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Characterization of Brazilian *Cordyceps fumosorosea* isolates: Conidial production, tolerance to ultraviolet-B radiation, and elevated temperature

Víctor Manuel Arévalo Rojas [a,b,*], Natasha SantAnna Iwanicki [a,*], Celeste Paola D'Alessandro [a], Maíra Blumer Fatoretto [c], Clarice Garcia Borges Demétrio [c], Italo Delalibera Jr. [a]

[a] Department of Entomology and Acarology, "Luiz de Queiroz" College of Agriculture (ESALQ), University of São Paulo (USP), Av. Pádua Dias, 11 – P.O. Box 9 – CEP, 13418-900 Piracicaba, São Paulo, Brazil

[b] Department of Engineering, College of Agricultural Engineering, University National of Barranca, Av. Toribio de Luzuriaga N°376, Mz J, Urbanización La Florida, Barranca, Lima, Peru

[c] Department of Exact Sciences, "Luiz de Queiroz" College of Agriculture (ESALQ), University of São Paulo (USP), Av. Pádua Dias, 11 – P.O. Box 9 – CEP, 13418-900 Piracicaba, São Paulo, Brazil

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ABSTRACT

Cordyceps fumosorosea is an entomopathogenic fungus with a global distribution and is used for the biological control of agricultural pests. High conidial productivity and tolerance to abiotic stresses such as elevated temperature and ultraviolet radiation (UV-B) are desired characteristics in candidate isolates for commercial products. Our goal in this study was to characterize promising isolates of *C. fumosorosea* from five Brazilian biomes regarding conidial production, tolerance to UV-B, and elevated temperature (45°). Seventy-two isolates out of 172 were chosen visually, based on growth and sporulation in culture medium, and grown on parboiled rice. Next, fourteen isolates were selected, based on productivity on rice and origin of isolation, for production in polypropylene bags and submitted to UV-B for 2, 4, 6, and 8 h or to 45 °C for 30, 60, and 90 min. High variations in conidial production were observed among isolates, and a positive correlation was observed between UV-B and heat tolerance. The isolates ESALQ4556 and ESALQ4778 showed the highest yields of conidial production in polypropylene bags (3.51×10^9 conidia/g dry rice), while ESALQ1296, an isolate recovered from insects, was the most tolerant to UV-B and 45 °C. Exposure to radiation for more than 4 h and placed directly at 45 °C for more than 30 min significantly reduced conidial germination for all *C. fumosorosea* isolates. These results contribute to a better understanding of the tolerance to abiotic factors of Brazilian isolates of *C. fumosorosea*.

1. Introduction

The entomopathogenic fungus *Cordyceps fumosorosea* (Wize) Kepler, B. Shrestha & Spatafora formerly *Isaria fumosorosea* (Kepler et al., 2017) (Hypocreales: Cordycipitaceae) has been exploited in some countries as a biocontrol agent of agricultural pests such as aphids, whiteflies, psyllids, mites, coleopterans and lepidopterans (Faria and Wraight, 2007; Hussein et al., 2013; Maluta et al., 2022; Mascarin et al., 2018a). In Brazil, the first product based on *C. fumosorosea* aerial conidia named Challenger®, was registered in 2018 by the company Koppert. Since then, other companies (e.g. Simbiose, Vital Brasil) have registered products based on *C. fumosorosea* conidia to control insects such as the Asian citrus psyllid *Diaphorina citri* (Hemiptera: Psyllidae) and the corn leafhopper, *Dalbulus maidis* (Hemiptera: Cicadellidae), the whitefly (*Bemisia tabaci*) and the cotton bollworm (*Helicoverpa armigera*).

Aerial conidia are the main active ingredient in bioproducts based on fungi such as *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae), *Beauveria bassiana* (Hypocreales: Cordycipitaceae), and *C. fumosorosea* (Hypocreales: Cordycipitaceae). Aerial conidia are traditionally produced using parboiled rice or wheat by solid-state fermentation (Faria and Wraight, 2007). Fungus-sporulated grains are then dried, and conidia are removed via mechanical means. Dried spores are generally formulated on an industrial scale to improve the product's physical and chemical characteristics and protect conidia against abiotic stresses

* Corresponding authors at: Department of Entomology and Acarology, "Luiz de Queiroz" College of Agriculture (ESALQ), University of São Paulo (USP), Av. Pádua Dias, 11 – P.O. Box 9 – CEP, 13418-900 Piracicaba, São Paulo, Brazil (N.S. Iwanicki). Department of Engineering, College of Agricultural Engineering, University National of Barranca, Av. Toribio de Luzuriaga N°376, Mz J, Urbanización La Florida, Barranca, Lima, Peru (V.M.A. Rojas).

E-mail addresses: varevalo@unab.edu.pe (V.M.A. Rojas), natasha.iwanicki@usp.br (N.S. Iwanicki).

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(Burges, 1998).

In tropical countries, such as Brazil, high temperature, lower relative humidity, and ultraviolet radiation are the main abiotic factors that negatively affect the performance of entomopathogenic fungi in field conditions (Braga et al., 2001a, 2001b; Fernandes et al., 2015, Bernardo et al., 2020). Conidia can be inactivated by solar UV-A (400–320 nm) and UV-B (320–280 nm) (Braga et al., 2001b) radiations. UV-B radiation is more harmful than UV-A to microorganisms by affecting DNA replication (Nascimento et al., 2010, Braga et al., 2015). Conversely, heat stress increases the production of reactive oxygen species (ROS), membrane lipid peroxidation, and membrane fluidity (Mittler, 2002; Apel and Hirt, 2004). Braga et al. (2001a, 2001b) showed that four hours of exposure to UV-B radiation could significantly reduce conidial viability and germination of the entomopathogenic fungus *Metarhizium*. In contrast, isolates of *C. fumosorosea* exposed to 40 °C showed a limited mycelial growth rate (Vidal et al., 1997). However, several studies argued that susceptibility to abiotic stress is inter- and intraspecific determined, emphasizing the importance of isolate screening studies (Fargues et al., 1996; Vidal et al., 1997; Braga et al., 2001a; Mascarin et al. 2018b; Acheampong et al., 2020; Bernardo et al., 2020; Couceiro et al., 2020). Ascomycete fungi of the genera *Metarhizium*, *Beauveria,* and *Cordyceps* (*Isaria*) grow well on solid substrates at temperatures between 24 °C and 30 °C (Zimmermann (2007a,b,2008; Jaronski, 2023). The favorable temperature ranges for the development of *M. anisopliae*, *B. bassiana*, *M. rileyi,* and *C. fumosorosea* are 24–30 °C, 22–26 °C, 20–30 °C, and 22–30 °C, respectively (Alves and Pereira 1986; Vidal et al., 1997; Zimmermann, 2008). Several isolates of *C. fumosorosea* from France grew between 11 and 30 °C (Fargues et al., 1992), while Mietkiewski et al. (1994) reported growth of *C. fumosorosea* between 5 and 32 °C, and an optimum of 25 °C. However, it is worth noting that most studies evaluated the mycelial growth of isolates subjected to different temperatures rather than the viability of conidia simulating sprayer tank conditions, which is another concept.

To be considered a good candidate for biological pest control programs, isolates of entomopathogenic fungi should meet several requirements, such as high conidial production on a low-cost substrate, high virulence, and tolerance to abiotic stresses, such as elevated temperature and UV-B radiation. Nonetheless, these characteristics should be weighted for selecting an isolate for commercial purposes, as productive isolates might not be the ones with higher tolerance to abiotic stress or with high virulence or vice versa (Acheampong et al., 2020).

Whereas there is extensive literature on the effect of UV radiation and temperature on conidia of the fungi *B. bassiana* and *Metarhizium* spp. (Inglis et al. 1995, 1996; Fargues et al., 1997; Couceiro et al., 2020; Bernardo et al., 2021, Acheampong et al., 2020, Braga et al., 2001a, 2001b; Rangel et al., 2015, 2018), few studies investigated those on the conidia of *C. fumosorosea* (Vidal et al.,1997, Fargues et al., 2002) or *C. javanica* (Mascarin et al. 2018b). In addition, no study has correlated conidia yield with responses to heat and UV radiation stresses. In this context, this study aimed to investigate conidial production, tolerance to UV-B radiation, and tolerance to elevated temperature of *C. fumosorosea* isolates from five biomes in Brazil. We hypothesized that: (1) Isolates that show increased tolerance to UV-B also had tolerance to elevated temperature. (2) There was no correlation between conidial production and tolerance to UV-B and elevated temperature.

2. Material and methods

2.1. Fungal strains

One hundred seventy-two isolates of *Cordyceps* spp. morphologically identified, according to Humber (2012), were chosen from the Entomopathogenic Fungal Collection from ESALQ-University of São Paulo (Piracicaba, Brazil). The main morphological characteristics of *C. fumosorosea* were: phialides with smooth, uncolored walls; conidia long ovoid, 4 mm long, rosy-tan to smoky pink (or gray) in mass (Humber 2012).

We selected isolates from soil of five Brazilian biomes (Amazon, Caatinga, Cerrado, Atlantic Forest, and Pampa) and two isolates, ESALQ 1296 and ESALQ 1409, both from insects (Suppl. Table S1). Isolates were grown in Petri dishes containing Sabouraud Dextrose Agar enriched with yeast extract (SDYA: 2.5 g/L bacteriological peptone; KASVI®, São José dos Pinhais, PR, Brazil), 10 g/L dextrose (Synth®, São Paulo, SP, Brazil), 2.5 g/L yeast extract (KASVI®, São José dos Pinhais, PR, Brazil), 10 g/L agar (Synth®, São Paulo, SP, Brazil) and incubated in a climatic chamber (26 ± 1 °C and 12 h photophase) up to 10 days.

A total of 72 isolates previously grown in Petri dishes presenting high sporulation based on visual observations (usually those with darker colors) were chosen for production in borosilicate glass bottles with screw caps (Schott®) and a capacity of 250 mL. Isolates with high mycelial growth (light coloration) and low sporulation were not selected.

2.2. Solid fermentation

2.2.1. Production in glass bottles

The screening for highly productive isolates was done in glass bottles. In each bottle, 50 g of parboiled rice were soaked in 100 mL of distilled water for 50 min to hydrate the substrate. Next, rice was sieved to remove excess water and returned to bottles following autoclaving for 20 min at 120 °C and 110 kPa. Conidia were scraped from sporulated Petri dishes and mixed in a sterile aqueous solution of Tween 80 (0.05%) to prepare a suspension of 1×10^7 conidia mL^{-1}. Each bottle was inoculated with 5 mL of that suspension, homogenized, and incubated at 26 ± 1 °C, 12 h of photophase for ten days.

Conidial yield was quantified by adding 150 mL of a sterile aqueous Tween 80 (0.05%) solution to each bottle and manually shaken for 5 min. Then, bottles were placed in a rotatory incubator shaker (Marconi®, MA 140 CFT) at room temperature (20 °C to 26 °C) and 300 rpm for 30 min. Serial dilutions were prepared, and conidial concentrations were determined using a Neubauer hemacytometer under phase microscopic. Next, we calculated the concentration of conidia per gram of rice for each isolate. Four bottles per isolate were used, and three experiments were done using new fungal preparations on different dates. The viability of harvested conidia was not determined.

2.2.2. Production in plastic bags

The experiment in plastic bags included 14 isolates out of 72 previously produced in glass bottles (Table 1). We selected ten isolates with the highest conidia yield, three isolates with the lowest conidial yield, and one isolate originally obtained from a naturally infected insect. Conidial suspensions were prepared as described in 2.2.1 and inoculated in a volume of 10 mL per polypropylene plastic bag containing 180 g of parboiled rice. After inoculation, the plastic bags were manually shaken, stapled, and incubated at 26 °C ± 1 °C and 12 h of photophase for ten days. After incubation, 540 mL of a sterile aqueous solution of Tween 80 (0.05%) were added to each bag and manually shaken for 5 min. Conidial suspension and colonized rice were poured into a 250 mL bottle glass and placed in a rotatory incubator shaker (Marconi®, MA 140 CFT) at room temperature (20 °C to 26 °C) and 300 rpm for 30 min. Conidial yield was calculated as described in 2.2.2. Three plastic bags per isolate were used, and two experiments were done using new fungal preparations on different dates. The viability of harvested conidia was not determined. The nuclear translation elongation factor 1-alpha (EF1-α) of 14 selected isolates was amplified and sequenced as described by Rehner and Buckley (2005) for species confirmation. The amplification and sequencing were performed with primers: 983F (5́-GCYCCYGGH-CAYGGTGAYTTYAT-3) and 2218R (5́-ATGACACCRACRG-CRACRGTYTG-3ó). Sequencing was performed by the Plant Genomics and Molecular Biology Laboratory of the University of São Paulo, Piracicaba, SP, Brazil. The sequences obtained were manually edited and the multiple alignments were constructed using the ClustalW tool with

Table1
List of Brazilian selected isolates investigated from the Entomopathogen Collection "Prof. Sérgio Batista Alves" ESALQ/USP, Piracicaba, State of São Paulo.

Strain	Biome / City, State, year of isolation	Origin
ESALQ1296	Atlantic Forest / Jaboticabal, São Paulo, 2001	Whitefly *B. tabaci* biotype B
ESALQ1609	Caatinga / Rio Verde, Goiás, 2012	Native vegetation soil
ESALQ1741	Caatinga / Delmiro Gouveia, Alagoas, 2012	Palm cultivation soil
ESALQ1998	Caatinga / Delmiro Gouveia, Alagoas, 2012	Palm cultivation soil
ESALQ2667	Caatinga / Delmiro Gouveia, Alagoas, 2012	Native vegetation soil
ESALQ2778	Amazon / Sinop, Mato Grosso, 2012	Corn cultivation soil
ESALQ3300	Atlantic Forest / Teotônio Vilela, Alagoas, 2013	Native vegetation soil
ESALQ3302	Caatinga / Delmiro Gouveia, Alagoas, 2013	Bean cultivation soil
ESALQ3307	Caatinga / Delmiro Gouveia, Alagoas, 2013	Palm cultivation soil
ESALQ3415	Atlantic Forest /Santa Barbara Ôoeste São Paulo, 2013	Citrus soil
ESALQ3422	Atlantic Forest / Itirapina, São Paulo, 2013	Citrus soil
ESALQ3430	Atlantic Forest / Itirapina, São Paulo,2013	Citrus soil
ESALQ4556	Pampa / Aceguá, Rio Grande do Sul, 2012	Native vegetation soil
ESALQ4778	Amazon / Sinop, Mato Grosso, 2012	Native vegetation soil

BioEdit (Hall, 1999). The Maximum Likelihood analysis (MV) was performed using GUI RAxML v.1.366 implementing the evolutionary model GTR + G. The analysis was conducted considering "gaps" as missing data and support for the obtained relationships was accessed with 1000 replicates of the "rapid bootstrap" algorithm. The generated tree was viewed and edited with MEGA X (Kumar et al., 2018). The nodes were considered to have good statistical support when the bootstrap values were ≥70%.

2.3. UV-B radiation tolerance of C. Fumosorosea isolates

Fourteen *C. fumosorosea* isolates were grown in Petri dishes containing Sabouraud Dextrose Agar enriched with yeast extract (SDAY) as described in topic 2.1. After incubation, conidia were harvested, and a suspension of 1×10^6 mL^{-1} was prepared using a sterile aqueous solution of Tween 80 (0.05%). A volume of 150 μL was inoculated in the central area of Rodac® Petri dishes (Replicate Organism Detection and Counting, 60 × 10 mm; J Prolab, São José dos Pinhais, PR, Brazil) containing Potato Dextrose Agar (PDA)(Difco®, Sparks, MD, U.S.A.) supplemented with 0.1% (v/v) Derosal® 500 SC (Carbendazim, Bayer CropScience, S.P., Brazil) (Oliveira et al. 2015). Rodac® Petri dishes were kept open inside a laminar flow chamber until the liquid evaporated. The UV-B radiation tolerance experiment was conducted in a wooden box containing four UV-B-313EL fluorescent lamps from Q-Lab Corporation (USA), with a peak at 313 nm (corresponding to UV-B radiation) and a mean irradiation value of 659.54 mW m^{-2} or 2.38 kJ m^{-2} measured using a spectroradiometer (Ocean optics USB2000 + rad) connected to a portable computer. Each lamp was covered with a 0.13 mm–thick cellulose diacetate film with a cutoff point at 290 nm. This permitted the passage of most UV-B and UV-A (290–400 nm), but prevented exposure to UV-C (>280 nm) and short-wavelength UV-B (>290 nm). Lamps were switched on 30 min before the experiment started to generate a stable irradiation level.

The Rodac® plates were placed inside a wooden box and covered with a diacetate sheet to avoid exposure to UV radiation. The temperature inside the box was uncontrolled. Plates were placed 68 cm away from lamps and were exposed to five treatments representing different exposure times to UV-B radiation 0, 2, 4, 6, or 8 h (irradiation dose corresponding to 0, 4.76, 9.52, 14.28, and 19.04 kJ m^{-2}, respectively, calculated by multiplying 2.38 kJ m^{-2} by the exposure time). Then, plates were incubated at 26 ± 1 °C and 24 h for control treatment and 48 h for exposed plates to allow DNA repair and germination of conidia. Percent germination was determined for at least 200 conidia at 400x magnification. Conidia were considered germinated if the germ tube was equal to or more than half the diameter of the conidia. Three repeated trials were performed with new batches of fungal preparations on different dates.

2.4. Heat tolerance of C. Fumosorosea isolates

Fourteen selected *Cordyceps* spp. isolates were grown in Petri dishes containing Sabouraud Dextrose Agar enriched with yeast extract (SDAY) as described in topic 2.1. Conidia were scraped from sporulated Petri dishes and mixed in a sterile aqueous solution of Tween 80 (0.05%) to prepare a suspension of 1×10^6 conidia mL^{-1}. Next, 10 mL of fungal suspensions were placed in sterile glass tubes (2.5 cm diameter × 8.5 cm height) and sealed with plastic film. Under agitation, all tubes were heat-treated in a water bath at 45 °C (±1°C) for 30, 60, and 90 min. Control treatments were kept at room temperature (20 °C to 26 °C). Then, aliquots of 150 μL of each fungal suspension were inoculated in Rodac® Petri dishes containing PDA (Difco®, Sparks, MD, U.S.A.) supplemented with 0.1% (v/v) Derosal® 500 SC (Carbendazim, Bayer CropScience, S. P., Brazil) and 5 mg·mL-1 Pentabiotic® (Fontoura-Wyeth, SP, Brazil). Rodac® Petri dishes were kept open inside a laminar flow chamber until the liquid evaporated; then, plates were incubated at 26 ± 1 °C, 12 h of photophase for 24 h for control treatment, 48 h for other treatments. Percent germination was determined for at least 200 conidia at 400x magnification. Conidia were considered germinated if the germ tube was equal to or more than half the diameter of the conidia. The experiment was repeated five times with new fungal preparations.

2.5. Statistical analyses

Conidial production on rice and conidial germination after exposure to UV-B radiation, and elevated temperature were analyzed with generalized linear models (GLM) for non-normal distribution (Nelder and Wedderburn 1972). As an extension of the generalized linear models, the generalized linear mixed model (GLMM) combines GLM and random effects that allow for the accommodation of existing dependency structures between observations (Breslow and Clayton (1993). The model that best fitted the proportion data of UV-B and temperature was the binomial-normal model with a link-logit function for different exposure times and ultraviolet radiations (Demétrio et al., 2014; Fatoretto et al., 2018). The conidial production data were fitted using the quasi-Poisson model due to overdispersion (Hinde and Demétrio, 1998). To test the similarity between isolates, we used likelihood-ratio tests. This test compares different nested models and hence tests groups of one or more effects. If at significance level α, the null hypothesis was rejected, it indicates that some, or all, of the omitted terms need to be retained; otherwise, the levels can be grouped.

Correlation plots were used to verify a correlation between the conidial production and conidial germination after exposure to UV-B and elevated temperature. For this study, the average results obtained for each isolate were used. For the UV-B radiation tolerance, we used data from 6 h of exposure to UV-B and the exposure time of 30 min at 45 °C. Data analyzes were conducted using the statistical program R Core Team (2020).

3. Results

3.1. Conidial production on rice

There was a variation in conidial production among *Cordyceps* spp. isolates grown in flasks, indicating that isolates exhibited different sporulation (Suppl. Fig. S1).

Four groups of isolates produced in plastic bags were defined at a

level of 5% significance (p-value = 0.2646) according to their production. Group 1 consisted of the isolates ESALQ4556 and ESALQ4778, which showed higher production than other isolates, estimated at 3.51 $\times$ 10^9 conidia/g. Group 2 was composed of the isolates ESALQ1296, ESALQ1741, ESALQ1998, ESALQ2778, ESALQ3302, ESALQ3415, ESALQ3422, ESALQ3430, with an estimated production of 2.07 $\times$ 10^9 conidia/g. The third group consisted of ESALQ1609 and ESALQ3300 with an estimated production of 0.93 $\times$ 10^9 conidia/g, and the least productive isolates were ESALQ 2667 and ESALQ3307 with an estimated production of 0.14 $\times$ 10^9 conidia/g (Fig. 1).

3.2. Effects of UV-B radiation on the germination of C. Fumosorosea isolates

The best-fitted model for conidial viability data was the binomial-normal model with a logit connection function and three random effects. In this model, conidial viability is described by separate curves, in which each fungus has distinct initial proportions and different slopes for each time. Next, we fitted clusters using the likelihood ratio test for isolates with similar curve patterns. Four groups were defined at a level of 5% significance (Fig. 2).

The conidial viability of plates exposed to UV-B radiation for 2 h was little affected. After 4 h of exposure, the viability was still above 75%. The most significant differences in UV-B radiation tolerance between the isolates could be noticed from 6 h of exposure, in which groups 1 and 2 showed an estimated germination rate greater than 75%. In contrast, groups 3 and 4 showed an estimated 60% and 37% germination rate, respectively (Fig. 2). The most tolerant isolate was ESALQ1296, the only isolate from group 1, with 68% germination after 8 h of exposure to UV-B. Group 2, which consisted of the isolates ESALQ3422, ESALQ1998, ESALQ1741, ESALQ2778, ESALQ3430, and ESALQ3415, showed germination rates ranging between 60% and 17%. Group 3, which consisted of the isolates ESALQ4778, ESALQ1609, ESALQ3300, ESALQ2667, ESALQ4556, and ESALQ3302 showed germination rates ranging between 37% and 6.% after 8 h of exposure to UV-B. Lastly, the isolate ESALQ3307, the only isolate from group 4, was the most sensitive and showed a decline in the germination rates after 4 h of exposure and only 3% germination rate after 8 h of exposure to UV-B. (Fig. 2). The germination rate of control not exposed to UV-B ranged between 96 and 98%. The most tolerant isolate (ESALQ1296) attained the highest half-life of 9.07 h. In contrast, the most susceptible isolate (ESALQ-3307) attained for lowest half-life of 5.42 h (Table 2).

3.3. Effects of elevated temperature on the germination of C. Fumosorosea conidia

We found that all isolates of *C. fumosorosea* showed a fast reduction in germination during the first 30 min of exposure to 45 °C, except for two isolates from group 1 (Fig. 3). Following the similar behavior of *C. fumosorosea* isolates (proximity between curves), several clusters were tested using the likelihood ratio test. The fungal isolates were grouped into three groups significantly different (Fig. 3). The isolates ESALQ1296 and ESALQ3415 (group 1) were the most tolerant to exposure to 45 °C for 30 min, obtaining viability values of 64.6% and 55.2%, respectively. The isolates ESALQ1741, ESALQ2778, ESALQ3430, ESALQ1998, ESALQ4778, ESALQ3302, ESALQ3300, ESALQ3422, ESALQ1609, ESALQ4556, and ESALQ2667 were classified in group 2 and showed germination values that varied from 54.6% to 27.8%. The ESALQ3307 isolate (group 3) was the most susceptible to 45 °C, with 22.7% germination after 30 min. The time that resulted in a loss of 50% viability ranged from 21.5 min to 38.4 min for isolates ESALQ3307 and ESALQ1296, respectively (Table 3).

3.4. Correlation analysis

Here we performed a correlation analysis to determine the relationship between conidial productivity and conidial germination after exposure to UV-B radiation, and elevated temperature. The exposure time to UV-B radiation of 6 h and exposure to 45 °C for 30 min was defined based on the greater diversity of responses observed for the isolates for those times (Figs. 2 and 3). In general, we found no correlation between conidial production and tolerance to UV-B (Fig. 4A) and elevated temperature (Fig. 4B), as most tolerant isolates showed a medium conidial production (Fig. 4A and 4B). The less productive isolate, ESALQ3307, was also the least tolerant to UV-B and elevated temperature. In contrast, the most productive isolate, ESALQ4556, was the second least tolerant to UV-B and elevated temperature. On the other hand, we found a clear positive correlation between UV-B radiation and elevated temperature tolerance (Fig. 4C). The most tolerant isolate, ESALQ1296, was the most tolerant to UV-B and elevated temperature, while ESALQ3307 was the least tolerant to both abiotic stresses.

4. Discussion

High conidial yield is a desirable attribute to help select

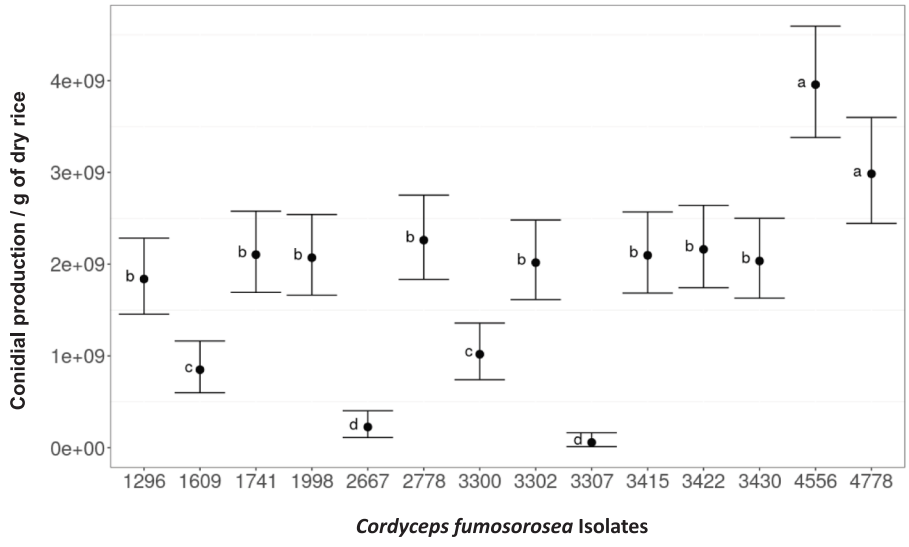

Fig. 1. Estimated means and confidence intervals for conidial production of 15 *Cordyceps fumosorosea* isolates grown on parboiled rice and incubated at 26 ± 1 °C and 12 h of photophase for ten days. Isolates with different letters indicate different clusters considering the likelihood-ratio test (alpha = 0.05).

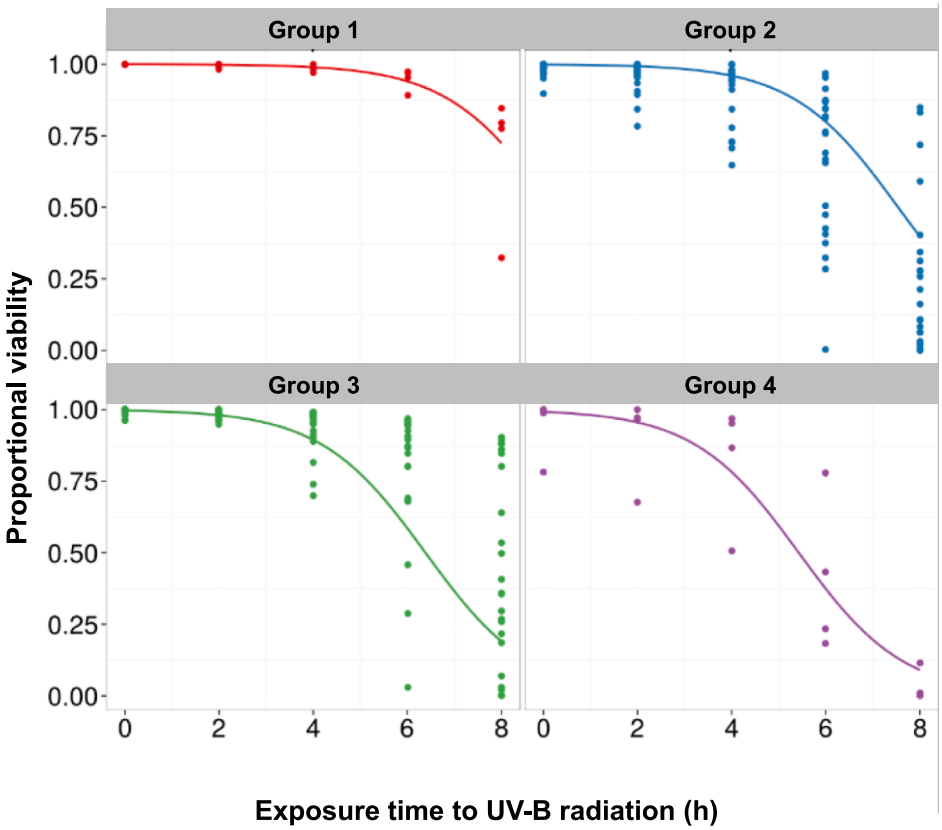

Fig. 2. Predicted proportion of germinated conidia of *Cordyceps fumosorosea* isolates after UV-B exposure for 2, 4, 6, or 8 h. Group one: ESALQ1296; Group two: ESALQ3422, ESALQ1998 ESALQ1741, ESALQ2778, ESALQ3430, and ESALQ3415. Group three: ESALQ4778, ESALQ1609, ESALQ3300, ESALQ2667, ESALQ4556, and ESALQ3302. Group four: ESALQ3307.

Table 2
Estimated time (h) of *Cordyceps fumosorosea* conidial germination exposed to UV-B light. Confidence interval (95%) with upper and lower bounds.

50% germination					75% germination				90% germination			
ESALQ Strains	Estimative	CI (95%)			Estimative	CI (95%)			Estimative	CI (95%)		
1296	9.07	7.56	;	10.58	7.85	6.33	;	9.36	6.62	5.11	;	8.14
3422	7.71	6.31	;	9.10	6.48	5.09	;	7.88	5.26	3.86	;	6.65
1998	7.58	6.20	;	8.96	6.36	4.97	;	7.74	5.13	3.75	;	6.51
1741	7.51	6.12	;	8.91	6.29	4.89	;	7.69	5.07	3.67	;	6.46
2778	7.51	6.11	;	8.91	6.29	4.89	;	7.68	5.06	3.67	;	6.46
3430	7.47	6.04	;	8.90	6.25	4.82	;	7.68	5.03	3.60	;	6.46
3415	7.42	6.03	;	8.80	6.19	4.81	;	7.57	4.97	3.59	;	6.35
4778	6.89	5.53	;	8.25	5.67	4.31	;	7.03	4.44	3.08	;	5.80
1609	6.48	5.14	;	7.81	5.25	3.91	;	6.59	4.03	2.69	;	5.37
3300	6.37	4.99	;	7.75	5.15	3.77	;	6.53	3.93	2.55	;	5.31
2667	6.24	4.91	;	7.57	5.01	3.68	;	6.34	3.79	2.46	;	5.12
4556	6.21	4.90	;	7.53	4.99	3.67	;	6.30	3.76	2.45	;	5.08
3302	6.14	4.83	;	7.45	4.91	3.60	;	6.22	3.69	2.38	;	5.00
3307	5.42	4.09	;	6.75	4.20	2.87	;	5.52	2.97	1.65	;	4.30

entomopathogenic isolates for biological control programs, reflecting lower industrial production costs. The present study determined that *C. fumosorosea* isolates differ in conidial production, ESALQ4556 and ESALQ4778 being the most productive, yielding up to 3×10^9 conidia/g. We consider productive isolates producing more than 1×10^9 conidia/g, which in practice means that it would require around 1–2 kg of colonized rice ($1–2 \times 10^{12}$ conidia) to produce the recommended dose of conidia per hectare for most pests and crops in Brazil. We determined in this study that most *C. fumosorosea* isolates have higher than 1×10^9 conidia/g, which means they meet industrial requirements.

Similar results were obtained by Murillo-Alonso et al. (2015) and Kim et al. (2010) for two different *C. fumosorosea* isolates yielded 5.3×10^9 and 1.4×10^9 conidia/g of rice, respectively. Although the solid fermentation of the fungus *C. fumosorosea* has been studied by other researchers, conidial production comparison frequently cannot be made directly due to variations in the methodologies used for calculating conidial yield, e.g., per gram of moist rice or per ml, to different substrates, such as barley and wheat and production methods. In this study, we present the values of conidia produced per gram of dry rice measured at the beginning of production before hydration and autoclaving, contrasting with most studies showing the yield of conidia per gram of moist rice at the end of production.

Furthermore, we determined a significant difference in conidial production in flasks and plastic bags, being conidial yield lower in the

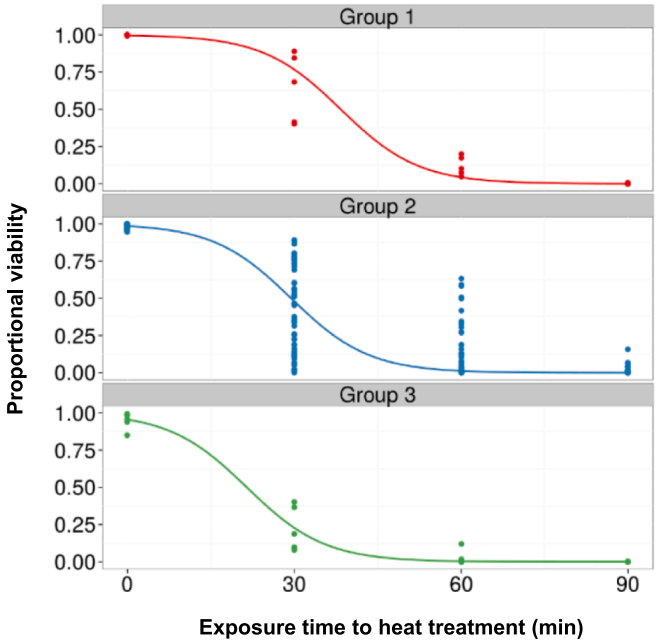

Fig. 3. Predicted proportion of germinated conidia of *Cordyceps fumosorosea* isolates after 45 °C exposure for 30, 60, or 90 min. Group one: ESALQ1296 and ESALQ3415. Group two: ESALQ1741, ESALQ2778, ESALQ3430, ESALQ1998, ESALQ4778, ESALQ3302, ESALQ3300, ESALQ3422, ESALQ1609, ESALQ4556, and ESALQ2667. Group three: ESALQ3307.

latter condition. This observation could be explained by more extensive contact with atmospheric air inside the bottle flasks, which favors higher oxygenation and, consequently, higher conidial production. On the other hand, results from conidial yield in plastic bags reflect those obtained in biofactories as fungi are grown under those conditions. Besides conidial production, other factors should be considered for choosing isolates for biological control programs, including tolerance to elevated temperature and UV-B radiation. These are the main abiotic factors affecting entomopathogenic fungal survival in field conditions (Braga et al. 2001a, 2001b, Bernardo et al. 2020).

This report showed high variability across *C. fumosorosea* isolates regarding tolerance to UV-B radiation, especially after six hours of exposure. The harmful effect of solar radiation depends on the type of ultraviolet radiation and the amount received by fungal isolates. Fargues et al. (1997) determined that the conidia of *C. fumosorosea* are highly susceptible to UV-B radiation (280–320 nm), although UV-A radiation (320–400 nm) is also dangerous, and UV-C is the most harmful. We found that the viability of *C. fumosorosea* isolates ranged from 82.3 to 98.6% after 4 h of UV-B exposure of 313 nm = 0.6 Wm^{-2} UV-B. These results differ from Fargues et al. (1996) for 33 isolates of *C. fumosorosea*, who observed that exposure to simulated sunlight at 295 nm = 0.3 Wm^{-2} UV-B killed conidia after 4 h of irradiation. The authors considered *C. fumosorosea* a low radiation tolerant species, followed by *B. bassiana*, *M. anisopliae*, and *M. flavoviride*. However, the authors observed that *C. fumosorosea* isolates from tropical regions were more tolerant to irradiation than isolates from temperate areas. This result agrees with Braga et al. (2001), who showed a tolerance to ultraviolet radiation in *Metarhizium* spp. isolates might be associated with geographic origin. The authors determined a significant quadratic relationship between decreasing UV-B tolerance with increasing latitude.

In this present study, groups 1 and 2 had isolates with conidial viability greater than 75% after 6 h of UV-B exposure, which corresponds to the doses of 14.28 kJ/m^2. In contrast, Mascarin et al. (2018) determined for *C. javanica* isolates that the median effective doses (ED_{50} ± standard error) of UV-B radiation ranged from 3.79 ± 0.36 to 7.86 ± 6.12 kJ/m^2. These results show that all isolates used in our study might be highly tolerant to UV-B radiation, and this feature should be investigated deeper. The isolate (ESALQ1296), which was most tolerant to UV-B radiation attained the highest half-life of 9.07 h, while the most susceptible isolate (ESALQ3307) achieved the lowest half-life of 5.42 h. Although the isolates selected in this study are highly tolerant to UV-B radiation compared to other studies, a similar pattern of tolerance to UV-B radiation was also obtained by Couceiro et al. (2021) for conidia of *M. brunneum*, *M. robertsii,* and *M. anisopliae* isolates. After 4 h of exposure to UV-B most isolates showed >75% of viability. Only after 6 h of exposure (total dose of 14.28 kJ/m^2) were the germination rates reduced for almost all 12 isolates of *Metarhizium* spp.

Interestingly, the isolate most tolerant to UV-B and high-temperature (ESALQ1296) is the only one used in this study that came from an infected insect, *Bemisia tabaci*, whereas other isolates came from the soil. In contrast, the least thermotolerant isolate, ESALQ3307, came from the Caatinga Biome, a region characterized by higher temperatures, followed by median tolerant isolates classified in Group 2 from different Biomes: Caatinga, Amazon, Atlantic Forest, and Pampa, the latter biome characterized by milder temperatures than the others. These results indicate no relationship between warm climate areas and higher thermotolerance, which contrasts with other authors such as Vidal et al. (1997), who showed that isolates of *C. fumosorosea* from warmer climates, such as in India have greater tolerance to elevated temperatures than those isolates from temperate climates (Europe). However, it is worth mentioning that the authors measured mycelial growth rates instead of the conidial germination of isolates submitted to elevated temperature, which might lead to another conclusion. Additionally, those isolates were from very different geoclimatic regions with temperate, subtropical, and tropical areas. In our research, isolates were

Table 3
Estimated time (min) of *Cordyceps fumosorosea* conidial germination exposed to 45 °C. Confidence interval (95%) with upper and lower bounds.

50% germination					75% germination				90% germination			
ESALQ Strains	Estimative	CI (95%)			Estimative	CI (95%)			Estimative	CI (95%)		
1296	38.43	30.87	;	45.99	30.75	23.19	;	38.31	23.07	15.52	;	30.63
3415	34.61	27.76	;	41.47	26.94	20.08	;	33.79	19.26	12.41	;	26.12
1741	32.08	25.56	;	38.60	24.40	17.88	;	30.92	16.73	10.21	;	23.25
2778	31.66	25.25	;	38.07	23.98	17.57	;	30.39	16.31	9.90	;	22.72
3430	31.54	24.96	;	38.12	23.86	17.28	;	30.44	16.19	9.61	;	22.77
1998	30.49	24.28	;	36.70	22.82	16.61	;	29.03	15.14	8.93	;	21.35
4778	30.21	23.78	;	36.65	22.54	16.1	;	28.97	14.86	8.43	;	21.29
3302	28.58	22.25	;	34.90	20.90	14.58	;	27.23	13.23	6.90	;	19.55
3300	28.23	21.9	;	34.56	20.55	14.22	;	26.88	12.88	6.54	;	19.21
3422	27.52	21.45	;	33.59	19.84	13.77	;	25.92	12.17	6.10	;	18.24
1609	26.87	20.82	;	32.92	19.19	13.14	;	25.24	11.51	5.46	;	17.56
4556	26.39	20.21	;	32.58	18.72	12.53	;	24.9	11.04	4.85	;	17.23
2667	25.89	19.85	;	31.94	18.22	12.17	;	24.27	10.54	4.49	;	16.59
3307	21.51	15.88	;	27.13	13.83	8.20	;	19.46	6.15	0.53	;	11.78

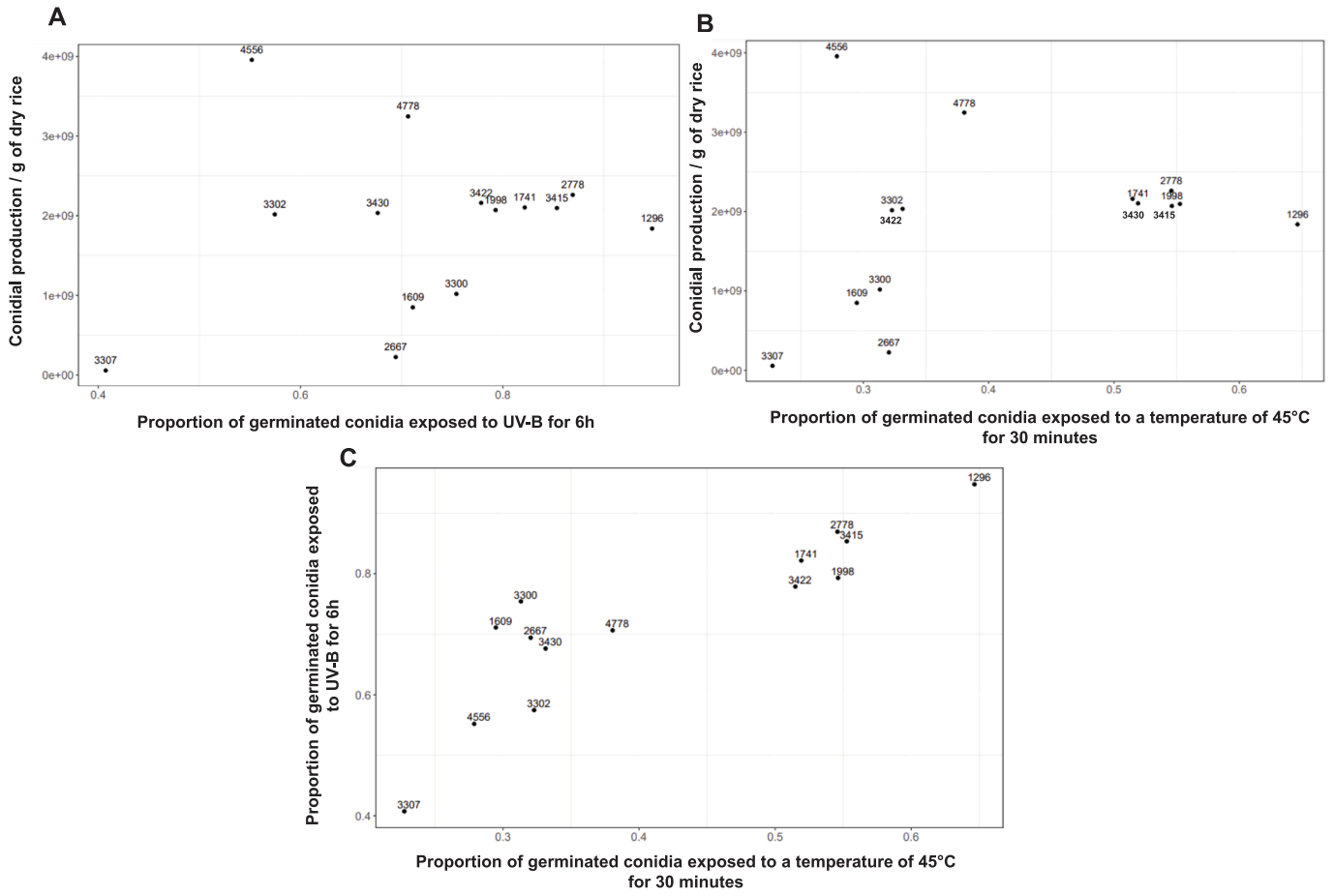

Fig. 4. Relationship between: Conidial production and germinated conidia exposed to UV-B for 6 h (A); Conidial production and germinated conidia exposed to 45 °C for 30 min (B); Germinated conidia exposed to UV-B for 6 h and germinated conidia exposed to 45 °C for 30 min (C).

from the warm regions, mostly tropical but also subtropical climates. Cross-tolerance to different abiotic stresses has been reported in the literature and is associated with how microorganisms respond to reactive oxygen species (R.O.S) produced as a response to biotic and abiotic stress (Wong et al. 2019; Pradhan et al., 2021). This report demonstrated a clear positive correlation between tolerance to heat and UV-B but not between conidial yield and tolerance to heat and UV-B. In practice, these results indicate that we can infer that a tolerant isolate to heat treatment is also tolerant to UV-B radiation.

It has been shown that *C. fumosorosea* isolates are also less tolerant to elevated temperatures than *B. bassiana*, *M. robertsii,* and *M. anisopliae* isolates (Rangel et al., 2005; Li and Feng, 2009; Fernandes et al., 2010; Souza et al., 2014). Souza et al. (2014) evaluated the conidia thermotolerance of one isolate of each fungi: *C. fumosorosea*, *B. bassiana*, *M. robertsii,* and *M. anisopliae* conidia at 38°, 41°, and 45 °C. The results showed that *C. fumosorosea* isolate had the lowest thermotolerance (TL_{50} = 2.06 h) after exposure to 41 °C. Superior results were found for conidia of *B. bassiana* exposed to 45 °C (TL_{50} = 3.5 h) and conidia of *M. robertsii* (TL_{50} = 2.8 h) and *M. anisopliae* (TL_{50} = 2.5 h). Although generalizations can be made at the species level, many studies have shown that conidial thermotolerance varies significantly between isolates. Here, *C. fumosorosea* isolates were classified into three groups with very different tolerance to heat treatment, mainly after 30 min of exposure, with viability ranging from 25% to 87%. Mascarin et al. (2018b) determined that the time to achieve 50% conidial viability (ET_{50}) among *Cordyceps javanica* isolates varied from 0.48 to 1.25 h based on 48 h germination readings after exposed to 45 °C. These isolates are more tolerant than those used in our study, which showed an estimative time to achieve 50% viability of 0.35–0.64 h (Table 3). Studies conducted by Fernandes et al. (2008) reported high variability in the thermotolerance of *Beauveria* spp. isolates, after 2 h of exposure to 45 °C, obtaining different germination rates: low (0–20%), medium (20–60%), and high (60–80%). In addition, Varela and Morales (1996) determined the viability of *B. bassiana* conidia from 10 to 20% and 80–90% for 10 min of exposure at 50 °C and 45 °C, respectively. Results for ESALQ3307 and ESALQ1296 isolates agreed with previous findings for *B. bassiana* isolates. It is important to highlight that fungi are exposed to temperatures as high as 45 °C in the spray tank but also in the industry where rice colonized by the fungus is exposed to heated air currents to facilitate drying. Our results highlight the importance of adjusting the drying temperature and exposure time for drying equipment in order to preserve conidial viability.

In summary, we determined that isolates of *C. fumosorosea* show variations between conidial production and tolerance to UV-B radiation and elevated temperature. There is a positive correlation between tolerance to elevated temperature and UV-B light, but there is no between these factors and conidial production. These results contribute to a better understanding of the tolerance to abiotic factors of Brazilian isolates of *C. fumosorosea* and highlight the importance of knowing these factors for selecting isolates for a biological pest control program. The virulence of many of these isolates was later evaluated against different pests, and some are now registered or under registration in Brazil.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jip.2023.107888.

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