Evaluation of Isolates of Entomopathogenic Fungi in the Genera Metarhizium,Beauveria, and Isaria, and Their Virulence to Thaumastocoris peregrinus (Hemiptera: Thaumastocoridae)

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Evaluation of isolates of entomopathogenic fungi in the genera *Metarhizium*, *Beauveria,* and *Isaria,* and their virulence to *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae)

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Abstract

Thaumastocoris peregrinus Carpintero and Dellapé, or bronze bug (Hemiptera: Thaumastocoridae), is a pest found in eucalyptus plantations worldwide. Use of entomopathogenic fungi has emerged recently as an alternative means of control for *T. peregrinus*. Here we report laboratory experiments conducted to select isolates of entomopathogenic fungi for use to control this pest. Seven isolates of *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae), 3 of *Metarhizium anisopliae* (Metschn.) Sorokīn (Hypocreales: Clavicipitaceae), 2 of *Metarhizium robertsii* J.F. Bisch., S.A. Rehner & Humber (Hypocreales: Clavicipitaceae), and 2 of *Isaria fumosorosea* Wize (Hypocreales: Cordycipitaceae) at a concentration of 1×10^8 conidia per mL were assessed for their virulence to adults of *T. peregrinus*. In vitro growth and sporulation of the isolates also were evaluated through the analysis of colony development and conidia production in PDA culture medium, at 26 ± 1 °C, 70% RH, and a 12:12 h (L:D) photoperiod. All tested isolates were found to be pathogenic to *T. peregrinus*. The isolates Bb3, Bb4, Bb7, Ma1, Ma2, Mr2, and If1 exhibited significant virulence to the insect. The greatest growth (longest mean diam of colony) was for the isolate Ma2, whereas the highest sporulation rates were observed in the isolates If2 and Ma2. This study demonstrated that isolates of *Beauveria*, *Metarhizium*, and *Isaria* can cause infection in *T. peregrinus*. The Ma2 isolate of *Metarhizium* stood out for its pathogenic effect on the adult insect, as well as for its capacity to grow rapidly and produce large numbers of conidia on an artificial medium, indicating its potential for use as a microbial control agent for *T. peregrinus*, and its suitability for mass production.

Key Words: eucalyptus; eucalyptus pests; biological control

Resumen

Thaumastocoris peregrinus Carpintero and Dellapé, ou percevejo-bronzeado do eucalipto (Hemiptera: Thaumastocoridae), é uma praga nas plantações de eucalipto em todo o mundo. O uso de fungos entomopatogênicos emergiu recentemente como meio alternativo de controle de *T. peregrinus*. Aqui nós relatamos experimentos de laboratório feitos para selecionar isolados de fungos entomopatogênicos para uso contra esta praga. Sete isolados de *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Clavicipitaceae), 3 de *Metarhizium anisopliae* (Metschn.) Sorokīn (Hypocreales: Clavicipitaceae), 2 de *Metarhizium robertsii* J.F. Bisch., S.A. Rehner & Humber (Hypocreales: Clavicipitaceae), e 2 de *Isaria fumosorosea* Wize (Hypocreales: Cordycipitaceae) na concentração de 1×10^8 conídios por mL foram avaliados em relação a sua virulência contra adultos de *T. peregrinus*. O crescimento e a esporulação in vitro dos isolados também foram avaliados, através da análise do desenvolvimento de colônias e produção de conídios em meio de cultura de BDA, a 26 ± 1 °C, 70% de UR, e fotoperíodo de 12:12 h (L:D). Todos os isolados testados foram patogênicos para *T. peregrinus*. Os isolados Bb3, Bb4, Bb7, Ma1, Ma2, Mr2, e If1 exibiram significativa virulência ao inseto. O maior crescimento (maior diâmetro médio de colônia) foi para o isolado Ma2, enquanto as maiores taxas de esporulação foram observadas nos isolados If2 e Ma2. Este estudo demonstrou que isolados de *Beauveria*, *Metarhizium* e *Isaria* podem causar infecção em *T. peregrinus*. O isolado Ma2 de *Metarhizium* destacou-se pelo seu efeito patogênico no inseto adulto, bem como por sua capacidade de crescer rapidamente e produzir um grande número de conídios em meio artificial, indicando seu potencial de uso como agente de controle microbiano contra *T. peregrinus* e sua adequação para produção em massa.

Palabras Claves: eucalyptus; pragas de eucalyptus; controle biológico

Thaumastocoris peregrinus Carpintero & Dellapé (Hemiptera: Thaumastocoridae) (Carpintero & Dellapé 2006) (previously called *Thaumastocoris australicus* Kirkaldy) is an important pest found in eucalyptus trees in Africa, Latin America, Europe, and Oceania (EPPO 2015).

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Adults of *T. peregrinus* have a flat body, approximately 3 mm in length, with a broad head, developed mandibular plates, and an antenna composed of 4 segments, the apical one being darker in color. It has a light brown body and some areas with a darker coloration (Nadel & Noack 2012).

Eggs of *T. peregrinus* are deposited in groups on the leaves and branches of the host plants (Carpintero & Dellapé 2006). Under laboratory conditions at 17 to 22 °C, *T. peregrinus* can complete its development in 20.4 d. Adults can live for about 40 d, and during this period produce about 60 eggs per female in a lifetime (Noack & Rose 2007).

Thaumastocoris peregrinus attacks a number of species of *Eucalyptus* and some species of *Corymbia* (both Myrtaceae) (EPPO 2015) that they damage by sucking plant sap that in turn leads to leaf abscission, reduced photosynthetic capacity, and at high infestation rates, plant death. Leaves that have been fed upon first have a silvery and then a bronzy coloration. The rapid spread of *T. peregrinus* in several parts of the world is likely due to the commercial transport of eucalyptus wood contaminated with the eggs of this insect (Wilcken et al. 2010).

Whereas the chemical control of *T. peregrinus* has been used effectively in small areas (Noack et al. 2009), chemical insecticide residues remain in the soil and groundwater and some products so used are harmful to human or animal health (Gerage et al. 2017). Biological control, especially the use of entomopathogenic fungi, is a promising alternative for controlling several pests of eucalyptus, but remains little used for *T. peregrinus*. Entomopathogenic fungi have some features that increase their value for pest control, especially the ones that allow the high dispersal of their spores under field conditions and the long persistence of their spores in the environment (Khan et al. 2012).

Previous studies have documented the occurrence of natural fungal infestations in nymphs and adults of this pest (Mascarin et al. 2012); however, few studies have looked at the deliberate selection from nature of potentially superior isolates, or have characterized the features of such isolates. Nevertheless, in order to use entomopathogenic fungi effectively in biological control programs, it is important to understand their rates of development, their capacity to infect and kill the target host, and find isolates suitable for efficient artificial production. The present study aimed at identifying isolates of entomopathogenic fungi useful for the biological control of *T. peregrinus* through the analysis of the biological characteristics, including colony growth rates, abundance of fungal sporulation, and fungal virulence to the target pest.

Materials and Methods

FUNGAL ORIGIN AND INSECT REARING

Seven isolates of *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae), 3 of *Metarhizium anisopliae* (Metschn.) Sorokīn (Hypocreales: Clavicipitaceae), 2 of *Metarhizium robertsii* J.F. Bisch., S.A. Rehner & Humber (Hypocreales: Clavicipitaceae), and 2 of *Isaria fumosorosea* Wize (Hypocreales: Cordycipitaceae) were used in this study, all from the mycology collection of the Laboratory of Plant Health (Embrapa Coastal Tablelands-CPATC/SE) and of the Laboratory of Insect Pathology (ESALQ-USP) (Table 1).

Adults of *T. peregrinus* used in experiments were from a colony held in the Laboratory of Forestry Entomology at the Federal University of Sergipe, Aracaju, Sergipe, Brazil. Adult bugs were maintained on foliage of *Eucalyptus grandis* W. Hill ex Maiden (Myrtaceae) at 25 ± 1.2 °C, 60 ± 10% RH, and a 12:12 h (L:D) photoperiod. Pathogenicity bioassays with the entomopathogenic fungi used unsexed adult insects.

Table 1. List and origin of the entomopathogenic fungal isolates used in the bioassays.

Fungus / Isolate	Host	Locality (State/Country)
Beauveria bassiana		
Bb1*	*Plutella* sp.	São Paulo/Brazil
Bb2	*Diaphorina citri*	Sergipe/Brazil
Bb3	Soil	Sergipe/Brazil
Bb4	Soil	Sergipe/Brazil
Bb5	Soil	Sergipe/Brazil
Bb6	Soil	Sergipe/Brazil
Bb7	Soil	Sergipe/Brazil
Ma1	Soil	Sergipe/Brazil
Metarhizium anisopliae		
Ma2	Soil	Sergipe/Brazil
Ma3	Soil	Sergipe/Brazil
Metarhizium robertsii		
Mr1	Soil	Sergipe/Brazil
Mr2	Soil	Sergipe/Brazil
Isaria fumosorosea		
If1	Soil	Sergipe/Brazil
If2	Soil	Sergipe/Brazil

*Isolate from the Laboratory of Insect Pathology (ESALQ–USP)

PRODUCTION OF SUSPENSIONS OF ENTOMOPATHOGENIC FUNGI

Conidia of each of the fungal isolates were produced in a semi-solid medium (rice) as described by Alves and Pereira (1998). Fifteen d after inoculation of the growing medium, fungal suspensions were prepared for each isolate by washing 50 g of the production medium (rice, containing conidia of the fungi) with 100 mL of Tween 80 solution (0.05%), shaking with a glass stick for 5 min. Subsequently, the resulting suspension was filtered and the conidia number quantified in a Neubauer chamber (Alves & Pereira 1998). All conidial suspensions were adjusted to 1×10^8 conidia per mL, a concentration that provided significant mortality in *T. peregrinus* adults in preliminary bioassays. Conidial viability was measured by placing a 100 µL aliquot of the fungal suspension in the center of the Rodac plate, containing Potato Dextrose Agar + Chloramphenicol (antibiotic), and incubating the plate in an incubator Bio-Oxygen Demand (model SL-225, Solab, Piracicaba, São Paulo, Brazil) for 12 h at 26 ± 1 °C and 70% RH. After this period, the plates were observed under a compound microscope at 400×, and the proportions of germinated and non-germinated conidia in the plate were determined.

PATHOGENICITY AND VIRULENCE

Eucalyptus leaves (*E. grandis*) with their petioles wrapped in cotton were placed individually in 50 mL Falcon tubes containing water, which preserved the foliage. Each Falcon tube with 1 leaf was inverted and attached to the lid of a transparent plastic cup (700 mL) to form the experimental arena. Each arena received 10 test insects, and each cup + 10 insects constituted 1 replicate of the experiment. A manual sprayer (air brush, 30 atm) was used to spray 1 mL of the fungal suspension, holding the application nozzle at a distance of 10 cm from the leaf and insects. A control group was treated with 1 mL of the Tween 80 solution (0.05%). Each treatment was replicated 4 times. After fungal application, treatments were maintained at 26 ± 1 °C, 70% RH, and a 12:12 h (L:D) photoperiod for 10 d. Dead insects were collected from test arenas daily and transferred to a humid chamber to detect fungal sporulation and confirm fungus infection as the cause of death.

IN VITRO GROWTH AND SPORULATION

Isolates were separately inoculated onto media in Petri dishes (70 × 15 mm) containing Potato Dextrose Agar + Chloramphenicol (antibiotic) culture medium and incubated in a Bio-Oxygen Demand germination chamber for 7 d at 26 ± 1 °C, 70% RH, and a 12:12 h (L:D) photoperiod. After this period, a disc 5 mm in diam was removed from the culture medium containing the fungal colony. This disc was then transferred to the center of a Petri dish (70 × 15 mm) containing 20 mL of culture medium Potato Dextrose Agar + Chloramphenicol (antibiotic), with the side of the disc that contained the mycelium facing down. The plates were incubated in Bio-Oxygen Demand germination chambers for 15 d under the same conditions as above. Three replicates were made for each isolate. The diameter of the resultant fungal colonies then was measured using a caliper at 3, 6, 9, 12, and 15 d of incubation. Fungal colony was measured in 4 positions with a difference of 45 degrees between them. Fungal colony growth was considered the mean of the diam measurement at these 4 positions performed on the same d.

After evaluation of the radial growth of fungal colonies, a sample of the fungus on the culture medium was collected and transferred to a test tube, in which it was homogenized with 10 mL of Tween 80 solution (0.05%). The spore count of each such suspension was then determined using a Neubauer chamber. Spore counts were performed in triplicate for each isolate.

STATISTICAL ANALYSIS

Rates of insect mortality for the different fungal treatments were corrected for control mortality using Abbott's formula (Abbott 1925), and treatment means were analyzed using ANOVA followed by a Tukey's test ($P < 0.05$). Analyses of insect survival as a function of the LT_{50} of the fungal isolates were performed using Kaplan-Meier estimators and a Log-rank test (Mantel-Cox), using the Statistical Package for the Social Sciences, version 15.0 (2006).

The results from both the growth rate and conidial production evaluations were subjected to analysis of variance (ANOVA), followed by a Tukey's test ($P < 0.05$), using the SPSS version 15.0 (2006).

Results

ISOLATE PATHOGENICITY

Mean conidial viability of materials used in bioassays of isolate pathogenicity and virulence to the target pest was 95%. All tested isolates, in the concentration of 1×10^8 conidia per mL, were pathogenic to *T. peregrinus* adults; however, at 10 d post-application there were significant differences ($F_{13,42} = 5.833$, $P < 0.001$) in the rate of mortality among the genera of the isolates, with isolates in the genera *Isaria* (88.9%) and *Metarhizium* (79.7%) being more pathogenic to *T. peregrinus* than isolates from *Beauveria* (71.4%). The mean rate of corrected mortality of the pest varied among fungal isolates from 52.2% (Bb5) to 100% (Mr2) (Fig. 1). The isolates Mr2 (100%), If1 (97.5%), Ma2 (90%), Bb7 (85%), If2 (80.3%), Ma3 (80%), Bb2 (80%), Bb1 (74.2%), Bb3 (73.1%), and Bb4 (73.1%) were moderately to highly pathogenic, but did not differ significantly among themselves (Tukey $P < 0.05$). The isolates Mr1, Bb6, Ma1, and Bb5 showed confirmed mortality equal to or lower than 70%.

LETHAL TIME VALUES (LT_{50}) FOR TESTED ISOLATES

Lethal time values (LT_{50}) varied among genera of tested isolates (Log-Rank $\chi^2 = 9.167$; df = 2; and $P = 0.010$), with *Metarhizium* isolates
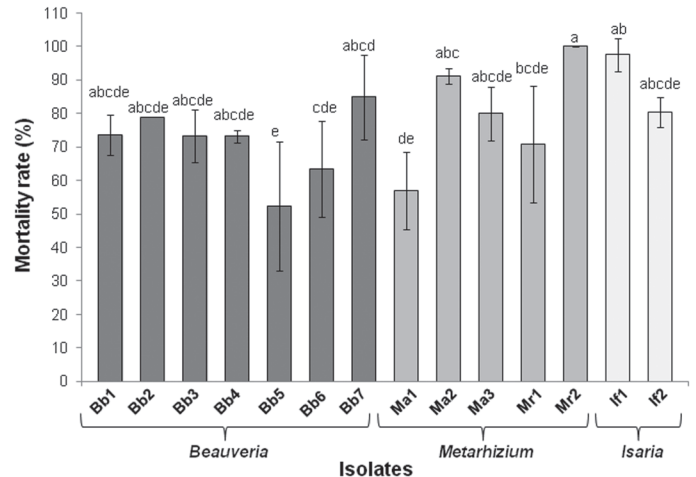

Fig. 1. Percentage of confirmed mortality of *Thaumastocoris peregrinus*, treated with suspensions of 1×10^8 conidia per mL of entomopathogenic fungal isolates. Different letters above the bars indicate differences between treatments by Tukey's test ($P < 0.05$). Bars represent the error $\pm 1 \times$ SEM (df = 100).

having shorter average lethal time values ($LT_{50} = 2$ d) than isolates of *Beauveria* ($LT_{50} = 2.3$ d) or *Isaria* ($LT_{50} = 2.5$ d) (Fig. 2).

Among isolates, we found significant differences in pest insect survival times (LT_{50}) (Log-Rank $\chi^2 = 146.884$; df = 14; and $P = 0.000$). Isolates Bb3, Bb4, and Bb7 (*Beauveria*), Ma2, Ma3, and Mr2 (*Metarhizium*), and If1 (*Isaria*) showed the lowest LT_{50} values, but did not differ significantly among themselves based on Tukey's test (Table 2). The other isolates all showed longer pest survival times, with LT_{50} values varying from 3 d (Bb5 and Ma1) to 3.6 d (Bb1) (Table 2).

IN VITRO GROWTH AND SPORULATION RATES AMONG ISOLATES

The mean growth rates of colonies of different fungal isolates varied throughout the evaluation period and showed significant differences at the end of the evaluation period (at 15 d) ($F = 12.841$; df = 13; $P = 0.000$) (Table 3). Among the isolates of *B. bassiana*, Bb5 (50.79 mm), Bb7 (55.58 mm), Bb2 (58.79 mm), Bb3 (59.71 mm), and Bb4 (60.38 mm) showed the highest mean values of colony diam on the last day
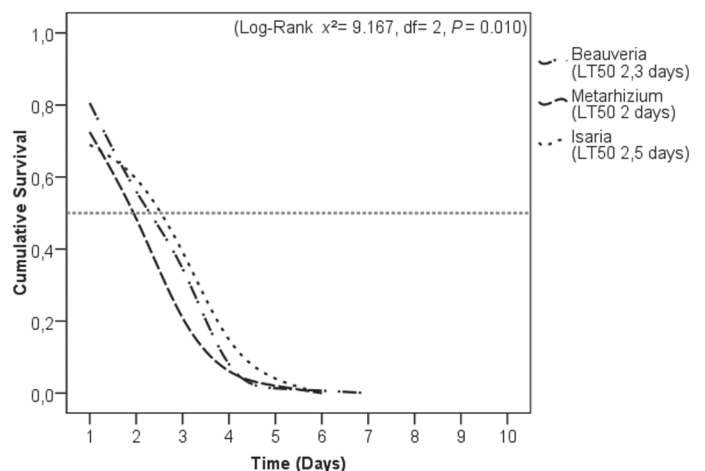
Fig. 2. Daily mean survival curves for adult *Thaumastocoris peregrinus* exposed to entomopathogenic fungal isolates of the genera *Beauveria*, *Metarhizium*, and *Isaria* for a period of 10 d. The horizontal dotted line indicates insect mortality of 50%.

Table 2. Mean lethal times (LT_{50}), in d, for the mortality of *Thaumastocoris peregrinus* treated with entomopathogenic fungal suspensions, at the concentration of 1×10^8 conidia per mL, through Kaplan-Meier estimators, Log-Rank test.

Treatment	$LT_{50} \pm SE^*$	Lower limit	Upper limit
Bb1	3.6 ± 0.21 f	3.2	4.0
Bb2	2.9 ± 0.16 bcdef	2.6	3.2
Bb3	2.0 ± 0.17 ab	1.7	2.4
Bb4	2.4 ± 0.19 abcde	2.0	2.8
Bb5	3.0 ± 0.16 def	2.7	3.4
Bb6	3.4 ± 0.20 f	3.0	3.8
Bb7	2.0 ± 0.13 a	1.7	2.2
Ma1	3.0 ± 0.21 cdef	2.6	3.4
Ma2	2.2 ± 0.17 abcd	1.9	2.6
Ma3	2.1 ± 0.19 abc	1.7	2.5
Mr1	3.2 ± 0.22 e	2.7	3.6
Mr2	2.0 ± 0.13 ab	1.7	2.3
If1	2.4 ± 0.21 abcde	1.9	2.8
If2	3.4 ± 0.27 e	2.8	3.9

*SE = Standard Error

of evaluation but did not differ significantly among themselves based on Tukey's test (*P* < 0.05). Among *Metarhizium* isolates, Ma2 had the highest growth (84.5 mm). *Isaria* isolates If1 (62.67 mm) and If2 (75.42 mm) did not differ at the end of the evaluation.

Isolates If2, If1, Mr1, Bb4, Bb3, Bb2, and Mr2 showed no significant differences in colony growth, varying from 58.2 mm (Mr2) to 75.4 mm (If2). Considering the growth in each evaluation period (3, 6, 9, 12, and 15 d), the isolate Ma2 stood out for exhibiting a higher growth rate than the others on all d of the evaluation (Table 3).

Based on counts of conidia, we found that isolates of *Beauveria* did not differ significantly in their sporulation rate, with values from 0.06×10^8 conidia per mL (Bb6) to 1.53×10^8 conidia per mL (Bb2 and Bb3) (Table 3). Among isolates of *Metarhizium*, Ma2 showed the highest sporulation rate (3.4×10^8 conidia per mL). There was significant variation in the sporulation rates of isolates of *I. fumosorosea*, with If2 reaching the highest rate of 4.37×10^8 conidia per mL.

In general, after 15 d of incubation, the isolate If2 showed the highest mean sporulation rate among all tested isolates, but did not differ significantly from that of Ma2 (Table 3).

Discussion

Whereas all tested genera of entomopathogenic fungi exhibited infectivity to *T. peregrinus*, isolates in *Isaria* showed mean pathogenicity higher than other tested genera (Fig. 1). In general, we observed high variation in pathogenicity rates among entomopathogenic fungal isolates, likely due to the biology of each fungal isolate, since the environmental conditions did not vary.

Previous studies have reported mortality levels similar to those observed for most of the *B. bassiana* isolates that we tested. Lorencetti (2013) found confirmed mortality rates from 37 to 80.1% to *T. peregrinus* in a similar experiment for 4 *B. bassiana* isolates.

In our study, *Metarhizium* isolates Mr2 and Ma2 at the same concentration caused 100 and 90% mortality, whereas somewhat lower mortality (80%) in adults of *T. peregrinus* was caused by a commercial product based on *M. anisopliae*, at 1×10^8 conidia per mL (Soliman et al. 2009). In this study, the *Isaria* isolates If1 and If2 caused mortality of 97.5 and 80.3% to *T. peregrinus*, respectively, whereas a previous study that analyzed the action of an isolate of *Isaria* sp. on *T. peregrinus* observed a mortality of 87% (Lorencetti 2013).

Pathogenicity and virulence are intrinsic characteristics of fungal isolates, and they both have a genetic origin. These traits also vary in response to the infected host species, the growth medium, abiotic factors, types of formulations used, and methods of handling and application of the pathogen (Khan et al. 2012). Pathogenicity demonstrates the capacity of the isolate of an entomopathogenic fungus to infect the insect causing disease, whereas virulence quantifies such effect based on the time (post-infection) of mortality or the degree of colonization of host tissues (Alves & Lecuona 1998).

The virulence of the entomopathogenic fungal isolates tested in this study was evaluated through the analysis of mean lethal time (LT_{50}) of the pest insect after its exposure to the pathogen. In general, there was a high mortality of *T. peregrinus* in the first few d after inoculation

Table 3. Radial growth rate (mm per d⁻¹) of the colonies and conidia production (conidia per mL) of the entomopathogenic fungal isolates cultivated in PDA medium, at 26 ± 1 °C, 70% RH, and 12:12 h (L:D) photoperiod.

Isolates	Radial growth (mm) per d					Sporulation (× 10⁸)
	3	6	9	12	15	
Bb1	13.17 ± 1.4 cde	21.96 ± 1.66 fg	30.75 ± 8.24 ef	35.79 ± 10.28 de	40.29 ± 11.48 de	0.26 ± 0.15 c
Bb2	13.92 ± 0.59 cd	28.38 ± 1.12 cde	46.00 ± 0.79 bc	53.58 ± 0.29 bc	58.79 ± 1.08 bc	1.53 ± 0.46 bc
Bb3	14.17 ± 0.83 c	28.79 ± 1.82 cde	40.38 ± 4.52 cde	51.46 ± 5.59 c	59.71 ± 3.68 bc	1.53 ± 0.28 bc
Bb4	17.25 ± 0.49 b	32.00 ± 1.02 bc	44.17 ± 0.08 bcd	54.88 ± 1.71 bc	60.38 ± 1.81 bc	0.68 ± 0.41 c
Bb5	10.79 ± 1.19 e	23.88 ± 2.55 efg	33.42 ± 3.55 def	42.63 ± 0.86 cde	50.79 ± 3.83 cde	1.51 ± 1.36 bc
Bb6	11.75 ± 1.36 cde	20.79 ± 3.29 g	27.63 ± 7.38 f	32.42 ± 10.13 e	37.13 ± 11.39 e	0.06 ± 0.03 c
Bb7	13.25 ± 0.65 cde	27.21 ± 1.56 cdef	38.83 ± 0.86 cdef	48.04 ± 4.34 cd	55.58 ± 8.07 cd	1.05 ± 1.27 c
Ma1	11.50 ± 1.27 de	22.13 ± 1.81 fg	37.42 ± 3.02 cdef	45.08 ± 3.16 cde	50.33 ± 2.06 cde	0.17 ±0.03 c
Ma2	31.67 ± 1.51 a	63.29 ± 3.64 a	77.75 ± 7.59 a	83.33 ± 4.94 a	84.50 ± 5.51 a	3.40 ± 0.20 ab
Ma3	12.75 ± 0.88 cde	25.21 ± 2.27 defg	36.38 ± 0.98 cdef	46.33 ± 3.86 cde	57.29 ± 6.79 cd	1.77 ± 0.40 bc
Mr1	12.92 ± 0.59 cde	26.96 ± 2.77 cdef	36.63 ± 2.36 cdef	50.13 ± 3.07 c	62.46 ± 4.95 bc	0.28 ± 0.32 c
Mr2	13.63 ± 0.37 cd	28.04 ± 0.08 cde	44.58 ± 2.83 bcd	52.25 ± 2.73 bc	58.29 ± 4.64 bc	0.24 ± 0.37 c
If1	18.25 ± 0.13 b	30.79 ± 0.46 bcd	47.88 ± 3.98 bc	55.54 ± 6.65 bc	62.67 ± 9.07 bc	0.01 ± 0.26 c
If2	17.92 ± 0.33 b	35.75 ± 3.26 b	53.08 ± 3.16 b	66.13 ± 4.32 b	75.42 ± 5.11 ab	4.37 ± 2.25 a
F	122.439*	86.935*	29.769*	20.618*	12.841*	9.729*
df	13	13	13	13	13	13

Results followed by the same letter in the column do not differ significantly (Tukey, P < 0.05). *P = 0.0001

of the fungal suspensions. The small body size of the insect in question may be one of the factors promoting the rapid action of the fungus. Attributes of the host, such as the presence or absence of wings, also may influence the development of entomopathogenic fungi on the host and, consequently, mortality in the initial d of evaluation (Alves & Lecuona 1998).

The estimated LT_{50} value overlaps the data of pathogenicity, since the mean lethal time of the isolates of *Metarhizium* (LT_{50} = 2 d) is shorter than those of *Beauveria* (LT_{50} = 2.3 d) and *Isaria* (LT_{50} = 2.5 d) (Fig. 2), suggesting that although the genus *Isaria* was more pathogenic to *T. peregrinus*, the genus *Metarhizium* was more virulent. The results also demonstrated significant variation in virulence among isolates of the tested genera (Table 2) that can be related to the genetic variation existing among the isolates of the genera. The isolates Bb3, Bb4, Bb7, Ma2, Ma3, Mr2, and If1 showed the lowest LT_{50} values (Table 2). Similar virulence levels were found for the isolates of *Beauveria* in our study to that reported by Soliman et al. (2009) (LT_{50} = 2.64 d) using a commercial *B. bassiana* product at 1×10^8 conidia per mL under laboratory conditions. Soliman (2014) reported higher LT_{50} values (between 5 and 7 d) for *T. peregrinus* for a commercial *M. anisopliae* product under similar experimental conditions.

Variation in the virulence of the tested isolates of entomopathogenic fungi to *T. peregrinus* can be related to the capacity of certain fungi to secrete enzymes that degrade insect cuticle, allowing the pathogen conidia to more easily penetrate the host, increasing the speed of penetration and colonization, and the subsequent death of the insect. This attribute has a genetic origin and is related to the coevolution between the pathogenic microorganisms and the hosts present in their natural habitat. This varies between species and between different isolates, suggesting a high specificity of the isolate to the target pest (Charnley & St. Leger 1991; Vilcinskas 2010; Ortiz-Urquiza & Keyhani 2013). Previous studies have found a relationship between enzymatic production and the virulence of some isolates of *B. bassiana* on *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Kaur & Padmaja 2009) and, similarly, between proteases and destruxins production and virulence of isolates of *M. anisopliae* (St. Leger et al. 1987). The modulation of these enzymes is believed to be responsible for the variations in the virulence and pathogenicity rates observed in this study.

Frequently, studies to select entomopathogenic fungi for the biological control of pest insects are based on the analysis of mortality and virulence of available isolates (Barta 2010; Lo Verde et al. 2015). However, the analysis of the biological aspects of these microorganisms, such as radial growth and sporulation, provides more information on the physiology and behavior of the isolates, expanding the characteristics evaluated for the selection of fungi with potential in the large-scale utilization for the biological control of pests. In this study, isolates of *B. bassiana* showed a mean colony diameter ranging from 40.29 mm (Bb1) to 60.38 mm (Bb4). When evaluating the development of 15 isolates of *B. bassiana* in PDA medium, Gandarilla-Pacheco et al. (2012) observed mean colony diameter of the isolates varying from 34 to 59 mm. Meanwhile, isolates of the genus *Isaria* showed cumulative growth of 62.67 mm (If1) and 75.42 mm (If2) in the current study, but there are no reports in the literature of similar analyses for other *Isaria* isolates. The *Metarhizium* isolate Ma2 showed the highest cumulative radial growth (84.5 mm) among all tested isolates regardless of genus, and this value was superior to those found in the literature (between 57 and 78.1 mm) (Gandarilla-Pacheco et al. 2012).

Regarding the sporulation rate, we observed that the isolate Ma2 showed the highest mean sporulation rate (3.4×10^8 conidia per mL) (Table 3) among the tested isolates. Previous studies observed variations in the sporulation rates of *M. anisopliae* isolates grown in PDA, with mean values between 8×10^7 and 1.8×10^8, after 18 d of incubation (Cito et al. 2014).

Radial growth and sporulation of entomopathogenic fungi show different rates of development among distinct isolates and can be modulated by changing the nutritional components of the production medium (Smith & Grula 1981), by abiotic factors (Ekesi et al. 1999; Mustafa & Kaur 2008; Borisade & Magan 2014), by their capacity to produce secondary metabolites (Calvo et al. 2002), and the production methodology used (Gouli et al. 2013). In the present study, all tested isolates of entomopathogenic fungi were subjected to the same nutritional and storage conditions; however, we believe that the variation observed in the rates of colony growth and sporulation indicates the genetic variability between the isolates.

Among the tested fungi, these bioassays sought to select 1 isolate with the potential to cause the greatest possible mortality to adults of *T. peregrinus*, but also an isolate that has the capacity to rapidly colonize the insects and produce conidia that can be transmitted horizontally, infecting the pest population as a whole. Also, in selecting an optimal isolate for use in a biological control program based on the mass production of entomopathogenic fungi, it is important to evaluate its potential for mass rearing, especially mycelial growth rates and conidia production levels.

Acknowledgments

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