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Selection of native isolates of *Beauveria bassiana* (Ascomycota, Hypocreales) for the control of the coffee borer beetle *Hypothenemus hampei* (Scolytinae) in Brazil

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Abstract: *Hypothenemus hampei* (coffee borer beetle or coffee berry borer) is the main pest of coffee *Coffea* spp. in the world. The aim of this study was to select native isolates of the ascomycete *Beauveria bassiana* for biological control of this pest. We collected 27 isolates on *H. hampei* from the state of Espírito Santo in Brazil. Isolates CCA-UFES/Bb-15, Bb-11, Bb-4 and Bb-18 were selected, with confirmed beetle mortality of > 60% after spraying with a suspension of 10^5 conidia/mL. The median lethal concentration (LC_{50}) of these isolates varied from 4.0×10^4 to 7.9×10^4 conidia/mL. The standard isolate (ESALQ-447) showed the highest conidiogenesis, with 8.5×10^6 conidia, followed by CCA-UFES/Bb-18, Bb-11, Bb-15 and Bb-4, all exceeding 4×10^6. Isolates CCA-UFES/Bb-4, Bb-11, Bb-15, and Bb-18 have a potential to control *H. hampei*.

Keywords: Coffee, conidiogenesis, entomopathogenic fungus, biological control

INTRODUCTION

The coffee borer beetle *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera: Scolytinae), also known as coffee berry borer, is the main coffee pest in the world. This insect causes a reduction in coffee yield and quality due to losses in mature berries, caused by fungal infection after the attack of the insect pest, i.e. the boring by adults and larvae (Bustillo 2005). Moreover, the control of this insect pest is difficult due to the entire life cycle hidden within the seed of the coffee berry (Damon 2000).

Broad-spectrum insecticides are used to reduce the damage caused by this insect, but this has a negative impact on the agroecosystem and leads to the development of resistant pest populations. Moreover, the behavior of *H. hampei* can reduce the efficiency of broad-spectrum insecticides (Brun et al. 1989; Wegbe et al. 2003; Rehner et al. 2006).

Natural enemies may be an alternative approach to control this insect pest (Santoro et al. 2005; Jaramillo et al. 2006). Explorations for natural enemies of *H. hampei* have revealed the presence of various parasitoids (Dalvi et al. 2008). However, the difficulty of rearing them has limited their use in biological control. A better candidate seems to be the entomopathogenic fungus *Beauveria bassiana* (Ascomycota: Hypocreales), which is the most prevalent fungus that attacks *H. hampei* wherever this insect pest has spread (Alves 1998; La Rosa et al. 1997), with infection rates from 20 to 90% in Colombia (Bustillo 2005). However, the exploitation of *B. bassiana* against arthropod pests depends on its virulence, which limits the market share of this entomopathogenic fungus (St Leger & Wang 2009).

The aims of this work were: (1) to recognize the infective potential of native *B. bassiana* strains, by screening its local isolates; and (2) to estimate their usefulness in the microbial control of this insect.

MATERIAL AND METHODS

Immature berries of coffee *Coffea* spp., with signs of boring and feeding activities of *H. hampei* adults, were collected on 53 coffee plantations at the municipalities of Alegre, Jerônimo Monteiro, Ibitirama, Irupi, and Vargem Alta in the South and Região Serrana of the state of Espírito Santo in eastern Brazil. In each plantation, 500 coffee berries were collected.

From these materials, berries with sporulating mycelium were selected, and the fungi were isolated on potato-dextrose agar (PDA). The cultures were kept in a greenhouse for 10 days and observed daily. Subsequently, the samples were examined under a microscope and identified. Twenty-seven isolates of *B. bassiana* were confirmed and received the codes CCA-UFES/Bb-1 to Bb-7 and Bb-9 to Bb-28. They were stored in a freezer at -20°C. The highly virulent isolate ESALQ-447 of the same species was used as the standard (Habib et al. 1998).

Larvae of the coffee borer beetle *Hypothenemus hampei* were reared as outlined by Hirose & Neves (2002).

Cultures of the wild-type strain of *B. bassiana* were maintained on PDA + yeast 10%. After sporulation of the fungi, conidia of each isolate were sprayed onto healthy, newly emerged adult beetles. After 10 days, conidia of the same fungi were again isolated in culture media (PDA + yeast 10%). These plates were incubated in growth chambers at 25±2°C and photophase of 12 h for 10 days. After this period, conidia were collected and again spread over the culture media for their use in testing. Suspensions containing conidia were inoculated in Petri dishes with water agar medium for 20 h, to determine the germination percentage of the isolates under a microscope with Neubauer chamber.

Petri dishes (8 cm) were lined with discs of tissue paper to pre-select the isolates. A cotton ball soaked in distilled water was placed on top of the plate to maintain humidity. Each plate (replicate) contained 30 young adults of *H. hampei*.

Five replicates were performed for each treatment. The experimental setup was a completely randomized design and the means were submitted to the Scott-Knott test at a probability level of $P < 0.05$ (Neves & Hirose 2005).

An aliquot containing 0.5 mL of conidial suspension in sterile water and Tween 80 (0.02%) was sprayed on the borers in each plate to pre-screen the isolates. The concentration was 1×10^5 conidia/mL, to obtain isolates with high virulence. Coffee powder (0.25 g) was provided as food for *H. hampei* on each plate. Sterile water + Tween 80 (0.02%, 0.5 mL) was sprayed on the insects as a control.

The dishes were kept in a growth chamber at 25±2°C, relative humidity of 80±10%, and photophase of 12 h for 6 days. After this period, dead insects were counted, separated, washed in sterile water, and kept in a moist chamber for 4 days. The corpses with symptoms of conidiogenesis were counted. All mortality data (%) were corrected for mean control mortality *by* using Abbott's (1925) formula. We calculated also the confirmed mortality, i.e. the percentage of insects showing signs of conidiogenesis after death.

The median lethal concentration (LC_{50}) was determined with selected isolates at concentrations of 1×10^5, 7.5×10^4, 5×10^4, 2.5×10^4, 1×10^4, 5×10^3, and 1×10^3 conidia/mL. The confirmed mortality data were subjected to the Probit analysis to estimate LC_{50}, using the POLO-PC program (LeOra Software 1987).

Conidiogenesis in *H. hampei* was estimated after the application of 1×10^5 conidia/mL. Fifty *H. hampei* corpses were collected and divided randomly into 5 groups. Each group was placed in a test tube containing water (10 mL) with Tween 80 (0.02%), and shaken. Counting of conidia was then performed in a Neubauer chamber. The potential production increase (PPI) was estimated by dividing the number of conidia of the most productive isolates by the number of conidia produced by isolate CCA-UFES/Bb-4. The means were compared using the Tukey test with a probability level of for $P < 0.05$.

RESULTS

The confirmed mortality of *H. hampei* ranged from 70.83 to 18.48% in the pre-screen for isolates CCA-UFES/Bb-15 and Bb-19, respectively, and the corrected mortality varied from 89.32 to 27.21% for the same isolates. Isolates CCA-UFES/Bb-4, Bb-11, Bb-15 and Bb-18, whose confirmed mortality was greater than 60% (Table 1), were selected for the further investigations of LC50 and conidiogenesis.

The results showed the accuracy of the Probit model for the estimation of the lethal concentration (LC_{50}), with no significant χ^2 and low heterogeneity. The confidence intervals indicated significant differences between the isolates (Table 2). Isolate CCA-UFES/Bb15, with a LC_{50} of 4.00×10^4, differed from the standard ESALQ-447, with a LC_{50} of 11.85×10^4.

Conidiogenesis of the native isolates ranged from 4.07×10^6 to 5.02×10^6 conidia/insect. These values were lower than for the standard isolate (ESALQ-447),

Table 1. Mortality (mean ± SE) of adults of *Hypothenemus hampei* (Coleoptera: Scolytidae) caused by *Beauveria bassiana* (10^5 conidia/mL, 25±1°C, photophase 12 h)

Isolate	Mortality (% of initial number of beetles)	
	Corrected	Confirmed
CCA-UFES/Bb-15	89.32 ± 4.55 a	70.83 ± 4.10 a
CCA-UFES/Bb-11	85.06 ± 4.11 a	67.77 ± 4.09 a
CCA-UFES/Bb-18	82.96 ± 1.85 a	64.23 ± 1.13 a
CCA-UFES/Bb-4	80.17 ± 1.01 a	62.43 ± 1.28 a
CCA-UFES/Bb-17	63.70 ± 1.72 b	49.25 ± 1.12 b
CCA-UFES/Bb-5	62.83 ± 0.86 b	48.42 ± 1.16 b
CCA-UFES/Bb-3	61.67 ± 4.37 b	47.49 ± 2.85 b
CCA-UFES/Bb-6	55.45 ± 2.39 c	43.63 ± 3.30 b
CCA-UFES/Bb-1	55.26 ± 4.09 c	41.24 ± 1.83 c
CCA-UFES/Bb-10	53.47 ± 4.39 c	40.11 ± 3.13 c
CCA-UFES/Bb-28	53.33 ± 2.35 c	47.33 ± 2.98 b
CCA-UFES/Bb-9	52.79 ± 2.25 c	38.86 ± 1.39 c
CCA-UFES/Bb-16	52.58 ± 5.85 c	40.30 ± 3.76 c
CCA-UFES/Bb-2	51.02 ± 3.18 c	36.10 ± 3.76 c
CCA-UFES/Bb-24	50.90 ± 4.79 c	29.03 ± 4.65 d
CCA-UFES/Bb-13	49.21 ± 3.77 c	34.14 ± 3.59 c
CCA-UFES/Bb-20	44.05 ± 6.87 d	29.33 ± 5.07 d
CCA-UFES/Bb-25	43.19 ± 5.24 d	27.76 ± 3.84 d
CCA-UFES/Bb-14	41.20 ± 1.79 d	29.07 ± 1.42 d
CCA-UFES/Bb-7	40.77 ± 4.01 d	25.66 ± 2.90 d
CCA-UFES/Bb-22	40.69 ± 2.93 d	28.66 ± 1.59 d
CCA-UFES/Bb-12	40.65 ± 2.69 d	28.31 ± 1.80 d
CCA-UFES/Bb-26	40.14 ± 5.06 d	31.11 ± 4.81 d
CCA-UFES/Bb-23	36.28 ± 7.37 d	24.88 ± 4.83 d
CCA-UFES/Bb-27	34.76 ± 2.59 d	21.81 ± 1.40d
CCA-UFES/Bb-21	31.68 ± 4.21 d	21.92 ± 3.62 d
CCA-UFES/Bb-19	27.21 ± 5.63 d	18.48 ± 5.19 d

Based on 5 replicates of 30 insects each. Means followed by the same letter do not differ significantly (Scott Knott test, $P < 0.05$).

Table 2. Median lethal concentration (LC_{50}, with CI 95%) and slope (± SE) for *Hypothenemus hampei* (Coleoptera: Scolytidae) sprayed with the most virulent isolates of *Beauveria bassiana*

Isolate	*N*	LC_{50} ($\times 10^4$ conidia/mL)	Slope	d.f.	χ^2
ESALQ-447	150	11.85 (8.71-17.80)	0.86 ± 0.08	4	3.60
CCA-UFES/Bb-4	150	6.90 (5.05-10.40)	1.00 ± 0.08	4	5.95
CCA-UFES/Bb-11	150	5.75 (4.03-9.25)	1.05 ± 0.08	4	9.74
CCA-UFES/Bb-15	150	4.00 (2.81-6.08)	0.95 ± 0.07	4	8.78
CCA-UFES/Bb-18	150	7.90 (5.23-14.46)	1.01 ± 0.08	4	10.20

Based on 5 replicates of 30 insects each. *N* = number of coffee berry borers tested; d.f. = degrees of freedom

Table 3. Number of conidia of *Beauveria bassiana* (mean ± SE) produced by selected isolates and their potential production increase (PPI) on corpses of *Hypothenemus hampei* (Coleoptera: Scolytidae)

Isolate	*N*	Conidia/insect ($\times 10^6$)	PPI
ESALQ-447	50	8.54 ± 0.20 a	2.12
CCA-UFES/Bb-18	50	5.07 ± 0.13 b	1.26
CCA-UFES/Bb-11	50	4.46 ± 0.14 bc	1.11
CCA-UFES/Bb-15	50	4.16 ± 0.14 c	1.03
CCA-UFES/Bb-4	50	4.02 ± 0.19 c	1.00

Based on 5 replicates of 10 corpses each. Means followed by the same letter do not differ significantly (Tukey test, $P < 0.05$). *N* = number of corpses tested; PPI = potential production increase (number of conidia produced by the isolate / number of conidia produced by isolate CCA-UFES/Bb-4).

whose production was estimated at 8.54×10^6 conidia/insect (Table 3). Thus the native isolates have a lower capacity to produce conidia than the standard isolate.

DISCUSSION

The confirmed and corrected mortality of *H. hampei*, caused by the studied *B. bassiana* isolates, showed wide variation in pre-screening (Table 1). The values of mortality were similar to those reported for the coffee berry borer by other authors

(La Rosa et al. 1997; Neves & Hirose, 2005). This is due to genetic variability between those entomopathogenic isolates, with effects on virulence (Alves 1998; Cruz et al. 2006; Carneiro et al. 2008; Monzón et al. 2008). Moreover, the phylogenic complexity of *B. bassiana* results in changes in the epidemiological relationship between the entomopathogen and its host (Rehner et al. 2006), with lower mortality due to the insect's immune system (Read & Taylor 2001; Narayanan 2004). Moreover, environmental factors constitute the primary selective forces shaping the adaptive evolution of *B. bassiana* (Bidochka et al. 2002). This indicates the importance of screening of native isolates for biological control.

The native isolates showed a greater instability between conidiogenesis and virulence, which was inversely proportional to the estimated LC_{50} (Table 2). The concentration of conidia in the suspension was directly related to the mortality of all isolates. High concentrations of conidia of *B. bassiana* imply a faster colonization of *H. hampei*, avoiding the proliferation of competing microorganisms (Neves & Hirose 2005).

The PPI shows the ability to increase the horizontal transmission of the fungus, which results in repetition of the cycle of the disease in the host population. A high rate of sporulation is important and necessary for the maintenance and dissemination of the pathogen in the field, contributing to the occurrence of epizooties (Oliveira et al. 2002).

The native isolates that cause the greatest mortality produce less conidia on the host (Table 3). This may reflect their low ability to persist in the field, indicating a trade-off between *H. hampei* colonization and conidiogenesis of *B. bassiana*. Tests to evaluate the performance of these isolates in the field are needed to determine their potential with other tactics of integrated management of this insect pest.

In conclusion, isolates CCA-UFES/Bb-4, Bb-11, Bb-15, and Bb-18 can be tested further to develop products for biological control of the coffee borer.

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