Uganda Journal of Agricultural Sciences, 2004, 9,445-451
ISSN 1026-0919

Merging biotechnology with biological control: Banana Musa tissue culture plants enhanced by endophytic fungi

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Abstract

Among the major constraints to highland cooking banana (*Musa* spp. AAA-EA) production in Uganda is the high pests infestation levels. Most important are the banana weevil *Cosmopolites sordidus* and a complex of plant parasitic root nematodes of which *Radopholus similis* is the most important. Clean planting material, such as tissue culture derived plants, is now being promoted in Uganda for establishments of new fields, although pest re-infestation remains a vital concern. Fungal endophytes are microorganisms that colonize plant tissue internally for at least part of their life cycle to form mutualistic relationships with their host plants, including antagonism to pests and diseases. These endophytes can be artificially inoculated into tissue culture plants and thus reduce banana weevil and nematode pressure. A survey for endophytes in highland cooking banana revealed that most strains belong to *Fusarium* spp. *In vitro* bioassays against *C. sordidus* eggs and *R. similis* juvenile and adult stages showed that, depending on the endophytic strain, mortality can reach up to 100.0%. *In vivo* screenhouse experiments using tissue culture plants revealed that the endophytic strain *V5w2* decrease *R. similis* reproduction by 22.9 and 60.6% in the banana cultivars Enyeru and Kibuzi (AAA-EA), respectively, compared to control plants. The use of endophytic fungi is a novel yet promising biological control strategy that can be used against the banana weevil and parasitic nematodes.

Key words: *Cosmopolites sordidus*, fungal endophytes, *Fusarium*, *Radopholus similis*,

Introduction

Highland cooking banana (*Musa* sp., AAA-EA) is the most important staple in the Eastern Africa Great Lakes region, serving both rural and urban populations. Since the 1970s, large areas of highland banana have experienced accelerated yield decline associated with decreased management standards, high levels of pests, and declining soil nutrient status (Gold *et al*., 1999). The banana weevil (*Cosmopolites sordidus*) and a complex of nematodes (*Radopholus similis*, *Helicotylenchus multicinctus*, *Pratylenchus* spp. and *Meloidogyne* spp.) are major constraints to banana production in Uganda (Speijer *et al*., 1999; Gold *et al.*, 2004). Banana weevils and nematodes are soil-borne pests that affect the roots and corm, damaging vascular tissue, impeding nutrient and water uptake and weakening plant anchorage. Yield reductions result from plant toppling, delayed maturation, reduced bunch size, diminished vitality of followers and decreased plantation life (Speijer *et al.*, 1999; Gold *et al.*, 2004). Yield losses of up to 100% have been reported (Gold *et al*., 2001).

Banana weevils and nematodes are notoriously difficult to manage. Cultural control methods contribute to the control of banana weevils and nematodes, although associated costs often limit farmer adoption (Gold *et al*., 2001). Most promising is the use of clean planting material such as tissue-culture plants (Gold *et al*., 2001). Re-infestation still remains a primary concern: banana weevils readily invade from neighbouring fields, while nematodes are often already present in the soil in fields. Research at the International Institute of Tropical Agriculture (IITA) is focusing on enhancing tissue-culture plants with microbial antagonists, such as endophytes, that are important biological control agents against banana weevils and nematodes (Niere, 2001; Gold *et al*., 2003).

Most plants harbour endophytes, which are microbial organisms that live part of their life cycle within the plant without producing disease symptoms. Endophytes in banana are common and many show antagonistic activity against banana weevils and nematodes (Griesbach, 1999; Niere, 2001). Due to the sterile nature of the production system, tissue-culture bananas lose the endophytes they

are naturally associated with (Pereira *et al*., 1999). By reintroducing these endophytes into the sterile plant, natural plant health will be restored before the plants are transferred to farmers.

Schuster *et al*. (1995) isolated a number of fungal endophyte strains from healthy banana roots and corms from the major banana growing regions in Uganda Similarly, Griesbach (1999) isolated 200 fungal endophyte strains from Ugandan banana roots and corms. Most of the strains were identified as *Fusarium* spp. Some of these strains might be effective against both *C. sordidus* and *R. similis*. When artificially inoculated into tissue culture plants, *Fusarium* endophytes might therefore constitute a single means for biocontrol of both pests.

The purpose of this study was to test the efficacy of some of these strains against both *C. sordidus* eggs and vermiform *R. similis* stages (males, females and juveniles) in laboratory bioassays. Strain *V5w2*, the strain with the highest activity against *C. sordidus* and *R. similis*, was then inoculated into tissue-culture banana (*Musa*) plants and further tested in a screenhouse. Endophyte-enhanced tissue-culture plants belonging to three different cultivars (Enyeru AAA-EA, Kibuzi AAA-EA and Kayinja ABB) and containing strain *V5w2* were tested in screenhouse experiments to test their effect on two different population densities of *R. similis*.

Material and methods

Location of experiments, and source of endophyte strains, C. sordidus, R. similis and banana plants

All experiments were carried out at the IITA Research Station at Sendusu-Namulonge, Uganda. Adult banana weevils were trapped using banana pseudostem traps. A colony of *R. similis* is being maintained by multiplication on carrot discs in the laboratory according to Speijer and De Waele (1997). Banana plants were micropropagated from sword suckers in tissue culture using standard shoot-tip culture protocols for banana as outlined by Vuylsteke (1998). All endophyte strains were originally isolated from root and corm tissues of East African highland banana in Uganda (Schuster et al., 1995; Griesbach 1999) and are being preserved in soil tubes at 5°C at the Station (Table 1).

***C. sordidus* bioassays**

Two *in vitro* bioassays were carried out to test the effect of 15 fungal endophytes on banana weevil eggs. Each bioassay used a different banana weevil population and was made up of four separate experiments carried out in four weeks. In each bioassay, each treatment consisted of two petri dishes containing weevil eggs. In each bioassay, the same fungal endophyte strains were tested except strain *Kib5.1o* (only tested in the first bioassay) and strain *Emb2.4o* (only tested in the second bioassay).

For each strain, small quantities of soil containing fungus from soil tubes was sprinkled on fresh synthetic nutrient agar medium (SNA) (1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 7H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose, 0.6 ml NaOH (1 M), 13.2 g agar in 1000 ml distilled water) in 85 mm diameter petri dishes and incubated in the laboratory at room temperature (± 25°C) with a photoperiod of ± 12: 12 L: D hrs for 7-10 days to allow them sporulate. For each strain, 4 blocks of SNA (± 0.5 cm^3) containing spores and mycelia were inoculated into 100 ml sterile potato dextrose broth medium (PDB) (12 g potato dextrose broth in 1000 ml distilled water) in a 250 ml erlenmeyer flask and the medium was incubated for 7 days. Erlenmeyer flasks containing autoclaved (121°C for 15 min) PDB yielded the control treatments and were not inoculated. During incubation, the flasks were shaken once daily to ensure even spore growth. A fungal spore suspension was obtained by filtering the fungal slurry through a 1 mm diameter sieve into a 500 ml sterile beaker. Spore densities were subsequently determined using a hemacytometer under a light microscope. For each strain, spore concentration was adjusted to 1.50-1.75 x 10^6 spores ml^{-1}.

A freshly harvested corm from a sucker (Enyeru or Kibuzi, AAA-EA) was pared and placed in a 5 L bucket. About 50 banana weevils (males and females in unknown proportions) were then placed on the corm and the bucket lid sealed with masking tape. For each experiment, four buckets were set up. After 24 hrs the corms were removed and the eggs extracted by paring the outer layer of the pseudostem base and the cortex using a kitchen knife. The eggs were taken to the laboratory and sterilized in a laminar flow cabinet by dipping in 75% EtOH for 2 min and rinsing thrice in sterile distilled water.

While working in the laminar flow cabinet, sterile filter papers were placed in sterile 90 mm diameter petri dishes and each filter paper was moistened with ± 1 ml sterile distilled water. Banana weevil eggs were then placed on the moistened filter paper, using 20 and 15 banana weevil eggs per petri dish for the first and second bioassay, respectively. A 5 µl drop of spore suspension or sterile PDB was added onto each weevil egg using a sterile pipette tip, after which the petri dishes were sealed with parafilm. Of the eggs that failed to hatch after 10 days, those that were discolored and/or colonized by fungal growth were considered to be dead while those that appeared creamy in color were considered alive (Griesbach, 1999).

***R. similis* bioassays**

Culture filtrates of nine endophyte strains were evaluated for their effect on *R. similis* mortality in three bioassays. Strains were grown for two weeks in banana corm broth. Corm tissue was obtained from banana suckers (Enyeru) and the broth was prepared by boiling 500 g of corm tissue in 1 L tap water until soft. The mixture was filtered through cheesecloth. The resulting filtrate was topped with distilled water to 1 L, dispensed into 250 mm erlenmeyer flasks, and autoclaved.

Table 1. The identity of fungal endophyte strains used in bioassays against banana weevil *Cosmopolites sordidus*) eggs and *Radopholus similis*, and in screenhouse experiments against *R. similes*

Strains	Species	Origin	Original cultivar
Emb2.4o	*Fusarium oxysporum*	Corm	Embiire (AAA-EA)
Eny7.41	*Fusarium* sp.	Corm	Enyeru (AAA-EA)
Eny7.11o	*Fusarium oxysporum*	Corm	Enyeru (AAA-EA)
Kib4.4o	*Fusarium solani*	Corm	Kibuzi (AAA-EA)
Kib5.1o	*Fusarium* sp.	Corm	Kibuzi (AAA-EA)
Eny1.31i	*Fusarium oxysporum*	Corm	Enyeru (AAA-EA)
Eny7.42	*Fusarium* cf. *concentricum*	Corm	Enyeru (AAA-EA)
V1w7	*Fusarium oxysporum*	Root	Unknown AAA-EA
V4w4	*Fusarium oxysporum*	Root	Unknown AAA-EA
III3w3	*Fusarium oxysporum*	Root	Unknown AAA-EA
III4w1	*Fusarium oxysporum*	Root	Unknown AAA-EA
V2w2	*Fusarium oxysporum*	Root	Unknown AAA-EA
V4w5	*Fusarium oxysporum*	Root	Unknown AAA-EA
V5w2	*Fusarium oxysporum*	Root	Unknown AAA-EA

Adopted from Griesbach (1999) and Niere (2001).

The broth was allowed to cool and inoculated with agar discs of the different strains according to the method described above. Flasks were shaken every day to disperse spores throughout the broth. Culture filtrates were obtained by centrifugation (10000 rpm for 15 min) and vacuum filtration. Un-inoculated broth served as a negative control. To remove the effect of pH among treatments, an additional control was incorporated in which the pH was adjusted to the same pH level as the broth containing the fungal strains. A nematode suspension (100 nematodes ml^{-1} of mixed stages) in 0.1 ml sterile distilled water was added to 1 ml of undiluted culture filtrate in sterile 30 mm diameter petri dishes. Un-inoculated broth served as the control treatment. Three petri dishes were used per treatment. Mortality was determined by rinsing nematodes after 24 hr with sterile distilled water and incubating them for an additional 24 hr. Nematodes that did not move after probing with a fine needle were recorded as dead.

Effect of three different banana tissue culture cultivars enhanced with strain V5w2 on two different population densities of R. similis

Three screenhouse experiments were carried out, each containing 28 tissue culture plants of each of three cultivars (Kayinja (AB), Enyeru and Kibuzi). The 28 plants per cultivar were divided into two groups of 14 plants each. Plants of one group per cultivar were inoculated with the fungal strain *V5w2* whereas the plants in the second group were dipped in pure PDB to serve as controls. Each group of 28 plants was inoculated with two different nematode densities (low or high).

A fungal spore suspension of strain *V5w2* of 1.5-1.75 x 10^6 spores ml^{-1} in sterile water was prepared as described above. Plants were inoculated as follows. The roots were washed free of the modified MS medium and cut back to ± 2 cm in length. Plants treated with endophytes were dipped in the spore suspensions for a period of 2 hr. Control plants were dipped in PDB medium. The inoculated plants were potted in 150 ml plastic cups having four holes at the bottom and containing about 150 ml of steam-sterilized soil. The cups were placed on trays in a humidity chamber in a screen house at ± 27°C. After 4 weeks, plants were transplanted into 350 ml polythene bags containing ± 3 l of steam-sterilized soil in the screenhouse and watered daily. Two different nematode suspensions were prepared, containing 200 *R.similis* ml^{-1} or 100 *R. similis* ml^{-1}. The plants were inoculated with *R. similis* 42 days after fungus inoculation by pipetting the nematode suspension into the soil through holes punched in the soil close to the roots using a sterile pointed nail.

Plants were uprooted on day 41, 53 and 48 after nematode inoculation for experiment 1, 2 and 3, respectively. After the roots were washed to remove soil, the number of intact and dead roots per plant was recorded and the fresh root weights determined using a digital weighing balance. Root necrosis was scored according to the method described in Speijer and Gold (1996). Roots were randomly selected per plant and 2.5 g was chopped into small pieces and macerated in a kitchen blender. The macerated suspension was poured through a 200 mm sieve and the suspension recovered in a plastic dish positioned below the sieve. The whole set up was allowed to stand for 24 hrs after which the contents of the dishes were rinsed with tap water into 100 ml glass jars. After the nematodes had settled, the volumes of

the suspensions were adjusted to 25 ml by siphoning off the water layer from the top and the number of female, juvenile and male *R. similis* were counted in three aliquots of 2 ml using a dissecting microscope.

Data analysis

To obtain a normal distribution with equal variances among treatments, some parameters were transformed prior to analysis. For the bioassays, percentage banana weevil egg mortality and percentage *R. similis* mortality were arcsine-squareroot transformed. For all screenhouse experiments, *R. similis* density, percentage root necrosis and percentage dead roots were squareroot (+0.5) transformed. Differences among treatments were detected using analysis of variance (ANOVA). In case of interactions, separate ANOVAs were performed. Multiple mean comparisons were performed using Tukey's studentized range test (SAS institute, 1989).

Results

Cosmopolitus sordidus egg bioassays

In both bioassays, weevil egg mortality in the controls (5.4 ± 1.32% and 0.8 ± 0.8% for bioassay 1 and 2, respectively) was significantly lower ($F = 13.03$, df = 12, $P < 0.0001$ and $F = 7.99$, df = 13, $P < 0.0001$ for bioassay 1 and 2, respectively) than in the fungal treatments (Table 2). Overall, weevil egg mortalities caused by fungal endophyte strains in bioassay 1 was higher (38.93 ± 1.32%) than in bioassay 2 (28.14 ± 1.55%). Fungal endophyte strains previously isolated from the roots caused higher mortalities (36.46 ± 1.27%) than those from the corms (34.66 ± 1.40%) ($F = 3.06$, df = 1, $P = 0.050$).

Within the fungal treatments in bioassay 1, there were significant differences in weevil egg mortality among the different strains screened ($F = 2.37$, df = 11, $P = 0.014$). Mortality due to fungal suspension of the different strains was between 32.78 ± 2.74 and 50.28 ± 2.93%. In bioassay 2, there were no significant differences in weevil egg mortalities among fungal treatments ($F = 0.84$, df = 12, $P = 0.66$). Egg mortalities caused by spore suspensions of the endophyte strains was between 25.83 ± 5.41 and 37.50 ± 4.35 %.

In both bioassays, different endophyte strains performed consistently across experiments (data not shown). However, the efficacy of the endophyte strains to cause mortality was not consistent between both bioassays. Strains *V2w2* and *V5w2* were the best strains in bioassay 1 and 2, respectively. Only two strains (*V2w2* and *III3w3*) ranked among the five best in both bioassays.

***Radopholus similis* bioassays**

Percentage nematode mortality was highly different among bioassays ($F = 28.11$, df = 2, $P < 0.0001$), and a significant interaction between bioassays and treatments ($F = 16.24$, df = 20, $P < 0.0001$) revealed that strains performed different across bioassays. Within each bioassay, percentage nematode mortality in both controls was significantly lower than in the fungal treatments (Tukey's studentized range test, $P < 0.05$). Only two strains (*V4w5* and *V5w2*) ranked consistently among the five best across the three bioassays (Table 3).

Effect of three different banana tissue culture cultivars enhanced with strain V5w2 on two different population densities of R. similis

The total number of nematodes per 100 g root varied across experiments (Fig 1). Across all experiments, the total number of nematodes per 100 g of root tissue was highly significantly lower in control plants than in endophyte-treated plants (*F e"* 11.76; df = 1; *P d"* 0.0002). The total number of nematodes per 100 g of root tissue was marginally non-dependent on banana cultivar in the first experiment ($F = 2.61$, df = 2, $P = 0.081$), but in the second and third experiments, significant differences were observed across cultivars ($F = 5.93$, df = 2, $P = 0.0043$ and $F = 3.00$, df = 2, $P = 0.050$, respectively) with significantly higher nematode densities in Kibuzi and Enyeru than in Kayinja. In none of the experiments was there any difference in the total number of nematodes per 100 g of root tissue between plants that had received a high nematode dose and plants that had received a low nematode dose (*F* d" 3.31, df = 1, *P* e" 0.074).

Fresh root weight was highly significantly different between cultivars in experiments 1 and 3 (*F e"* 11.51, df = 2, $P < 0.0001$) but not in experiment 2 ($F = 1.35$, df = 2, $P = 0.27$) (Table 4). Endophyte treatment did not affect the fresh root weight in all experiments (*F* d" 0.01, df = 1, *P e"* 0.093). Percentage root necrosis differed significantly among cultivars in experiment 2 ($F = 3.87$, df = 2, $P = 0.026$) but not in the experiments 2 and 3 (*F d"* 0.20, df = 2, *P e"* 0.24). Endophyte treatment did not affect percentage root necrosis in all experiments (*F d"* 0.010, df = 1, *P e"* 0.11). Percentage dead roots was not significantly different across cultivars in all experiments (*F* d" 0.75, df = 2, *P* e" 0.39) and averaged 8.1 ± 1.1 (n = 83), 2.9 ± 0.5 (n = 80) and 3.9 ± 0.8 (n = 73) for experiments 1, 2 and 3, respectively. Endophyte treatment

Discussion

Results from the C. sordidus egg bioassay indicate the capacity of spore suspensions of fungal endophytes to cause mortality to banana weevil eggs. Banana weevil egg mortality in controls was negligible, whereas all strains caused 26.7-50.0% mortality. This is in agreement with earlier results obtained by Griesbach (1999). The generally higher weevil egg mortalities recorded in bioassay 1 as compared to bioassay 2 may be due to the differences in weevil egg vitality. Eggs for bioassay 1 and 2 were obtained from two different weevil populations. This possibility is further supported by the fact that, in bioassay 1 where the mortality due to spore suspensions were higher, mortality for eggs inoculated with sterile PDB were higher too.

Table 2. Percentage mortality of banana weevil (*Cosmopolites sordidus*) eggs by spore suspensions of *Fusarium* spp. Endophytes

Strain	% mortality (Mean±	
	Bioassy 1	Bioassy 1
Control	5.4 ± 1.9	0.8 ± 0.8
Eny1.31i	39.0 ± 5.2ab	35.8 ± 3.1a
Eny7.11o	37.3 ± 4.0ab	30.8 ± 4.7 a
Eny7.41	34.0 ± 5.0b	28.3 ± 6.0 a
Eny7.42	50.0 ± 3.8a	30.0 ± 3.7a
III3w3	45.0 ± 4.6a	37.5 ± 4.3a
III4w1	43.7 ± 3.5ab	27.5 ± 5.6a
Kib4.4o	32.3 ± 2.0b	25.8 ± 5.4a
Kib5.1o	41.2 ± 2.4ab	-
Emb2.4o	-	30.8 ± 3.8a
V1w7	41.0 ± 4.4ab	30.0 ± 3.7a
V4w4	32.8 ± 2.7b	28.3 ± 6.9a
V5w2	34.6 ± 3.7b	37.5 ± 5.2a
V2w2	50.3 ± 2.9a	35.0 ± 3.7a
V4w5	42.9 ± 3.6ab	26.7 ± 4.2a

Means in the same column followed by different letters are significantly different at $P < 0.05$ (Tukey's studentized range test).
N = 8 for each strain within a bioassay.

Table 3. Percentage mortality of male, female and juvenile *Radopholus similis* by culture filtrates of *Fusarium* spp. Endophytes

Strain		% mortality (Mean±SE	
	Bioassay 1	**Bioassay 2**	**Bioassay 3**
pH-adjusted control	14.8 ± 1.2	14.2 ± 2.1	36.9 ± 4.8
Unadjusted control	79.1 ± 3.6	41.2 ± 2.7	46.7 ± 12.4
Emb2.4o	97.7 ± 0.6ab	97.4 ± 1.5ab	96.1 ± 1.1abc
Eny1.31i	99.0 ± 0.5ab	99.1 ± 0.9ab	77.6 ± 5.1dc
Eny7.11o	99.6 ± 0.4a	100.0 ± 0.0a	84.2 ± 3.0dc
III3w3	100.0 ± 0.0a	76.4 ± 1.1c	88.2 ± 2.6bcd
III4w1	98.6 ± 0.7ab	98.5 ± 0.8ab	84.8 ± 4.8dc
V1w7	94.2 ± 0.5b	95.3 ± 1.3b	97.5 ± 0.4ab
V2w2	98.0 ± 1.3ab	97.0 ± 1.6ab	100.0 ± 0.0a
V4w5	99.6 ± 0.4a	100.0 ± 0.0a	89.9 ± 2.5bcd
V5w2	99.2 ± 0.8ab	100.0 ± 0.0a	99.0 ± 1.0a

Means in the same column followed by different letters are significantly different at $P < 0.05$ (Tukey's studentized range test).
N = 3 for each strain within a bioassay.

Enyeru Kibuzi Kayinja

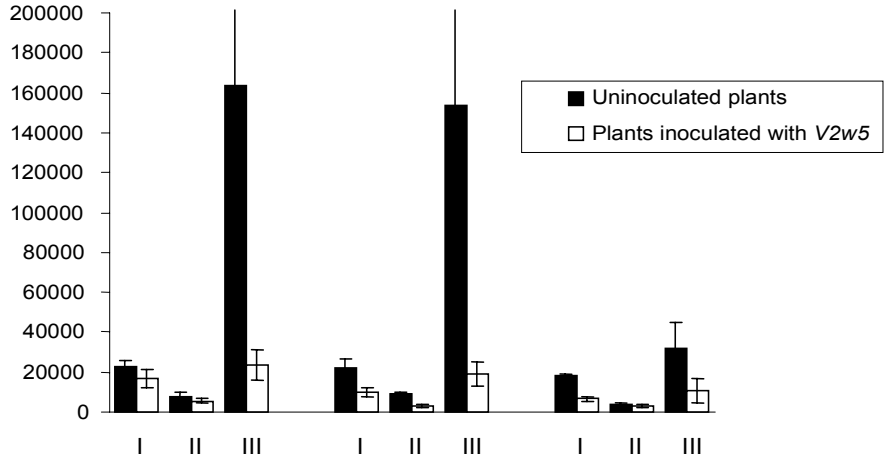

Fig. 1. *Radopholus similis* densities in Enyeru, Kibuzi (AAA-EA) and Kayinja (AB) banana plants with or without endophytic *Fusarium* strain *V5w2*.

Table 4. Percentage root necrosis and fresh root weight in banana cultivars Enyeru, Kayinja and Kibuzi.

Cultivar	Root necrosis (%)								Fresh root weight (g)									
	Exp. 1			Exp. 2			Exp. 3			Exp. 1			Exp. 2			Exp. 3		
	n	Mean ± SE		n	Mean ± SE		N	Mean ± SE		n	Mean ± SE		N	Mean ± SE		N	Mean ± SE	
Enyeru	27	5.1 ± 1.9	a	26	8.5 ± 2.5	a	2	2.5 ± 1.2	a	2	3.2 ± 0.3	b	2	11.0 ± 0.7	a	26	7.0 ± 1.0	b
							6			8			6		b			
Kayinja	28	4.2 ± 1.8	a	27	2.9 ± 1.2	b	2	1.1 ± 0.4	a	2	5.6 ± 0.4	a	2	13.1 ± 0.8	a	26	9.2 ± 0.5	a
							6			7			7					
Kibuzi	28	5.2 ± 2.0	a	24	6.6 ± 1.6	a	2	3.6 ± 1.4	a	2	3.2 ± 0.2	b	2	10.4 ± 0.8	b	26	4.8 ± 0.3	b
						b	6			8			4					

Means within each column followed by the same letter are not significantly different (Tukey's studentized range test, $P > 0.05$)
Data from treatments with or without endophyte *V5w2* were pooled.

In all *R. similis* bioassays, fungal filtrates of all strains caused 84.2-100% mortality, whereas *R. similis* mortality in pH-adjusted control medium was only 14.2-36.9%. Because fungal filtrates do not contain spores or mycelium, these mortality rates are a strong indication of the production of nematicidal metabolites by the strains tested. Strains are continuously being isolated and screened against *R. similis* at IITA. Since all nine strains tested against *R. similis* caused near 100% mortality, modifications have to be incorporated in subsequent bioassays to differentiate the best strains for further tests in the screenhouse. In future bioassays, culture filtrate dilutions will be used that will more closely mimic the natural conditions in banana plants encountered by *R. similis* and that will be able to differentiate strains.

Interestingly, strain *V5w2* seems to be adapted to cause mortality in both *C. sordidus* and *R. similis*. The effectiveness of a single strain against both the banana weevil and *R. similis* opens up the possibility of using a single endophyte strain to target the two pests that usually occur simultaneously in the field.

When strain *V5w2* was inoculated into tissue culture plants, *R. similis* densities were always much lower in endophyte-inoculated plants than in control plants. The exact mechanism employed by the fungal strain to limit nematode population reproduction inside the root tissues of the inoculated banana plants was not investigated in this experiment. However, production of metabolites with possible nematicidal properties was demonstrated in the *R. similis* bioassays and has been reported to occur among fungi that inhabit plant tissues as endophytes (Sikora and Schuster, 1999). It is likely that the metabolites produced by these endophytes interfered with the reproduction of the nematodes hence leading to lower population densities inside the roots. The activity of strain *V5w2* against *R. similis* reproduction was not influenced by initial *R. similis* density. This demonstrates that endophytes have the potential to be equally effective against a wide range of pest densities.

The three banana cultivars (Enyeru, Kibuzi and Kayinja) showed major differences in the densities of *R. similis* extracted from their root tissues. Kayinja registered lower *R. similis* densities as compared to Enyeru and Kibuzi. Speijer and Bosch (1996) reported variations in nematode susceptibility among banana cultivars based on studies with the nematode *Pratylenchus goodeyi*. Their results suggested that the East African highland banana cultivars were more susceptible to nematodes than an exotic *Musa* cultivar such as Kayinja. However another reason that may explain the low *R. similis* densities in Kayinja could be the higher colonization ability of the endophytes in this cultivar compared to Enyeru and Kayinja.

The fresh root weight of control plants was not consistently different from the fresh root weight in endophyte-inoculated plants, indicating that dry matter accumulation and possibly water storage in banana root tissues were not affected by the endophytes. Fresh root

weights of Kayinja plants were statistically higher than those of Enyeru and Kibuzi in the majority of the experiments because Enyeru and Kibuzi are genetically closely related as opposed to Kayinja, a brewing type.

Two damage parameters that indicate *R. similis* activity, percentage dead roots and percentage root necrosis, did not differ consistently between endophyte-inoculated plants and control plants. The duration of the experiments was probably too short for the two nematode damage symptoms to manifest since *R. similis* was not allowed to multiply in such numbers that they could cause considerable damage to the root tissues. Challenge periods that are longer than 8 weeks are probably required for root damage assessments in future experiments. Nevertheless, the percentage of dead roots following fungal strain inoculation seemed to be numerically higher in plants inoculated with single fungal strains and relatively lower in those plants that were inoculated with multiple strains.

Mortality rates obtained in the bioassays were a good indicator that fungal endophytes have the potential to be used as a microbial antagonist against *C. sordidus* and *R. similis*, and that some strains, such as strain *V5w2*, can act against both pests. In addition, we demonstrated in screenhouse experiments that strain *V5w2* reduces *R. similis* densities when inoculated into tissue culture plants. The effect of endophyte-enhanced tissue culture plants on *C. sordidus* is presently being studied at IITA. In addition to detailed field studies, the mechanisms used by these endophyte strains towards controlling banana weevils and *R. similis* and the potential of these fungal strains to stimulate plant growth will also be investigated at IITA.

Acknowledgements

This study was funded by a grant from the Bundesministerium für Wirtschaftliche Zusammenarbeit (BMZ).

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