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RESEARCH PAPER

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UV-B tolerances of conidia, blastospores, and microsclerotia of *Metarhizium* spp. entomopathogenic fungi

Amanda R. C. Corval[1] | **Emily Mesquita**[1] | **Thaís A. Corrêa**[1] | **Cárita de S. R. Silva**[2] | **Ricardo de O. B. Bitencourt**[1] | **Éverton K. K. Fernandes**[2] | **Vânia R. E. P. Bittencourt**[1,3] | **Donald W. Roberts**[4] | **Patrícia S. Gôlo**[1,3]

[1]Programa de Pós-Graduação em Ciências Veterinárias, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, Rio de Janeiro, Brazil

[2]Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, Goiás, Brazil

[3]Departamento de Parasitologia Animal, Instituto de Veterinária, Seropédica, Rio de Janeiro, Brazil

[4]Department of Biology, Utah State University, Logan, Utah, USA

Correspondence
Patrícia S. Gôlo, Departamento de Parasitologia Animal, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Rodovia BR-465, Km 07, s/n, Seropédica, RJ 23897-000, Brazil.
Email: patriciagolo@gmail.com

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Abstract

The aim of the present study was to analyze ten native *Metarhizium* spp. isolates as to their UV-B tolerances. Comparisons included: different fungal propagules (conidia, blastospores, or microsclerotia [MS]); conidia in aqueous suspensions or in 10% mineral oil-in-water emulsions; and conidia mixed with different types of soil. The UV-B effect was expressed as the germination of conidia or culturability of blastospores and MS relative to nongerminated propagules. *Metarhizium anisopliae* LCM S05 exhibited high tolerance as blastospores and/or MS, but not as conidia; LCM S10 and LCM S08 had positive results with MS or conidia but not blastospores. The formulations with 10% mineral oil did not always protect *Metarhizium* conidia against UV-B. Conidia of LCM S07, LCM S08, and LCM S10 exhibited the best results when in aqueous suspensions, 24 h after UV-B exposure. In general, conidia mixed with soil and exposed to UV-B yielded similar number of colony forming units as conidia from unexposed soil, regardless the soil type. It was not possible to predict which type of propagule would be the most UV-B tolerant for each fungal isolate; in conclusion, many formulations and propagule types should be investigated early in the development of new fungal biocontrol products.

KEYWORDS
abiotic effects, biological control, conidium, formulations, fungal propagules

1 | INTRODUCTION

Entomopathogenic fungi, such as *Metarhizium* spp. and *Beauveria* spp., are widely used in insect pest control programs, and they have great potential in the regulation of arthropod species [1–4]. *Metarhizium anisopliae* has been used in Brazil for more than 40 years in the control of spittlebugs (Hemiptera: Cercopidae) in sugarcane, representing the largest microbial control program based on a mycoinsecticide worldwide [5]. Nevertheless, environmental factors, including solar irradiance, high temperatures, and low humidity can negatively influence the action of these fungi and thereby limit their ability to control arthropod pests [3].

Solar irradiation is of extreme importance to the environment, and its positive effects, particularly on plants, have been widely studied [6–8]; its ultraviolet components (UV-A and UV-B), however, can be harmful to living

organisms, including fungi, and nonliving matter [9–12]. Exposure to direct solar radiation (particularly the UV-B fraction) for a few hours can inactivate most fungal conidia [10,13,14]; moreover, sublethal doses of UV irradiation may damage germination and decrease the virulence of entomopathogenic fungi [14–16].

Atmospheric ozone drastically reduces the penetration of solar radiation wavelengths shorter than 320 nm, and completely excludes those below 290 nm [10,17]. Accordingly, only the UV-A (320–400 nm) and UV-B (280–320 nm) fractions reach the Earth's surface. Within these spectra, the UV-B fraction is more harmful to entomopathogenic fungi [8,15,18,19]. Although the UV-A fraction is also harmful, it can stimulate the recovery of fungi from irradiation damage. When fungi are exposed to simulated sunlight (which contains both UV-B and UV-A radiation), they can benefit from the UV-A fraction. Accordingly, the harmful effects of exposure to simulated solar radiation depends primarily on the amount of UV-B in the radiation received by the fungus. Therefore, the dose of UV-B usually is considered the most suitable variable in evaluating the effect of sunlight on the persistence of entomopathogenic fungi in irradiated environments [16].

Metarhizium can produce at least three different types of propagules under natural or artificial culture conditions, and the most commonly occurring type is conidia. These cells germinate to produce the structures that are responsible for causing the infection of arthropod hosts and for dispersing the fungus in the environment [20]. *Metarhizium* spp. conidia are usually 5–9 μm in length, not septate, ovoid, and often with a slight central narrowing [21]. Aerial conidia are produced by structures named conidiophores on the infected hosts' surface [22], or on solid substrates in the laboratory. Conidia are generally resistant to desiccation and may remain in the soil, in latent state, for long periods [23]. After penetration to the host's hemocoel, the fungus produces hyphal bodies; these vegetative yeast-like cells also can be produced in liquid medium, in which case they are called blastospores.

Blastospores are thin-walled, pleomorphic, hydrophilic propagules which generally germinate faster than conidia [24]. Blastospores production use to be cheaper than conidial production because they can be produced in large amounts in liquid media, and in less time and space [22,25]. Under proper storage conditions, they may remain viable for up to 12 months [26,27]. Also, they can be more virulent than conidia, but usually more vulnerable to environmental stress conditions [5,20,22,24,28]. Conidia is, in general, more thermotolerant than blastospores; conidia are naturally resistant structures commonly found in the environment, whereas blastospores are yeast-like vegetative cells, analogs to the ones produced in the host's hemocoel during fungal infection [29].

Many fungi, especially plant pathogens, naturally produce microsclerotia (MS); these are pseudoparenchymatous aggregations of hyphae that enhance persistence of these fungi in soil [30,31]. These compact hyphal aggregates are generally composed of only a few cells and measure from 50 to 600 μm in diameter. They become melanized and desiccated as they develop, and they contain endogenous nutritional reserves for fungal growth when conditions are favorable again. Unlike plant-pathogen MS, there are no reports of MS production in nature by entomopathogenic fungi [31]. *Metarhizium*, however, can be induced to make MS propagules in liquid culture [32]. On appropriate solid substrates, MS start growing and produce conidia that are capable of infecting host arthropods [31,33].

The effects of UV-B on *Metarhizium* spp. conidia have been examined extensively [13,16,18,34–39]; however, information on blastospore tolerance to UV is scarce. It was reported a reduced viability of *M. anisopliae* blastospores exposed to UV radiation, although the UV dose was not clearly documented and the wavelength tested was below 280 nm (i.e., UV-C), a range that is naturally blocked by the atmosphere [40]. Furthermore, as far as we know, there is no literature addressing the tolerance of *Metarhizium* MS to UV-B irradiation.

Most mycoinsecticides produced currently in Brazil are based on aerial conidia produced by solid substrate fermentation technologies [5]. In 2007, on a global scale, biopesticides represented about 2.4% (around US$ 512 million) of the general pesticide market. Although the market for biological pesticides is experiencing constant progress in comparison to chemical pesticides [41], the performance of entomopathogenic fungi in the field needs to be constantly improved to support this development. Biological control programs of pests that need improvement may include more effective formulations and selection of the most effective fungal propagules which are best suited to the target environmental conditions. Accordingly, in the present study, the UV-B tolerance of different propagules of 10 native Brazilian *Metarhizium* spp. isolates were examined.

Differences in the UV-B tolerance of different propagules of the same fungal isolate can provide critical tools for improving the success of biological control of arthropods pests using fungi. As far as we know, this is the first study that compares the UV-B tolerance of three different propagules of individual fungal isolates. In addition, we analyzed the UV-B tolerances of aerial conidia prepared in aqueous solution, oil-in-water emulsion, or different soil types. Determining the influence of different soil types on the protection of fungal propagules against UV-B is crucial since different particles in the soil may protect the microbiota from UV radiation, for example, by absorbing the UV radiation by anionic dyes in clays [42].

2 | MATERIALS AND METHODS

2.1 | *Metarhizium* spp. isolates

Ten *Metarhizium* spp. strains were isolated from 234 soil samples collected in Rio de Janeiro State, from March 2015 to April 2016 (Table 1). *Metarhizium* isolates were macro- and micro-morphologically identified by culturing in Petri dishes containing Potato Dextrose Agar (PDA) medium. Nine isolates (LCM S01 to LCM S09) were molecularly identified [43]. The isolates ARSEF 2575 and ARSEF 324 were obtained from the Agricultural Research Service Collection of Entomopathogenic Fungal Cultures (ARSEF; USDA-US Plant, Soil, and Nutrition Laboratory). The isolates were cultivated on PDA medium supplemented with 0.1 g/L yeast extract (PDAY) under controlled temperature and relative humidity (RH; 25 ± 1°C and RH ≥ 80%).

2.2 | UV-B tolerance of *Metarhizium* aerial conidia

2.2.1 | UV-B tolerance of *Metarhizium* conidia suspended in water or in oil-in-water emulsion

Fresh conidia from 14 to 16-day-old cultures of 10 isolates (Table 1) were scraped from PDAY culture plates and suspended in 1% (vol/vol) polyoxyethylene sorbitan monooleate (Tween 80®) sterile distilled water solution. Suspensions were filtered through hydrophilic cotton in sterile glass funnels to remove conidial aggregates; the suspensions were adjusted to 1.0×10^5 conidia/ml. The oil-in-water formulation were prepared as follows: conidia were added to mineral oil (Proquimios Comércio e Industria Ltd) and one part of this was added to nine parts of Tween 80® sterile distilled aqueous solution at 1% (vol/vol).

TABLE 1 Fungal isolates used in the experiments

Isolate code[a]	Species[b]	Origin		Isolation date
		Location (Country/state)	Geographical coordinates	
ARSEF 324	*Metarhizium acridum*	Australia/ Queensland	19°00′S	Feb 1979
ARSEF 2575	*Metarhizium robertsii*	USA/South Carolina	34°00′N	Jul 21, 1988
LCM S01	*Metarhizium anisopliae*	Brazil/Rio de Janeiro	22°45′54″S 43°41′58″W	Dec 15, 2015
LCM S02	*M. anisopliae*	Brazil/Rio de Janeiro	22°46′05″S 43°40′39″	Dec 15, 2015
LCM S03	*M. anisopliae*	Brazil/Rio de Janeiro	22°46′04″S 43°40′41″W	Dec 15, 2015
LCM S04	*M. anisopliae*	Brazil/Rio de Janeiro	22°45′58″S 43°40′48″W	Dec 15, 2015
LCM S05	*M. anisopliae*	Brazil/Rio de Janeiro	22°45′58″S 43°40′49″W	Dec 15, 2015
LCM S06	*M. anisopliae*	Brazil/Rio de Janeiro	22°45′58″S 43°40′49″W	Dec 15, 2015
LCM S07	*Metarhizium pingshaense*	Brazil/Rio de Janeiro	22°45′58″S 43°40′48″W	Dec 15, 2015
LCM S08	*M. anisopliae*	Brazil/Rio de Janeiro	22°45′58″S 43°40′49″W	Dec 15, 2015
LCM S09	*M. pingshaense*	Brazil/Rio de Janeiro	22°49′07″S 43°12′09″W	Dec 15, 2015
LCM S10	*Metarhizium* sp.	Brazil/Rio de Janeiro	22°49′07″S 43°12′09″W	Dec 21, 2015

[a]ARSEF–USDA Agriculture Research Service Collection of Entomopathogenic Fungal Cultures. LCM–Laboratório de Controle Microbiano, Departamento de Parasitologia Animal, Federal Rural University of Rio de Janeiro (UFRRJ).

[b]Species of LCM isolates were identified by Mesquita et al. [43].

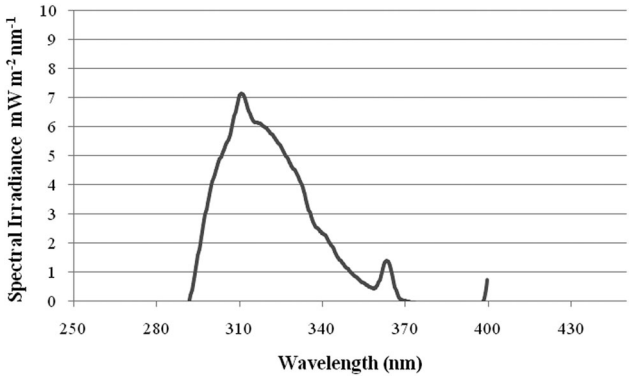

FIGURE 1 Irradiance spectrum of the cellulose diacetate-filtered lamps of the UV-B irradiation chamber. The lamps provided 856.97 mW/m^2 of UV-B irradiation at the exposure-shelf level in the chamber, based on Quaite-weighted irradiance [44]. Readings were taken at the level of the fungal colonies within the culture Petri plates with a sheet of cellulose diacetate in the light path

This suspension was vortexed vigorously to create an oil-in-water emulsion. A 20-µl aliquot of aqueous suspension or oil-in-water emulsion of each isolate was inoculated onto 4-ml PDAY medium + 0.002% (wt/vol) benomyl with 25% active ingredient (Hi-Yield Chemical Company) [13,18] (benomyl allows germination of conidia but does not allow development of mycelium, permitting the counting of germinated and nongerminated conidia) in Petri plates (35 × 10 mm, Kasvi). The experiments were conducted in a UV chamber, with UV irradiation provided by two TL 20 W/12 RS fluorescent lamps (Philips). Conidia in aqueous suspensions or oil-in-water emulsions were exposed to UV-B radiation for approximately one hour (total dose of 4.0 kJ/m^2). During irradiation, plates were covered with a 0.13-mm thick cellulose diacetate film (JCS Industries), which blocks UV-C radiation (below 280 nm) and the UV-B short-wavelength (280–290 nm), but permits the passage of most UV-B (290–320 nm) and the minimal UV-A (320–400 nm) radiation emitted by the lamps (Figure 1). Control plates also were placed in the chamber but covered with aluminum foil to block all UV radiation. Temperature inside the chamber was controlled (25 ± 1°C).

The germination of unformulated conidia was evaluated on PDAY + 0.002% (wt/vol) benomyl. Nevertheless, the mineral oil in the oil-in-water emulsions inoculated on agar Petri plates seemed to interfere with conidial germination. Accordingly, the oil was removed before evaluating conidial germination in oil-in-water emulsions by using Solub'Oil® (General Chemicals) according to Paixão et al. [45]. Conidia were washed off the plates with 1 ml of 2% Solub'Oil® aqueous solution and then transferred to a 15-ml centrifuge tube. The samples were vigorously vortexed for two min and centrifuged for 8 min at 6800 rpm. The supernatant was discarded, and the conidia were resuspended in Tween 80® 1% (vol/vol) aqueous solution. Germination was evaluated by placing 20-µl conidial suspension (at 1.0×10^5 conidia/ml) on PDAY + 0.002% (wt/vol) benomyl. *Metarhizium robertsii* ARSEF 2575 and *Metarhizium acridum* ARSEF 324 were tested as standard isolates to validate the assays since their conidial tolerances to UV-B are already known.

The DNA damage action spectrum (pyrimidine dimerization) normalized for the unit at 300 nm was used to calculate UV irradiance [44]. The irradiation spectrum was measured using the Ocean Optics USB 2000 Spectroradiometer (Du germination using a microscope at ×400 after staining with methyl blue. Conidia with germ tubes longer than the maximum nedin, FL). After irradiation, the plates were incubated for 24 or 48 h at 27°C in the dark. All experiments were repeated three times using new batches of conidia. Conidia were observed for conidial diameter were considered germinated. Relative germination was calculated as previously described by Braga et al. [13].

2.2.2 | UV-B tolerance of *Metarhizium* spp. conidia in soil samples

Three types of soil mixes were tested to compare the effects of soils, particularly their clay component, on *Metarhizium* conidial susceptibility to UV-B radiation. Soil type I: commercial planter soil (Natus Solos do Brasil®); soil type II (native clayey soil); and soil type III (native sandy soil). Table 2 shows the granulometric characteristics of each soil. Aqueous conidial suspensions were prepared as described previously at 1.0×10^5 conidia/ml. Fifty microliters of the suspensions were inoculated into 3 g of sterile soil in plastic bags (7 × 10.5 cm; Talge©). After homogenization by shaking the bags, soil samples were individually transferred to Petri dishes (35 × 10 mm) and reached 8-mm height, approximately. Soil samples were exposed to UV-B irradiation for 1 h (total dose 4.0 kJ/m^2) as described previously. Control plates were covered with aluminum foil. Immediately after exposure, 0.35 g of irradiated soil were transferred to a 1.5-ml microtube, plus 1-ml 0.01% (vol/vol) Tween 80® sterile distilled water solution and homogenized (vortex) for 30 s. A 50-µl aliquot of each suspension was removed from each soil type and spread on plates with CTC culture medium [46]. The plates were held under controlled conditions (27 ± 1°C, RH > 80%) in the dark. Colony-forming units (CFUs) were counted 7 days after UV-B exposure. The experiments

TABLE 2 Granulometric characteristics of each soil type

Soil type	Total clay (%)	Total sand (%)	Thin sand (%)	Coarse sand (%)	Silt (%)
I	36.9	62.7	20.5	42.2	0.04
II	40.0	46.2	25.7	20.5	13.8
III	18.5	78.5	21.3	57.2	0.3

Note: The soils were analyzed by the Soil Department of Federal Rural University of Rio de Janeiro. Soil type I: commercial planter soil purchased from Natus Solos do Brasil®, Taubaté, São Paulo, Brazil; soil type II: native clay soil collected in Seropédica city, Rio de Janeiro State, Brazil; soil type III: native sandy soil collected in Seropédica city, Rio de Janeiro State, Brazil.

were performed three times with different batches of conidia.

2.3 | UV-B tolerance of *Metarhizium* blastospores suspended in water

For blastospore production, *Metarhizium* spp. conidia were produced on PDAY (25 ± 1°C; RH > 80%) for 14 days. Conidial aqueous suspensions of each isolate (Table 1) were prepared at 1×10^8 conidia/ml. Three milliliters conidial suspensions were inoculated into 250-ml Erlenmeyer flasks containing 50 ml of potato dextrose broth (Kasvi) supplemented with 0.1 g/L yeast extract. The flasks were capped with hydrophobic cotton and placed on an orbital shaker (TE-424; Tecnal®) at 200 rpm for 72 h at 25 ± 1°C. After 72 h, the medium was filtered through a funnel with sterile gauze to remove the mycelium produced during the culture. The resulting blastospore suspensions transferred to 50-ml centrifuge tubes. The medium containing blastospores were centrifugated twice at 3410 *g* for 5 min (Rotina 380 R; Hettich®). After the first centrifugation cycle, the supernatant was discarded and the pellet suspended in 10-ml 0.01% (vol/vol) Tween 80® sterile distilled water solution, followed by vortex homogenization and centrifugation. After the second centrifugation cycle, the supernatant was discarded and the pellet suspended in 5 ml 0.01% (vol/vol) Tween 80® aqueous solution, followed by vortex homogenization. Suspensions were adjusted to 1.0×10^4 blastospores/ml. A 20-µl aliquot of blastospores aqueous suspension was inoculated onto Petri plates with PDAY and spread by using a glass rod. Immediately after inoculation, the plates were exposed to UV-B radiation (total dose 4.0 kJ/m^2), as previously described. Control plates were placed in the UV irradiation chamber but covered with aluminum foil. After irradiation, the plates were incubated at 27 ± 1°C in the dark. CFUs were quantified for each *Metarhizium* isolate 72 h after UV-B exposure. The relative culturability was calculated as previously described by Braga et al. [13]. The experiments were performed three times with different batches of blastospores.

2.4 | UV-B tolerance of *Metarhizium* spp. MS propagules

MS from the 10 *Metarhizium* spp. isolates (Table 1) were prepared according to Mascarin et al. [47]. The liquid medium for MS production had the following composition (per liter): glucose 36 g; yeast extract 3.64 g; KH_2PO_4, 4.0 g; $CaCl_2 \cdot 2H_2O$, 0.8 g; $MgSO_4 \cdot 7H_2O$, 0.6 g; $FeSO_4 \cdot 7H_2O$, 0.1 g; $MgSO_4 \cdot H_2O$, 0.016 g; $ZnSO_4 \cdot 7H_2O$, 0.014 g. The carbon:nitrogen (C/N) ratio was 50:1. One mililiter solution containing MS was added to 9-ml sterile saline solution (0.9% NaCl). This solution was centrifuged twice at 1792 *g* for 10 min, and once for 15 min. After each centrifugation cycle, the supernatant was discarded, and the pellet was resuspended in 9-ml sterile saline solution. A 50-µl aliquot of the final MS suspension was placed on a slide with a glass cover for MS quantification under a light microscope. All MS in 50 µl were counted. One hundred MS were inoculated onto water–agar medium and spread with a glass rod. The plates were exposed to UV-B radiation (total dose 4.0 kJ/m^2) as described previously. Control plates were placed in the chamber and covered with aluminum foil. The presence or absence of hyphal growth from each MS was evaluated with a dissecting microscope 6 days after the UV-B exposure. MS granules were considered germinated upon hyphal development around the MS granule (MS granule hyphal germination). Experiments were considered valid when control plates had at least 95% germinated MS. The relative culturability was calculated as previously described by Braga et al. [13]. The experiments were performed three times with different batches of MS.

2.5 | Statistical analysis

All data were submitted to the Kolmogorov–Smirnov test for distribution of normality. The UV-B tolerance of *Metarhizium* conidia suspended in water or in oil-in-water, UV-B tolerance of *Metarhizium* blastospores, and UV-B tolerance of *Metarhizium* MS were analyzed using analysis of variance (ANOVA) followed by the Skott-Knott test. The nonparametric data (conidia mixed with different

soil types) were analyzed using the Kruskal–Wallis test, followed by a Dunn's test. The ANOVA were performed using the RStudio software (version 1.1.463) and the Kruskal–Wallis test was performed using GraphPadPrism, v.8.4.0, Inc (GraphPad Software).

3 | RESULTS

3.1 | UV-B tolerance of *Metarhizium* aerial conidia

3.1.1 | Conidia suspended in water or in oil-in-water emulsion

The effects of UV-B exposure on conidial germination of the 12 *Metarhizium* spp. isolates (10 LCM isolates + 2 standard ARSEF isolates) at 24 and 48 h are shown in Figure 2 and Table S1 h after the exposure to UV-B; but only three isolates (i.e., LCM S01, LCM S08, and LCM S09) exhibited higher relative germination when aqueous solutions were compared to oil-in-water emulsions 48 h after exposure to UV-B. A considerable delay in the relative germination caused by the UV-B exposure was observed for some isolates when the germination at 24-h incubation was compared with 48 h incubation (Figure 2).

3.1.2 | UV-B tolerance of *Metarhizium* spp. conidia in soil samples

The effects on culturability of conidia of the 10 *Metarhizium* spp. isolates when mixed with different types of soil and exposed to UV-B are shown in Table 3. Conidia from the soils that were exposed to UV-B yielded a similar number of CFUs as conidia from unexposed soil, regardless the soil type, except LCM S05 mixed with soil type I and LCM S09 mixed with soil type III. The soil type III (with less clay) did not protect the conidia as the soil types I and II for isolates LCM S01 or LCM S03, or the soil type II for LCM S09.

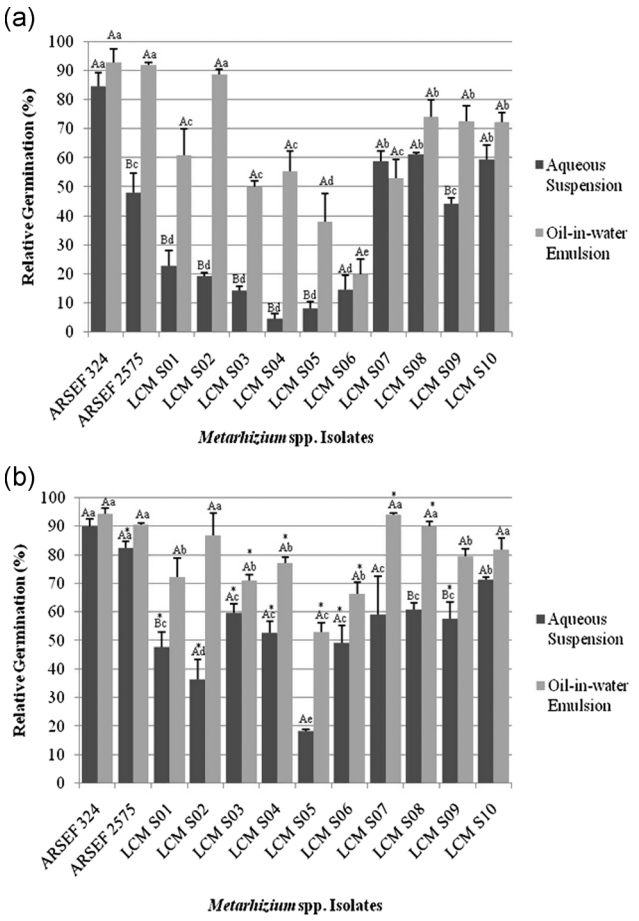

FIGURE 2 Average relative germination and standard error of *Metarhizium* spp. conidia in aqueous suspension or in oil-in-water emulsion, and incubated at (A) 24 h or (B) 48 h after exposure to UV-B for 1 h (total dose 4.0 kJ/m^2). Bars with the same uppercase letter in the same isolate and incubation period, and lowercase letter in the same suspension type (aqueous or oil-in-water) and incubation period do not differ statistically by Skott-Knott test ($p \geq .05$). *Significant difference between the incubation periods (24 or 48 h) in a same fungal isolate. The experiments were performed three times with different batches of conidia

3.2 | UV-B tolerance of *Metarhizium* blastospores suspended in water

The effects of the UV-B exposure on the culturability of *Metarhizium* spp. blastospores are shown in Figure 3 and Table S2. The average relative culturability of blastospores ranged from 1.31 ± 0.5% to 63 ± 0.9% among the isolates, with most of the isolates exhibiting low tolerance to UV-B. Only *M. anisopliae* LCM S05 blastospores demonstrated high culturability after exposure. Isolates LCM S01, LCM S02, LCM S07, LCM S08, and LCM S09 were the most susceptible (Figure 3).

3.3 | UV-B tolerance of *Metarhizium* spp. MS propagules

The effects of the UV-B exposure on the culturability of *Metarhizium* spp. MS are shown in Figure 4 and Table S3. The average relative culturability of the tested isolates as MS varied considerably, ranging from 9.2 ± 2.5% to 100 ± 0.0%. Isolates LCM S05, LCM S08, and LCM S10 had the best relative culturability among the tested isolates (Figure 4).

TABLE 3 Average of colony-forming units (CFUs) and standard error of conidia of *Metarhizium* spp. isolates exposed to UV-B (total dose 4.0 kJ/m^2) in different types of soil

Metarhizium isolate	CFUs in each soil type			Sources of variation	Degrees of freedom
	I	II	III		
LCM S01				Isolates (I)	9
Not exposed	3.3 ± 1.11Aab	7.6 ± 2.37Aa	3.8 ± 1.00Ab		
Exposed	3.3 ± 0.98Aa	7.6 ± 2.61Aa	0.6 ± 0.21Ab	Soil (S)	2
LCM S02				Exposure to UV-B (UV-B)	1
Not exposed	2.4 ± 1.60Aa	5.2 ± 3.14Aa	5.8 ± 1.13Aa		
Exposed	2.4 ± 1.17Aa	5.2 ± 3.69Aa	1.8 ± 0.54Aa		
LCM S03				I × S	18
Not exposed	3.8 ± 2.31Aa	5.1 ± 1.47Aa	4.8 ± 2.68Aa		
Exposed	3.8 ± 1.57Aa	5.1 ± 1.49Aa	1.6 ± 0.55Ab	I × UV-B	9
LCM S04				S × UV-B	2
Not exposed	3.4 ± 1.40Aa	5.2 ± 2.93Aa	6.8 ± 1.13Aa		
Exposed	3.3 ± 0.55Aa	4.6 ± 1.54Aa	4.6 ± 1.28Aa	I × S × UV-B	18
LCM S05				Error	120
Not exposed	6.8 ± 20.98Aa	5.5 ± 0.88Aa	13 ± 4.76Aa		
Exposed	2.1 ± 0.47Ba	5.5 ± 2.12Aa	5 ± 2.77Aa	Corrected total	179
LCM S06					
Not exposed	3.8 ± 1.10Aa	2.1 ± 1.13Aa	1.5 ± 0.22Aa	CV (%)	27.33
Exposed	3.8 ± 1.07Aa	2.1 ± 0.87Aa	1.3 ± 0.49Aa		
LCM S07					
Not exposed	3.5 ± 0.84Aa	2.8 ± 0.79Aa	4.5 ± 1.99Aa		
Exposed	3.5 ± 1.68Aa	2.8 ± 1.11Aa	4.5 ± 2.84Aa		
LCM S08					
Not exposed	6.5 ± 1.47Aa	2.5 ± 3.21Aa	7 ± 2.92Aa		
Exposed	5.5 ± 1.17Aa	2.5 ± 2.81Aa	2 ± 2.80Aa		
LCM S09					
Not exposed	3.75 ± 0.85Aa	6.3 ± 12.6Aa	5 ± 0.93Aa		
Exposed	2.8 ± 0.30Aab	3.7 ± 8.71Aa	1 ± 0.44Bb		
LCM S10					
Not exposed	4.3 ± 1.70Aa	4.3 ± 1.47Aa	5.5 ± 0.88Aa		
Exposed	2.5 ± 1.23Aa	4.3 ± 2.11Aa	2.3 ± 1.05Aa		

Note: Each assay was conducted three times, on different days, using new conidial preparations each day. Means obtained from unexposed plates and from UV-B exposed plates for the same isolate were statistically compared for each soil type. The same uppercase letters in the same column do not differ significantly at $p \geq .05$ (Kruskal–Wallis test followed by a Dunn's test). Means obtained from unexposed plates or UV-B exposed plates for each isolate were statistically compared in the three different soil types (the same lowercase letters for the same isolate and group in the same row do not differ significantly at $p \geq .05$, Kruskal–Wallis test followed by Dunn's test).

Abbreviation: CV, coefficient of variation.

4 | DISCUSSION

Metarhizium spp. are entomopathogenic fungi that are widely studied and used for insect-pest control in agriculture. The effectiveness of fungi in controlling arthropod pests depends not only on their high virulence to the target pest but also on their tolerances to certain environmental conditions that may inhibit their viability. Since these conditions are critical to the bioactivity of entomopathogenic fungi, their tolerances to them must be considered during their development as biocontrol products [3]. In the present study, three types of *Metarhizium* propagules (i.e., conidia,

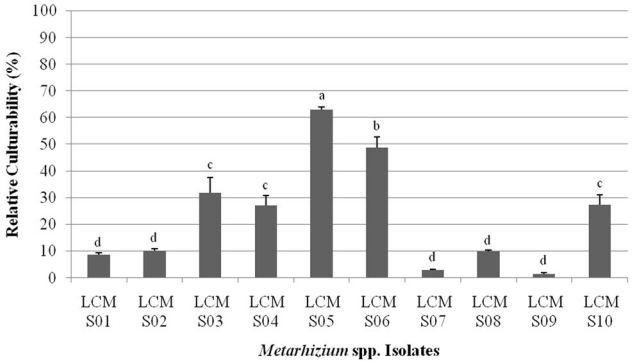

FIGURE 3 Average and standard error of *Metarhizium* spp. blastospore relative culturability 72 h after the UV-B exposure for 1 h (total dose 4.0 kJ/m^2). Bars with the same lowercase letter do not differ by Skott-Knott test ($p \geq .05$). The experiments were performed three times with different batches of blastospores

blastospores, and MS) of several fungal isolates were investigated as to their tolerances to UV-B irradiation; and these findings were used to identify the most appropriate type of propagule for each isolate for various field conditions.

Mineral and vegetable oils adjuvants that are often used to formulate chemical or biological pesticides improve the ease of application and even protect the active constituent of a biological product against abiotic factors [36,45,46,48,48,49]. We expected that conidia in oil-in-water emulsion when exposed to UV-B would have higher relative germination than conidia in aqueous suspension, as observed in previous studies [50,51]. Nevertheless, in the present study, conidia of several isolates (Figure 2) had the same tolerance whether in aqueous suspensions or in oil-in-water emulsions. Conidial germination 24 h after the UV-B exposure suggested that isolates that exhibited low innate tolerance were more responsive to the addition of oil in the fungal formulation (except LCM S06, that did not have its low tolerance in aqueous suspension improved in comparison to the tolerance of conidia in oil-in-water emulsion; Figure 2). Delayed conidial germination after UV-B exposure probably is due to the fungal cells requiring time to repair damage caused by environmental stress, before germination [52]. In the present study, some isolates exhibited considerable delay (expressed by their significantly different relative germination) when the results at 24 and 48 h post UV-B exposure were compared (Figure 2). Under field conditions, however, this improved in the conidial germination, observed 48 h after the UV-B exposure, may be inhibited if the conidia are exposed repeatedly (each day) to solar irradiation each day, and thereby totally inhibit germination. Analyzing the growth responses of fungal strains to solar irradiation present in the target pest's natural environment is fundamental to selecting the strains appropriate for development as biological control agents for that pest.

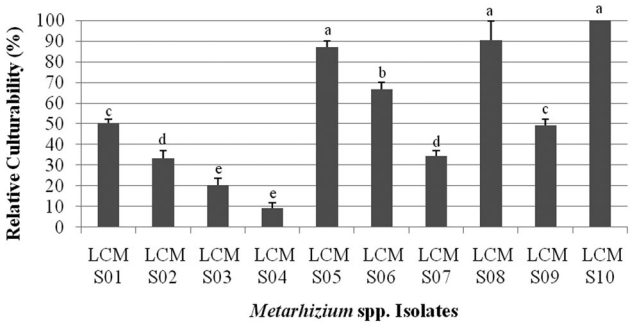

FIGURE 4 Average and standard error of *Metarhizium* spp. microsclerotia relative culturability 6 days after UV-B exposure (4.0 kJ/m^2). Microsclerotia granules were considered germinated upon hyphal development around the microsclerotia granule. Bars with the same lowercase do not differ by Skott-Knott test ($p \geq .05$). The experiments were performed three times with different batches of microsclerotia propagules

To analyze if different soil types enhance or inhibit UV-B tolerance of conidia, three types of soil were mixed with *Metarhizium* conidia and the soil-fungus mixtures exposed to UV-B irradiation. The definition of a soil's structure involves its network of pores; these pores are the habitat of soil microbiota [53], which may include *Metarhizium* spp. Our results suggested that the tested soils (regardless the soil type) afforded physical UV-B protection to *Metarhizium* spp. isolates (except LCM S05 mixed with soil type I and LCM S09 mixed with soil type III; Table 3). Conidial photoprotection may be explained by the absorption of UV radiation by anionic dyes in clay and by physical attenuation of UV irradiance by the soil particles. Photostabilization of entomopathogenic fungi by composite clay matrices has been reported [42]; but in the present study, soil type III (with less clay) exhibited similar protection as soil types I and II, except for the isolates LCM S01, LCM S03, and LCM S09 (Table 3). The photoprotection associated with clay is attributed to its UV scattering properties, which attenuate the intensity of irradiation [42]. On the other hand, sandy soil contains a large number of mesopores and macropores, which help mycelial growth. Soils with high amounts of clay have less porosity and increased water retention, which may make it difficult for mycelia to grow and penetrate the soil. In addition, soil compaction reduces the size and opening of pores, and thereby hinders the growth of fungi. As a result, clay soil may have reduced availability of water, circulation of nutrient-bearing solutions, and limited gas exchange [54].

In comparison to conidia, blastospores are more susceptible to abiotic stresses, probably because they are

vegetative (growing) cells [5,20]. In studies with conidia and blastospores of *Cordyceps fumosorosea* (formerly, *Isaria fumosorosea*), conidia were more tolerant to high temperatures than blastospores [55]. This difference may be due to these two structures being produced in different ways; also, conidia are generated by phialic conidiogenesis and contain metabolites resistant to environmental stresses [56], whereas blastospores, on the other hand, are produced by budding of hyphae and have weak membranes [55]. In the present study, *Metarhizium* spp. blastospores in aqueous suspensions were very susceptible to UV-B, except *M. anisopliae* LCM S05 (Figure 3). Although the majority of the isolates tested here as blastospores had medium or low tolerance to UV-B, a recent study showed that conidia and blastospores from two *Metarhizium* isolates had similar tolerance to UV-B irradiation [29]. In addition, Bernardo et al. [20] suggests that both types of propagule, conidia and blastospores, are promising for tick control. Using blastospores formulated in adjuvants that provide protection against negative environmental factors may support their use against arthropod pests in the field, rather than propagules that are less virulent.

Sclerotia of fungal plant-pathogens are reported to be less likely affected by unfavorable temperatures than the more active phases of these fungi [30]. Small sclerotia (MS) have been successfully artificially induced also in entomopathogenic fungi, such as *Metarhizium* spp. [32,57,58] and *Akanthomyces lecanii* (formerly, *Lecanicillium lecanii*) [59]. Characterization of entomopathogenic fungi MS as to their tolerance to abiotic factors is fundamental to the successful development of these propagules for field use. *Metarhizium* spp. isolates surveyed here varied widely in their responses to UV-B exposure (Figure 4). These results suggested that even though *Metarhizium* MS are considered resistant structures, they do not always have high tolerance to UV-B.

Several factors influence the UV-B tolerance of entomopathogenic fungi, including their pigment and metabolite production, culture history (nutrients and light quality), water imbibition (dry or wet), and presence or absence of protective adjuvants. In the present study, the culture history of each type of propagule was different; that is, aerial conidia were produced on solid medium (PDAY), blastospores in a simple liquid medium (potato dextrose broth plus yeast extract), and MS in a different liquid medium (glucose, yeast extract, and salts). Interestingly, it was not possible to predict which type of propagule (i.e., aerial conidia, blastospores, or MS) would be the most UV-B tolerant for each fungal isolate. For example, LCM S10 and LCM S08 exhibited excellent UV-B tolerance as MS and good results with conidia when suspended in water and analyzed 24 h after the UV-B exposure, but these isolates were not tolerant as blastospores. While LCM S05 was tolerant to UV-B as blastospores or MS, but not as conidia. This suggests that each propagule type may have different strategies to resist and/or recover from exposure to solar irradiance, and that the intrinsic UV-B tolerance of one propagule type does not guarantee the same tolerance for other propagule types.

The UV-B tolerance of single fungal isolates when observed as different propagules or formulations provides important information about each isolate's intrinsic tolerance, as well as variations in UV-B tolerances of propagule types from the same isolate. Accordingly, when seeking the most appropriate propagule form of a new fungal isolate for use in new biocontrol studies, as many formulations and propagule types as practicable should be investigated early in the development of the new product.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Amanda R. C. Corval https://orcid.org/0000-0001-6926-7916
Emily Mesquita https://orcid.org/0000-0002-4263-1332
Thaís A. Corrêa https://orcid.org/0000-0001-6909-2925
Cárita de S. R. Silva https://orcid.org/0000-0002-3081-8191
Ricardo de O. B. Bitencourt https://orcid.org/0000-0002-6298-2869
Éverton K. K. Fernandes https://orcid.org/0000-0001-7062-3295
Vânia R. E. P. Bittencourt https://orcid.org/0000-0001-8473-8501
Patrícia S. Gôlo http://orcid.org/0000-0003-1854-7488

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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