SCIENTIFIC NOTE

Abundance of *Beauveria* spp. and *Metarhizium* spp. in maize and banana agroecosystems in central Cuba

Abundancia de *Beauveria* spp. y *Metarhizium* spp. en agroecosistemas de maíz y banano en el centro de Cuba

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ABSTRACT

Entomopathogenic fungi are an ecological alternative for the control of agricultural pests. These fungi live in organic matter in the soil and can cause natural epizootics in many arthropods associated with the rhizosphere. The aim of this study was to evaluate the abundance of *Beauveria* and *Metarhizium* spp. in maize and banana agroecosystems in central Cuba. Selective medium and insect baiting methods were used to isolate the entomopathogenic fungi from the soil. *Metarhizium* spp. were significantly more abundant than *Beauveria* spp. in both types of fields of agroecosystems. The abundance of *Metarhizium* spp. was higher in Sagua la Grande than in Santa Clara and Camajuaní municipalities. The insect bait method resulted as the most successful way to isolate entomopathogenic fungi from soil. These results show the composition of the entomopathogenic fungi in different agroecosystems, and they are an advance in the understanding of their ecology.

Key words: entomopathogenic fungi, fungal diversity, insect bait method, selective medium.

RESUMEN

Los hongos entomopatógenos son una alternativa ecológica para el control de plagas agrícolas. Estos hongos viven en la materia orgánica contenida en el suelo y pueden causar epizootias naturales a muchos artrópodos asociados a la rizosfera. El objetivo de este estudio fue evaluar la abundancia de *Beauveria* y *Metarhizium* spp. dentro de los agroecosistemas de maíz y banano en el centro de Cuba. Se utilizaron los métodos de medio selectivo e insecto cebo para aislar los hongos entomopatógenos del suelo. *Metarhizium* spp. fue significativamente más abundante que *Beauveria* spp. en ambos agroecosistemas. La abundancia de *Metarhizium* spp. fue mayor en Sagua la Grande que en los municipios de Santa Clara y Camajuaní. Además, el método de insecto cebo constituye el más apropiado para aislar hongos entomopatógenos. Estos resultados muestran la composición de los hongos entomopatógenos en diferentes agroecosistemas y constituyen un avance en la comprensión de su ecología.

Palabras clave: hongos entomopatógenos, diversidad fúngica, método del insecto cebo, medio selectivo.

Introduction

Entomopathogenic fungi constitute an important biotic component in the natural regulation of arthropod populations (Meyling & Eilenberg, 2007). *Beauveria* spp. have been found in several ecosystems worldwide including forest, seminatural habitats, and agricultural fields (Clifton *et al*., 2015). In contrast, *Metarhizium* spp. are more abundant in temperate regions, but not in colder regions (Steinwender e*t al*., 2015).

These entomopathogenic fungi show potential as microbial control agents against different agricultural pests, and they can be artificially reproduced. Among the attributes of these fungi, we can mention a high mortality of the targeted pest population, high genetic diversity across a wide number of strains, infection of multiple life stages, penetration through the integument, and capacity for both horizontal and vertical transmission (Destefano *et al*., 2004; Jaronski, 2014).

The environmental and ecological variations within ecosystems have become a major factor influencing the biocontrol effects of *Beauveria* and *Metarhizium* species. A more detailed understanding of environmental and ecological

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interactions, especially the distributions of these fungi in different ecological areas, is needed to improve consistency in the control capacity of these fungi. In this sense, particular stages in the life cycle of *Beauveria* spp. and *Metarhizium* spp., including their persistence and dispersal in the environment, are unresolved in Cuba. The aim of this study was to evaluate the abundance of *Beauveria* spp. and *Metarhizium* spp. in maize (*Zea mays* L.) and banana (*Musa paradisiaca* L.) fields or agroecosystems. These are the most important crops in Cuba.

Materials and methods

Field sampling

Field samplings were conducted from April to July 2018 in three maize (*Zea mays* (L.), cv. 'Jibara') and three banana (*Musa paradisiaca* (L.), cv. 'Grande Naine') fields located in three municipalities in Villa Clara province, Cuba. The selected municipalities were Camajuaní (22°28'4" N, 79°43'26" W), Santa Clara (22°24'49" N, 79°57'58" W) and Sagua la Grande (22°48'24" N, 80°4'32" W), where five collection points spaced 20 m apart were selected in each of the maize and banana fields. Two soil samples of 500 g each were collected with a garden spade around selected points to a depth of about 15 cm after removal of surface litter. The garden spade was disinfected with 70% ethanol between every collection to avoid contamination (Klingen *et al*., 2002). The soil samples from each point were placed into polyethylene bags and transferred to the Microbiology Laboratory at the Universidad Central "Marta Abreu" de Las Villas. Collected soil samples were thoroughly homogenized by hand and stored at 4°C until processing.

Isolation methods

The selective medium and the insect bait methods were used to isolate entomopathogenic fungi from soil samples. The first method was used through serial dilutions of soil in a culture medium, and the insect bait method employed the use of *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae. *Galleria mellonella* larvae were used for this purpose due to their high susceptibility to many fungal pathogens and because they are commercially reproduced in the Entomophagous and Entomopathogenic Reproduction Center in Cuba. A growth selective medium for *Beauveria* spp. and *Metarhizium* spp. was formulated using saboraud dextrose agar (SDA) (BioCen, Cuba) according to Meyling and Eilenberg (2007). The SDA culture medium was mixed with 1 mg L^{-1} (w/v) of thiabendazole, 0.05% streptomycin sulfate, and 250 mg L^{-1} (w/v) of chloramphenicol to avoid bacterial and some saprophytic fungi.

One g of each soil sample was placed in 20 ml of sterile distilled water with 0.01% Tween 80® in a 40 ml flat bottom glass tube. The tubes were mixed by vortexing for 1 min, and 100 µl of the soil solution was serially diluted to 10^{-3} conidia/ml and then inoculated into Petri dishes (9 cm diameter) with the selective medium described above. The Petri dishes with the soil dilution were incubated at 25 ± 1°C, and 75% relative humidity (RH) in the dark, for the emergence of fungal colonies. There were four replicates for each sample.

The insect bait method was conducted with the use of *G. mellonella* larvae. Soil samples (500 g) were placed in glass containers (500 ml) and five healthy 5-week-old *G. mellonella* larvae, obtained from the Entomophagous and Entomopathogenic Reproduction Center in Santa Clara, Cuba, were added. To prevent cocoon production and further webbing, *G. mellonella* larvae were conditioned before they were added by immersion of the larvae in water at 56°C for 15 sec, followed by the pouring of cold water at 4°C for 30 sec. Finally, the immobile larvae were placed on paper towels until they regained their movement (Woodring & Kaya, 1988). Containers were covered with lids perforated with 15 holes for aeration and placed at 25°C, 90% RH in the dark. No food was provided for the larvae. Containers were inverted every day to ensure that the larvae remained exposed to the soil. They were checked every two days for mortality until all larvae were dead. All cadavers were rinsed with distilled water and transferred to a moist chamber in Petri dishes (9 cm diameter) with moistened filter paper to stimulate fungal growth. A total of 150 larvae were used and the evaluations lasted 24 d. When larvae showed external fungal growth, the fungi were isolated on SDA chloramphenicol (250 mg L^{-1} (w/v)) and incubated at 25 ± 1°C and 90% RH in the dark. Colony colors were treated according to Kornerup and Wanscher (1984).

Entomopathogenic fungi identification

Entomopathogenic fungal isolates obtained from the soil were mounted on standard microscope slides (7.5 x 2.5 cm) and then mixed with a drop of lactophenol. Glass coverlips (2.5 x 5.0 cm and 0.16 cm thick) were then attached to the slide and sealed with resin. Fungal isolates were morphologically identified under a compound microscope (Motic, USA, 400x magnification) according to morphological characteristics described by Humber (2012) for each fungal species. The fungal isolates were kept in refrigeration at 4°C in tubes with SDA in the culture collections at the Departamento de Agronomía, Universidad Central "Marta Abreu" de Las Villas and the Instituto de Investigaciones Fundamentales en Agricultura Tropical "Alejandro de

Humboldt" (WDCM 853). Abundance was determined through the number of samples in which *Beauveria* and *Metarhizium* were found.

Statistical analysis

Analysis of variance (ANOVA) was applied to evaluate differences in frequencies of entomopathogenic fungi in maize and banana fields as well as to compare the effectiveness of the isolation methods. Means of entomopathogenic fungi were separated using Fisher's least significant difference (LSD) test. ANOVA were run using STATGRAPHICS Plus 5.1 (Manugistics Inc.) with significance level of 0.05.

Results and discussion

A total of 151 fungal isolates were obtained from the different maize and banana fields with both selective medium and insect bait methods. The identified entomopathogenic fungi are described below:

Beauveria spp.

Colonies on SDA attaining 50 mm in 7 d at 25°C, cottony at center, radially sulcate to filamentous toward the filiform margin, white (Fig. 1). Reverse colonies were reddish at the center and yellow around the periphery. Mycelium superficial and immersed. Hyphae septate, branched, hyaline, smooth, 1-2 µm wide. Conidiogenous cells polyblastic, lageniform, integrated or discrete, indeterminate ampulliform to subcylindrical at the base, geniculate, sympodial extended forming a rachis, with several distinct or inconspicuous denticles at the conidiogenous loci, arise from aerial hyphae. Conidia solitary, acropleurogenous, globose, unicellular, smooth-walled, hyaline, dry with 3.1 µm of diameter.

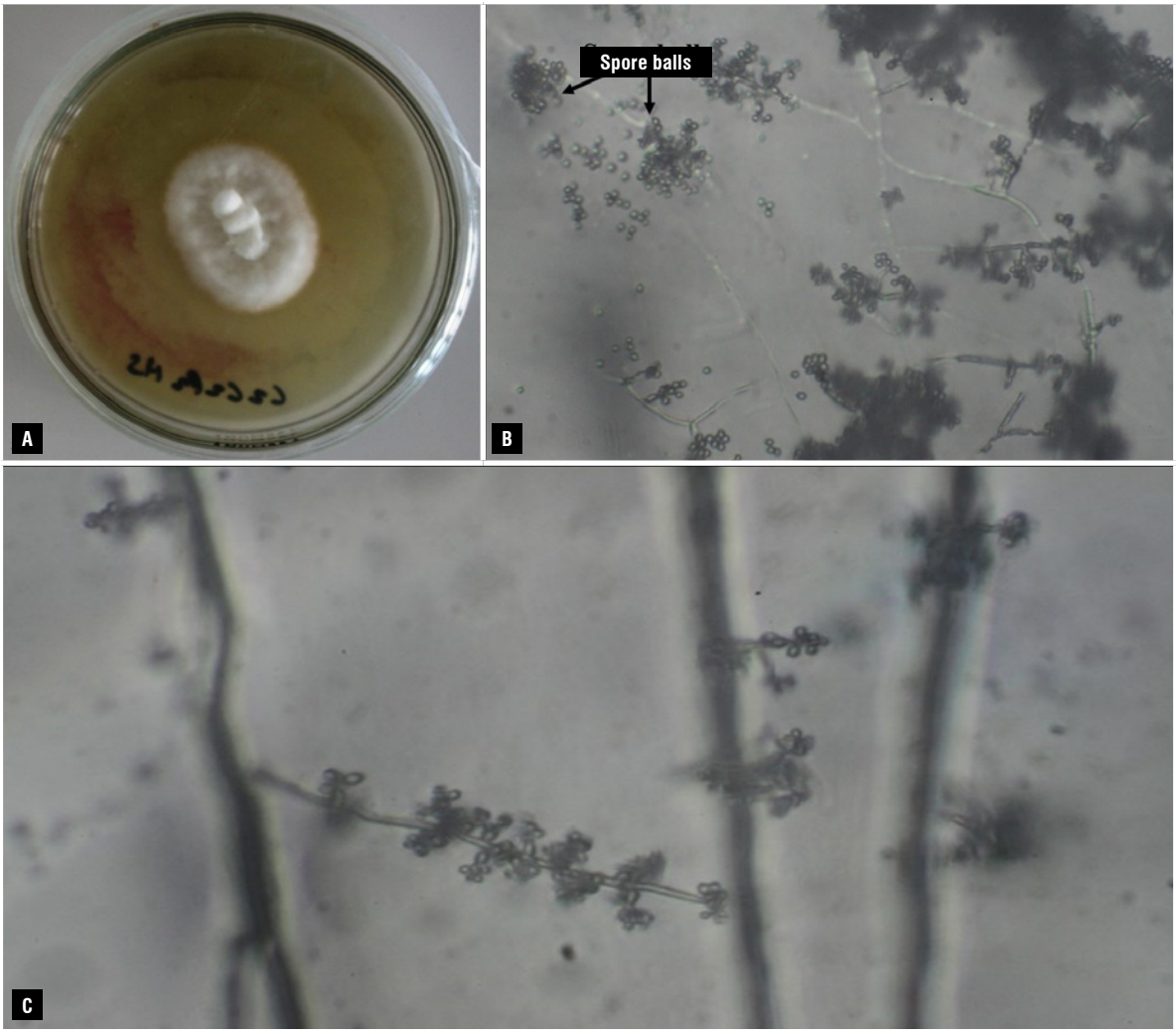

FIGURE 1. *Beauveria* sp. obtained from maize and banana fields. A) Colony of *Beauveria* sp. on SDA culture medium 7 d after inoculation at 25ºC. B) Spore balls representing dense clusters of large numbers of conidiogenous cells and conidia. C) Conidium formed successively on each denticle.

Metarhizium spp.

Colonies on SDA attaining 80 mm in 7 d at 25ºC, cottony to floccose at center, curled toward the slightly filiform margin that is colored white, with several sporodochial conidiomata, green or olivaceous (Fig. 2). Reverse was brownish. Mycelium was superficial and immersed. Hyphae were septate, branched, hyaline, smooth, 1-2 µm wide. Conidiomata were sporodochial, columnar, scattered or confluent, green, olivaceous to olivaceous brown. Conidiophores were macronematous, septate, penicillate or irregularly branched, hyaline, smooth, forming a compact cluster or clumps in the sporodochial conidiomata. Conidiogenous cells were monophialidic, cylindrical, discrete, determinate, smooth, hyaline. Conidia were basocatenulate, cylindrical, truncated at the ends, unicellular, pale olivaceous 6-8 × 1.5-2 µm, accumulating in a columnar, dark olivaceous masses.

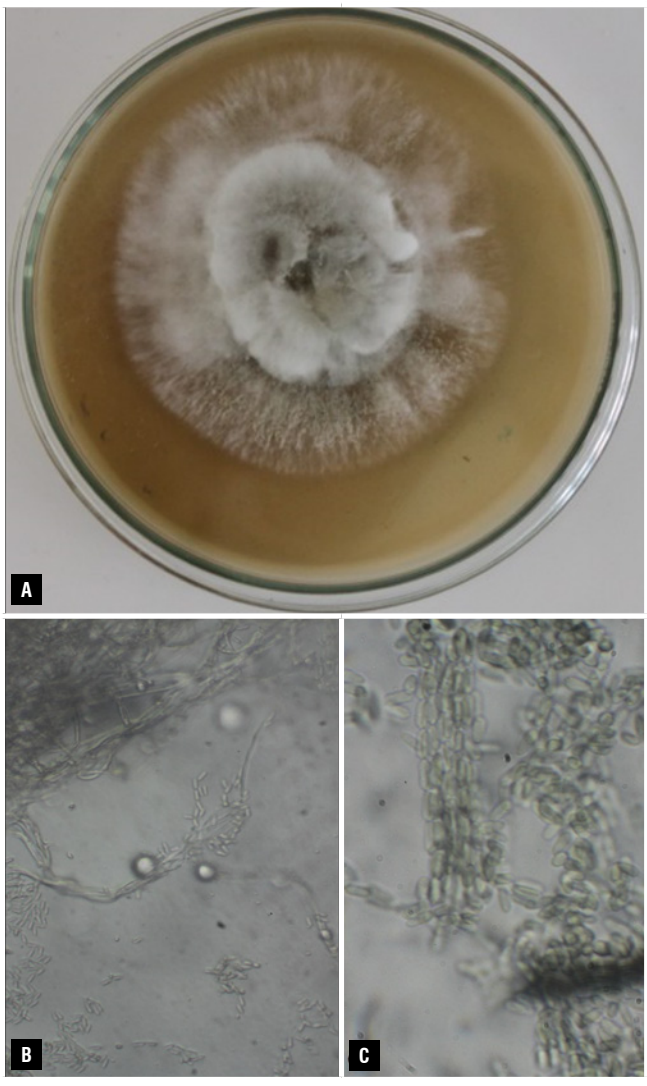

FIGURE 2. *Metarhizium* spp. obtained from maize and banana fields. A) Colony of *Metarhizium* spp. on SDA culture medium 7 d after inoculation at 25ºC. B) Branched conidiophore. C) Conidial chains.

A total of 36 and 52 *Metarhizium* spp. isolates were obtained from maize and banana fields, respectively. This fungal species was significantly (F=15.30; *df*=1; *P*=0.0001) more abundant than *Beauveria* spp., which were represented by 25 and 38 isolates in both the maize and banana fields. *Beauveria* spp. and *Metarhizium* spp. were the most frequently found entomopathogenic fungi in Mexican agroecosystems, and *Beauveria bassiana*, *Beauveria pseudobassiana* and *Metarhizium robertsii* were widely distributed (Pérez-González *et al*., 2014). Our results were in accordance with the results obtained by Korosi *et al*. (2019) who obtained more *Metarhizium* spp. (33%) than *Beauveria* spp. (26) in Australian vineyard soils. The abundance and diversity of entomopathogenic fungi have not been reported in maize and banana fields in Cuba before and, thus, constitutes a new record for the country.

Beauveria spp. isolates obtained from Santa Clara (10), Camajuaní (10) and Sagua la Grande (13) municipalities did not show significant differences (*P*>0.05) in abundance. However, *Metarhizium* spp. isolates in Sagua la Grande (19) were higher in number of infected larvae (F=10.18; *df*=2; *P*=0.0001) than in Santa Clara (13) and Camajuaní (12) (Tab. 1).

TABLE 1. Abundance (number of infected larvae) of *Beauveria* spp. and *Metarhizium* spp. obtained from maize and banana fields in three municipalities in Villa Clara, Cuba.

Location	Entomopathogenic fungi	
	***Beauveria* spp. (mean ± SE)**	***Metarhizium* spp. (mean ± SE)**
Santa Clara	10 ± 0.77 ab	13 ± 1.88 b
Camajuaní	10 ± 0.94 ab	12 ± 1.61 b
Sagua la Grande	13 ± 1.86 a	19 ±1.74 a

Different letters in the same column indicate significant differences in the abundance of *Beauveria* spp. and *Metarhizium* spp. isolates according to the Fisher's test (*P*<0.05).

These results can be supported by the fact that *Metarhizium* is reported to be more abundant than other entomopathogenic fungi in cultivated fields (Tkaczuk *et al*., 2014). In contrast, Pérez-González *et al*. (2014) obtained 112 *Beauveria* spp. and 9 *Metarhizium* spp. isolates from the soil of 11 locations of Guanajuato State, Mexico. These results demonstrated that the abundance and distribution of entomopathogenic fungi is still unclear, and more studies are needed to clarify this aspect. However, the abundance of *Metarhizium* spp. over *Beauveria* spp. in banana and maize fields in Cuba could be explained

through the hypothesis that the association of *Metarhizium* spp. with insect host species has a tropical origin. In addition, *Metarhizium* comprises an assemblage of cryptic species, many of which traverse large geographical barriers (Bidochka & Small, 2005).

Biotic (interaction with other species) and abiotic factors (mainly temperature) are considered primary determinants of abundance and population genetic structure of *Metarhizium* (McGuire & Northfield, 2020). According to these data we infer that the tropical conditions of Cuba allowed a greater abundance of *Metarhizium* spp. in banana and maize fields compared with *Beauveria* spp.

The mean of *Beauveria* spp. (22) and *Metarhizium* spp. (30) isolates recovered with the insect baiting method were higher (F=25.12; *df*=1; *P*=0.0018) than those obtained with the selective medium (10 *Beauveria* spp. and 15 *Metarhizium* spp. isolates) (Tab. 2).

TABLE 2. Abundance of *Beauveria* spp. and *Metarhizium* spp. obtained by selective medium and insect bait methods.

Isolation method	Entomopathogenic fungi	
	***Beauveria* spp. (mean ± SE)**	***Metarhizium* spp. (mean ± SE)**
Selective medium	10 ± 0.70 b	15 ± 0.60 b
Insect bait	22 ± 0.99 a	30 ± 1.35 a

The selective medium indicates the number of colonies per Petri dish, while insect bait shows the number of infected larvae. Different letters in the same column indicate significant differences in the abundance of *Beauveria* and *Metarhizium* isolates obtained with selective medium and insect bait methods according to the Fisher's test ($P<0.05$).

Different results have been obtained about methods of isolating entomopathogenic fungi in the same soil sample (Hernández-Domínguez *et al*., 2016). Our results demonstrated that the insect bait method is better for obtaining entomopathogenic fungi. The selective medium is targeted at particular fungal species, while insect baiting could detect a larger number of species (Keller *et al*., 2003). However, Tkaczuk *et al*. (2014) did not find difference in *Metarhizium* spp. from organic fields using the insect baiting and selective medium methods. The possible explanation for this result is focused on the insect bait method. The absence of water within the plastic boxes could limit the growth of the entomopathogenic fungi. In a similar study conducted by Ramos *et al*. (2017) the authors used sterile water to moisten the soil before introducing it to the plastic boxes. Water contents in the soil helps to maintain a high relative humidity which in turn helps the growth of the entomopathogenic fungi (Lazzarini *et al*., 2006; Jaronski, 2009).

Conclusion

According to our results, the entomopathogenic fungi *Metarhizium* spp. were significantly more abundant than *Beauveria* spp. in both maize and banana plots. The abundance of *Metarhizium* spp. in Sagua la Grande was higher than in Santa Clara and Camajuaní. The insect bait method resulted in the most appropriate method to isolate entomopathogenic fungi from soil. These results contribute to a better understanding of hypocrealean fungi ecology and their composition in both maize and banana fields in central Cuba.

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Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

YR and OP designed the experiment. YR, ADT, CA and AA conducted the experiment. YR performed the statistical analysis. ADT, CA, ALA and RCR wrote the initial draft. YR, RCR and OP wrote the final version of the manuscript. All authors have reviewed the manuscript.

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