Chapter 4

Effect of endophytic *Fusarium oxysporum* isolates on host preference, attraction, root penetration and reproduction of *Radopholus similis* in tissue culture banana plants

Abstract

The effects of three endophytic *Fusarium oxysporum* isolates, *V5W2*, *Eny1.31i* and *Eny7.11o*, on host preference and attraction, root penetration and reproduction of *Radopholus similis* in tissue culture banana plants *cv*. Enyeru (*Musa* spp., AAA-EA) were evaluated under laboratory and screen house conditions. Treatment of tissue culture banana plants with the three isolates did not alter host preferences and attraction of *R. similis*, as similar numbers of nematodes migrated towards plants and root segments from both endophyte-inoculated and uninoculated plants. The number of *R. similis* that penetrated the roots of endophyte-inoculated and un-inoculated plants was not influenced by the three endophytic fungal isolates, either in the laboratory or in the screen house. *Radopholus similis* reproduction was, however, highly influenced by endophyte infection. Differences in *R. similis* reproduction rates between endophyte-inoculated and un-inoculated plants were observed at 50 and 75 days after nematode inoculation. No differences were observed 25 days after inoculation. Endophyte-un-inoculated plants supported higher nematode densities than endophyte-inoculated plants, and the nematode reproduction rates in control plants were 10, 4 and 3 times higher than in plants treated with isolates *V5W2*, *Eny7.11o* and *Eny1.31i* at 75 days after inoculation, respectively. Isolate *V5W2* resulted in the highest reduction in *R. similis* reproduction for all nematode stages (females, males and juveniles). The results of this study imply that the early processes of banana root infection by *R. similis* are not affected by endophytes and that the main endophyte effects are post-infectional and occur only during the nematode reproduction phase.

Introduction

Radopholus similis (Cobb) Thorne is a migratory endoparasite that completes its life cycle in the roots and rhizomes of banana plants (*Musa* spp.) in 20 to 25 days (Gowen and Quénéhervé, 2005). Only female and juvenile stages are infective, as males have degenerate stylets. *Radopholus similis* penetrates banana roots at the root apex mainly, but penetration can also occur at any position along the root (Sarah *et al*., 1996). After root penetration, the nematodes occupy the intercellular spaces of the cortical parenchyma. Nematodes may migrate both within and between cells in the root cortex to feed on cell cytoplasm. This results in collapsed cell walls, cavities and tunnels in the root (Sarah *et al*., 1996). Symptoms of nematode damage become visible as reddish brown necrotic patches that are confined to the cortex region. In the rhizome, necrosis appears as a reddish-brown discoloration that begins where roots attach to the rhizome (Speijer and De Waele, 1997). Nematode feeding destroys root and rhizome tissue, reduces water and mineral uptake, and results in a reduction of plant growth and development. This leads to a severe reduction of bunch weight and a significant increase in time between successive harvests (Sarah *et al.,* 1996; Gowen *et al*., 2005).

During their initial life stages nematodes migrate through the rhizosphere to the plant roots (Kaplan and Keen, 1980). Host recognition involves signals from plant roots that influence egg hatch, attraction towards roots and root penetration (Zhao *et al*., 2000). Plant-parasitic nematodes are attracted to plant roots by a variety of factors, which may operate over considerable distances (Prot, 1980; Prot and Van Gundy, 1980; Zuckerman and Jansson, 1984; Spiegel *et al*., 2001; Luc *et al*., 2005). The major factors of nematode attraction to plant roots are chemostatic factors and secretions emanating from the host plant, while minor factors include thermal, vibratory and tactile stimuli (Prot, 1980; Zuckerman and Jansson, 1984; Perry, 1996). Following attraction, nematodes penetrate the host plant cells by using their stylets where they feed and reproduce.

Differences in the response of the host plant to nematode infection have been used to classify plants as resistant, tolerant, intolerant, host or non-host. Resistant plants allow for nematode penetration but not reproduction. Tolerant plants suffer little damage even when heavily infested, while susceptible plants suffer heavy damage under light nematode infestations. Plants are further classified as hosts or non-hosts depending on whether nematode

reproduction occurs or not. Plants that allow high nematode reproduction are classified as good hosts, while those that allow for low nematode reproduction are classified as non-hosts (Luc *et al*., 2005).

Antagonistic microorganisms can reduce early root infection by nematodes. Oostendorp and Sikora (1989; 1990) reported a reduction in egg hatch and early root infection of sugar beet (*Beta Vulgaris* L.) by the sugar beet nematode *Heterodera schachtii* Schmidt after seed treatment with antagonistic rhizobacteria. However, the application of rhizobacteria to the root surface of sugar beet seedlings did not alter migration of *H. schachtii* second stage juveniles. The authors concluded that bacterial alteration of root exudates might have influenced nematode hatch, attraction and root penetration behavior.

Endophytic *Fusarium oxysporum* Schlecht.: Fries reduced *R. similis* populations in tissue culture banana plants (Chapter 3; Pocasangre, 2000; Niere, 2001; Gold and Dubois, 2005). The mechanism through which nematode populations were reduced, however, is unknown. The objectives of this study, therefore, were to determine the effect of endophytic *F. oxysporum* isolates on (i) host preferences and relative attractiveness of endophyte-inoculated plants to *R. similis* (ii) root penetration by *R. similis* and (iii) reproduction of *R. similis* in tissue culture banana plants.

Materials and methods

Site description

Experiments were carried out in the laboratory or screen house at the International Institute of Tropical Agriculture (IITA) Research Station in Namulonge-Uganda, approximately 30 km Northeast of Kampala, Uganda. The screen house air temperatures ranged from 19.1 to 32.8ºC, while the soil temperature in the polythene bags ranged from 20.4 to 26.3ºC. The site is situated at 1150 m above sea level, 32º 34'N latitude, with a mean annual rainfall of 1255 mm and an average temperature of 22ºC.

Fungal isolates and nematode cultures

Three *F. oxysporum* isolates (*V5W2*, *Eny1.31i* and *Eny7.11o*) were included in all experiments. These isolates were obtained from healthy East African highland cooking banana plants roots and rhizomes in Uganda (Schuster *et al*., 1995) and are preserved in soil tubes (Niere, 2001). The isolates were selected on their ability to consistently reduce nematode population build-up in banana plants in *in vivo* screening experiments (Chapter 3). Pure *R. similis* cultures maintained on carrot disks were used as the source of nematode inoculum (Chapter 1; Speijer and De Waele, 1997).

Tissue culture plants

Tissue culture banana plants of the cultivar Enyeru (*Musa* spp. AAA-EA) were used in this study. The plants were micropropagated from sword suckers using standard shoot-tip culture protocols for banana (Vuylsteke, 1998). When ready for weaning, plants were transferred to an aquaculture system comprised of 250-ml lidded plastic pots filled with 200 ml of nutrient solution to allow for root development prior to inoculation with the endophytes (Fig. 1). The nutrient solution was prepared by dissolving a commercial fertilizer in sterilized (autoclaved at 121ºC for 15 min) tap water. In the root penetration experiments, plants were fertilized with Multifeed Classic (Gouws and Scheepers Ltd., Witfield, South Africa) at a rate of 2.5 g/L (Table 1). For the *R. similis* host preference, attraction and reproduction experiments, plants were grown in 1 g/L of Poly-Feed (Haifa Chemicals, Haifa Bay, Israel) (Table 1). In each pot, a plant was supported by the lid with the roots submerged in the nutrient solution. Strips of sterile sponge were used to stabilize the plants through a hole made in the middle of the lids. To simulate dark soil conditions and to discourage growth of algae on the roots, the pots were wrapped with a brown paper bag. The plastic pots then were transferred to a humidity chamber (Chapter 3) for a 1-month acclimatization period, during which the nutrient solution was renewed weekly.

Inoculation of plants with fungal isolates

The fungal isolates were pre-grown on synthetic nutrient agar (SNA) (1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_47H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose, 0.6 ml NaOH (1 M) and 13.2 g agar/L distilled water) in 65-mm-diameter Petri dishes at ± 25°C and 12 hrs light, 12 hrs darkness photoperiod (laboratory conditions) for 1 week. The SNA medium was supplemented with 10 mg chlortetracycline, 100 mg penicillin G and 50 mg streptomycin-sulphate/L to prevent bacterial contamination. Half strength potato dextrose broth (PDB) (Sigma-Aldrich, MO, USA) was prepared by dissolving 12 g of PDB in 1 L of distilled water. One hundred-ml aliquots of PDB were dispensed into 250-ml Erlenmeyer flasks and sterilized. After cooling, flasks were inoculated with 4 to 5 disks of agar of each fungal isolate. Uninoculated PDB was used as the control treatment. Duplicate flasks were prepared for each fungal isolate and the control. Inoculated flasks were incubated in the laboratory for 7 days to allow for fungal growth and sporulation. Fungal spore suspensions were filtered through a 1-mm-diameter sieve to remove mycelial fragments. The spore suspensions were then adjusted to provide a final spore count of 1.5×10^6 spores/ml.

For the inoculation of banana tissue culture plants with endophytic *F. oxysporum* isolates, 1-month-old plants were removed from the nutrient solution and the roots cut back to 10 cm in length. Plants were selected for uniformity according to size and subdivided among the different treatments. To inoculate the plants with the different fungal isolates, the root systems were dipped in the different fungal spore suspensions for 2 hrs. Control plants were dipped in sterile un-inoculated PDB for the same duration of time. After fungal inoculation, plants were transplanted in steam-pasteurized heavy loamy soil either into 3-L polythene bags for the root penetration and reproduction experiments, or into 250-ml plastic pots for the host preferences and attraction experiments.

To determine colonization of plant roots by the fungal isolates, three healthy primary roots were randomly selected from each plant at harvest and surface sterilized in 75% ethanol for 1 min, followed by sterilization in 2% NaOCl for 30 s. Root pieces were blotted dry on sterile tissue paper and cut into ca. 0.25 cm-long segments. Six segments per root were randomly selected and placed on SNA in 65-mm-diameter Petri dishes. The plates were incubated in the laboratory for 7 days under laboratory conditions. Fungal colonies growing from the root

pieces were identified under a light microscope (magnification x 400) as *F. oxysporum* based on the presence of microconidia carried in false heads on short phialides, the shape of macroconidia and the presence of chlamydospores (Nelson *et al*., 1983). The number of root pieces with *F. oxysporum* colonies were recorded and the percentage recovery of the fungus calculated.

Host preference and attraction experiments

Two experiments were conducted to investigate host preference and relative attractiveness of endophyte-inoculated and un-inoculated (control) banana plants to *R. similis*. Each experiment was repeated once. These included a detached root bioassay where root segments from endophyte-inoculated and uninoculated banana plants were paired in a Petri dish, and an intact plant bioassay using endophyte-inoculated and un-inoculated banana plants paired in a polyvinyl chloride (PVC) tube apparatus. Both experiments had seven treatments that involved combinations of the three endophyte-inoculated *V5W2* (E1), *Eny1.31i* (E2) and *Eny7.11o* (E3) and uninoculated (C) banana plants in the following pairs: C-C, C-E1, C-E2, C-E3, E1-E1, E2-E2 and E3-E3. The detached root bioassay and the intact plant bioassay were replicated four and five times, respectively.

Detached root bioassay for host preference and attraction

Endophyte-inoculated and uninoculated plants were uprooted 1 month after being transplanted into the 250-ml pots and washed free of soil. In the laboratory, healthy roots of the same age and size were selected from the plants and cut into 1-cm-long segments. For each replication, root segments were obtained from the same plant. Root segments from endophyte-inoculated and uninoculated plants were paired and placed on opposite sides in 90-mm-diameter Petri dishes filled with a 50-mm thick layer of moistened sterile sand (Fig. 2). The bottom of the Petri dish was divided into two equal sections, A and B, and two root segments from either an endophyte-inoculated or uninoculated plant placed in each of the sections. The distance between the root segments was 70 mm, and each root segment was placed 10 mm away from the wall of the Petri dish. Petri dishes were covered and left to stand for 12 hrs. Approximately 500 *R. similis* mixed stages (females, males and juveniles) in 0.3 ml water were inoculated in the middle of the Petri dish equal distances from the root

segments using a micropipette. After 24 hrs, nematodes were extracted from the sand and from root segments on either side of the inoculation point by means of the modified Baermann technique (Hooper *et al*., 2005). Extraction from the sand was carried out for 48 hrs by spreading the sand on the tissue paper in the modified Baermann funnel apparatus. Nematodes were extracted for 24 hrs from the root segments following maceration in a Waring blender (Waring, Connecticut, USA) at low speed for 15 s.

Intact plant bioassay for host preference and attraction

A PVC tube apparatus was designed to determine whether *R. similis* is differentially attracted to endophyte-inoculated or uninoculated (control) banana plant roots (Prot, 1979). The PVC apparatus consisted of a 22.5–cm-long and 5-cm-diameter PVC tube divided into seven sections, each 2.5 cm long (Fig. 3). The sections were filled with moistened sterile heavy loam soil and joined together with adhesive tape. The use of PVC pipes not only reduced the amount of soil from which nematodes had to be extracted, but also restricted nematode movement from the point of inoculation to either of the two direction.

Endophyte-inoculated and uninoculated plants transplanted into 250-ml plastic pots were grown until they were 8 weeks old. An endophyte-inoculated plant was then paired with an uninoculated plant on opposite sides of the PVC apparatus. The bottom parts of the pots were first removed to expose the roots, and the pots were then fixed to the ends of the PVC tubes in section number 1 and number 7 (Fig 3). Treatments were paired in such a way that the first plant which is either endophyte-inoculated (E1, E2 or E3) or uninoculated (C) in each treatment pair was placed in PVC section number 1 and the last in section number 7. A 3-mm-diameter opening was drilled at the top of the middle of each PVC section to facilitate watering and nematode inoculation.

One week after transplanting the banana plants into the PVC apparatus, 2000 mixed stages of *R. similis* were pipetted into the soil in the middle section (section 4) that was an equal distance from both plants. The tubes were maintained horizontally at laboratory conditions. The PVC sections were separated 5 days after introduction of the nematodes by cutting through the adhesive tape using a knife. The number of nematodes in each section was determined by extracting the nematodes from the soil over 48 hrs using the modified

Baermann funnel method (Hooper *et al*., 2005). Nematode extraction from the plant's roots in sections number 1 and 7 was conducted for 24 hrs after maceration in a Waring blender for 15 s. Nematode suspensions were concentrated on a 28-µm sieve prior to counting.

Root penetration experiments

To study the effect of endophytes on root penetration by *R. similis*, two experiments were conducted on 8-week-old banana plants inoculated with three different fungal endophytes (*V5W2*, *Eny1.31i* and *Eny7.11o*). The first experiment involved the *in vitro* root penetration of endophyte-inoculated and un-inoculated plants by nematodes in the laboratory. The second experiment was an *in vivo* root penetration experiment in the screen house, conducted as a time course study. Nematode inoculation was performed on the whole root system and plants were sampled to determine the number of nematodes that had penetrated into the roots 3, 6 10 and 14 days after inoculation. Six plants were randomly harvested after each time interval. Each experiment was repeated twice.

In vitro *root penetration experiments*

Endophyte-inoculated and un-inoculated banana plants were uprooted from their polythene bags and their roots washed free of soil. These plants were then transplanted to rectangular plastic trays (90 x 143 x 230 mm) that were filled with *ca*. 500 cm^3 moistened sterile sand (Fig 4). A hole was cut on the side of the trays, and a slit made from the rim to the hole to facilitate placement of the plant. A strip of sponge was used to stabilise the plants and avoid damage to their pseudostems. Three 65-mm-diameter Petri dishes in which two V-shaped cuts were made on the opposite sides were placed inside the trays and filled with moistened sterile sand. Three healthy primary roots of the same size and age were randomly selected from the transplanted plants, and each one of roots placed inside one of the three Petri dishes.

A nematode suspension containing a total of 50 *R. similis* mixed stages in *ca*. 0.1 ml of water was pipetted directly on the root segments in each Petri dishes 24 hrs after transfer of the plants. The roots were then covered with sterile sand. The plastic trays were covered with a lid to prevent dehydration of the plants and root segments. Inoculated root segments were excised after 24 hrs and stained with acid fuschin to observe nematodes that had penetrated

the roots (Byrd *et al*., 1983). After staining, root segments were flattened between two microscope slides and the number of nematodes visible in the root tissue was recorded. There were five plants for each treatment, with three roots selected per plant, each representing a single replication (i.e. a total of 15 replications per treatment).

In vivo root penetration experiments

Eight-week-old endophyte-inoculated and un-inoculated banana plants grown in 3-L polythene bags were inoculated with a nematode inoculum containing 500 mixed stages of *R. similis* per 2 ml water as follows. Three holes were made at the base of the plants around the roots. To each hole, *ca*. 0.6 ml of the nematode suspension was pipetted, and the hole covered with soil. Six plants from each treatment were then uprooted 3, 6, 10 and 14 days after nematode inoculation. Nematodes were extracted from 5 g of each root samples by first macerating them in a Waring blender at low speed for 15 s, and subsequently collecting the nematodes for 24 hrs using a modified Baermann technique (Hooper *et al*., 2005). Nematode suspensions were transferred into sample bottles and kept in the fridge at 4ºC until counted. Prior to counting, nematode suspensions were concentrated on a 28-µm sieve.

Radopholus similis reproduction experiments

A single-root inoculation technique developed for screening banana plants for resistance to *R. similis* (De Schutter *et al*., 2001) was adopted to study the effects of endophytes on *R. similis* reproduction. One-month-old endophyte-inoculated and un-inoculated plants were grown for a further 2 months in 3-L plastic polythene bags after acclimatization in the humidity chamber. The 3-month-old plants were then uprooted and their roots separated. The plants were placed into 24 x 54 cm plastic buckets filled three quarters with sterile sawdust (Fig. 5). Three equally developed primary roots were selected from each plant and planted in 150-ml plastic pots filled with moistened sterile sand. After transplanting, the roots not planted in the pots were covered with sterile soil. A nematode suspension containing 50 *R. similis* in approximately 0.3 ml of sterile water was pipetted directly onto each of the root segments planted inside the plastic pots. The inoculated roots were then also covered with moistened sterile sand. Following inoculation, the banana plants were maintained in the screen house (20.4 to 26.3ºC) and watered two times per week.

Nematodes were extracted from inoculated banana roots 25, 50 and 75 days after nematode inoculation. At each harvest date, five plants were randomly selected and the plastic pots excavated from the sawdust. The root segments inside the pots were removed and washed with tap water. The percentage root necrosis was determined by slicing each root segment lengthwise and estimating the percentage necrotic area (Speijer and De Waele, 1997). The sliced root segment was then cut into 0.5-cm pieces and macerated in a Waring blender at low speed for 15 s. Nematodes were extracted from the macerated root tissue over 24 hrs using the modified Baermann funnel method (Hooper *et al*., 2005). All *R. similis* stages (females, males, juveniles) were counted. Nematode reproduction rates (RR) were calculated by dividing the final population density (*Pf*) with the initial population density (*Pi*). Reisolation of fungal endophytes was conducted on the root segments adjacent to nematode inoculated root segment as described above.

Data analysis

Nematode data in each experiment were tested for normality and homogeneity of variances using the Shapiro-Wilkinson, Levene-Welch and Kolmogorov tests. Normal probability plots, box plots and stem leaf plots were additionally used to confirm normality of data. If not normally distributed with equal variances across treatments, various transformations were tested until the most suitable transformation was obtained.

For the host preference and nematode attraction bioassays using detached roots in Petri dishes, statistical analysis was performed on untransformed data. One-way ANOVA was used to demonstrate differences in the total number of nematodes in each treatment pair. If different, treatment means were separated using Tukey's Studentized range test. Within each treatment pair, differences between treatments were evaluated using a paired t-test.

For the bioassay using intact plants in the PVC tube apparatus and for the *R. similis* reproduction experiments, nematode counts were square root (sqrt) (x+0.5)-transformed prior to analysis. Within each treatment, differences between the proportion of nematodes attracted to either endophyte-inoculated or uninoculated plants were evaluated using a paired t-test. For the reproduction experiments, a two-way ANOVA was conducted to demonstrate variability among treatments (control and fungal isolates) and days after nematode inoculation. Prior to

analysis, the nematode RR was sqrt-transformed x+0.5 to normalise data. Percentage root necrosis and colonization were arcsine sqrt-transformed x+0.5 prior to analysis. If different, means were separated using Tukey's studentized range test (SAS Institute, 1989).

Results

Host preference and attraction experiments

Detached root bioassay for host preference and attraction

Similar numbers of nematodes were attracted to banana roots, whether they were inoculated or uninoculated with *F. oxysporum* endophytes (Fig. 6 and 7) Comparison between the total numbers of *R. similis* that migrated towards opposite sections of the Petri dish containing either an endophyte-inoculated or un-inoculated root segment revealed no significant differences between any of the treatments pair (Table 2). When two endophyte-uninoculated root segments (treatment pair C-C) were paired in opposite sides of a Petri dish, the number of nematodes attracted to either root segment did not differ significantly (Table 2). Likewise, when an endophyte-uninoculated root segment was paired with an endophyte-inoculated root segment (treatment pairs C-E1, C-E2 and C-E3), the number of nematodes that migrated towards either root segment did not differ significantly. When two endophyte-inoculated root segments were paired (treatment combinations E1-E1, E2-E2 and E3-E3), no differences were observed in the number of *R. similis* that migrated towards either root segment (Table 2). The only exception was observed in experiment 2, when two root segments infected with isolate *Eny1.31i* (E2-E2) and isolate *Eny7.11o* (E3-E3) were paired, where significant differences in the number of *R. similis* that migrated to either root segment were observed (Table 2).

Intact plant bioassay for host preference and attraction

THE EXPERIMENTS USED TO DETERMINE NEMATODE ATTRACTION SHOWED THAT THE NUMBER OF NEMATODES (EXPRESSED AS A PERCENTAGE OF THE TOTAL NEMATODES RECOVERED PER TREATMENT) THAT MIGRATED FROM THE POINT OF INOCULATION IN PVC TUBES DID NOT DIFFER BETWEEN THE TREATMENT PAIRS. IN EXPERIMENT 1 (*P*=0.1544), THE PROPORTION OF NEMATODES THAT MIGRATED RANGED FROM 10.2 ± 3.5% IN TREATMENT PAIR E3-E3 (TWO *ENY7.11O*-INFECTED PLANTS PAIRED) TO 35.9 ± 3.5% IN C-C (WHEN TWO CONTROL PLANTS WERE PAIRED). IN BIOASSAY 2 (*P*=0.3776) THE PROPORTION OF NEMATODES THAT MIGRATED FROM THE POINT OF INOCULATION RANGED FROM 40.4 ± 1.6 WHEN TWO *ENY7.11O*-TREATED PLANTS WERE PAIRED TO 57.7 ± 10.1% WHEN A CONTROL PLANT WAS PAIRED WITH A PLANT TREATED WITH *ENY.11O*. PAIRED T-TESTS DEMONSTRATED THAT THE PROPORTION OF

NEMATODES THAT MIGRATED TO OPPOSITE SIDES OF THE INOCULATION POINT TOWARDS EITHER AN ENDOPHYTE-INOCULATED PLANT OR UNINOCULATED PLANT DID NOT DIFFER AMONG THE TREATMENT PAIRS IN BOTH BIOASSAYS EXCEPT IN THE TREATMENT WERE TWO *ENY7.11O*-INFECTED PLANTS WERE PAIRED (TABLE 3).

In both bioassays, the total number of nematodes present in each section of the PVC tube differed significantly between the sections ($P<0.0001$) and was influenced by the distance from the nematode inoculation point. In all treatment pairs, most nematodes were found in the middle section (section 4), the point of nematode inoculation (Fig. 8 A and B), with less nematodes in sections further from the point of nematode inoculation. The number of nematodes recorded in sections at the same distance from the point of nematode inoculation was not different within a given treatment ($P<0.05$).

Root penetration experiments

In vitro experiments

No significant difference was found in the number of nematodes that penetrated root segments of endophyte-inoculated and uninoculated plants 24 hrs after inoculation with *R. similis* ($P=0.6064$, 0.2528 and 0.8752 for experiment 1, 2 and 3 respectively) (Fig. 9). Nematodes were observed in the cortex region of the root segments only and not the stele.

In vivo experiments

No significant differences in the penetration of banana roots by *R. similis* were observed between the endophyte-inoculated and uninoculated banana plants planted in polythene bags (Table 4). There were also no significant differences in the number of nematodes that penetrated the roots 3, 6, 10 and 14 days after nematode inoculation for any of the treatments.

Radopholus similis reproduction

Endophyte treatment significantly reduced the number of *R. similis* females, males, juveniles and the total nematode density at 75 days after nematode inoculation (DAI) in the first

experiment, but not the second one (Table 5). In the former, the percentage reduction in *R. similis* females was 65.0%, 67.4% and 35.5% after 75 days, whilst juvenile numbers compared to the control were lower by 55.2%, 77.4% and 90.8% in plants infected with *Eny1.31i*, *Eny7.11o* and *V5W2*, respectively. The reduction in the number of *R. similis* males was 63.4%, 83.6% and 92.8% and the reduction in the total nematode density was 65.7%, 76.5% and 90.2% in plants treated with the fungal isolates *Eny1.31i*, *Eny7.11o* and *V5W2* respectively compared to control plants (Table 5). In the second experiment, only isolate *V5W2* resulted in less females, males, juveniles and the total nematode density although this did not differ significantly from the control. Isolates *Eny1.31i* and *Eny7.11o*-treated plants had more nematode than the controls 75 DAI (Table 5).

No differences were observed in the number of females, juveniles, males and the total nematode density between endophyte-inoculated and un-inoculated plants at 25 and 50 DAI, (Table 5). Although not significantly different, *R. similis* female numbers were lower by 71.1, 40.5 and 23.0% in plants infected with the fungal isolates *Eny1.31i*, *Eny7.11o* and *V5W2* 50 DAI, respectively, compared to the un-inoculated plants. Similarly, the number of *R. similis* juveniles was lower by 50.7%, 51.7% and 18.6% in plants treated with the fungal isolates *Eny1.31i*, *Eny7.11o* and *V5W2*, respectively (Table 5). The most important reduction in the second experiment, although not significant, was achieved with *Eny1.31i* that reduced nematode numbers by 62.9% after 25 DAI, and after 50 days, juvenile numbers by 78.5%, 24.3% and 43.4%, and males were reduced by 74.2, 15.2 and 37.7% in plants treated with isolates *Eny1.31i*, *Eny7.11o* and *V5W2* respectively.

The DAI affected the female, male, juvenile and total *R. similis* density significantly influenced ($P<0.0001$) in both endophyte-inoculated and uninoculated plants. No differences were observed in the nematode numbers at 50 and 75 DAI in both endophyte-treated plants while the number of nematodes in both endophyte-inoculated and un-inoculated plants at 25 DAI were significantly lower than those at 50 and 75 DAI ($P<0.0001$).

Treatment with endophytes did not significantly reduce the damage caused by nematodes to banana roots (percentage root necrosis) ($P=0.5593$), but the damage was reduced significant over time ($P=0.0239$) (Table 6). At 25 and 75 DAI, plants had similar levels of damage, whether they were inoculated with endophytes or not. The only significant differences in

percentage root necrosis were observed in experiment 1 50 DAI where plants treated with *V5W2* and *Eny1.31i* had significantly more necrosis than control plants, but not in experiment 2 (Table 6).

Nematode reproduction rate (RR) was significantly reduced by endophyte treatment after 75 days in experiment 1, but not experiment 2 (Table 6). At 25 and 50 DAI, no significant differences were observed in the RR between endophyte-inoculated and un-inoculated plants. For both endophyte-inoculated and un-inoculated plants, the nematode RR increased with an increase in DAI, except for *V5W2*-treated plants where the RR was higher at 50 DAI than at 75 DAI (Table 7).

Endophytic colonization of banana roots

In the *in vivo* root penetration experiments, the percentage colonization of plants treated with isolates *Eny1.31i* (16.9 ± 6.8%), *Eny7.11o* (15.5 ± 5.9%) and *V5W2* (20.5 ± 5.5%) was higher than in the control plants (3.6 ± 2.9%). Root colonization increased with increase in the days after nematode inoculation (DAI). In experiments 1 and 3, colonization was significantly influenced by treatment (P=0.0003) but not by DAI (P=0.6210). Lowest root colonization was recorded in endophyte-un-inoculated plants compared to colonization rates in roots inoculated with the various fungal isolates.

At all harvest dates in the first *R. similis* reproduction experiment, percentage colonization of roots by endophytic *F. oxysporum* did not differ between endophyte-inoculated and un-inoculated plants (Table 6). Significant differences, however, were found between un-inoculated and endophyte-inoculated plants 25 (P=0.0002) and 75 (P=0.0004) DAI. Although *F. oxysporum* was re-isolated from control plants 50 DAI, no isolations were made 25 and 50 DAI in either experiment. Still, the difference between the control and endophyte treatments was not significant, even where control yielded 0% re-isolation, and the treated plants 61% re-isolation.

Discussion

This study has demonstrated that endophyte-inoculated roots of tissue culture banana plants did not influence host preferences, attraction and penetration by *R. similis*. This is evident from the lack of repulsion or attraction of *R. similis* in the host preference and attraction experiments. Similar penetration rates into plant roots indicate that the initial processes of host recognition and root penetration, and invasion are not affected by the endophytes tested. The results suggest that, once in the field, roots of plants infected with the endophytic *F. oxysporum* isolates used in this study will still be infected by *R. similis*. However, endophytes inhibit reproduction of the nematode over several generations.

The results obtained in the current study are in contrast to those obtained with other fungal endophytes. Bernard and Gwinn (1991) reported that more nematodes (*Pratylenchus scribneri* Steiner) migrated towards endophyte-free tall fescue (*Festuca arundinacea* Schreb) root segments when both endophyte-infected and endophyte-free root segments were paired in a petri dish. Evidence that biological control agents other than endophytes affect early root infection by nematodes was demonstrated by Oostendorp and Sikora (1989; 1990). These authors reported a reduction in egg hatch and early root infection of sugar beet by the sugar beet nematode, *H. schachtii* after seed treatment with antagonistic rhizobacteria. Treatment of sugar beet seedlings with the rhizobacteria did not alter migration of *H. schachtii* second stage juveniles. These examples, however, involve different crops and nematode species and thus the response in banana may be different.

The results obtained in this study are in contrast to results obtained by Pocasangre (2000) who reported reduction in root penetration by *R. similis* in Cavendish banana plants treated with endophytic isolates of *Fusarium* spp. The contradiction in results may be due to the fact that the banana cultivars, *R. similis* populations and endophytic isolates in the two studies were different and experiments were conducted under different environmental conditions. In grasses, the presence of morphological barriers such as thickening of the endodermis in tall fescue (*Festuca arundinacea* Shreb.) infected by the endophyte *Neotyphodium coenophialium* ([Morgan-Jones and Gams] Glenn, Bacon, and Hanlin) was associated with reduced root penetration of root-knot nematodes *Meloidogyne marylandi* Jepson and Golden (Gwinn and Bernard, 1993) but not of the migratory nematode *Pratylenchus scribneri* Steiner. Reduced

root penetration subsequently led to reduced growth and reproduction of *M. marylandi* in the endophyte-infected grasses. In the current study, penetration into roots of endophyte-treated as well as untreated plants may be an indication that the endophytes did not induce morphological and structural changes that limit nematode penetration.

The complete life cycle of *R. similis* takes 20-25 days a t a temperature range of 24-32ºC (Loos, 1962). In the current study, nematodes reproduced both in endophyte-inoculated and un-inoculated plants. The nematode reproduction rates were, however, lower in endophyte-inoculated plants compared to uninoculated plants. Endophyte-inoculation did not have an effect on the first *R. similis* generation; reduction in nematode populations was observed at the second and third generation only. It is probable that, at 25 DAI, the nematodes that were found in the roots were those that survived after initial inoculation. The lower reproduction rates of *R. similis* in endophyte-treated plants during the second and third generations may probably be due to inhibition of nematode migration inside the plant roots, interference with the feeding processes or reduction in nematode reproduction capacity (Oostendorp and Sikora, 1989; 1990; Jones and Bernard, 1997). These conclusions, however, require further investigation. Another implication of these results is that endophytes may protect banana plants against *R. similis* for multiple nematode generations. Banana is a perennial crop and protection for several seasons by endophytes would greatly benefit the plant (Sikora *et al*., 2003). Hunt *et al*. (2005) defined resistant plants as those that allow for nematode penetration but restrict nematode reproduction. Based on this definition, endophyte infection of tissue culture banana plants restricted nematode reproduction and thus conferred some degree of resistance to the host plant. The lack of differences in *R. similis* reproduction between endophyte-inoculated and uninoculated plants in one of the experiments may have been due to differences in the vigour of both the plants and nematode inoculum used. Large variation in the data may also be responsible for the observed lack of differences. Nevertheless, endophyte-inoculated plants supported less nematodes than uninoculated plants.

Endophytic colonization was higher in the endophyte-inoculated plants than in the non-inoculated plants, but not significantly different. The true identity of the re-isolated endophytic *F. oxysporum* strains, however, is unknown, since endophytic *F. oxysporum* strains have also been re-isolated from the control treatment. The endophytic *F. oxysporum* from non-inoculated plants could have come from the soil, water or screen house

environment. Before it is possible to deduce whether the inoculated *F. oxysporum* indeed contributed to the reduction in reproduction rates, and also to the non-significance of endophyte treatment in host preference, attraction and penetration, it would be necessary to conduct these experiments under greenhouse conditions that prevent the re-introduction of endophytes into control plants. Colonization of control plants by *F. oxysporum*, however, is a common occurrence in screen house studies, and has been reported before by Niere (2001) and Paparu (2005). Niere (2001) reported colonization of uninoculated plants of up to 40%, while Paparu (2005) reported up to 15% colonization of uninoculated plants.

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Table 1. Nutrient composition of Multifeed Classic (Gouws and Scheepers Ltd., Witfield, South Africa) and Polyfeed fertilizers (Haifa Chemicals, Haifa Bay, Israel) used to enhance root development of tissue culture plants prior to fungal inoculation.

Ingredient	Multifeed Classic (g/kg)	Polyfeed (g/kg)
Nitrogen	190	0.19
Phosphorus	82	0.19
Potassium	158	0.19
Iron	0.75	1
Manganese	0.3	0.5
Boron	1	0.2
Zinc	0.35	0.15
Copper	0.075	0.11
Molybdenum	0.07	0.07
Magnesium	0.9	0

Table 2. Paired *t*-tests for the differences in the total number of *Radopholus similis* present in the sand, roots and in both sand and roots, which migrated towards root segments obtained from endophyte-inoculated or un-inoculated plants paired in a Petri dish.

		Bioassay 1						Bioassay 2					
		Sand		Root		Sand + Root		Sand		Root		Sand + Root	
Treatment pair *	Difference**	T value	Pr>|t|	T value	Pr>|t|	T value	Pr>|t|	T value	Pr>|t|	T value	Pr>|t|	T value	Pr>|t|
C-C	A-B	2.37	0.0986	0.28	0.7999	2.23	0.1119	1.29	0.2861	-0.34	0.7587	1.09	0.3538
C-E1	A-B	0.64	0.5684	-2.71	0.0729	0.45	0.6860	0.41	0.7116	1.36	0.2671	0.65	0.5641
C-E2	A-B	0.63	0.5723	-0.90	0.4339	0.35	0.7492	-0.49	0.6749	-0.8	0.4798	-1.31	0.2809
C-E3	A-B	1.28	0.2918	-0.42	0.7016	0.92	0.4236	1.43	0.2474	-0.72	0.5239	1.26	0.2982
E1-E1	A-B	1.62	0.2033	1.68	0.1920	2.11	0.1253	1.36	0.2666	-0.61	0.5848	1.16	0.3315
E2-E2	A-B	-1.77	0.1749	0.11	0.9173	-2.06	0.1312	-4.01	0.0279	-0.78	0.4917	-3.46	0.0407
E3-E3	A-B	-0.50	0.6512	0.28	0.7987	-0.42	0.7017	-3.35	0.044	0.73	0.5158	-2.97	0.0589

*C= control, E1=endophyte isolate *V5W2*, E2= endophyte isolate *Eny1.31i*. E3=endophyte isolate *Eny7.11o*, root segments from either control or endophyte-infected plants were paired in a Petri dish as indicated above.

**The Petri dishes were divided into two sections, A and B, with the first and second treatment in each treatment pair placed in section A and B respectively (Fig. 2).

Table 3: Paired *t*-tests for the differences between the proportion of nematodes that migrated towards an endophyte-inoculated or un-inoculated plant in the intact plant host preference and attraction bioassays conducted using PVC tube apparatus.

		Bioassay 1		Bioassay 2	
Treatment pair*	Difference**	T Value	P>|t|	T Value	P>|t|
C-C	A-B	2.17	0.0958	-0.59	0.5877
C-E1	A-B	0.76	0.4879	-1.40	0.2344
C-E2	A-B	-1.05	0.3517	-1.70	0.1644
C-E3	A-B	0.26	0.8042	0.34	0.7507
E1-E1	A-B	-0.09	0.9305	0.91	0.4321
E2-E2	A-B	-2.49	0.0673	-0.37	0.7234
E3-E3	A-B	4.54	0.0105	2.79	0.0493

*C= control, E1= endophyte isolate *V5W2*, E2= endophyte isolate *Eny1.31i*. E3= endophyte isolate *Eny7.11o*.

**Control and endophyte-infected plants were paired in a polyvinyl chloride (PVC) tube divided into 7 sections (Fig. 3), with the first and second treatment in each treatment pair placed on section 1 and 7, respectively. Nematodes were inoculated at the middle section (4) of the tube equidistant from the two plants. A and B represents sections on either side of the nematode inoculation point and correspond to the plant (treatment) on that side of the inoculation point.

Table 4. Average number of *Radopholus similis* in 5 g banana root samples of endophyte-inoculated and un-inoculated plants 3, 6, 10 and 14 days after nematode inoculation in in vivo root penetration experiments in the screen house.

Treatment	Days after nematode inoculation				*P*-Value[2]
	3	6	10	14	
Experiment 1					
Control	13.5 ± 0.5	19.2 ± 0.6	15.6 ± 1.9	14.8 ± 5.7	0.1028
V5W2	9.2 ± 4.6	6.6 ± 2.1	13.6 ± 0.6	8.3 ± 5.9	0.7772
Eny1.31i	5.8 ± 1.1	8.8 ± 2.8	7.6 ± 3.4	13.1 ± 3.7	0.8447
Eny7.11o	6.1 ± 2.4	4.8 ± 1.4	7.6 ± 3.4	13.1 ± 3.7	0.3514
P-value[1]	0.6771	0.6340	0.1301	0.7300	
Experiment 2					
Control	4.1 ± 1.1	5.2 ± 2.2	3.4 ± 1.3	1.8 ± 0.3	0.5584
V5W2	1.7 ± 0.4	4.2 ± 1.1	6.0 ± 1.6	3.9 ± 0.8	0.2099
Eny1.31i	1.0 ± 0.5	10.9 ± 9.5	4.0 ± 0.6	3.8 ± 1.1	0.5859
Eny7.11o	2.8 ± 0.9	1.5 ± 0.4	5.9 ± 3.3	3.3 ± 0.8	0.3147
P-value[1]	0.1179	0.7025	0.6780	0.1909	
Experiment 3					
Control	6.0 ± 1.4	4.2 ± 1.3	5.5 ± 1.4	13.7 ± 5.4	0.1531
V5W2	2.8 ± 0.6	5.5 ± 1.4	6.2 ± 2.9	26.5 ± 11.9	0.0693
Eny1.31i	5.3 ± 3.1	3.3 ± 0.5	5.5 ± 1.7	4.6 ± 1.8	0.6586
Eny7.11o	12.1 ± 6.7	2.2 ± 0.4	4.5 ± 2.4	4.8 ± 0.6	0.4473
P-value[1]	0.5981	0.2858	0.7552	0.0643	

P-Value[1] is the *P*-value for treatments along columns in each experiment. *P*-Value[2] is the *P*-value for days after inoculation along rows within each treatment.

Table 5. Effect of endophyte treatment of tissue culture banana plants cv. Enyeru (*Musa* spp. AAA-EA) with fungal isolates *V5W2*, *Eny1.31i* and *Eny7.11o* on the reproduction of *Radopholus similis* 25, 50 and 75 days after inoculation of individual primary roots with 50 *Radopholus similis* females.

Treatment	Females			Juveniles			Males			Total*		
	Days after nematode inoculation			Days after nematode inoculation			Days after nematode inoculation			Days after nematode inoculation		
	25	50	75	25	50	75	25	50	75	25	50	75
Experiment 1												
Control	1.1 ±0.3^{a}	115.4±44.7^{a}	270.0±55.1^{a}	1.0±0.3^{a}	143.3±53.7^{a}	581.8±110.9^{a}	0.1 ±0.1^{a}	80.4±33.7^{a}	268.5±47.8^{a}	2.0 ±0.6^{b}	339.2 ±130.6^{a}	1120.6 ±196.7^{a}
Eny1.31i	7.5±3.3^{a}	33.4 ±11.3^{a}	94.5±47.1^{b}	4.3±1.9^{a}	70.6±28.7^{a}	190.5±98.7^{b}	2.1 ±0.8^{a}	36.0±13.2^{a}	98.5±56.5^{b}	12.8 ±5.6^{ab}	140.0 ±49.7^{a}	383.5 ±194.1^{b}
Eny7.11o	4.2±2.1^{a}	68.6±32.3^{a}	87.9±47.1^{b}	1.1±0.5^{a}	69.2±39.1^{a}	130.6±63.8^{b}	2.1 ±1.6^{a}	57.2 ±31.2^{a}	43.8±18.2^{b}	5.5 ±3.0^{ab}	195.0 ±101.8^{a}	262.9 ±127.5^{b}
V5W2	12.5±3.9^{a}	88.8±36.2^{a}	35.5±16.8^{b}	6.3±2.3^{a}	116.6±49.9^{a}	53.5±18.8^{b}	5.5 ±1.9^{a}	114.7±47.6^{a}	19.1±6.7^{b}	24.5 ±7.9^{a}	320.2 ±128.2^{a}	109.0 ±41.9^{b}
P-value	0.0756	0.7151	0.0034	0.0756	0.8610	0.0003	0.0662	0.7920	0.0001	0.0226	0.8561	0.0003
Experiment 2												
Control	19.3±1.6^{a}	228.6±73.4^{a}	443.0±25.6^{a}	33.0±1.9^{a}	453.8±165.6^{a}	381.1±67.4^{a}	13.0±1.1^{a}	159.8±56.4^{a}	255.8±132.2^{a}	65.3±2.9^{a}	842.2±258.4^{a}	1080.0±174.1^{a}
Eny1.31i	9.2±4.6^{a}	84.6±38.3a	536.9±247.3^{a}	12.8±6.5^{a}	97.3±47.1^{a}	408.2±199.1^{a}	7.0±4.0^{a}	44.1±21.4^{a}	319.7±189.3^{a}	29.1±15.2^{a}	226.1±104.3^{a}	1265.0±627.5^{a}
Eny7.11o	18.1±5.1^{a}	195.7±56.7^{a}	887.5±350.2^{a}	26.8±7.6^{a}	343.1±110.4^{a}	509.3±140.2^{a}	14.4±5.5^{a}	135.5±39.6^{a}	344.5±100.6^{a}	59.3±16.9^{a}	674.3±202.5^{a}	1741.4±544.4^{a}
V5W2	17.2±5.4^{a}	222.5±68.6^{a}	436.2±188.9^{a}	28.8±8.8^{a}	256.8±88.3^{a}	278.1±84.8^{a}	13.8±5.1^{a}	102.6±37.4^{a}	221.3±70.6^{a}	59.8±17.3^{a}	582.1±181.8^{a}	935.7±305.3^{a}
P-value	0.7740	0.6775	0.6416	0.7719	0.1001	0.6665	0.0814	0.8551	0.7416	0.8745	0.3213	0.7778

*Total *R. similis* density (females + males + juveniles). In columns within an experiment, means followed by the same letter (superscript) are not different at $P<0.05$ according to Tukey's studentized range test. Values represent the mean ± S.E., n= 15.

Table 6. Percentage root necrosis, reproduction rates (Pf/Pi) of *Radopholus similis* and endophytic colonization in roots of tissue culture banana plants cv. Enyeru (*Musa* spp. AAA-EA) 25, 50 and 75 days after inoculation of individual primary roots with 50 *Radopholus similis* females.

Treatment	Root necrosis (%) Days after nematode inoculation			Reproduction ratio (*Pf/Pi*) Days after nematode inoculation			Root colonization (%) Days after nematode inoculation		
	25	50	75	25	50	75	25	50	75
Experiment 1									
Control	26.4 ±11.5^{a}	8.4 ±2.9^{b}	11.7 ±2.5^{a}	0.1 ±0.0^{b}	6.7 ±2.6^{a}	22.4 ±3.9^{a}	0.0 ±0.0^{a}	26.8 ±12.1^{a}	0.0 ±0.0^{a}
Eny1.31i	22.7 ±6.4^{a}	18.4 ±1.0^{a}	12.9 ±1.7^{a}	0.2 ±0.1^{ab}	2.8 ±0.9^{a}	7.6 ±3.8^{b}	13.8 ±7.3^{a}	39.5 ±6.3^{a}	50.0 ±41.6^{a}
Eny7.11o	32.5 ±9.9^{a}	13.2 ±3.1^{ab}	5.7 ±1.5^{a}	0.1 ±0.1^{ab}	3.9 ±2.0^{a}	5.2 ±2.5^{b}	61.1 ±26.4^{a}	21.6 ±7.7^{a}	0.0 ±0.0^{a}
V5W2	20.5 ±7.1^{a}	19.7 ±0.1^{a}	12.1±2.6^{a}	0.4 ±0.1^{a}	6.4 ±2.5^{a}	2.1 ±0.8^{b}	16.6 ±8.3^{a}	38.8 ±8.0^{a}	33.3 ±14.2^{a}
P-value	0.7525	0.0047	0.0630	0.0218	0.8583	0.0004	0.1257	0.5924	0.1796
Experiment 2									
Control	20.8 ±5.8^{a}	100.0 ±0.0^{a}	97.5 ±1.7^{a}	1.3 ±0.1^{a}	16.8 ±5.1^{a}	21.6 ±3.4^{a}	0.0 ±0.0^{b}	12.5 ±7.2^{a}	0.0 ±0.0^{b}
Eny1.31i	15.8 ±7.6^{a}	81.6 ±9.5^{a}	98.3 ±1.6^{a}	0.5 ±0.3^{a}	4.5 ±2.1^{a}	25.3 ±12.5^{a}	45.2 ±5.7^{a}	48.2 ±12.0^{a}	43.7 ±21.3^{a}
Eny7.11o	29.1 ±7.0^{a}	95.0 ±1.8^{a}	95.0 ±3.1^{a}	1.1 ±0.3^{a}	13.4 ±4.1^{a}	34.8 ±10.8^{a}	52.1 ±10.4^{a}	36.4 ±9.6^{a}	66.6 ±5.4^{a}
V5W2	31.6 ±8.7^{a}	76.1±10.5^{a}	91.1 ±5.4^{a}	1.1 ±0.3^{a}	11.6 ±3.6^{a}	18.7 ±6.1^{a}	53.5 ±9.7^{a}	46.1 ±7.5^{a}	53.3 ±6.2^{a}
P-value	0.4214	0.1392	0.2826	0.0895	0.7271	0.4813	0.0002	0.5616	0.0004

In columns within an experiment, means followed by the same letter are not significantly different at $P<0.05$ according to Tukey's studentized range test. Values represent the mean ± S.E., n= 15.

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Figure 1. Banana tissue culture plants growing in an aquaculture system in 250-ml plastic pots filled with nutrient solution.

Figure 2. Experimental set up used to test for host preference and attraction of *Radopholus similis* to either root segments from endophyte-inoculated or un-inoculated banana plants by means of the detached root bioassay (figure not drawn to scale).

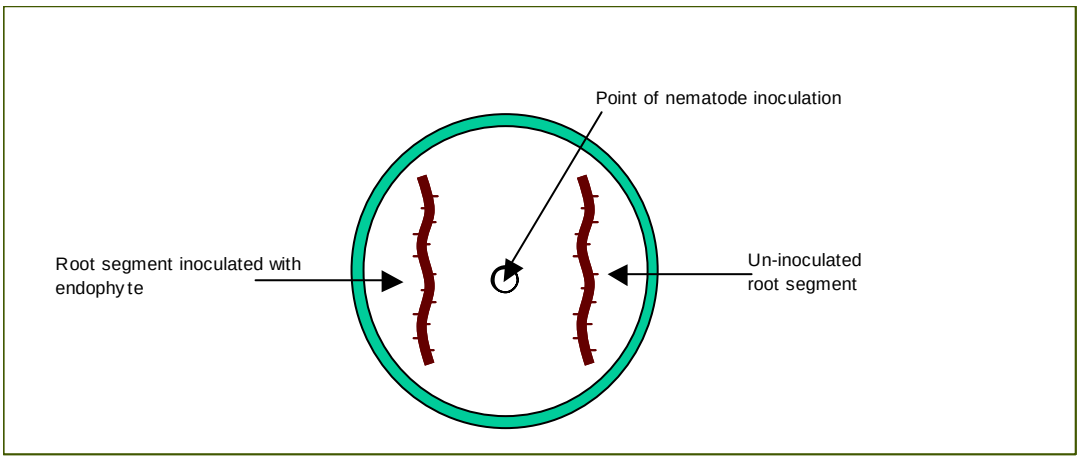


Figure 3. The polyvinyl chloride (PVC) tube apparatus used for *Radopholus similis* host preference and attraction bioassays involving intact endophyte-inoculated and un-inoculated (control) plants (A), and a schematic presentation of the PVC apparatus (B) (figure not drawn to scale).

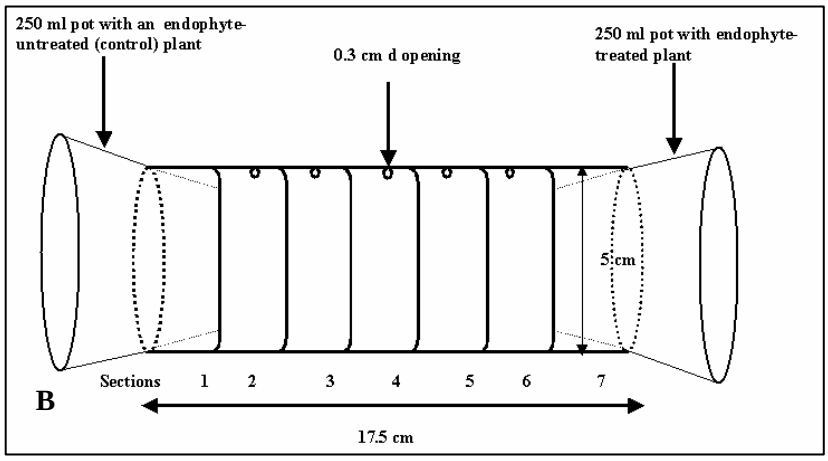


Figure 4. Experimental set up used to assess penetration of endophyte-inoculated and un-inoculated banana roots by *Radopholus similis* in vitro. (A), comprising a rectangular plastic container in which the plants were placed with three roots from each plant placed in Petri dishes. Nematode inoculation was done directly on the root sections inside the Petri dishes and extraction performed 24 hrs later. (B) represents a schematic presentation of the experimental set up (figure not drawn to scale).

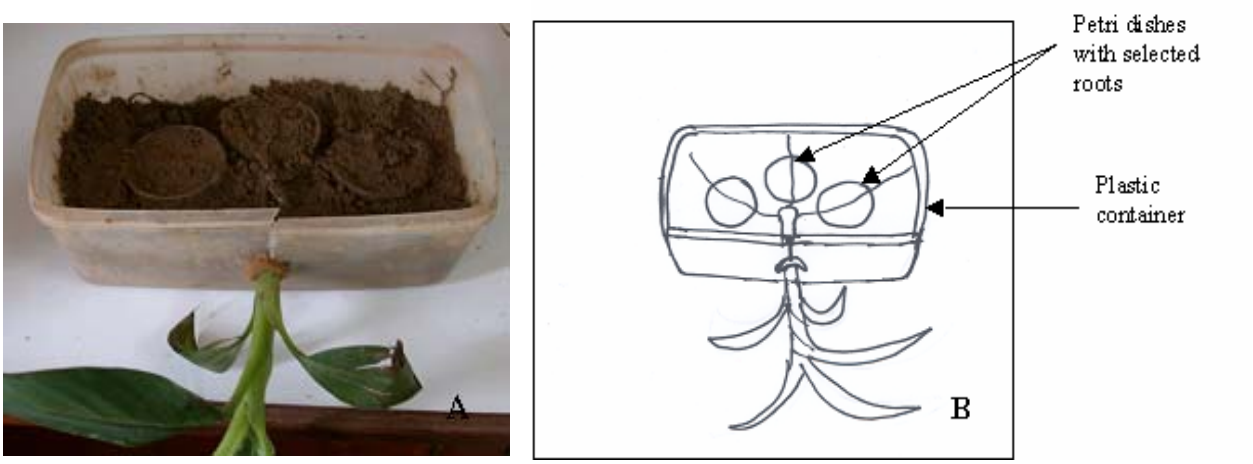


Figure 5. Experimental set up used to assess *Radopholus similis* reproduction in individual roots (A) and an excavated pot with the nematode inoculated root segment inside the plastic pot flanked by two root pieces (B).

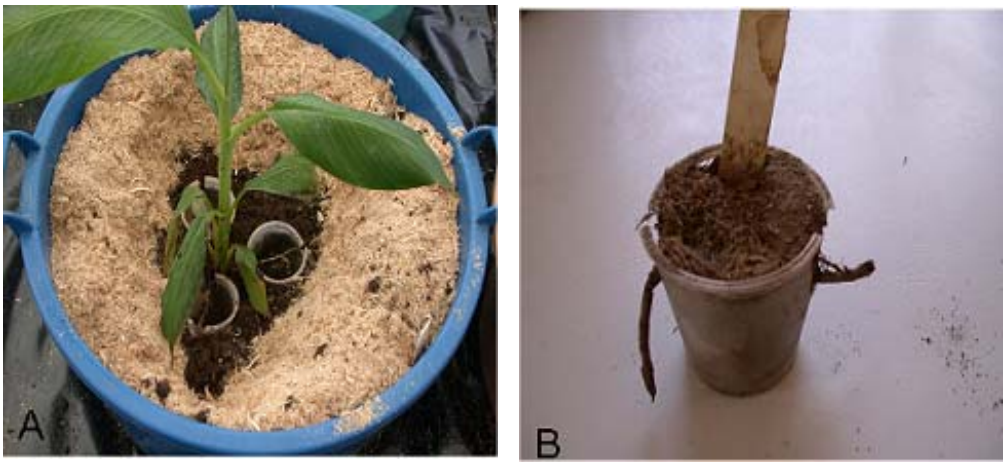
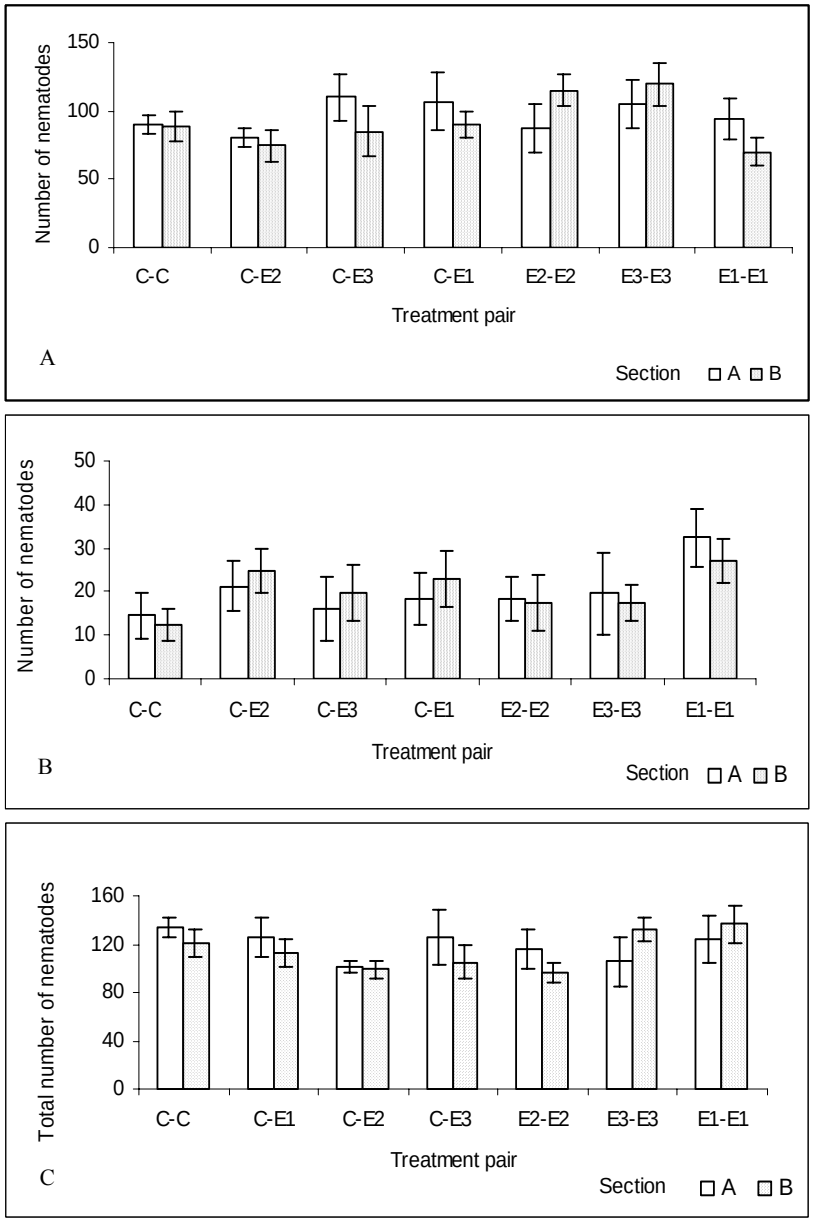
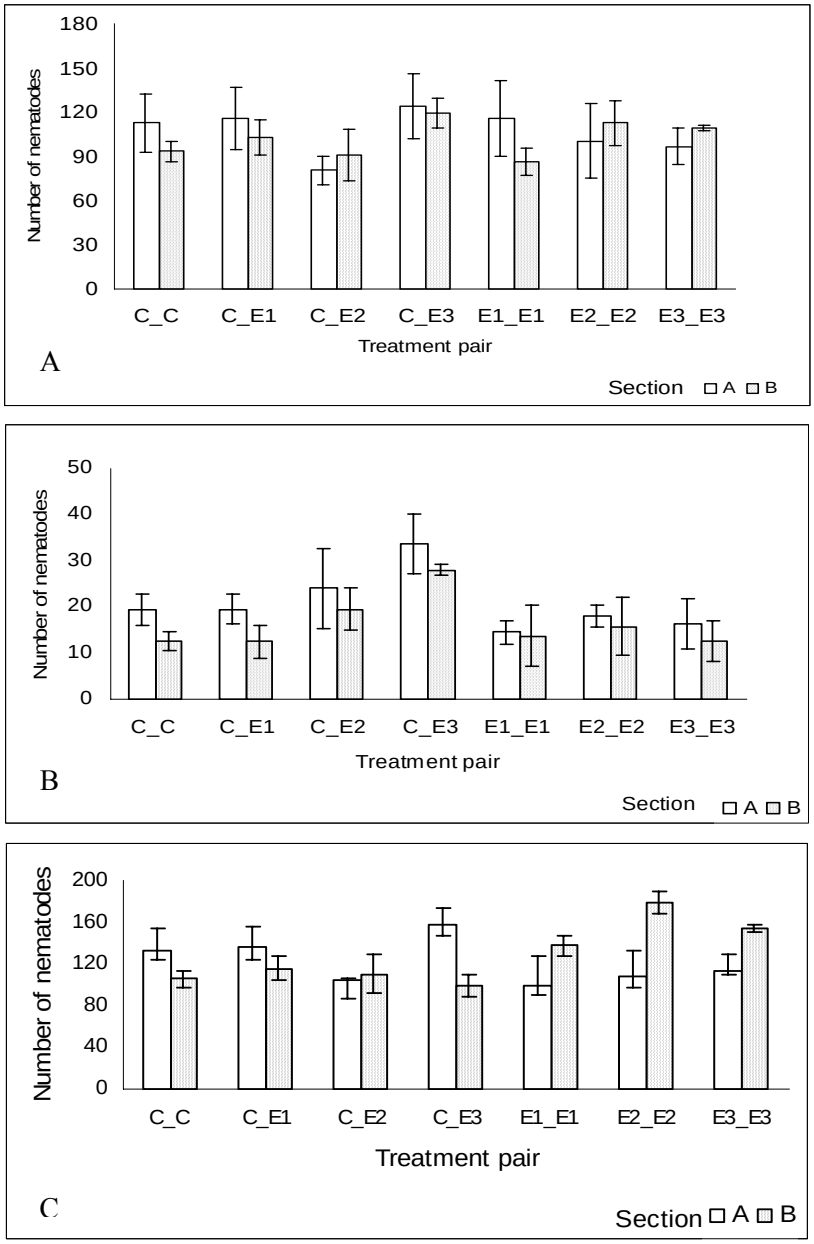


Figure 6. Average number of *Radopholus similis* that migrated towards root segments excised from endophyte-inoculated or un-inoculated banana roots in the detached root bioassay (Bioassay 1). Total nematodes recovered from the sand (A), number from the root segments (B) and the total nematodes from both the sand and root segment (C) in opposite sides of a Petri dish containing each respective treatment pair.



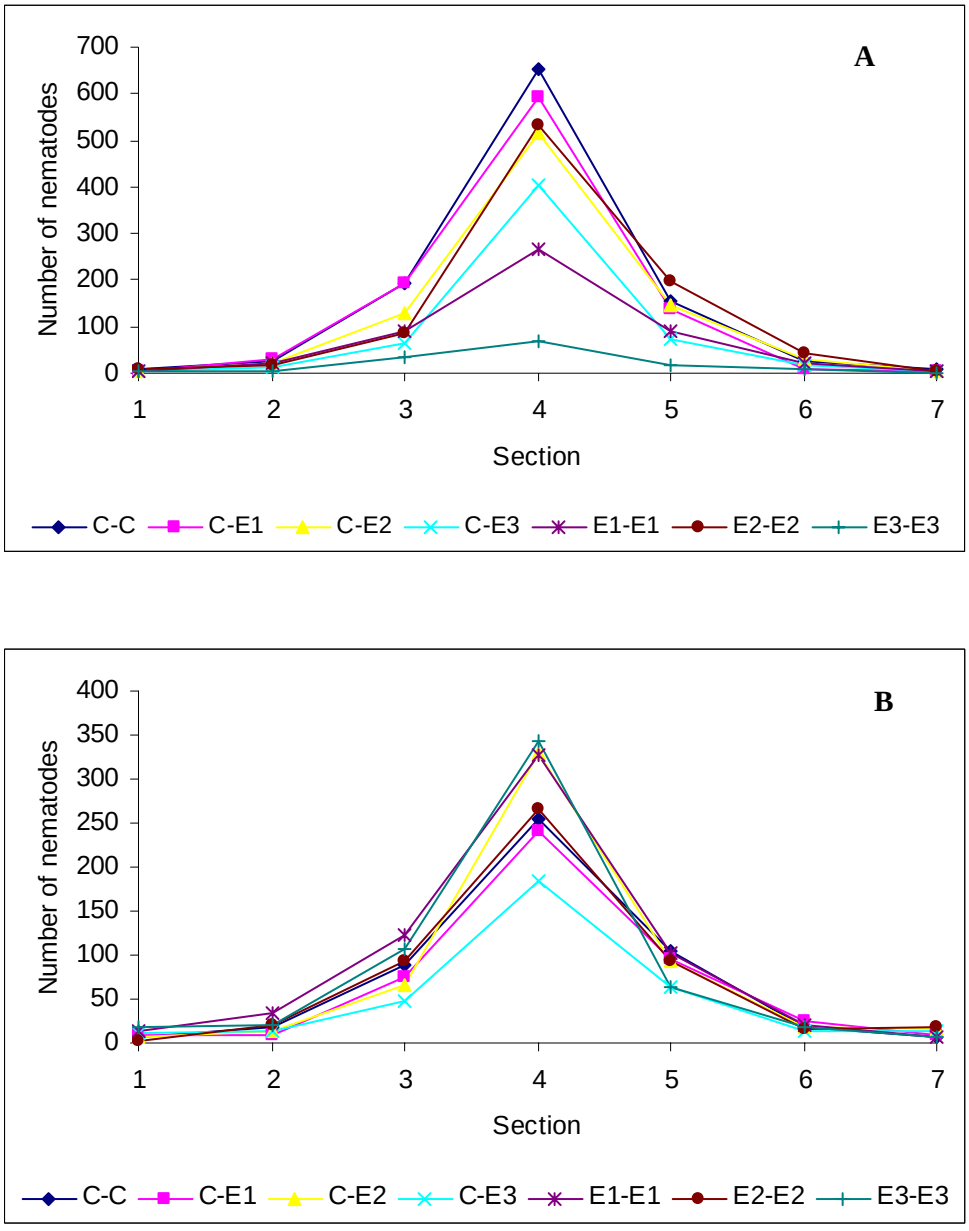
C=control, E1=endophyte isolate *V5W2*, E2= endophyte isolate *Eny1.31i*, E3= endophyte isolate *Eny7.11o*. Root segments from either endophyte-inoculated or un-inoculated plants were paired in 90-mm-diameter Petri dishes. The first treatment in each treatment pair was placed in section A and the second in section B. Nematodes were inoculated at the middle of the Petri dish equidistant from the two root segments. Nematode extraction was carried out 24 hrs later from both sand and the root segment in both sections of the Petri dish. Error bars represent standard errors of the mean, n=4

Figure 7: Average number of *Radopholus similis* that migrated towards root segments excised from endophyte-inoculated or un-inoculated banana roots in the detached root bioassay (Bioassay 2). Total nematodes recovered from the sand (A), number from the root segments (B) and the total nematodes from both the sand and root segment (C) in opposite sides of a Petri dish containing each respective treatment pair.



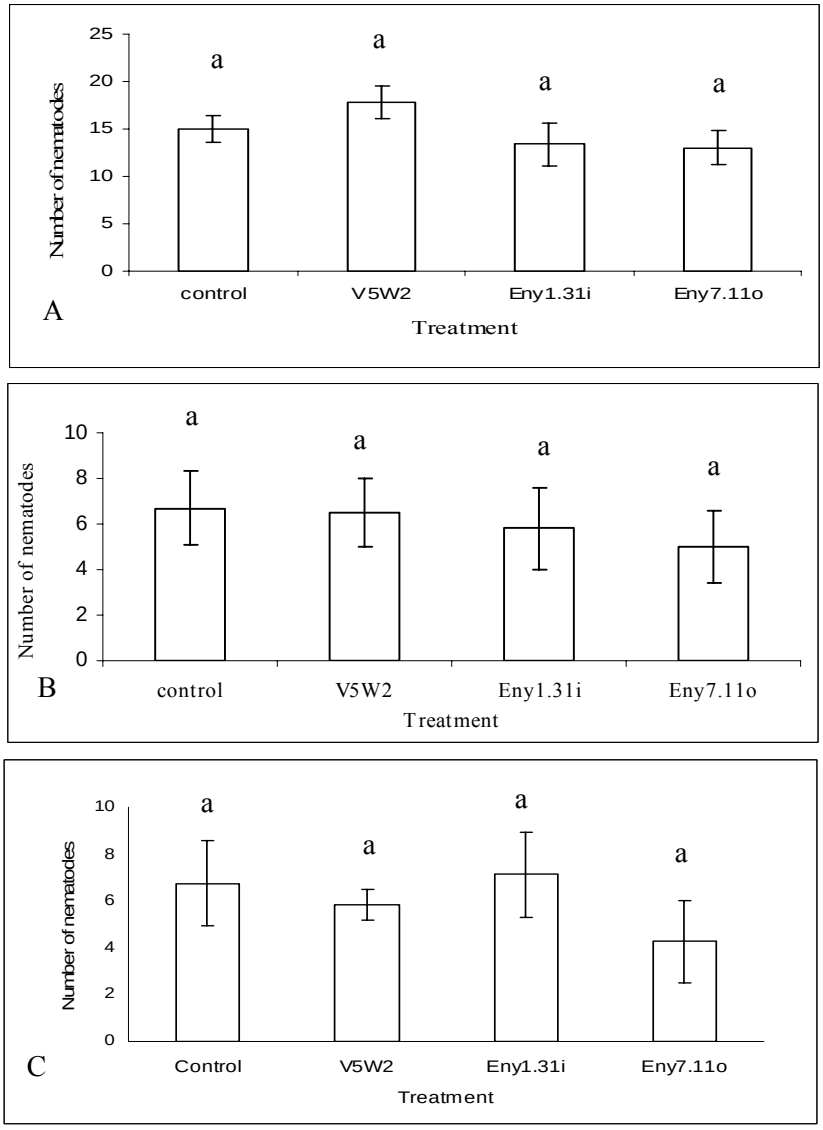
C=control, E1=endophyte isolate *V5W2*, E2= endophyte isolate *Eny1.31i*, E3= endophyte isolate *Eny7.11o*. Root segments from either endophyte-inoculated or un-inoculated plants were paired in 90-mm-diameter Petri dishes. The first treatment in each treatment pair was placed in section A and the second in section B. Nematodes were inoculated at the middle of the Petri dish equidistant from the two root segments. Nematode extraction was carried out 24 hrs later from both sand and the root segment in both sections of the Petri dish. Error bars represent standard errors of the mean, n=4

Figure 8: Distribution of nematodes in the polyvinyl chloride (PVC) sections showing migration of nematodes towards endophyte-inoculated and un-inoculated banana plants paired in a PVC tube apparatus divided into seven sections (A, bioassay 1; B, bioassay 2). *Radopholus similis* (2000 mixed stages) were introduced in the middle section of the PVC apparatus (section 4) equal distances from both plants placed in sections 1 and 7, which contained the either an endophyte-inoculated or un-inoculated plant.



C=control, E1= endophyte isolate *V5W2*, E2= endophyte isolate *Eny1.31i*, E3= endophyte isolte *Eny7.11o*.

Figure 9. Number of nematodes present in 8-cm-long root segments of endophyte-inoculated and un-inoculated banana plants 24 hrs after direct inoculation of root segments with 50 *Radopholus similis* females in the *in vitro* root penetration experiments. A, B and C are repeat experiments.



Control plants were not treated with any fungal isolate; endophyte-inoculated plants were treated with any of the three endophyte isolates viz. *V5W2*, *Eny1.31i* and *Eny7.11o*. Error bars represent standard errors of the mean, n=15. Bars with the same letters are not significantly different at *P*=0.05 according to Tukey's studentized range test.