SCREENING OF *BEAUVERIA BASSIANA* ISOLATES TO THE BANANA WEEVIL AND HORIZONTAL TRANSMISSION UNDER LABORATORY CONDITIONS

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Abstract

The effective use of the entomopathogen *Beauveria bassiana* for the management of banana weevil needs evaluations of isolates under laboratory conditions for the most virulent strains. For screening work, 20 adult weevils were contaminated by dipping into a conidial suspension titrated $1x10^8$ conidia ml^{-1} for 11 seconds. The excess of suspension was drained and banana corm was introduced as food in 250ml plastic containers. All the screened isolates of *B*. *bassiana* tested were found to be pathogenic to the adult *C. sordidus* causing mortalities of between 20-51% by 40 days post exposure. ICIPE 273 was the most pathogenic killing 51% of adults, followed by ICIPE 645 36% and ICIPE 281 30%. The rest ICIPE 603, ICIPE 289, ICIPE 50, ICIPE 284, ICIPE 283, ICIPE 647 and ICIPE 279 had a kill of less than 30% with ICIPE 279 being the least pathogenic to the adult *C. sordidus*. From the screening work done, the three best isolates ICIPE 273, ICIPE 645 and ICIPE 281 were tested at a concentration of 1×10^9 and they caused mortalities varying from 50-70%, 40 days after exposure. In disease transmission experiment it was possible to transmit infection from two weevils dosed with conidia at 1×10^9 from the three strains of *Beauveria bassiana* to a group of 18 non infected banana weevils in 250ml plastic containers mainly through contact. The rate of transmission from infected to non infected weevils caused mortalities of between 24 – 26% of horizontal infection for the tested isolates. Incubation of dead weevils in clean petri dishes with moist sterile filter papers led to development of mycelia on the surface starting from intersegmental junctions, confirming that the mortality was caused by fungus. Dead weevils from the control had no fungal growth. Based on these results, ICIPE 273 shows promise for the control of banana weevil and should be subjected to further studies.

Keywords*:* biocontrol*, Cosmopolites sordidus,* entomopathogens, pest management

Introduction

Bananas constitute a major staple food crop for millions of people in developing countries providing energy as well as important vitamins and minerals. The majority of producers are small-scale farmers growing the crop either for home consumption or for local markets as an important source of rural income (Karamura, 1998). Banana production in Kenya has been on the decline due to environmental stresses, declining soil fertility, poor crop management as well as lack of clean planting material (Seshu *et al.*, 1998). Pest and disease pressures have also increased, reducing the life span of banana orchards (ISAAA, 1996). Pests of economic importance are the banana weevil, *Cosmopolites sordidus* and the parasitic nematodes *Radopholus similis, Pratylenchus spp.* and *Helicotylenchus multicinctus*. Both the weevil and nematode infestation interfere with nutrient uptake and transport, resulting in slow growth, reduced fruit filling and susceptibility to wind lodging, hence the need to device control measures for sustainable banana production (Gold *et al.,* 2003).

The yield losses associated with the weevil range from 40% to 100% in severe infestations (Seshu *et al.*, 1998). Banana weevil attack in newly-planted banana stands can lead to poor crop establishment. In established fields, weevil develop within the tree trunk destroying its vascular system and eventually causing collapse and death of the plant (Gold *et al.,* 2003).

The use of pesticides such as aldrin and carbofuran have been used mainly by farmers to control the banana weevil, but heavy use of insecticide has resulted in weevil resistance, high costs and adverse effects of pesticides on the environment (Allard *et al.,*1991).

The most commonly used cultural control methods include the use of clean planting material, crop sanitation, agronomic methods to improve plant vigor and tolerance to weevil attack, treatment with neem extract and mass trapping. Application of these methods has cost implications and adoption by resource poor subsistence farmers is often limited (Gold *et al.,* 1998).

There is need therefore to develop safer and cheaper control alternatives that can be used to complement existing control methods (Nankinga *et al.,* 1998). Entomopathogenic fungi have been used successfully to control various agricultural pests, (Kaaya and Hassan, 2000), including banana weevils (Gold *et al*., 2003). *Beauveria bassiana* has been researched extensively as an alternative means for controlling the banana weevil. When spores of the fungus come into contact with the body of an insect host, they germinate and the hyphae that emerge penetrate the cuticle. The fungus then develops inside the body eventually killing the insect after a few days; this lethal effect is very likely aided by the production of insecticidal cyclic peptides (destruxins). The potential of entomopathogenic fungus such as

B.bassiana as biological control agents against several banana weevil species has been evaluated. Isolates often perform well in short-term laboratory bioassays causing high mortality of >90.0% within 2 weeks but efficacy in the field has not been clearly demonstrated (Kaaya *et al*., 1993; Nankinga *et al*. , 1994; Godonou *et al*., 2000).

Few reports are available in the literature on evaluation of entomopathogens for the control of *Cosmopolites sordidus.* Laboratory pathogenicity tests using different strains of *B.bassiana* in Uganda (Nankinga *et al.,* 1994) and in West Africa (Godonou *et al.,* 2000) produced 50% - 100% mortality in banana weevil adults in 14 days. It is evident therefore that *B. bassiana* is highly effective under laboratory conditions (Magara *et al.,* 2004).

Horinzontal transmission occurs when a pathogen is transferred from individual to individual either through integument contact or natural body openings while vertical transmission is whereby the fungus is transferred directly from parent to the offsprings (Rath, 2000). Horizontal transmission may play an important role in the management of banana weevils since they are gregarious, found in clusters in cavities and depressions in the outer sheaths of the banana close to the ground surface and also below the surface.

Entomopathogenic fungi have the potential to grow, multiply and persist on the insect they kill. Infected individuals can move away from the infected point thus carrying the pathogen throughout the pest habitat leading to an epizootic situation (Ferron, 1981).

Little is known about transmission of *B. bassiana* from infected banana weevils to non infected banana weevils. Studies conducted by Godonou *et al*. (2000) showed a possible dissemination of *B. bassiana* conidia from infected to non infected weevils. Details on how *B. bassiana* can be transmitted from infested individuals to non infected ones will be important in developing an effective delivery system for the pathogen.

The aim of this study was therefore to evaluate efficacy of *B. bassiana* isolates to the banana weevil, *C. sordidus* and also to quantify transmission of *B. bassiana* from artificially infected weevils to non infected weevils under laboratory conditions.

2.0 Materials and Methods

2.1 Insects

Approximately one thousand adult banana weevils were obtained from naturally infested banana plants in Kenya Agriculture Research Institute, Mwea. The weevils were maintained in 250ml plastic containers at room temperature in the laboratory for one week before being used in the experiments. The covers of the containers

were perforated for ventilation. Banana suckers of susceptible variety were pared and 200g pieces of corm tissue placed in the containers and used as food.

2.2 Fungal Isolates

The following isolates of *B. bassiana* were used in the bioassays: ICIPE 273, ICIPE 279, ICIPE 50, ICIPE 284, ICIPE 603, ICIPE 289, ICIPE 283, ICIPE 645, ICIPE 647 and ICIPE 281. They were obtained from the ICIPE's Arthropod Germplasm Centre, Duduville, Nairobi, Kenya. Most of the isolates were selected at random because of their virulence to other arthropod pests in previous studies. The original cultures were stored at –85^{o}C in 10% sterile glycerol. The isolates were cultured on 65g Sabouraud Dextrose Agar (SDA) medium in 90ml petri dishes for three weeks for complete sporulation in an incubator (27°C). Antibiotic chloramphenical 250mg was added to the medium to keep off any bacterial contamination.

2.3 Inoculum preparation

Conidia stock solution was prepared according to Inglis *et al*.(1996) were gently scrapped from petri dishes and suspended in 10ml of 0.01% Tween-20 until all the spores had been harvested. The conidial suspension was transferred by using a sterile pasteur pipette into 20 ml sterile universal bottles with glass beads. Conidial density for each strain was determined using an improved Neubauer haemocytometer after vortexing for 5min to produce a homogenous conidial suspension. The viability of conidia was then determined by spread plating 0.1ml of suspension (titrated 3.0 x 10^{6}) on SDA plates.A sterile microscope coverslip was placed on each plate.The plates were incubated at 26 ± 2^{o}C and examined after 16h. Conidial germination was assessed by counting the total number of conidia that germinated plus those that had not germinated in four different fields under a dissecting microscope. Conidial germination was characterized by germ tube development and these were categorized as viable conidia while the non-germinated conidia that lacked the germ tube were categorized as non-viable. A total of 100 spores were counted for each plate and four replicates were used for each isolate (Inglis *et al*.,1996).

2.4 Bioassays

From the stock solution a concentration of 1 x 10^{8} conidia ml^{-1}was prepared (standard concentration used for large insects in ICIPE) for the efficacy trials. For counting the spores a dilution of x100 was prepared by removing 0.1ml using a pasteur pipette from 10ml sterile distilled water with Tween-20. The same quantity of 0.1ml was picked from the stock solution and added into the diluent. Vortexing was done for three minutes. Conidial density for each strain was then estimated by placing 0.1ml on the improved Neubauer haemocytometer and counting the spores. For the different concentrations of 1 x 10^{8} and 1 x 10^{9} the concentration formula was used. The spores counted from x100 dilution were multiplied by a constant 2.5 x 10^{5} and x 100. This gives the spores in the standard 1 x 10^{8}. From the standard

concentration C_1V_1 and C_2V_2 the number of spores needed for 1 x 10^9 were then calculated. Based on the results from screening, ICIPE 273, ICIPE 645 and ICIPE 281 isolates were selected for the transmission experiment. A higher concentration of 1 x 10^9 was prepared for the transmission experiment since the infected banana weevils would be used to transmit the fungus to the non infected weevils and a control treatment of sterile distilled water containing 0.01% Tween 20 was also prepared.

2.5 Inoculation of weevils

Twenty weevils were infected by dipping them into 20 ml fungal suspension titrated 1 x 10^8 conidia ml^{-1} for 11 seconds for the efficacy trials. The excess of suspension was drained and the weevils were transferred to 250ml plastic containers. Banana corms approx 200g were then placed in the containers as food. Control weevils were dipped in sterile distilled water containing 0.01% Tween 20. Mortality rate was recorded after every 3days for 40 days. The experiment consisted of five replicates for ICIPE 279, ICIPE 284, ICIPE 273 and ICIPE 603 and four replicates for ICIPE 50, ICIPE 289,ICIPE 281, ICIPE 283 ICIPE 645, ICIPE 647and control. For the transmission experiment each batch of banana weevils was placed in a petri dish and 10ml of the appropriate conidial suspension 1 x 10^9 of ICIPE 273, ICIPE 645 and ICIPE 281 were gently poured in immersing the banana weevils. For control sterile distilled water with tween 20 was poured on the banana weevils. To obtain rapid immersion the petri dish was shaken gently for 11s after which the suspensions were poured out and the infected weevils introduced into plastic containers with 18 non infected banana weevils with a 200g piece of banana corm as a source of food. To assess mycosis, dead insects were surface-sterilized in 2% sodium hypochlorite, 70% alcohol and two rinses of sterilized water for 15 seconds before placing them in clean Petri dishes with moist sterile filter papers. Dead insects were monitored for fungal growth for two weeks and observations recorded. Only dead insects with fungal growth which was observed under a dissecting microscope were considered to have been killed by the fungus.For control weevils no fungal growth was observed.

3.0 Statistical analysis

All data for efficacy was analysed with ANOVA using the PROC GLM of SAS statistical software and the treatment means were separated using Student-Newman-Keuls (SNK) test at P= 0.05. Percent conidial germination was calculated as: (viable conidia/total conidia) x100. Data on horizontal transmission in the laboratory was arcsine square root transformed and analysed using the GLM procedure of SAS. Mean comparisons were done using Student-Newman-Kuels at P = 0.05. All analysis was done on SAS (SAS, 1999).

4.0 Results

Table 1: *Beauveria bassiana* isolates used in the study and their percent viability

Isolates	Year of Isolation	Host/ Substrate	Locality/ Country	% Viability ± SE
ICIPE 273	2006	Soil	Mbita/Kenya	92.2 ± 3.2
ICIPE 279	2005	Coleopteran larvae	Kericho/Kenya	86.8 ± 4.5
ICIPE 284	2005	Soil	Mauritius	85.4 ± 3.7
ICIPE 603	2007	Hymenoptera	Taita hills/Kenya	86.6 ± 3.5
ICIPE 289	2005	Soil	Mauritius	82.3 ± 3.7
ICIPE 283	2005	Soil	Mauritius	81.3 ± 3.5
ICIPE 645	2005	Soil	Mauritius	92.7 ± 2.1
ICIPE 281	2005	Soil	Mauritius	88.0 ± 2.6
ICIPE 50	1996	*Rhipicephalus appendiculatus*	Rusinga island Kenya	87.3 ± 2.6
ICIPE 647	2005	Soil	Mauritius	89.0 ± 2.4

ICIPE's Arthropod Germplasm Centre

4.1 Efficacy of Isolates

Percent conidial germination ranged between 81.2-92.7% for all the *B. bassiana* strains (Table 1). There was no significant difference (P=0.37) in percentage conidial germination among all the isolates.

All the ten isolates of *B. bassiana* were pathogenic to the adult *C. sordidus,* causing mortalities varying from 6-51% by 40 days post-exposure depending on the fungal isolate. ICIPE 273 was the most pathogenic killing 51% of adults followed by ICIPE 645 36%, ICIPE 281 30%,the rest ICIPE284, ICIPE 603, ICIPE 289,ICIPE 283, ICIPE 50, ICIPE 647 and ICIPE 279 had a kill of less than 30% with ICIPE 279 being the least pathogenic to *C. sordidus* (Figure 1).

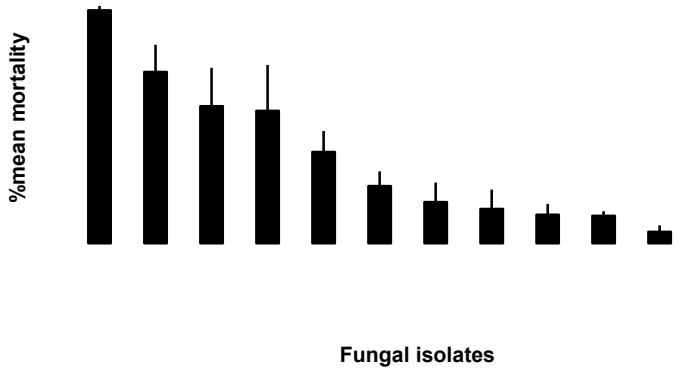

Figure 1: Mortalities in adults infected with 10 isolates of Beauveria bassiana fungal isolates

Table 2: Percent mortality (mean ± SE) of Adult banana weevil at different spore concentrations

Isolate	**Spore Concentrations**	
	1 x 10^8	1 x 10^9
ICIPE 273	50 ± 0.9a	69.3 ± 4.1a
ICIPE 645	39.3 ± 2.5b	65.6 ± 4.6ab
ICIPE 281	30.0 ± 4.2c	55.6 ± 2.9b
CONTROL	3.7 ± 1.2d	3.7 ± 1.2c

Means followed by similar letter in each column are not significantly different at P =0.05 by Student Newmans Kuels test.

An increase in the inoculum level was accompanied by an increase in mortality, (Table 2).There was a significant difference among the treatments, (P = 0.0001) for the concentration of 1 x 10^8 and (P = 0.0002) for 1 x 10^9.

4.2Transmission in the laboratory

There was a significant difference ($P \leq 0.05$) between the fungal isolates and the control (P = 0.0001) in the mortality (Figure 2), while there was no significant difference among the fungal isolates for mycosis.

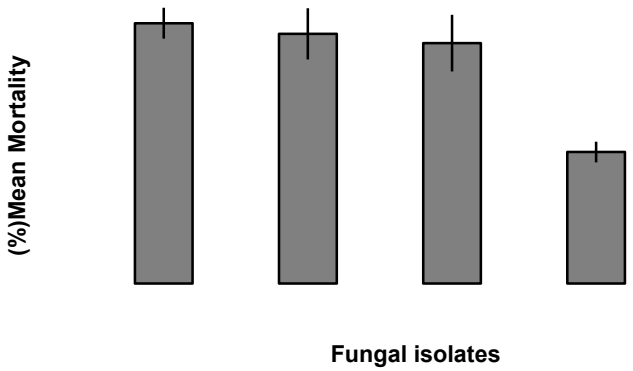

Figure 2. Adult banana weevil mortality due to horizontal transmission in the laboratory.

4.3 Development of surface mycosis

Incubation of dead *B. bassiana*-treated insects in humidified chambers resulted in development of mycelia on the surface of the cadaver, starting from the intersegmental junctions of the body and legs (plate 1). No fungi grew on dead weevils in the controls.

Plate 1: Adult C.sordidus infected with B.bassiana.Notice white fungal growth at intersegmental junctions (arrows)

5.0 Discussion and conclusions

The results clearly demonstrate the ability of *B. bassiana* to control the banana weevil. All the tested isolates were pathogenic to the banana weevil even though there were significant variations among the tested isolates. Intraspecific differences in pathogen activity may exist between isolates of *B. bassiana* and *Metarhizium anisopliae* in many arthropod pests (Maniania, 1992)

From the efficacy tests carried out the results suggest that banana weevils are susceptible to isolates of *B. bassiana* with varying mortalities of between 6%-51% when using a standard concentration of 10^8spores/ml. ICIPE 273 was identified as the best biocontrol agent for *C. sordidus* since it killed 51% of the adult weevils within 40 days under laboratory conditions. This is acceptable for entomopathogens when used as biological control agents since they target reducing the pest population to 50% and above. The level of effectiveness obtained in this study compares favourably with those of (Nankinga *et al.,* 1994; Godonou *et al*., 2000; Pena *et al.,* 1991).

An increase in spore concentration to 1 x 10^9 spores/ml and the mortality increased to 69% for ICIPE 273, 65% for ICIPE 645 and 55% for ICIPE 281. Efficacy of fungal isolates against banana weevils is influenced by culturing method, spore dose, substrate, formulation and method of application (Gold et al.,2002). The search for effective strains of entomopathogenic fungi should include natural isolates from the target insect because such isolates may have higher virulence than those from unrelated hosts (Maniania, 1992 ;Geden *et al.,* 1995).

A major limitation in the development of fungi for insect control is the lack of a readily available formulation technology for improved shelf life, persistence, efficacy and field targeting (Gitonga, 1996).Transmission of infection from infected to non infected weevils in the laboratory gave (24%-26%) mortalities which compares favourably with Schoeman et al. (1998) who obtained mortalities of between 24.4%-

26.83% when he infected 15 banana weevils and introduced them to a container with 15 uninfected weevils in the laboratory for 37 days in South Africa.

Laboratory results indicated that weevils can transfer the pathogen from infected to uninfected individuals (Gitonga, 1996). Transmission takes place any time there is physical contact between infected and non infected individuals. There were no significant differences in percent transmission in the laboratory. However more virulent strains of this pathogen are needed that lead to higher rates of transmission and infection.

ICIPE 273 was identified as the best biocontrol agent for *C. sordidus* since it gave the highest mortalities when the two concentration levels were used and should be considered for further studies to assess its potential as a biological control agent for this pest.``

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