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A Novel Approach to the Biological Control of Banana Nematodes

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Abstract

Plant parasitic nematodes cause considerable losses in both commercial and subsistence banana production systems and their control remains difficult. Control measures are mainly based on the use of nematicides and clean planting material such as tissue culture plantlets. Tissue cultured bananas, however, have been found to be more susceptible to nematodes when compared to conventional planting material, especially in the first crop cycle. Isolation of endophytic fungi from healthy banana rhizome tissue showed that *Fusarium oxysporum* is the predominant fungal species colonizing banana rhizome tissue. Isolates of *F. oxysporum* were inoculated onto tissue cultured bananas prior to weaning. Endophyte inoculated bananas showed less nematode reproduction and damage when compared to non-inoculated plants. Under field conditions, beneficial effects were observed during the first 4 months after transplanting in fields heavily infested with nematodes. The results showed that inoculation of fungal endophytes improves plant health of banana in the first cycle and as such contributes to a sustainable banana production and reduced pesticide use.

Keywords: banana, endophytes, *Fusarium oxysporum*, nematode control, tissue culture.

Introduction

Bananas (*Musa* spp.) are known to be a major commodity in international fruit trade, but are far more important as a starchy staple in local food economies. This is reflected by the fact that only 10% of the annual world production of approximately 90 million tons enters the export trade. In Sub-Saharan Africa bananas and plantains provide food to over 100 million people. This is also the region were the highest per capita consumption of bananas in the world is found. Banana and plantain are one of the cheapest foods to produce;commercial production and subsistence cultivation of banana, however, is threatened by a complex of pests and diseases.

Destruction of banana roots is caused by a complex of different nematode species. The burrowing nematode (*Radopholus similis*), root lesion nematodes (*Pratylenchus* spp.), and the spiral nematode (*Helicotylenchus multicinctus*) may affect banana production depending on the location either alone or as a complex. Disease severity is affected by a number of environmental factors but usually correlates with the number of nematodes present in the roots. The burrowing nematode *R. similis* is one of the major pests limiting banana production worldwide and is considered the primary cause of banana root rot. The nematode causes

reddish brown lesions in the cortex and affected roots may finally die. The damaged root system results in reduced water and nutrient uptake and poor anchorage of the plant. Yield is reduced and the vegetative cycle is lengthened (Gowen and Quénéhérvé, 1990). Severely damaged plants may finally topple, leading to total loss of the bunch.

Chemical control of nematodes is environmentally unfriendly, hazardous to human health and too expensive for small scale farmers serving local markets in Africa. Breeding for nematode resistance seems to be favorable, however, little nematode resistance within the common commercial *Musa* cultivars is known (Gowen, 1995). Therefore, the development of biological control agents as an alternative nematode control component is necessary (Kerry, 1990; Sikora, 1992).

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Tissue cultured planting material is propagated under sterile conditions and all organism, including beneficials, associated with banana plants are lost during tissue culture initiation. Banana grown from shoot-tip culture are disease free and provide clean planting material. However, tissue culture derived planting material is more susceptible to pests and diseases when compared to conventional planting material. This is especially true for young banana plants being transplanted from nursery sheds to infested fields. Plantation establishment is the most labour and cost intensive part of banana production, hence, protection of the planting material at an early stage is extremely important.

Fungal endophytes are potentially effective biological control agents for plant parasitic nematodes management (Hallmann and Sikora, 1994; Schuster et al., 1995). Many fungi have been described to be associated with nematode lesions in banana roots mostly increasing disease severity (Stover, 1966; Sikora and Schlösser, 1973; Pinochet and Stover, 1980; Mateille and Folkertsma, 1991). Nevertheless, it has been shown that some fungi colonising banana root tissue are inhibitory to migratory endoparasites (Sikora, 1992; Schuster et al., 1995; Niere et al., 1999). Fungi from healthy banana roots have been isolated and previously tested for their nematode controlling ability *in vitro* using culture filtrates (Schuster et al., 1995).

Biological control of banana nematodes has been demonstrated *in planta*, whereby damage and multiplication was reduced in roots of endophyte inoculated tissue cultured bananas (Niere et al., 1999). Furthermore, plant height of some banana cultivars was increased by some endophytic isolates (Niere et al., 1999). In addition, strains of *Fusarium oxysporum* are capable of reducing damage caused by the banana weevil, *Cosmopolites sordidus*, (Griesbach et al., 1996), and seems to protect against the banana wilt pathogen, *Fusarium oxysporum* f.sp. *cubense*, (Gerlach, K., personal communication).

Methodology

Endophytic fungi were isolated from healthy banana corm and root tissue. Surface sterilisation of plant tissue was carried out using standard protocols (Gams et al., 1998). Tissue was cut in small pieces, plated on Synthetic Nutrient Agar (SNA) described by Nirenberg (1976), and incubated for 4 to 10 days. Petri dishes were viewed daily for fungal growth and outgrowing fungi were transferred to sterile petri dishes containing SNA. Pure

cultures were either stored on soil tubes (Schneider, 1958) or at -80°C. Isolates of *Fusarium oxysporum* from healthy tissue were chosen for inoculation of tissue cultured bananas. Fungal cultures were grown in potato dextrose broth on a rotary shaker and spores harvested by filtering the fungal slurry through a KLEENEX tissue placed in a filter holder. Spore suspensions of 10^6 –10^7 spores/ml were used for inoculation of tissue cultured banana plants. Banana plants, cultivars Gros Michel (*Musa* AAA) and Enyeru (*Musa* AAA-EA), were produced in tissue culture using standard shoot-tip culture protocols (Vuylsteke, 1998). Inoculation with endophytes took place immediately after removing the plants from the test tubes. Roots were first trimmed and washed free of culture medium, then dipped in spore suspensions of the respective fungal isolate prior to weaning. Control plants were dipped in liquid culture media only. Plants were potted in plastic cups filled with sterilised soil and hardened in a humidity chamber for 2 weeks. Four weeks old plants were transferred to 2 L polythene bags filled with sterile soil. Plants growth parameters were taken every 4 weeks and at the termination of each experiment.
Banana plants were challenged with plant-parasitic nematodes using two different methods:

i), plants grown in bags were infested with a nematode suspension following the method for nematode resistance screening of banana (Speijer and De Waele, 1997)

ii), banana plants were transplanted to nematode infested fields at the IITA Sendusu Farm Station in Namulonge/Uganda (1150 masl).

Nematode damage was assessed (Speijer and Gold, 1996) and nematodes were extracted overnight using the extraction dish method (Oostenbrink, 1960) from either the whole root system in test (i), or from a 5 g sub-sample of randomly selected roots in test (ii).

Results

None of the plants inoculated with endophytic fungal isolates showed symptoms of wilting or disease. Plant growth of endophyte inoculated plants was not affected in five months old East African Highland Bananas, cv. Enyeru (syn. Nabusa, *Musa* AAA-EA) grown in bags. Nine months old plants of the dessert cultivar Gros Michel (syn. Bogoya, *Musa* AAA) grown in fields heavily infested with nematodes were also not affected when compared to non-inoculated control plants.
Differences in nematode multiplication were detected between endophyte inoculated and control plants. Biological control effects among plants inoculated with different endophytic fungal isolates as well as under varying challenge situations were observed. In the cultivar Nabusa, development of females, although not significant, was affected in plants inoculated with fungal endophyte B 1. The number of females in endophyte-inoculated plants was reduced by 50% compared to control plants, whereas the number of males and juveniles was not altered considerably (Table 1).

Table 1. Nematode densities in roots of six months old endophyte-free (control) and endophyte-inoculated tissue cultured banana plants, cv. Enyeru (syn. Nabusa, *Musa* AAA-EA) eight weeks after inoculation of 1,000 vermiform *Radopholus similis* per plant

Treatment[1]	*Fungal species*	Radopholus similis*/root system*			
		female	***male***	***juveniles***	***total***
Control	-	209	7	78	294
B 1	Fusarium oxysporum	103	5	95	203

[1] Following treatments were applied: control (Potato Dextrose Broth only) and spore suspension of *Fusarium oxysporum* isolate V5w2 (B 1). No significant differences between treatments, n =7.

In fields heavily infested with nematodes, fungal endophyte inoculation of tissue cultured *Musa* plants, cv. Gros Michel, accounted for lower nematode numbers in roots of all endophyte treated plants compared to control plants four months after transplanting to the fields. The number of spiral nematodes, *Helicotylenchus multicinctus*, was significantly lower in plants inoculated with endophyte B 1 over the control plants. In this treatment, nematode numbers were reduced by 75% compared to untreated plants (Table 2). Other nematode species present at this site were not affected by the various treatments.

Table 2. Densities of the spiral nematode, *Helicotylenchus multicinctus*, in roots of endophyte-free (control) and endophyte-inoculated tissue cultured banana plants, cv. Gros Michel (*Musa* AAA) four months after transplanting to nematode infested field.

Treatment[1]	*Fungal species*	**nematodes/100 g roots**
Control	-	14063 a
B 6	Fusarium oxysporum	8260 a
B 10	*Fusarium oxysporum*	9063 a
B 11	*Fusarium oxysporum*	4060 a
B 1	*Fusarium oxysporum*	3524 b

[1] Following treatments were applied: control (Potato Dextrose Broth only) and spore suspensions of *Fusarium oxysporum* isolates V4w5 (B 6) III3w3 (B 10), III4w1 (B 11), and V5w2 (B1). Means in column followed by the same letter are not significantly different at $P < 0.05$ (Duncan's Multiple Range Test), n = 8.

Conclusion

Growth promotion of plants inoculated with fungal endophytes has been reported for grasses (e.g. Clay, 1988), tomato (Hallmann and Sikora, 1994), and banana (Niere et al., 1999). The isolates of *F. oxysporum* used for inoculation of tissue cultured bananas in these experiments, however, did not alter plant growth compared to control plants. Interactions between non-pathogenic fungal isolates and banana cultivars are not yet fully understood, genetic and environmental factors are suspected to contribute to the variation in growth response. Important was the fact that none of the fungal endophytes tested reduced plant growth. Furthermore, the isolates of *F. oxysporum* used for inoculation did not induce wilting

symptoms or discoloration of the vascular strands in the highly Fusarium wilt susceptible cultivar Gros Michel during the course of the experiments, again demonstrating the non-pathogenic nature of these fungal isolates.

Nematode densities were reduced in plants evaluated using international standard nematode resistance screening protocols. Although the reduction of 30% was not significant, the number of *R. similis* females was reduced by 50%. The influence of fungal isolates on the developmental stages affect nematode population composition and may thereby slow down the build-up of disease pressure. This is especially important when plants are transplanted to fields with a history of plant-parasitic nematode infestations. Nematode population could be affected in a way that nematode numbers remain below a threshold level of economic importance.

Nematode control was also observed in endophyte inoculated plants transplanted to nematode infested fields. All endophyte treatments reduced *H. multicinctus* numbers nine months after endophyte inoculation and four months after transplanting to plots where high numbers of nematodes were present. Earlier results suggested that the reduction in nematode numbers was not due to direct antagonistic activity of the fungal isolate on the nematode in the soil system, but was caused by endophyte activity inside the root tissue of plants (Niere et al., 1999). This observation is strengthened by the fact that nematode control could be observed nine months after fungus inoculation, several transplantations, and that no additional fungus, other than the initial inoculum used, was applied during the course of the experiment.

The fungal application system used - dipping tissue culture plants at weaning stage in a spore suspensions - can be effectively used in mass propagation of biological enhanced tissue cultured planting material before it is placed in the field. Production of fungal spores is easy, inexpensive, and the weaning process is only slightly altered by the inoculation of the fungal isolates prior to weaning. Furthermore, only small amounts of inoculum are needed and no subsequent applications are necessary.
The above mentioned findings, as well as results from other working groups, suggest that fungal endophytes are involved in host plant responses to pests and diseases. Reintroduction of beneficial micro-organisms to sterile tissue cultured plants may substantially improve state-of-the-art biotechnology and as such could contribute to sustainable banana production.

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