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Fusarium oxysporum and soil nutrient amendments provide short-term inhibition of *Cosmopolites sordidus* raising questions on biopesticide and plant nutrition potentials in tissue culture banana

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ABSTRACT

Endophytic *Fusarium oxysporum* strain V5w2 has been suggested to offer the ecosystem service of suppressing *Cosmopolites sordidus* and other pests that attack tissue culture banana plants in agroecosystems. The effects of endophytic *F. oxysporum* V5w2 and nutrient supply on *C. sordidus* in potted tissue culture banana plants were investigated. In the screenhouse, rhizome damage by *C. sordidus* larvae was lower in *F. oxysporum* V5w2-inoculated plants than in non-inoculated ones. Banana plants inoculated with *F. oxysporum* V5w2 were larger and suffered less rhizome damage but with low chlorophyll content. Weights of *C. sordidus* larvae were not different between those reared on *F. oxysporum* V5w2-inoculated banana plants and the non-inoculated ones. Larval *C. sordidus* from nutrient-treated plants had lower weight than those that fed on plants that did not receive nutrients. In the field, fewer adult *C. sordidus* were found on *F. oxysporum* V5w2-inoculated banana plants than on non-inoculated plants 12 h after insect release. The number of adult *C. sordidus* and their eggs did not vary between *F. oxysporum* V5w2-inoculated banana plants and controls at the end of the experiment. Adult *C. sordidus* did not discriminate between nutrient-treated banana plants and those without nutrient treatment. However, non-beneficial interactions between *F. oxysporum* V5w2 and plant-parasitic nematodes negate the chances of its application as an endophytic biological control agent. In conclusion, while *F. oxysporum* V5w2 is not quite viable for application as an endophytic biological control agent for *C. sordidus* and other banana pests, this fungus may still have some potential to offer alternative ecosystem services through the provisioning of pest-inhibitive organic compounds.

Introduction

The banana weevil *Cosmopolites sordidus* (Coleoptera: Curculionidae) inflicts heavy damage on banana plants [36] (Fig. 1).

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Rhizome feeding by *C. sordidus* results in the snapping of banana plants [2,12,13,21], with yield losses ranging from 44 % to 100 % being reported [41,59]. Banana weevil *C. sordidus* has been reported to have the potential of dispersing infective structures of the highly pathogenic *Fusarium oxysporum* f. sp. cubense tropical race 4 attached to their exoskeletons and those released through faeces [25,40,61]. However, managing *C. sordidus* is difficult due to its cryptic feeding behaviour within the banana rhizome and pseudostem tissues [44,68]. Tissue culture is a technique that is currently employed for the rapid production of pest-free banana planting material [28,70]. However, tissue culture banana plants are vulnerable to re-infestation by field pests, since protective endophytic microbes are eliminated during the stringent aseptic production [35]. Tissue culture banana plants are also grown in sterilized soil [46,47,50], which lacks protective rhizosphere microbes and those that help in nutrient uptake [24,29,69]. Therefore, tissue culture banana plants are produced through aseptic processes that render them devoid of endosphere and rhizosphere microbes that offer pest suppression and nutrient cycling ecosystem services [51].

Tissue culture banana plants are being inoculated with endophytic and rhizosphere microbes to restore protection against invertebrate pests and microbial pathogens while enhancing nutrient uptake [7,11,69]. Various strains of *F. oxysporum* are among microbes that have been recommended for such beneficial purposes in tissue culture banana plants [14,16,65,74]. For instance, *F. oxysporum* strain V5w2 that was obtained from banana plants in Uganda by Schuster et al. [63], has been associated with protection of tissue culture banana plants against the banana weevil *C. sordidus* and several species of plant-parasitic nematodes [23,30,33,53,54,71,72]. The technology of inoculating tissue culture banana plants with *F. oxysporum* V5w2 has already been transferred to farmers in Kenya and Uganda [14,15,71,74]. Conversely in the year 2010, it was concluded that 'data on the effect of nutrients, soil microorganisms and mulching do not support the transfer of *F. oxysporum* V5w2-treated banana plants to farmers, because the plants suffer from reduced performance' [46]. *F. oxysporum* V5w2 was found not to have close genetic relationship with pathogenic races of *F. oxysporum* f. sp. *cubense* [34], while its pathogenicity to banana plants has remained unresolved [34,46]. It was noted that the concept of utilizing *F. oxysporum* V5w2 among other strains as endophytic microbial biological control agents of banana pests is relatively new while information on this subject is still scarce [65]. November 2021, *F. oxysporum* V5w2 was reported to be a non-beneficial endophyte that interacts with *Radopholus similis* in a wilt disease complex of banana [48]. January 2022, it was recommended that future research is still necessary to elucidate the mechanisms underpinning nematode suppression and increased banana yield observed after treatment with *F. oxysporum* V5w2 [33]. Such inconsistent observations have created regulatory challenges in the registration of *F. oxysporum* V5w2 among other microbial agents as biopesticides in line with the International Standards for Phytosanitary Measures [5,17,27]. Therefore, the effects of *F. oxysporum* V5w2 on tissue culture banana plants and *C. sordidus* among other pests still require further studies and evidences [33,47,48].

The present study investigated the effects of *F. oxysporum* V5w2 and soil nutrient amendments on the behaviour and fitness of *C. sordidus* in tissue culture plants. The outcomes would contribute towards understanding the possible ecosystems services of *F. oxysporum* V5w2 in banana-based agroecosystems.

Fig. 1. The banana weevil *Cosmopolites sordidus* and rhizome damage. **(A)** Adult female with eggs. **(B)** Larva and young adult. **(C)** Healthy-looking banana rhizome *(left)* and a banana rhizome damaged by *C. sordidus* tunneling *(right)* (Source: Own work).

Materials and methods

Tissue culture banana plants and F. oxysporum V5w2 inoculation

East African highland cooking banana plants (cv. *Kibuzi*) for the experiments (300 plants) were obtained from the Banana Tissue Culture Laboratory at the Sendusu Field Station of the International Institute of Tropical Agriculture (IITA-Uganda). The plants had been produced by shoot-tip micropropagation [70], and maintained for six weeks on nutrient solution (1 g/L, Polyfeed™ fertilizer, Haifa Chemicals, Israel). *F. oxysporum* V5w2 (*nit*3) was obtained from a sterile soil culture maintained at 4 °C in the Microbiology Laboratory at the Sendusu Field Station (IITA-Uganda). *F. oxysporum* V5w2 inoculum prepared comprised of a spore suspension (1.5×10^6 spores/mL) [46]. The 300 plants were randomly assigned for 2×2 factorial treatments namely; plants inoculated with endophytic *F. oxysporum* V5w2 (E+) or without endophyte inoculation (E-); either supplied with complete nutrient solution (N+), or with only water (N-), hence four treatment combinations (i.e. N-/E-, N-/E+, N+/E-, N+/E+). Plants meant for the endophyte treatment were inoculated by dipping the roots into the spore suspension of *F. oxysporum* V5w2 for 4 h [46].

The banana plants were grown in steam-sterilized loamy soil in 2.5 L plastic pots. All plants were supplied with 100 mL of complete nutrient solution (1650 mg NH_4NO_3, 1900 mg KNO_3, 440 mg $CaCl_2{\cdot}2H_2O$, 370 mg $MgSO_4{\cdot}7H_2O$, 170 mg KH_2PO_4, 37.3 mg $Na_2EDTA{\cdot}2H_2O$, 27.8 mg $FeSO_4{\cdot}7H_2O$, 6.2 mg H_3BO_3, 22.3 mg $MnSO_4{\cdot}4H_2O$, 8.6 mg $ZnSO_4{\cdot}7H_2O$, 0.83 mg KI, 0.25 mg $Na_2MoO_4{\cdot}2H_2O$, 0.025 mg $CuSO_4{\cdot}5H_2O$ and 0.025 mg $CoCl_2{\cdot}6H_2O$ per litre) [43], once weekly until they had attained a suitable girth size ($\geq$ 3 cm diameter) at eight weeks for infestation with *C. sordidus*. Water (100 mL per plant) was supplied daily. Nutrient deficiency was induced by not providing nutrient solution for four weeks. Nutrient deficiency was indirectly assessed using a chlorophyll meter, which indirectly measures leaf nitrogen content in SPAD units (SPAD 502, Spectrum Technologies, Illinois, USA) [6,58].

Adult and larval C. sordidus

Three months before the experiments, adult *C. sordidus* were collected over different times without determining their sexes, from old banana fields at IITA, Namulonge using pseudostem traps [66]. The adult *C. sordidus* from mixed collections were reared on banana rhizomes in buckets to produce larvae.

Experiment I: host plant suitability to larval C. sordidus

This experiment had four treatments (N-/E-, N-/E+, N+/E-, N+/E+), each having 15 potted plants replicated three times resulting in 45 plants per treatment. Plants within each treatment replicate were grouped together to facilitate the application of the complete nutrient solution, and the 12 groups (i.e. 4 treatments $\times$ 3 replicates) were arranged in a randomized block (RBD) design in the screenhouse. In the rearing, *C. sordidus* larvae were removed from rhizome pieces, weighed and placed separately in glass Petri dishes containing wet filter paper to minimize desiccation. Since *C. sordidus* larvae were not produced uniformly, their selection for experiments was only based on visual judgement for smaller ones, and their weights ranged between 0.001 g and 0.09 g. Plants were infested by making a downward notch at the juncture of the pseudostem and the rhizome using a sterile surgical blade, then placing a randomly selected larva into the notch (Fig. 2). *C. sordidus* larvae that did not start burrowing into the plants within 30 s were discarded and replaced with fresh ones. The notches were covered with masking tape to keep the larvae inside the plant and to protect them from adverse conditions. All 180 plants were arranged in a completely randomized design in the same screenhouse. At the time of infestation with *C. sordidus* larvae, mean plant size (girth: height) for the treatments were; N-/E- (8.2 cm: 25.3 cm); N-/E+ (8.9 cm: 29.9 cm); N+/E- (9.5 cm: 32.8 cm); and N+/E+ (9.8 cm: 37.4 cm).

Banana plants infested with *C. sordidus* larvae were maintained for 30 days. Weekly supply of the nutrient solution (100 mL) was provided to plants assigned to the nutrient treatment, and water supplied daily to all plants. The 30-day period had been selected after pre-tests verifying that *C. sordidus* larvae did not pupate; which fell within the larval duration of 12-165 days in *C. sordidus* [21]. Plant height (the distance from the base of the plant to the youngest leaf axil) and girth (circumference at the base of the pseudostem) were recorded after 30 days. At the end of the experiment, the plants were removed from the pots and their roots were cut off. Plant damage assessment was based on the methods described by Ortiz et al. [52] and Gold et al. [22]. Peripheral damage (PD) was estimated by paring the rhizomes then marking them vertically into four approximately equal sections that represented 25 % (quarter) of the surface

Fig. 2. Infestation of a potted tissue culture banana plant with larval *Cosmopolites sordidus* through a notch at the base of the pseudostem to assess host plant suitability (Source: Own work).

area. The amount of rhizome surface tissue consumed by *C. sordidus* larvae in each quarter was estimated and the sum scores for the four sections were expressed as a percentage. The rhizomes were split transversely into two pieces. Internal injury on the lower rhizome was estimated in 25 % sectors as a percentage cross sectional area covered by damaged tissues on rhizome (CD). The number of tunnels on the lower rhizome was counted as an estimate of tunnelling damage (TD). During damage assessment, *C. sordidus* larvae that were inside the rhizomes and in the soil were recovered and weighed, and the number of recovered larvae expressed as a percentage of the initial sample size.

Experiment II: host plant preference and suitability to adult C. sordidus

This study was conducted in the field at IITA Namulonge in August 2008. The experiment consisted of four treatments (N-/E-, N-/E+, N+/E-, N+/E+), each comprising 10 plants replicated three times. Each replicate consisted of a land strip (2 × 20 m), spaced 30 m from each other. In each strip, 20 plants were placed in holes, without removing them from their pots, in two rows, 1 m apart, and at 1 m distance within the row (Fig. 3A). The plants were arranged in a way that a *F. oxysporum* V5w2-inoculated plant and its corresponding control within a nutrient treatment were paired directly across rows (Fig. 3B). This arrangement was repeated ten times while systematically exchanging the positions of paired treatments subsequently within the strip. A thin layer of dry grass was laid at the base of each plant to create a conducive environment for the adult *C. sordidus*. The adult *C. sordidus* in the rearing buckets were sexed based on the punctation patterns on the rostrum and the angle of inclination of the 9th abdominal segment [20,21]. Thirty-two (32) adult *C. sordidus* (a mixture of 12 males and 20 females) were released in the centre of each group of four plants at 18:00 hrs (Fig. 3C). This imbalanced sex ratio was adopted since there were fewer *C. sordidus* males than females in the rearing. The next morning (12 h after release), the number of adult *C. sordidus* at the base of each plant (within ~15 cm) was counted. The adult *C. sordidus* were allowed to lay eggs for five days. Plants were removed from the holes and the number of adult *C. sordidus* attached to the pseudostems and in the soil counted. Plant damage in the form of wounds caused by the adult *C. sordidus* was categorized as high or low (i.e. high >x; low < x; x = a quarter of pseudostem basal circumference damaged). Eggs of *C. sordidus* were counted by scraping rhizome and pseudostem tissues using a surgical blade.

Plant tissue nutrient analyses

To obtain information on the effect of *F. oxysporum* V5w2 on plant nutrient content, chemical analyses were done using 100 days old harvested plants obtained from a concurrent experiment with similar treatments (n=15), but excluding *C. sordidus* [46].

Colorimetric assessment of root extracts was conducted to indirectly determine the effect of *F. oxysporum* V5w2 on the biochemical composition of plant tissues. Five roots from three plants per treatment were chopped together into a composite sample. This was conducted in triplicate. Ten grams of each composite sample were mixed with 100 mL distilled water, and macerated using a Waring Laboratory Blender (Christison Scientific, Gateshead, UK). The root extracts were diluted (10 ×) and their optical density (absorbance)

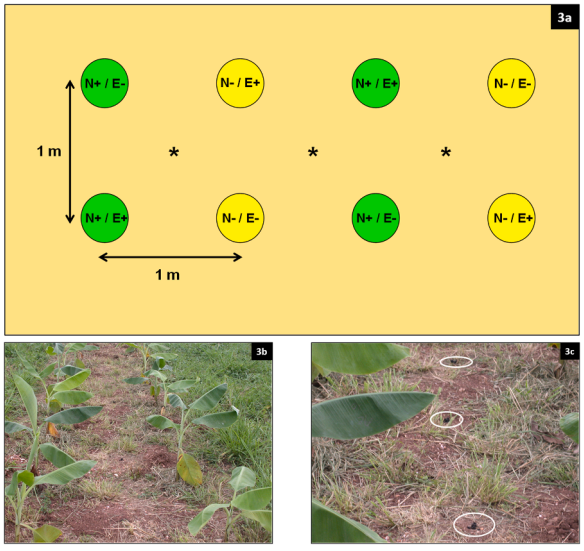

Fig. 3. Arrangement of a potted tissue culture banana plants in the field for assessment of host plant preference and suitability by adult *Cosmopolites sordidus*. **(A)** Green circles and yellow circles represent potted tissue culture banana plants that received nutrient solution (N+) or those that did not receive nutrient solution (N-), either inoculated with the endophyte *Fusarium oxysporum* V5w2 (E+) or left without endophyte inoculation (E-), respectively. The asterisks marks between quartets of banana plants spaced at 1 × 1 m represent the point of release of adult *C. sordidus*. **(B)** Field arrangement of tissue culture banana plants expressing variations in leaf coloration as per their nutritional and *F. oxysporum* inoculation status, just after release of adult *C. sordidus* **(C)** (Source: Own work).

was measured at 320 nm using a spectrophotometer (Genesys 10 UV, Thermo Fisher Scientific, USA).

Direct nutrient analysis was done to confirm the chemical composition of banana leaves and roots, but only for plants that received nutrient solution. Two youngest open leaves and 10 g of roots from seven randomly selected banana plants per treatment were oven-dried at 70°C for 14 days. Samples for each banana plant were mixed and analyzed separately for nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and Zinc (Zn) in the Soil and Plant Analytical Laboratories at Kawanda Agricultural Research Institute, Kampala, Uganda.

Determination of endophytic and rhizosphere colonization by F. oxysporum V5w2

Endophytic growth of *F. oxysporum* V5w2 was determined by the percentage root piece colonization method [55], using 100 days old harvested plants obtained from a concurrent experiment with similar treatments, but excluding the insect pest [46]. Three roots were selected per plant and surface-sterilized by dipping it into 96 % ethanol followed by flaming. This ensured that only microbes within the tissues remained alive. Each root was then cut into six pieces. The root pieces were plated on 60 mm glass Petri dishes containing PDA enriched with $KClO_3$ for 7 days. Growth of *Fusarium* strains including V5w2 on PDA was characterized by whitish mycelia with pink pigmentation [46]. The number of root pieces with growth of *Fusarium* species among six assessed pieces was recorded as fungal endophyte colonization. Root piece colonization was the percentage of all root pieces yielding *Fusarium* among all assessed pieces within a treatment.

For determination of rhizosphere colonization by *F. oxysporum* V5w2, 10 tissue culture banana plants were inoculated with a chlorate-resistant strain of *F. oxysporum* V5w2 by the root-dip method [55]. Another 10 plants were left without inoculation to serve as controls. The banana plants were grown in 750 mL transparent plastic cups containing sterilized loam soil. Thirty (30) sterile wooden toothpicks soaked in 1g/L NH_4NO_3 solution to enhance their nutritional content were randomly inserted through the surface of each cup as rhizosphere probes (Fig. 4). Seven days later, the tips of the toothpicks in contact with the rhizosphere were plated in Petri dishes containing $KClO_3$-enriched Potato Dextrose Agar (PDA) for detection of the chlorate-resistant *F. oxysporum* V5w2.

Data analysis

All statistical analyses were performed using SAS 9.1 software (SAS Institute Inc., 2004). Two-way analyses of variance for ranks and LS-means in Proc GLM were used in comparisons for chlorophyll content, plant damage by *C. sordidus* larvae (peripheral rhizome damage, cross-sectional rhizome damage, number of tunnels) and for number of *C. sordidus* eggs. Two-way ANOVA with LSD was used for comparison of log-transformed plant height and girth, and for untransformed *C. sordidus* larval weights between treatments. Percentages of recovered *C. sordidus* larvae and adults on plants in Experiment I and Experiment II, respectively, and pseudostem damage in Experiment II were analyzed using Proc Genmod (binomial distribution); Bonferroni-adjusted p-values were obtained by Proc Multtest from raw p-values in Proc Genmod and used for pairwise comparisons between treatments. Pearson Correlations between parameters in Experiment I were obtained using Proc Corr.

Results

Experiment I

Chlorophyll content was higher in nutrient-treated banana plants than in nutrient-deficient ones (Table 1). In the nutrient-treated banana plants, those that were inoculated with *F. oxysporum* V5w2 had a lower chlorophyll content than the ones without the endophyte. In the nutrient-deficient banana plants, there was no difference in chlorophyll content between *F. oxysporum* V5w2-inoculated and the non-inoculated ones. Banana plants that were inoculated with *F. oxysporum* V5w2 suffered significantly lower rhizome damage than those that were not inoculated (Table 1). *F. oxysporum* V5w2-inoculated banana plants were taller and had wider girths than those that were not inoculated; while nutrient-treated plants were significantly taller and thicker than those without nutrients (Table 1). The percentages of recovered larvae between the four treatments were not significantly different and averaged 41 % ($p > 0.05$). *F. oxysporum* V5w2 inoculation status negatively correlated with chlorophyll content, rhizome damage (PD, CD, TD), and percentage of recovered *C. sordidus* larvae ($p < 0.05$). The nutrient treatment was positively correlated with chlorophyll content, plant

Fig. 4. Detection of *Fusarium oxysporum* V5w2 in the rhizosphere of potted tissue culture banana plants using rhizosphere probes made from nutrient-enriched wooden toothpicks (Source: Own work).

Table 1
Chlorophyll content, rhizome damage by *Cosmopolites sordidus* larvae, plant size and larval weight in tissue culture banana plants either inoculated with the endophyte *Fusarium oxysporum* V5w2 or not, and either treated with nutrient solution or without nutrient solution (water only).

Source of variation	Df [x]	Chlorophyll	Rhizome damage by larvae			Plant size		Larval weight	
			PD[4]	CD[5]	TD[6]	height	girth	initial	final
		F values							
Endophyte[1]	1	1.5	16.4***	17.8***	9.8**	109***	57***	0.5	3.3
Nutrient[2]	1	296***	1.8	1.8	0.2	399***	439***	0.4	8.8**
Endophyte × Nutrient	1	9.0*	0.1	0.4	0	7.1**	0.1	0.4	0
Df[y]		176	176	176	176	175	175	176	69
		SPADs [3]	percent	percent	Means count	cm	cm	gram	gram
Overall mean		51.7	27.3	12.9	2.5	34.9	9.1	0.01	0.12
Endophyte		51.4	21.2 b	8.8b	2.2 b	37.9 a	9.3 a	0.01	0.11
No endophyte		52.0	33.4 a	16.9a	2.9 a	32.1 b	8.8 b	0.01	0.12
Nutrients		57.8 a	29.2	10.3	2.5	40.9 a	9.6 a	0.01	0.10 b
No nutrients		45.7 b	25.4	15.5	2.5	20.0 b	8.6 b	0.01	0.13 a
Endophyte + Nutrients		56.5 b	25.3	7.2	2.1	43.6 a	9.8	0.01	0.09
Endophyte only		46.3 c	17.1	10.5	2.2	32.3 c	8.9	0.01	0.12
Nutrients only		59.1 a	33.1	13.3	3.0	38.3 b	9.5	0.01	0.11
Control (no treatment)		45.0 c	33.8	20.4	2.8	25.8 d	8.2	0.01	0.14

[1] *Fusarium oxysporum* strain V5w2;
[2] Nutrient solution used was PolyFeed™ nutrient solution;
[3] SPAD units [58];
[4] peripheral rhizome damage,
[5] internal rhizome damage expressed as cross-sectional area covered by injured tissues,
[6] number of tunnels;
[x] treatment and
[y] error degrees of freedom. Means with the same letter are not significantly different ($p > 0.05$), asterisks indicate the level of significance (***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$)

height and girth ($p < 0.0001$), and negatively correlated with rhizome damage (CD) ($p = 0.01$).

Initial weights *C. sordidus* larvae did not vary between the treatments (Table 1). However, larvae from nutrient-treated banana plants had significantly lower weights than those from nutrient-deficient plants. Weights of *C. sordidus* larvae were not statistically different between *F. oxysporum* V5w2-inoculated plants and the non-inoculated ones (Table 1). Weights of *C. sordidus* larvae were negatively correlated with chlorophyll content ($R^2 = -0.24$, $p = 0.04$). There was no correlation between *C. sordidus* larval weight and other measured parameters (number of inner and outer tunnels, percentage damage of inner and outer rhizome, and percentage peripheral damage) ($p > 0.05$).

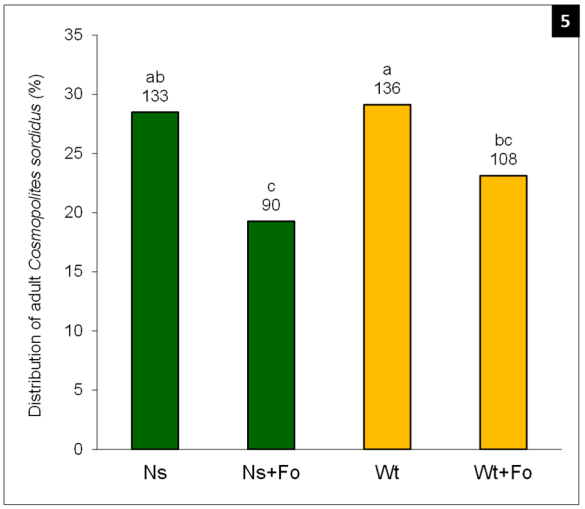

Fig. 5. Distribution of 467 adult *Cosmopolites sordidus* on banana plants inoculated with endophytic *Fusarium oxysporum* V5w2 or not inoculated, each group either having receive nutrient solution or water only, after a period of 12 hours after release of 1824 the weevils under field conditions. Bars represent the overall percentages (and numbers above bars are the actual populations) of adult *C. sordidus* on the four plant treatments; those with the same letter are not significantly different ($\chi^2 = 16.56$, d.f. = 3, P = 0.0009).

Experiment II

Twelve hours after release of adult *C. sordidus* in the field, fewer adult weevils were found on banana plants inoculated with *F. oxysporum* V5w2 than on non-inoculated plants, but their number was not different between nutrient-treated and nutrient-deficient plants ($p < 0.005$) (Fig. 5). The total number of adult *C. sordidus* recovered from plants at the end of the experiment was 270 (recapture rate of 15 %); their distribution did not vary between treatments ($p > 0.05$). There was no difference in the percentage of plants that had pseudostem damage among the four treatments (average: 28 %) ($p > 0.05$). There was no difference in the number of recovered *C. sordidus* eggs among treatments (average = 3 eggs/plant) ($p > 0.05$).

Plant tissue chemical content and F. oxysporum V5w2 colonization

Root extracts from *F. oxysporum* V5w2 inoculated plants had lower optical density than those not inoculated with the fungus (Fig. 6). Plants that received nutrient solution had roots extracts with lower optical density than those that received water only (Fig. 6).

The content of nutrient elements (N, P, K, Ca, Mg and Zn) in leaves and roots did not show clear trends of quantitative differences between plants inoculated with *F. oxysporum* V5w2 and those not inoculated with the fungus (Table 2). However, nutrient contents seemed to vary considerably more prominently between leaves and roots, with Zn being much higher in the roots than leaves, while N, P and Mg being higher in the leaves than in the roots (Table 2).

Banana plants inoculated with *F. oxysporum* V5w2 had a higher percentage root piece colonization with mycelia that resembled those of the inoculated fungus than those not inoculated with the fungus (Fig. 7). Banana plants that received nutrient solution had a higher percentage root piece colonization with mycelia that resembled those of the inoculated fungus than those that only received water only (Fig. 7). Detection of these fungi whose mycelia appeared like the inoculated *F. oxysporum* V5w2 even from the non-inoculated plants was not conclusive, and hence they were generally considered to be *Fusarium* spp. Mycelia that appeared similar to those of the inoculated *F. oxysporum* V5w2 were detected in 70 % of toothpicks from the rhizospheres of plants inoculated with fungus but not in those not inoculated (0 %) (Fig. 8).

Discussion

In the present study, *F. oxysporum* V5w2 instigated short-term effects on the behaviour of adult *C. sordidus*. This was evident in the field, where the number of adult *C. sordidus* was low on *F. oxysporum* V5w2-inoculated plants 12 h after release of the weevils. Yet, at the end of the experiment there was no difference in the number of adult *C. sordidus*, their eggs and pseudostem damage between treatments. In olfactometer studies, adult *C. sordidus* were found not to show clear discrimination between plants inoculated with *F. oxysporum* V5w2 and controls, or between the two groups of plants and clean air [46]. Therefore, *F. oxysporum* V5w2 would not provide consistent volatile-mediated protection of banana plants from infestation by adult *C. sordidus*. *Fusarium* species vary in odour emissions [18,62], with *Fusarium odoratissimum* (*F. oxysporum* f. sp. *cubense* TR4) that infects banana being among such odour emitters [8,38]. Such odours may be linked with the low preference of banana plants inoculated with *F. oxysporum* V5w2 by *C. sordidus*. However, it is not quite clear on whether the short-term low preference of *C. sordidus* towards *F. oxysporum* V5w2-inoculated banana plants results due to *in planta* infection or rhizosphere colonization by the fungus. Despite trends in root piece and rhizosphere

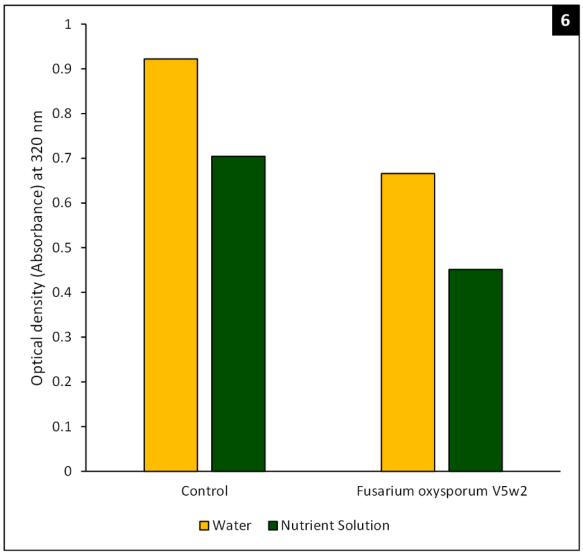

Fig. 6. Optical density of root extracts from tissue culture banana plants inoculated with *Fusarium oxysporum* V5w2 and non-inoculated ones supplied with nutrient solution or water only.

Table 2
Nutrient content in tissue culture banana leaves and roots from plants either inoculated with *Fusarium oxysporum* V5w2 or left without inoculation with the fungus and grown in steam-sterilized soil supplied with complete nutrient solution.

Tissue	Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Zn (ppm)
Leaves	Control	2.97	0.13	3.76	1.13	0.65	5.61
	F. oxysporum V5w2	2.95	0.13	3.71	1.48	0.70	5.66
Roots	Control	1.45	0.08	4.65	1.07	0.24	29.3
	F. oxysporum V5w2	1.46	0.06	4.78	1.10	0.31	23.8

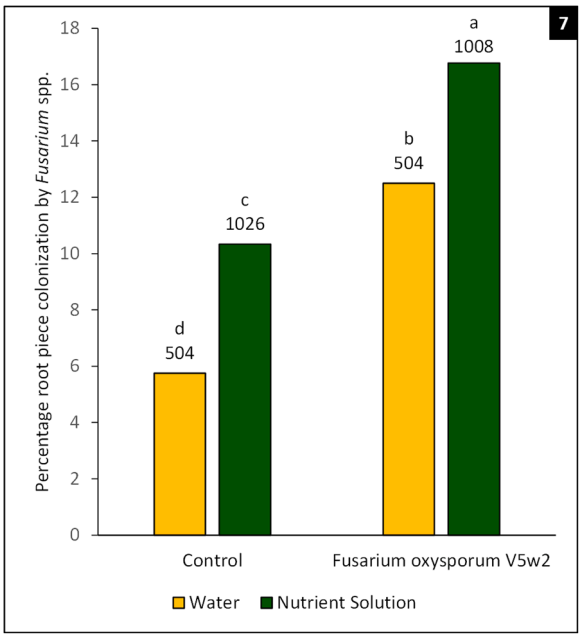

Fig. 7. Percentage root piece colonization by *Fusarium* spp. in tissue culture banana plants inoculated with *Fusarium oxysporum* V5w2 and non-inoculated ones supplied with nutrient solution or water only.

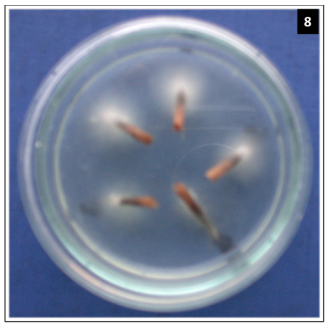

Fig. 8. Growth of *Fusarium* spp. mycelia from tips of toothpicks in potato dextrose agar after being removed from the rhizosphere of potted tissue culture banana plants inoculated with *Fusarium oxysporum* V5w2.

colonization having consistence with *F. oxysporum* V5w2 inoculation, the lack of reliable identification of the *Fusarium* sp. especially in control plants is a draw-back in making meaningful conclusions [30,46,53,54]. Separate assessments of host preference in female and in male *C. sordidus* would have provided a better view of their sex-specific behaviours towards *F. oxysporum* V5w2-inoculated banana plants. This is because female and male weevils having sex-specific physiological differences may exhibit behavioural disparities towards host plants in terms of the need for food, oviposition sites, shelter and response towards pheromones.

F. oxysporum V5w2 expressed some potential to inhibit plant damage by *C. sordidus* larvae. There were some indications that *F. oxysporum* V5w2-inoculated plants exhibited lower rhizome damage by *C. sordidus* larvae than those that were not inoculated, and were larger in terms of height and girth. However, the validity of the plant damage assessment methods remain debatable. This is

because a wide range of *C. sordidus*-related damage assessment methods exist but with no agreed upon assessment protocols [22]. Furthermore, the apparent large size of banana plants inoculated with *F. oxysporum* V5w2 cannot be fully attributed to plant growth promotion. This is because chlorophyll content was low in leaves of *F. oxysporum* V5w2-inoculated banana plants. However, other studies have found that inoculation of banana plants with *F. oxysporum* V5w2 results in yellowing of banana leaves and stunted growth [46–48,50,51]. *Fusarium* sp. infection has been associated with reduced chlorophyll content and impaired photosynthetic efficiency in host plants [57]. Furthermore, reduction in the optical density of root extracts of banana plants inoculated with *F. oxysporum* V5w2 in a similar manner to root extracts from nutrient-starved plants may imply biochemical changes due to exploitation of host plant nutrient resources. In the present study, it is also possible that saprophytic growth of *F. oxysporum* V5w2 in the rhizosphere led to improved acquisition of phosphorus and other nutrients by banana roots leading to faster growth of the inoculated potted plants [56]. First growth rate of banana plants increases the uptake of rhizospheric nitrogen [67], whose depletion results in reduced chlorophyll synthesis [4]. However, in the present study, data on leaf and root tissue nutrient analysis does not provide clear trends in N, P, K, Ca, Mg and Zn. Effects of *F. oxysporum* V5w2 on banana plant growth would have been clearer if initial plant growth-related parameters had been recorded. Inclusion of banana plants without *C. sordidus* or *F. oxysporum* V5w2, and those inoculated with only *F. oxysporum* V5w2 would have provided more information to address this knowledge gap on possible plant growth promotion. Direct quantification of chlorophyll alongside nutrient content analysis (e.g. N, P, K, Ca, Mg, Fe) in soil and plant tissues would have added more evidence to speculations in the present study. Consequently, this would have helped address concerns over the negative effects of *F. oxysporum* V5w2 in banana plants as observed in similar studies on *Radopholus similis* [47].

Organic nitrogen content in plant tissues is important for insect growth [60]. In the present study, nitrogen content was low in banana plants that received reduced levels of nutrient solution (nutrient-deficient), as also indirectly indicated by the low chlorophyll concentration and reduced optical density of root extracts. However, *C. sordidus* larvae from the nutrient-deficient banana plants had greater weights than those from nutrient-treated plants. This contradicted the expectation that nutrient-deficient plants are less suitable food for insect growth. However, there was no difference in *C. sordidus* larval feeding damage between nutrient-treated and nutrient-deficient plants, which may indicate similar levels of plant tissue hardness to the larvae. Ortiz et al. [52] suggested that investigations of resistance mechanisms in the banana plant should consider either the absence of essential nutrients for *C. sordidus* or compounds that inhibit its development.

There are three possible hypotheses for the low *C. sordidus* larval weights from nutrient-treated plants. First hypothesis, is richness in nutrients with a low concentration of defensive compounds [10,31,49], which allowed faster larval development to surpass the growth peak that is characterized by pre-pupal weight loss [19,45], earlier than those from nutrient-deficient plants. Second hypothesis, nutrient supply may have enhanced the synthesis of plant defensive compounds that limited larval growth [3,26,73]. Third hypothesis, nutrient supply may have created an imbalance in plant nutrient content leading to the accumulation of toxic inorganic compounds such as nitrates [39,42]. Although the first hypothesis is fundamental, the other two hypotheses may be strengthened by the fact that the multiplication of another banana pest *R. similis* seemed to be slower in nutrient-treated banana plants than in nutrient-deficient ones [46]. In the present study, the olfaction behaviour of adult *C. sordidus* was not influenced by host plant nutrition. It is likely that the chemical cues from banana plants that are utilized by adult *C. sordidus* were not affected by fertilizer application [1,9]. However, plant nutrition has been found to affect host preference in some curculionid pests [37].

In summary, inoculating tissue culture banana plants with *F. oxysporum* V5w2 partly interfered with host infestation by adult *C. sordidus*, and tended to suppress rhizome damage by their larvae. Larval *C. sordidus* appeared to grow better on nutrient-deficient plants, but soil fertility did not affect host-derived cues for the adults. Although *F. oxysporum* V5w2 appears to offer beneficial ecosystem services related to suppression of *C. sordidus*, the effects were very short-lived and hence having remote applicability. The plant growth promoting potential of *F. oxysporum* V5w2 is undermined by reduction in nitrogen and chlorophyll content, alongside non-beneficial interactions between this fungal endophyte and plant-parasitic nematodes [46–48,50,51]. Besides this, the studies were conducted using banana plants inoculated with a single fungal isolate and grown in sterilized soil only, and may therefore not provide sufficient information in the context of the complex banana endosphere and rhizosphere that are rich in microbial biodiversity [32,47,51,64]. The short-term inhibitory effects of *F. oxysporum* V5w2 towards adult and larval *C. sordidus* raises further questions on the potential of this insect pest dispersing the fungal endophyte among other related phytopathogens of banana [25,40,61]. However, the inhibitory effects of *F. oxysporum* V5w2 towards *C. sordidus* adults may point to the possibility of developing pest-inhibitive innovative products based on fungal volatile organic compounds (VOCs) [36]. In conclusion, while *F. oxysporum* V5w2 is not quite viable for application as an endophytic biological control agent for *C. sordidus* and other banana pests, this fungus may still have some potential to offer alternative ecosystem services through the provisioning of pest-inhibitive organic compounds.

Credit author statement

The information presented in this article is my own work derived from the PhD Thesis of Dr. Dennis M.W. Ochieno [46]. All contributors to these studies have been acknowledged.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

These datasets were derived from a common experiment that comprised *F. oxysporum* V5w2 treatments ([46], page 28-30), and have therefore been collectively disassociated from studies that propose this phytopathogenic fungus as an Endophytic Biological

Control Agent alongside data in these other related articles [47,48,50,51]. The Scientific Integrity Committee of Wageningen University & Research (CWI) has already advised on these issues.

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