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Virulence and horizontal transmission of selected Brazilian strains of *Beauveria bassiana* against *Cosmopolites sordidus* under laboratory conditions

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Abstract

Virulence of Brazilian selected strains of *Beauveria bassiana* (Balsamo) Vuillemin, their infectivity at different temperatures and horizontal transmission potential on adults of *Cosmopolites sordidus* (Germar) (Coleoptera Curculionidae) were investigated. No relation was observed between groups formed by molecular analysis and the biological activity of tested *B. bassiana* against *C. sordidus* in preliminary screening bioassay. LC_{50} after a ten-day period was 3.9×10^7 and 2.6×10^8 conidia ml^{-1} for the selected strains CG1013 and CG1027, respectively. LT_{50} at 21, 25 and 29 °C was less than 7.4 days for a 2×10^8 conidia ml^{-1} suspension of CG1013. LT_{50} for CG1027 were up to 11 days for the same temperatures. Fungal transmission occurred among infected and healthy individuals. Density increase of inoculated insects in the population increased the mortality of non-inoculated insects for both strains. The number of conidia produced per cadaver was similar for the two strains. However, horizontal transmission efficacy of CG1013 was higher than that observed for CG1027. Mortality of non-inoculated adults at a ratio of 2:10 of infected to healthy adults was 10% for CG1027, after 20 days of exposure. At the same ratio, mortality of non-inoculated insects was 34% for CG1013. Our results indicate the potential of the strain CG1013 of *B. bassiana* as a biocontrol agent against the banana weevil.

Key words: biological control, microbial control, banana weevil, entomopathogenic fungi, screening.

Introduction

Banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera Curculionidae) is present in all banana-producing regions worldwide and is a serious pest of this crop (Gold *et al*., 2001). Populations of this pest grow slowly, becoming a serious problem after successive cycles of cultivation. The larvae feed in the rhizome, mainly in the central cylinder and cortex, leading to production losses (Gold *et al*., 2001; 2005) through bunch size reduction, plant weakening and snapping as well as increasing susceptibility to rot-causing fungi attacking the rhizome (Mesquita, 2003; Fancelli, 2004).
Studies in laboratory conditions have shown susceptibility of the insect for particular isolates of the fungus *Beauveria bassiana* (Balsamo) Vuillemin. Gold *et al.* (2001) compiled references from several studies with *B. bassiana* on banana weevil, mainly from Africa and the Americas, from the 1970s to the 1990s. However, the occurrence of natural epizootics of *B. bassiana* in banana-producing areas is not reported in the literature. Although frequently occurring in the insect's population, natural infection is low (Gold *et al*., 2001). Thus, applications are necessary to establish higher levels of the disease for long-term control of the pest. Some studies advocate the use of pseudostem baits combined with the pathogen for adult control (Brenes and Carballo, 1994; Godonou *et al*., 2000; Castrillón *et al*., 2002), despite some limitations and controversial results (Gold *et al*., 2001).

Inoculum introduction or fungi increase using pseudostem baits have been assessed for the control of the banana weevil in Brazil since the late 1980s (Batista Filho *et al*., 1991; 1995; Fancelli *et al*., 2004). Mass production of *B. bassiana* is being carried out in this country since the 1990s in solid fermentation systems, allowing large amounts of fungi to be released in the field. In 2007, the estimated domestic production was approximately 100 tons of biopesticides for the control of several agricultural pests, including *C. sordidus* (Alves *et al*., 2008).

Many factors may affect the dynamics of the disease on the banana weevil population under field conditions following introduction of *B. bassiana*, such as the abiotic factor predominating in the different cultivation regions and seasons of the year. The capacity of an isolate to cause high levels of infection and transmit the disease among individuals of a population can determine the success of the microorganism's self- dissemination system (Gold *et al*., 2001). Horizontal transmission of *B. bassiana* is a phenomenon occurring in several orders of insects, including the coleopterans (Long *et al*., 2000; Kreutz *et al*., 2004; Klinger *et al*., 2006). Some studies indicate the occurrence of horizontal transmission also among adults of *C. sordidus* in laboratory (Godonou *et al*., 2000) and in the field (Tinzaara *et al*., 2004; 2007). However, virulent strains which provide the formation of secondary outbreaks by the production of new infective structures in the field should be selected. First, this work aimed to evaluate the patho-

genicity of *B. bassiana* isolates on two different insect populations and the correlation between their biological activity and genetic variability using molecular markers. Secondarily, we studied the virulence of two Brazilian isolates of *B. bassiana* previously selected by means of response-concentration curves, their ability to produce conidia on dead insects and their transmission potential among infected and healthy adults of *C. sordidus*, under laboratory conditions.

Materials and methods

Collection and maintenance of *C. sordidus* in the laboratory

Pseudostem traps or "pitfall-type" traps were installed in commercial banana plantations in Tancredo Neves-BA (northeast region) and Ibiporã-PR (south region), Brazil, to collect adults and to determine the natural occurrence of entomopathogenic fungi within the population. Pseudostem traps consists in pieces of pseudostem with 50 cm long cut in the middle lengthwise (Mesquita, 2003). The traps, placed at the base of the plants, were inspected every 10 days and the adults collected were transferred to the laboratory. The collections were made between September 2008 and February 2009. Groups of 200 to 250 insects remained in quarantine in plastic containers containing sterile vermiculite and feed (pieces of pseudostem replaced weekly). Populations, from each collection site and time, were kept separately for a period no longer than four months. Inspections were made every three or four days and all the dead insects found in the containers were transferred to a wet chamber for disease confirmation to determine the level of natural infection by *B. bassiana* in each population. No infected insects were observed in the containers after the quarantine period. The healthy insects submitted to quarantine were used in the bioassays.

Preliminary selection of *B. bassiana* strains

Pathogenicity studies were carried out with 15 *B. bassiana* strains against the banana weevil populations collected in both regions described above. These strains originated from different regions of the country and from different hosts (table 1). These strains are housed in the Invertebrate Fungi Collection of Embrapa Genetic Resources and Biotechnology, Brasília, Brazil.

The strains used in the bioassays were obtained after cultivation of cryopreserved conidia on potato dextrose agar medium (PDA Difco Laboratories, Detroit, USA), followed by inoculation of banana weevil adults by immersion during 90s in a suspension of each isolate and posterior re-isolation from cadavers presenting fungal growth and conidiation. After 10 days of incubation (25 ± 0.5 °C and 12 h photophase), conidia were scraped off with a sterile metal spatula and immediately suspended in sterile water with Tween 80® (0.01% v/v) at a concentration of 1×10^9 conidia ml^{-1}, which allowed the separation of the isolates in groups. Twenty microliters of the conidia suspension for each isolate was placed on PDA medium and after 20 h of incubation (25 ± 0.5 °C and 12 h photophase) germination was assessed microscopically at 400× magnification by examining 300 conidia per replicate. Conidia were considered germinated when germ tubes were longer than conidial length. Conidia viability was above 93% for all isolates.

The insects were washed in sterile water and later inoculated for 90s by immersion in a suspension of each isolate. Control insects were immersed only in sterile water. Groups of 10 or 15 inoculated individuals remained in Petri dishes without feed for 24 h. After this period, the insects were transferred into plastic cups (150 ml) with a screened lid, and half-filled with sterilized vermiculite. Four replicates were prepared for each strain, totalling 50 or 60 insects. Pieces of pseudostem were used as feed. The plastic cups remained in a climatic chamber at 25 ± 0.5 °C and 24h in dark through-

Table 1. Origin of *B. bassiana* strains used in the screening studies for *C. sordidus* control.

Strain [1]	Original host	Location
CG1026	*Membracis* sp.	Cruz das Almas - BA
CG1027 (=ESALQ PL63 [2])	*Atta* sp.	Piracicaba - SP
CG1013	*C. sordidus*	Brasília - DF
CG1024	*Metamasius hemipterus* (L.)	Cruz das Almas - BA
CG1037 (=IBCB146)	Soil	Cascavel - PR
CG17	*Hypothenemus hampei* (Ferrari)	Piracicaba - SP
CG1036 (=IBCB 74)	*Lissorhoptrus oryzophilus* Kuschel	Japan
CG451 (=ESALQ 447)	*Solenopsis invicta* Buren	Mato Grosso - MT
CG1032	Unknown	Cruz das Almas - BA
CG1022 (=CNPMF 01)	Unknown	Cruz das Almas - BA
CG919 (=IBCB 66)	*H. hampei*	São José do Rio Preto - SP
CG1031 (=CNPMF 30)	*Castnia invaria volitans* Lamas	Cruz das Almas - BA
CG1034 (=CPATSA 01)	*C. sordidus*	Petrolina - PE
CG1030 (=CNPMF 20)	*C. sordidus*	Cruz das Almas - BA
CG1033	Unknown	Cruz das Almas - BA

[1]Code in other collections.
[2]*B. bassiana* strain available in Brazilian market.

out the experimental period. Insect mortality was assessed every five days during a period of 35 days and the cadavers were transferred to wet chamber for disease confirmation.

Assessment of the genetic variability of *B. bassiana* strains

For genetic variability assessment studies by means of random molecular markers (RAPD and AFLP) analysis, monosporic colonies of the 15 strains were prepared in PDA medium using the material obtained from re-isolation of the dead insect, described above. Ten day-culture discs were inoculated into Erlenmeyer flask with 250mL of SDAY liquid media (glucose 1%; malt extract 0.3%; peptone 0.5%; yeast extract 0.3%) and incubated at 25 ± 0.5 °C at 250 rpm for 4 days. Mycelium samples were collected on a filter-paper using vacuum filtration, frozen at –70 °C, lyophilized and stored at –20 °C. Around 25 mg of lyophilized mycelia of each sample were frozen in liquid nitrogen, crushed in a mortar and the total genomic DNA extracted using a modification of the cetyltrimethylammonium bromide (CTAB) DNA extraction protocol described by Boucias *et al.* (2000). PCR reactions were performed in 30 µl volume, with 15 ng of each template, using the PTC-100 programmable thermal controller (MJ Research), and a temperature profile described by Tigano-Milani *et al.* (1995). Amplifications were performed using the following reaction mix: 1 µm of 10 mer primer (Operon Technologies), 200 µm of each dNTP (Pharmacia Biotec), 2 units of Taq DNA Polymerase (Cenbiotec) and 1× of the recommended polymerase buffer. The following twenty-six random 10 mer oligonucleotide primers were selected for this study: A4, A7, A8, AB11, AB17, B6, B7, B20, C9, C16, C18, D5, D7, D16, E4, E9, G2, G3, G5, J10, J17, J19, K1, K4, K6, K7, K20, L8, M20, N7, P1, P2, R7 and R8. Primers codes indicate a specific short sequence of nucleotides.

For AFLP analysis, 0.3-2.0 µg of genomic DNA was digested with *EcoRI* and was ligated to *EcoRI* adaptors in a single overnight reaction at 37 °C (Suazo and Hall, 1999). The digestion-ligation reactions were diluted with TE buffer to a final volume of 200 µl and stored at –20 °C. Twenty-two primers were used, consisting of the *EcoRI* adapter sequence GACTGCGTACCAATTC plus three or six 3' selective nucleotides (AGT, ACT, ATT, CAG, CCT, TCG, AGTAGG, AGTATT, AGTAAC, AGTCGA, AGTGGG, AGTCTC, AGTCAT, AGTTTA, AGTTTG, AGTGAC, AGTGTG, AGTCCG, AGTTCT, AGTGAG, AGTTGC and AGTCAC). Amplification reactions were conducted in a volume of 25 µl containing the anchor-annealed *EcoRI* fragments, primer and Taq DNA polymerase using the conditions established by Suazo and Hall (1999). Amplified products were separated by electrophoresis in 2% agarose gel prepared in 0.5× Tris-borate-EDTA (TBE) buffer, and visualized by staining with ethidium bromide and photographed under UV light.

DNA fingerprints were scored directly from the photographs. Only well-resolved products were scored. The presence or absence of each fragment was considered as an independent character. RAPD markers were analyzed using NTSYS-pc V1.8 (Rohlf, 1993). A similarity matrix was calculated using Jaccard similarity coefficient. Clustering was performed using the non-weighted mean pair group arithmetic mean method (UPGMA).

Determination of LC_{50} and LT_{50} for *B. bassiana* strains CG1013 and CG1027

The strains CG1013 and CG1027 were produced in PDA medium. Suspension preparation, insect inoculation, and recipients used were the same as already described. For the response-concentration assays, the concentrations of 2×10^7, 6.3×10^7, 2×10^8, 6.3×10^8 and 2×10^9 conidia ml^{-1} were established based on preliminary isolate selection tests, at temperature of 25 ± 0.5 °C and 24 h in dark, with mortality assessment and remove of the cadavers carried out every two days until the 10^{th} day. In the response-time studies, the concentration 2×10^8 conidia ml^{-1} was used and each bioassay was submitted to temperatures of 21, 25 or 29 ± 0.5 °C, and 24 h scotophase throughout the experimental period, with daily mortality being assessed from the fourth and up to the seventeenth day after inoculation. For determination of LC_{50} and LT_{50} of each strain, 16 adult insects were used, with four replicates. To estimate the number of conidia adhered to the body of the insects, four adult insects were immersed for 90s at concentrations of 2×10^8 and 2×10^9 conidia ml^{-1}, and, after one hour, washed individually in 5 ml of sterile water with Tween 80® 0.1% v/v, with the suspension being stirred for 3 minutes in vortex and 20 minutes in ultrasound bath. The suspension obtained was diluted when necessary and conidia count performed in a Neubauer chamber. The bioassays conducted to determine LC_{50} and LT_{50} and to estimate the number of conidia adhered to the body of the inoculated insects collected in the northeast region were repeated twice each, at different dates.

Horizontal transmission of *B. bassiana* among *C. sordidus* adults

The studies of transmission of the strains CG1013 and CG1027 among *C. sordidus* adults collected in Bahia were carried out under controlled laboratory conditions (25 ± 0.5 °C and 24 h scotophase). Conidia production of the strains, suspension preparation and insect inoculation were performed as previously described, using the concentration of 2×10^9 conidia ml^{-1} in all treatments.

The treatments simulated different proportions of individuals infected in one population, and consisted of 0, 10, 20, 30, 40 and 50% of infected individuals in groups of 30 insects, with four replications. The infected adults were marked before inoculation, with adhesive paint (Colorama L´Oreal, Rio de Janeiro, RJ, Brazil) on the elytra to differentiate them from the healthy ones. After 24 h of inoculation, both the treated and healthy insects were released at opposite corners of plastic Gerbox (11 × 11 × 3.5 cm), with 2/3 of the volume filled with sterilized vermiculite. To avoid manipulating the insects during feed exchange and interfering in the transmission dynamics, feed consisted of small pieces of pseudostem (15 g) replaced every five days. The number of marked and non-marked dead insects was assessed at day 20, by transferring the cadavers to a wet chamber for confirma-

tion of the disease caused by the pathogen. All the live insects remained for 10 extra days in plastic cups containing sterilized vermiculite and feed. After this period, the dead insects were also transferred to the wet chamber to confirm the disease. The transmission bioassays for both isolates were repeated twice each, at different dates.

In addition, 15 insects inoculated with each strain were maintained over the same 20 day period, and under the same conditions of the transmission experiments. Twelve dead insects of each strain presenting typical signs of the pathogen (mycelia growth and conidiogenesis) were selected to determine the number of conidia produced per cadaver. Washing was performed in 5 ml of sterile water with Tween 80® (0.1% v/v), by stirring the suspension for 3 minutes in vortex and 10 minutes in ultrasound bath. The suspension was diluted 100× and conidia were counted in a Neubauer chamber.

Statistical analysis

All experiments had a completely randomized design (CRD). Confirmed mortality data (only cadavers presenting fungal growth and conidiation) from the preliminary strain selection bioassays were submitted to analysis of variance and the means compared by the Scott-Knott test ($p < 0.05$, data transformed $\sqrt{}$ of $x + 1$). Confirmed mortality data from concentration and time-response were analyzed by a generalized linear model (GLM) of mixed effects fit by a restricted maximum likelihood procedure, attributing to the variable response a binomial distribution (*logit*) in the statistical language program R (R version 2.8 2006). To study the transmission potential between infected and healthy insects under laboratory conditions, a generalized linear model was used (McCullagh and Nelder, 1989), where binomial distribution was attributed to the response. Based on the estimates of this model, the number of infected insects sufficient to infect the population with 50% of the disease was calculated. The quality of the fit model was assessed by observing the existence of correlation among the observations and the influence of atypical points. To correct the super dispersion present in the data, the quasi-verisimilitude method was used to estimate the heterogeneity factor (McCullagh and Nelder, 1989).

Results

Preliminary selection of *B. bassiana* strains

The mean levels of natural infection caused by *B. bassiana* in the populations collected in Ibiporã-PR and Tancredo Neves-BA were 0.70% and 0.64%, respectively, during the quarantine period. Only a single adult insect collected in Bahia (< 0.01%) presented infection caused by *Metarhizium* sp. After quarantine, no insects were found to present infection in the populations kept in the laboratory.

Strains CG1013, CG1027, CG1026 and CG1024 resulted in the highest mortality of weevils collected from northeast region (BA state), i.e., > 73%. Similarly, high mortality, i.e., > 75% was obtained with these same strains, except CG1026, against banana weevils from south region (PR state) (table 2). Overall, the strains grouped similarly in relation to mortality of the geographically distinct populations, with variations below 25%. No dead insects were observed among the controls.

Evaluation of genetic variability

In the analyses of the molecular markers, 210 fragments were selected for RAPD and 223 fragments for AFLP. Since the grouping analyses were similar for both markers, the results were analyzed together, showing high variability among the strains (similarity > 69%), but without any observation being made of groupings related to pathogenicity for banana weevil or origin of the strains (figure 1).

Table 2. Mortality of *C. sordidus* adults from two populations exposed to different *B. bassiana* strains under laboratory conditions after 35 days (concentration of 1×10^9 conidia ml^{-1}; 25 ± 0.5 °C and dark).

Strains	Mortality (%)* ± SEM	
	Population BA[1]	Population PR[2]
CG1026	88.3 ± 5.4 a	61.7 ± 4.0 c
CG1027	85.6 ± 6.7 a	75.0 ± 9.6 b
CG1013	75.0 ± 6.7 a	91.7 ± 2.1 a
CG1024	73.3 ± 4.8 a	96.7 ± 2.1 a
CG1037	65.0 ± 3.9 b	80.0 ± 5.8 b
CG17	63.3 ± 1.9 b	61.7 ± 4.0 c
CG1036	63.3 ± 4.5 b	86.7 ± 2.1 b
CG451	46.7 ± 5.2 b	53.3 ± 6.4 c
CG1032	40.3 ± 3.9 c	60.0 ± 5.3 c
CG1022	29.5 ± 5.4 c	60.0 ± 3.2 c
CG919	28.3 ± 4.0 c	53.3 ± 4.1 c
CG1031	10.3 ± 2.3 d	48.3 ± 3.1 c
CG1034	10.0 ± 3.5 d	73.3 ± 4.2 b
CG1030	6.0 ± 2.4 d	28.3 ± 8.7 d
CG1033	6.0 ± 2.4 d	73.3 ± 6.7 b
CV (%)	23.9	19.9

* Scott-Knott (5%). Data transformed (sqrt of x + 1).
[1]BA – adults collected in Tancredo Neves, Bahia (BA) state.
[2]PR – adults collected in Ibiporã, Paraná (PR) state.

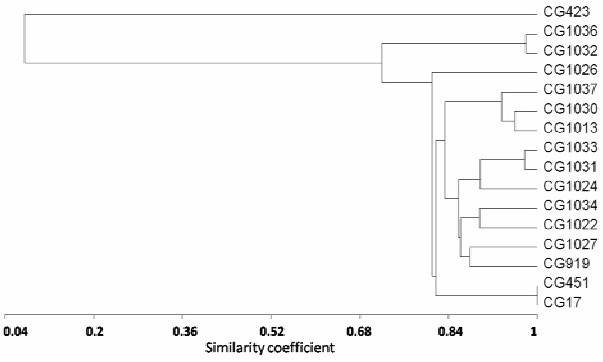

Figure 1. Dendrogram (UPGMA) built with data analyzed by RAPD and AFLP indicating the relationship between *B. bassiana* tested strains.

Determination of LC_{50} and LT_{50} for pre-selected isolates of *B. bassiana*

Strains CG1013 and CG1027 were selected for response-concentration and response-time studies for the banana weevil. Besides confirmed mortality, other criteria, such as domestic market availability and conidia productivity in the culture medium, were used to select the strains for the next steps.

Strain CG1013 was the most virulent, compared to CG1027, with estimates of LC_{50} of 3.9×10^7 conidia ml^{-1} (d = 58.08, P = 2.00e-12, df = 35, FL = $3.4 \times 10^7 - 4.4 \times 10^7$) and 2.6×10^8 conidia ml^{-1} (d = 54.57, P = 2.26e-13, df = 35, FL = $2.1 \times 10^8 - 3.2 \times 10^8$), respectively (figure 2). Increased mortality on the 10th day for strain CG1013 was 28.9%, from a concentration of 2×10^8 conidia ml^{-1} (66.4%) to 2×10^9 conidia ml^{-1} (95.3%). For isolate CG1027, increase was 29.7% (from 45.3% to 75%).

Strain CG1013 also presented a better performance for the three temperatures tested, with LT_{50} of 7.3 days for 21 °C; 6.2 days for 25 °C and 29 °C. For strain CG1027, LT_{50} values varied from 11.4 to 13.7 days between the lowest and highest temperatures (table 3). Temperatures 25 °C and 29 °C were more favourable to the action of strain CG1013 than temperature 21 °C. In the case of CG1027, originated from a region with lower mean temperatures (southeast), temperatures of 21 °C and 25 °C were more favourable than 29 °C. Conidia production per cadaver was similar for strains CG1013 and CG1027, with mean values of $5.07 \times 10^8 \pm 0.41$ and $4.83 \times 10^8 \pm 0.64$ conidia per cadaver, respectively.

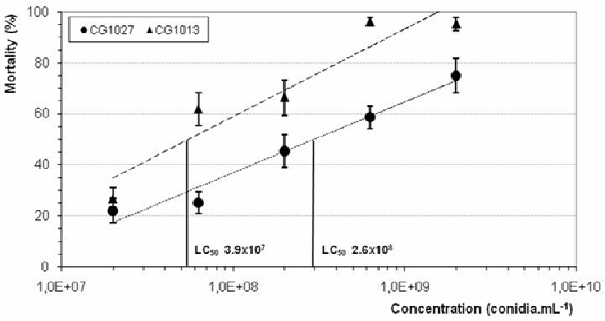

Figure 2. Mortality of *C. sordidus* adults treated with different conidia concentrations of the strains CG1013 and CG1027 of *B. bassiana*, 10 days after inoculation (25 ± 0.5 °C and dark). Symbols depict mean ± SEM.

Table 3. Lethal-time (LT_{50}) for *C. sordidus* adults inoculated with *B. bassiana* strains CG1013 and CG1027 at temperatures of 21, 25 and 29 ± 0.5 °C (concentration of 2×10^8 conidia ml^{-1} and dark).

	Temperature	Days after inoculation		
		LT_{50}	FL[1]	
CG1013	21 °C	7.33	7.16	7.50
	25 °C	6.21	6.04	6.38
	29 °C	6.17	5.92	6.42
CG1027	21 °C	11.44	11.16	11.73
	25 °C	11.31	10.98	11.64
	29 °C	13.71	13.19	14.24

[1] FL – fiducial limits.

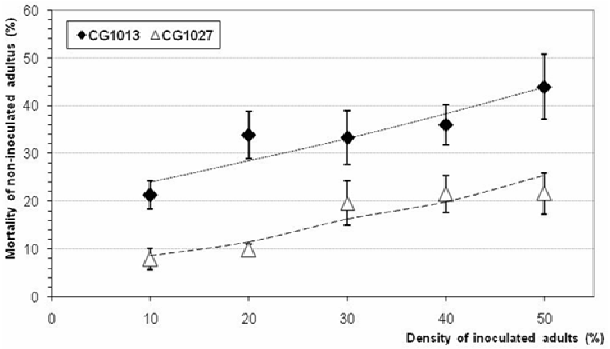

Figure 3. Relationship between densities of *B. bassiana* inoculated adults and mortality of *C. sordidus* non-inoculated adults after 20 days (25 ± 0.5 °C and dark). Lines indicate the fit models. Symbols depict mean ± SEM.

Horizontal transmission of *B. bassiana* among *C. sordidus* adults

Mortality of the insects marked and inoculated with strains CG1013 and CG1027 was above 93.7%, with, on average, 90.4% and 95.7% of the cadavers showing fungal growth and conidiation from intersegmental regions. Most of the dead insects killed by the pathogen (95.5%) were found on the vermiculite surface. Control mortality was below 5.5% and no dead control insect showed mycosis.

Figure 3 shows that both linear models fit well, because the ratio of infected insects is very close to the estimated ratio. Transmission of the disease from the inoculated to the non inoculated insects occurred under laboratory conditions for strains CG1013 and CG1027, with P values of 0.00230 and 0.00012, respectively. However, CG1013 was more effective in transmitting the fungus from inoculated insects (marked) to non inoculated insects. Significant differences were observed between the two strains regarding the proportion of insects that contracted the infection (P = 2.14e-08). Strain CG1027 is estimated to be approximately 38% less effective in transmitting the disease than isolate CG1013. With only 10% of inoculated insects in the population, around 20% of the non-inoculated insects acquired the disease for strain CG1013. This index reaches 34%, when the proportion of infected insects increases to 20%. As for strain CG1027, this index reaches 20% only in the proportion of 30% of infected individuals.

Discussion

The results of this study suggest that horizontal transmission of the disease among individuals in a population is directly related to the biological activity or virulence of the isolate and activity under a wide range of temperature may be determinant in selecting the fungal strain to be used in the field. Other factors, such as isolate location or the number of propagules produced on the cadaver were less important for select a potential strain.

Several works have confirmed the virulence of strains of *B. bassiana* for *C. sordidus*; however, comparisons

among the results obtained are difficult to make due to the variation among the methodologies applied in the laboratory bioassays. Adult mortality levels between 73 and 100% were obtained by Brenes and Carballo (1994) in studies on selection of isolates of *B. bassiana* aiming to control *C. sordidus*, for the same concentration and inoculation method, similar to those obtained in this study. Gold *et al.* (2001) reviewed several studies, with few indicating comparative parameters, such as dose or concentration-response and time-response. Of the results compiled in the review using adult immersion in conidia suspension, mortalities between 15% and 100% were observed for suspensions between 1×10^7 and 1×10^9 conidia ml^{-1}. Strains with better performance promoted up to 50% banana weevil mortality at concentrations between 1.1×10^7 and 4.6×10^7 conidia ml^{-1}, similar to that observed for the strain CG1013.

Pathogenic action of the fungus *Beauveria* occurs within a wide range of temperature. Infections caused by the fungus also occur at various temperatures, with different intensity for different isolates and host. *Scolytus scolytus* (F.) adults are susceptible to infection caused by *B. bassiana* when exposed to temperatures between 15 °C and 25 °C (Doberski, 1981). Similarly to fungus growth *in vitro*, temperatures close to 25 °C are more favorable to disease occurrence. Increase from 26 °C to 32 °C affected the activity of isolate UEL25 of *B. bassiana* against adults of *Alphitobius diaperinus* (Panzer), reducing mortality from 82.2% to 26.6% (Alexandre *et al*., 2006). In this study, this effect was visible for strain CG1027, in which LT_{50} increased significantly to 29 °C, but not for CG1013, originating from a warmer region. Such information is important when deciding to use a particular isolate in function of the place where it will be applied. This is a factor to be considered for banana production in Brazil because this crop is grown in cooler regions below the Tropic of Capricorn as well as in the Amazon region.

We do not observe any relation between the groups formed by molecular analysis and the biological activity of tested strains against *C. sordidus*. In comparative evaluations between native Brazilian isolates, groupings formed by analyzing RAPD markers also do not present any relation with virulence against *Triatoma infestans* Klug (Luz *et al*., 1998). Similarly, other studies with *B. bassiana* show this lack of groupings correlated with the host or geographic region of the isolate (Coates *et al*., 2002a; 2002b). However, studies using molecular markers to evaluate the occurrence of groupings of isolates of *Beauveria* for different geographic regions, host specificity and virulence against a particular target are still controversial. Other works suggest such correlation (Wang *et al*., 2003; 2005; Estrada *et al.*, 2007; Santoro *et al*., 2008). Variation in susceptibility to entomopathogenic fungi among populations of different geographic regions is an aspect scarcely studied as are the mechanisms that may be involved in this process. In some cases, no difference is found between the actions of an isolate for distinct populations, as observed by Poprawski and Walker (2001) for *B. bassiana* on populations of *Bemisia* in laboratory. Other studies show the susceptibility of insect populations to *Beauveria*, as suggested by Keller *et al.* (1999) for the scarabaeid pest *Melolontha melolontha* (L.) and by Tinsley *et al.* (2006) for *Drosophila melanogaster* Meigen. In both cases, the authors indicate that the difference in susceptibility of geographically distinct populations results from the coevolution between the pathogen and the host, which occurred under specific conditions of the regions of origin and due to the continuous exposure of the population to the fungus. However, in this study it was observed a low incidence of *B. bassiana* occurring naturally for the two populations and at quite similar levels. Fungus applications for the control of banana weevil in Brazil are in most cases sporadic, with no report on pathogen releases being available for the two areas where the insects were collected.

Although the experimental conditions in this study do not represent the insect and fungus dynamics in the field, horizontal transmission was clear for both selected isolates. Under self-inoculation and dissemination of entomopathogenic fungi, horizontal transmission is a decisive factor for disease evolution in the pest population. This aspect is very relevant in banana culture, in which individual inoculation is performed using traps, with the inoculated individuals dying after leaving the trap, disseminating the fungus. Trap characteristics, association with attractive compounds, pest habits, host density, microbial product formulation, etc., may interfere in the transmission rate. As observed in this study, a positive correlation between cadaver density and infection of non-inoculated individuals was also reported by Long *et al.* (2000) and by Klinger *et al.* (2006) for *Leptinotarsa decemlineata* (Say) in laboratory bioassays with *B. bassiana*. The capacity a strain has of causing high mortality levels, reproducing on the host, and making its infective structures available, can also have a direct relation with its horizontal transmission potential. However, in this study, conidia production per cadaver was similar for strains CG1013 and CG1027. Long *et al.* (2000), studying larvae of *Leptinotarsa*, observed that the greater number of conidia of *B. bassiana* produced on the cadaver may not influence the transmission rate. A reduction of 86.1% of the conidia on the cadavers of the infected larvae did not cause lower mortality of the non-inoculated insects placed in the same arena. The best performance of strain CG1013 in the transmission of the disease was probably on account of its higher virulence, as confirmed in the concentration and response- time bioassays. Thus, although the mortality of the marked (inoculated) insects and the inoculum produced by the cadavers was the same for both strains, the transmission rate for CG1013 was higher.

Death of the infected insects on the surface and close to the feeding sites favored pathogen reproduction and was important for the transmission of the disease, since the cadavers covered with conidia remain in places where the non-inoculated insects were present for feeding and mating. Under field conditions, Tinzaara *et al.* (2004; 2007) observed fungus transmission from infected adults to healthy ones, and verified that most cadavers were found in the leaf sheath and at the base of the banana plants, feeding and oviposition sites.

An important aspect related with the use of *B. bassiana* to control *C. sordidus* in commercial banana cultivations is the economic viability of the proposed method. Initial evaluations detailing the potential of the pathogen strains for a particular target are indispensable for the definition of the best material to be used, as shown in this study for strain CG1013. Besides the greater virulence of this strain, the inoculated adults in a population were found to effectively transmit the disease to non-inoculated individuals, under laboratory conditions. Further studies are being conducted aiming to determine a practical and low-cost method of fungus release in banana production areas, taking into account the specifics of the fruit production model in Brazil.

References

ALEXANDRE T. M., ALVES L. F. A., NEVES P. M. O. J., ALVES S. B., 2006.- Efeito da temperatura e cama do aviário na virulência de *Beauveria bassiana* (Bals.) Vuill. e *Metarhizium anisopliae* (Metsch.) para o controle do cascudinho (*Alphitobius diaperinus*) (Panzer) (Coleoptera: Tenebrionidae).- *Neotropical Entomology*, 35 (1): 75-82.

ALVES S. B., LEITE L. G., BATISTA FILHO A., ALMEIDA J. E. M., MARQUES E. J., 2008.- Produção massal de fungos entomopatogênicos na América Latina, pp. 215-237. In: *Controle microbiano de pragas na América Latina: avanços e desafios* (ALVES S. B., LOPES R. B., Eds).- Felaq, Piracicaba, SP, Brazil.

BATISTA FILHO A., SATO M. E, LEITE L. G., RAGA A., PRADA W. A., 1991.- Utilização de *Beauveria bassiana* (Bals.) Vuill. no controle do moleque da bananeira *Cosmopolites sordidus* Germar, 1824 (Coleoptera: Curculionidae).- *Revista Brasileira de Fruticultura*, 13: 35-40.

BATISTA FILHO A., LEITE L. G., RAGA A., SATO M. E., 1995.- Enhanced activity of *Beauveria bassiana* (Bals.) Vuill. associated with mineral oil against *Cosmopolites sordidus* (Germar) adults.- *Anais da Sociedade Entomolológica do Brasil*, 24 (2): 405-408.

BOUCIAS D., STOKES C., SUAZO A., FUNDERBURK J., 2000.- AFLP analysis of the entomopathogen *Nomuraea rileyi*.- *Mycologia*, 92: 638-648.

BRENES S., CARBALLO V. M., 1994.- Evaluación de *Beauveria bassiana* (Bals) para el control biológico del picudo del plátano *Cosmopolites sordidus* (Germar).- *Manejo Integrado de Plagas*, 31: 17-21.

CASTRILLÓN C., URREA C. F., CARDONA J. E., ZULUAGA L. E., MORALES H., ALZATE G., 2002.- Potencial del hongo nativo entomopatógeno *Beauveria bassiana*, como un componente de manejo integrado del Picudo Negro (*Cosmopolites sordidus*) en Colômbia, pp.278-283. In: *XV Memórias de la Reunion de la Acorbat*, Medellín, Colombia.

COATES B. S., HELLMICH R. L., LEWIS R. C., 2002a.- Allelic variation of a *Beauveria bassiana* (Ascomycota: Hypocreales) minisatellite is independent of host range and geographic origin.- *Genome*, 45: 125-132.

COATES B. S., HELLMICH R. L., LEWIS R. C., 2002b.- *Beauveria bassiana* haplotype determination based on nuclear rDNA internal transcribed spacer PCR-RFLP.- *Mycological Research*, 106 (1): 40-50.

DOBERSKI J., 1981.- Comparative laboratory studies on three pathogens of the elm beetle *Scolytus scolytus*. Effect of temperature and humidity on infections by *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces farinosus*.- *Journal of Invertebrate Pathology*, 37: 195-200.

ESTRADA M. E., CAMACHO M. V., BENITO C., 2007.- The molecular diversity of different isolates of *Beauveria bassiana* (Bals.) Vuill. as assessed using inter-microsatellites (ISSRs).- *Cellular & Molecular Biology Letters*, 12: 240-252.

FANCELLI M., 2004.- Pragas e seu controle, pp.195-208. In: *O cultivo da bananeira* (BORGES A. L., SOUZA L. S., Eds).- Embrapa Mandioca e Fruticultura, Cruz das Almas, BA, Brazil.

FANCELLI M., DIAS A. B., JESUS S. C., DELALIBERA JÚNIOR I., NASCIMENTO A. S., SILVA S. O., 2004.- *Controle biológico de* Cosmopolites sordidus *(Germ.) (Coleoptera: Curculionidae) pelo fungo* Beauveria bassiana *(Bals.) Vuill.*- Comunicado Técnico 102, Embrapa Mandioca e Fruticultura, Cruz das Almas, BA, Brazil.

GODONOU I., GREEN K. R., ODURO K. A., LOMER C. J., AFREH-NUAMAH K., 2000.- Field evaluation of selected formulations of *Beauveria bassiana* for the management of the banana weevil (*Cosmopolites sordidus*) on plantain (*Musa* sp., AAB group).- *Biocontrol Science and Technology*, 10: 779-788.

GOLD C. S., PENA J. E., KARAMURA E. B., 2001.- Biology and integrated pest management for the banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae).- *Integrated Pest Management Review*, 6: 79-155.

GOLD C. S., RAGAMA P. E., COE R., RUKAZAMBUGA N. D. T. M., 2005.- Selection of assessment methods for evaluating banana weevil *Cosmopolites sordidus* (Coleoptera: Curculionidae) damage on highland cooking banana (*Musa* spp., genome group AAA-EA).- *Bulletin of Entomological Research*, 95: 115-123.

KELLER S., SCHWEIZER C., SHAH P., 1999.- Differential susceptibility of two *Melolontha* populations to infections by the fungus *Beauveria brongniartii*.- *Biocontrol Science and Technology*, 9: 441-446.

KLINGER E., GRODEN E., DRUMMOND F. A., 2006.- *Beauveria bassiana* horizontal infection between cadavers and adults of the Colorado Potato Beetle, *Leptinotarsa decemlineata* (Say).- *Environmental Entomology*, 35 (4): 992-1000.

KREUTZ J., ZIMMERMANN G., VAUPEL O., 2004.- Horizontal transmission of the entomopathogenic fungus *Beauveria bassiana* among the spruce bark beetle, *Ips typographus* (Coleoptera: Scolytidae) in the laboratory and under field conditions.- *Biocontrol Science and Technology*, 14: 837-848.

LONG D. W., GRODEN E., DRUMMOND F. A., 2000.- Horizontal transmission of *Beauveria bassiana* (Bals.) Vuill.- *Agricultural and Forest Entomology*, 2: 11-17.

LUZ C., TIGANO M. S., SILVA I. G., CORDEIRO C. M. T., ALJANABI S. M., 1998.- Selection of *Beauveria bassiana* and *Metarhizium anisopliae* isolates to control *Triatoma infestans*.- *Memórias do Instituto Oswaldo Cruz*, 93 (6): 839-846.

MC CULLAGH P., NELDER J. A., 1989.- *Generalized Linear Models*. 2nd ed.- Chapman & Hall, New York, USA.

MESQUITA A. L. M., 2003.- *Importância e métodos de controle do "moleque" ou broca-do-rizoma-da-bananeira*.- Circular Técnica 17 Embrapa Agroindústria Tropical, Fortaleza, CE, Brazil.

POPRAWSKI T., WALKER J., 2001.- Host plant effects on activity of the mitosporic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* against two populations of *Bemisia* whiteflies (Homoptera: Aleyrodidae).- *Mycopathology*, 151 (1): 11-20.

R DEVELOPMENT CORE TEAM, 2006.- *R: a language and environment for statistical computing*.- R Foundation for Statistical Computing, Vienna, Austria.

ROHLF F. J., 1993.- *NTSYS-pc v. 1.8. Numerical taxonomy and multivariate analysis system*.- Applied Biostatistics Inc., Setauket, NY, USA.

SANTORO P. H., NEVES P. M. O. J., ALEXANDRE T. M., SARTORI D., ALVES L. F. A., FUNGARO M. H. P., 2008.- Selection of *Beauveria bassiana* isolates to control *Alphitobius diaperinus*.- *Journal of Invertebrate Pathology*, 97: 83-90.

SUAZO A., HALL H. G., 1999.- Modification of the AFLP protocol applied to honey bee (*Apis mellifera* L.) DNA.- *BioTechniques*, 26: 704-709.

TIGANO-MILANI M. S., HONEYCUTT R. J., LACEY L. A., ASSIS R., MCCLELLAND M., SOBRAL B. W. S., 1995.- Genetic variability of *Paecilomyces fumosoroseus* isolates revealed by molecular markers.- *Journal of Invertebrate Pathology*, 65: 274-282.

TINSLEY M. C., BLANFORD S., JIGGINS F. M., 2006.- Genetic variation in *Drosophila melanogaster* pathogen susceptibility.- *Parasitology*, 132: 767-772.

TINZAARA W., GOLD C. S., NANKINGA C., DICKE M., VAN HUIS A., RAGAMA P. E., KAGEZI G. H., 2004.- Integration of pheromones and entomopathogenic fungus for the management of the banana weevil.- *Uganda Journal of Agricultural Sciences*, 9: 621-629.

TINZAARA W., GOLD C. S., DICKE M., VAN HUIS A., NANKINGA C., KAGEZI G. H., RAGAMA P. E., 2007.- The use of aggregation pheromone to enhance dissemination of *Beauveria bassiana* for the control of the banana weevil in Uganda.- *Biocontrol Science and Technology*, 17 (2):111-124.

WANG C., SHAH F. A., PATEL N., LI Z., BUTT T. M., 2003.- Molecular investigation on strain genetic relatedness and population structure of *Beauveria bassiana*.- *Environmental Microbiology*, 5 (10): 908-915.

WANG S., MIAO X., ZHAO W., HUANG B., FAN M., LI Z., HUANG Y., 2005.- Genetic diversity and population structure among strains of the entomopathogenic fungus, *Beauveria bassiana*, as revealed by inter-simple sequence repeats (ISSR).- *Mycological Research*, 109 (12): 1364-1372.

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