



Endophytic *Beauveria bassiana* in banana (*Musa* spp.) reduces banana weevil (*Cosmopolites sordidus*) fitness and damage

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ABSTRACT

The effect of endophytic *Beauveria bassiana* in banana (*Musa* spp.) plants against the banana weevil *Cosmopolites sordidus* was examined in a screenhouse study in Uganda. Tissue-cultured banana plants (cv. Kibuzi, genome group EA-AAA) were inoculated by dipping roots in a *B. bassiana* suspension of 1.5×10^7 conidia/ml for 2 h. *C. sordidus* larvae were introduced 2 months later. Two weeks after larval infestation, endophytic *B. bassiana* significantly reduced larval survivorship (23.5–88.9% mycosis), resulting in 42.0–86.7% reduction of plant damage. This study has demonstrated for the first time that endophytic *B. bassiana* can be used to target the cryptic and damaging stage of *C. sordidus*, and offers an alternative, effective delivery mechanism for this biological control agent.

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1. Introduction

The banana weevil *Cosmopolites sordidus* (Germar) is regarded as the most damaging arthropod pest of bananas (*Musa* spp.) worldwide (Gowen, 1995). In East Africa, *C. sordidus* remains a serious threat to the production of highland cooking bananas (genome group EA-AAA), causing up to 100% yield losses (Koppenhofer et al., 1994; Gold et al., 2004). The pest causes significant physiological damage to bananas by feeding and tunneling in the rhizomes and pseudostems, which results in reduced nutrient uptake, premature leaf senescence, reduced bunch filling or plant snapping (Rukazambuga et al., 1998).

Currently, *C. sordidus* management focuses on using cultural practices, insecticides, biological control and host plant resistance, which do not always provide adequate control (Gold et al., 2001). Microbial agents, including the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin, have also been tested to suppress adult populations of *C. sordidus* (Nankinga, 1999; Godonou et al., 2000; Schoeman and Botha, 2003). Though very effective in the laboratory, application of *B. bassiana*, used as a conventional bio-pesticide, fares less favourably in the field due to high costs and inadequate application technology. Also, *C. sordidus* adults are targeted, as opposed to the damaging larval stage, which occurs inside the plant.

These limitations led to our attempts to use *B. bassiana* as an artificial endophyte. Compared with conventional bio-pesticides, endophytes have the advantage of targeting *C. sordidus* larvae within the rhizome, at reduced application costs because little inoculum is required. Furthermore, endophytic *B. bassiana* is protected inside the banana plant from abiotic and biotic factors that limit its use under field conditions.

Recently, studies demonstrated that *B. bassiana* can survive as an artificial endophyte in several plant species (Posada and Vega, 2005, 2006; Gómez-Vidal et al., 2006; Quesada-Moraga et al., 2006). In maize (*Zea mays* L.), endophytic *B. bassiana* was shown to provide protection against *Ostrinia nubilalis* (Hübner) and *Sesamia calamistis* (Hampson) (Bing and Lewis, 1991; Cherry et al., 2004). When tissue-cultured banana plants were inoculated using a *B. bassiana* suspension, up to 78.7% endophytic colonization was achieved, which persisted for at least 4 months (Akello et al., 2007a, 2007b). The purpose of this study was to determine the effect of endophytic *B. bassiana* in tissue-cultured plants against banana weevil larvae and the damage they cause.

2. Materials and methods

2.1. Experimental site and design

A screenhouse experiment was conducted at the International Institute of Tropical Agriculture (IITA), Namulonge, Uganda, located 28 km northeast of Kampala (0°32'N and 32°35'E) at 1260 m above

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sea level. The site receives a mean annual rainfall of 1255 mm and has an average temperature of 22 °C. The experiment consisted of two treatments: (1) plants dipped in a 1.5×10^7 conidia/ml *B. bassiana* suspension and (2) control plants dipped in 0.01% sterile Tween 80. Tissue-cultured banana plants derived from the East African highland cultivar Kibuzi were used in the study. The experimental design was completely randomized with 25 plants per treatment and repeated twice.

2.2. Fungal inoculum

B. bassiana strain G41, originally isolated from soil from banana plantations and provided by the National Agricultural Research Organization (NARO), Kawanda, Uganda, was selected based on its high sporulation ability, pathogenicity against *C. sordidus* and high plant colonization rate (Nankinga, 1999; Akello et al., 2007b). The fungus was cultured on Sabouraud dextrose agar medium supplemented with yeast extract (SDAY) (200 g glucose, 20 g peptone, 5 g yeast extract and 15 g agar/l distilled water) and containing antibiotics (0.1 g penicillin, 0.2 g streptomycin and 0.05 g chlortetracycline/l SDAY) in 90 mm diameter plastic Petri dishes after retrieval from storage in silica gel. The Petri dishes were incubated in the laboratory (~ 25 °C and a natural photoperiod of $\sim 12:12$ h L:D) for 21 days. The Petri dish lids were removed in a laminar air flow cabinet and the cultures air dried for 48 h. Conidia were harvested under sterile conditions by scraping the mycelium and conidia from the surface of the dried medium into a sieve (150 μ m aperture) and collecting the conidia into aluminium foil. The conidia were suspended in 200 ml 0.01% Tween 80 in a 500 ml bottle. Conidial density was determined using an improved Neubauer haemocytometer and adjusted to 1.5×10^7 conidia/ml.

2.3. Plant inoculation

Tissue-cultured banana plants were produced according to Vuylsteke (1998). At the deflasking stage, plants were suspended singly in a 250 ml nutrient solution containing 1 g/l Poly-Feed (Haifa chemicals, Haifa, Israel) in 300 ml lidded plastic cups and placed in a humidity chamber. A sponge wrapped around the pseudostem base through a hole made in the lid provided support when plants were placed in the nutrient solution. The nutrient solution was changed weekly. After 4 weeks, plants, which possessed well developed roots and shoots (5–6 cm tall with 3–4 standing leaves), were randomly assigned to the treatments. Plant roots and rhizomes were dipped in a 300 ml *B. bassiana* suspension containing 1.5×10^7 conidia/ml for 2 h and then planted singly in sterile loamy soil in 20 l plastic buckets. Control plants were dipped in 300 ml sterile 0.01% Tween 80. The plants were watered daily and grown in the screenhouse for 2 months prior to infestation with *C. sordidus* larvae.

2.4. Banana weevil larvae

Three weeks prior to larval infestation, 300 adult banana weevils of mixed sex (1:2 male:female ratio) were released into a 10 l plastic bucket containing five banana rhizomes. The weevils remained in the buckets for 24 h, covered with perforated lids, to lay eggs in the rhizomes. Rhizomes were transferred to empty buckets and maintained in the laboratory for 3 weeks. Hatched larvae were exposed by slicing the rhizomes piece by piece with a knife. Each exposed larva was carefully removed by hand and placed singly in 55 mm diameter plastic Petri dishes. The larvae were weighed and randomized according to weight prior to plant infestation.

At plant infestation, three holes (~ 10 mm deep and a diameter of ~ 20 mm) were cut at equidistal sites on the pseudostem, ~ 2 cm

above the pseudostem base. A larva was introduced into each hole before sealing with masking tape. Plant height (the distance from the base of the plant to the youngest leaf axil), number of fully developed leaves, and leaf width (widest part of the lamina) and length (the distance from the leaf apex to the leaf stalk) of the youngest fully opened leaf were recorded.

2.5. Harvest

The plants were harvested 2 weeks following larval infestation and for each plant, plant height, number of leaves, and leaf length and width were recorded. The plants were removed from the buckets, their roots and rhizomes rinsed with tap water to remove soil, and the total number of roots and pseudostem base girth was recorded. The rhizomes were pared by removing roots, and banana weevil damage to the rhizome, pseudostem base and the rhizome periphery scored according to Gold et al. (1994). Peripheral rhizome damage was assessed by dividing the outer surface of the pared rhizome into four portions of 25% each. The rhizome surface area tunneled by banana larvae was scored from 25% for each portion and the total percentage peripheral damage obtained from the sum of the four portions. For pseudostem base and rhizome damage, cross-sections were made through the collar (the junction between the rhizome and the pseudostem) and through the rhizome ~ 2 cm below the collar, respectively. In each cross-section, the central cylinder and the cortex were divided into four equal-sized portions, each representing 25% of the surface area. The inner damage (corresponding to the central cylinder) and the outer damage (corresponding to the cortex) of both the rhizome and the pseudostem base were calculated as described above. Rhizome and pseudostem base colonization by *B. bassiana* was determined according to Akello et al. (2007b). Root and shoot weights of each plant were determined and after drying the shoots in an oven at 60 °C for 48 h, dry shoot weight was recorded.

For each plant, the number of live, dead and mycosed larvae was recorded. Living larvae were reared single on pieces of fresh clean banana rhizome for 15 days while dead larvae without *B. bassiana* mycosis were incubated in sterile 90 mm glass Petri dishes lined with moistened filter paper for 15 days. Percentage dead, mycosed and live larvae and pupae were calculated as: (number/total number recovered per plant) $\times 100$.

2.6. Data analysis

All plant growth parameters were analyzed using a pooled *t*-test, except for number of leaves, which was analyzed using a Kruskal–Wallis test. For parameters with unequal variances between treatments (tested using the folded *F* statistic at a 5% level of significance), a Statterthwaite's approximation *t*-value was used to compare sample means. For each experiment, multifactor data for plant damage was subjected to analysis of variance (ANOVA). Before carrying out ANOVA, inner and outer rhizome, inner and outer pseudostem base, and peripheral rhizome damage were $\log_{10}(x + 1)$ -transformed to obtain normally distributed data with equal variance across treatments. Differences in the mean percentage damage to the various plant parts were separated using Tukey's studentized range test. Within each plant part, a pooled *t*-test was performed to examine differences in banana weevil damage between treatments. Percentage colonization and number of living and dead larvae were analyzed using logistic regression. Prior to analysis, percentage rhizome and pseudostem base colonization were calculated as: (number of segments exhibiting *B. bassiana* outgrowth/total number of segments) $\times 100$. Differences in percentage colonization of different plant parts were separated using a Dunn–Sidak correction (SAS Institute, 1995; Sokal and Rohlf, 1995).

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