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Endophytic Non-Pathogenic *Fusarium oxysporum*-Derived Dual Benefit for Nematode Management and Improved Banana (*Musa* spp.) Productivity

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Abstract: The banana weevil (*Cosmopolites sordidus*) and the burrowing nematode *Radopholus similis* represent two of the most important pests of bananas. Previously, colonization of banana plants by the non-pathogenic *Fusarium oxysporum* (isolate V5w2) and the entomopathogenic *Beauveria bassiana* (isolate WA) have been shown to increase host resistance to various banana pests and diseases. However, there is limited data on how the combined inoculation of these isolates would affect field performance of bananas. In this study, the fungal endophytes were inoculated separately and in combination. Tissue cultured plantlets of cooking banana cultivar Mbwazirume and dessert banana cultivar Grande Naine were inoculated by root drenching with a suspension of 1.0×10^7 spores mL^{-1} of the endophytes on three occasions, separated 4 weeks apart, before transplanting into the field. Each plantlet was further inoculated with 1800 nematodes, composed primarily of *R. similis*. Inoculation of banana plants with the fungal endophytes significantly reduced nematode densities by >34%. Similarly, plant toppling was lower in the endophyte-enhanced plants (<16.5%) compared with the control (23.3%). We also observed improved yield of the first crop cycle in the endophyte-enhanced plants, which yielded >11 t ha^{-1} $year^{-1}$ versus 9 t ha^{-1} $year^{-1}$ achieved in the non-inoculated plants. These findings demonstrate the benefits of fungal endophytes in improving the yield of both cooking and dessert bananas via suppression of nematode densities and nematode-related damage.

Keywords: banana nematodes; biological control; endophytes; microbial antagonists; bio-enhanced plants; *Cosmopolites sordidus*

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1. Introduction

Banana (*Musa* spp.) is a major food and cash crop in more than 135 countries and territories across the tropics and subtropics. The crop ranks second in fruit production with a global production estimated at approximately 167 million metric tons (t) [1] feeding more than 400 million people. Banana is mainly cultivated by smallholder farmers. The cooking bananas are produced primarily for home consumption, local and regional markets, with over 85% consumed in-country. On the other hand, dessert bananas are majorly cultivated for commercial purposes, and sold both on the local and international markets. In 2016, the estimated trade value for bananas was US$11.5 billion [2], with Africa responsible for approximately 33% of the global banana production. In 2018, approximately 10% of the

global banana production was produced in the East African region [1]. In some East African countries, banana provides up to 60% of the daily calorie intake. Uganda has the highest consumption at >0.5 kg person^{-1} day^{-1}.

There has been a global decline in banana production for the past 40 years [1] with the current yield being a fraction of its potential [3,4]. The banana weevil (*Cosmopolites sordidus*) and a multifaceted array of plant-parasitic nematodes (PPNs) are among the critical biotic constraints to banana production [5]. *Radopholus similis* is generally viewed as the most damaging nematode pest of bananas [6–8], but in East Africa a complex of species, comprising *R. similis*, *Helicotylenchus multicinctus*, *Pratylenchus* spp. and *Meloidogyne* spp. create a combined community that affects bananas [9–11]. The community composition varies by locality, climate and banana genotype [10]. Most of the developmental stages of the weevil (eggs, larvae and pupa) are found in the corms of infested plants, while the adults are found in crop residues, sheltering in or around the corms, and between the leaf sheaths in close association with the banana corms. In contrast, all development stages of the nematodes reside in the corm and roots of infested plants [12]. Despite the free movement of adult weevils and their ability to infest new fields, the dissemination of both weevils and nematodes is primarily through contaminated planting materials. Most banana farms in the region are plagued with both weevils and PPNs, usually in combination. Heavy infestation of banana fields by both pests can result in up to 100% yield losses [13].

The physical damage caused by weevils and nematodes is also a crucial entry point for banana pathogens, while weevils are also known to act as a vector for some banana diseases [14,15]. A key strategy to improve banana production therefore relies on developing appropriate and effective management options against both the weevil and the PPNs.

The use of fungal endophytes in the management of both the weevil and nematodes has previously been shown to be effective. The non-pathogenic *Fusarium oxysporum* isolate V5w2 effectively colonized banana plants without penalty to the host, and even improved plant growth [16–18]. Similarly, the entomopathogen *Beauveria bassiana* isolate WA effectively colonized banana plants without hampering plant vigor or development [19]. Endophytic colonization of *F. oxysporum* also led to reduced nematode damage and infection levels [17,20–22], while colonization by *B. bassiana* reduced weevil density and damage [23,24]. The majority of studies, however, have been undertaken in pots in the screenhouse, although some limited field work has been conducted [25]. Field studies in Uganda demonstrated that following inoculation with *F. oxysporum*, colonization persisted for up to 6 months [26], and that bunch weight and yield during the first crop cycle was higher when enhanced with *F. oxysporum* compared with uninoculated controls [25]. There is limited information about the dual inoculation of *F. oxysporum* and *B. bassiana* in banana and the potential effect on plant pest and disease resistance and effect on yield. The current study was consequently designed to assess the performance of single and combined inoculation of *F. oxysporum* and *B. bassiana* into tissue-cultured banana plants to determine endophyte colonization persistence, effect on weevil damage and PPN infection levels, plant growth and yield under field conditions, simulating smallholder farming conditions in Uganda.

2. Materials and Methods

2.1. Fungal Cultures

Fusarium oxysporum isolate V5w2, previously isolated from roots of healthy banana plants in central Uganda [27], and the *B. bassiana* isolate WA, previously isolated from a mycosed banana weevil from Mbarara, Western Uganda [28,29] were sourced from the Stellenbosch University, South Africa and the microbial culture collection unit at the Centre for Agriculture and Bioscience International (CABI), United Kingdom, respectively. The two isolates were sub-cultured from cultures on synthetic nutrient agar (SNA) media by placing SNA media plugs containing the actively growing mycelia onto freshly prepared half-strength potato dextrose agar (PDA) media in 90 mm glass Petri dishes. Cultures

were maintained in the laboratory at ambient temperature (approximately 25 °C) and a natural photo period (12:12 h, light: dark) for 3 weeks until sporulation. After sporulation, 3–4 media blocks (approximately 0.5 cm^3) from each isolate were placed into 300 mL Erlenmeyer flasks containing 150 mL of sterile potato dextrose broth (PDB) media, and was incubated for 3 days on a rotary shaker (300 rpm) [30,31].

2.2. Mass Production of Fungal Spores and Inoculum Preparation

Long grain rice (*Oryza sativa*) (2 kg) was soaked in boiling water for 30 min, placed on a 1 mm pore sieve to drain off excess water and then rinsed under running tap water to eliminate all the starch. The washed rice was transferred into self-aerating Milner bags (600 mm × 350 mm) and autoclaved at 121 °C for 1 h, then left overnight to cool down. Each bag was individually inoculated with 25 mL of the 3-day old PDB mycelial culture of the fungal isolates. The bags were aseptically sealed and incubated at ambient temperature (25 ± 2 °C) for 2–3 weeks with daily massaging to enhance uniform fungal growth and spore formation [31].

Following sporulation, the rice substrate was placed on an 850 µm-aperture pore sieve, the spores gently washed off the grains using sterile distilled water and were collected in a 1 L beaker placed below the sieve. The viability of the fungal spores was determined as described by Inglis et al. [32] and the fungal inoculum was prepped by adjusting the spore concentration of each individual isolate to 1.5×10^7 spores mL^{-1} using a haemocytometer under a compound microscope. Additionally, an inoculum containing spores of both fungal isolates was prepared by mixing equal volumes of the *F. oxysporum* isolate V5w2 and *B. bassiana* isolate WA, to result in a spore concentration of 7.5×10^6 spores mL^{-1} per isolates and a combined spore concentration of 1.5×10^7 spores mL^{-1}.

2.3. Tissue Cultured Banana Plants

The East African Highland cooking banana cultivar Mbwazirume (EA-AAA) and the dessert banana cultivar Grande Naine (AAA) were used for the study. The banana cultivars Mbwazirume and Grande Naine are among the nematode susceptible cultivars grown in the Great Lakes region of Africa, primarily for cooking and dessert purposes, respectively. Tissue-cultured (TC) plants at the deflasking stage were sourced from two separate commercial tissue culture laboratories. Plants of cultivar Mbwazirume were sourced from Agro-Genetic Technologies Ltd. (AGT), Buloba, Uganda, while plants of cultivar Grande Naine were sourced from Agromax Uganda Ltd., Kampala, Uganda. The plant roots were washed free of rooting media with tap water and planted in 66 multi-cell plug plastic propagation trays containing a steam sterilized potting mixture of forest soil: composted manure (2:1, *v*/*v*). Plants were maintained in a humidity chamber (relative humidity > 80%, Temp = 25 ± 2 °C) for 5 weeks, before transferring into 2 L plastic potting bags filled with the same steam sterilized potting mixture. The plants were placed in the greenhouse for acclimatisation and watered daily for 10 weeks before transplanting into the field.

2.4. Inoculation of Tissue Cultured Plants

Plants were inoculated with the fungal endophytes on three occasions: (1) at the deflasking stage, (2) at 4 weeks after deflasking, (3) and at 4 weeks after transfer into the 2 L potting bags (8 weeks old plants). For each inoculation, the plant roots were drenched with approximately 10% (*v*/*v*) of the fungal suspension, which was equivalent to~4 mL per plant in the seedling trays and ~200 mL per plant in the potting bags. The volume used for plant drenching was based on the maximum volume of suspension that the potting substrate could absorb without leaking. The control plants were drenched with distilled water drained through rice grains not inoculated with the fungal isolates.

2.5. Field Site and Experimental Design

The field trial was established in November 2018 at the International Institute of Tropical Agriculture (IITA) research station, Namulonge, Uganda (00°31′49.6″ N, 32°36′42.2″ E) at 1128 m above sea level.

The field trial was conceived as a two-factorial experiment, where each banana cultivar (cultivar Mbwazirume and cultivar Grande Naine) was assessed vis-à-vis each endophyte treatment. Treatments were arranged in a randomized complete block design (RCBD) comprising four blocks with eight experimental plots per block, each plot corresponding to a different experimental treatment. Each experimental plot contained 16 plants with a 3 × 3 m inter-plant spacing, and a 5 m inter-plot and inter-block spacing. A border row containing an untreated mixture of banana cultivars was planted around the experimental area, spaced 4 m from the trial plots.

2.6. Nematode Inoculation, Field Establishment and Maintenance

Prior to the transplant of the banana plants in the field, nematode assessment was undertaken from two soil samples per plot. Soil per plot were bulked and nematodes extracted using 100 mL sub-samples to determine nematode presence and density [33]. No PPNs associated with banana were recovered from the trial area and so the plants were each inoculated with 1800 nematodes 8 weeks after transfer into the 2 L potting bags, and 2 weeks before transplanting to the field. The inoculated nematodes included, 1000 pure *R. similis* cultured on carrot discs [34], which was supplemented with 800 individuals (males, females, second-stage juveniles) of a mixed population (*R. similis* (62%), *H. multicinctus* (36%) and *Meloidogyne* spp. (2%)) using 11 g of chopped, infected fresh banana roots collected from a neighbouring field, after assessing density using a modified Baermann method [33,35]. This was undertaken to resemble the multi-species distribution frequently observed in naturally infested fields. To inoculate plantlets, a layer of topsoil was removed from the potted plantlets exposing the topmost banana roots. Four holes were created around each plantlet's roots with a pencil and the *R. similis* pipetted into the holes, and the chopped banana roots placed around the banana roots before replacing the topsoil over the roots.

The banana plants were planted into planting holes measuring approximately 60 × 60 × 60 cm (L × W × H) filled with 20 kg of composted manure mixed with top-loam soil [36,37]. After transplanting, no supplementary irrigation was provided during the first month as it was the wet season. From December onwards, during the dry season, each plant received 10 L of water twice a week until February 2019, and dry grass was additionally applied as mulch around each plant to reduce evaporation. Plants that died due to transplant shock in the first month following transplanting were replaced with plants of the same treatment. The field was weeded regularly, and the trial was maintained through two cropping cycles (~24 months).

2.7. Data Collection

2.7.1. Plant Growth and Yield Data

Plant growth parameters were recorded for plant height (from the soil level to the point of the youngest leaf emergence), pseudostem girth (measured at the soil level and 100 cm above ground) and the number of functional leaves were recorded for each plant at transplanting (0-), 3-, 6- and 9-months post transplanting (MPT), at flowering and at harvest stages. In addition, root samples were collected at the same time points to assess fungal colonization and nematode damage and density estimations.

The trial was monitored 3 times a week and dead, snapped (broken at the pseudostem-corm junction) and toppled (uprooted) plants were recorded. Plant loss was attributed to weevils if plants were dead with characteristic weevil symptoms, i.e., stunted plant growth with a dead central cylinder [38], or snapped, or attributed to nematodes if toppled.

Bunches were harvested upon the ripening of the first finger and the weight recorded. The date was recorded and the number of days from planting to harvest and flowering to maturity calculated.

2.7.2. Banana Weevil Damage

Banana weevil assessment was undertaken for all plants at harvest or when snapped, toppled or recorded as dead according to Viljoen et al. [39]. Cross-sections were cut through the corm at the collar to assess damage to the pseudostem and at 10 cm below the collar to assess damage to the corm. The cortex and the central cylinder were divided into 4 equal-sized portions at each cross-section, each representing 25% of the surface area and the percentage of tissue consumed by the weevil larvae (i.e., galleries) was scored out of 25% for each quarter. The total damage to the pseudostem and corm was calculated as the sum from all 4 quarters.

2.7.3. Nematode Infection and Endophyte Colonization from Root Samples

At three-month intervals (3-, 6- and 9-MPT), 4 plants per treatment per block were randomly selected for assessment of PPN densities and damage, and endophyte colonization. At field establishment, 5 randomly selected plants were sampled from each banana cultivar × endophyte treatment to assess for endophytic root colonization. For each sampling, 10 roots were randomly collected from a hole (5 × 5 × 5 cm) excavated at the base of the mat. Five roots were used for assessment for nematode damage and densities, and five roots for endophyte colonization. Furthermore, 5 roots were sampled from each dead, snapped and toppled plant, and for each plant at flowering and harvest of the mother crop (R0) and ratoon crop (R1) for nematode damage and densities.

To assess nematode damage each root was cut to 10 cm length and sliced lengthwise. One half of the sliced root was scored for necrotic root damage, each root damage was scored out of 20% according to Speijer et al. [40], and the total percentage of root necrotic damage calculated as the sum of the five root scores. These roots were then chopped into ca. 0.5 cm pieces and thoroughly mixed, and a 5 g sample used to extract nematodes over a 48-h period using the modified Baermann technique [26,28]. Nematode suspensions were reduced to 25 mL and nematode densities quantified from 3 × 2 mL aliquots using a compound microscope (×20 magnification) (Leica Microsystems, Wetzlar, Germany).

The roots for endophyte colonization were thoroughly washed under running tap water, then blotted dry with tissue paper. In the laminar flow cabinet, roots were disinfected by dipping in 400 mL of 15% (*v*/*v*) commercial bleach (3.85% *m*/*v* sodium hypochlorite) for 1 min, followed by dipping in 400 mL of 75% ethanol for 1 min before rinsing thrice in 400 mL sterile distilled water and blotting dry with sterile tissue paper. Two pieces approximately 0.5 cm long were cut from the tip, middle and base of each root. The 6 root pieces per root were inserted halfway into half-strength PDA media supplemented with streptomycin sulphate (0.2 g L^{-1}), penicillin G (0.1 g L^{-1}) and chlortetracycline (0.05 g L^{-1}) in 90 mm diameter sterile Petri dishes. These were incubated at ambient temperature (25 ± 2 °C) for 10–14 days. Regrowth of the characteristic fungal isolate from plated root piece(s) signified colonization by the respective fungal isolate(s), the root pieces were monitored and those exhibiting growth of the fungal isolate(s) were quantified and used to calculate percentage fungal colonization (Equation (1)). Identification of the fungal isolates was conducted based on the morphometric characteristics of the fungal colony, whereas a white aerial mycelia tinged with purple to reddish-brown color, short microconidia produced on false heads was assigned to *F. oxysporum* [41], and the white mycelia with a creamy to powdery appearance and rounded to single ovoid conidia was attributed to *B. bassiana* [42].

$$\text{Percentage fungal colonization per plant} = \frac{\text{A}}{\text{B}} \times 100\% \quad (1)$$

where:

A = Total number of root pieces exhibiting characteristic growth of the fungal isolate per plant;

B = Total number of root pieces plated per plant.

2.8. Data Analysis

Due to the binary nature of the endophyte colonization data: colonized vs. non-colonized, nematode damage: necrotized vs. non-necrotized root tissue, weevil damage: damaged vs. non-damaged banana corm tissue, plant survival at harvest: snapped or toppled vs. healthy plants, the data were fitted using generalized linear models (GLM) with binomial distribution [43] to check the effect of endophyte inoculation on each of the variables. The significance of the model was established using an analysis of deviance (Wald chi-square test) followed by computation of least-square means using the 'emmeans' package [44], and group mean separation was performed by Tukey multiple comparisons.

Analysis of variance was performed on nematode, plant growth, bunch weight and plant yield data fitted with linear mixed-effects models using 'lmer' function of the package 'lme4' [45]. Cropping cycle and/or banana cultivar and/or endophyte treatment were specified as fixed variables, while the block was specified as the random variable. Model significance was established using an analysis of deviance (Wald chi-square test). When significant differences were observed between group means of the explanatory variable(s), computation of least-square means was performed using the 'lsmeans' function of the package 'emmeans' and group means were separated using the adjusted Tukey's method executed using the 'cld' function from the 'multicomp' package.

All data analyses were performed using R, Version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria) statistical software [46].

3. Results

3.1. Endophyte Colonization of Plant Roots

No difference in root colonization was observed between banana cultivars for the fungal isolates V5w2 ($\chi^2 = 0.24$, $p = 0.62$) or WA ($\chi^2 = 0.15$, $p \geq 0.59$) and, therefore, data were pooled across banana cultivars for analysis.

Isolation of fungal isolates from banana roots prior to transplanting confirmed the successful colonization of both fungal isolates V5w2 (>30%) and WA (11%). Differences in fungal colonization of roots at the sampling occasions were observed for V5w2 ($\chi^2 = 299.4$, $df = 2$, $p < 0.001$) and WA ($\chi^2 = 199.6$, $df = 2$, $p < 0.001$), with the colonization effect reducing with plant age (Figure 1).

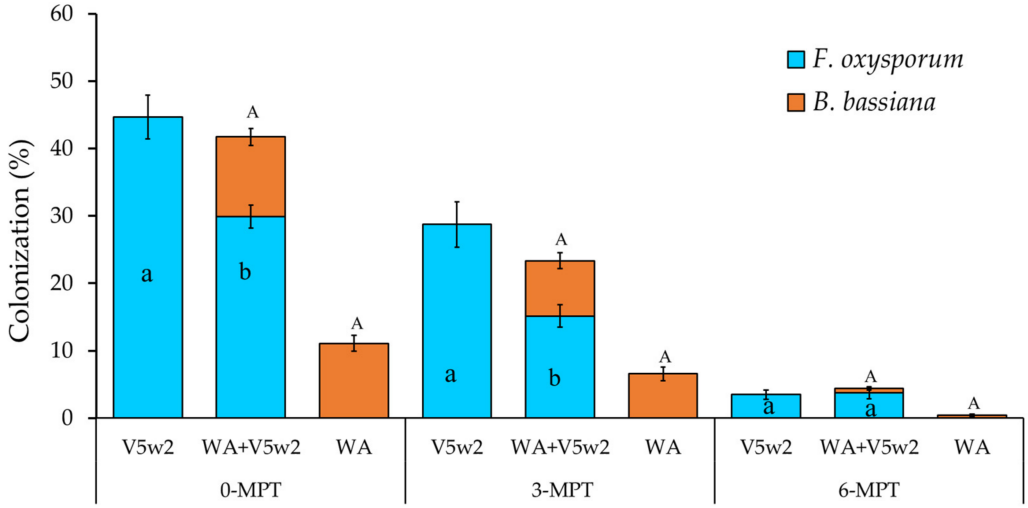

Figure 1. Colonization of banana root tissue of cultivars Mbwazirume and Grande Naine (data pooled) by the fungal endophytes *Fusarium oxysporum* isolate V5w2 and *Beauveria bassiana* isolate WA at 0-, 3-, and 6-months post transplanting. V5w2: plants singly inoculated with *F. oxysporum* isolate V5w2, WA: plants singly inoculated with *B. bassiana* isolate WA, WA + V5w2: plants inoculated with a combination of the two fungal isolates. For each time point, bars followed by different letter(s) indicate significant difference in colonization by *F. oxysporum* (lower case letters) and *B. bassiana* (upper case letter) between treatments. MPT: months post-transplanting.

There was a significant effect of the fungal treatment on the root colonization by the isolate V5w2 at transplanting (χ^2 = 16.94, $p < 0.001$) and 3 months post transplanting (χ^2 = 14.62, $p < 0.001$). For both sampling occasions, the root colonization was significantly higher in plants where the V5w2 isolate was inoculated singly, compared to plants in which the two fungal isolates (WA and V5w2) were simultaneously inoculated. However, there was no effect of fungal treatment on V5w2 colonization of plant roots at 6-months post transplanting in the field (χ^2 = 0.06, p = 0.80). On the contrary, there was no treatment effect on root colonization by isolate WA at 0-, 3- and 6-MPT ($\chi^2 \leq 1.05$, $p \geq 0.30$) (Figure 1). None of the fungal isolates were recovered from the banana roots from either cultivar at 9-MPT.

3.2. Plant Growth, Survival, Toppling and Snapping

Plant height, girth at the base of banana pseudostem, girth at 100 cm above pseudostem base, and the number of functional leaves did not differ between endophyte treatments across all growth stages ($p \geq 0.11$). However, these growth parameters differed between banana cultivars ($p < 0.001$). Except for the number of functional leaves, which was significantly higher in cultivar Grande Naine, plant height, girth at the pseudostem base, and girth at 100 cm above pseudostem base were significantly higher for cultivar Mbwazirume compared to cultivar Grande Naine (Table 1).

Table 1. Effect of single and combined inoculation of *Fusarium oxysporum* isolate V5w2 and *Beauveria bassiana* isolate WA on banana plant development in the field at six growth stages.

Banana Cultivar	Treatment	Banana Growth Stage					
		0-MPT	3-MPT	6-MPT	9-MPT	Mother Crop (Harvest)	Ratoon Crop (Harvest)
		Plant height (cm)					
Grande Naine	Control	13.1 ± 0.3 bB	57.6 ± 1.7 aB	103.3 ± 3.0 aB	162.0 ± 3.5 aB	181.2 ± 3.9 aB	207.9 ± 4.4 aB
	V5W2	18.0 ± 0.4 aB	58.7 ± 2.2 aB	106.3 ± 3.1 aB	161.3 ± 3.2 aB	175.8 ± 3.3 aB	202.9 ± 4.3 aB
	WA	14.7 ± 0.5 bB	58.4 ± 2.1 aB	101.1 ± 3.2 aB	158.4 ± 3.6 aB	177.1 ± 4.2 aB	197.3 ± 3.9 aB
	WA + V5w2	16.6 ± 0.3 aB	59.5 ± 1.9 aB	103.8 ± 3.2 aB	166.2 ± 3.1 aB	183.4 ± 2.8 aB	214.9 ± 4.4 aB
Mbwazirume	Control	24.0 ± 0.6 abA	78.9 ± 1.8 aA	132.9 ± 2.7 aA	217.2 ± 3.7 aA	260.9 ± 3.2 aA	284.7 ± 4.7 aA
	V5W2	24.2 ± 0.6 abA	77.8 ± 2.1 aA	133.9 ± 3.3 aA	212.3 ± 4.3 aA	257.8 ± 3.3 aA	284.3 ± 3.6 aA
	WA	22.6 ± 0.6 bA	79.6 ± 2.2 aA	135.4 ± 3.2 aA	219.9 ± 4.6 aA	263.4 ± 2.0 aA	291.7 ± 3.5 aA
	WA + V5w2	24.8 ± 0.5 aA	79.1 ± 1.6 aA	132.2 ± 2.8 aA	217.7 ± 4.3 aA	256.5 ± 3.4 aA	279.9 ± 5.3 aA
		Girth (cm) at pseudostem base					
Grande Naine	Control	na	24.4 ± 0.8 aB	42.2 ± 1.2 aB	63.8 ± 1.3 aB	63.3 ± 0.9 abB	69.7 ± 1.2 abA
	V5W2	na	25.2 ± 0.8 aB	44.4 ± 1.2 aB	63.5 ± 1.0 aB	60.2 ± 0.6 bB	67.3 ± 1.2 bB
	WA	na	24.8 ± 0.9 aB	41.0 ± 1.2 aB	61.6 ± 1.1 aB	60.8 ± 0.8 abB	65.5 ± 1.0 bB
	WA + V5w2	na	24.8 ± 0.8 aB	42.3 ± 1.3 aB	64.7 ± 1.1 aB	63.5 ± 0.9 aB	73.7 ± 1.0 aA
Mbwazirume	Control	na	27.7 ± 0.7 aA	49.7 ± 1.0 aA	69.4 ± 0.9 aA	67.0 ± 1.1 aA	71.5 ± 1.3 aA
	V5W2	na	27.7 ± 0.9 aA	48.4 ± 1.3 aA	68.8 ± 1.1 aA	66.6 ± 1.0 aA	72.0 ± 1.0 aA
	WA	na	27.8 ± 0.8 aA	48.5 ± 1.1 aA	69.8 ± 0.9 aA	69.0 ± 0.9 aA	75.7 ± 1.2 aA
	WA + V5w2	na	26.8 ± 0.5 aA	47.7 ± 1.0 aA	67.0 ± 1.1 aA	66.7 ± 0.9 aA	72.7 ± 1.2 aA
		Girth (cm) at 100 cm above pseudostem base					
Grande Naine	Control	na	na	36.4 ± 0.6 aA	45.8 ± 0.9 aA	46.2 ± 0.8 aB	56.9 ± 1.1 bA
	V5W2	na	na	35.3 ± 0.6 aA	44.8 ± 0.6 aA	44.8 ± 0.7 aB	54.6 ± 1.0 bA
	WA	Na	na	35.0 ± 0.9 aA	43.7 ± 0.7 aB	45.6 ± 0.7 aB	53.8 ± 0.8 bB
	WA + V5w2	na	na	35.3 ± 0.6 aA	45.1 ± 0.8 aA	46.8 ± 0.8 aB	61.1 ± 1.0 aA
Mbwazirume	Control	na	na	34.2 ± 0.5 aB	47.1 ± 0.8 abA	48.8 ± 0.8 aA	53.5 ± 1.3 bA
	V5W2	na	na	34.1 ± 0.6 aA	46.4 ± 0.9 abA	47.9 ± 0.8 aA	54.5 ± 1.0 abA
	WA	na	na	35.0 ± 0.5 aA	48.7 ± 1.0 aA	50.1 ± 0.8 aA	57.7 ± 1.1 aA
	WA + V5w2	na	na	33.5 ± 0.6 aA	45.1 ± 1.0 bA	47.6 ± 0.7 aA	53.5 ± 1.1 bB
		Number of functional leaves					
Grande Naine	Control	6.4 ± 0.1 aA	8.7 ± 0.2 aA	11.8 ± 0.3 aA	8.4 ± 0.2 abA	0.1 ± 0.1 aB	0.6 ± 0.2 aB
	V5W2	6.5 ± 0.1 aA	8.9 ± 0.3 aA	12.4 ± 0.2 aA	8.0 ± 0.2 bcA	0.4 ± 0.1 aB	0.4 ± 0.2 aB
	WA	6.0 ± 0.1 bA	8.6 ± 0.2 aA	12.0 ± 0.2 aA	7.9 ± 0.2 cA	0.3 ± 0.1 aB	0.6 ± 0.2 aB
	WA + V5w2	5.6 ± 0.1 bA	8.4 ± 0.2 aA	12.4 ± 0.2 aA	8.6 ± 0.1 aA	0.3 ± 0.1 aB	0.6 ± 0.2 aB
Mbwazirume	Control	5.3 ± 0.1 aB	7.2 ± 0.1 aB	10.9 ± 0.1 aB	7.5 ± 0.1 aB	2.9 ± 0.2 aA	1.1 ± 0.3 aA
	V5W2	5.4 ± 0.1 aB	7.0 ± 0.2 aB	10.6 ± 0.2 aB	7.4 ± 0.1 aB	3.2 ± 0.2 aA	0.9 ± 0.2 aA
	WA	5.2 ± 0.1 aB	7.0 ± 0.2 aB	10.3 ± 0.2 aB	7.5 ± 0.1 aA	3.0 ± 0.2 aA	1.7 ± 0.2 aA
	WA + V5w2	4.5 ± 0.1 bB	6.8 ± 0.1 aB	10.6 ± 0.2 aB	7.4 ± 0.1 aB	3.4 ± 0.2 aA	1.0 ± 0.3 aA

Values represent means ± standard error. At each growth stage and per each banana cultivar, means followed by the same lower-case letter(s) indicate no treatment difference (per column). While for each endophyte treatment, means with the same upper-case letter(s) indicate no difference between banana cultivars at p = 0.05. na: data not applicable to the specified stage of plant growth. MPT: months post transplanting (per row).

The proportion of flowered plants did not differ between cropping cycles (χ^2 = 1.85, df = 1, p = 0.17), banana cultivar (χ^2 = 0.12, df = 1, p = 0.72) and endophyte treatment (χ^2 = 7.39, df = 3, p = 0.06). Similarly, there was no interaction effect of cropping cycle, banana cultivar and endophyte treatment on the proportion of flowered plants (χ^2 = 81.28, df = 3, p = 0.73) (Table 2). However, the proportion of harvested plants was significantly different between cropping cycle (χ^2 = 23.60, df = 1, $p < 0.001$) and endophyte treatment (χ^2 = 17.83, df = 3, $p < 001$). Similarly, there was a significant interaction effect of the cropping cycle and endophyte treatment on the proportion of harvested plants (χ^2 = 10.74, df = 3, p = 0.01). In the mother crop, inoculation of the banana plants, with either the single *F. oxysporum* isolate V5w2 or a combination of V5w2 and WA, resulted in more harvested plants (≥89.1%) compared to the non-inoculated plants (73.4%) (Table 2).

Table 2. The effect of single and combined inoculation of *Fusarium oxysporum* isolate V5w2 and *Beauveria bassiana* isolate WA on the proportion of flowered, harvested, toppled and snapped banana cultivars Mbwazirume and Grande Naine (data pooled) along two cropping cycles.

Crop Cycle	Treatment	Flowered Plants (%)	Harvested Plants (%)	Toppled Plants (%)	Snapped Plants (%)
Mother crop (harvest)	Control	90.6 ± 3.1 aA	73.4 ± 3.0 cA	23.3 ± 3.2 aA	3.3 ± 1.2 aB
	V5w2	95.3 ± 2.0 aA	93.6 ± 1.7 aA	2.3 ± 1.6 cB	4.1 ± 1.2 aB
	WA	95.3 ± 2.3 aA	80.4 ± 3.4 bcA	16.5 ± 4.1 abA	3.1 ± 1.7 aB
	WA + V5w2	92.2 ± 1.6 aA	89.1 ± 2.6 abA	7.8 ± 1.6 bcB	3.1 ± 1.7 aB
Ratoon crop (harvest)	Control	88.9 ± 1.7 aA	66.5 ± 5.1 aA	25.7 ± 3.7 aA	7.7 ± 1.9 aA
	V5w2	93.1 ± 2.3 aA	75.5 ± 4.8 aB	16.5 ± 3.3 aA	8.0 ± 2.3 aA
	WA	94.0 ± 2.8 aA	75.8 ± 6.2 aA	18.2 ± 4.5 aA	6.0 ± 3.2 aA
	WA + V5w2	87.9 ± 6.9 aA	67.1 ± 8.2 aB	24.9 ± 4.8 aA	8.0 ± 4.3 aA

Values represent means ± standard error. For each parameter and crop cycle, means followed by the same lower-case letter(s) indicate no treatment difference (per column). While for each treatment, means with the same upper-case letter indicate no difference between cropping cycles (per row) at p = 0.05.

There was a significant effect of the cropping cycle on the proportion of snapped plants (χ^2 = 8.38, df = 1, p = 0.003), where more plants snapped in the ratoon crop (7.4%) compared to the mother crop (3.4%). However, the proportion of snapped plants did not differ between banana cultivar (χ^2 = 3.10, df = 1, p = 0.07) or endophyte treatment (χ^2 = 0.35, df = 3, p = 0.94). Furthermore, no interaction effect between cropping cycle, banana cultivar and endophyte treatment was observed (χ^2 = 1.34, df = 3, p = 0.71) (Table 2).

The length of the cropping cycle (days from planting to harvest) was shorter for banana cultivar Mbwazirume (≤433) compared to cultivar Grande Naine (≥476) in both the mother crop (F = 97.37, df = 1, $p < 0.001$) and the ratoon crop (F = 34.65, df = 1, $p < 0.001$). However, there was no effect of endophyte treatment on the cropping cycle length in mother crop (F = 2.32, df = 3, p = 0.07) and the ratoon crop (F = 0.60, df = 3, p = 0.61). Similarly, no interaction effect of endophyte treatment and banana cultivar was observed in both cropping cycles ($F \leq 2.00$, df = 3, $p \geq 0.11$).

The proportion of toppled banana plants between the two cropping cycles differed (χ^2 = 13.67, df = 1, $p < 0.001$), with more toppled plants observed in the ratoon crop (21.3%) compared to the mother crop (12.5%). However, no difference was recorded between cultivars (χ^2 = 0.39, df = 1, p = 0.53). However, a significant interaction effect between crop cycle and endophyte treatment was observed (χ^2 = 14.13, df = 3, p = 0.002). Consequently, data on the proportion of toppled plants were analysed independently for each cropping cycle but it was pooled across banana cultivars within each cropping cycle. In the mother crop, endophyte inoculation with V5w2 or a combination of V5w2 and WA reduced plant toppling (≤7.8%) compared to non-inoculated plants (23.3%) (χ^2 = 32.17, df = 3, $p < 0.001$). However, plant toppling in the ratoon crop did not differ between endophyte inoculated and non-inoculated plants (χ^2 = 5.35, df = 3, p = 0.14) (Table 2).

3.3. Nematode Densities

At 3-, 6- and 9-MPT, nematode densities were similar between banana cultivars ($F \leq 2.59$, $df = 1$, $p \geq 0.11$), with no interaction observed between banana cultivar and the endophyte treatment ($F \leq 1.26$, $df = 1$, $p \geq 0.28$). Consequently, data on nematode density were pooled across banana cultivars for each sampling occasion. The densities of *R. similis* differed between endophyte treatments at 3-($F = 2.68$, $df = 1$, $p = 0.04$), 6-($F = 5.41$, $df = 1$, $p = 0.001$) and 9-($F = 3.44$, $df = 1$, $p = 0.01$) MPT. Similarly, the density of *H. multicinctus* varied between endophyte treatments at 6-MPT ($F = 2.70$, $df = 1$, $p = 0.04$), but not at 3-($F = 0.19$, $df = 1$, $p = 0.90$) or 9-MPT ($F = 1.37$, $df = 1$, $p = 0.25$) (Table 3). While inoculation of the banana plants with V5w2 significantly suppressed the total nematode densities at all the three sampling occasions ($F \geq 2.95$, $df = 3$, $p \leq 0.03$), the combined inoculation of plants with both V5w2 and WA significantly reduced the total nematode density at 6-MPT ($F = 5.65$, $df = 3$, $p = 0.001$) and 9-MPT ($F = 11.44$, $df = 3$, $p = 0.009$) (Table 3). The proportion of samples from which *Meloidogyne* spp. were recovered were few (<7%), so data on *Meloidogyne* spp. infection were not separately analysed but incorporated in the computation and statistical analysis of the total nematode density.

Table 3. Nematode densities (nematodes/100 g root weight) and percentage root damage (root necrosis) of banana plants cultivar Mbwazirume and cultivar Grande Naine (data pooled) inoculated with endophytes *Fusarium oxysporum* isolate V5w2 and *Beauveria bassiana* isolate WA individually and in combination at five growth stages after establishment of banana plants in the field.

Growth Stage	Treatment	*R. similis*	*H. multicinctus*	Total Nematode *	Root Necrosis (%)
3-MPT	Control	3319 ± 645 a	122 ± 50 a	3528 ± 657 a	24.2 ± 1.1 a
	V5w2	1528 ± 308 b	81 ± 30 a	1622 ± 324 b	12.8 ± 1.0 c
	WA	2800 ± 619 ab	116 ± 38 a	2934 ± 626 ab	17.7 ± 0.9 b
	WA + V5w2	2175 ± 373 ab	109 ± 44 a	2341 ± 395 ab	14.5 ± 0.7 bc
6-MPT	Control	5567 ± 780 a	205 ± 78 a	5780 ± 796 a	33.0 ± 1.4 a
	V5w2	2892 ± 410 b	43 ± 16 b	2954 ± 415 b	26.4 ± 1.9 b
	WA	3350 ± 502 b	76 ± 24 ab	3485 ± 512 ab	29.5 ± 1.9 ab
	WA + V5w2	3129 ± 281 b	112 ± 28 ab	3281 ± 291 b	28.0 ± 1.5 ab
9-MPT	Control	8203 ± 1064 a	275 ± 103 a	8559 ± 1072 a	34.3 ± 1.9 a
	V5w2	5409 ± 610 ab	125 ± 78 a	5550 ± 613 b	22.1 ± 1.4 b
	WA	7238 ± 811 ab	94 ± 34 a	7366 ± 806 ab	34.2 ± 2.9 a
	WA + V5w2	5113 ± 588 b	131 ± 54 a	5269 ± 601 b	24.0 ± 1.7 b
Mother crop (harvest)	Control	8024 ± 509 a	2867 ± 373 a	11069 ± 561 a	52.6 ± 1.6 a
	V5w2	5301 ± 614 b	1761 ± 265 a	7202 ± 666 b	41.8 ± 1.5 b
	WA	6200 ± 646 ab	3187 ± 534 a	9531 ± 867 ab	50.8 ± 1.6 a
	WA + V5w2	4489 ± 368 b	2559 ± 317 a	7153 ± 440 b	42.8 ± 1.3 b
Ratoon crop (harvest)	Control	9158 ± 1063 a	1050 ± 167 a	10375 ± 1194 a	49.6 ± 1.9 a
	V5w2	8685 ± 813 a	940 ± 119 a	9679 ± 892 a	48.3 ± 2.2 a
	WA	9320 ± 1151 a	1072 ± 169 a	10517 ± 1288 a	52.1 ± 1.5 a
	WA + V5w2	8148 ± 700 a	1160 ± 152 a	9450 ± 796 a	49.7 ± 1.9 a

Values represent means ± standard error. At each growth stage, means with the same letter(s) for each variable are not significantly different by Tukey's honestly significant difference (HSD) test at $p < 0.05$. V5w2: plants inoculated with *F. oxysporum* isolate V5w2, WA: plants inoculated with *B. bassiana* isolate WA, WA + V5w2: plants inoculated with a combination of WA and V5w2, Control: Non-endophyte inoculated plants. * Total nematode is the sum of *R. similis*, *H. multicinctus* and *Meloidogyne* spp. MPT: months post transplanting.

For the mother plant, inoculation with V5w2 and a mixture of V5w2 and WA suppressed *R. similis* and the overall total nematode densities at harvest ($F \geq 2.57$, $df = 3$, $p \leq 0.05$). Nevertheless, neither the individual nematode species nor the total nematode densities differed between the endophyte inoculated and non-inoculated plants at harvest for the ratoon crop ($F \leq 3.94$, $df = 3$, $p \geq 0.26$).

3.4. Nematode Root Necrosis Damage

At 3-, 6- and 9-MPT, nematode root necrosis damage between the two banana cultivars was similar ($\chi^2 \leq 0.77$, $df = 1$, $p \geq 0.37$), with no significant interactions observed between cultivar and endophyte treatment at each of the sampling occasions ($\chi^2 \leq 1.55$, $df = 1$, $p \geq 0.67$) and so the data were pooled across banana cultivars for each sample occasion. The inoculation of endophytes resulted in lower root necrosis damage at 3-($\chi^2 = 81.17$, $df = 3$, $p < 0.001$), 6-($\chi^2 = 8.21$, $df = 3$, $p = 0.04$) and 9-($\chi^2 = 29.45$, $df = 3$, $p < 0.001$) MPT (Table 3).

At plant harvest, necrosis damage of the ratoon crop was higher than for the mother crop ($\chi^2 = 5.99$, $df = 1$, $p = 0.01$). There was also a significant interaction effect between cropping cycle and endophyte treatment ($\chi^2 = 11.65$, $df = 3$, $p = 0.008$), but no effect of banana cultivar ($\chi^2 = 0.92$, $df = 1$, $p = 0.33$). Thus, nematode necrosis damage data at crop harvest was split between the two cropping cycles but pooled across cultivars within each cycle. At harvest, the nematode necrosis damage differed between endophyte treatments for the mother crop ($\chi^2 = 40.68$, $df = 3$, $p < 0.001$), but not for the ratoon crop ($\chi^2 = 1.98$, $df = 3$, $p = 0.57$) (Table 3).

3.5. Banana Weevil Damage

Tunneling damage caused by the banana weevil larvae to the outer corm (OC), inner corm (IC), outer pseudostem (OP) and inner pseudostem (IP) differed between the two banana cultivars ($\chi^2 \geq 4.35$, $df = 1$, $p < 0.03$). Overall, more damage was recorded on banana cultivar Mbwazirume (10.4%) compared to cultivar Grande Naine (6.7%). Similarly, there was a significant effect of crop cycle on OC ($\chi^2 = 303$, $df = 1$, $p < 0.001$), IC ($\chi^2 = 63.55$, $df = 1$, $p < 0.001$), OP ($\chi^2 = 63.1$, $df = 1$, $p < 0.001$) and IP ($\chi^2 = 12.83$, $df = 1$, $p < 0.001$). Weevil larvae tunneling damage was significantly greater in the ratoon crop (10.6%) than in the mother crop (7.4%). However, there was no significant difference in damage between the endophyte inoculated and non-inoculated plants in the mother crop ($\chi^2 \leq 3.28$, $df = 3$, $p \geq 0.34$) or ratoon crop ($\chi^2 \leq 7.02$, $df = 3$, $p \geq 0.07$) (Table 4).

Table 4. Percentage weevil damage to banana plants cultivar Mbwazirume and cultivar Grande Naine (data merged) inoculated with endophytes *Fusarium oxysporum* isolate V5w2 and *Beauveria bassiana* isolate WA individually and in combination at the harvest stage of two cropping cycles.

Growth Stage	Treatment	Outer Corm (OC)	Inner Corm (IC)	Outer Pseudostem (OP)	Inner Pseudostem (IP)
Mother crop (harvest)	Control	5.80 ± 0.28 aB	2.35 ± 0.25 aB	2.98 ± 0.23 aB	1.35 ± 0.19 aB
	V5w2	6.56 ± 0.57 aB	2.27 ± 0.46 aB	3.43 ± 0.47 aB	1.43 ± 0.40 aB
	WA	5.28 ± 0.55 aB	2.14 ± 0.48 aB	2.73 ± 0.39 aB	1.01 ± 0.25 aB
	WA + V5w2	5.68 ± 0.48 aB	1.81 ± 0.28 aB	2.32 ± 0.37 aB	1.04 ± 0.27 aB
Ratoon crop (harvest)	Control	15.27 ± 1.29 aA	5.40 ± 0.89 aA	8.36 ± 1.85 aA	2.96 ± 1.66 aA
	V5w2	12.06 ± 2.26 aA	3.52 ± 1.27 aA	7.02 ± 1.83 aA	3.54 ± 0.93 aA
	WA	16.26 ± 1.77 aA	4.77 ± 1.80 aA	8.35 ± 2.57 aA	2.55 ± 1.39 aA
	WA + V5w2	15.90 ± 2.56 aA	6.57 ± 2.30 aA	9.93 ± 2.57 aA	3.67 ± 1.18 aA

Values represent means ± standard error. For each plant part and within each crop cycle, means followed by the same lower-case letter indicate no significant difference between endophyte treatments (per column). While for each treatment, means with the same upper-case letter indicate no difference between the cropping cycle. Means separated by Tukey HSD test at $p = 0.05$ (per row).

3.6. Plant Yield

Bunch weight and crop yield differed between cropping cycles ($F \geq 16.77$, $df = 1$, $p < 0.001$); in addition, there was a significant interaction between cropping cycle and endophyte treatment for bunch weight ($F = 3.44$, $df = 3$, $p = 0.01$). Consequently, data on bunch weight and crop yield were analysed independently for each cropping cycle. In the mother crop, banana plants inoculated with either V5w2 or a combination of V5w2 and WA produced heavier bunches and a higher yield compared to the non-inoculated plants ($F \geq 5.32$, $df = 3$, $p \leq 0.006$). However, neither bunch weight nor crop yield were significantly different between the two banana cultivars ($F \leq 2.89$, $df = 1$, $p \geq 0.08$). Conversely, neither bunch weight nor yield of the ratoon crop differed between endophyte inoculated

and non-inoculated plants ($F \leq 0.93$, $df = 3$, $p \geq 0.42$) (Figure 2). Bunch weights for the banana cultivar Grande Naine were heavier than cultivar Mbwazirume in the ratoon crop ($F = 7.33$, $df = 1$, $p = 0.007$).

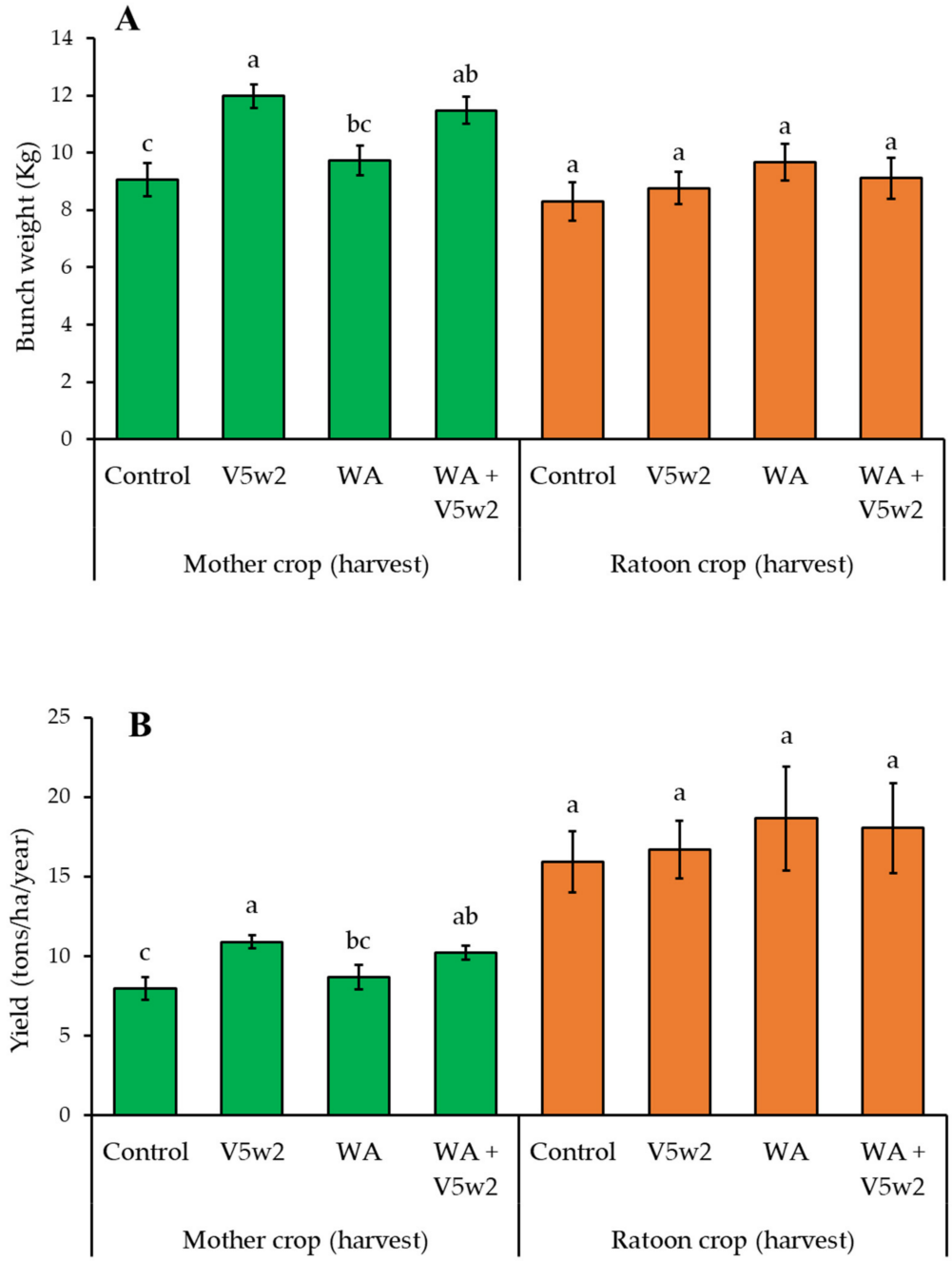

Figure 2. Effect of endophyte inoculation on bunch weight (**A**) and yield (**B**) of banana plants cultivars Mbwazirume and Grande Naine (data pooled) across two growth cycles (mother crop and ratoon crop). Within each crop cycle, means followed by the same letter(s) are not significantly different by Tukey's honestly significant difference (HSD) test at $p < 0.05$.

4. Discussion

The inoculation of banana tissue cultured plants with *F. oxysporum* isolate V5w2 individually or in combination with the *B. bassiana* isolate WA protected plants against nematode infection and damage, and increased the bunch weight and overall yield of the mother crop, under field conditions in Uganda. However, the dual inoculation of plants with *F. oxysporum* and *B. bassiana* did not significantly reduce nematode infection nor

improve yield beyond the inoculation with *F. oxysporum* alone, an indication that the single inoculation of plants with *F. oxysporum* is optimal. Furthermore, there was no observed carry-over effect of the endophytes on nematode suppression and yield on the ratoon crop. Nematode infection of banana can cause up to 30% reduction in bunch weight, more than 60% plant toppling [47], extended crop cycle duration to harvest and reduced annual yields by over 50% [48]. Furthermore, Ssango et al. [49] showed that root necrosis is negatively correlated with bunch weight and yield. In our study, inoculation of the banana plants with the fungal isolate V5w2 resulted in more than 34% reduction in nematode infection levels and decreased nematode root necrosis by >19%, while the total number of harvested plants increased by up to 27%, demonstrating the protective effect of endophytes against PPN infection and related damage in banana.

Keeping other factors constant, a well-developed and healthy banana root system supports optimum water and nutrient uptake, leading to healthy plant growth and vigor and consequently large bunches. Enhancement of plants with endophytes by inoculating with V5w2 reduced nematode infection levels and root necrosis damage. This protection translated into bigger bunches than in the control plants. Furthermore, the healthier root system meant better plant anchorage, which explains the reduced toppling observed in plants inoculated with endophytes and the higher number of harvested plants compared with the controls. The reduction in nematode densities on V5w2-inoculated plants, however, did not significantly influence the proportion of flowered plants between treatments. In banana, nematode damage is most obvious after flowering, at the fruit filling stage when the banana plants rely on an efficient root system to supply nutrients and water to the developing bunch and support the weight of the filling fruit, hence the plant topples at the weight of its own fruit.

In Kenya, Waweru et al. [25] reported a reduction in *Pratylenchus goodeyi* infection, reduced root necrosis damage and increased bunch weight and yield of Giant Cavendish and Grand Naine dessert banana cultivars following enhancement with endophytic *F. oxysporum* isolate V5w2. In addition to testing the fungal endophyte in a different location from where Waweru et al. [25] conducted their study, our study demonstrates the potential use of endophytes to manage multiple nematode species (*R. similis*, *H. multicinctus* and *Meloidogyne* spp.) in dessert banana cultivars as well as in cooking bananas (EAHB), indicating the broader spectrum of applicability for fungal endophyte(s). Consequently, these studies complement each other and can be used to fast track the registration and incorporation of the *F. oxysporum* isolate V5w2 into IPM management strategies against PPNs in bananas in the East African region.

Yield is a factor of bunch weight, crop cycle length, and plant density (the number of harvested plants per hectare per year) [50,51], where bunch weight and plant density are positively correlated with yield, while crop cycle length is negatively correlated. Our study's highest reported yield value (18.6 t ha^{-1} $year^{-1}$), following endophyte enhancement, is lower than the reported potential yield value of 60 t ha^{-1} $year^{-1}$. This low yield could be attributed to the deliberate and uniform infection of all plantlets with PPNs before their establishment in the field, which generally would not happen under farmer's practice. While our findings revealed no effect of endophyte treatment on the crop cycle length, inoculation of the plants with V5w2 improved bunch weight and increased plant survival (number of harvested plants) in the mother crop. This observation ultimately explains the higher yield of the endophyte treated plants compared to control plants. The lack of yield differences between endophyte-treated and control plants in the ratoon crop indicates that the influence of endophyte inoculation wanes and reduces over time and that repeated applications may be necessary to boost endophyte enhancement. Inoculation of banana plants with *B. bassiana* isolate WA alone, however, had no effect on the number of harvested plants, or bunch weight. Moreover, while we reported no effect of fungal treatment on plant growth parameters, the observed difference in growth parameters between the two banana cultivars could largely be attributed to agronomic and genomic differences between the two banana cultivars.

Previous studies have indicated a high colonization of banana plants by the *B. bassiana* isolate WA with no penalty to plant growth [19], coupled with high virulence against the banana weevil and a high sporulation potential on culture media [28,29]. In our assessment of endophyte colonization at the transplanting stage, we confirmed the establishment of both fungal isolates (*B. bassiana* and *F. oxysporum*) in the banana root system. Furthermore, both isolates persisted in the banana roots for 6-months after transplanting into the field. Other studies have also reported successful colonization of banana roots following inoculation with single [20,21] and multiple [22] isolates of non-pathogenic *F. oxysporum*, and single isolates of *B. bassiana* [19,52] under screenhouse conditions. Studies assessing the colonization potential of non-pathogenic *F. oxysporum* isolates in tomato (*S. lycopersicon*) [53], and maize (*Zea mays*) [54], among other crops, have also been demonstrated. Similarly, studies have demonstrated the endophytic potential of *B. bassiana* isolates in a range of crops, such as pine (*Pinus* spp.) [55], cocoa (*Theobroma cacao*) [56] corn [57], tomato [58]. Such reports indicate the potential application of endophytic *F. oxysporum* and *B. bassiana* across plant species.

Upon inoculation, fungal spores of non-pathogenic *F. oxysporum* endophytic isolates are induced to germinate, and fungal hyphae are triggered to produce branches upon contact with the plant root system. The fungal hyphae invade the root system through cracks and wounds in the epidermis or a direct penetration at the root tip. Hyphae of non-pathogenic *F. oxysporum* colonize and establish in the cortical region of the plant root without extending into the stele [59]. Studies have demonstrated a downregulation of plant defence pathways and mechanisms during the initial colonization of the plant by the mutualistic microbes [60]. However, later induction of the defence signaling pathways prevents the microbe from transcending the 'mutualistic' limits [61], which may lead to a gradual reduction in the level of mutualistic endophytes in the plant system. The observed gradual reduction, with time, in colonization levels of both V5w2 and WA fungal isolate could be explained by a possible induction of signaling pathway(s) with a suppressive action against the mutualistic endophytes. Paparu et al. [62] demonstrated an upregulation of the phenylpropanoid enzymes phenylalanine ammonia lyase (PAL) and peroxidase (POX) in roots of banana plants inoculated with the non-pathogenic *F. oxysporum* isolate V5w2. However, the same enzymes were upregulated in leaves of banana plants challenged with the pathogenic *F. oxysporum* f. sp. *cubense* [63]. While PAL and POX have been shown to play a positive role in the plant defence against the pathogenic *F. oxysporum* f. sp. *cubense* tropical race four [64], it can be assumed that the exact defence mechanism can be applied against the non-pathogenic endophytic fungus, leading to a gradual reduction in colonization level. Furthermore, with the increased growth and development of the plant root system, it is possible that the fungal hyphae do not easily establish in the newly formed roots, and thus the chance of recovering the inoculated fungal isolate(s) diminishes with the increased mass of the root system.

While our study confirmed recovery of the fungal endophytes from the banana roots until 6-months post field establishment, several studies have demonstrated the persistence of defence mechanisms and production of metabolites initiated by fungal endophytes even beyond the time when the fungal endophyte can be recovered from the plant tissue [65]. These post-exposure mechanisms could explain the continued suppression of nematode infection levels until the harvest stage of the mother crop cycle in plants singly inoculated with V5w2 or in combination with WA. In addition, the continued suppression of nematode numbers could explain the observed reduction in the number of toppled plants, which consequently increased the proportion of harvested plants in the mother crop.

While, for fear of instant death of the experimental banana plants due to banana weevil infestation, there was no direct infection of the young plantlets with the banana weevils. Assessment through pseudostem trapping [30] at 6-months after transplanting into the field confirmed natural infestation of the experimental plants by the banana weevil. We suspect that this natural infestation came from the mature weevil-infested banana fields that neighboured the trial field.

Previous screenhouse studies have demonstrated the potential use of endophytic *B. bassiana* for the management of the banana weevil [23,24]. Akello et al. reported a reduction in weevil damage to tissue-cultured banana plants inoculated with endophytic *B. bassiana* and challenged with banana weevil larvae [24] and adults [23] in two separate studies. However, in these studies, they also observed a significant reduction in the survivorship of all growth stages of the weevil with respective mycosis, an indication that *B. bassiana* mainly acted against the banana weevil as an endophytic entomopathogen. In fact, Akello et al. [23] concluded that: "Our study therefore demonstrates for the first time that through direct parasitism, endophytic *B. bassiana* kills the eggs, larvae, pupae, and adults of *C. sordidus*". The failure to observe differences in banana weevil damage between the different endophyte treatments in our study could be attributed to the fact that using fungal endophytes against the coleopteran banana weevil would only be effective if the fungus persists in the plant tissue to entomopathogenically infect the weevil. Consequently, a repeated in-field application of the endophytic entomopathogen *B. bassiana* could help boost its inoculum levels, which would help to entomopathogenically infect the weevil, reducing their field population and damage to banana. Thus, the best way to test the efficacy of fungal isolates against the banana weevil might be when used as entomopathogens or endophytic entomopathogens. Studies about the endophytic use of *B. bassiana* for the management other plant pests for example: aphid (*Myzus persicae*) infection in tobacco (*Nicotiana tabacum*) [66], and the leaf miners (*Liriomyza sativae* and *L. trifolii*) in French beans (*Phaseolus vulgaris*) [54,67] resulted in improved plant vigour and development. Similarly, the use of endophytic *B. bassiana* resulted in the management of the Asian corn borer (*Ostrinia furnacalis*) in maize [68] and diamond back moth (*Plutella xylostella*) infection of cabbage (*Brassica oleracea*) [69]. However, studies on the endophytic potential of *B. bassiana* against coleopteran insects are very limited.

5. Conclusions

This study demonstrated that inoculation of the East African highland banana cultivar Mbwazirume and the dessert banana cultivar Grande Naine with the non-pathogenic endophytic fungus *F. oxysporum* isolate V5w2 suppresses nematode infection and increases banana productivity. We found that application of V5w2 alone was sufficient in nematode suppression and boosting banana yield, indicating high potential for its development into an environmentally friendly biopesticide. However, future research will be necessary to elucidate the mechanisms underpinning nematode suppression and increased banana yield observed after treatment with V5w2. The influence of single or combined application of the assessed endophytes on soil microbiome and soil health should also be assessed to better understand other benefits that could be derived from this potential biopesticide. Furthermore, repeat in-field application of the fungal isolates should be assessed for boosting the fungal inoculum following field establishment over successive crop cycles. Lastly, due the low yield value reported in our study in comparison to the reported potential yield value, it would be appropriate that future similar trials include a control treatment with no pest challenge; this will help depict farmer management practice.

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