DYNAMICS OF *BEAUVERIA BASSIANA* AND *METARHIZIUM ANISOPLIAE* INFECTING *HYPOTHENEMUS HAMPEI* (COLEOPTERA: SCOLYTIDAE) POPULATIONS EMERGING FROM FALLEN COFFEE BERRIES

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ABSTRACT

The aim of this research was to evaluate the effect of soil sprays of the entomopathogens *Beauveria bassiana* and *Metarhizium anisopliae* on coffee berry borer (cbb) adults, *Hypothenemus hampei*, emerging from fallen berries through time. Each fungus was applied to a plot 5000 m^2 in size of the Colombian variety in the third harvest year. The experimental plot was formed with 9 trees, and the experimental unit was the central tree. In this tree all the green uninfested berries were left and the whole tree covered with a screen cage to avoid further cbb infestation or escape. Nine treatments replicated ten times were arranged in a complete randomized design. Conidia of each fungus were suspended in emulsified oil and water and applied on the base of the trees at a dosage of 1×10^9 conidia/tree. Under each experimental tree 350 cbb-infested coffee berries were placed on the soil to serve as a source for aerial infestation of the trees. Infested berries were applied the same day of the spray and at 2, 5, 10, 15, 20, 25 and 30 days after fungus application. Results showed that infection levels of both fungi on cbb were the highest during the first five days after application, reaching nearly 30% for *B. bassiana* and 11% for *M. anisopliae*. However, the infection decreased for 20 days but peaked again at 25 days post-treatment with 24.3% for *B. bassiana* and 7.7% for *M. anisopliae.* These results are explained by the formation of propagules in the soil by these fungi, due probably to the accumulation of infective conidia on infected insects which infect other insects leaving the fruits. The two species were recovered from the soil even after two months and fluctuation in numbers of colony forming units was attributed to the rainfall during the study period and the fungus conidiation. *B. bassiana* was shown to be more infective than *M. anisopliae*, considering that the latter is more frequently associated to soil habitats. The authors believe efficiency of these fungi can be increased if improvements are made to the formulations, e.g., using a granulated formulation to avoid leaching of the conidia from the soil during heavy rainy seasons. During this study it was found that *H. hampei* is a new host of *Paecilomyces lilacinus*.

Key Words: Coffee berry borer, *Hypothenemus hampei*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces lilacinus*

RESUMEN

Este estudio evaluó el efecto de aspersiones de *Beauveria bassiana* y *Metarhizium anisopliae* al suelo sobre la broca del café, *Hypothenemus hampei*, que emerge de frutos caídos, a medida que transcurre el tiempo después de depositar el hongo. Se seleccionaron dos lotes de café variedad Colombia de tercera cosecha con un área de 5000 m^2 y se evaluaron los dos hongos en lotes experimentales diferentes. La parcela se formó con 9 árboles y el árbol central se escogió como la unidad experimental. A este árbol se le dejó solo frutos verdes sin infestación por broca y se cubrió con una jaula entomológica para evitar nuevas infestaciones o escape de broca. Las conidias de los hongos utilizados se suspendieron en aceite emulsionable y agua usando una dosis de 1×10^9 conidias/árbol. En la base del árbol que sirvió como unidad experimental se asperjaron los hongos al plato de cada árbol y se depositaron 350 frutos brocados el mismo día de la aspersión y 2, 5, 10, 15, 20, 25 y 30 días después de la aspersión. Los resultados muestran niveles máximos de infección durante los cinco primeros días posterior a la aspersión de los hongos, cercanos al 30% para *B. bassiana* y 11% para *M. anisopliae*. Sin embargo la infección disminuyó y de nuevo alcanzó un nuevo pico hacia el día 25 de la evaluación. Después de este tiempo la infección se incrementó nuevamente hasta niveles similares a los alcanzados en los 5 primeros días, 24,3% y 7,7% para *B. bassiana* y *M. anisopliae* respectivamente. Estos resultados se pueden explicar por la formación de propágulos en el suelo por estos hongos, debido probablemente a la acumulación de conidias infectivas sobre insectos atacados que infectan otros insectos que salen de los frutos. Las dos especies se recuperaron del suelo aún después de dos meses y la fluctuación en el número de las unidades formadoras de colonia se atribuyó a la precipitación y a la conidiación del hongo. *B. bassiana* mostró un efecto superior al de *M. anisopliae*, si se tiene en cuenta que este último está más asociado a condiciones del suelo. La eficiencia de estos hongos se podría mejorar con formulaciones granuladas del hongo que permitan una mayor permanencia en el suelo para disminuir la lixiviación causada por las lluvias. Durante el estudio se constató que *H. hampei* es un nuevo huésped de *Paecilomyces lilacinus*.

The coffee berry borer (cbb), *Hypothenemus hampei* (Ferrari), was introduced into Colombia in 1988 and now is widespread in the major coffee producing area where it is the most important insect pest (Bustillo 1991). Infested coffee berries that fall to the soil are the main source of reinfestation of the coffee plantations at the end of the harvest period. Traditionally, the berries that fall during the harvest period are not harvested because this practice is very tedious and expensive. In Colombia, about 10% of the coffee berries are not harvested, resulting in berries ending up on the soil eventually (Chamorro et al. 1995).

Understanding the dynamics of cbb in the soil is important to the development of a control strategy. Studies carried out in Mexico (Baker 1984) and Colombia (Ruiz 1996) have demonstrated that high humidity caused by rainfall is the main trigger of cbb emergence from fallen berries. On the other hand, soil moisture stimulates expulsion and death to the immature stages inside the berry (Baker et al. 1994). When the soil is dry, the cbb remains in the berries in the soil and continues to reproduce. When the rainy season arrives, massive adult emergence occurs.

Several attempts have been made throughout the world to use mass-produced biopesticides based on entomopathogenic fungi. In Brazil, mass production of *Metarhizium anisopliae* (Metsch.) Sorokin has resulted in an intensive use to control a sugarcane pest, *Mahanarva posticata* (Stal) (Ferron 1981, Alves 1986). In several countries of Eastern Europe, *B. bassiana* is recommended to control *Leptinotarsa decemlineata* (Say) (Ferron 1981, Lipa 1990). To replace the use of chemical insecticides due to an embargo on trade with Cuba, Cuba has been forced to move in the development of biopesticides, especially with entomapathogenic fungi, to control different insect pests (Jaffé & Rojas 1993). In Africa, international attempts to develop more ecological control practices has resulted in the formulation of a commercial product "green muscle" based on *M. flavoviride* (Gams et Rozsypal) to control several species of the desert locust, *Schistocerca gregaria* Forskal (Lomer et al. 1997). In Colombia an intensive research program with entomopathogenic fungi is been conducted to control *H. hampei* (Bustillo & Posada 1996).

Beauveria bassiana (Balsamo) Vuillemin is the main natural mortality factor of cbb and is found in all the Colombian coffee regions infested by this insect (Bustillo &

Posada 1996, Ruiz 1996). This fungus is being investigated as a control tool in our coffee IPM programs. *M. anisopliae* is also a potential entomopathogen that could infect the cbb in the soil (Bernal et al. 1994). Our research was conducted to determine the role of both *B. bassiana* and *M. anisopliae* on the regulation of cbb adult populations that emerge from the fallen berries, and on persistence of fungi in the soil.

Materials and Methods

This study was conducted during 1996 at the Experiment Substation Maracay of Cenicafé near Armenia, Colombia. Two large plots each of 5000 m^2 with 2500 coffee plants of the Colombia variety were selected, one planting for each fungus. Soil pH was 5.1 with an organic matter content of 12% for the *B. bassiana* plot and16.5% for the *M. anisopliae* plot. The experimental plots had enough susceptible green berries in an optimal developmental stage for borer infestation. Plots contained nine trees in square with a three-row border in all directions. The central tree of each plot served as the sample unit. The tree was covered with a screen cage of translucid nylon cloth to prevent movement of borers. Prior to the study, trees were left with uninfested green berries suitable for borer infestation, and the berries on the ground were removed from the soil surface beneath the trees.

B. bassiana isolate Bb 9205 originally from *Diatraea saccharalis* (Fabricius) and *M. anisopliae* isolate Ma 9236 obtained from the CIAT fungi collection (accession #1773) maintained in liquid nitrogen, were inoculated and then reisolated from cbb adults and produced on rice (Antia et al. 1992). Previous studies (Bustillo & Posada 1996, Bernal et al. 1994) had shown effect of these fungi against cbb populations under field conditions. To assure good quality of fungi produced on rice medium, concentration and viability was checked, and pathogenicity on cbb adults was performed following the protocol established by Vélez et al. (1997). Fungal conidia were suspended in Tween-20® and an emulsified oil Carrier® in equal parts. Water was added to the mixture to give a concentration of 2×10^7 conidia/ml. The fungi were sprayed onto the ground at the base of the trees using a volume of 50 ml/tree with a manual backpack sprayer at a constant pressure of 40 psi, and a final dose of 1×10^9 conidia/tree.

Treatments consisted of a liquid application of fungus to the ground of the trees, and subsequent deposition of 350 infested berries on the ground immediately after fungal application or 2, 5, 10, 15, 20, 25 and 30 days after application. The study followed a completely randomized design for both experiments with 10 replications and control consisting of infestation with cbb but no fungal application. Hypothetically, cbb emerging from the infested berries will contact the fungus. Then adults will fly to the trees and infest the healthy berries and die from fungal infection. Infested berries were previously disinfected with NaOCl at 2.75% to avoid natural fungal contamination, and then dried for 12 h with the help of a fan.

Following infestation, on each tree 15 branches were randomly marked. Mycosis to cbb was made 30 days after berry infestation by recording the number of infected and healthy adults found in the 15 branches. The infested berries were dissected to confirm cbb adult mortality, and dead adults without signs of fungal infection were placed individually in humid chambers (90% RH, 25°C) for eight days to allow fungal expression on the cadavers.

To determine fungal persistence in the soil following application of conidia a dilution method was used to recover fungal propagules from the treated soil. Soil samples were collected weekly for two months from each tree by taking 10 g of soil/tree randomly from 5 sites. The 5 samples were pooled and homogenized and a subsample of 1 g was placed to reach a 10-ml suspension with sterile distilled water. From this suspension, a 10^{-3} dilution was prepared and two 0.1 ml aliquots were used for counting. Isolation of *B. bassiana* and *M. anisopliae* was made using a selective media (Rivera & López 1992). This medium was prepared by adding to the one liter Saubouraud dextrose agar a mixture of 12 mg copper oxychloride, 26.6 µl cyproconazol and 1 ml of a 44% of lactic acid solution. Fungal spore density was estimated from the average of 10 counts per treatment and recorded as colony forming units (CFUs) /g of soil. Analysis of variance was made and Tukey's test ($P = 0.05$) to determine treatment differences using SAS statistical package version 6.11.

Results and Discussion

In all treatments, coffee berry borer infestation occurred in the aerial part of the tree as a consequence of the adult emergence from the infested berries on the soil (Tables 1 & 2). Although 350 berries were placed under each tree, different levels of infestation occurred in different plots. Levels of *B. bassiana* and *M. anisopliae* were significantly higher in treatments than in controls, demonstrating that the borers contacted the conidia in the soil. Maximum adult infection was 29.3% for *B. bassiana* (Table 1) and 11% for *M. anisopliae* (Table 2) when cbb infestation was made 0, 2, and 5 days after fungus application. Levels of infection decreased in the subsequent treatments (10, 15 and 20 days after spray), but at 25 days post-treatment an increase in infection was detected to levels similar to the ones registered at the initial treatments. The reason for this increase may be the conidiation of fungi on cadavers or the formation of new propagules from the existing inoculum, which is common when fungi are sprayed into the soils, as suggested by Fargues & Robert (1985). Similar results were found by López et al. (1995) with the isolate Bb9205 of *B. bassiana* active against the cbb. Under laboratory conditions propagules of this fungus were recovered from sterile and nonsterile soil even after 218 days of soil inoculation, and an unexpected increase of CFUs was recorded 41 days after inoculation.

TABLE 1. *BEAUVERIA BASSIANA* INFECTION OF *HYPOTHENEMUS HAMPEI* ON COFFEE TREES TREATED WITH FUNGAL APPLICATION TO THE SOIL.

Infestation interval (days after spray)	Average number of cbb adults[1] ± S. E[3]	Infection of cbb with *B. bassiana* (%)	Infection of cbb with *M. anisopliae* (%)	Infection of cbb with *P. lilacinus* (%)
0	551.4 ± 52.3	24.7 ab[2]	0.1 b	0.7 b
2	221.4 ± 35.9	29.3 a	0.5 a	0.2 a
5	46.8 ± 6.4	21.7 abcd	0.2 b	0.1 a
10	74.3 ± 9.7	10.3 cde	0.0 b	1.0 b
15	93.0 ± 9.9	8.4 de	0.2 b	1.6 b
20	91.9 ± 10.4	11.3 cde	0.0 b	0.8 b
25	243.8 ± 24.5	24.3 abc	0.0 b	0.0.a
30	49.1 ± 10.2	7.2 de	0.0 b	0.0.a
Control	170.7 ± 18.9	6.0 e	0.0 b	0.0.a

[1]Average number of coffee berry borers (cbb) adults in 15 branches/treated tree.
[2]Numbers followed by the same letter do not differ significantly according to the Tukey test ($P = 0.05$).
[3]Standard Error.

TABLE 2. *METARHIZIUM ANISOPLIAE* INFECTION OF *HYPOTHENEMUS HAMPEI* ON COFFEE TREES TREATED WITH FUNGAL APPLICATION TO THE SOIL.

Infestation interval (days after spray)	Average number of cbb adults[1] ± S. E[3]	Infection of cbb with *M. anisopliae* (%)	Infection of cbb with *B. bassiana* (%)	Infection of cbb with *P. lilacinus* (%)
0	527.7 ± 58.6	7.9 abc[2]	8.6 d	1.2 a
2	109.5 ± 14.5	11.0 a	15.0	1.5 a
5	60.1 ± 6.7	9.2 ab	4.5 cd	1.0 a
10	86.9 ± 9.5	6.7 abc	8.8 bc	1.2 a
15	102.7 ± 8.2	4.5 bc	3.4 cd	0.3 a
20	79.1 ± 7.1	4.9 abc	1.8 d	0.8 a
25	250.7 ± 26.2	7.7 abc	17.0 a	1.8 a
30	74.9 ± 11.6	4.7 bc	3.9 cd	0.6 a
Control	207.9 ± 31.2	1.7 c	4.1 cd	0.0 a

[1]Average number of coffee berry borers (cbb) adults in 15 branches/treated tree.
[2]Numbers followed by the same letter do not differ significantly according to the Tukey test ($P = 0.05$).
[3]Standard Error.

M. anisopliae infection of cbb differed from *B. bassiana* in that levels of infection were lower (Table 2). Infection fluctuated between 7.9% and 11% at 0-5 days post-treatment but decreased gradually thereafter. Another peak of infection was reached at the 25-day post-treatment (7.7%). These results for both *B. bassiana* and *M. anisopliae* are similar to those reported by Müller-Kogler & Zimmermann (1986); in studies to control *L. decemlineata* using *B. bassiana*, they found an unexpected increase in number of conidia after several months of fungus spray in the soil.

The incidence of *M. anisopliae* and *B. bassiana* was measured by quantifying the infection on *H. hampei* adults on the trees, but it is possible that a part of this population is not quantified since they may die before reaching the trees and are difficult to locate. Low levels of Infection of *B. bassiana, M. anisopliae* and *P. lilacinus* were recorded on cbb populations in plots where they were not sprayed (Tables 1 and 2). The same was observed from the soil samples (Figs. 1 and 2).

It was possible to recover *B. bassiana* and *M. anisopliae* from the soil even two months after application (Figs. 1 and 2). Interestingly, both species were recovered in each plot; however, *B. bassiana* was more abundant in both plots. This can be explained by previous use of *B. bassiana* and *M. anisopliae* at this Research Station in programs to control the borer.

No direct relationship was found between the abundance of fungi CFUs and rainfall, but a reduction in quantity of propagules was registered after heavy rains. In the case of *B. bassiana* in the soil, two peaks were observed, one at the beginning and other later in the experimental period, which corresponded to the high levels of fungal infection in cbb on the trees (Fig. 1). The dynamics of *M. anisopliae* in the soil was similar to *B. bassiana*, but with significantly lower recovery (Fig. 2).

During soil sampling in both plots, another entomopathogen was isolated frequently and identified as *Paecilomyces lilacinus* (Thom.) Samson by Harry C. Evans from IIBC in England. This is the first record of this fungus attacking *H. hampei* adults under natural conditions. *P. lilacinus* has been isolated previously from coffee soils in Colombia with high nematode (*Meloidogyne* spp.) populations, to which this fungus is

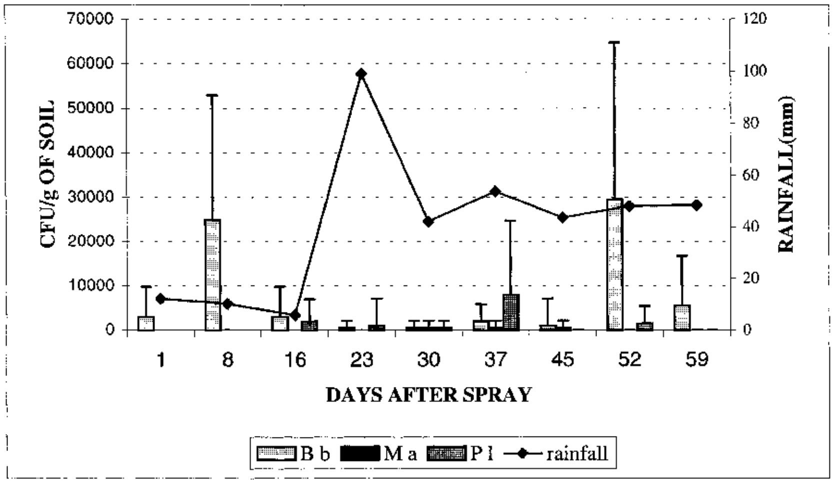

Fig. 1. Abundance (CFU/g) of *Beauveria bassiana* (*Bb*), *Metarhizium anisopliae* (*Ma*) and *Paecilomyces lilacinus* (*Pl*) in soil treated with *B. bassiana*. Vertical bars represent standard errors of the mean.

pathogenic (Cardona 1995). Although *P. lilacinus* was never applied, it was recovered from the soil in higher proportion than *M. anisopliae* in both plots (Figs. 1 and 2).

Successful infection of susceptible soil-inhabiting insects by soil-applied entomopathogenic fungi is largely dependent upon survival of an infective inoculum in the

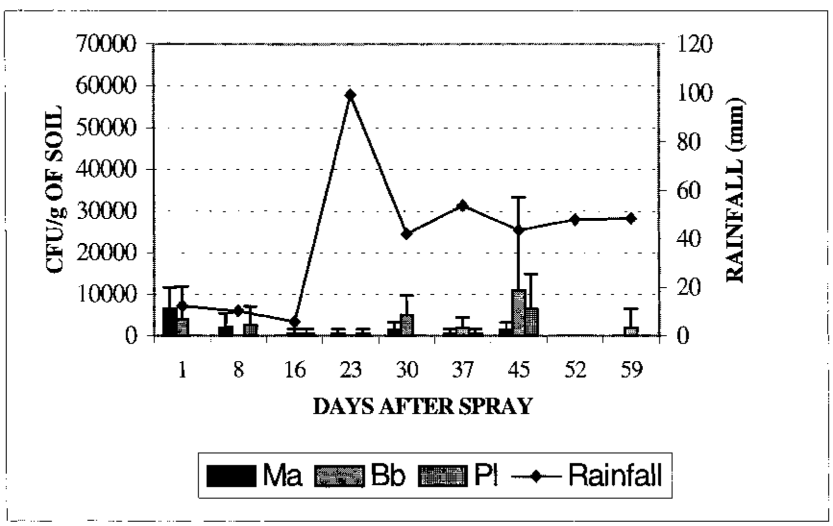

Fig. 2. Abundance (CFU/g) of *Metarhizium anisopliae* (*Ma*), *Beauveria bassiana* (*Bb*) and *Paecilomyces lilacinus* (*Pl*) in soil treated with *M. anisopliae*. Vertical bars represent standard errors of the mean.

soil. This study shows that conidia of both *M. anisopliae* and *B. bassiana* can persist for short periods of time in the soil. Other studies (Fargues & Robert 1985, Gaugler et al. 1989, Li & Holdom 1993, Studdert et al. 1990, Su et al. 1988) have shown that survival of these fungi may vary depending on fungal strain, type of soil, pH, microbial fauna present, and soil management. Lingg & Donaldson (1981) demonstrated that survival of *B. bassiana* conidia was primarily dependent on temperature and soil water content. In addition, microcyclic conidiation could be implicated in the high survival of conidia (Fargues & Robert 1985, Müller-Kogler & Zimmermann 1986). Due to the high variability of conidial survival of entomopathogenic fungi, its potential as a microbial insecticide is much greater in some soil environments than in others.

Fungal formulations play important roles in the persistence in soils. Propagules penetrate vertically in the soil when liquid formulations are used (Storey & Gardner 1988). Recovery of CFUs from *B. bassiana* in treated plots was 10 times greater using granular formulations than when liquid formulations were used (Storey et al. 1989). The efficacy of *B. bassiana* and *M. anisopliae* to control *H. hampei* could be improved by the use of more appropriate formulations such as a granular formulation. This kind of formulation could avoid the loss of conidia trough rainfall, maintain high conidia viability, and prevent movement from the upper layers of the soil where contact with the borer takes place.

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