Research

Infection of *Anastrepha ludens* (Diptera: Tephritidae) adults during emergence from soil treated with *Beauveria bassiana* under various texture, humidity, and temperature conditions

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Abstract

Anastrepha ludens (Loew) (Diptera: Tephritidae) is a major pest of mango and citrus in Mexico. It is usually controlled by applying several strategies in a holistic approach. The entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuill. (Cordycipitaceae) is a biocontrol agent that infects adults of many tephritids pests including *A. ludens*. In this work, we carried out 4 experiments with the objective of estimating the mortality of *A. ludens* adults when different conidial concentrations of the fungus *B. bassiana* were applied to the soil at different humidity, texture, and temperature conditions under controlled laboratory conditions. In a first experiment, mortality ranged from 38.3 to 79.8%, with the highest mortality occurring at a concentration of 800 mg of formulated conidia per kg of soil, while the lowest mortality was recorded at a concentration of 400 mg/kg. In a second experiment, when soils with different texture were investigated at 12% humidity, the pathogenicity of *B. bassiana* on *A. ludens* adults was similar in sandy clay soil and sandy loam (64.0% mortality), but was lower in sandy soil with 36.0% mortality. In a third experiment on humidity tests, fly mortality of *A. ludens* adults ranged from 43.0 to 79.8%, showing the highest mortality at 12% soil humidity and the lowest at 21%. Mortality was similar between 15 to 35 °C (>83%). Emergence of *A. ludens* adults in the controls ranged between 94.5 and 98.3%. The soil humidity was the major factor that significantly affected fungal efficacy. The study demonstrated the potential of using *B. bassiana* applied to the soil for management of *A. ludens* and other tephritids. However, *B. bassiana* must be evaluated under field conditions before recommendations can be made.

Key Words: microbial control; entomopathogenic fungus; Mexican fruit fly; mycosis; key factor

Resumen

Anastrepha ludens (Loew) (Diptera: Tephritidae) es la principal plaga del mango y cítricos en México. Actualmente para su manejo se aplican varias estrategias de control con un enfoque holístico. El hongo entomopatógeno, *Beauveria bassiana* (Bals.-Criv.) Vuill. (Cordycipitaceae) es un agente de control biológico que ataca adultos de muchas tefrítidos que son plagas, incluyendo *A. ludens*. En este trabajo, realizamos cuatro experimentos y el objetivo fue estimar la mortalidad de adultos de *A. ludens* con diferentes concentraciones de conidios del hongo *B. bassiana*, que fueron aplicados en suelo con diferentes humedades, texturas y temperatura, en condiciones controladas de laboratorio. En el primer experimento, la mortalidad varió de 38.3 a 79.8%, ocurriendo la mortalidad más alta a la concentración de 800 mg del formulado de conidios/kg de suelo, mientras que la mortalidad más baja se registró en la concentración de 400 mg/kg de suelo. En el segundo experimento, cuando se evaluaron diferentes texturas de suelo con 12% de humedad, la patogenicidad de *B. bassiana* sobre adultos de *A. ludens* fue similar en suelo areno-arcilloso y areno-limoso (64.0% de mortalidad), pero fue más bajo en suelo arenoso donde hubo 36.0% de mortalidad. En el tercer experimento, cuando se evaluó la humedad del suelo, la mortalidad de adultos de *A. ludens* varió de 43.0 a 79.8%, donde la mayor mortalidad se registró en suelo con 12% de humedad y la menor mortalidad fue en suelo con 21% de humedad. En el cuarto experimento, la mortalidad fue similar cuando la temperatura del suelo varió de 15 a 35 °C, siendo siempre >83%. La emergencia de adultos en los testigos varió de 94.5 a 98.3%. La humedad del suelo fue el mayor factor que afectó significativamente la eficacia del

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hongo. En este estudio se demostró el potencial del hongo *B. bassiana* aplicado al suelo para el control de *A. ludens* y otros tefrítidos. Sin embargo, es necesario que *B. bassiana* sea evaluado bajo condiciones de campo antes de recomendar su uso.

Palabras Clave: control microbiano; hongos entomopatógenos; mosca Mexicana de la fruta; micosis; factor clave

A pest management program with an integrated approach involving sterile insect technique, chemical, biological, and cultural control practices has been followed to control *Anastrepha ludens* (Loew) (Diptera: Tephritidae), an important fruit fly pest of most citrus (except for sour lemon) and mango (*Mangifera indica* L.; Anacardiaceae) in Mexico, (Aluja 1994; Reyes et al. 2000). The strategies are effective individually and when implemented together (Reyes et al. 2000). However, the strategies can be improved, as their efficiency can still be increased and, more importantly, their environmental impacts can be reduced, with the development of new alternatives. Among these alternatives, fungal entomopathogens, such as *Beauveria bassiana* (Bals.-Criv.) Vuill. (Cordycipitaceae), play an important role, as several strains and isolates have shown high potential to be used as a biological control agent against *A. ludens* (Toledo et al. 2008).

Beauveria bassiana infection occurs by the germinated conidia penetrating throughout the cuticle (exoskeleton) of the insect, reaching the hemocoel, where the fungus proliferates (Feng et al. 1994; Vega et al. 2012). Therefore, infection is independent of the ingestion of propagules, although horizontal and transovarial infection also may occur (Ferron 1978; Feng et al. 1994; Toledo et al. 2007). During the infection phase, the penetrating hyphae produce a series of enzymes such as proteases, lipases, and chitinases, all related to the adherence and penetration throughout the insect cuticle (exoskeleton), which are the most important factors in this process (Samšiňáková et al. 1971).

A diversity of strains and isolates of *B. bassiana* (Bb4, Bb16, Bb18, Bb24, Bb25 and Bb26) and *Metarhizium anisopliae* (Metsch.) Sorokin (Clavicipitaceae) (ICIPE 18, ICIPE 20, ICIPE 32, ICIPE 40, ICIPE 41, ICIPE 62, ICIPE 63, and ICIPE 68) has been reported to show biological activity against adults of *A. ludens* and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (De la Rosa et al. 2002; Dimbi et al. 2003; Muñoz et al. 2009; Toledo et al. 2008). However, when the same strains of *B. bassiana* were tested against the larval stage, mortality was low (2 to 8%), and pupae were resistant to the infection by the fungus, resulting in a high percentage of emergence of flies (De la Rosa et al. 2002).

Therefore, the use of fungi to control fruit flies must be based on an appropriate strategy to allow the adults to come in contact with the fungal inoculum. For this purpose, 3 strategies have been used: 1) incorporating the inoculum in the soil or spraying it on the soil surface to target adults emerging from soil (Ekesi et al. 2005); 2) using baits impregnated with the inoculum (Navarro-Llopis et al. 2015); and 3) releasing sterilized adults, previously inoculated with the fungus, as vectors of conidia (Toledo et al. 2007). A similar approach has been used in the selection of efficient fungal strains using "self-disseminators". This technique employs baits covered by a piece of cloth previously impregnated with conidia in such a way that the attracted flies become inoculated and distribute the spores in the field.

Application of fungal entomopathogens to the soil as fly control agents show several advantages compared with their application directly on the plant (e.g., on leaves, fruits, flowers, etc.). Soil is a protective environment to several abiotic factors for the survival of conidia, such as solar UV radiation and desiccation. The use of soil inoculated with *B. bassiana* below the tree canopy has proven to be an efficient method to obtain significant levels of fruit fly mortality (Ekesi et al. 2005). One of the advantages of this method is that conidia remain viable for longer periods of time (Gaugler et al. 1989). However, the effectiveness of fungi as control agents depends on several biotic and abiotic factors (Jaronski 2010). For example, there are many microbial antagonists in the soil, on leaf surfaces, and even in the insect integument. Also, self-grooming and age of the insect pest may reduce the effectiveness of a potential fungal infection (Baverstock et al. 2010). Among the abiotic factors, soil texture, temperature, humidity, and inoculum density may limit the effectiveness of the fungus as a control agent (Jaronski 2010; Garrido-Jurado et al. 2011). For example, temperature can directly affect the penetrating hypha, the development of disease, and conidia production (Carruthers & Hural 1990). This is why it is important to determine the optimum conditions for an efficient infection, evaluating potentially important factors such as inoculum density, temperature, humidity, and soil texture. In this study, we investigated the capacity to infect emerging adults of *A. ludens* from soil treated with *B. bassiana* under different texture, humidity, and temperature conditions.

Materials and Methods

BIOLOGICAL MATERIAL AND ENVIRONMENTAL CONDITIONS

This study was carried out at El Colegio de la Frontera Sur (ECOSUR) in Tapachula, Chiapas, Mexico. Environmental conditions in the laboratory during the experiments were maintained at 26 ± 1 °C, with 70 ± 5% relative humidity (RH), under a 12:12 h L:D photoperiod. Conidia of *B. bassiana* were obtained from the commercial product Bassianil®, a strain from Cali, Colombia, mixed with sterile microtalc at 2×10^9 conidia per g of commercial product (Laverlam, International Corp., Cali, Colombia). This product is highly virulent to adults of *A. ludens*, with a concentration of 1×10^8 conidia per mL causing a mortality of 99.3% along with a LT_{50} of 4.04 (3.73–4.37) d ($p = 0.95$), and the LC_{50} was estimated at 2.69×10^7 (1.76×10^7 to 4.15×10^7) ($p = 0.95$) per ml (Toledo et al. 2007). The viability of conidia was assessed before the experiments by using the microculture method. This was done by plating 100 µL of the conidial suspensions on Saboraud Dextrose Agar (Becton Dickinson de Mexico S. A. de C.V., Mexico) and counting the germinated conidia 30 h later. Viability was confirmed by the presence of germinating hyphae, which in all cases exceeded 95% (De la Rosa et al. 2002). The product was stored at 12 °C and 80% RH until further use. *Anastrepha ludens* pupae (2 d before they would emerge as adults) were obtained from the Moscafrut (SAGARPA-IICA) mass rearing facility located in Metapa de Domínguez, Chiapas, Mexico. The larvae of the following generations were reared on artificial diet according to the standard procedures described by Domínguez et al. (2010).

DOSE RESPONSE OF ADULTS EMERGING FROM SOIL TREATED WITH CONIDIA

Virulence of the *B. bassiana* strain was determined by testing 5 concentrations of conidia in the soil: 400, 600, 800, 1,200, and 1,600 mg formulated conidia per kg soil added in 120 mL water to keep soil humidity at 12% (w/w). For the control, only 120 mL of water was added. Each concentration and control was replicated 6 times for a total of 36 experimental units.

Sandy clay soil was obtained from the 'Viva México' mango orchard (14.911667°N, 92.334722°W) at 78 m asl between the towns of Tapachula and Huixtla in Chiapas State, Mexico. This soil, used in 3 experiments, represents the area cultivated with mango in the region. It was

composed of 74% sand, 14% silt, 12% clay, and 2% organic material and had a pH of 6.4. Soil was sieved in a #8 mesh and autoclaved prior to use. It was first dried in 50 × 50 × 10 cm aluminum pans under the sun for 2 d, then dried at 95 °C for 24 h in an oven. Once dried, 3 kg of dried soil were separately treated by adding 120 mL of conidia suspension per kg and thoroughly mixing the treated soil. Control soil samples received water only.

Afterwards, each experimental unit (20 cm L × 10 cm W × 8 cm H plastic containers) first received a 1 cm layer of the corresponding treatment. Subsequently, 100 pupae were homogeneously placed on this layer of soil and then a 4 cm layer of the remaining soil was added on top. Each experimental unit contained a 5 cm soil layer and 500 g soil.

Each container was covered with a cone-shaped mesh to capture the emerged adults. Adults were transferred daily to a 30 × 30 × 30 cm cage. They were fed with a 1:3 mixture of hydrolyzate protein to sugar and water contained in 200 mL plastic containers (8.2 cm H × 6.5 cm in diameter) covered with cotton. Cages were kept under laboratory conditions (see above) for a period of 5 to 10 d. Flies were examined daily and mortality was recorded until the last fly died. Dead flies were removed daily from cages, and they were immediately surface disinfected with sodium hypochlorite (1%) for 5 s, followed by 3 rinses with sterile distilled water. Finally, the dead flies were transferred to a humidity chamber (glass Petri dish with a wet filter paper and sealed with Parafilm®) and kept in a room at 26 °C to promote the growth of the fungus on the cadavers to corroborate that *B. bassiana* infection was the cause of death.

INFECTION OF ADULTS EMERGING FROM SOILS OF VARIOUS TEXTURES

The concentration of conidia that caused the highest mortality in the first experiment was evaluated in 3 textures of soil: sand (96.2% sand, 0.7% silt, 3.1% clay, and 0.2% organic material with a pH of 6.6) obtained from Campo Agricola Experimental Rosario Izapa (14.974444°N, °92.156111°W), sandy loam (80.3% sand, 13.7% silt, 6.1% clay, and 11.4% organic material with a pH of 6.3) obtained nearby (14.895833°N, 092.323611°W), and sandy clay (described previously). Each soil texture was considered a treatment and each treatment had 5 replicates. In addition, a control (without the fungus) was included for each soil texture, again with 5 replicates per texture. In total, there were 20 experimental units distributed randomly. All experimental units consisted of the same plastic containers with 500 g of soil with a gravimetric moisture content of 12% (w/w). The insects used were in the pupal stage 2 d prior to the emergence of adults.

The conidia concentration used per treatment was 800 mg of formulated conidia per kg of soil diluted in 120 mL of distilled water. The control soils were treated with water only. Processing of soils, preparation of bioassay units, processing of emerged adults, and recording of results followed the same procedures described in the dose response section. This activity was carried out for 10 consecutive days or until the final fly died.

EFFECT OF HUMIDITY ON THE INFECTION OF FLIES

The concentration of conidia (800 mg/kg soil) that caused the highest mortality in the previous experiment was used to assess the role of humidity in the soil and the ability of the fungus to infect the emerging flies from the soil. The sandy clay texture of soil was used as described above under the following humidity conditions: 6, 9, 12, 15, 18, and 21% (w/w). Each humidity level was considered as a treatment, and each treatment was replicate 6 times, totaling 36 different experimental units distributed randomly. Processing of soils, preparation of bioassay units, processing of emerged adults, and recording of results were done following the procedures described in the dose response section.

EFFECT OF TEMPERATURE ON THE INFECTION OF FLIES

A 4th experiment was carried out using the same conidia concentration (800 mg/kg soil) used before, as well as the same soil texture (sandy clay), and 12% humidity, as it showed to be the optimum to achieve the highest percentage of infection in the previous experiment. Five temperature conditions were tested: 15, 20, 25, 30, and 35 °C, and each treatment had a range of temperature of ± 0.5 °C. The 35 °C treatment was performed in an incubator (Lab-line Instrument, Inc., Illinois). Each temperature was considered a treatment and each treatment was replicated 4 times, totaling 20 experimental units distributed randomly. The experimental units were prepared as described in the dose response section, and mortality recording and microscopic examination were carried out as described at the end of the dose response section.

DATA ANALYSES

Mortality data recorded in each experiment from bioassays with the fungus was subjected to analysis of variance (ANOVA) tests, and the discrimination of means was analyzed with the Tukey–Kramer honest significant difference (HSD) test. Data analyses were conducted with the statistical program JMP version 5.0.1. Statistical Discovery Software (SAS Institute 2003). Data consisted of the mortality accumulated throughout the experiment, using only the individuals that showed the mycosis. Abbott's correction of mortality (Abbott 1925) was not performed, as natural mortality in the controls was practically the same as in the treatments with the fungus, that is, individuals not infected with the fungus, which were not included in the analysis. In other words, the results of the analysis were the same without the Abbott's correction. However, if the Abbott's correction was performed taking into account only the control mortality and including only the infected individuals of the treatments, then a statistical artifact might have been introduced, as the natural mortality within the treatments was not included. Additionally, natural mortality was very low (between 2.2 and 4.2%), which might have had very little influence in the results.

Results

DOSE-RESPONSE OF ADULTS EMERGING FROM SOIL TREATED WITH *BEAUVERIA BASSIANA*

All the tested conidia concentrations of *B. bassiana* induced some level of mortality in adults of *A. ludens* emerging from the treated soil. Mortality ranged from 38.3 to 74.7% (Fig. 1). However, the concentration of 800 mg/kg soil showed the highest mortality. These results indicated a lack of dose-response relationship, precluding analysis by programs such as Probit, which establishes the relationship between the mortality levels and the entomopathogen concentration tested in the experiment. A statistical significance from this relationship would have allowed us to estimate a mean lethal concentration (LC_{50}), which is the main parameter to measure the pathogen virulence. However, almost all mean mortality values showed significant differences (F = 22.76; df = 4, 25; $P < 0.001$). During the 10 d of observation, the mortality of control flies was low (4.2%), and there were no dead flies with signs of fungal infection. No significant differences were observed in

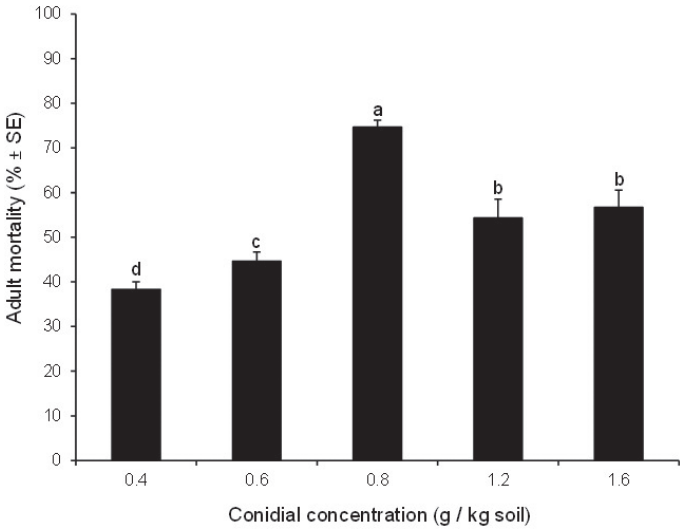


Fig. 1. Adult mortality of *Anastrepha ludens* infected with different concentrations of *Beauveria bassiana* conidia, after emerging from treated soil. Different letters indicate significant differences among treatments based on 1-way ANOVA followed by the Tukey Honest Significant Difference test, $P < 0.05$).

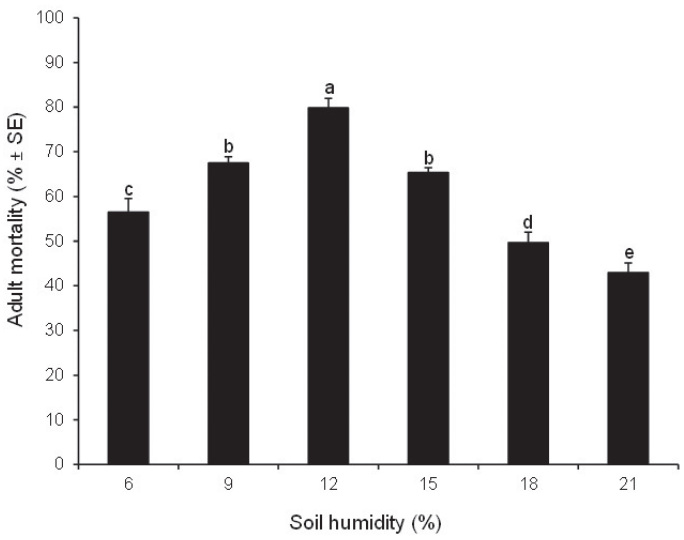


Fig. 2. Adult mortality of *Anastrepha ludens* when emerged from treated soil with *Beauveria bassiana* conidia at different levels of soil moisture. Different letters indicate significant differences among treatment based on 1-way ANOVA followed by the Tukey Honest Significant Difference test ($P < 0.05$).

terms of emerging adults (92.8 ± 0.4%) between the treatments, which may indicate that pupal survival was not affected by the conidia concentrations tested.

INFECTION OF ADULTS EMERGING FROM SOILS OF VARIOUS TEXTURES TREATED WITH *BEAUVERIA BASSIANA*

Soil texture showed a significant effect on the infection of adults ($F = 21.28$; df = 2, 12; $P < 0.001$). The highest percentage of mortality caused by infection was observed in adults emerging from the soils of sandy clay and sandy loam texture, but it was significantly lower in adults emerging from sand (Table 1). Emergence of flies in the control treatment was 95% on average, and the natural mortality at 24 d of observation of control adults emerging from the sandy clay soil was 10.7 ± 5.2%. In control group adults emerging from the sandy loam and sandy soils, natural mortality at 24 d was 10.3 ± 2.3% in both cases, but there were no signs of infection by fungus. During the 10 d of observation, there were no dead flies with signs of fungal infection in this control group.

EFFECT OF HUMIDITY ON THE INFECTION OF FLIES

A significant variation in infectivity of the fungus on emerging adults of *A. ludens* was observed when the conidia concentration that showed the highest percentage of mortality in the previous experiment (800 mg conidia/kg soil) was tested under different humidity concentrations. Adult mortality varied from 43.0 to 79.8% (Fig. 2), with emerged adults from the 12% soil humidity treatment having the highest percentage of mortality. The percentage of mortality differed significantly among treatments ($F = 39.24$; df = 5, 30; $P < 0.001$). As in the previous experiment, no significant difference was observed in the mean emergence of adults among the treatments (96.3 ± 0.5%). In this case, during the 8 d of observation, the mortality of flies in the control was low (2.2%), and no mycosis was detected in the dead flies.

Table 1. Mortality (± SE) of *Anastrepha ludens* adults emerging from 3 soil textures treated with *Beauveria bassiana* conidia at 12% humidity. (N = 500 flies per treatment).

Soil Texture	Mortality (% ± SE)[a]
Sandy clay	64.0 ± 4.6 a
Sandy loam	63.4 ± 2.6 a
Sand	36.0 ± 2.9 b

[a]Values in the same column and followed by the same letter are not significantly different (Tukey HSD test, $P > 0.05$).

EFFECT OF TEMPERATURE ON THE INFECTION OF FLIES

When the formulated product of *B. bassiana* was tested at 800 mg/kg soil and 12% soil humidity under a range of temperatures between 15 and 35 °C, mortality ranged between 83.5 and 91.0%, indicating that all tested temperatures showed high levels of mortality. However, mortality was quite similar across the treatments, with the highest percentage of mortality being recorded at 25 °C. There were no statistically significant differences detected in regards to mortality ($F = 2.91$; df = 4, 15; $P = 0.057$). Also, no differences were observed in mean emergence (92.4 ± 0.8%) of adults among the tested temperatures, indicating that the evaluated temperatures had no measurable effect on the emergence of adults or the infectivity of conidia. Adult emergence in the controls ranged from 89.4 to 93.8%. Natural mortality in control flies was low (3.4%) in the 10 d of observation, and there were no dead flies with signs of infection.

Discussion

The effectiveness of entomopathogenic fungi as biological control agents is influenced by biotic and abiotic factors under field conditions, and some are more important than others. Some of these relevant factors include soil texture, inoculum density, temperature, and soil humidity. Soil humidity and texture, especially the size distribution of pore spaces, affect the infectivity of spores by potentially mediating physical contact (Kessler et al. 2003). To improve the use of this fungus as a biological control agent, there should be further testing of the influence of these abiotic factors under controlled conditions, so that valid extrapolations can be made to identify the best product for field evaluations.

Several products, strains, and isolates of *B. bassiana* have been tested for their efficiency in infecting and killing larvae and adults of fruit flies during their soil-related life stages (pupating larvae within the soil and adults emerging from pupae). Other tests have been conducted either by immersion in conidia suspensions or by using vectors (De la Rosa et al. 2002; Toledo et al. 2008; Muñoz et al. 2009). The application of conidia to soil shows potential as a pest control technique, by applying conidia to the soil in such a way that the emerging adults from the buried pupae come in contact with the conidia as they emerge from the soil (Hodgson et al. 1998). This technique may decrease the pest population and may promote the infection of other *A. ludens* adults during mating (Toledo et al. 2007).

The effect of temperature and humidity on the virulence of a fungal entomopathogen has been widely studied in other hosts (Hajek & St. Leger 1994; García-Gutiérrez et al. 2007). During the infection process, there is a relationship between the pathogen and its host where several endogenous and exogenous factors interact (Jaronski 2010). Physiological and genetic factors of the host influence its resistance, tolerance, or susceptibility to the pathogen. However, these factors are also highly influenced by exogenous factors, such as diet, climate, and population density among others, potentially changing the host's response to a possible infection threat (Watanabe 1987; Jaronski 2010). Moreover, the effectiveness of the fungus as a control agent is also influenced endogenously by factors such as specificity, virulence, toxin production, cuticular extracts, and other similar factors, and by environmental factors such as temperature, humidity, solar radiation, rain, pH, and soil texture (Jaronski 2010). For example, temperature and soil moisture affected the efficacy of fungi on pupal mortality of African fruit flies, upon application of an entomopathogenic fungus (Ekesi et al. 2003).

Although several reports indicated a negative effect of certain temperatures on conidia survival, limiting the effectiveness in infecting pupae (González-Reyes 2003; Keyser et al. 2014), temperature in our study had little influence on the survival of both conidia and pupae. However, the temperature range we tested was within the temperatures usually found in our semi-tropical environment. Extreme temperatures (i.e. lower than 5 °C or higher than 40 °C) may have a negative influence on both conidia and pupal survival, as reported earlier (Eskafi & Fernandez 1990; Keyser et al. 2014). However, the effectiveness of *Beauveria brongniartii* (Saccardo) Petch was affected by soil temperatures above 27 °C, as well as high clay content of soil (Kessler et al. 2003).

The lower levels of soil humidity we tested at 25 °C resulted in the highest infectivity of the fungus, contrary to our expectations, as normally a high fungal infection is associated with high humidity in the environment (Walstad et al. 1970). It is very important to note that humidity retained by sandy soils is expected to be lower, in comparison with soils with a higher proportion of clay that retain more water. Interestingly, it has been reported before that when strains of *M. anisopliae* were tested against *A. ludens* in sandy loam soil with humidity ranging between 10 and 20%, lethal mycosis was around 65%, while in the same soil type with 5% humidity, infection increased to ~90% (González-Reyes 2003). The author suggested that adult flies are able to move more freely in a less humid soil, thereby increasing the possibility of infection. Therefore, soil texture and moisture may interact in a complex relationship in relation to the size and movement behavior of the insect. Also, it is known that the emergence from soil of fruit fly adults is influenced by humidity, usually observed when larvae select soils with different humidity levels (Montoya et al. 2008). Additionally, persistence of infective conidia in soil is a prerequisite for successful control, because its persistence is related to the retention and viability of conidia in the soil (Garrido-Jurado et al. 2011).

In conclusion, a concentration of 800 mg of formulated product per kg of sandy clay soil or sandy loam kept at 12% humidity under normal temperatures of this region (from 20 to 30 °C) caused the highest levels of infection of *A. ludens* by *B. bassiana* under controlled conditions. These results lead us to predict that *B. bassiana* may be adopted as another control strategy against fruit fly pests, especially in tropical ecosystems where optimal conditions found in this study are frequent. The potential of this approach as control method requires confirmation in field tests.

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