophytes has mostly emphasized resident or mutualistic fungi of grasses, which render hosts unpalatable to herbivores and insects (Clay, 1988; 1989). Detrimental effects of grass endophytes on fungal pathogens have also been demonstrated. For example, isolates of *Acremonium lolii* Link ex

Fries, and *A. coenophialum* Morgan-Jones & W. Gams showed antibiosis against a range of fungal plant pathogens in culture (White and Cole, 1985). Research on grass endophytes has clearly demonstrated the nature and extent of protection afforded to the host plants by the interactions, with mutualistic associations between grasses and endophytic fungi benefiting the host plants in most circumstances (Clay, 1990). In mutualistic associations, endophyte-infected plants are protected from attack by some species of insects, nematodes and fungi while, in return, the endophyte is provided with shelter and nutrition by the host plant (Latch, 1993; Saikkonen *et al*., 1998; Schardl *et al*., 2004).

Although most reports on host plant infection by endophytes concern grass endophytes, symptomless infections of other plants by endophytic fungi belonging to diverse taxonomic groups have been known for many years (Carroll, 1988). The presence of endophytes has been demonstrated in many plants, including important crops such as banana (Brown *et al*., 1998; Pereira *et al*., 1999; Cao *et al*., 2004a,b; Cao *et al*., 2005), maize (*Zea mays* L.)(Fisher *et al*., 1992) rice (*Oryza sativa* L.)(Fisher and Petrini, 1992), and tomato (*Lycopersicon esculentum* L.) (Hallman and Sikora, 1994c; Cao *et al*., 2004a). Some of the principal groups of root colonizing plant beneficial fungi, which have developed symbiotic relationships with the host plants, belong to *Fusarium* and *Trichoderma* spp. (Haas and Defago, 2005). Endophytic bacterial species have been found in many plant species (McInroy and Kloepper, 1995; Hallman *et al*., 1997b; Li *et al*., 2002). Several studies have shown that the interaction between plants and endophytic bacteria may be beneficial through plant growth promotion and biological control against plant pathogens (Lalande *et al*., 1989; Chen *et al*., 1995; Hallman *et al*., 1998). Chen *et al.* (1995) demonstrated reduction of root rot caused by *Rhizoctonia solani* Kühn and vascular wilt caused by *Fusarium oxysporum* f. sp. v*asinfectum* W.C. Synder & H.N. Hans in cotton (*Gossypium hirsutum* L.) plants by endophytic bacteria. M'piga *et al*. (1997) demonstrated that an endophytic isolate of *Pseudomonas fluorescens* Migula increased resistance to *F. oxysporum* f. sp. *radicis-lycopersici* Garvis & Shoemaker in tomato plants. Intensity of colonization by the pathogen was markedly reduced together with accumulation of electron dense material in the epidermal and outer cortex layers, and callose cell wall appositions in bacterized plants.

In this review, the role of endophytic microorganisms in the management of plant-parasitic nematodes in agricultural crops is discussed. Since limited information is available on the use of endophytes for control of the banana nematode *Radopholus similis* (Cobb) Thorne, the

review will focus on existing literature on the interactions between endophytes and plant-parasitic nematodes in grasses and other crops, highlight the implications of infection of plants by endophytes, and discuss the beneficial effects of endophytic microorganisms in the management of plant-parasitic nematodes. Finally, banana production in Uganda, production constraints and nematode control options, including the potential of endophytes, are discussed.

## 2. Why endophytes?

Several definitions for endophytism have been proposed (Carroll, 1988; Clay, 1990). For the purpose of this review, the term endophyte will refer to fungi or bacteria, which for all or part of their life cycle, invade and live inside tissues of living plants without causing any disease symptoms in or any apparent injury to the host plant (Petrini, 1991; Wilson, 1995). Epiphytes are bacteria and fungi that colonize plant surfaces. In contrast to epiphytes, endophytes are contained entirely within plant tissues, are asymptomatic and may be described as mutualistic (Clay, 1990). Bacteria associated with roots and the rhizospheres of many plant species known to benefit the plants through growth promotion and biological protection from pests and diseases (Rammamorthy *et al*., 2001) are referred to as plant growth promoting rhizobacteria (PGPR). Most PGPR belong to the genus *Bacillus* and *Pseudomonas* and are capable of proliferating not only around the plants roots but also inside (Van Peer *et al*., 1990; Pleban *et al*., 1995; Hallman *et al*., 1997b; Shishido *et al*., 1999). Similarly, fungi associated with roots and rhizospheres of the plants are referred to as plant growth promoting fungi (PGPF). Some of the important PGPF belong to the genus *Trichoderma* and *Gliocladium* (Altomare *et al*., 1999) and the arbuscular mycorrhizal fungi (AMF), which form symbiotic associations with plant roots and are also capable of colonizing the roots of their hosts (Gera Hol and Cook, 2005).

The use of endophytes for the control of plant-parasitic nematodes is a relatively new approach. Since endophytes spend most of their life cycle inside plant tissues they are less exposed to the external environmental factors and thus do not entirely depend on the environment for their multiplication and survival (Siddiqui and Shaukat, 2003a). Endophytes occupy a similar niche as the pests and are thus in close contact with the pests which makes them an edge over other biological control agents (Hallman *et al*., 1996b; 1997b). Inside the plant tissue, the host plant provides a relatively uniform and protected environment enabling

the endophytes to avoid microbial competition and extreme environmental conditions such as fluctuations in temperature and moisture (Ramamoorthy *et al*., 2001). They are easy to culture *in vitro* and can be applied as seed treatments or on transplants, reducing the inoculum levels required (Sikora, 1992; Musson *et al*., 1995; Sikora and Schuster, 1999). Another advantage is that once developed, farmers will not need to apply the control product themselves as this may be done by public/private organizations engaged in commercial tissue culture production (Dubois *et al*., 2006). Inspite of these advantages over other biological control agents, the potential of bacterial and fungal endophytes in pest and disease management in crops remains largely unexplored.

## 3. Colonization of plants by endophytes

The processes of colonization of plant tissue by endophytes are complex and include host recognition, spore germination, penetration and colonization. Endophytes penetrate their host plants through natural openings or wounds or actively using hydrolytic cellulases and pectinases (Hallman *et al*., 1997b; Rutherford *et al*., 2002), forming inconspicuous infections within healthy plant tissues for all or part of their life cycles (Siegel *et al*., 1987). Plant wounding induced by biotic factors such as plant-parasitic nematodes also constitute a major factor for entry of bacterial endophytes (Hallmann *et al*., 1998).

For many years, endophytic microorganisms colonizing plants have been thought to be weakly virulent pathogens residing latently within plant tissues (Sinclair and Cerkauskas, 1996). The distinction between endophytic and latent infections is not very clear. In latent infections, the host plant does not show any symptoms, with the infection persisting latently until symptoms are prompted to appear by environmental or nutritional stress conditions. The state of the host plant and the pathogen may also provide signals for symptom expression. Since the production of disease symptoms is dependent upon the interaction between the host, parasite and environment over time, endophytic colonization is considered not to cause any disease (Sinclair and Cerkauskas, 1996).

To detect endophytic colonization of plants, several methods for *in-situ* detection of fungal endophytes in plant tissues have been developed. A simple method involves microscopic examination of differentially stained samples of endophyte-infected plants (Saha *et al*., 1988). This method is, however, time consuming and less reliable since histological staining is not

endophyte-specific (Hahn *et al*., 2003). Other methods for *in situ* detection of endophytes have been developed. Some examples include the use of monoclonal antibodies (Hiatt *et al*., 1997; Hiatt *et al*., 1999), tissue print immunoblotting (Gwinn *et al*., 1991), tissue print immunoassays (Hahn *et al*., 2003), electron microscopy (Sardi *et al*., 1992) and autoradiography (You *et al*., 1995).

## 4. Methods for isolation of endophytes from plants

The growth of fungal hyphae outwardly from internal tissues of surface-sterilized plant tissues is considered the main evidence for endophytism (Petrini, 1986; Petrini, 1991). Several surface sterilization methods can be used depending on the plant tissue (Petrini 1991; Hallman *et al*., 1996a; Cao *et al*., 2005). The most common procedure involves surface sterilization of plant tissue using various disinfectants. Some of the disinfectants used include various concentrations of sodium hypochlorite (Fisher *et al*., 1992, Hallman *et al*., 1997b), ethanol (Fisher *et al*., 1992; Dong *et al*., 1994) and hydrogen peroxide (McInroy and Kloepper, 1995). Surface sterilization using ethanol involves dipping the plant tissue into ethanol and flaming the surface. This method is advantageous in that it is simple, fast and allows for processing of a large number of samples. Concentrations of the disinfectants and the length of sterilization times differ according to the plant species, age and the plant part. Afterwards, the samples are rinsed in sterile water and dried on sterile tissue paper. The surface sterilized samples are then placed on microbiological media and pure cultures are made followed by identification. Complete surface sterilization is often difficult to achieve and more advanced techniques for isolation of endophytes from plants have been developed. For example, techniques such as vacuum and pressure extraction of plant sap (Hallman *et al*., 1997a) and centrifugation of intercellular fluid of plant tissue (Dong *et al*., 1994) have been used successfully to isolate endophytic bacteria from plants. These two methods have been shown to bypass the problems associated with surface sterilization techniques.

## 5. Endophytic fungi and nematode control

The first report of nematode antagonism by fungal endophytes was described on tall fescue plants (*Festuca arundinacea* Schreb.) infected by *Pratylenchus scribneri* Steiner. West *et al*., (1988) compared nematode populations of migratory endoparasitic *P. scribneri* present in the soil surrounding endophyte free roots to those in roots of tall fescue plants infected with *A.*

*coenophialium*. Their results showed a reduction in nematode populations both in pots and field experiments and higher yield in tall fescue plots infected by *A. coenophialium*.
The effects of endophyte infection of tall fescue by root knot nematodes (*Meloidogyne* spp.) are probably best studied. Endophytic fungi adversely affected *Meloidogyne marylandi* Jepson and Golden, a root-knot nematode commonly associated with pasture grasses. A reduction in *M. marylandi* and *M. graminis* Sledge & Golden populations in tall fescue roots infected by the endophytic fungus *A. coenophialium* has been reported (Kimmons *et al*., 1989; Kimmons *et al*., 1990; Elmi *et al*., 2000). Juvenile emergence from eggs, the number of egg masses per pot and the number of eggs per egg mass were found to be lower than in endophyte-free tall fescue plants. Similarly, Ball *et al*. (1997) demonstrated higher numbers of *M. marylandi* in roots of perennial ryegrass plants free of the endophytic fungus *Neotyphodium lolii*. *Pratylenchus scribneri* penetrated roots of *N. coenophialum*-infected and endophyte-free tall fescue plants equally well but reproduction of the nematodes was hindered in the presence of the endophyte (Kimmons *et al*., 1990).

Endophyte effects on nematodes in other crops have also been demonstrated. For example, Hallman and Sikora (1994a, 1994b, 1994c, 1996c) demostrated that endophytic *F. oxysporum* isolated from tomato roots had detrimental effects on *Meloidogyne incognita* (Kofoid and White). Colonization of tomato roots by the endophyte resulted in 60% reduction of *M. incognita* infection.

The response to fungal endophytes by plant parasitic nematodes depends on the particular way in which the nematode species feeds (Cook and Lewis, 2001). Ectoparasitic nematodes remain in the soil or on the root surfaces feeding on the outer cells. Migratory endoparasites penetrate roots and feed on internal cells. Sedentary endoparasitic nematodes form specialised feeding cells in the plant tissue and remain embedded in the tissue. Due to protection by surrounding plant tissue, they are difficult to control by soil and rhizosphere microorganisms. Therefore, endophytic microorganisms colonizing plant root tissues may be better able to manage sedentary endoparasitic nematodes due to the fact that both occupy the same ecological niche and are in close contact (Hallman *et al*., 1996b; 1997b; Siddiqui and Shaukat, 2003a).

The length of time that the nematode spends feeding on plant cells may influence the chances of contact between the nematode and the endophyte or its by-products. The more intimate the

relationship between the nematode and plant, the more sensitive the nematode species are to fungal metabolites (Hallman and Sikora, 1996c). Selectivity for trophic groups has also been demonstrated by fungal endophytes. *Fusarium oxysporum* isolate 162 inactivated nematode species in similar trophic groups (Hallman and Sikora, 1996c). Between 60 to 100% of plant-parasitic nematodes were inactivated within 24 hrs of exposure, whereas the mobility of mycophagous and bacteriophagous nematodes was not altered (Hallman and Sikora, 1996c). The nematode stage may also influence the sensitivity to fungal metabolites and juvenile stages may be more sensitive than more advanced stages.

Although endophytic fungi have been shown to protect plants from nematode attack and damage, not all nematode species are affected by endophyte infection. Results obtained range from reduced root and soil nematode populations to no effect on nematode populations and increased levels of nematode infestation (Kimmons *et al*., 1990; Cook *et al*., 1991; Sikora *et al*., 2003). Populations of plant parasitic nematodes in three grassland sites on perennial ryegrass infected or not infected by the endophytic fungus *A. lolii* were studied over a 2-year period. In two of the three sites, nematodes were not reduced, but rather increased in the endophyte-infected plants, while no effect on nematode populations was observed at the third site (Cook *et al*., 1991).

## 6. Endophytic bacteria and nematode control

Information on the potential use of endophytic bacteria for nematode control is scarce. Hallman *et al*., (1995) provided evidence that endophytic bacteria may contribute to the control of *M. incognita* in tomato. Some endophytic bacterial isolates from cotton also resulted in a significant reduction of root galling by *M. incognita* (Hallman *et al*., 1998). Production of 2,4-diacetylpholoroglucinol, a bacterial secondary metabolite from an endophytic *P. fluorescens* isolate in culture, reduced egg hatching and resulted in substantial mortality of *Meloidogyne javanica* (Treub) Chitwoodi juveniles. Under glasshouse conditions, application of this isolate reduced root-knot development in tomato plants compared to untreated plants (Siddiqui and Shaukat, 2003b).

## 7. Mechanisms of action of endophytes against nematodes

Although endophytes have long been known to protect plants from both biotic and abiotic stresses, little is known on how they suppress pests and diseases. A thorough understanding of the mechanisms of action by endophytes, though, is needed to maximize the use, efficiency and consistency of biological control. Various mechanisms of action by fungal endophytes have been suggested (Clay, 1987). These include production of nematicidal metabolites (Hallmann and Sikora, 1994a, b; Cook and Lewis, 2001; Siddiqui and Ehteshamul-Haque, 2001; Li *et al*., 2002), changes in the host plant physiology (West *et al*., 1988; 1993; 1994; Assuero *et al*., 2000; Elmi *et al*., 2000) and the induction of general plant defense responses (Kimmons *et al*., 1990; Fuchs *et al*., 1999; Schulz *et al*., 1999, Siddiqui and Shaukat, 2003b). Direct parasitism of nematodes by endophytes could not be demonstrated (Vu *et al*., 2004) and may thus not represent an important effect of endophytes on nematodes.

### *7.1 Production of nematicidal secondary metabolites*

The production of toxic compounds (antibiosis) is an important mechanism of action of beneficial endophytic microorganisms against plant parasitic nematodes. Grass endophytes, mainly those belonging to *Neotyphodium* spp., produce a wide range of metabolites both in culture and in plants (Cook and Lewis, 2001). The production of alkaloids toxic to both insects and herbivores by grass endophytes has been documented (Breen, 1994). These toxins have been isolated successfully from both plants and pure cultures of grass endophytes. Infection of tall fescue plants by *N. coenophialum* resulted in both qualitative and quantitative differences in the production of volatile compounds between endophyte-infected and endophyte-free plants (Yue *et al*., 2001). Other examples of production of toxic metabolites by endophytic fungi have also been reported. Ma *et al*. (2004) reported production of rhizoctonic acid, momomethylsulochrin, ergosterol and 3β,5α,6β-trihydroxyergosta-7,22-diene by an endophytic isolate of *Rhizoctonia* sp. in Bermuda grass *Cynodon dactylon* (L.), which were toxic to the bacterium *Helocibacter pylori* Warren & Marshall.

Despite strong evidence for the production of toxic metabolites by grass endophytes, information regarding nematicidal compounds from non-grass fungal endophytes is scarce. Köpcke *et al*. (2002a; b) reported the production of pregaliellalactone and other related lactones by non-graminaceous endophytes, which had nematicidal activity. Production of 3-

hydroxypropionic acid (3-HPA) in culture by endophytic fungi isolated from above-ground plant organs has been demonstrated (Schwarz *et al*., 2004). This compound showed selective nematicidal activity against *M. incognita* and *Caenorhabditis elegans* (Maupas). Dead juveniles of *M. incognita* incubated for 12 hours in purified 3-HPA appeared stiff, turgid and straight. Culture filtrates of an endophytic *F. oxysporum* isolated from tomato roots reduced mobility of *M. incognita* within 10 minutes of exposure (Hallman and Sikora, 1994a; b). Ninety-eight percent of juveniles were inactivated after 60 minutes exposure to the culture filtrates, demonstrating the production of toxic metabolites in culture by this isolate.

Bacterial endophytes have also been shown to produce metabolites toxic to plant-parasitic nematodes. Li *et al*. (2002) reports production of toxic metabolites in culture filtrates by an endophytic *Burkholderia ambifaria* sp. novel (Coenye *et al*., 2001) isolate from corn roots. Culture filtrates inhibited egg hatch and mobility of second-stage juveniles of *M. incognita*. Results from purified culture filtrates showed that a <3kDa fraction was responsible for inhibition of *M. incognita*. *Burkholderia cepacia* Palleroni & Holmes has also been shown to be antagonistic to *M. incognita* (Meyer *et al*., 2000). An endophytic isolate of *Pseudomonas aeruginosa* (Schroeter) Migula produced toxic compounds *in vitro,* which resulted in substantial mortality of *M. javanica* juveniles (Siddiqui and Ehteshamul-Haque, 2001). Production of 2,4-diacetylpholoroglucinol, by an endophytic *Pseudomonas fluorescens* Migula isolate in culture reduced egg hatch and resulted in substantial mortality of *M. javanica* juveniles (Siddiqui and Shaukat, 2003b).

The ability of endophyte-infected plants to produce biologically active compounds may depend on the location and concentration of the endophyte in the plant. Distribution of these compounds in the plant may also vary depending on the compound itself and the season (Cook and Lewis, 2001). Toxins produced in endophyte-infected plants may be translocated elsewhere (Watson *et al*., 1993) and exuded into the surrounding soil, affecting nematode populations. For example, smaller populations of root lesion nematodes in the roots and rhizosphere of endophyte-infected grasses were associated with translocation of lolitrem and peramines from the stems and leaves to the roots (Kimmons *et al*., 1990).

Although toxic metabolites produced by most endophytes in culture may show antagonistic activity against nematodes *in vitro,* the role of these compounds in nematode reduction in the plant can only be shown if they are present in detectable concentrations in the plant tissues

and rhizosphere of plants that are infected by the endophyte, compared to endophyte-free plants (Lopez-Llorca and Olivares-Bernabeu, 1998; Kerry, 2000). Great uncertainty exists between the products of endophytes in culture compared with in nature. Production of bioactive compounds by endophytes may facilitate domination of a biological niche within the host plant or provide protection to the plant (Tan and Zhou, 2001). Although microbial endophytes may be a source of novel natural products that could be used as alternatives to chemical treatments in agriculture, very few plant species have been explored for the identification of endophytes and, subsequently, their associated products (Tan and Zhou, 2001; Strobel and Daisy, 2003).

Both the type and quantity of secondary toxic metabolites produced in endophyte-infected plants might depend on the fungal genotype (Cook and Lewis, 2001). For example, tall fescue endophytes grown *in vitro* differed in the production of ergot alkaloids (Bacon, 1988). Hill *et al*. (1990) also found that different isolates of *A. coenophialum* from tall fescue plants differed in the amounts and types of ergopeptine alkaloids produced. The host plant may also affect production and concentration of secondary metabolites. For example, concentrations of lolitrem B, ergovaline and peramine were found to vary among tall fescue cultivars infected with the same isolate of *A. lolli,* suggesting that phenotypic differences in tall fescue plants may have contributed to differences in endophyte-grass interactions (Hill *et al*., 1990). The results obtained suggest that the production of toxic compounds in the host plant by fungal endophytes may require some kind of plant-endophyte recognition. Additionally, the host-endophyte symbiosis is affected by the environmental conditions under which the host is growing, especially temperature. Breen (1994) suggested that the compatibility of an endophyte and its host may be important in determining production of toxic metabolites by the endophyte. It is, thus, important to determine compatible host-endophyte-genotype combinations in order to maximize the benefits of the association (Hill *et al*., 1990; Breen, 1994; Siddiqui and Shaukat, 2003a).

### *7.2 Changes in host plant physiology*

Endophyte-infected grasses have improved physiological responses to adverse environmental conditions. Probably the most documented feature of abiotic stress tolerance in endophyte-infected grasses is tolerance to, and recovery from, drought stress. Reduced nematode parasitism in endophyte infected tall fescue plants has been associated with enhanced root

growth and osmotic adjustment in growing points of the plant, thereby reducing the effects of drought on the host plant (Elmi *et al*., 2000). Endophyte-infected tall fescue plants were shown to be more drought tolerant than their endophyte-free counterparts (West *et al*., 1988; West *et al*., 1993; West, 1994). Morse *et al*. (2002) demonstrated that infection by *N. coenophialum* was beneficial to the growth of Arizona fescue (*Festuca arizonica* Vasey) under low water availability. Endophyte infection of grasses has been demonstrated to have positive effects on the plant's growth during water stress. For example, endophyte infected tall fescue plants had a higher net growth rate during water deficit than endophyte-free plants (Assuero *et al*., 2000). Under water stress, the tall fescue endophyte was associated with increased cell wall elasticity, which in turn conferred the drought tolerance response in the endophyte-infected grass (White *et al*., 1992).

While most studies have shown that endophyte-infected grasses are more drought-tolerant than endophyte-free counterparts, this may not always be true. For example, Cheplick (2004) demonstrated that endophyte-free perennial ryegrass under drought conditions had more tillers, greater leaf area and a higher total mass than grasses infected by the endophytic fungus *N. lolii*. After drought recovery, endophyte-free plants showed an equal or greater allocation to tiller bases than endophyte-infected grasses. Similarly, Ahlholm *et al*. (2000) demonstrated that endophyte-infected *Festuca pratensis* Huds. Syn. plants had less tillers and lower biomass production in low nutrient and water conditions than endophyte-free plants. Other results with *N. coenophialum* in *F. arizonica* showed that, under water and nutrient stress conditions in the field, the endophytes decreased growth and seed production in the host (Faeth and Sullivan, 2003).

Endophyte infection may improve the growth, size and quality of the host plants. Cook *et al.* (1991) demonstrated that endophyte-infected ryegrass grew much better than uninfected plants. The enhanced plant growth could lead to increased nematode populations compared to uninfected ryegrass, as a consequence of better growth of endophyte-infected plants. Enhanced growth of endophyte-infected tall fescue and perennial ryegrass compared to endophyte-free plants has been demonstrated. Malinowski and Belesky (2000) and Clay and Schardl (2002) showed that endophyte infected plants of *Lolium multiforum* Lam had more vegetative tillers and allocated more biomass to roots and seeds than endophyte free plants.

Endophytes have also been shown to influence photosynthesis rates in host plants. For example, tall fescue plants infected by *N. coenophialum* photosynthesized faster and flowered earlier than uninfected plants (Newman *et al*., 2003). Also, endophyte-infected tall fescue plants exhibited higher survival and flowering frequency (Hill *et al*., 1991). Such attributes of endophyte infection confer an ecological advantage to the endophyte-infected plants, enabling their survival and dominance over endophyte-free plants (West *et al*., 1988; Vila-Aiub *et al*., 2005).

Despite strong evidence for improved host physiology and tolerance to adverse abiotic stresses by endophyte-infected grasses, the mechanisms underlying this phenomena is still unknown. Research focused on this aspect is still needed and may help in selection of endophyte isolates that improve the plant's tolerances to both biotic and abiotic stresses. Apart from evidence gathered from research with grass endophytes, little to nothing is known about the influence of non-grass endophytes on their hosts' physiological responses.

*7.3 Induced plant defense responses*

Induction of systemic resistance by non-pathogenic microorganisms against pests and diseases is a well-documented phenomenon (Rammamorthy *et al*., 2001; Compant, 2005a). For example, a non-pathogenic *F. oxysporum* isolate (Fo47) induced resistance in tomato plants to *F. oxysporum* f. sp. *lycopersici* Jarvis et Shoem. when inoculated prior to infection with the pathogen (Fuchs *et al*., 1999). The endophyte *Acremonium kiliense* Grütz. induced resistance to *F. oxysporum* and *Clavibacter michiganense* pv *michiganense* (Smith), leading to delayed wilt symptoms, reduction of disease severity, and diminished loss of plant biomass in tomato plants (Bargmann and Schonbeck, 1992).

Induced systemic resistance (ISR) is defined as the resistance in plants induced by localized infection or treatment with microbial components or their products, or chemical compounds (Kuc, 2000; Ramamoorthy *et al*., 2001). ISR is different from systemic acquired resistance (SAR). SAR develops in plants in response to both biotic (pathogen attack) and abiotic factors (chemicals) and depends on the accumulation of salicylic acid (Van Loon *et al*., 1998). The onset of SAR is characterized by expression of genes for pathogenesis-related proteins (PR proteins) such as PR-1, PR-2, chitinases and peroxidases (M'Piga *et al*., 1997; Ramamoorthy

*et al*., 2001; Jeun *et al*., 2004). ISR, on the other hand is dependent on the jasmonic acid and phenylpropanoid pathways (Pieterse *et al*., 1998; Van Loon *et al*., 1998; Ramamoorthy *et al*., 2001). ISR leads to synthesis of plant defense products including peroxidases, polyphenol oxidases and phenylalanine ammonia-lyases (PAL). Polyphenol oxidases catalyse the formation of lignin through polymerization of phenols while PAL are involved in synthesis of phytoalexins and of phenolic compounds. It has not been demonstrated that endophytes induce SAR, which involves the hypersensitive reaction (Ramamoorthy *et al*., 2001).

Plant growth-promoting rhizobacteria (PGPR) are one of the most widely studied inducers of resistance in host plants against pest and pathogen attack (Ramamoorthy *et al*., 2001). Some PGPR may enter the interior of plant roots and establish endophytic associations (Kloepper *et al*., 1999). The ability of PGPR to establish endophytic relationships with their host plants indicate the possibility that naturally occurring endophytes may be able to induce resistance responses similar to those induced by PGPR. Endophytes may be suitable inducers of plant defense mechanisms, because by colonizing internal plant tissues they establish intimate relationships over a long period of time with their host plants (Siddiqui and Shaukat, 2003b). Induction of plant defense responses may occur through enhancement of the physical and mechanical strength of the plant cell wall, as well as biochemical changes in the host reaction, which leads to the synthesis of defense-related chemicals against the pest or pathogen (Ramamoorthy *et al*., 2001).

### *7.3.1 Induced physical and mechanical defense responses*

Few studies have been conducted on the host physical changes induced by endophyte infection. Nevertheless, there are a few indications that plants undergo structural changes due to infection by endophytes. Kimmons *et al*. (1990) noted a correlation between the thickness of the wall of the root endodermis and the presence of *P. scribneri* in tall fescue roots. Induced structural changes in the roots, mainly thickening of the endodermal cell walls, were also associated with endophyte infection in tall fescue, which in turn led to reduced *M. marylandi* populations. Thickening of the endodermis might reduce the ability of *M. marylandi* to penetrate the stele, induce giant cells and reproduce (Kimmons *et al*., 1990; Gwinn and Bernard, 1993).

### *7.3.2 Induced biochemical defense responses*

Induction of biochemical changes by PGPR is well documented. Accumulation of phenolic compounds, which occurs after challenge inoculation with the pathogens in plants, may be one of the mechanisms through which fungal endophytes become beneficial to the host plant (Biggs, 1992). For example, the endophytic bacterium *Burkholderia phytofirmans* Sessitsch *et al.* induced phenolic compound accumulation and strengthening of the cell walls in the exodermis in grapevines (*Vitis vinifera* L.) (Compant *et al*., 2005b). Schulz *et al*. (1999) demonstrated a higher phenolic metabolite concentration in roots of larch (*Larix larcina* (Duroi) K.Koch) and barley (*Holdeum vulgare* L.) plants infected by endophytic *Cryptosporiopsis* sp. and *Fusarium* sp. than in endophyte-free plants. Increased chitinase activity observed in endophyte-infected clones of tall fescue was associated with reduced nematode effects (Roberts *et al*., 1992). Although the authors could not directly link increased chitinase activity and nematode response, they concluded that, since chitinase is a PR-protein (Van Loon *et al*., 1998), it may have some effect on the resistance mechanism of the plants leading to reduced nematode populations. Chitinases indirectly release oligosaccharide signal molecules capable of activating a variety of plant defenses against pests and diseases (Ryan, 1988).

### *7.3.3 How to detect ISR*

The evidence for ISR is mainly obtained by separation of the biological control agent from the infection courts and the subsequent decrease of disease upon challenge inoculation with the pathogen. The split-root technique provides a convenient method to assess ISR as it gives spatial separation of the inducing agent and the pathogen for the duration of the experiment (Van Loon, 1997). Since split-root experiments prevent direct interaction between the biological control agent and the pest/pathogen, the observed pest or disease reduction is usually attributed to increased defense responses due to colonization of the roots by the biological control agent (Fravel *et al*., 2003). For example, Duijff *et al*. (1998) used split root experiments to demonstrate that induced resistance was a mechanism of control by endophytic *F. oxysporum* in the suppression of fusarium wilt of tomato. Similarly, Reitz *et al*. (2000), using split-root experiments, showed that soil treatments on one half of the root system with lipopolysacharides (LPS) of the rhizobacteria *Rhizobium etli* sp. novel L. Segovia**,** Young and Martinez-Romero resulted in a reduction of root penetration by the cyst

nematode, *Globodera pallida* (Stone) on the untreated half of the split root system on potato. Their results showed that LPS of *R. etli* act as the inducing agent of systemic resistance against *G. pallida* in potato roots. Although the antagonistic bacterium used was isolated from the rhizosphere, these results lead to the assumption that endophytic bacteria would induce similar responses in other plants. Spatial separation of the pathogen or pest and the inducing agent can also be achieved when the pest is applied on the roots and the inducing agent in the foliage, or by inoculating the pest and inducing agent on different leaves of the same plant. For example, Bargabus *et al*. (2004) demonstrated a reduction in Cercospora leaf spot symptoms by inoculating the biological control agent *Bacillus pumilus* Meyer and Gottheil on different leaves of sugar beet (*Beta vulgaris* L.) than the pathogen.

### *7.3.4 Benefits of ISR*

Biological pest and disease management by endophytes mediated through induction of resistance is advantageous because sustained pest and disease suppression can be observed, even when the population of the inducing bacteria or fungus declines over time. Additionally, activation of plant defense mechanisms may be maintained for prolonged periods of time and may be effective against multiple pathogens (Ramamoorthy *et al*., 2001). Non-specificity of ISR has been demonstrated, although the effectiveness of ISR against different pathogens may vary (Kúc, 2000). If ISR is the main mechanism of action by endophytes against nematodes, then the need for complete colonization of the plant root system is unnecessary. Threshold colonization rates capable of stimulating the plant to defend itself would need to be determined (Hallman, 2001).

## 8. Banana production in Uganda

The National Agricultural Research Organization (NARO) reported that bananas occupied the largest cultivated area among staple food crops in Uganda (NARO, 2001) with more than 75% of all farmers growing the crop (Gold *et al*., 1993). *Per capita* annual consumption of bananas in Uganda is the highest in the world, estimated at around 250 kg / year / person (INIBAP, 2000; NARO, 2001). Bananas are consumed as fruit, prepared by cooking, roasting or drying, or fermented for the production of alcoholic beverages as well as for non-alcoholic banana juice (Karamura, 1993).

Bananas are primarily grown as a subsistence crop for both rural and urban consumption with surplus production sold in local markets (Karamura, 1993; Mugisha and Ngambeki 1994; Karamura *et al*., 1996). Most banana production takes place on small subsistence farms (plots of less than 0.5 ha) with low input farming methods (Gold *et al*., 1998; Karamura, 1999). The life span of banana plantations depends on agro-ecological conditions and management practices, and may range from as low as 4 years in Central Uganda to over 30 years in Western Uganda (Speijer *et al*., 1999a).

Most of the banana varieties grown in Uganda are endemic to the East African highlands. The clones are triploid hybrids belonging to the East African highland bananas (*Musa* AAA-EA). NARO (2001) also reports that an estimated 85% of bananas grown in the country are East African highland bananas. These endemic banana varieties (AAA-EA genomic group) consist of two types based on their use: cooking bananas (*matooke*) and beer bananas (*mbidde*). They are classified by morphological characteristics into five clone sets: Musakala, Nakabululu, Nakitembe, Nfuuka and Mbidde (Karamura and Karamura, 1994; Gold *et al*., 1998). The non-endemic bananas grown in Uganda have their origins in Southeast Asia and include exotic beer and sweet bananas (AB, ABB and AAA genomic groups), and roasting bananas or plantains (AAB genomic group). Banana production is characterized by wide cultivar diversity with farmers growing 10 to 15 different banana cultivars in stands of less than 200 banana mats (Karamura, 1993; Karamura *et al*., 1996; Gold *et al*., 1998).

Geographic shifts of banana production towards new growing areas (Southwestern Uganda) and the abandonment of the crop in traditional areas (Central Uganda) have occurred in recent years. In the traditional banana growing areas of Central Uganda, production of endemic East African highland bananas has stimulated an increase in the production of non-endemic bananas and other food crops (e.g. maize, sweet potato, cassava). Combinations of pest and disease pressure, declining soil fertility, and socio-economic constraints (reduced labor availability and management of the plants) have been cited as the causes for this trend (Gold *et al*., 2000). The major pest constraints to banana production are the banana weevil *Cosmopolites sordidus* (Germar) and a complex of plant-parasitic nematodes.

## 9. Nematode problems of banana

A complex of plant-parasitic nematodes comprising *Radopholus similis* (Cobb) Thorne, *H. multicinctus* (Cobb) Golden and *Pratylenchus goodeyi* (Sher and Allen) have been identified as major nematode species affecting banana production in Uganda (Gold *et al*., 1993, Karamura, 1993, Speijer *et al*., 1999a). Geographic distribution of the main nematode species is governed by temperature and elevation. *Radopholus similis* and *H. multicinctus* are the predominant species between 1000-1350 m above sea level (Kashaija *et al*., 1994) while at higher elevations and cooler temperatures *P. goodeyi* is the predominant species (Speijer *et al*., 1994). Mixed populations occur at lower elevations. Cultivar, cropping systems, farm management and sources of planting material influence abundance of individual nematode species, while species composition varies among sites and between farms within sites (Kashaija *et al*., 1994). All three nematode species are migratory endoparasites with their life cycles being completed in the root tissues. Among these nematode species, the burrowing nematode *R. similis* is considered to be most important economically in Uganda (Kashaija *et al*., 1994) and worldwide (Sarah *et al*., 1996).

## 10. Biology of *Radopholus similis*

*Radopholus similis* is a migratory endoparasitic nematode completing its life cycle in 20-25 days in root and corm tissue under optimal temperature conditions of 30ºC. Embryonic development occurs within 4-10 days and the subsequent juvenile stages are completed in 10-15 days. They attack almost all banana cultivars as well as abáca (*Musa textilis* L.) and other seeded *Musa* spp. (Gowen and Quénéhervé, 2005). Distribution of *R. similis* is governed by preferences for temperature fluctuations between 24ºC and 32ºC with optimum reproduction occurring around 30ºC (Sarah *et al.,* 1996). Being primarily endoparasitic, *R. similis* is hardly found in the soil.

Penetration of nematodes in banana roots occurs mainly at the root apex though invasion can occur at any portion along the root length (Sarah *et al*., 1996). After root penetration, the nematode occupies an intercellular position in the cortical parenchyma and migrates in and between cells in the root cortex feeding on cell cytoplasm. This results in collapsed cell walls, cavities and tunnels. On the corm, lesions begin to develop where infested roots are attached and then spread outwards. Necrosis can extend to the whole cortex of the corm and the roots

but the root stele is rarely damaged. *Radopholus similis* females lay eggs inside roots and all development occurs within the root. All juvenile stages and females of *R. similis* are infective and cause damage to the roots while males lack a developed stylet and are not plant-parasitic (Speijer and De Waele, 1997). Though primary, secondary as well as tertiary roots can be affected, *R. similis* were found to occur in higher numbers in primary roots than in lateral roots (Kashaija, 1996). Spatial distribution of nematodes in banana root parts revealed the highest *R. similis* numbers in the cortical parenchyma and less in the epidermis and vascular cylinder (Araya and De Waele, 2001).

## 11. Damage and economic importance of *Radopholus similis*

Damage due to *R. similis* infection occurs because of its migration and feeding activity inside the plant roots. Symptoms of nematode damage can be identified early in longitudinal sections of roots as reddish brown necrotic patches usually confined to the cortex region and extending from the root surface to the centre. On corms, necrosis appears as reddish brown discoloration, usually beginning where roots leave the corm (Speijer and De Waele, 1997). Nematode feeding destroys root and corm tissue, reducing water and mineral uptake, resulting in a reduction of plant growth and development. This leads to a severe reduction of bunch weight and a significant increase in time period between two successive harvests. Due to weakening of the root systems, plants can easily be blown over by strong winds, a condition referred to as toppling (Sarah *et al.,* 1996; Jones, 2000; Gowen and Quénéhervé, 2005).

Nematodes and banana weevils frequently occur together on the same plant (Speijer *et al*., 1994). On young suckers, nematode and banana weevil damage are highly associated, which aggravates the loss of banana plants (Speijer *et al*., 1993). Due to the association between banana nematodes and weevils on banana plants, it's often very difficult to estimate the actual yield losses caused by nematodes alone (Speijer *et al*., 1993). Damage caused by weevils and nematodes is often confused leading to underestimation of nematode damage with most of the damage on the corm attributed to the weevil (Gold *et al.,* 1993).

*Radopholus similis* can cause yield losses of up to 30-50% per cycle in on-station trials (Speijer *et al*., 1999b; Speijer and Kajumba, 2000). The East African highland banana cv. Mbwazirume infected by both *R. similis* and *P. goodeyi* had bunch weight reductions of up to 30% with the percentage of toppled plants being four times higher when both nematodes were

present compared to when either of the two occurred alone (Talwana *et al*., 2003). Nematode-induced losses are a result of an increase in the number of dead roots, root necrosis, reduced number of standing leaves, reduced flower production, increased plant toppling and reduction in bunch weight (Speijer *et al*., 1999b; Speijer and Kajumba, 2000; Talwana *et al*., 2003).

## 12. Management of *R. similis* in banana

*Radopholus similis* control has mainly relied on the use of chemical nematicides in commercial plantations and although these nematicides are very effective, the feasibility for use is beyond the economic means of most small-scale farmers in developing countries such as Uganda (Gowen, 1991). Conventional methods of establishing plantations using nematode-infested suckers as planting material are the main avenues for introducing these pests to new fields (O'Bannon, 1977; Sarah, 1989). Dissemination of the banana nematodes through infested planting material continues to occur unabated as farmers continue to use infested suckers. Most farmers in Uganda perceive control strategies against banana pests (nematodes, weevils and diseases) to be too costly, labour consuming and/or ineffective (Gold *et al*., 1993). Nevertheless, several nematode management options are available.

### *12.1 Cultural control*

The most important measure to control nematodes in banana stands is the use of healthy planting material (Sarah, 2000). Using pest and disease-free planting material reduces the spread of nematodes to new fields (Speijer *et al*., 1995). Clean planting material can be obtained in several ways. Corm paring, which involves removal of nematode infested roots and corm tissue, can reduce initial infestation (Speijer *et al*., 1995; Gold *et al*., 1998). Additional hot water treatment of corm-pared suckers by dipping in hot water at a temperature of 53ºC for 20 min rids the plants of nematodes (Speijer *et al*., 1995; Gold *et al*., 1998) leading to crop yield improvements of about 30% in the first crop cycle (Speijer *et al*., 1999a). Though these methods have proven effective and feasible, they only offer temporary control as re-infestation of the plants readily occurs in the fields (Speijer *et al*., 1995; Speijer *et al*., 2001). Acceptance of the hot water treatment technology at the farmer level is also constrained by problems of transportation of the suckers and hot water tank (Speijer *et al*., 1999b).

Mulching can also mitigate the effects of nematodes on banana plants. Obiefuna (1991) demonstrated that mulching with crop residues effectively decreased *R. similis* populations and increased yield in plantain. Mulching reduced the population densities and damaging effects of *R. similis* on cv. Mbwazirume (*Musa* spp. AAA-EA). Banana mats in mulched plots had lower soil temperatures than unmulched plants, which may have slowed down *R. similis* reproduction (Talwana *et al*., 2003). In contrast, McIntyre *et al*. (2001) obtained higher nematode numbers and root necrosis in mulched banana plots. Mulching may reduce the impacts of nematodes through increased soil nutrient availability, increased soil porosity and increased surface rooting and may also has beneficial effects on plant growth (Speijer *et al*., 1999a; McIntyre *et al*., 2000).

Use of legume intercrops was found to have no effect on nematode and the banana weevil populations and damage. Though intercrops may not reduce the effects of nematodes they may be incorporated in banana farming systems to increase land use efficiency and provide food security (McIntyre *et al*., 2001). Use of intercrops is mainly hampered by the ability of *R. similis* to reproduce in many plant species as well as weeds (O'Bannon, 1977).

### *12.2 Host plant resistance*

Strong resistance to *R. similis* has been identified in Pisang jari buaya clones (*Musa* spp. AA group) (Pinochet and Rowe, 1979) and in the banana cultivars Yangambi KM5 (AAA), Gros Michel (AAA) (Fogain and Gowen, 1997; Sarah *et al*., 1997) and Kunnan (AB) (Collingborn *et al*., 2000). Studies to investigate the resistance mechanisms in Yangambi KM5 revealed greater amounts of preformed phenolic compounds (Valette *et al*., 1998) and while cultivar Kunnan was found to possess high amounts of condensed tannins (Collingborn *et al*., 2000). Resistance may offer a long-term intervention against nematodes for resource poor farmers in Africa. However, resistance to nematodes has not been identified in highland cooking banana cultivars. Banana improvement by means of conventional plant breeding has proved extremely difficult due to the genetic complexity of the crop and the long period required to evaluate crossings for resistance to different nematode collections (Stover and Buddenhagen, 1986; Tripathi, 2003). Additionally, most banana varieties are triploid genotypes that are almost or fully sterile, which further complicates the situation.

### *12.3 Transgenic banana*

Due to difficulties involved in conventional breeding methods, there has been increased research interest in the potential of genetic engineering to tackle pressing biotic problems in banana production. Banana plants may be genetically transformed to express resistance genes for protein inhibitors such as cystatins (Tripathi, 2003). Cystatins are naturally occurring proteinase inhibitors used by plants as a defense against insects and pests (Atkinson *et al*., 1995). They impair digestion of dietary protein by nematodes and suppress nematode multiplication (Atkinson *et al*., 2004). Cystatins are effective against a wide range of nematodes and can protect banana from different combinations of pest species that occur in plantations. Atkinson *et al.* (2004) successfully transformed Grand Naine plants (Cavendish subgroup-AAA) with a rice cystatin. Transformed plants achieved a reduction of 70% in *R. similis* population densities. The acceptance and adoption of transgenic banana in developing countries such as Uganda may be undermined by lack of biosafety rules and regulations. Although developing transgenic banana may offer a long term control option against banana pests and diseases, it may take a long time before such cultivars are freely available to farmers (De Waele, 2000).

### *12.4 Tissue culture banana plants*

Use of tissue culture banana plants is one means to provide clean planting material (Mateille *et al*., 1994; Sarah, 2000). Tissue culture plants are produced axenically, making them pest- and disease-free. Additional advantages are the rapid multiplication, higher yields and uniformity (Robinson, 1996). Advocacy for the use of tissue culture plants is on the increase, though adoption by farmers in Uganda remains limited, especially due to the low availability and relatively high cost. Loss of beneficial microorganism such as endophytes through the axenic production of tissue culture plants may probably make them more vulnerable to nematode attack in the field (Pereira *et al*., 1999). Research has shown that tissue culture plants are more susceptible to nematode and disease attack in the field than plants derived from suckers (De Waele *et al*., 1997; Stanton, 1999; Viaene *et al*., 2003; Blomme *et al*., 2004). Tissue culture plants infested with *R. similis* had more nematodes and higher root damage than plants derived from suckers (Viaene *et al*., 2003). Although tissue culture plants may offer temporary solutions to nematode problems in banana, there is a need to develop affordable, sustainable and environmentally friendly management strategies that complement

the benefits of clean planting material offered by tissue culture. The artificial introduction of beneficial microorganisms such as endophytic fungi in these sterile plants at the hardening phase may offer protection to the young plants in the early growth stages and extend the life of planting material (Sikora and Schuster, 1999).

### *12.5 Biological control*

Biological control of plant-parasitic nematodes may offer an alternative to nematicides due to environmental and health concerns associated with the chemicals. However, biological control should not be used in isolation but should form part of an integrated nematode management approach. Several biological control agents have been used to reduce the effects of *R. similis* in banana.

#### *12.5.1 Rhizosphere bacteria and Actinomycetes*

Four isolates of rhizosphere fluorescent bacteria (three *P. fluorescens* and a *P. putida* Trevisan Migula isolate) showed *in vitro* repellent effects to *R. similis* and resulted in lower nematode invasion and development in greenhouse plants (Aalten *et al*., 1998). Application of *Streptomyces costaricanus* sp. nov. Esnard**,** Potter and Zuckerman resulted in improved plant growth and lower *R. similis* populations (Esnard *et al*., 1998). Combining these cultures with wheat mash further improved results. Pre-inoculation of banana plantlets using a commercial water dispersible *Paecilomysces lilacinus* (Thom) Samson product (strain 251) resulted in decreased *R. similis* activity in the field, with a positive correlation between rates of application and degree of *R. similis* control (Mendoza *et al*., 2004)

#### *12.5.2 Mycorrhizae*

The use of arbuscular mycorrhizal fungi (AMF) to control nematodes in banana in pot experiments has also been demonstrated. AMF have also been shown to promote plant growth. Declerck *et al*. (1995) reported a significant increase in growth of tissue culture banana plantlets inoculated with AMF. Inoculation of plantain tissue culture plants with the AMF *Glomus mosseae* (Nicolson & Gerdemann) Gerd during the weaning phase significantly improved plant growth and reduced *R. similis* populations compared to non-mycorrhized

plants. Similar results were reported by Elsen *et al*. (2003) and Fogain and Njifenjou (2003). Elsen *et al*. (2003) demonstrated that mycorrhization of different banana genotypes differing in root morphology resulted in better plant growth even in the presence of nematodes.

### 12.5.3 *Endophytes*

Endophytes offer a novel and environmentally friendly means of nematode management in banana. Since *R. similis* is an endoparasite completing its life cycle in banana root and corm tissues, the prospects for using endophytes, which occupy the same niche, are promising. Additionally, application of endophytes in vegetatively propagated crops such as banana may ensure continued pest and disease suppression in later ratoon crops if the endophytes are transmitted to the sucker plants (Ramamoorthy *et al*., 2001). Banana is a perennial herb and the introduction of endophytes into tissue culture plantlets at an early stage of growth is feasible. Since tissue culture plants contain no microorganisms, introduced endophytes should easily establish in the plant tissues (Cao *et al*., 2005). Many effective endophytes have been found in other crops for the management of nematodes, which makes biological control of banana nematodes using fungal endophytes a promising option.

Evidence for the presence of fungal endophytes in banana plants has been reported. Endophytic fungi have been isolated from healthy banana plants growing in nematode and banana weevil infested plantations in Uganda (Schuster *et al*., 1995; Griesbach, 1999; Niere 2001), Central America (Pocasangre, 2000) and South Africa (Athman *et al*., unpublished). The main fungi isolated as endophytes from roots and corms of banana plants mainly belonged to the genus *Fusarium*. *Fusarium oxysporum* was the predominant species in Uganda, Central America and South Africa.

Artificial inoculation of 1-month-old tissue culture banana plants has successfully been achieved through root and corm dip. Tissue culture plants are deflasked and dipped in the fungal spore suspension for variable time periods allowing spore attachment and the subsequent endophytic colonization of the roots and corm tissues (Griesbach, 2000; Niere, 2001; Paparu *et al*., 2004). Fungal isolates inoculated into banana plants are able to colonize the plants roots, which has led to enhanced plant growth and lower nematode infection (Griesbach, 2000; Sikora *et al*., 2000; Niere, 2001). Inoculation of endophytes into tissue

culture plants may serve to biologically protect the plants at a crucial stage before they are transplanted to the field. The treatment of tissue culture plants provides the advantage of lower costs due to the low levels of inoculum required (Sikora *et al*., 2003). Application of the endophyte pre-grown on a solid substrate such as maize bran also offers an alternative method for artificially introducing the endophyte in tissue culture plants by mixing the fungal inoculum with the potting soil at transplanting time (Paparu, 2005).

Various isolates of endophytic *F. oxysporum* have been shown to produce nematostatic and nematicidal compounds against *R. similis* in culture filtrates. Pocasangre (2000) and Niere (2001) demonstrated inactivation of *R. similis* motile stages in culture filtrates of endophytic *Fusarium* spp. isolates from banana and related this effect to possible toxic metabolites in the culture filtrates. Although these toxins were not identified, it was concluded that the fungal isolates tested produced secondary metabolites in culture, which resulted in inactivation and mortality of *R. similis*. *In vitro* tests for parasitism of four endophytic *F. oxysporum* isolates in the absence of banana plants demonstrated a lack of direct parasitism on *R. similis* although nematode activity decreased significantly (Vu *et al*., 2004).

Positive effects of endophyte infection of banana plants in screen house experiments have been demonstrated. Pocasangre (2000), working on fungal endophytes isolated from banana plants in Central America, found a lower number of *R. similis* in banana roots. A reduction in penetration of nematodes in root tissue of up to 74%, and increased shoot and root weights in endophyte-inoculated banana plants was also noted. Speijer (1993) reported decreased penetration and development of *P. goodeyi* in banana plants colonized by a non-pathogenic isolate of *F. oxysporum*.

Plant growth promotion effects of banana plants manifested in higher root and shoot weights in endophyte-treated plants than in non endophyte-plants, although this was not consistent for all isolates tested (Pocasangre, 2000; Niere, 2001). Griesbach (2000) reported increased banana biomass production in field plants of the cultivars Nakyetengu and Nfuuka (AAA-EA) inoculated with the fungal endophyte *Fusarium concentricum* Nirenberg & O'Donnell. Reduced nematode populations in endophyte-treated plants may depend on the fungal isolate and banana clone (Niere *et al*., 2004). The endophytic *F. oxysporum* isolate V5W2 has been earmarked for its biocontrol potential against the banana nematode *R. similis*. *In planta* screening experiments using tissue culture plants have shown this isolate to reduce *R. similis*

reproduction by between 22.9 and 60.6% in the banana cultivars Enyeru and Kibuzi (AAA-EA), respectively, compared to control plants (Dubois *et al*., 2004).

Colonization and persistence of introduced endophytic fungal isolates in tissue culture banana plants has been extensively studied through re-isolation from infected roots and corm tissues of banana plants after surface sterilization (Paparu, 2005). The identity of re-isolated fungal isolates was further confirmed with vegetative compatibility tests. No differences were observed among endophyte isolates or banana cultivars, indicating a lack of interaction between isolate and cultivar (Paparu, 2005). One month after inoculation of plants with the fungal isolates, corms were colonized to a higher extent compared to roots. However, colonization rates declined over time and there was substantial decline in percentage colonization after field planting of the plants. Decline in the colonization rates of corm tissue was more pronounced than in roots up to 33 weeks after plants had been inoculated with the endophytes. Different methods of introducing fungal endophytes resulted in differences in percentage colonization (Paparu, 2005). The use of a solid substrate medium for introducing endophytes into banana roots gave the best results, followed by root and corm dip of plants with a bigger root biomass in a conidial suspension. To enhance formation of a bigger root biomass, plants were first grown in a nutrient solution for 1 month before being inoculated with the endophyte. The lowest colonization rates were observed in plants that were inoculated with the conidial suspensions after being removed straight from rooting medium.

## 13. Conclusions

Endophytes have important implications for their host plants, in providing protection against biotic and abiotic stresses. Presently, little is known of the potential benefits of endophytic microorganisms. Endophytic microorganisms are potentially attractive biocontrol agents and could be utilized to protect tissue culture plants before they are transplanted to the field. This would expose the plant to microorganisms that could provide protection through systemically induced plant responses. While the use of endophytic microorganisms for nematode and pest control in crops is relatively new and unexplored, it is an environmentally friendly alternative to pesticides at a time that the latter become increasingly restricted. Information on the diversity of endophytes and mutualistic interactions with crops other than grasses remains scanty, and more research is needed to understand their full potential as biocontrol agents.

Culture filtrates of endophytic fungi have shown *in vitro* activity against nematodes, suggesting that these fungi produce secondary metabolites that are nematicidal. However, only a limited number of research projects have focused on isolation and identification of such compounds and their effects. Additionally, production of these compounds in culture (*in vitro*) does not necessarily reflect what would happen in the plant. Their effects on nematodes in the plants may only be proven if the compounds can be extracted from endophyte-infected plants. Screening isolates and banana cultivars for the production of toxins is necessary to determine the role of antibiosis in nematode suppression in the plant.

Although research on the use of endophytes remains recent, there is strong evidence that they could provide a source of novel nematicidal compounds capable of protecting plants from pest and disease attack. Little is known about the interactions between the host plant, endophyte and the pest. Understanding the host-endophyte-pest interactions is essential for the successful development of biological control as it would help in selection of the most suitable candidate isolates and also help in designing control programmes that maximally utilize their nematode controlling potentials. Elucidation of different mechanisms of action would also facilitate combination of isolates, thus providing a broader spectrum of control.

Decline in colonization of banana plants by introduced fungal endophytes with continued pest suppression provides strong evidence for the presence of other mechanisms of action acting through the host plant. Continued nematode population reduction, even when endophyte colonization rates fall substantially, is an indication that additional mechanisms are involved, especially induced resistance mechanisms. It is, therefore, important to determine threshold colonization rates that are capable of eliciting effective and durable plant defense responses in plants.

The use of endophytes for inducing systemic resistance may be more beneficial to vegetatively propagated crops such as banana. As a perennial crop banana remains in the field for relatively long periods and is thus continuously challenged by pest and disease attack. Since the frequency of re-isolation of the endophyte isolates on banana plants decreases over time, long-term protection in banana may not be high. How long the plant remains in the induced state is also unknown. Earlier studies so far suggest that introduced endophytes can provide protection throughout the first growth cycle (Niere *et al*., unpublished). Although initial introduction of endophytes in tissue culture plants may provide

protection during this vulnerable stage, what happens at later stages of the plants growth is unknown. The possibility of maternal transmission of introduced endophytes to suckers needs to be investigated. This would minimize the need for additional or frequent applications as the introduced endophytes become self-perpetuating and sustaining. The effect and necessity for booster applications following initial inoculation, especially when plants are in the field, needs to be investigated.

Finally, while endophytes may be successfully used in plant-parasitic nematode management programmes, they are unlikely to achieve the levels of control provided by pesticides and, therefore, are unlikely to replace pesticides (Kerry, 1990; Van Loon *et al*., 1998). Their performance is inconsistent under screen house conditions (Niere, 2001). Their use must, therefore, be maximized in an integrated nematode management programme that include cultural practices and field management (Sikora *et al*., 2003). Additionally, combinations of biocontrol agents with complementary or synergistic mechanisms of action may be used to further reduce pest and disease pressure. Biological control of plant-parasitic nematodes using endophytes may be an additional option for environmentally friendly and benign nematode management.

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