Non-pathogenic *Fusarium oxysporum* endophytes provide field control of nematodes, improving yield of banana (*Musa* sp.)

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Abstract

Endophytic colonization by the fungus *Fusarium oxysporum* can result in increased host resistance to pests and diseases, and greater biomass production. However, few studies have assessed the field performance of this fungus for biological control of pests and diseases in banana. Further to greenhouse assessment, studies were carried out to evaluate the performance of *F. oxysporum* strains against plant-parasitic nematodes on banana (*Musa* sp., cv. Giant Cavendish and cv. Grand Nain) in the field using tissue-cultured plants. Plants were inoculated separately with one of three strains (*V5W2, Eny 7.11o* and *Emb2.4o*) before being inoculated with *Pratylenchus goodeyi* and *Helicotylenchus multicinctus* in an on-station trial and in an on-

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farm trial planted in a field naturally infested with the same nematodes. All three endophytic strains significantly suppressed *P. goodeyi* and *H. multicinctus* densities and damage in the field. On-station, nematode population densities were reduced by>45% in endophyte-inoculated plants compared to non-inoculated plants, while percentage root necrosis was reduced by>20%. Similarly, on-farm, nematode damage to roots and densities were also significantly lower in endophyte-inoculated plants compared with control plants. Significantly improved yields were observed for plants inoculated with endophytes when compared to the control plants, with inoculation with strains *Emb 2.4o* and *V5W2* resulting in up to 35% and 36% increased banana yields, respectively, for the on-station trial. For the on-farm trial, up to 20% increase in yields were observed for strain *Eny 7.11o* compared to control plants. This study provides the first report from the field in Africa on the reduction of nematode populations and damage, and the increase in banana production by fungal endophytes. The study shows that endophytes have potential to enhance yields of tissue-cultured banana plants and protect them against pests.

Keywords:

Africa

Biological control

Endophyte inoculation

Microbial antagonist

Plant-parasitic nematode

Tissue culture

1. Introduction

Bananas (*Musa* sp.) are produced in many districts of Kenya. In the higher altitude western regions of Kenya, the East African highland bananas (genome group AAA-EA, cv. Matooke and cv. Mbidde) are most common, while in the central and eastern highlands and coastal areas, dessert cultivars (genome group AAA) are the most popular, especially cv. Cavendish and cv. Gros Michel (Wambugu and Kiome, 2001). Banana production is faced by a number of constraints, although a complex of plant-parasitic nematodes, banana weevils (*Cosmopolites sordidus* (Germar)), poor agronomic practices, diseases and poor soil fertility combine to adversely affect yields in Kenya (Inzaule et al., 2005) and East Africa (Blomme et al., 2013). Progressive yield decline in plantations of banana is a problem in the small plots of resource-limited farmers of East, Central and West Africa. Plant-parasitic nematodes cause serious crop losses worldwide and are among the most important agricultural pests (Koenning et al., 1999). Several species of plant-parasitic nematodes have been associated with banana in Kenya. The most important are *Pratylenchus goodeyi* (Cobb) Sher and Allen, *Helicotylenchus multicinctus* (Cobb) Golden and *Meloidogyne* spp., which have a varied distribution (Kung'u, 1995; Seshu Reddy et al., 2007). Yield losses associated with banana nematodes range between 30-60% (Brooks, 2004). Roots damaged by nematodes are less able to supply plants with needed water and nutrients. This damage slow plant growth, lengthen the time to fruiting, reduce bunch weight and decrease the productive life of the plantation. Top-heavy plants may topple due to poor anchorage from damaged root systems.

The key to high banana productivity lies in the effective management of pests and diseases. The management of nematodes is often more difficult than that of other pests because nematodes

attack the underground parts of the plants (Stirling, 1991). Although chemical nematicides are effective, easy to apply and show rapid effects, concerns about public health and environmental safety have been raised (Schneider et al., 2003). Furthermore, nematicides are often too costly and difficult to access for smallholder farmers. Therefore, efficient approaches to control nematodes that utilize a range of biological control options are needed (Sikora et al., 2003).

The potential of non-pathogenic *Fusarium oxysporum* (Schlecht.: Fries), naturally occurring within banana plants as endophytes, has gained attention as an alternative to nematicides (Sikora et al., 2003; Athman et al., 2006; Dubois et al., 2006). There are a number of ways through which endophytes are reported to protect host plants against nematodes, including: improvement of plant physiology through, for example, enhanced tillering and root growth, and increased drought tolerance (Malinowski et al., 1997; Elmi et al., 2000); induction of systemic resistance (Paparu et al., 2006; Vu et al., 2006; Paparu et al., 2013); and production of nematicidal metabolites (Cook and Lewis, 2001; Dubois et al., 2004; Athman et al., 2006).

In East Africa, banana plants are increasingly being produced through tissue culture by small- to medium-sized private companies. The technology has great potential in Kenya, although pests and disease re-infestation in the field may hamper its uptake by smallholder farmers, because tissue-cultured plantlets are more fragile than conventional planting material (Kabunga et al., 2012a,b; Dubois et al., 2013; Niere et al., 2014). Enhancement of tissue-cultured plantlets with *F. oxysporum* endophytes is especially promising from a commercial point of view, since the endophytes are introduced into the plants before they are sold to farmers and the know-how is easily transferable to a commercial tissue culture laboratory.

Research in Costa Rica, Kenya and Uganda (Dubois et al., 2006; Pocasangre et al., 2007; Zum Felde, 2008; Machungo et al., 2009) has shown that endophytes are adapted to local

conditions and that, therefore, locally isolated endophytes may be more suitable in controlling nematodes. In Kenya, fungal endophytes originating from Kenya and Uganda have been demonstrated to lower populations of banana nematodes (*P. goodeyi* and *H. multicinctus*) both *in vitro (*Mwaura et al., 2009; 2010) and in the greenhouse (Machungo et al., 2009; Waweru et al., 2013). However, the potential of endophytic *F. oxysporum* to effectively manage banana nematodes in the field as a biological control agent has yet to be clearly demonstrated, with only a conference abstract (Pocasangre et al., 2006) and an MSc dissertation (Menjivar Barahova, 2005) reporting on field results of banana inoculated with *Fusarium oxysporum* endophytes in Costa Rica.

Thus, the objectives of the current study were to evaluate the efficacy of locally adapted endophytic strains inoculated into tissue-cultured banana plants for biological control of banana nematodes (*P. goodeyi* and *H. multicinctus*) in the field, using both on-station and on-farm trials.

2. Materials and methods

2.1. Site description

Two trials were conducted in Kenya between 2007-2009, one on-station at Jomo Kenyatta University Agriculture Technology (JKUAT) farm, Juja (1,537 m above sea level, 01º05’25.6”S, 037º00’45.5”E), and one on-farm in Maragua district, a key banana production area (1,346 m above sea level, 00º47’17.3”S, 037º08’18.3”E).

2.2. Experimental design

The endophytic *F. oxysporum* strains *V5w2, Emb.2.4o* and *Eny 7.11o*, originally isolated from East African highland cooking banana plants in Uganda (Schuster et al., 1995), were used as they were identified as the most effective strains against *R. similis in vitro* and *in vivo* in Kenya and Uganda (Athman et al., 2006; Mwaura et al., 2009, 2010; Machungo et al., 2009). The trials included two factors: banana cultivar and endophyte treatment. Cultivars included the dessert bananas cv. Giant Cavendish and cv. Grand Nain. Endophyte treatments included the three *F. oxysporum* endophytes (*V5w2, Emb.2.4o* or *Eny 7.11o*) and a negative control (non-inoculated plants), yielding a total of eight treatments. On-station, nine plants were used per treatment, which were all inoculated with nematodes. On-farm, fifteen plants were used per treatment but not inoculated with nematodes because fields were already found to be infested with nematodes. Both trials were laid out in split-plot design where the main plot factor was cultivar and the subplot factor was endophyte treatment.

2.3. Plant preparation and endophyte inoculation

Two month old tissue-cultured plants (post-weaning stage) of each cultivar were obtained from JKUAT's tissue culture laboratory in Nairobi, Kenya. The plants were micropropagated using a standard shoot-tip culture protocol for banana according to Vuylsteke (1998). Upon deflasking, plant roots were rinsed free of media in tap water and selected for uniformity in size before planting in weaning trays (60 × 30 cm). Plants were allowed to grow for one month before being transferred to 3 l plastic potting bags (5 × 9 × 4 cm) containing steam-sterilized sandy loam soil. The plants were watered daily and maintained on raised benches in a greenhouse for one month before endophyte inoculation. Inoculation of plants with endophytes and nematodes was carried out according to Machungo (2009).

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134 *2.4. Preparation of fungal inoculum*

135 For each strain, pieces of filter paper containing mycelium were plated on fresh synthetic
136 nutrient agar (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
137 sucrose, 0.6 ml NaOH (1 M), 13.2 g agar, 0.1 g penicillin G, 0.2 g streptomycin sulphate and
138 0.05 g chlortetracycline/l SDW) in 90 mm diameter Petri dishes in the laboratory at room
139 temperature (~25°C) under sterile conditions. The Petri dishes were maintained under a natural
140 photoperiod of (12:12 h; L:D) for 7-10 to allow sporulation. For each strain, four blocks of SNA
141 (~0.5 cm^3) containing mycelia and spores were inoculated into 100 ml sterile potato dextrose
142 broth (PDB) medium (12 g PDB/l SDW; Sigma-Aldrich, St. Louis, USA) in a 250 ml
143 Erlenmeyer flask, and the medium was incubated for 7 days. To obtain a solid substrate, 200 g of
144 maize bran was placed in 500 ml Erlenmeyer flasks, moistened by adding water, autoclaved for
145 30 min at 121^{o}C and allowed to cool overnight before re-autoclaving for 30 min. Thirty ml of the
146 fungal suspension was transferred into each of the flasks with maize bran, and the medium was
147 incubated for 10 days at room temperature. Non-inoculated maize bran was used for the control
148 treatment. The spore concentration in the solid substrate was standardized to 3.3×10^6 spores/ml
149 and plants were inoculated by adding 2 g maize bran containing the fungal inoculum into three 4
150 cm deep pencil-made holes around the plant roots. Holes were covered with soil and plants were
151 maintained in the greenhouse.

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153 *2.5. Nematode inoculation*

154 At the onset of both trials, soil sampling was carried out to assess the densities and species of
155 nematodes in the soil. Ten ~200 g soil samples were randomly collected within each field.

Samples from the same field were combined to make a composite sample. The samples were placed in plastic bags and transported to the laboratory for nematode extraction. Nematodes were extracted from a 100 g subsample using the Baermann tray method (McSorley, 1987). Nematode species were identified using morphological characteristics.

In the on-station site, nematode species that are known to attack banana were absent. Therefore, roots were obtained from visibly diseased banana plants in a nematode-infested plantation as follows. A hole measuring approximately 5 × 5 × 5 cm was excavated at 10 cm distance from the base of the mother plant. All banana roots showing symptoms of nematode damage were selected and used for nematode extraction. These roots were cut to ~1 cm in length, thoroughly mixed, and nematodes extracted from a 25 g subsample using a modified Baermann tray method (Hooper et al., 2005) over a 24 h period. The suspensions were reduced to 5 ml and nematode densities estimated from a 1 ml subsample. Each plant was inoculated with 1,200 nematodes/plant (95% *Pratylenchus goodeyi*, 3% *Radopholus similis* (Cobb) Thorne and 2% *H. multicintus*) four weeks after endophyte inoculation into three 4 cm deep pencil-made holes around the plant. Holes were covered with soil and plants were maintained in the greenhouse.

Plants for the on-farm trial were not inoculated with nematodes. Nematode populations in the soil of the on-farm trial averaged 373/100 g of soil (96 *P. goodeyi*, 174 *H. multicinctus* and 103 *Melodoigyne* sp.).

2.6. Planting

Nine month old plants were planted in the field in mid-May (on-station) and mid-November (on-farm) 2007. Plants were placed in holes measuring 1 × 1 m and spaced at 3 × 3 m between and within the rows, respectively, and covered by a mixture of topsoil with 40 kg of compost

manure. Compost manure (40 kg/plant) was also added five months after planting. Weeds were controlled manually as necessary to maintain weed-free plots. The fields were irrigated depending on weather conditions: during the dry seasons, irrigation was conducted thrice weekly (20 l/plant).

2.7. Plant growth assessment

Plant height (distance from the base of the plant to the point of the youngest leaf emergence), girth of the pseudostem (measured at base of the plant) and number of healthy functional leaves (leaves were considered healthy when>75% of the leaf area was green as opposed to yellow, brown or dry), were measured on a monthly basis. At harvest, yield (t/ha), bunch weight, number of hands and number of fingers per bunch were determined. Bunches were considered mature when fingers of the second hand attained a round shape. The number of days to flowering and harvest was recorded for each plant. Data were collected for the first crop cycle of each trial only. No toppling was observed in either trial. Yield (kg/ha/year) was calculated as: average bunch weight/treatment × percentage of plant harvested/treatment × plants/ha (1,111 plants/ha) × (365/number of days from planting to harvest).

2.8. Nematode densities and damage assessment

Root samples were collected at 3, 6, 9 and 12 months after transplanting (MAT) to assess for nematode damage and population. A hole measuring 5 × 5 × 5 cm was excavated at 10 cm distance from the base of the mother plant. All banana roots within the hole were collected, and five roots were randomly selected and used to assess for nematode damage according to Speijer and De Waele (1997). Nematode damage was expressed as percentage necrotic root tissue. The

five roots were cut to a length of 10 cm, sliced length-wise and the percentage root cortex showing necrosis of each root estimated to a maximum of 20% each. The percentage root necrosis comprised the sum of each of the five pieces. These roots were further cut to ~1 cm in length, thoroughly mixed, and nematodes extracted from a 25 g subsample using the modified Baermann tray method (Hooper et al., 2005) over 24 h. The suspensions were reduced to 5 ml and nematode densities estimated from a 1 ml subsample. *Pratylenchus goodeyi* and *H. multicintus* were counted per life stage (female, male and juvenile).

2.9. Data analysis

Nematode counts were $\log_{10}(x+1)$-transformed, while percentage nematode damage data were arcsine-square root-transformed before analysis of variance (ANOVA). Plant growth and yield data were left untransformed prior to ANOVA. A generalized linear model was used to test for factor effects and their interactions. When factor interactions were significant, effects of one factor were analyzed at each level of the other factor. Means were separated using least significant difference tests (LSD) (SAS Institute, 2001).

3. Results

3.1. Plant growth and yield

Plant growth parameters (height, girth and number of functional leaves) were not significantly different ($P>0.05$) between the two banana cultivars and neither was there a significant interaction ($P>0.05$) between cultivar and endophyte treatment for either trial (Table 1).

Endophyte-inoculated plants exhibited enhanced growth compared to non-inoculated plants, although this was not statistically significant ($P>0.05$).

For both trials, days to flowering and days to harvest were not significantly different ($P>0.05$) between the two banana cultivars nor endophyte treatments (Table 2). There was no significant interaction ($P>0.05$) between cultivar and endophyte treatment for either trial.

Bunch weight and yield were not significantly different between banana cultivars ($P>0.05$), nor was there a significant interaction between cultivar and endophyte treatment ($P>0.05$) for either trial. Therefore bunch weight and yield data were pooled across cultivars. In both trials, bunch weight and yield of plants inoculated with endophytic *F. oxysporum* strains were significantly higher ($P<0.05$) than those of control plants (Table 3). In the on-station trial, plants treated with *V5W2* produced significantly heavier bunches (22%) than untreated plants, and plants treated with *V5W2* and *Emb 2.4o* had significantly higher yields (36-37%) compared to control plants. In the on-farm trial, *Eny 7.11o*-treated plants produced significantly heavier bunches (20%) and higher yields (20%) compared to untreated plants.

3.2. Nematode damage

For either trial, nematode damage was not statistically significant between banana cultivars ($P>0.05$), nor was there a significant interaction between cultivar and endophyte treatment ($P>0.05$). Therefore, root necrosis data was pooled across cultivars for both trials. In the on-station trial, endophyte inoculation significantly reduced nematode damage in inoculated plants at 6 ($P<0.05$), 9 ($P<0.01$) and 12 ($P<0.005$) MAT compared with non-inoculated plants (Table 4). In the on-farm trial, nematode damage remained low in all treatments up to 6 MAT, but

246 became more evident over time, with significantly lower necrosis observed in endophyte-
247 inoculated plants compared with control plants at 9 MAT (P <0.05) and 12 (P<0.005).
248
249 *3.3. Nematode population densities*
250 Nematode density data were pooled across cultivars for both trials, because nematode
251 densities were not significantly different (P<0.05) between cultivars, nor was there an interaction
252 between cultivar and endophyte treatment (P>0.05) for either trail.
253 In the on-station trial, although endophyte non-inoculated plants contained numerically higher
254 nematode densities (*P. goodeyi* and *H. multicinctus*) at 3 and 6 MAT, the differences were not
255 significant (P>0.05) among treatments (Table 5). At 9 and 12 MAT, endophyte-inoculated plants
256 had significantly fewer (P<0.001) nematode densities in roots compared with non-inoculated
257 plants. No *R. similis* nematodes were recovered from the roots.
258 In the on-farm trial, few nematodes were recovered from the roots by 3 and 6 MAT, with no
259 significant differences observed in densities among treatments. At 9 and 12 MAT, endophyte-
260 inoculated plants had significantly lower (P<0.05) densities of *P. goodeyi* compared with non-
261 inoculated plants. At 9 MAT, *Helicotylenchus multicinctus* densities were relatively, but not
262 significantly, lower in endophyte-inoculated plants. After 12 MAT, significant differences
263 (P=0.028) were observed in *H. multicinctus* densities between endophyte-inoculated and non-
264 inoculated plants.
265 In the on-station trial, *Meloidogyne* sp. was not observed. In the on-farm trial, the population
266 of *Meloidogyne* sp. was not significantly (P>0.05) different between cultivars or endophyte
267 treatments, and ranged from 325-477 at 3 MAT to 109-230 at 12 MAT.

4. Discussion

The current study provides strong evidence of the protective effects of fungal endophytes against plant-parasitic nematodes on banana under field conditions. A significant reduction in nematode densities and associated damage to banana roots was observed on-station and also in a naturally infected farm following inoculation with *F. oxysporum* strains, which translated into improved bunch weights and yields in the first, mother crop cycle. Use of fungal endophytes to protect against plant-parasitic nematodes has previously been demonstrated in the field. Grasses infected by *Neotyphodium* sp. and *Acremonium coenophialium* endophytes, for instance, inhibited some species of migratory and sedentary endoparasites, leading to a higher yield in *Festuca arundinacea* Schreb. plants (West et al., 1988; Bacetty et al., 2009). In other crops, non-pathogenic *Fusarium* spp. strains isolated from roots reduced *Meloidogyne incognita* (Kofoid & White) Chit. populations on tomato *Solanum lycopersicum* L. (Hallmann and Sikora, 1994), and *Meloidogyne graminicola* Golden & Birchfield populations on rice (*Oryza sativa* L.) (Le et al., 2009).

A number of studies have demonstrated the beneficial effect of *F. oxysporum* endophytes on banana, primarily against *R. similis* (Niere, 2001; Athman et al., 2006; Dubois et al., 2006; Paparu et al., 2006; Zum Felde, 2008). However, there are few studies that have assessed their effect in the field. Menjivar Barahova (2005) demonstrated reduction in *R. similis* populations of up to >50% in large dessert banana (cv. Valery) plantations seven months after tissue-cultured plantlets had been inoculated with locally adapted *F. oxysporum* strains and planted in the field in Costa Rica. These reductions are higher than what we found for *H. multicinctus* and *P.*

goodeyi 12 MAP in Kenya. Although Menjivar Barahova (2005) did not find large differences in functional root weight between *F. oxysporum*- and non-inoculated banana plants, we found a large reduction in root damage caused by *H. multicinctus* and *P. goodeyi*. Interestingly, Menjivar Barahova (2005) observed large increases in plant height, circumference and number of standing leaves, which we failed to detect statistically. However, both Menjivar Barahova (2005) and the present study found that *F. oxysporum* endophytes significantly increased bunch weights and yields compared to non-inoculated plants.

Inoculation of other hypocrealean fungal endophytes such as *Trichoderma viride* Pers. has equally shown to reduce nematode populations in banana fields. Pocasangre et al. (2007) reported that *R. similis* populations in Costa Rica and the Dominican Republic were reduced to levels similar of those obtained by chemical control after tissue-cultured plantlets had been inoculated with *T. viride*, although no effects were found in field trials in Venezuela. Also, high reduction of nematodes has been demonstrated in naturally suppressive fields in Guatemala, where banana plants were left uninoculated but soils were found to be colonized by high levels of *F. oxysporum* and *T. viride* (Sikora et al., 2010).

The current study demonstrates that *F. oxysporum* endophytes, inoculated into tissue-cultured banana plants, are beneficial to the farmer. Non-pathogenic endophytic *F. oxysporum* strains (*V5W2, Eny7.11o* and *Emb 2.40*) reduce populations of *P. goodeyi* and *H. multicinctus*, thereby reducing root necrosis, and increasing bunch weight and ultimately yield.

The results are derived from the first crop cycle only, however, and so as a perennial crop, the benefits may extend further into following crop cycles. Nematode pests manifest over time and build up on perennial crops such as banana, where they tend to be more damaging in following

313 crop cycles. Referring to *P. goodeyi* on East African highland bananas in Uganda, Speijer et al.
314 (1999) suggested that long-term studies are needed for its damage potential to be realized.
315 Improved yields following endophyte enhancement could also be attributed to growth
316 enhancement as a response to endophytic colonization. In our study, a slight increase in plant
317 height, girth of the banana pseudostems and number of functional leaves for endophyte
318 inoculated plants was observed, although these were not significant. In our study, plants
319 enhanced with endophytes were harvested sooner than untreated plants, but this was also not
320 statistically significant. In other studies, however, banana growth was stimulated following
321 inoculation with *F. oxysporum* endophytes (Niere, 2001), while Waller et al. (2005) observed
322 similar responses following *Piriformospora indica* Sav. Verma colonization of barley (*Hordeum*
323 *vulgare* L.). Endopyhtic fungi produce phytohormones (Tan and Zou, 2001; Nassar et al., 2005),
324 which could contribute to improved growth.
325 Although endophytic fungi have been shown to protect plants from nematode attack and
326 damage, not all nematode species are similarly affected by endophyte infection. *In vitro*
327 assessment of a range of banana nematode species showed marked differences in their reaction to
328 fungal metabolites (Van Dessel et al., 2011). In a greenhouse study, Niere et al. (1999) also
329 reported that *H. multicinctus* densities were reduced by 75% by endophytes, while other species
330 present were unaffected. In the current study, *Meloidogyne* sp. in the on-farm trial was not
331 significantly affected by the endophyte infection, by comparison to other species.
332 This study provides the first report from the field in Africa on the effects of fungal endophytes
333 on reducing nematode populations and damage, and increasing banana production. Bio-
334 enhancement of tissue-cultured plantlets with endophytic strains *V5W2, Eny 7.11o* and *Emb2.4o*
335 increased their protection against nematode pests, when treated prior to field release. Such

treatment of seedling material provides a targeted application ahead of distribution to farmers and field planting, which helps to optimize use and efficiency of these biological control options. In the first crop cycle, application of the endophytes led to higher bunch weights and yields of banana. Further studies are needed to determine the longer-term effect of endophytes on growth and yields over successive crop cycles, as well as their interaction with other factors such as management practices.

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Non-pathogenic *Fusarium oxysporum* endophytes provide field control of nematodes, improving yield of banana (*Musa* sp.)

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Abstract

Endophytic colonization by the fungus *Fusarium oxysporum* can result in increased host resistance to pests and diseases, and greater biomass production. However, few studies have assessed the field performance of this fungus for biological control of pests and diseases in banana. Further to greenhouse assessment, studies were carried out to evaluate the performance of *F. oxysporum* strains against plant-parasitic nematodes on banana (*Musa* sp., cv. Giant Cavendish and cv. Grand Nain) in the field using tissue-cultured plants. Plants were inoculated separately with one of three strains (*V5W2, Eny 7.11o* and *Emb2.4o*) before being inoculated

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with *Pratylenchus goodeyi* and *Helicotylenchus multicinctus* in an on-station trial and in an on-farm trial planted in a field naturally infested with the same nematodes. All three endophytic strains significantly suppressed *P. goodeyi* and *H. multicinctus* densities and damage in the field. On-station, nematode population densities were reduced by>45% in endophyte-inoculated plants compared to non-inoculated plants, while percentage root necrosis was reduced by>20%. Similarly, on-farm, nematode damage to roots and densities were also significantly lower in endophyte-inoculated plants compared with control plants. Significantly improved yields were observed for plants inoculated with endophytes when compared to the control plants, with inoculation with strains *Emb 2.4o* and *V5W2* resulting in up to 35% and 36% increased banana yields, respectively, for the on-station trial. For the on-farm trial, up to 20% increase in yields were observed for strain *Eny 7.11o* compared to control plants. This study provides the first report from the field in Africa on the ~~the~~ reduction of nematode populations and damage, and the increase in banana production by fungal endophytes~~negative and positive effects of fungal endophytes on nematodes and banana production, respectively~~. The study shows that endophytes have potential to enhance yields of tissue-cultured banana plants and protect them against pests.

Keywords:

Africa

Biological control

Endophyte inoculation

Microbial antagonist

Plant-parasitic nematode

Tissue culture

1. Introduction

Bananas (*Musa* sp.) are produced in many districts of Kenya. In the higher altitude western regions of Kenya, the East African highland bananas (genome group AAA-EA, cv. Matooke and cv. Mbidde) are most common, while in the central and eastern highlands and coastal areas, dessert cultivars (genome group AAA) are the most popular, especially cv. Cavendish and cv. Gros Michel (Wambugu and Kiome, 2001). Banana production is faced by a number of constraints, although a complex of plant-parasitic nematodes, banana weevils (*Cosmopolites sordidus* (Germar)), poor agronomic practices, diseases and poor soil fertility combine to adversely affect yields in Kenya (Inzaule et al., 2005) and East Africa (Blomme et al., 2013). Progressive yield decline in plantations of banana is a problem in the small plots of resource-limited farmers of East, Central and West Africa. Plant-parasitic nematodes cause serious crop losses worldwide and are among the most important agricultural pests (Koenning et al., 1999). Several species of plant-parasitic nematodes have been associated with banana in Kenya. The most important are *Pratylenchus goodeyi* (Cobb) Sher and Allen, *Helicotylenchus multicinctus* (Cobb) Golden and *Meloidogyne* spp., which have a varied distribution (Kung'u, 1995; Seshu Reddy et al., 2007). Yield losses associated with banana nematodes range between 30-60% (Brooks, 2004). Roots damaged by nematodes are less able to supply plants with needed water and nutrients. This damage ~~ese~~ slow plant growth, lengthen the time to fruiting, reduce bunch weight and decrease the productive life of the plantation. Top-heavy plants may topple due to poor anchorage from damaged root systems.

The key to high banana productivity lies in the effective management of pests and diseases. The management of nematodes is often more difficult than that of other pests because nematodes

attack the underground parts of the plants (Stirling, 1991). Although chemical nematicides are effective, easy to apply and show rapid effects, concerns about public health and environmental safety have been raised (Schneider et al., 2003). Furthermore, nematicides are often too costly and difficult to access for smallholder farmers. Therefore, efficient approaches to control nematodes that utilize a range of biological control options are needed (Sikora et al., 2003).

The potential of non-pathogenic *Fusarium oxysporum* (Schlecht.: Fries), naturally occurring within banana plants as endophytes, has gained attention as an alternative to nematicides (Sikora et al., 2003; Athman et al., 2006; Dubois et al., 2006). There are a number of ways through which endophytes are reported to protect host plants against nematodes, including: improvement of plant physiology through, for example, enhanced tillering and root growth, and increased drought tolerance (Malinowski et al., 1997; Elmi et al., 2000); induction of systemic resistance (Paparu et al., 2006; Vu et al., 2006; Paparu et al., 2013); and production of nematicidal metabolites (Cook and Lewis, 2001; Dubois et al., 2004; Athman et al., 2006).

In East Africa, banana plants are increasingly being produced through tissue culture by small- to medium-sized private companies. The technology has great potential in Kenya, although pests and disease re-infestation in the field may hamper its uptake by smallholder farmers, because tissue-cultured plantlets are more fragile than conventional planting material (Kabunga et al., 2012a,b; Dubois et al., 2013; Niere et al., 2014). Enhancement of tissue-cultured plantlets with *F. oxysporum* endophytes is especially promising from a commercial point of view, since the endophytes are introduced into the plants before they are sold to farmers and the know-how is easily transferable to a commercial tissue culture laboratory.

Research in Costa Rica, Kenya and Uganda (Dubois et al., 2006; Pocasangre et al., 2007; Zum Felde, 2008; Machungo et al., 2009) has shown that endophytes are adapted to local

conditions and that, therefore, locally isolated endophytes may be more suitable in controlling nematodes. In Kenya, fungal endophytes originating from Kenya and Uganda have been demonstrated to lower populations of~~control~~ banana nematodes (*P. goodeyi* and *H. multicinctus*) both *in vitro (*Mwaura et al., 2009; 2010) and in the greenhouse (Machungo et al., 2009; Waweru et al., 2013). However, the potential of endophytic *F. oxysporum* to effectively manage banana nematodes in the field as a biological control agent has yet to be clearly demonstrated, with only a conference abstract (Pocasangre et al., 2006) and an MSc dissertation (Menjivar Barahova, 2005) reporting on field results of banana inoculated with *Fusarium oxysporum* endophytes in Costa Rica.

Thus, the objectives of the current study were to evaluate the efficacy of locally adapted endophytic strains inoculated into tissue-cultured banana plants for biological control of banana nematodes (*P. goodeyi* and *H. multicinctus*) in the field, using both on-station and on-farm trials.

2. Materials and methods

2.1. Site description

Two trials were conducted in Kenya between 2007-2009, one on-station at Jomo Kenyatta University Agriculture Technology (JKUAT) farm, Juja (1,537 m above sea level, 01º05’25.6”S, 037º00’45.5”E), and one on-farm in Maragua district, a key banana production area (1,346 m above sea level, 00º47’17.3”S, 037º08’18.3”E).

2.2. Experimental design

The endophytic *F. oxysporum* strains *V5w2, Emb.2.4o* and *Eny 7.11o*, originally isolated from East African highland cooking banana plants in Uganda (Schuster et al., 1995), were used as they were identified as the most effective strains against *R. similis in vitro* and *in vivo* in Kenya and Uganda (Athman et al., 2006; Mwaura et al., 2009, 2010; Machungo et al., 2009). The trials included two factors: banana cultivar and endophyte treatment. Cultivars included the dessert bananas cv. Giant Cavendish and cv. Grand Nain. Endophyte treatments included the three *F. oxysporum* endophytes (*V5w2, Emb.2.4o* or *Eny 7.11o*) and a negative control (non-inoculated plants), yielding a total of eight treatments. On-station, nine plants were used per treatment, which were all inoculated with nematodes. On-farm, fifteen plants were used per treatment but not inoculated with nematodes because fields were already found to be infested with nematodes. Both trials were laid out in split-plot design where the main plot factor was cultivar and the subplot factor was endophyte treatment.

2.3. Plant preparation and endophyte inoculation

Two month old tissue-cultured plants (post-weaning stage) of each cultivar were obtained from JKUAT's tissue culture laboratory in Nairobi, Kenya. The plants were micropropagated using a standard shoot-tip culture protocol for banana according to Vuylsteke (1998). Upon deflasking, plant roots were rinsed free of media in tap water and selected for uniformity in size before planting in weaning trays (60 × 30 cm). Plants were allowed to grow for one month before being transferred to 3 l plastic potting bags (5 × 9 × 4 cm) containing steam-sterilized sandy loam soil. The plants were watered daily and maintained on raised benches in a greenhouse for one month before endophyte inoculation. Inoculation of plants with endophytes and nematodes was carried out according to Machungo (2009).

134

135 *2.4. Preparation of fungal inoculum*

136 For each strain, pieces of filter paper containing mycelium were plated on fresh synthetic
137 nutrient agar (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
138 sucrose, 0.6 ml NaOH (1 M), 13.2 g agar, 0.1 g penicillin G, 0.2 g streptomycin sulphate and
139 0.05 g chlortetracycline/l SDW) in 90 mm diameter Petri dishes in the laboratory at room
140 temperature (~25°C) under sterile conditions. The Petri dishes were maintained under a natural
141 photoperiod of (12:12 h; L:D) for 7-10 to allow sporulation. For each strain, four blocks of SNA
142 (~0.5 cm^3) containing mycelia and spores were inoculated into 100 ml sterile potato dextrose
143 broth (PDB) medium (12 g PDB/l SDW; Sigma-Aldrich, St. Louis, USA) in a 250 ml
144 Erlenmeyer flask, and the medium was incubated for 7 days. To obtain a solid substrate, 200 g of
145 maize bran was placed in 500 ml Erlenmeyer flasks, moistened by adding water, autoclaved for
146 30 min at 121^oC and allowed to cool overnight before re-autoclaving for 30 min. Thirty ml of the
147 fungal suspension was transferred into each of the flasks with maize bran, and the medium was
148 incubated for 10 days at room temperature. Non-inoculated maize bran was used for the control
149 treatment. The spore concentration in the solid substrate was standardized to 3.3×10^6 spores/ml
150 and plants were inoculated by adding 2 g maize bran containing the fungal inoculum into three 4
151 cm deep pencil-made holes around the plant roots. Holes were covered with soil and plants were
152 maintained in the greenhouse.

153

154 *2.5. Nematode inoculation*

155 At the onset of both trials, soil sampling was carried out to assess the densities and species of
156 nematodes in the soil. Ten ~200 g soil samples were randomly collected within each field.

Samples from the same field were combined to make a composite sample. The samples were placed in plastic bags and transported to the laboratory for nematode extraction. Nematodes were extracted from a 100 g subsample using the Baermann tray method (McSorley, 1987). Nematode species were identified using morphological characteristics.

In the on-station site, nematode species that are known to attack banana were absent. Therefore, roots were obtained from visibly diseased banana plants in a nematode-infested plantation as follows. A hole measuring approximately 5 × 5 × 5 cm was excavated at 10 cm distance from the base of the mother plant. All banana roots showing symptoms of nematode damage were selected and used for nematode extraction. These roots were cut to ~1 cm in length, thoroughly mixed, and nematodes extracted from a 25 g subsample using a modified Baermann tray method (Hooper et al., 2005) over a 24 h period. The suspensions were reduced to 5 ml and nematode densities estimated from a 1 ml subsample. Each plant was inoculated with 1,200 nematodes/plant (95% *Pratylenchus goodeyi*, 3% *Radopholus similis* (Cobb) Thorne and 2% *H. multicintus*) four weeks after endophyte inoculation into three 4 cm deep pencil-made holes around the plant. Holes were covered with soil and plants were maintained in the greenhouse.

Plants for the on-farm trial were not inoculated with nematodes. Nematode populations in the soil of the on-farm trial averaged 373/100 g of soil (96 *P. goodeyi*, 174 *H. multicinctus* and 103 *Melodoigyne* sp.).

2.6. Planting

Nine month old plants were planted in the field in mid-May (on-station) and mid-November (on-farm) 2007. Plants were placed in holes measuring 1 × 1 m and spaced at 3 × 3 m between and within the rows, respectively, and covered by a mixture of topsoil with 40 kg of compost

manure. Compost manure (40 kg/plant) was also added five months after planting. Weeds were controlled manually as necessary to maintain weed-free plots. The fields were irrigated depending on weather conditions: during the dry seasons, irrigation was conducted thrice weekly (20 l/plant).

2.7. Plant growth assessment

Plant height (distance from the base of the plant to the point of the youngest leaf emergence), girth of the pseudostem (measured at base of the plant) and number of healthy functional leaves (leaves were considered healthy when>75% of the leaf area was green as opposed to yellow, brown or dry), were measured on a monthly basis. At harvest, yield (t/ha), bunch weight, number of hands and number of fingers per bunch were determined. Bunches were considered mature when fingers of the second hand attained a round shape. The number of days to flowering and harvest was recorded for each plant. Data were collected for the first crop cycle of each trial only. No toppling was observed in either trial. Yield (kg/ha/year) was calculated as: average bunch weight/treatment × percentage of plant harvested/treatment × plants/ha (1,111 plants/ha) × (365/number of days from planting to harvest).

2.8. Nematode densities and damage assessment

Root samples were collected at 3, 6, 9 and 12 months after transplanting (MAT) to assess for nematode damage and population. A hole measuring 5 × 5 × 5 cm was excavated at 10 cm distance from the base of the mother plant. All banana roots within the hole were collected, and five roots were randomly selected and used to assess for nematode damage according to Speijer and De Waele (1997). Nematode damage was expressed as percentage necrotic root tissue. The

five roots were cut to a length of 10 cm, sliced length-wise and the percentage root cortex showing necrosis of each root estimated to a maximum of 20% each. The percentage root necrosis comprised the sum of each of the five pieces. These roots were further cut to ~1 cm in length, thoroughly mixed, and nematodes extracted from a 25 g subsample using the modified Baermann tray method (Hooper et al., 2005) over 24 h. The suspensions were reduced to 5 ml and nematode densities estimated from a 1 ml subsample. *Pratylenchus goodeyi* and *H. multicintus* were counted per life stage (female, male and juvenile).

2.9. Data analysis

Nematode counts were $\log_{10}(x+1)$-transformed, while percentage nematode damage data were arcsine-square root-transformed before analysis of variance (ANOVA). Plant growth and yield data were left untransformed prior to ANOVA. A generalized linear model was used to test for factor effects and their interactions. When factor interactions were significant, effects of one factor were analyzed at each level of the other factor. Means were separated using least significant difference tests (LSD) (SAS Institute, 2001).

3. Results

3.1. Plant growth and yield

Plant growth parameters (height, girth and number of functional leaves) were not significantly different ($P>0.05$) between the two banana cultivars and neither was there a significant interaction ($P>0.05$) between cultivar and endophyte treatment for either trial (Table 1).

Endophyte-inoculated plants exhibited~~perienced~~ enhanced growth compared to non-inoculated plants, although this was not statistically significant ($P>0.05$).

For both trials, days to flowering and days to harvest were not significantly different ($P>0.05$) between the two banana cultivars nor endophyte treatments (Table 2). There was no significant interaction ($P>0.05$) between cultivar and endophyte treatment for either trial.

Bunch weight and yield were not significantly different between banana cultivars ($P>0.05$), nor was there a significant interaction between cultivar and endophyte treatment ($P>0.05$) for either trial. Therefore bunch weight and yield data were pooled across cultivars. In both trials, bunch weight and yield of plants inoculated with endophytic *F. oxysporum* strains were significantly higher ($P<0.05$) than those of control plants (Table 3). In the on-station trial, plants treated with *V5W2* produced significantly heavier bunches (22%) than untreated plants, and plants treated with *V5W2* and *Emb 2.4o* had significantly higher yields (36-37%) compared to control plants. In the on-farm trial, *Eny 7.11o*-treated plants produced significantly heavier bunches (20%) and higher yields (20%) compared to untreated plants.

3.2. Nematode damage

For either trial, nematode damage was not statistically significant between banana cultivars ($P>0.05$), nor was there a significant interaction between cultivar and endophyte treatment ($P>0.05$). Therefore, root necrosis data was pooled across cultivars for both trials. In the on-station trial, endophyte inoculation significantly reduced nematode damage in inoculated plants at 6 ($P<0.05$), 9 ($P<0.01$) and 12 ($P<0.005$) MAT compared with non-inoculated plants (Table 4). In the on-farm trial, nematode damage remained low in all treatments up to 6 MAT, but

became more evident over time, with significantly lower necrosis observed in endophyte-inoculated plants compared with control plants at 9 MAT (P <0.05) and 12 (P<0.005).

3.3. Nematode population densities

Nematode density data were pooled across cultivars for both trials, because nematode densities were not significantly different~~lower~~ (P<0.05) between cultivars, nor was there an interaction between cultivar and endophyte treatment (P>0.05) for either trail.

In the on-station trial, although endophyte non-inoculated plants contained numerically higher nematode densities (*P. goodeyi* and *H. multicinctus*) at 3 and 6 MAT, the differences were not significant (P>0.05) among treatments (Table 5). At 9 and 12 MAT, endophyte-inoculated plants had significantly fewer (P<0.001) nematode densities in roots compared with non-inoculated plants. No *R. similis* nematodes were recovered from the roots.

In the on-farm trial, few nematodes were recovered from the roots by 3 and 6 MAT, with no significant differences observed in densities among treatments. At 9 and 12 MAT, endophyte-inoculated plants had significantly lower (P<0.05) densities of *P. goodeyi* compared with non-inoculated plants. At 9 MAT, *Helicotylenchus multicinctus* densities were relatively, but not ~~-~~ significantly, lower in endophyte-inoculated plants. After 12 MAT, significant differences (P=0.028) were observed in *H. multicinctus* densities between endophyte-inoculated and non-inoculated plants.

In the on-station trial, *Meloidogyne* sp. was not observed. In the on-farm trial, the population of *Meloidogyne* sp. was not significantly (P>0.05) different between cultivars or endophyte treatments, and ranged from 325-477 at 3 MAT to 109-230 at 12 MAT.

4. Discussion

The current study provides strong evidence of the protective effects of fungal endophytes against plant-parasitic nematodes on banana under field conditions. A significant reduction in nematode densities and associated damage to banana roots was observed on-station and also in a naturally infected farm following inoculation with *F. oxysporum* strains, which translated into improved bunch weights and yields in the first, mother crop cycle. Use of fungal endophytes to protect against plant-parasitic nematodes has previously been demonstrated in the field. Grasses infected by *Neotyphodium* sp. and *Acremonium coenophialium* endophytes, for instance, inhibited some species of migratory and sedentary endoparasites, leading to a higher yield in *Festuca arundinacea* Schreb. plants (West et al., 1988; Bacetty et al., 2009). In other crops, non-pathogenic *Fusarium* spp. strains isolated from roots reduced *Meloidogyne incognita* (Kofoid & White) Chit. populations on tomato *Solanum lycopersicum* ~~*Lycopersicon esculentum*~~ L. (Hallmann and Sikora, 1994), and *Meloidogyne graminicola* Golden & Birchfield populations on rice (*Oryza sativa* L.) (Le et al., 2009).

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A number of studies have demonstrated the beneficial effect of *F. oxysporum* endophytes on banana, primarily against *R. similis* (Niere, 2001; Athman et al., 2006; Dubois et al., 2006; Paparu et al., 2006; Zum Felde, 2008). However, there are few studies that have assessed their effect in the field. Menjivar Barahova (2005) demonstrated reduction in *R. similis* populations of up to >50% in large dessert banana (cv. Valery) plantations seven months after tissue-cultured plantlets had been inoculated with locally adapted *F. oxysporum* strains and planted in the field in Costa Rica. These reductions are higher than what we found for *H. multicinctus* and *P.*

goodeyi 12 MAP in Kenya. Although Menjivar Barahova (2005) did not find large differences in functional root weight between *F. oxysporum*- and non-inoculated banana plants, we found a large reduction in root damage caused by *H. multicinctus* and *P. goodeyi*. Interestingly, Menjivar Barahova (2005) observed large increases in plant height, circumference and number of standing leaves, which we failed to detect statistically. However, both Menjivar Barahova (2005) and the present study found that *F. oxysporum* endophytes significantly increased bunch weights and yields compared to non-inoculated plants.

Inoculation of other hypocrealean ~~similar~~ fungal endophytes such as *Trichoderma viride* Pers. has equally shown to reduce nematode populations in banana fields. Pocasangre et al. (2007) reported that *R. similis* populations in Costa Rica and the Dominican Republic were reduced to levels similar of those obtained by chemical control after tissue-cultured plantlets had been inoculated with *T. viride*, although no effects were found in field trials in Venezuela. Also, high reduction of nematodes has been demonstrated in naturally suppressive fields in Guatemala, where banana plants were left uninoculated but soils were found to be colonized by high levels of *F. oxysporum* and *T. viride* (Sikora et al., 2010).

The current study demonstrates that *F. oxysporum* endophytes, inoculated into tissue-cultured banana plants, are beneficial to~~can have a direct impact on~~ the farmer. Non-pathogenic endophytic *F. oxysporum* strains (*V5W2*, *Eny7.11o* and *Emb 2.40*) reduce populations of *P. goodeyi* and *H. multicinctus*, thereby reducing root necrosis, and increasing bunch weight and ultimately yield.

The results are derived from the first crop cycle only, however, and so as a perennial crop, the benefits may extend further into following crop cycles. Nematode pests manifest over time and build up on perennial crops such as banana, where they tend to be more damaging in following

crop cycles. Referring to *P. goodeyi* on East African highland bananas in Uganda, Speijer et al. (1999) suggested that long-term studies are needed for its damage potential to be realized.

Improved yields following endophyte enhancement could also be attributed to growth enhancement as a response to endophytic colonization. In our study, a slight increase in plant height, girth of the banana pseudostems and number of functional leaves for endophyte inoculated plants was observed, although these were not significant. In our study, plants enhanced with endophytes were harvested sooner than untreated plants, but this was also not statistically significant. In other studies, however, banana growth was stimulated following inoculation with *F. oxysporum* endophytes (Niere, 2001), while Waller et al. (2005) observed similar responses following *Piriformospora indica* Sav. Verma colonization of barley (*Hordeum vulgare* L.). Endopyhtic fungi produce phytohormones (Tan and Zou, 2001; Nassar et al., 2005), which could contribute to improved growth.

Although endophytic fungi have been shown to protect plants from nematode attack and damage, not all nematode species are similarly affected by endophyte infection. *In vitro* assessment of a range of banana nematode species showed marked differences in their reaction to fungal metabolites (Van Dessel et al., 2011). In a greenhouse study, Niere et al. (1999) also reported that *H. multicinctus* densities were reduced by 75% by endophytes, while other species present were unaffected. In the current study, *Meloidogyne* sp. in the on-farm trial was not significantly affected by the endophyte infection, by comparison to other species.

This study provides the first report from the field in Africa on the ~~negative and positive~~ effects of fungal endophytes on reducing nematode populations and damage,~~s~~ and increasing banana production~~, respectively~~. Bio-enhancement of tissue-cultured plantlets with endophytic strains *V5W2*, *Eny 7.11o* and *Emb2.4o* increased their protection against nematode pests, when

treated prior to field release. Such treatment of seedling material provides a targeted application ahead of distribution to farmers and field planting, which helps to optimize use and efficiency of these biological control options. In the first crop cycle, application of the endophytes led to higher bunch weights and yields of banana. Further studies are needed to determine the longer-term effect of endophytes on growth and yields over successive crop cycles, as well as their interaction with other factors such as management practices.

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Table 1

The effect of inoculation of endophytic *F. oxysporum* strains on growth of banana cv. Giant Cavendish and cv. Grand Nain (combined) in an on-station and an on-farm trial in Kenya.

Site	Treatment	Height (cm)	Girth (cm)	NOFL[a]
On-station	*V5W2*	111.2 ± 3.8 a	41.2 ± 1.2 a	15.0 ± 0.3 a
	Eny 7.11o	105.7 ± 3.8 a	39.9 ± 1.2 a	15.0 ± 0.3 a
	Emb 2.4o	108.2 ± 3.9 a	39.7 ± 1.8 a	14.7 ± 0.3 a
	Control	109.5 ± 3.8 a	40.9 ± 1.3 a	15.3 ± 0.3 a
On-farm	*V5W2*	134.0 ± 4.2 a	50.4 ± 1.5 a	14.6 ± 0.3 a
	Eny 7.11o	134.6 ± 4.0 a	51.0 ± 1.4 a	15.0 ± 0.3 a
	Emb 2.4o	129.1 ± 4.0 a	50.2 ± 1.5 a	14.7 ± 0.3 a
	Control	130.8 ± 4.2 a	49.5 ± 1.5 a	14.4 ± 0.3 a

Values represent means ± standard error. For each trial, means with same letters within a column are not significantly different ($P<0.05$, Tukey's Studentised range test). On-station: n=9 per treatment; on-farm: n=12 per treatment.

[a] NOFL=number of functional leaves.

Table 2

The effect of inoculation of endophytic *F. oxysporum* strains on duration to flowering and harvesting of banana cv. Giant Cavendish and cv. Grand Nain (combined) in an on-station and an on-farm trial in Kenya.

Site	Treatment	Days to flowering	Days to harvesting
On-station	*V5W2*	461.2 ± 5.2 a	641.6 ± 5.0 a
	Eny 7.11o	467.2 ± 9.1 a	645.0 ± 11.2 a
	Emb 2.4o	455.2 ± 6.2 a	635.2 ± 8.9 a
	Control	468.3 ± 7.6 a	645.3 ± 4.6 a
On-farm	*V5W2*	274.2 ± 56.6 a	403.3 ± 81.6 a
	Eny 7.11o	315.5 ± 8.0 a	478.8 ± 8.0 a
	Emb 2.4o	318.7 ± 4.3 a	486.3 ± 5.8 a
	Control	329.3 ± 14.1 a	494.2 ± 12.7 a

Values represent means ± standard error. For each trial, means with same letters within a column are not significantly different ($P<0.05$, Tukey's Studentised range test). On-station: n=9 per treatment; on-farm: n=12 per treatment.

Table 3

The effect of inoculation of endophytic *Fusarium oxysporum* strains on bunch weight and yield of banana cv. Giant Cavendish and cv. Grand Nain (combined) in an on-station and an on-farm trial in Kenya.

Site	Treatment	Bunch weight (kg)	Yield (t/ha)
On-station	*V5W2*	31.3 ± 1.2 a	19.6 ± 0.9 a
	Eny 7.11o	29.7 ± 1.5 ba	17.6 ± 1.3 ba
	Emb 2.4o	30.3 ± 1.0 ba	19.4 ± 1.4 a
	Control	25.7 ± 1.6 b	14.3 ± 1.4 b
On-farm	*V5W2*	28.9 ± 0.6 ba	20.1 ± 4.1 ba
	Eny 7.11o	30.3 ± 0.8 a	24.1 ± 1.0 a
	Emb 2.4o	26.9 ± 0.9 ba	21.5 ± 1.2 ba
	Control	25.3 ± 0.8 b	20.1 ± 1.5 b

Values represent means ± standard error. For each trial, means with same letters within a column are not significantly different ($P<0.05$, Tukey's Studentised range test). On-station: n=9 per treatment; on-farm: n=12 per treatment.

Table 4

Nematode root necrosis (%) of banana plants cv. Giant Cavendish and cv, Grand Nain (data combined) inoculated with endophytic *F. oxysporum* at four sample times after transplanting in an on-station and an on-farm trial in Kenya.

Site	Treatment	Months after transplanting			
		3	6	9	12
On-station	*V5W2*	26.4 ± 2.3 a	23.9 ± 2.3 b	39.0 ± 2.1 b	45.3 ± 3.9 b
	Eny 7.11o	24.4 ± 2.3 a	30.9 ± 2.2 b	37.8 ± 2.2 b	47.8 ± 2.2 b
	Emb 2.4o	22.8 ± 2.8 a	30.3 ± 2.5 b	37.3 ± 3.6 b	49.9 ± 2.9 b
	Control	27.5 ± 3.4 a	35.6 ± 2.9 a	49.5 ± 1.8 a	58.9 ± 1.9 a
On-farm	*V5W2*	1.8 ± 1.1 a	6.1 ± 3.3 a	13.7 ± 3.1 b	18.1 ± 4.0 b
	Eny 7.11o	2.4 ± 1.5 a	5.9 ± 2.5 a	11.8 ± 3.5 b	13.5 ± 3.6 b
	Emb 2.4o	4.4 ± 1.9 a	9.8 ± 3.4 a	20.8 ± 3.9 b	28.9 ± 4.0 b
	Control	7.7 ± 2.8 a	13.2 ± 4.3 a	25.4 ± 2.6 a	38.1 ± 4.6 a

Values represent means ± standard error. For each trial, means with same letters within a column are not significantly different ($P<0.05$, Tukey's Studentised range test). On-station: n=9 per treatment; on-farm: n=12 per treatment.

Table 5

Pratylenchus goodeyi and *H. multicinctus* densities (nematodes/100 g) in banana plants cv. Giant Cavendish and cv, Grand Nain (data combined) inoculated with endophytic *F. oxysporum* at four sample times after transplanting in an on-station and an on-farm trial in Kenya.

Site	Treatment	Months after transplanting							
		3		6		9		12	
		Pg	*Hm*	*Pg*	*Hm*	*Pg*	*Hm*	*Pg*	*Hm*
On-station	*V5W2*	223 ± 25 a	99 ± 12 a	280 ± 49 a	80 ± 15 a	238 ± 19 b	135 ± 14 b	1,300 ± 101 b	641 ± 49 b
	Eny 7.11o	234 ± 30 a	103 ± 12 a	299 ± 43 a	93 ± 17 a	235 ± 25 b	117 ± 17 b	1,322 ± 96 b	653 ± 51 b
	Emb 2.4o	293 ± 51 a	136 ± 27 a	318 ± 72 a	80 ± 18 a	303 ± 37 b	169 ± 35 b	1,394 ± 230 b	651 ± 91 b
	Control	346 ± 58 a	162 ± 30 a	408 ± 65 a	103 ± 16 a	625 ± 96 a	361 ± 63 a	2,752 ± 237 a	1,500 ± 157 a
On-farm	*V5W2*	2 ± 1 a	6 ± 4 a	75 ± 53 a	28 ± 24 a	43 ± 13 c	70 ± 23 a	277 ± 177 b	105 ± 35 b
	Eny 7.11o	23 ± 22 a	8 ± 7 a	36 ± 34 a	39 ± 32 a	65 ± 26 bc	47 ± 19 a	134 ± 70 b	82 ± 34 b
	Emb 2.4o	13 ± 12 a	16 ± 10 a	87 ± 62 a	89 ± 53 a	304 ± 157 ba	106 ± 49 a	507 ± 179 ba	248 ± 96 ba
	Control	61 ± 28 a	42 ± 19 a	207 ± 93 a	137 ± 63 a	317 ± 77 a	161 ± 94 a	898 ± 268 a	449 ± 173 a

Values represent means ± standard error. For each trial, means with same letters within a column are not significantly different ($P<0.05$, Tukey's Studentised range test). On-station: n=9 per treatment; on-farm: n=12 per treatment. *Pg=P. goodeyi*. *Hm=H. multicinctus*.