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Tolerance of Entomopathogenic and Phytopathogenic Fungi to Different Stressing Agents

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Orientador: Prof. Dr. Drauzio Eduardo Naretto Rangel

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"TOLERÂNCIA DE FUNGOS PATÓGENOS DE INSETOS E FUNGOS PATÓGENOS DE PLANTAS A RADIAÇÃO UV, ESTRESSE OSMÓTICO, ESTRESSE DE PAREDE CELULAR E ESTRESSE TÉRMICO"

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Tolerância de Fungos Entomopatogênicos e Fitopatogênicos a Diferentes Agentes Estressores

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“Creio firmemente em uma lei de compensação. As verdadeiras recompensas são sempre proporcionais ao esforço e aos sacrifícios feitos.”

Nikola Tesla

RESUMO

COSTA, T. P. C. **“Tolerância de Fungos Entomopatogênicos e Fitopatogênicos a Diferentes Agentes Estressores”**. 2020. 106f. Tese (Doutorado). Universidade Brasil – São Paulo, 2020.

No primeiro capítulo foram estudadas a germinação, crescimento micelial e produção de conídios dos fungos entomopatogênicos *Trichothecium roseum*, *Metarhizium robertsii* e *Metarhizium acridum* sob condições de estresse osmótico por KCl, à radiação UV, ao 4-nitroquinoline-1-oxide (4-NQO), ao calor e ao vermelho Congo. Ao confrontarmos o *T. roseum*, o *M. robertsii* e o *M. acridum*, sob condições de estresses, constatamos que o *T. roseum* é o mais tolerante em relação aos outros quando em situações de estresse osmóticos por KCL, estresse ao 4-NQO e, estresse ao vermelho Congo. Entretanto, sob as condições de radiação à UV as espécies de *Metarhizium* se mostraram mais tolerantes. Em resumo, concluímos que o fungo *T. roseum* é um agente promissor no controle biológico de insetos. No segundo capítulo foram estudados os efeitos dos comprimentos de onda da luz visível na germinação, crescimento radial micelial e produção de conídios dos fitopatógenos *Colletotrichum acutatum* e *Fusarium fujikuroi*. Em conclusão, não foram encontradas diferenças para germinação e crescimento para ambos os fungos sob diferentes regimes de luz e escuro; no entanto, diferenças significativas ocorreram tanto na produção quanto na radiação UV de conídios. No terceiro capítulo foram estudadas as sensibilidades diferenciais ao estresse da parede celular causado pelo vermelho do Congo foram observadas em muitas espécies de fungos. O fungo saprotrófico *Aspergillus niger* e o fungo micoparasita *Trichoderma atroviride* destacaram-se como as espécies mais resistentes ao estresse da parede celular causado pelo vermelho do Congo, seguidos pelos fungos fitopatogênicos e outros saprotróficos. Os insetos patógenos tiveram tolerância baixa ou moderada. Os insetos patógenos *Metarhizium acridum* e *Isaria fumosorosea* foram os mais sensíveis.

Palavras-chave: fungos entomopatogênicos, fotobiologia, fungos fitopatogênicos, fungos saprotróficos, fungos micoparasitas, tolerância ao estresse.

ABSTRACT

COSTA, T. P. C “Tolerance of Entomopathogenic and Phytopathogenic Fungi to Different Stressing Agents**”**. 2020. 106f. Thesis (Doctorate). Universidade Brasil – São Paulo, 2020.

In the first chapter, germination, mycelial growth, and conidia production of the entomopathogenic fungi *Trichothecium roseum*, *Metarhizium robertsii*, and *Metarhizium acridum* were studied under conditions of osmotic stress induced by KCl, UV radiation, 4-nitroquinoline-1-oxide (4-NQO), heat, and Congo red. *T. roseum* is more tolerant than the others when confronting osmotic stress due to KCL, stress due to the 4-NQO, and stress due to Congo Red. However, under UV radiation conditions, *Metarhizium* species were more tolerant. In summary, we conclude that the fungus *T. roseum* is a promising agent in the biological control of insects. In the second chapter, the effects of visible light wavelengths on germination, mycelial radial growth, and conidia production of the phytopathogens *Colletotrichum acutatum* and *Fusarium fujikuroi* were studied. No differences were found for germination and growth for both fungi under different light and dark regimes; however, significant differences occurred in both conidial production and UV radiation. In the third chapter, differential sensitivities to cell wall stress caused by Congo red were studied in several species of fungi. The saprotrophic fungus *Aspergillus niger* and the mycoparasitic fungus *Trichoderma atroviride* stood out as the species most resistant to cell wall stress caused by Congo red, followed by phytopathogenic and other saprotrophic fungi. The insect pathogens exhibited low or moderate tolerance. The insect pathogens *Metarhizium acridum* and *Isaria fumosorosea* were the most sensitive.

Keywords: entomopathogenic fungi, photobiology, phytopathogenic fungi, saprotrophic fungi, mycoparasitic fungi, stress tolerance.

LIST OF FIGURES

LIST OF TABLES

SUMMARY

1. INTRODUCTION

Over time, plants have faced of water and nutrient limitations, UV radiation, temperature stress, and harmful actions due to the oxidative atmosphere, and this adaptation influenced the diversity of fungi (Karányi et al., 2013). To continue to proliferate and develop in the environment, fungi have the ability to easily adapt to almost any environment, which has culminated in the existence of a great diversity of these organisms (Duran, Cary, and Calvo 2010).

Naturally, this disparity in the diversity of fungi is due in part to its exceptional arsenal that has evolved to withstand various types of stresses faced during its evolution (Orosz et al., 2018). Fungi have developed metabolites to withstand stress conditions, important for their survival in unfavorable environments (Day and Quinn 2019). Among the metabolites that fungi produce, trehalose, mannitol, and erythritol carbohydrates provide tolerance to numerous stress conditions (Rangel, 2011).

This ability to adapt is a fundamentally important characteristic of fungi in the face of the stresses imposed by the host and environment (Day et al., 2018). To survive, each species must feel and respond to the imposed changes (Brown et al., 2017).

Stresses such as changes in pH, environmental temperature, osmolarity, UV radiation, and oxidative process interfered in the evolution of ancient life forms in the various ecosystems. Therefore, essential conditions for cellular responses to these pressures are preserved in all kingdoms (Brown, Larcombe, Pradhan, 2020). For example, hyperosmotic stress stimulates molecular responses in fungi, leading to cell adaptation, including the accumulation of osmolytes that promote restoration of pressure, growth, and turgor (Brown, Larcombe, Pradhan, 2020). To resist the pressure of internal turgor, a fungal cell needs a resistant wall, because when confronted with cell wall degradation, fungal cells swell and burst (Nobel, Ende; Klis 2000). Another example of cell wall stress is exposure to chemicals, such as 4-nitroquinoline-1-oxide (4-NQO) and Congo red dye.

Entomopathogenic fungi play an essential role in the organization of insect populations in the environment (Wang and Wang 2017), and are used as

promising biopesticides (Bojke et al., 2018). The most evaluated organisms are the classes *Aschersonia*, *Beauveria*, *Entomophthora*, *Hirsutella*, *Metarhizium, Nomuraea*, *Paecilomyces, Tolypocladium*, and *Verticillium*. Biological control programs mostly used *Metarhizium* and *Beauveria* (Dias et al., 2018).

Phytopathogenic fungi are responsible for many plant diseases in the world, considerably reducing agricultural production (Chandrasekaran et al., 2016). Fungi, such as *Fusarium* spp., growing on plants, are capable of producing mycotoxins (Mahlo et al., 2016), and *Colletotrictum gleosporioides* is responsible for lychee anthracnose (Hu et al., 2019).

Reported losses in agricultural crops are due to the action of phytopathogenic fungi and pests. Thus, the interest in biological control agents in relation to agricultural production and pest and insect control motivates research involving entomopathogenic and phytopathogenic fungi in the face of various stress conditions.

This thesis is divided into three chapters. In the first chapter, we present the study about the tolerance of three entomopathogenic fungal isolates – *Trichothecium roseum*, *Metarhizium robertsii*, and *Metarhizium acridum* – under conditions of osmotic stress caused by KCl, stress due to UV radiation, stress from the mutagenic and genotoxic agent 4-NQO, heat stress, and cell wall integrity stress due to Congo red.

In the second chapter, we compare the different wavelengths of light in the production of conidia, mycelial growth, and tolerance to UV radiation stress of two phytopathogen isolates – *Colletotrichum acutatum* and *Fusarium fujikuroi.*

The third chapter is about the fungal tolerance to Congo red in a varied collection of fungal species grouped as insect pathogens, plant pathogens, saprotrophs, and mycoparasites.

Finally, in final remarks contains the main conclusions of this research and the implications for future research perspectives.

2. LITERATURE REVIEW

2.1 Biopesticides

Biopesticides are used to control pests as an alternative to pesticides composed of chemical substances such as organophosphates and organochlorines, which harm the health of humans and animals (Kumar; Singh 2015). Biopesticides act to control pests and are based on live microorganisms or natural products (Chandler et al., 2011). Most are microbial pathogens (fungi, bacteria, viruses) of the pest that requires control (Sommerville, 2002).

They were defined to include biochemical pesticides (such as pheromones and other attractions), microbial pesticides, and those resulting from genetic manipulations (Wakil, Brust; Perring 2018). For example, some fungi, such as *Colletotrichum gloeosporioides*, control certain weeds (Harding; Raizada 2015) and other fungi, such as *Metarhizium anisopliae*, kill insects (Tiago, Oliveira; Lima 2014).

Biopesticides are less toxic than conventional pesticides (Kumar 2012), generally affecting only the target pest and closely related organisms. Conventional broad-spectrum pesticides can affect various organisms and cause an imbalance in nature (Dannon et al., 2020).

They are effective even in very small quantities and decompose quickly and in an ecologically correct manner, resulting in lower exposures and avoiding the pollution problems caused by conventional chemical pesticides; nevertheless, the issue of a cultural acceptance remains (Kumar 2012). Their use as a component of Integrated Pest Management (IPM) programs helps to reduce the use of conventional pesticides and maintain good crop results (Dannon et al., 2020).

2.2 Entomopathogenic fungi – mechanism of action on pests control

The spores of the fungus, when they come into contact with the arthropod host, form a germ tube, which has an infective structure capable of exerting mechanical pressure through the cuticle (Dias, 2018). The spores produce degrading enzymes that assist the entry into the host, multiplying, and

invading the tissues with the production of more spores to continue the infection cycle (Dara, 2019) (Figure 1).

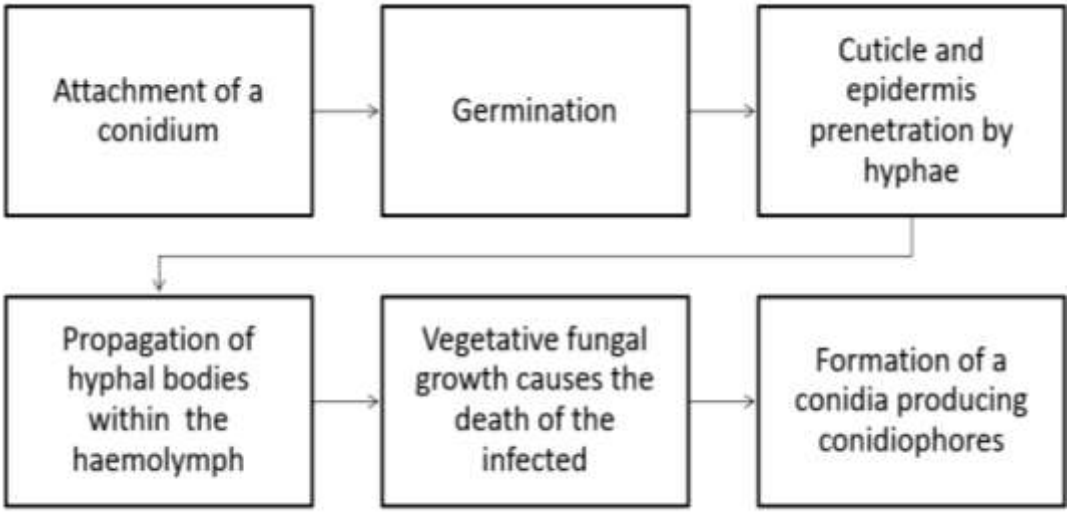

Figure 1 – Scheme of the mode of action of entomopathogenic fungi against lepidopteran insects. Adapted from Singh, Raina and Singh (2017).

The infection starts with the fixation of single-cell dispersive forms of the fungus, which may be conidia or blastospores, to the insect cuticle, which with the aid of hydrolytic enzymes –for example proteases, chitinases, and lipases – promote fungal germination and growth of the throughout the host, and consequently the penetration of into cuticular layers (Dara, 2019; Xiao et al., 2012). In this process, the fungus produces specialized infection structures, such as penetration pits and/or appressoria, which facilitate the penetration of growing hyphae into the integument of the host (Dara, 2019; Singh, Raina; Singh 2017; Zheng et al., 2011), then, the pathogen encounters the immune system of the host.

Entomopathogenic fungi can be applied in the form of conidia or mycelia that sporulate after application, and their use as an alternative to conventional pesticides can be very useful for managing pesticide resistance (Dannon et al., 2020; Ortiz-Urquiza and Keyhani, 2013).

2.3 Phytopathogenic fungi – mechanism of action

The plasticity of fungi allows the process of colonization and invasion through hyphae, formation of differentiated spores, infectious structures, andfruiting bodies that can assist in the dispersion process (Perez-Nadales et al., 2014). Phytopathogenic fungi are generally transmitted through spore dispersion. Some fungi launch mycelial to form a network within their habitat.

According to their lifestyle and interactions with the host, these fungi can be classified as biotrophic, necrotrophic, or hemibiotrophic. Necrotrophy is an infection resource in which the host plant dies (Ikeda, Park, Nakayashiki, 2019). Biotrophy is characteristic for the conservation of the host plant and consequently the absorption of nutrients (Perez-Nadales et al., 2014). In hemibiotrophy, the fungus not only invades, it colonizes host plants (Perez-Nadales et al., 2014).

Furthermore, hemibiotrophic fungi start with an asymptomatic phase, and then present the necrotrophic phase, which causes the death of the host plant that then undergoes colonization and sporulation (Spanu; Panstruga 2017). *Colletotrichum* sp. is an example of hemibiotrophic fungi.

2.4 Lengths of light waves in the production of conidia

Different types of light are used by fungi as environmental information to assist in the preparation against stressful conditions and in the ability to induce their progression in the environment (Dias et al., 2020). Some spectra induce the production of conidia, while others can kill them, decreasing the size of the population and limiting dispersion (Menezes et al., 2014).

UV radiation is detrimental to the viability of fungi, as some more tolerant isolates can survive for a few hours from direct exposure, delaying the germination of conidia and reducing development of the fungus (Fernandes et al., 2015). In addition, susceptible isolates cannot resist exposure (Fernandes et al., 2015; Menezes et al., 2014).

2.5 Osmotic stress

Tolerance to osmotic stress requires the composition of adequate solutes for osmotic adjustment, ion conduction, and melanin synthesis (Araújo et al., 2019), so that fungi can adjust intracellular water activity with compatible solute reserves, such as trehalose, mannitol, glycerol, erythritol, and arabitol (Rangel, 2011; Rangel et al., 2015).

Osmotic stress, mainly due to the action of the high osmolarity glycerol (HOG) pathway, leads to an critical response to adapt and survive hyperosmotic conditions (Duran, Cary, and Calvo, 2010), together with the cell wall integrity pathway (CWI) (Fuchs and Mylonakis, 2009). The role of mitogen-activated protein kinase (MAPK) is also fundamental.

This stress affects morphological variations of cells and colonies and may increase fatty acid unsaturation (Araújo et al., 2019). For example, fungal cells experience a hyperosmotic shock associated with a significant flow of water and cellular setback (Duran, Cary, and Calvo 2010; Hohmann 2002; Blomberg and Adler 1992). As a result, the response of fungus to variations in external water capacity involves the detection and transduction of signals through osmosensors, such as the Sln1p protein, which is a hypo-osmolarity-activated sensor that can directly detect osmotic changes (Hohmann 2002; Blomberg and Adler 1992).

2.6 Stress by UV radiation

Ambient light conditions are associated with the development and behavior of fungi, as are several segments of the light spectrum (Lee et al., 2006). Some species of fungi can respond to light characteristics ranging from 450 nm (blue) to 700 nm (red) (Fuller, Loros, and Dunlap 2015).

Studies have shown that several mechanisms indicated inactivation of conidia by UV radiation at wavelengths between (280 and 320 nm), which damages DNA and can cause mutations or transcription failures (Kaiser et al., 2018, Cadet and Wagner 2013; Griffiths et al., 1998). UV radiation delays the germination of surviving conidia (Fernades et al., 2007). UV-B radiation is absorbed by macromolecules, nucleic acids, lipids, and proteins, involving their

structure and function (KUNZ et al., 2007). Effects of indirect toxicity by UV-A radiation include indirect toxicity through reactive oxygen species (ROS) that damage nitrogenous bases, and consequently membrane proteins and DNA (Cadet and Wagner 2013; Griffiths et al., 1998).

2.7 Cell wall stress

The fungal cell wall is a competent organelle that provides structure, protection, and viability to the fungus, controlling interactions with the host during infection (Hopke et al., 2018). Many of the cell wall components are conserved in several species of fungi, while others are species specific. Cell wall probably has the more phenotypic diversity and plasticity than any part of the cell, in fungi this is a characteristic in relation to the ability to promote changes in responses to environmental stimuli (Gow, Letge, and Munro 2017).

The composition of the cell wall is made up of glycoproteins – which are widely modified with carbohydrates linked to nitrogen (N) and oxygen (O) and, in many cases, also contain an anchor of glycosylphosphatidylinositol (GPI) – and polysaccharides (Latgé 2007) – mainly glucan (predominantly beta1,3-glucan, long linear chains of glucose linked to beta-1,3, some with alternating double bonds, such as beta-1,6-glucan) and chitin (chains of N-acetylglucosamine residues linked to beta-1,4, normally less abundant than the glycoprotein or glucan portions) (Garcia-Rubio et al., 2020; Bowman and Free 2006). The β-glucan layer protects the fungus from osmotic and mechanical stresses, in addition to mediating interactions with the external environment through adhesins together with receptors that, after activation, trigger a cascade of signals inside the cell (Garcia-Rubio et al., 2020; Bleackley et al., 2019).

The cell wall integrity (CWI) pathway provides a way to sustain and restore damage to the cell wall that ensures its resistance to aggressive environments (Fuchs and Mylonakis 2009). The CWI signal is integrated by a mitogen activated protein kinase (MAPK) module. After the beginning of the phosphorylation cascade, the CWI signal leads to the expression of genes relevant to the cell wall that also regulate expression and production of specific molecules (for example cell wall synthesis genes), which are used by fungi for

maximum competitiveness in the environment when exposed to stress conditions (Valiante 2017).

2.8 Thermal Stress

Temperature triggers stress responses in fungi (Tiwari, Thakur, and Shankar, 2015; Papagianni 2004; Leache and Cowen 2013). To overcome stress, the body expresses heat shock proteins (Hsps) to perform biological functions such as transcription, translation, and post-translation modifications; protein folding; and protein aggregation and breakdown (Tiwari, Thakur, and Shankar, 2015).

Thermotolerance is closely described for the production of these essential proteins for heat tolerance and endogenous reserve of polyols and trehalose in fungi (Kim et al., 2010).

3. OBJECTIVES

General

To assess the tolerance of insect-pathogenic fungi and plant-pathogenic fungi to osmotic stress, UV radiation stress, cell wall stress, and thermal stress.

Specific

1) Study tolerance to osmotic stress caused by KCl, tolerance to UV radiation, tolerance to the mutagenic agent and cell wall stressor 4-NQO, and the integrity of the cell wall after exposure to Congo red, and thermal stress in three isolates of entomopathogenic fungi *Trichothecium roseum, Metarhizium robertsii,* and *Metarhizium acridum*;

2) Compare how the different wavelengths of light affect the production of conidia and mycelial growth, as well as the tolerance to UV radiation stress of two phytopathogen isolates –*Colletotrichum acutatum* and *Fusarium fujikuroi*;

3) Study the tolerance of fungi to Congo red in a varied collection of fungal species grouped as insect pathogens, plant pathogens, saprotrophic and mycoparasitic.

Trichothecium roseum a novel fungus for insect biological control agent

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Abstract

Germination, mycelial growth, and conidia production of the entomopathogenic fungi *Trichothecium roseum*, *Metarhizium robertsii*, and *Metarhizium acridum* were studied under osmotic stress by KCl, UV radiation, 4-nitroquinoline-1-oxide (4-NQO), heat, and Congo red. All fungi were grown on potato dextrose agar (PDA), and all suspensions were used immediately after being prepared for each of the stress conditions experiments. The germinations were observed, depending on the experiment, at 24 or 48 h after inoculation of the conidial suspensions in the medium and maintained at 26 °C in the dark for germination. At least 300 conidia were evaluated per plate. When *T. roseum* (ARSEF 1212), *M. robertsii* (ARSEF 2575) and *M. acridum* (ARSEF 324) were challenged with stress conditions, *T. roseum* (ARSEF 1212) was the most tolerant of the conditions evaluated osmotic stress by KCl, by 4-NQO and Congo red. However, in the conditions of UV radiation, *Metarhizium* species exhibited more tolerance. In summary, the fungus *T. roseum* is a promising agent for the biological control of insects.

Key Words: entomopathogenic fungi; photobiology; UV-B radiation; heat stress, osmotic stress, genotoxic stress

Introduction

Trichothecium roseum is a fungus distributed worldwide and generally found in decomposing plant substrates (Chasseur et al., 2001).

The fungus belongs to the Hipocreales order and Ascomycota division, reproduces asexually through the formation of conidia with unknown sexual stage (Batta, 2020), and is Saprophytic (Shamsi and Sultana 2008, Batta 2020). It is sometimes considered a secondary pathogen, causing pink rot in post-harvest fruits and vegetables (Batta, 2020), and/or an opportunistic pathogen of some *Aedes aegypti* mosquito larvae (Ramirez et al., 2018). It exhibits antagonistic activity towards other fungi (Freeman and Morrison 1948; Robertson, Smithies, and Tittenso 1949, Negri et al., 2003; May-De Mio, Negri, and Michailides 2014). Mycoparasitic with significant antifungal activity (Pan et al., 2006), *T. roseum* is an important cause of fruit rot (Wei et al., 2018; Dong and Bian, 2013; Hong and Michailides, 1997) with reports of infection in vegetables such as tomato (Hamid et al., 2014).

The colonies of *T. roseum* are diffuse, initially white, but soon deposit in a rosy pink color (Shamsi and Sultana 2008, Kendrick and Cole 1969). Conidiophores are up to 147 × 3.0–4.5 µm, hyaline conidia, slightly swollen at the ends, and in pink mass, septate (Barnett and Hunter, 1998), with thick wall, each with a flattened protuberance at the base, 13.5-27.0 × 8–11 µm, frequently grouped (Shamsi and Sultana 2008).

Trichothecium roseum produces a rich diversity of secondary metabolites and mycotoxins, such as trichothecenes, rosenonolactones (Batta, 2020, Zhang et al., 2010), and roseotoxins (Žabka et al, 2006), which can invade hosts such as plants and fruits (Batta, 2020 , Zhang et al., 2010). Secondary metabolites, such as polyketides, non-ribosomal peptides, and terpenes, perform biological activities that contribute to the survival of fungi in an occupied ecological niche (Boruta 2017). On the other hand, a part of the secondary metabolites of fungi has no known biological function and are commonly presented as host-selective toxins (HSTs) (Žabka et al, 2006). In plant pathogenic fungi that produce these HSTs (usually secondary low molecular weight metabolites), they act as effectors controlling pathogenicity or virulence in certain plant-pathogen interactions (Tsuge et al., 2013).

The mycotoxins of *T. roseum* include rosenonolactones, cyclic dipertene, which are formed by the migration of methyl C – 10 to C – 9 and are found in metabolites of *T. roseum* (Marcano and Hasegawa 2002, p. 303). Trichothecin (TCN) is a toxic metabolite belonging to the group of trichothecenes (name derived from this compound) and was isolated as an antifungal agent from a culture of *T. roseum*, which was the first occurrence of pure trichothecene isolated (Ishii et al., 1986). Roseotoxins are a group of lipophilic and neutral cyclohexadepsipeptides (Žabka et al, 2006).

The objectives of this study were to compare the tolerance of *Trichothecium roseum*, *Metarhizium robertsii*, and *Metarhizium acridum* for tolerance to osmotic stress; UV radiation; 4-nitroquinoline-1-oxide (4-NQO), and Congo red.

Materials and methods

Fungal isolates

All fungal isolates were obtained from the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF; Robert W. Holley Center for Agriculture & Health, Ithaca, NY, USA) (Table 1). Stock cultures were maintained at 4 ˚C in test tubes on slants of potato dextrose agar (Difco Laboratories, Sparks, MD, USA).

Table 1. Fungal species and geographic origin

Isolate	Species	Substrate/Host	Geographic Origin	Year
ARSEF 324	*Metarhizium acridum*	*Austracris guttulosa* [Orthoptera: Acrididae]	Queensland, Australia	1979
ARSEF 2575	*Metarhizium robertsii*	*Curculio caryae* [Coleoptera: Curculionidae]	South Carolina, USA	1988
ARSEF 1212	*Trichothecium roseum*	Nymph, *Adelphocoris* sp. [Hemiptera: Miridae]	Lazio, Italy	1983

Source: Environmental microbiology laboratory for fungi collection - Drauzio Eduardo Naretto Rangel.

Conidial production and harvesting

Conidia were produced on 23 ml of the following media: potato dextrose agar (Difco, Sparks, MD, USA) (PDA) in 95 mm polystyrene Petri dishes. The pH of all media was adjusted to 6.9. A conidial suspension (100 μl of 10^7 conidia ml^{-1}) was plated evenly with a glass spreader onto agar media. The cultures were incubated at 26 ± 1 °C and approximately 90% relative humidity (RH) for 14 days. Three different batches of conidia were produced, one for each replication of each type of stress experiment.

A few microbiological loops of conidia of all isolates (Table 1) were collected after 14 days of growth and suspended in 10 ml of sterile Tween 80 solution (0.01%) in screw cap Pyrex tubes (20 × 125 mm). The amount of conidia collected were enough to make a suspension of 10^5 conidia ml^{-1}. The suspensions (1×10^5 conidia ml^{-1}) were shaken vigorously using a vortex and 40 µl were inoculated (dropped, but not spread) on the center of the PDA medium. All suspensions were used immediately after prepared for each of the stress conditions experiments.

Conidial germination for stress experiments

The germinations were observed, depending on the experiment, at 24 or at 48 h after the conidial suspensions were inoculated on the medium. The plates were kept at 26 °C in the dark for germination. The agar plate at the point of the inoculation was stained with a drop of Methyl Blue solution (Braga et al., 2002) and it was covered with a coverslip and examined under light microscope at 400× magnification. Conidia were considered germinated when the germ tube showed a white projection from the conidium. At least 300 conidia per plate were evaluated, and the percent of germination was calculated as described by Braga et al. (2001). All germinating or non-germinating conidia were counted on a single coverslip. The scanning pattern for counting was around the margin of the conidial suspension drop, which is an area commonly less populated by conidia. Each treatment was repeated at least four times with a fresh batch of conidia produced for each repetition.

Tolerance to osmotic stress

To study the tolerances to osmotic stress of conidia produced in the treatments: light, dark, and nutritive stress, conidia were exposed to potassium chloride (Sigma-Aldrich Corp. St. Louis, Missouri, USA) according to Araújo et al. (2019).

The suspensions were inoculated (dropped, but not spread) on the center of the PDA on glass Petri dishes (12 × 40 mm Normax, Portugal) with 4 ml PDA medium (control 0 M) or PDA supplemented with potassium chloride in polystyrene Petri dishes (35 × 10 mm). For all isolates, the concentrations of KCl were used were 0.9, 1.0, 1.3, 1.4, 1.5, 1.7, 1.9, 2.1 and 2.4 M. The plates were incubated for 24 h at 26 °C.

Tolerance to UV radiation

To study the tolerances to UV radiation of conidia produced in the treatments: light, dark, and nutritive stress, conidia were exposed to UV radiation using the Xenon Test Chamber as described by Dias et al. (2018).

The conidial suspensions prepared as described earlier were inoculated (dropped, but not spread) on the center of the polyethylene plates (35 × 10 mm) containing 6 ml of PDA supplemented with benomyl 0.003% with 25% active ingredient (Hi-Yield Chemical Company, Bonham, TX). The plates were left open in the laminar flow for 30 minutes to dry the suspensions of conidia. After drying the suspensions they were exposed in a realistic test equipment for UV radiation using the Xenon Test Chamber QSUN XE-3-HC 340S (QLAB® Corporation, Westlake, OH USA) with a Daylight-Q filter. The plates were covered with a diacetate film to avoid desiccation of the medium. Petri plates containing the dried suspension of all isolates were exposed for 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200 and 210 minutes, equivalent to the irradiance of 3.5, 3.9, 4.3, 4.6, 5.0, 5.3, 5.7, 6.0, 6.4, 6.7, 7.1 and 7.5 kJ / m^2, respectively.

The Quaite weighted irradiance inside the chamber was 1335 mW m^{-2}. Spectral irradiance was measured as in (Dias et al., 2018; Rangel et al., 2004).

The DNA-damage (cyclobutane pyrimidine dimer formation) action spectrum developed by Quaite et al. (1992) and normalized to unity at 300 nm was used to calculate weighted UV irradiances in mW m^{-2}. Cellulose diacetate filters (JCS Industries, Le Miranda, CA) are used to exclude UV-C and short wavelength UV-B radiation. The irradiance and temperature of the equipment was adjusted according Dias et al. (2018).

The Petri dishes (one for each isolate) with the conidial suspension non exposed to UV radiation (control) were placed in the chamber for 210 minutes covered with aluminum foil.

The germinations were observed, depending on the experiment, at 24 h (Control plates) or at 48 h (UV-irradiated plates) after the conidial suspension was inoculated on the medium. The plates were kept at 26 °C in the dark for germination. The agar plate at the point of the inoculation was stained with a drop of Methyl Blue solution (Braga et al., 2002) and it was covered with a circular glass coverslip (15 mm diameter) to avoid air between the medium and the coverslip. The germination was examined under light microscope at 400× magnification. Conidia were considered germinated when the germ tube showed a white projection from the conidium. At least 300 conidia per plate were evaluated, and the percent of germination was calculated as described by Braga et al. (2001). All germinating or non-germinating conidia were counted on a single coverslip. The scanning pattern for counting was around the margin of the conidial suspension drop, which is an area commonly less populated by conidia. Each treatment was repeated at least four times with a fresh batch of conidia produced for each repetition.

Tolerance to 4-nitroquinoline-1-oxide (4-NQO)

To study the stress tolerances to mutagens of conidia produced in the treatments: light, dark, and nutritive stress, conidia were exposed to the mutagenic compound 4-nitroquinoline 1-oxide (4-NQO) according to (Araújo et al., 2018). The chemical compound 4-NQO is a potent carcinogen, and it has been used as a mutagen in bacteria and fungi for genetic studies on DNA damage and repair (Downes et al., 2014; Thomas et al., 1991; Yamamoto et al.,

1970). Also, 4-NQO causes microbial mutagenesis by DNA strand break and by producing charge-transfer adducts (Downes et al., 2014).

The conidial suspensions were prepared for 4-NQO exposure and conidia of each isolate were harvested as described earlier. The suspensions were inoculated (dropped, but not spread) on the center of the PDA medium (control 0 M) or PDA supplemented with potassium chloride in polystyrene Petri dishes (35 × 10 mm). Each Petri dishes contained 5 ml of medium. For all isolates, the concentrations of 4-NQO were used were 0.5, 0.7, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 μM. The plates were incubated for 24 h at 26 °C.

Tolerance to Congo red

A conidial suspension from 14-d-old cultures from each isolate was spot inoculated with 4 μl of the suspension to the center of PDAY medium in polystyrene Petri dishes (95 × 15 mm). The inoculated plates were incubated at 28 °C. For all three isolates, seven concentrations of Congo red were used: 0 (control), 50, 100, 200, 300, 400, 500, and 600 μg/ml, according to Nikolaou et al. (2009). The colony sizes were measured daily from the 5^{th} day to the 10^{th} day. The colony diameter (mm) was measured daily from the center of inoculation point towards the periphery of the colony in each plate. Two diameter measurements were made at right angles for each plate and then averaged. The measurement sites were done on the same axes each day. The experiment was repeated three times.

Tolerance to heat

To study the heat tolerances of conidia produced in the treatments: light, dark, and nutritive stress, the conidial suspensions were prepared for heat exposure, conidia of each isolate were harvested and suspended as described earlier and subjected to heat according to Rangel et al. (2005) and Souza et al. (2014).

The conidial suspensions were vigorously shaken and 2 ml of the suspension was transferred to screw cap Pyrex tubes (20 × 125 mm). The

tubes with the isolates suspensions were placed in a thermal bath for 0 (unexposed control), 2, 3, 4, and 5 h at 45 °C. After exposure to heat, 40 µl of the suspension was inoculated on the centre of a polystyrene Petri dish (60 × 15 mm) with 10 ml of PDA medium supplemented with 0.003% benomyl with 25% active ingredient (Hi-Yield Chemical Company, Bonham, TX), and conidial germination was evaluated after 24 (for the heat unexposed control) or 48 h (heat exposed fungus). The fungicide benomyl has been used in numerous studies to reduce the germination speed facilitating the germination counts after 24 and 48 h (Rangel et al., 2006). The percent germination was determined as described before. At least four independent repetitions were performed using a different culture for each repetition.

Conidial production

To measure conidial production under different treatment conditions, three agar plugs (per plate) were removed with a cork borer (5 mm diam) at places on the medium surface with an even coverage of conidia, and the conidia were suspended in 1 ml sterile Tween 80 (0.1 %) solution. After the conidial suspensions were vigorously shaken, the conidial concentrations were determined by haemocytometer counts. Each experiment was performed on three different dates, and each experiment used a new batch of cultures.

Statistical analyses

The effect on stress tolerance of conidia was assessed with analysis of variance of a one-way factorial. Significance levels of pair-wise mean comparisons among treatments were controlled for experiment-wise type I error using the Tukey method with overall $\alpha = 0.05$. All analyses were carried out with the statistical program Sisvar (Ferreira, 1999; Ferreira, 2011).

Results

Tolerance to osmotic stress

The isolate that demonstrated greatest tolerance to osmotic stress was *T. roseum* (ARSEF 1212) followed by *M. robertsii* (ARSEF 2575), these isolates had a behavior similar to 1.1 M KCl; however, *T. roseum* (ARSEF 1212) had a minimum inhibitory concentration of 3 M while *M. robertsii* (ARSEF 2575) had 1.8 M KCl. Being the lest tolerant, *M. acridum* (ARSEF 324) showed a minimum inhibitory concentration of 1.6 M KCl (Figure 2).

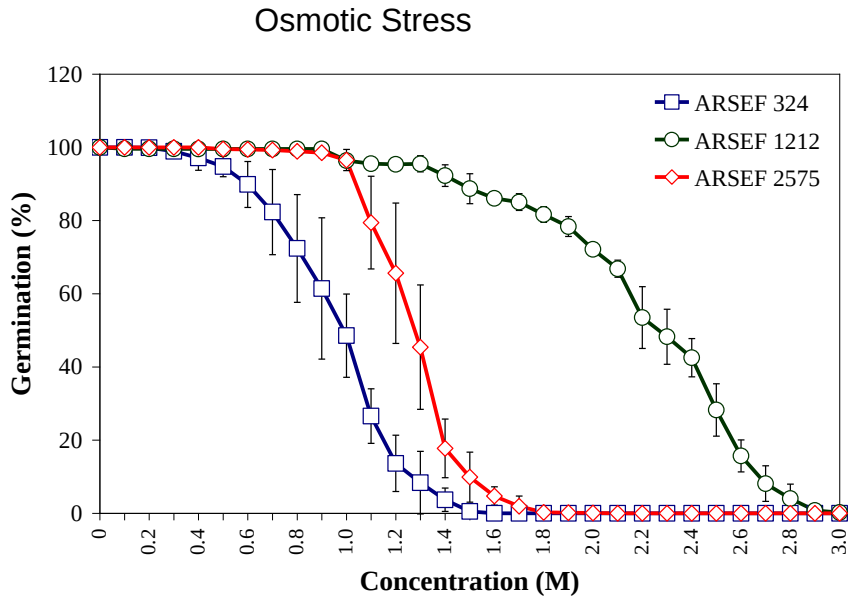

Figure 2 – Effect of KCl concentration on conidial germination of three entomopathogenic fungal species. The conidial germination was evaluated 24 h after inoculation on PDA (control) or PDA supplemented with KCl with 15 different concentrations from 0.2 to 3.0 M. The plates were incubated for 24 h at 26 ºC.

Tolerance to UV radiation

The mycelial germination of the three isolates was similar with 230 min of exposure to UV radiation. With 260 min of exposure, *M. acridum* (ARSEF 324) grew just over 80%, *T. roseum* (ARSEF 1212) grew about 70%, while *M. robertsii* (ARSEF 2575) grew less than 50%. When we analyzed 290 min of exposure, *M. acridum* (ARSEF 324) had about 65% germination, *T. roseum* (ARSEF 1212) grew about 30%, and the growth of *M. robertsii* (ARSEF 2575) reduced to about 10%. With 320 min of exposure, the isolates that grew were

M. acridum (ARSEF 324) and *T. roseum* (ARSEF 1212) with about 15% germination, *M. robertsii* (ARSEF 2575) did not exhibit any growth (Figure 3).

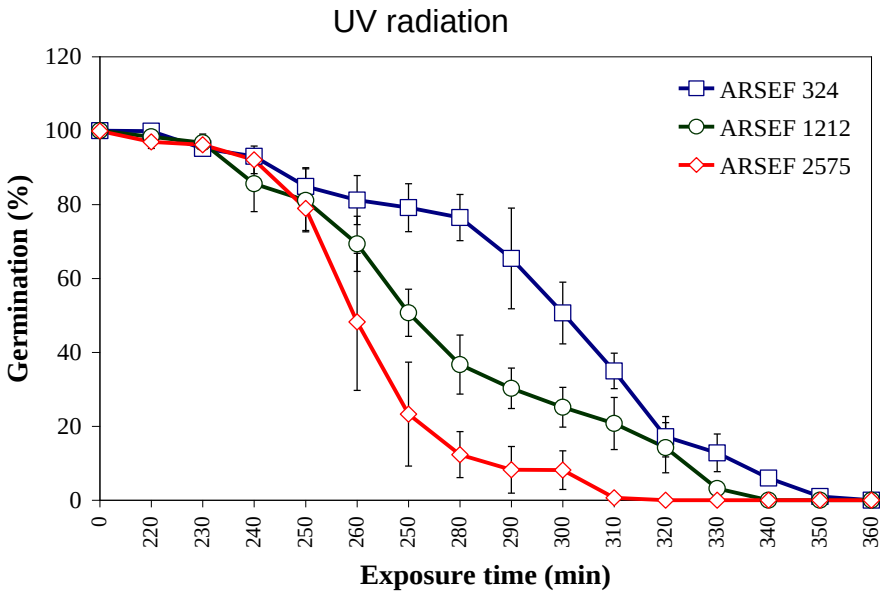

Figure 3 – Mean conidial germination of the three isolates *Metarhizium robertsii* (ARSEF 2575), *Metarhizium acridum* (ARSEF 324), and *Trichothecium roseum* (ARSEF 1212) after exposure to simulated full-spectrum solar radiation (QSUN). Petri plates containing the dried suspension of the isolates were exposed for to 0 (control), 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, and 360 min, equivalent to the irradiances of 0 (control), 17.62, 18.42, 19.22, 20.02, 20.82, 21.63, 22.43, 23.23, 24.03, 24.83, 25.63, 26.43, 27.23, 28.03, and 28.83 kJ m^{-2} , respectively. After the irradiation, the plates were maintained at 26 °C in the dark. Error bars are standard errors of at least three independente experiments with a fresh batch of conidia.

Tolerance to 4-nitroquinoline-1-oxide (4-NQO)

The germination of the three isolates exposed to the genotoxic agent 4-NQO was analyzed in 10 concentrations. At concentrations of 0.2 µM of 4-NQO, *T. roseum* (ARSEF 1212) achieved 100% germination, *M. robertsii* (ARSEF

2575) grew just under 100%, while *M. acridum* (ARSEF 324) was the least tolerant with less than 40% germination.

At concentrations of 0.5 µM, *T. roseum* (ARSEF 1212) exhibited 100% germination, *M. robertsii* (ARSEF 2575) grew less than 80%, while *M. acridum* (ARSEF 324) was the least tolerant with a germination just over 20%. At concentrations of 1.0 µM, *T. roseum* (ARSEF 1212) was more tolerant with growth of approximately 80%, *M. robertsii* (ARSEF 2575) grew about 75%, *M. acridum* (ARSEF 324) was the less tolerant with only 15% growth.

In higher concentrations such as 2.5 µM, *T. roseum* (ARSEF 1212) was more tolerant with a germination of 35%, *M. robertsii* (ARSEF 2575) grew less than 5%, and *M. acridum* (ARSEF 324) did not achieve any growth (Figure 4).

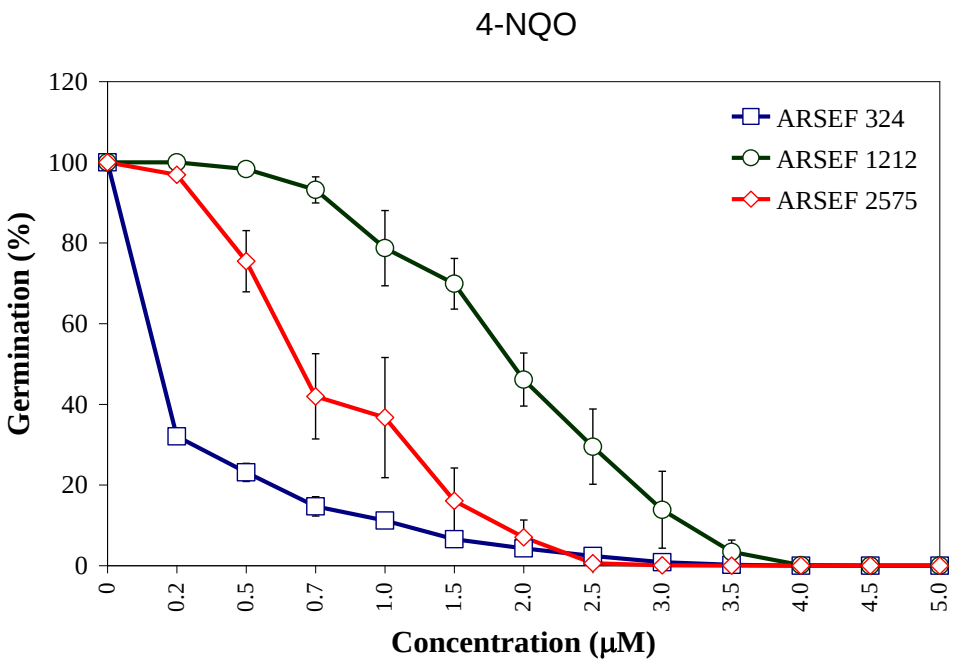

Figure 4 – Mean conidial germination of three species of entomopathogenic fungi. The conidial germination was evaluated 24 h after inoculation on PDA (control) or PDA supplemented with 4-nitroquinoline 1-oxide (4-NQO) with 10 different concentrations of 0.2; 0.5; 0.7; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; and 4.0 µM 4-NQO. The plates were maintained at 26 °C in the dark. Error bars are standard errors of at least four independent experiments with a fresh batch of conidia produced for each repetition.

Tolerance to Congo red

The mycelial growth of the three isolates was inhibited in the presence of Congo red, but *T. roseum* was the most tolerant of the three isolates. *T. roseum* (ARSEF 1212) grew about 38 mm, 30 mm, and 25 mm at concentrations of 50 μg/μl, 100 μg/μl, and 300 μg/μl, respectively. The *M. robertsii* isolate (ARSEF 2575) grew about 40 mm, 21 mm, and 22 mm at concentrations of 50 μg/μl, 200 μg/μl, and 300 μg/μl, respectively. The isolate *M. acridum* (ARSEF 324) exhibited sensitivity at concentrations greater than 100 and 200 μg/μl, with growth of 20 mm and 10 mm, respectively. At concentrations of 300 μg/μl, the *T. roseum* (ARSEF 1212) and *M. robertsii* (ARSEF 2575) isolates displayed similar behaviors (Figure 5).

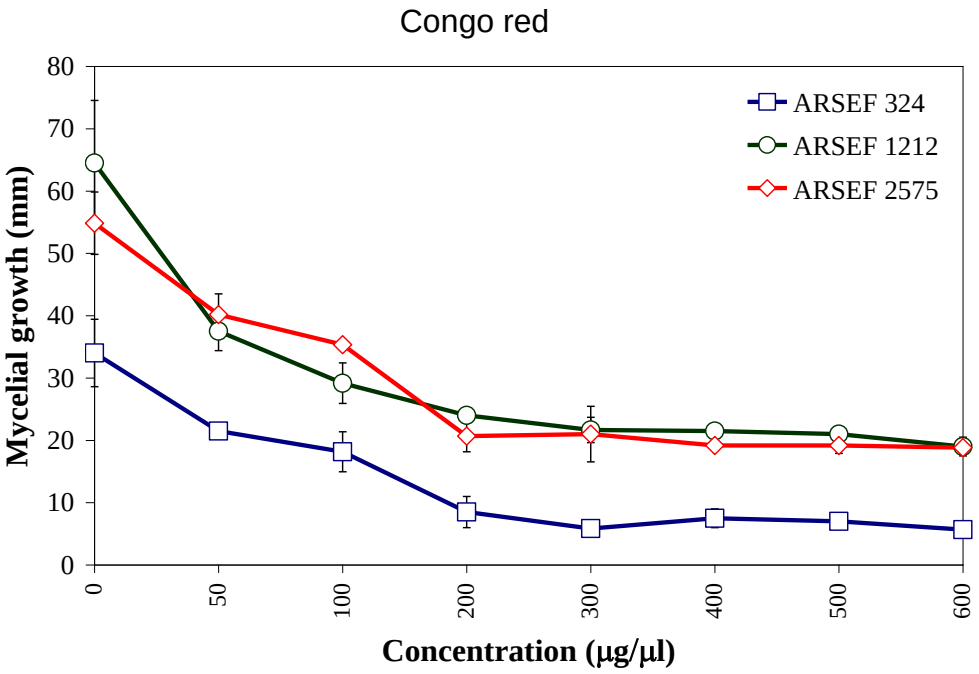

Figure 5 – Effect of Congo red on mycelial growth of 3 entomopathogenic fungi. The inoculated plates were incubated at 28 °C. For all three isolates, seven concentrations of Congo red were used: 0 (control), 50, 100, 200, 300, 400, 500, and 600 μg/ml, Error bars are standard errors of three trials. The experiment was repeated three times.

Tolerance to heat

In our study of thermal tolerance, with 1 h of exposure, the isolate *M. acridum* (ARSEF 324) achieved 100% growth, the isolate *M. robertsii* (ARSEF 2575) germinated 60% and *T. roseum* (ARSEF 1212) about 35%. With 2 h of

exposure, the isolates ARSEF 324, ARSEF 2575, and ARSEF 1212 germinated 90%, 20%, and 10% respectively. With 3 h of exposure the same isolates germinated 59%, 9%, and 10%, respectively. The ARSEF 1212 isolate remained viable until 4 hours of exposure, while ARSEF 2575 was almost unviable after 5 h. Therefore, *M. acridum* (ARSEF 324) is the most tolerant to heat, followed by *M. robertsii* (ARSEF 2575), and finally *T. roseum* (ARSEF 1212) (Figure 6).

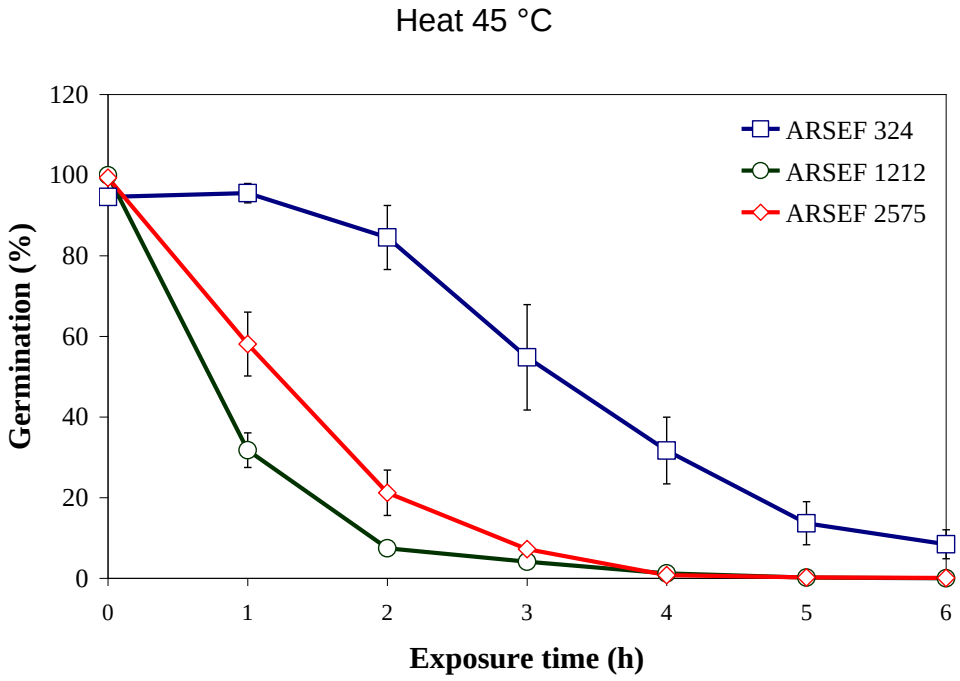

Figure 6 – Effect of the heat on germination of 3 entomopathogenic fungi *Metarhizium robertsii* (ARSEF 2575), *Metarhizium acridum* (ARSEF 324), and *Trichothecium roseum* (ARSEF 1212) after 1, 2, 4, 5 and 6h at temperature 45 °C. Error bars are standard errors of four trials.

Discussion

In this study, the fungus *Trichothecium roseum* (ARSEF 1212) exhibited greater tolerance to osmotic stress, the mutagenic 4-nitroquinoline 1-oxide (4-NQO), and Congo red than the studied isolates of *Metarhizium acridum*

(ARSEF 324) and *Metarhizium robertsii* (ARSEF 2575). However, when exposed to UV radiation, *T. roseum* presented moderate tolerance, and it was the least tolerant to heat.

In this study, *T. roseum* (ARSEF 1212) had the higher tolerance to osmotic stress, than the studied *Metarhizium* species. *M. robertsii* (ARSEF 2575) presented moderate tolerance and *M. acridum* (ARSEF 324) was osmosensitive.

Controlling changes in water balance is an important stimulus for fungi and hyperosmotic shock has been applied through the addition of salts such as sodium chloride (NaCl) and sorbitol (Brown et al., 2017). The solute used in our studies, KCl, was the same applied to induce osmotic stress by Araújo et. al., (2016), and they also studied *T. roseum*. Because of this solute, the fungus undergoes an occasional loss of pressure from the intracellular turgor, which is fundamental for its development, thus causing the concentration of intracellular osmolites, such as glycerol (Brown et al., 2017; Duran, Cary; Calvo 2010). The high tolerance of *T. roseum* to osmotic stress is associated with its high xerophilic capacity among entomopathogenic fungi (Araújo et al., 2019), because xerophilic fungi grow under conditions of reduced water activity (Vinnere Pettersson; Leong 2011). Among *Metarhizium* species, *M. robertsii* is more tolerant than *M. acridum*, corroborating the osmotolerance studies by Araújo et al., (2019). *M. robertsii* has the ability to act against cellular responses to high osmotic pressure (Lovett and St. Leger, 2015).

In the UV radiation tolerance experiment, *T. roseum* (ARSEF 1212) was less tolerant than *M. acridum* (ARSEF 324), and more tolerant than *M. robertsii* (ARSEF 2575). Differences between *Metarhizium* species have been found for tolerances to UV radiation (Braga et al., 2001, Dias et al., 2018). *M. acridum* is highly resistant to UV radiation (Azevedo et al., 2014; Braga et al., 2001; Dias et al. 2018) and has more trehalose and mannitol in its conidia, which provides greater resistance to UV radiation and heat than *M. robertsii* (Rangel et al., 2005, 2008, 2015).

In our study, *T. roseum* (ARSEF 1212) exhibited the most tolerance to the mutagenic 4-nitroquinoline 1-oxide (4-NQO). This agent is used to reproduce the biological effects of UV radiation on fungal organisms to study whether it would be a predictor of tolerance to UV radiation (Araújo et al., 2018).

However, in this study, *T. roseum* was sensitive to UV radiation, without correlation between tolerance to 4-NQO and tolerance to UV radiation. *M. robertsii* (ARSEF 2575) was presented moderate tolerance, whereas *M. acridum* (ARSEF 324) showed reduced tolerance to 4-NQO, wich in other studies it also showed lower tolerances to the fungicide dodine (Araújo et al., 2018, Azevedo et al., 2014) and other clinical antifungal agents (Bracini et al., 2018).

In our study, the best growth in stress for Congo red was the *T. roseum* isolate (ARSEF 1212), suggesting greater tolerance among the studied isolates. *M. robertsii* (ARSEF 2575) was considered moderately tolerant, the least tolerant was *M. acridum* (ARSEF 324) with a lower growth than the other isolates. Congo red causes damage to the cell wall of fungi when sublethal concentrations induce morphological changes, such as weakening of the cell wall (Ram and Klis, 2006; Pancaldi et al., 1984; Vannini et al., 1983). Its inhibitory capacity in chitin synthesis has been described (Bartnicki-Garcia et al. 1994), and studies have mentioned this effect also in chitin-binding enzymes to β-1,3-glucan (Ram and Klis 2006) interposing in the cell wall originating a chitin and β-1,3-glucan complex (Kopecká and Gabriel, 1992; Ogawa et al., 1994; Pancaldi et al., 1984; Vannini et al., 1983). Studies on chitinases from *T. roseum* are limited (Xian et al., 2012).

In the heat tolerance study, our results were different from those of Rangel et. al., (2006), which when analyzing *Metarhizium* species found results for the isolate ARSEF 2575 with a tolerance of approximately 50% after 2 h of exposure to 45 °C, and a tolerance of the isolate ARSEF 324 after 8 h of exposure at the same temperature. This very low biological activity may have been caused by a cross reaction due to an indirect exposure of UV-C lamps present in the laboratory.

Conclusion

When *T. roseum* (ARSEF 1212), *M. robertsii* (ARSEF 2575), and *M. acridum* (ARSEF 324) are challenged with different stress conditions, *T. roseum* (ARSEF 1212) displayed the more tolerance than others in situations of osmotic stress due to KCl, stress to the 4-NQO, and stress to the Congo

Red. However, under the conditions of UV radiation, the *Metarhizium* species were more tolerant. In summary, we conclude that the fungus *T. roseum* is a promising agent in the biological control of insects.

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Different wavelengths of light influence the conidial production and stress tolerance of the plant pathogens *Colletotrichum acutatum* and *Fusarium fujikuroi*

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Abstract

The effects of the visible light wavelengths on germination, mycelial radial growth, and conidial production of the plant pathogens *Colletotrichum acutatum* and *Fusarium fujikuroi* were studied. Both fungi were grown on potato dextrose agar medium (PDA) in the dark (control) or on PDA under continuous white, blue, green or red light. In addition, the conidia from each treatment were exposed to UV radiation. The germination and growth of both plant pathogenic fungi were not affected by any of the treatments. *C. acutatum* produced more conidia when the fungus grew under white and red light. *F. fujikuroi* produced more conidia in the dark. The tolerances to UV radiation of conidia produced on different light and dark treatments differed for both *C. acutatum* and *F. fujikuroi*. Conidia of *C. acutatum* were at least 30% more tolerant to UV radiation when

they were produced under white light than under blue and green light and at least 20% more tolerant than conidia produced in the dark. Conidia of *C. acutatum* produced under red light were the least tolerant. Conidia of *F. fujikuroi* produced under white and blue light were at least 30% more UV tolerant than conidia produced in the dark, green, and red light. In conclusion, no differences were found for germination and growth for both fungi under different light regimes and dark; however, significant differences occurred both in production and UV radiation of conidia.

Key Words: photobiology; stress tolerance; UV radiation; germination; mycelial growth

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Introduction

Most organisms on Earth sense visible light (380 to 740 nm) through the use of photoreceptive proteins specifically adapted to respond to it (Dasgupta et al. 2015). The response of fungi are reflected in different physiological responses such as conidial production (Yu et al. 2013; de Menezes et al. 2015), conidial stress tolerance (Idnurm and Heitman 2005; Rangel et al. 2011; Fuller et al. 2013; Aver'yanov et al. 2014; de Menezes et al. 2015; Rangel et al. 2015; Dias et al. 2019), conidial pigmentation (Fuller et al. 2013; Yu et al. 2013), virulence (Yu et al. 2013; Aver'yanov et al. 2014; Oliveira et al. 2018), germination speed (Fuller et al. 2013; Oliveira et al. 2018), and secondary metabolism (Tisch and Schmoll 2010; Fanelli et al. 2012). Fungal molecular biology studies have identified several genes that encode proteins which are involved in the detection of visible light, and have investigated the mechanisms that activate physiological and morphological responses (Chen et al. 2009; Fuller et al. 2016). Most fungi perceive and respond to blue light, and some

fungi can perceive and respond to wavelengths of light in the red, green, and ultraviolet regions of the spectrum (Idnurm et al. 2010; Yu and Fischer 2019). Blue light photoreceptors are White Collar 1 (WC-1 and homologous proteins), cryptochromes and photolyases. Red and green wavelengths are sensed by phytochromes and opsins, respectively (Idnurm et al. 2010).

The insect-pathogenic fungi *Metarhizium robertsii* and *M. acridum* grown under visible light up-regulate many stress-related genes (Brancini et al. 2019; Dias et al. 2019). In turn, the corresponding changes in transcription and protein accumulation play a role in tolerance to stress by inducing higher conidial tolerance to osmotic stress, UV-radiation, and heat (Rangel et al. 2011; Rangel et al. 2015; Dias et al. 2019). In addition, *M. robertsii* conidia produced under white light germinate faster and are more virulent than conidia produced in the dark (Oliveira et al. 2018). In the fungus *Botrytis cinerea,* the White Collar Complex (WCC) is required for coping with excessive light and oxidative stress, as well as achieving full virulence (Canessa et al. 2013). This physiological outcome suggested the possibility that individual components of white light can also produce conidia with increased stress tolerance in other fungal species.

Conidial production is greatly regulated by visible light, but not all fungal species, and even different isolates from the same species, respond in the same way to visible light. *Paecilomyces fumosoroseus* (currently *Isaria fumosorosea*) produces the most conidia under white and blue light, less conidia under green light, and the least conidia under red light (Sanchez-Murillo et al. 2004). *Aspergillus nidulans* produces more conidia under the white light than in the dark (Atoui et al. 2010). *Alternaria solani* produces more conidia in the far-red, followed by red light and in the dark. Growth of *A. alternata* under blue, white and green light produces very few conidia (Igbalajobi et al. 2019). *Beauveria bassiana* produces more conidia under white and blue light and less conidia under green, purple, yellow and red light, and darkness (Zhang et al. 2009). *Metarhizium robertsii* produces more conidia under blue light than under red light and darkness (Oliveira et al. 2018). *Neurospora crassa* produces four-fold more conidia under the visible light than in the dark (Lauter et al. 1997).

Studying the effect of radiation on the development of plant pathogenic fungi is important to discover ways to control them in the environment. For example, *Alternaria*, *Botrytis*, and *Stemphylium* can be controlled by eliminating

certain radiation wavelengths and they sporulate only when they receive radiation in the ultraviolet (UV) range below 360 nm (Agrios 2005). Diseases of greenhouse vegetables caused by several species of plant pathogenic fungi can be controlled by covering or constructing the greenhouse with a special UV-absorbing vinyl film that blocks the transmission of wavelengths below 390 nm (Agrios 2005).

Therefore, our objective was to study the effects of different radiation wavelengths on the physiology of plant pathogens *Colletotrichum acutatum*, the causing agent of fruit rots as well as shoot, leaf, and flower blights (Agrios 2005), and *Fusarium fujikuroi*, the causal agent of the bakanae disease of rice (Hossain et al. 2016).

The conidial production and germination as well as the mycelial radial growth of colonies and tolerance to UV radiation of these fungal species in different radiation wavelengths were studied.

Materials and methods

Fungal isolates

The *Colletotrichum acutatum*, isolate FDC 52, was provided by Gilberto U.L. Braga, Universidade de São Paulo. This fungus was isolated from orange plants by Fundecitrus in Taquarituba, SP, Brazil. This isolate is deposited in the Fundecitrus Collection.

The *Fusarium fujikuroi*, isolate FKMC 1995, was provided by Javier Avalos, Universidad de Sevilla, Spain. This fungus was isolated from rice in Taiwan and deposited in the Kansas State University Collection.

Stock cultures were maintained at 4 °C in test tubes on slants of potato dextrose agar (Difco Laboratories, Sparks, MD, USA) adjusted to pH 6.9.

Conidial production and harvesting

Conidia of *C. acutatum* and *F. fujikuroi* were produced on 23 ml of potato dextrose agar (Difco, Sparks, MD, USA) (PDAY) in 95 mm polystyrene Petri dishes in the dark. The pH was adjusted to 6.9 by using NaOH (1 M). A conidial suspension (100 μl of 10^7 conidia ml^{-1}) was inoculated evenly with a glass

spreader onto agar media. The cultures were incubated at 26 ± 1 °C and approximately 90% relative humidity (RH) for 14 days. Three different batches of conidia were produced, one for each replication of each type of stress experiment.

Light treatments

For the white light treatment, the Petri dishes with cultures on PDA medium with lids in place, in a single layer (not stacked) were maintained under continuous light provided by two 15 W cool white Philips (TL-D 15 W/75-650) broad-spectrum fluorescent light bulbs suspended at a distance of 25 cm above the samples. A sheet of 0.13 mm cellulose diacetate covered the plates to avoid medium dehydration. The integrated irradiance of the lamps that passed through the diacetate film and Petri dish lid was 5.0 W m^{-2} and 2230 lux (Figure 7A).

For blue, green, and red light treatments, the Petri dishes with cultures on PDA medium with lids in place, in a single layer (not stacked), were maintained under continuous blue, green or red light provided by three incubators that were adjusted to enable incubation of the cultures under different wavelengths of light. Incubator 1 contained four Color Led LLUM® E27 5W light bulbs (Jinli Lighting Co., China), set in the blue wavelength providing a maximum output of integrated irradiance of 4.8 W m^{-2} and 645.5 lux. Incubator 2 contained four Color Led LLUM® E27 5W light bulbs set in the green wavelength providing a maximum output of integrated irradiance of 2.2 W m^{-2} and 2602 lux. Incubator 3 contained four Color Led LLUM® E27 5W light bulbs set in the red wavelength providing a maximum output of integrated irradiance of 2.8 W m^{-2} and 53 lux. The permitted distance of the incubator between the LEDs and the agar plates was 6 cm and the temperature of the incubators were adjusted to 26 °C, and no heating effect by the LEDs were detected (Figure 7B).

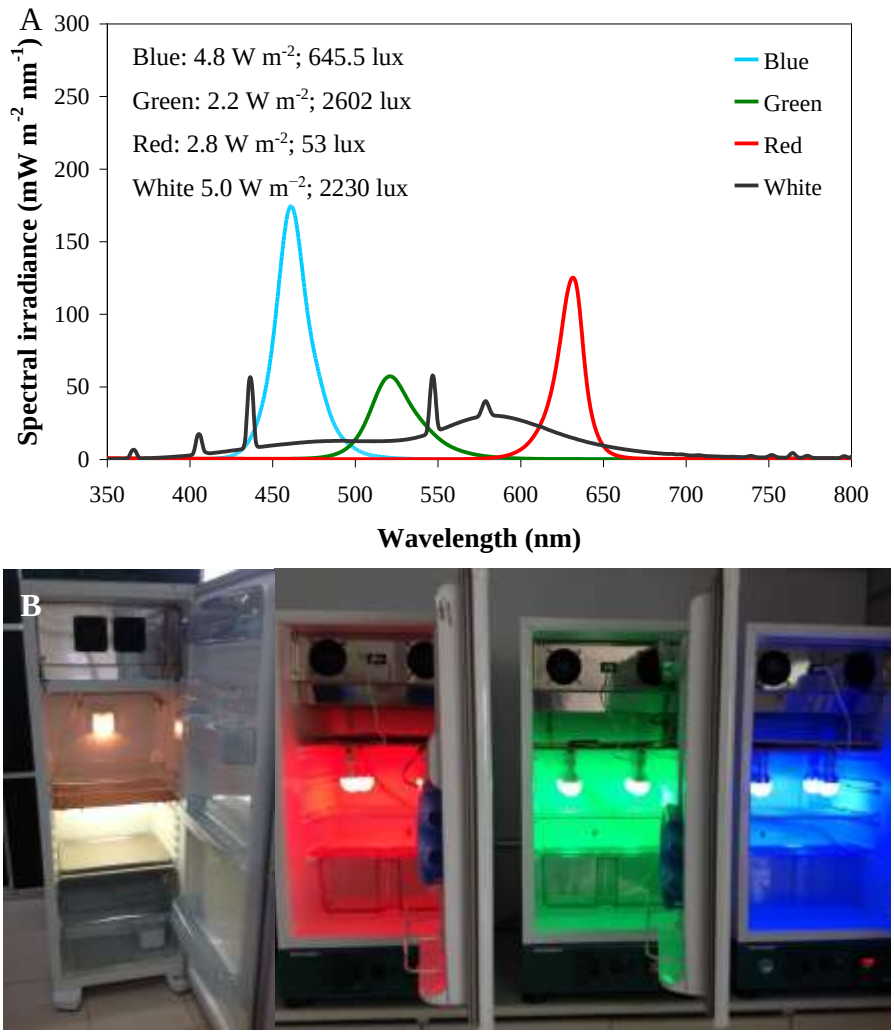

Figure 7 – (A) Spectral irradiances of the lamp setups used. For white light treatment, the Petri dishes with cultures on PDA medium with lids in place, in a single layer (not stacked) were maintained under continuous light provided by two 15 W cool white Philips (TL-D 15 W/75-650) broad-spectrum fluorescent light bulbs suspended at a distance of 25 cm above the samples. A sheet of 0.13-mm cellulose diacetate covered the plates to avoid medium dehydration. The integrated irradiance of the lamps that passed through the diacetate film plus the Petri dish lid was 4.98 W m^{-2} and 2230 lux. For blue, green, or red light treatment, the Petri dishes with cultures on PDA medium with lids in place, in a single layer (not stacked) with LED light provided by four Color Led LLUM® E27 5W (Jinli Lighting Co., China) bulbs each in three incubators. No heating effect by the LEDs was detected. (B) Incubators with white, red, green, and blue light irradiances.
Fonte: Autoria própria.

The spectral irradiances (in W m^{-2}) of the incubators were measured with a spectroradiometer Ocean Optics (Dunedin, FL, USA) Model USB2000 + Rad connected to a laptop (Figure 7) and the illuminance (in lux) of the incubators were measured with a Onset HOBO® data logger U12-012.

For dark treatments, all Petri dishes were maintained in the same incubator as the light treatment, but the Petri dishes were kept inside a perforated plastic box (to keep the cultures ventilated) and covered with a thick black cloth sleeve. Temperature and radiation inside the dark treatments were measured continuously using an Onset HOBO® data logger U12-012.

Conidial germination

Conidia from cultures grown for 14 days in PDA culture medium were collected with the aid of a microbiological loop and were suspended in 10 ml of sterile Tween 80 solution (0.01% v/v) in glass tubes. The suspensions were adjusted to a concentration of 10^5 conidia ml^{-1} and then stirred.

The conidial suspensions (40 μl) were inoculated in the center of the polyethylene Petri dishes (35 × 10 mm Greiner Bio-One) containing 6 ml of PDA. After inoculation, the plates were kept at 26 °C in the treatments: dark (control), white, blue, green or red light. Germination of *C. acutatum* and *F. fujikuroi* was evaluated for 2, 4, 6, 8, 10, 12, 14, and 16 h according to Rangel et al. (2004). After the incubation period, the conidia were stained with methylene blue solution (Braga et al. 2002) and germination was evaluated with 400 × magnification. Conidia were considered germinated when a projected germ tube from the conidia was visible (Milner et al. 1991). At least 300 conidia per plate were counted and the germination percentage was calculated. Conidia from both isolates and from all treatments were tested at the same time for each repetition. Three repetitions were done.

Mycelial radial growth

From the colonies of *C. acutatum* and *F. fujikuroi* grown on PDA medium in Petri dishes (90 × 15 mm), one disk of 5 mm diameter was removed with a

cork borer and inoculated in the center of Petri dish containing 23 ml of PDA. After inoculation, the plates were kept at 26 °C for the treatments: dark (control), and white, blue, green or red light. The fungi *C. acutatum* and *F. fujikuroi* were grown for 5 days. Colony diameter of mycelial growth was measured on the fifth day horizontally and vertically (at a perpendicular axis). For each treatment, three Petri dish replicates were prepared, and three repetitions were performed on different days.

Measurement of conidial production

To measure conidial production under different treatment conditions, three agar plugs (per plate) were removed with a cork borer (5 mm diam) at different places on the medium surface with an even coverage of conidia, and the conidia were suspended in 1 ml sterile Tween 80 (0.1 %) solution. After the conidial suspensions were vigorously shaken, the conidial concentrations were determined by hemocytometer counts. Each experiment was performed on three different dates, and each experiment used a new batch of cultures.

Conidial tolerance to UV radiation

Conidia from PDA medium from all four light treatments and the dark treatment were collected after 14 days of growth with a few passes of a microbiological loop (Decon Labs, Inc., PA, USA) and transferred to 10 ml of sterile Tween 80 (0.01 % v/v). The amount of conidia collected were enough to produce a suspension of 10^5 conidia ml^{-1}. The suspensions (1×10^5 conidia ml^{-1}) were shaken vigorously using a vortex, and 40 µl were inoculated (dropped, but not spread) on the center of the medium (polyethylene plates 35 × 10 mm) containing 6 ml of PDA supplemented with benomyl 0.003% with 25% active ingredient (Hi-Yield Chemical Company, Bonham, TX, USA) (Milner et al. 1991). All suspensions were exposed to UV radiation (290–400 nm) immediately after preparation (Dias et al. 2018).

The plates were left open in laminar flow for 30 min to dry the conidial suspensions (Dias et al. 2018). After drying, the suspensions they were

exposed in a realistic test equipment for UV radiation using the Xenon Test Chamber QSUN XE-3-HC 340S (QLAB® Corporation, Westlake, OH, USA) with a Daylight-Q filter. The plates were covered with a diacetate film to avoid desiccation of the medium. Petri plates containing the dried suspension of all isolates were exposed for 160, 170, 180, 190, 200 and 210 minutes, equivalent to the irradiance of 5.7, 6.0, 6.4, 6.7, 7.1 and 7.5 kJ/m^2, respectively. The exposure time and irradiances were used following previously published methods (Dias et al. 2018; Dias et al. 2020). The irradiance and temperature of the equipment was adjusted according to Dias et al. (2018).

The Quaite weighted irradiance inside the chamber was 1335 mW m^{-2}. Spectral irradiance was measured as in Dias et al. (2018). The DNA-damage (cyclobutane pyrimidine dimer formation) action spectrum developed by Quaite et al. (1992) and normalized to unity at 300 nm was used to calculate weighted UV irradiances in mW m^{-2}. Cellulose diacetate filters (JCS Industries, Le Miranda, CA, USA) are employed to exclude UV-C (230–280 nm) and short wavelength UV-B radiation (280-290 nm) (Rangel et al. 2006; Dias et al. 2018).

For the control, Petri dishes (one for each isolate and treatment) with the conidial suspension not exposed to UV radiation were placed in the chamber for 210 min covered with aluminum foil (Dias et al. 2018).

The germinations were observed, depending on the experiment, at 24 h (control plates) or at 48 h (UV-irradiated plates) after the conidial suspension was inoculated on the medium. The plates were kept at 26 °C in the dark for germination. The agar plate at the point of the inoculation was stained with a drop of Methyl Blue solution (Braga et al. 2002) and then covered with a circular glass coverslip (15 mm diameter) to avoid air between the medium and the coverslip. The germination was examined under light microscope at 400× magnification. Conidia were considered germinated when the germ tube showed a visible projection from the conidium. At least 300 conidia per plate were evaluated, and the percent of germination was calculated. All germinating or non-germinating conidia were counted on a single coverslip. The scanning pattern for counting was around the margin of the conidial suspension drop, which is an area commonly less populated by conidia. Each treatment was repeated at least four times with a fresh batch of conidia produced for each repetition.

Statistical analyses

The effect of white, blue, green or red wavelengths of radiation on germination, mycelial growth, conidial production, and conidial tolerance to UV irradiation was assessed with analysis of variance of a one-way factorial. Significance levels of pair-wise mean comparisons among treatments were controlled for experiment-wise type I error using the Tukey method with overall α = 0.05. All analyses were carried out with the statistical program Sisvar (Ferreira 1999; Ferreira 2011).

Results

Conidial germination

Continuous white, blue, green or red light did not affect the germination speed, compared to darkness for *C. acutatum* and *F. fujikuroi* conidia germinated on potato dextrose agar medium (Figure 8).

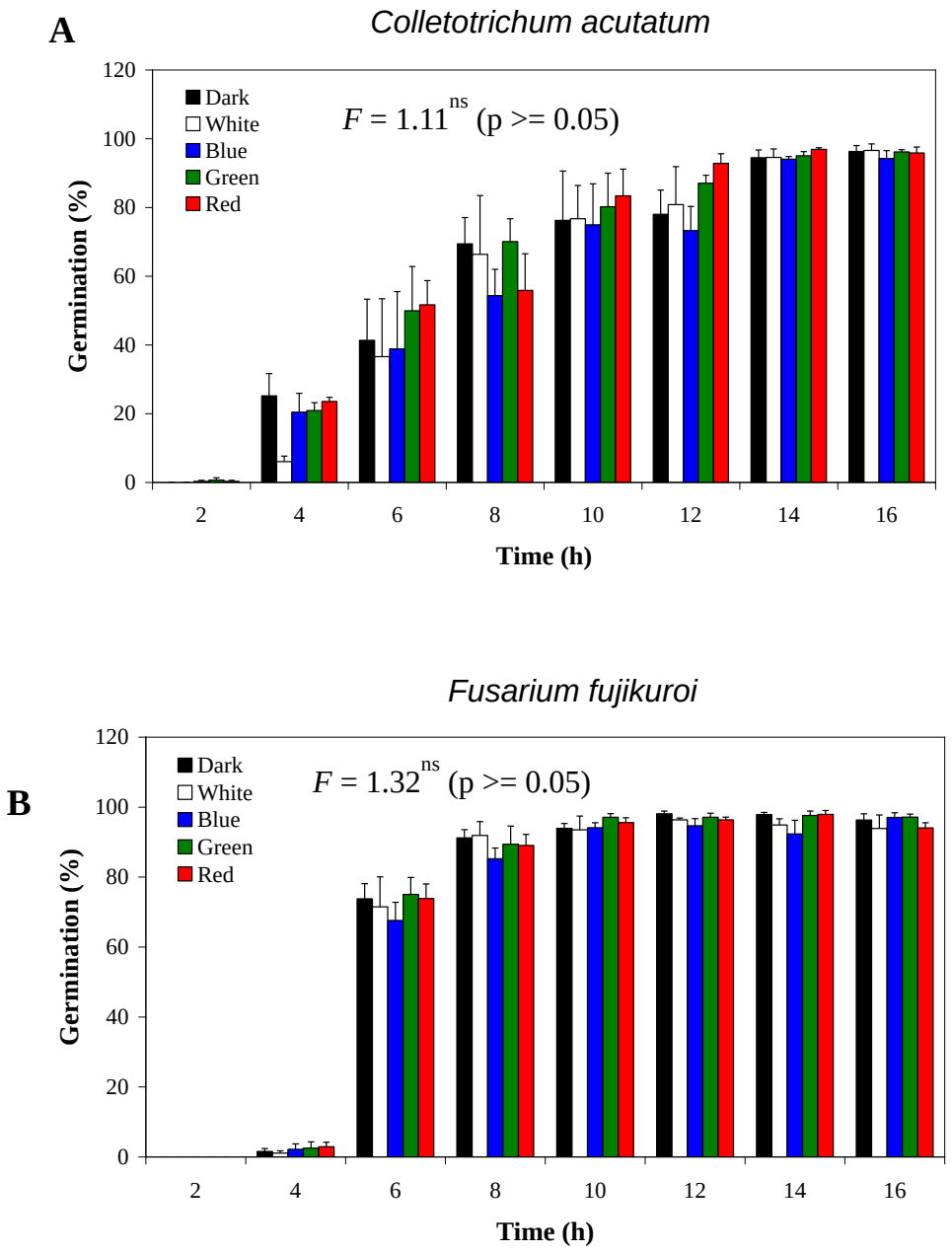

Figure 8 – Germination of (A) *Colletotrichum acutatum* and (B) *Fusarium fujikuroi* conidia in the treatments: dark (control), white, blue, green, and red light at 26 °C. Bar errors represent the standard deviation of at least three independent experiments at different times.

Mycelial radial growth

No statistical differences in mycelial growth were found for *C. acutatum* and *F. fujikuroi* grown on potato dextrose agar medium in the dark or under continuous white, blue, green or red light (Figure 9).

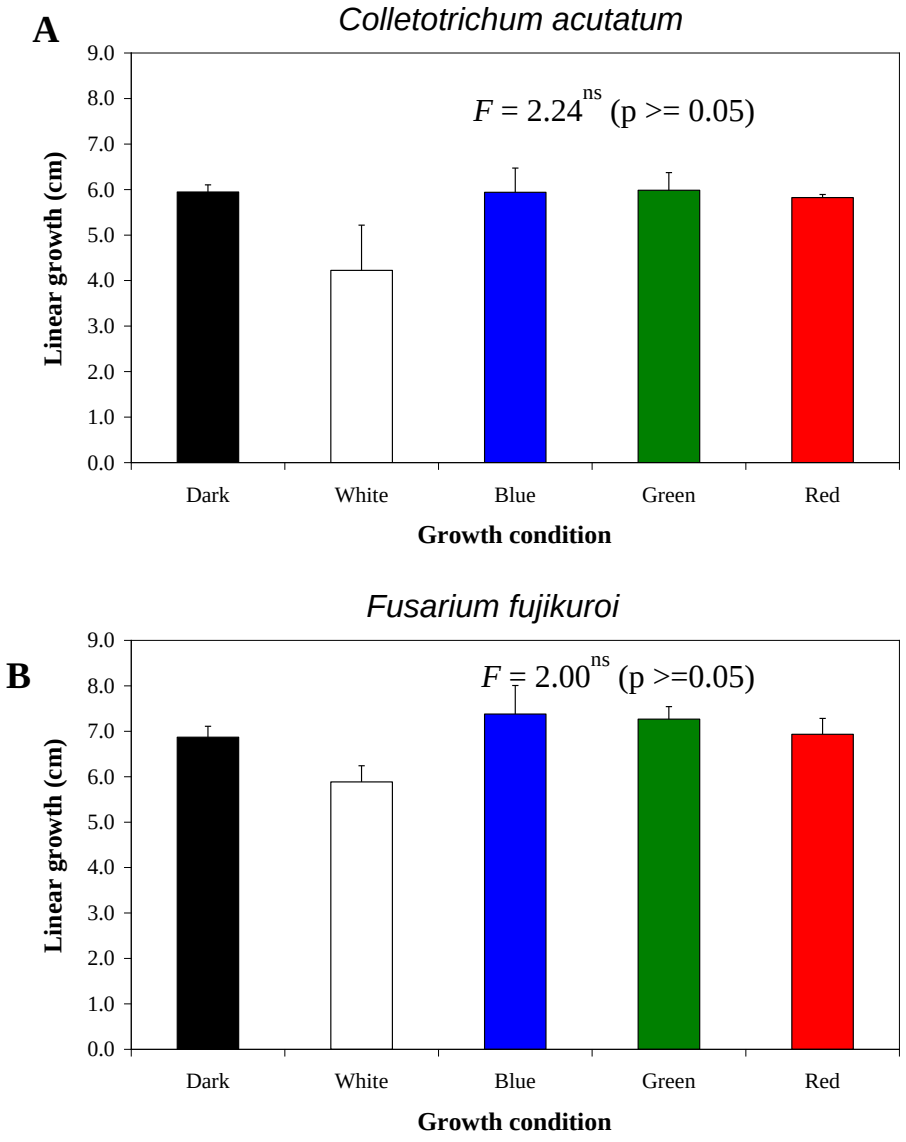

Figure 9 – Radial growth of (A) *Colletotrichum acutatum* and (B) *Fusarium fujikuroi* colonies in the treatments: dark (control), white, blue, green, and red light at 26 °C. Conidia were point inoculated at the center of the plate containing PDA medium. The plates were kept at 26 °C for five days. Error bars represent the standard deviation of the average of three repetitions.

Conidial production

C. acutatum produced more conidia when the fungus was grown under white and red light (Figure 10A). However, growth under the blue and green light produced 76.2 and 64.7% less conidia than the white light treatment.

Mycelial growth in the dark produced 42.1% less conidia than white light treatment (Figure 10A).

F. fujikuroi produced more conidia when grown in the dark than all light treatments. Growth under blue and green light produced 45.2 and 45.8% less conidia than growth in the dark (Figure 10B). Growth under red light produced 57.9% less conidia than growth in the dark. Growth under white light produced 77.7% less conidia than growth in the dark (Figure 10B).

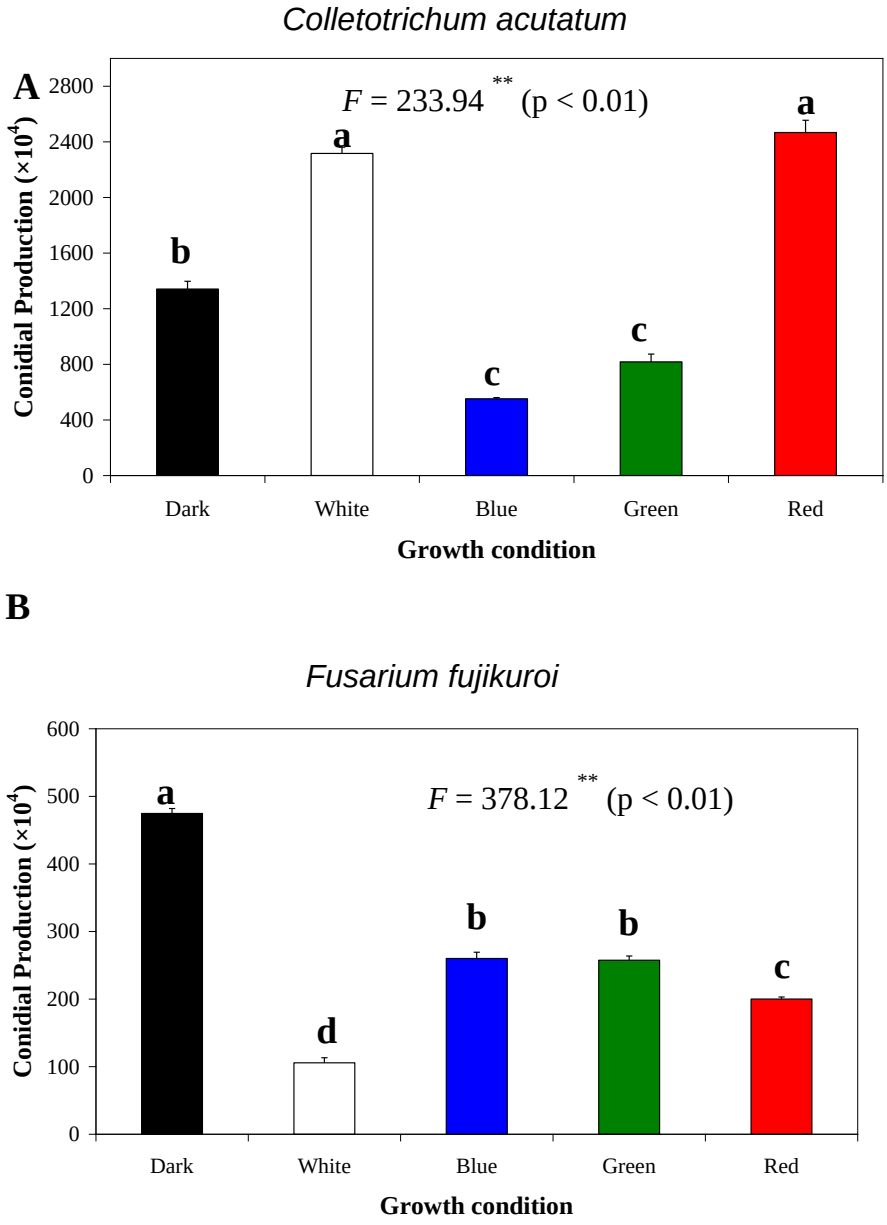

Figure 10 – Conidial production of (A) *Colletotrichum acutatum* and (B) *Fusarium fujikuroi* in the treatments: dark (control), white, blue, green, and red light. Error bars represent the standard deviation of the mean of three

repetitions performed with independent experiments. Graph bars with the same letter are not significantly different ($p < 0.01$).

Conidial tolerance to UV radiation

For *C. acutatum*, conidia produced under white light were significantly more tolerant to UV radiation than conidia produced under blue, green, red light and in the dark (Figure 11A). Conidia produced under white light and in the dark had similar tolerance to UV radiation. However, at the higher exposure time of 220 min, conidia produced under white light were more tolerant than conidia produced in the dark. Conidia produced under red light displayed the least tolerant to UV radiation (Figure 11A).

F. fujikuroi conidia produced under blue light were most tolerant to UV radiation, and statistically similar to the tolerance of conidia produced under white light. On the other hand, growth under green and red light produced less tolerant conidia and similar tolerance as conidia produced in the dark (Figure 11B).

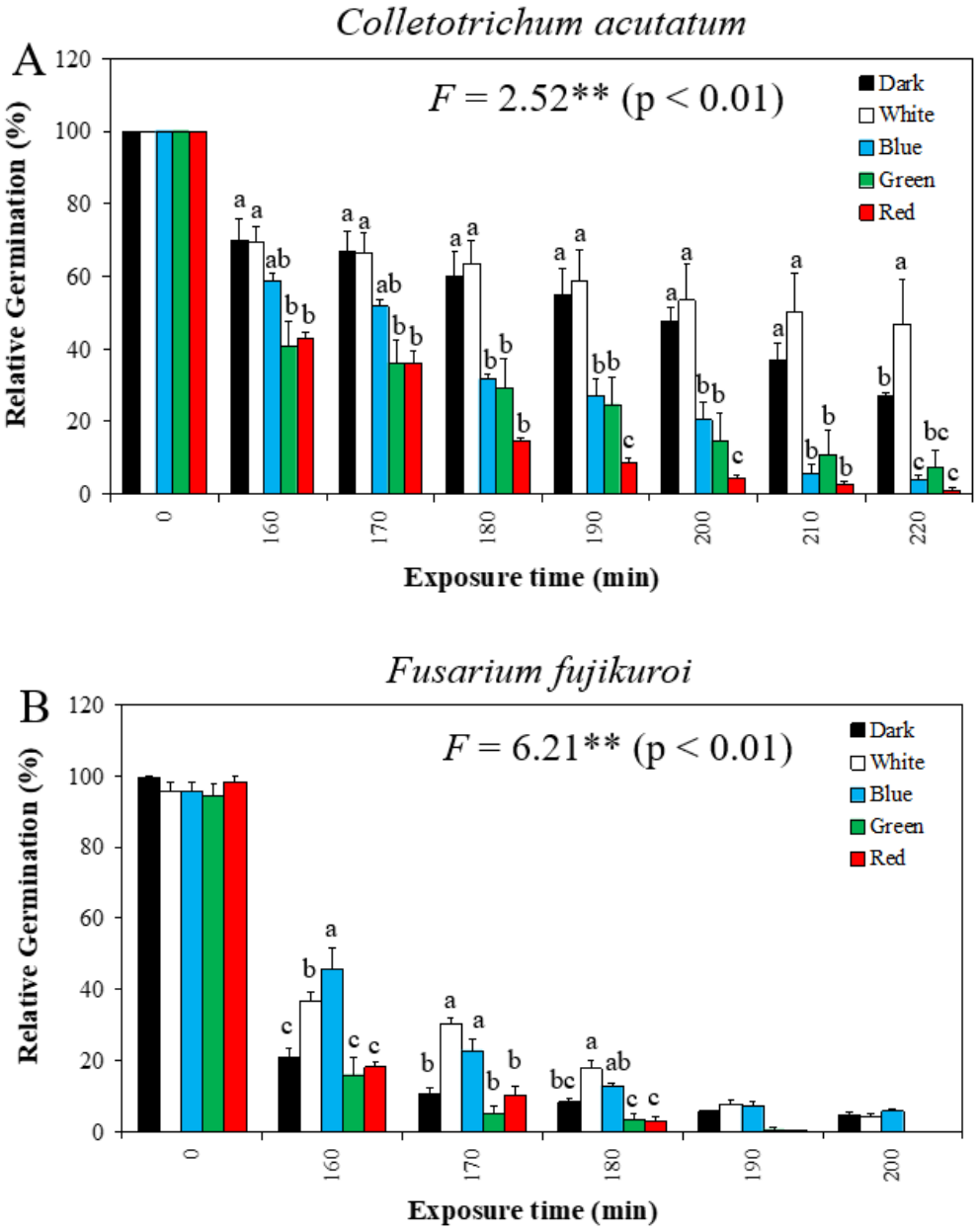

Figure 11 – Germination percentage of (A) *Colletotrichum acutatum* and (B) *Fusarium fujikuroi* after exposure to UV radiation for 160, 170, 180, 190, 200, 210, and 220 min, equivalent to the irradiance of 5.7, 6.0, 6.4, 6.7, 7.1, 7.5, 7.8, and 8.2 kJ m^{-2}, respectively. Error bars are the standard errors of at least three independent experiments conducted at different times. Graph bars with the same letter are not significantly different ($p < 0.01$).

Discussion

The plant-pathogenic fungi *Colletotrichum acutatum* and *Fusarium fujikuroi* control several aspects of their physiology in response to light. Light did

not affect the germination and mycelial growth of these fungi. It has long been recognized, with few exceptions e.g. in *Botrytis cinerea* that grows less under light (Canessa et al. 2013), that the effect of light on the fungi is more important for reproduction than vegetative growth (Gottlieb 1950; Lilly and Barnett 1951; Cochrane 1958). Conversely, the conidial production as well as the conidial stress tolerance differs according to the different light treatments during mycelial growth.

C. acutatum produced more conidia under white and red light than in darkness, and blue and green light generated the least conidia (Figure 10A), as also observed by de Menezes (2015). *Fusarium fujikuroi* produced more conidia in the dark (Figure 10B); however, *F. verticillioides* cultures grown under white, blue, yellow, green, and red wavelengths produce more conidia than the cultures grown in the dark (Fanelli et al. 2012). Conidiation is also stimulated by light in the wild type strain IMI58289 of *F. fujikuroi* (Avalos et al. 1985; Avalos and Estrada 2010), but in a different wild type strain of the same species mycelial growth in the dark produced more conidia than under light (Prado et al. 2004; Estrada and Avalos 2008; Estrada and Avalos 2009; Avalos and Estrada 2010). Estrada and Avalos (2008; 2009) found that growth in the dark of the same *F. fujikuroi* isolate we used (FKMC 1995) generated more conidia than growth in the light. The results of our study agreed with Estrada and Avalos, in which this isolate also produced abundant conidia in the dark (Figure 10B).

Growth under white light also improved conidial UV radiation tolerance of *C. acutatum* (Figure 11A) and *F. fujikuroi* (Figure 11B). Although *C. acutatum* conidia produced in the light were similarly tolerant to UV radiation as conidia produced in the dark in the lowest UV irradiances, at the highest UV irradiances, conidia produced under light were more tolerant than conidia produced in the dark. Mycelial growth under blue, green or red light generated conidia less tolerant than conidia produced in the dark (Figure 11B). Similar to our results, growth of *C. acutatum* colonies under low irradiance of white light increased conidia and mucilage production, and conidia produced under the light were two-times more tolerant to UV radiation (de Menezes et al. 2015). *C. acutatum* is more pigmented under white and blue light, while the least pigmentation was observed in mycelia incubated in the dark (Yu et al. 2013). Blue light also enhances melanin production, enhancing virulence of *C.*

acutatum (Yu et al. 2013), because appressorial wall melanin permits very high turgor pressure that the melanin deposition pattern directs into the penetration peg. Inhibition of melanin synthesis by tricyclazole is shown to prevent plant infection by fungal pathogens, and this process is usually attributed to inhibition of appressorial penetration (Butler et al. 2005). Moreover, the effect of green and red light stimulates less melanin production than blue light, leading to reduced disease severity (Yu et al. 2013). However, for *F. fujikuroi*, growth under white and blue light produces conidia twice as more tolerant to UV radiation than conidia produced in the dark and under green and red light. Growth under illumination up-regulates many stress genes that are important to produce conidia with increased stress tolerance (Wu et al. 2014; Brancini et al. 2019; Dias et al. 2019). Light also promotes higher resistance of *Aspergillus fumigatus* against exogenous oxidative stress and enhances resistance to acute ultraviolet radiation (Fuller et al. 2013). *M. robertsii* conidia produced under white light exhibit higher tolerance to osmotic stress (Dias et al. 2019), heat (Rangel et al. 2011; Rangel et al. 2015), and UV radiation (Rangel et al. 2011; Rangel et al. 2015; Dias et al. 2019).

F. fujikuroi and *C. acutatum* grown under red light produced conidia very susceptible to UV radiation compared to conidia of these fungi produced under white and blue light. Similar results were found for *M. robertsii* in which conidia produced under red light are less tolerant to osmotic stress caused by potassium chloride and UV radiation than conidia produced in the dark (Dias et al. 2019). In addition, exposure of fast-growing mycelia of *M. acridum* to white, blue or UV-A wavelengths induces tolerance to subsequent UV-B irradiation. However, red light induced lower mycelial tolerance to subsequent UV-B irradiation (Brancini et al. 2016). This observation may indicate that red-light represses genes for tolerance to stress. Therefore, pathogenic fungi use environmental cues to prepare their conidial offspring against challenges in the environment (Rangel et al. 2008; Rangel 2011; Rangel et al. 2012; Rangel et al. 2018; Rangel and Roberts 2018; Medina et al. 2020). By producing offspring more tolerant to the same or other stress conditions (Rangel et al. 2004; Rangel et al. 2011; Rangel et al. 2018) as well as enhancing their virulence (Oliveira et al. 2018; Oliveira and Rangel 2018). This will support greater dispersal distances under daytime conditions, consequently, conidia which are more

resistant to damage due to UV radiation exposure could also be an advantage in dispersal and infection, which could be a factor in the epidemiology of the diseases caused by these two pathogens.

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Fungal tolerance to Congo red, a cell wall integrity stress

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Abstract

Differential sensitivities to the cell wall stress caused by Congo red (CR) have been observed in many fungal species. In this study, the tolerances and sensitivities to CR was studied with an assorted collection of fungal species grouped in the ecological niches of insect pathogens, plant pathogens, saprotrophs, and mycoparasitics. The saprotroph *Aspergillus niger* and the mycoparasite *Trichoderma atroviride* stood out as the most resistant species to cell wall stress caused by CR, followed by the plant pathogenic fungi and other saprotrophs. The insect pathogens had low or moderate tolerance to CR. The insect pathogens *Metarhizium acridum* and *Isaria fumosorosea* were the most sensitive to CR.

Key Words: entomopathogenic fungi; plant-pathogenic fungi; saprotrophic fungi; mycoparasite fungi; stress tolerance

Introduction

Congo Red (CR; 3,3′-([1,1′-biphenyl]-4,4′-diyl)bis(4-aminonaphthalene-1-sulfonic acid)) is a water soluble diazo dye. Its color depends on the pH and changes from blue to red in the pH range of 3.0 – 5.2. CR is regularly used to induce cell wall integrity stress or to fungal cell wall mutants (Ram and Klis, 2006). Since its solubility is low at acidic pH, its application at pH lower than 5.5 is not recommended (Ram and Klis, 2006).

CR is thought not to be entering the cells due its two sulfonic-acid groups which are charged negatively at slightly acidic to alkali pH (Kopecká and Gabriel, 1992). Although CR has high structural stability and very resistant to biodegradation, certain *Aspergillus niger* strains can partially degrade it by extracellular lignin and manganese peroxidases (Asses et al., 2018).

The antifungal activity of CR is explained by that it can intercalates into the cell wall forming a complex with chitin and β-1,3-glucans (Kopecká and Gabriel, 1992; Levin, 2005; Ogawa et al., 1994; Pancaldi et al., 1984; Vannini et al., 1983). Its inhibitory effect on chitin synthesis has been reported (Bartnicki-Garcia et al., 1994) and on enzymes connecting chitin to β-1,3-glucan has also been suggested (Ram and Klis, 2006). Due to these effects CR induces morphological changes and weakens the cell wall in fungi at sublethal concentration (Pancaldi et al., 1984; Ram and Klis, 2006; Vannini et al., 1983).

Genome-wide transcriptional changes detected after CR stress treatments in *Saccharomyces cerevisiae* demonstrated that CR induced compensatory changes in cell wall structure through the Rlm1 transcription factor activated by the cell wall integrity (protein kinase C) MAPK pathway (García et al., 2004; Jung and Levin, 1999; Lagorce et al., 2003).

In *Aspergillus nidulans*, CR stress caused only minor changes in cell wall composition (Kovács et al., 2013). It transiently induced then repressed the transcription of the brlA gene (Kovács et al., 2013) encoding a transcription factor involved in the initiation of both condidiogenesis and autolytic cell wall degradation (Adams et al., 1988; Pócsi et al., 2009; Szilágyi et al., 2010). As a consequence, the production of extracellular cell wall hydrolases (including EngA β-1,3-endoglucanase, ChiB chitinase and NagA hexosaminidase) was induced (in submerged cultures), while conidiophore development was

suppressed (in surface cultures) (Kovács et al., 2013). Both RlmA dependent and independent regulatory pathways were involved in the regulation of the stress response initiated by CR (Kovács et al., 2013).

Regarding to other fungi, inactivation of genes (e.g. eki1, tmk3) encoding proteins of MAPK pathways regulating cell wall biosynthesis increased the CR sensitivity of *Trichoderma reesei* (He et al., 2015; Wang et al., 2013). Similarly, the copy number of mpkC orthologs in *Aspergilli* showed correlation with CR stress tolerance (Emri et al., 2018). Deletion of the MaChsV and MaChsVII genes encoding chitin synthases or MaFKS encoding β-1,3-glucan synthase in *Metarhizium acridum* (Yang et al., 2011; Zhang et al., 2019) as well as deletion of the cell wall protein gene Bbecm33 in *Beauveria bassiana* (but not of Mrecm33 in *Metarhizium robertsii*) (Chen et al., 2014) increased CR susceptibility. In contrast, deletion of the Mrmep1 and Mrmep2 metalloprotease genes increased the CR tolerance in *Metarhizium robertsii* (Zhou et al., 2018).

Differential sensitivities to the cell wall stress caused by CR have been observed in many fungal species (Nikolaou et al., 2009). In this study, the mycelial growth was used to quantify the tolerance/sensitivity to CR was studied with an assorted collection of fungal species grouped as insect pathogens, plant pathogens, saprotrophs, and mycoparasitics.

Materials and methods

Fungal isolates and conidia production

The saprotrophic fungal isolates were provided by Dr. Ely Nahas, Universidade Estadual Paulista (UNESP), by ATCC, and ARSEF collections (Table 1). Under the saprotrophic fungal isolates were included one xerophilic fungal isolate *Aspergillus pseudoglaucus* (JH06JPD) donated by John E. Hallsworth, Queen's University Belfast, Northern Ireland.

The plant pathogenic isolates were provided by Javier Avalos, Universidad de Sevilla Spain (Table 2).

Table 2 – Fungal species and geographic origin.

Isolate	Species	Substrate/Host	Geographic Origin	Year
ARSEF 23	*Metarhizium robertsii*	*Conoderus sp.* [Coleoptera: Elateridae]	North Carolina, USA	1961
ARSEF 324	*Metarhizium acridum*	*Austracris guttulosa* [Orthoptera: Acrididae]	Queensland, Australia	1979
ARSEF 1187	*Metarhizium brunneum*	*Oxycanus* sp. [Lepidoptera: Hepialidae]	Palmerston North, New Zealan	1966
ARSEF 2134	*Metarhizium robertsii*	*Phyllophaga ?anxia* [Coleoptera: Scarabaeidae]	Southern Quebec, Canada	1985
ARSEF 2341	*Metarhizium anisopliae* s.l.	*Scotinophara coarctata* [Hemiptera: Pentatomidae]	Palawan, Philippines	1986
ARSEF 2560	*Metarhizium robertsii*	*Atta sexdens rubropilosa* [Hymenoptera: Formicidae]	Sao Paulo, Brazil	1988
ARSEF 2575	*Metarhizium robertsii*	*Curculio caryae* [Coleoptera: Curculionidae]	South Carolina, USA	1988
ARSEF 3609	*Metarhizium acridum*	*Patanga succincta* [Orthoptera: Acrididae]	Thailand	1992
ARSEF 4343	*Metarhizium anisopliae* s.l.	Soil	Macquarie Island, Australia	1994
ARSEF 4570	*Metarhizium anisopliae* s.l.	Soil	Macquarie Island, Australia	1993
ARSEF 5626	*Metarhizium brunneum*	*Tenebrio molitor* [Coleoptera: Tenebrionidae] bait from soil	Hämeen Laani, Finland	1986
ARSEF 5749	*Metarhizium anisopliae* s.l.	*Schistocerca piceifrons* [Orthoptera: Acrididae]	Colima, Mexico	1992
ARSEF 7711	*Metarhizium brunneum*	I Earth BioScience F52, IMI 385045	Austria	2005
ARSEF 252	*Beauveria bassiana*	*Leptinotarsa decemlineata* [Coleoptera: Chrysomelidae]	Orono, Maine, USA	1978
ARSEF 1212	*Trichothecium roseum*	*Nymph, Adelphocoris* sp. [Hemiptera: Miridae]	Lazio, Italy	1983
ARSEF 3392	*Tolypocladium cylindrosporum*	Soil	Nepal	1991
ARSEF 3889	*Isaria fumosorosea*	*Bemisia tabaci* [Homoptera: Aleyrodadae]	Hawaii, USA	1993
ARSEF 4877	*Tolypocladium inflatum*	[Coleoptera: Scarabaeidae: Aphodiinae]	Danby, New York, USA	1994
ARSEF 4361	Cladosporium herbarum	Aphis gossypii [Hemiptera: Aphididae] Citrus.	Rio de Janeiro, Brazil	1994
ARSEF 6430	Simplicillium lanosoniveum	Leptopharsa heveae [Hemiptera: Tingidae]	French Guiana	2000
ARSEF 6433	*Lecanicillium aphanocladii*	Triangulo Agroindustrial S.A.	Mato Grosso, Brazil	2001
ARSEF 7200	*Clonostachys rosea*	*Oncometopia tucumana* [Hemiptera: Cicadellidae]	Tucuman, Argentina	2003
JH06JPD	*Aspergillus pseudoglaucus*	Antique wooden rice-scoop	Japan	
ATCC 10074	*Aspergillus nidulans*	Unknown substrate	Belgium	
F111	*Aspergillus niger*	Soil	Jaboticabal, SP, Brazil	
IMI 206040	*Trichoderma atroviride*	Associated organism *Picea excelsa*	Sweden, Europe	1970
SF 116	*Fusarium fujikuroi*	Carotenoid-overproducing strain. Mutation from FKMC1995	Spain	
FKMC 1995	*Fusarium fujikuroi*	Kansas State University Collection (Manhattan, KS)		
FGSC 7600	*Fusarium verticillioides*	Maize A-00149, M3125, MYA-4922	Visalia, CA, USA	

Source: Environmental microbiology laboratory for fungi collection - Drauzio Eduardo Naretto Rangel.

The entomopathogenic fungal isolates were obtained from ARSEF, USDA-ARS Collection of Entomopathogenic Fungal Cultures (Robert W. Holley Center for Agriculture and Health, Ithaca, NY, USA) (Table 2) (Humber, 2013).

The mycoparasitic fungal species were provided by ARSEF, and by Alfredo H. Herrera-Estrella, Laboratorio Nacional de Genómica para la Biodiversidad (CINVESTAV), isolate IMI 206040 (Table 2).

Stock cultures were maintained at 4 °C in test tubes on slants of potato dextrose agar (Difco Laboratories, Sparks, MD, USA) (PDA) adjusted to pH 6.9.

Conidia of each isolate were produced on 23 ml of PDA in polystyrene Petri dishes (95 × 15 mm). A conidial suspension (100 µl of 10^7 conidia ml^{-1}) from 14-days old cultures suspended in Tween 80 0.01% (v/v) was inoculated evenly with a glass rod spreader onto the agar media. The cultures were incubated in the dark at 26 × 1 °C for 14 days. Each treatment was repeated at least three times in different days as biological replications, with a new batch of conidia produced for each repetition.

Mycelial growth experiments under Congo red

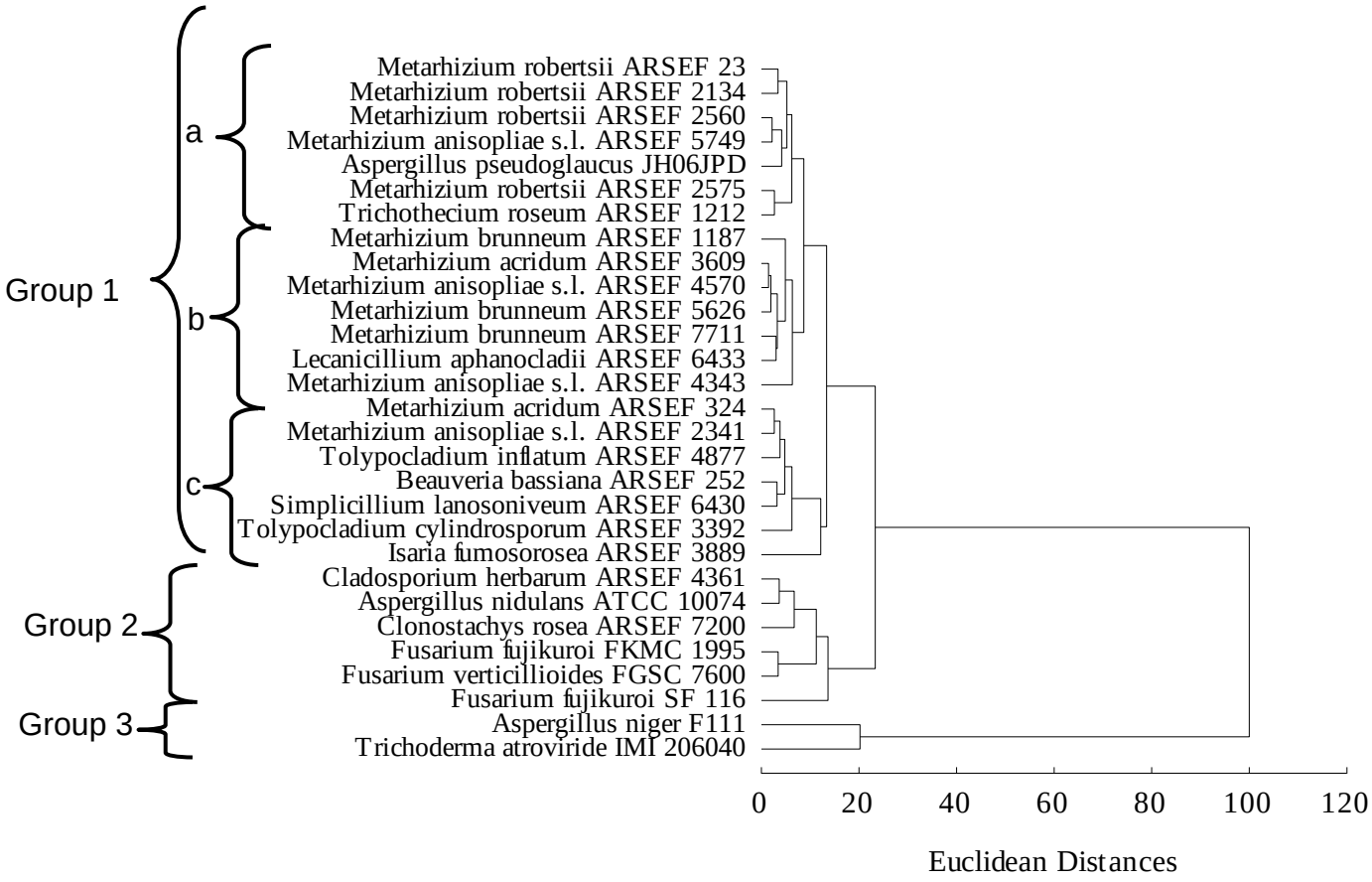
A conidial suspension from 14-d-old cultures from each isolate was spot inoculated with 4 μl of the suspension to the center of PDAY medium in polystyrene Petri dishes (95 × 15 mm). The inoculated plates were incubated at 28 °C. For all 29 isolates, seven concentrations of Congo red were used: 0 (control), 50, 100, 200, 300, 400, 500, and 600 μg/ml, according to Nikolaou et al. (2009). The colony sizes were measured daily from the 5th day to the 10th day. The colony diameter (mm) was measured daily from the center of inoculation point towards the periphery of the colony in each plate. Two diameter measurements were made at right angles for each plate and then averaged. The measurement sites were done on the same axes each day. The experiment was repeated three times.

Statistical analysis

The analysis of hierarchical cluster (Sneath and Sokal, 1973) was performed by calculating the Euclidean distance using the data of the growth on the 7th day and the colony sizes at the concentrations 50, 100, 300, and 600 μg/ml and by using Ward's algorithm to obtain similar access groups. The result of the analysis was presented in a graphical form (dendrogram), which helped to identify the groups of the isolates to compare the CR tolerance among the groups. The hierarchical cluster analysis was processed in the STATISTICA software version 10 (StatSoft, 2010).

Results and Discussion

The tolerances of the isolates were classified into three categories as follow: Group 1 – Low tolerance, Group 2 – Moderate tolerance, and Group 3 – High tolerance (Figure 12).



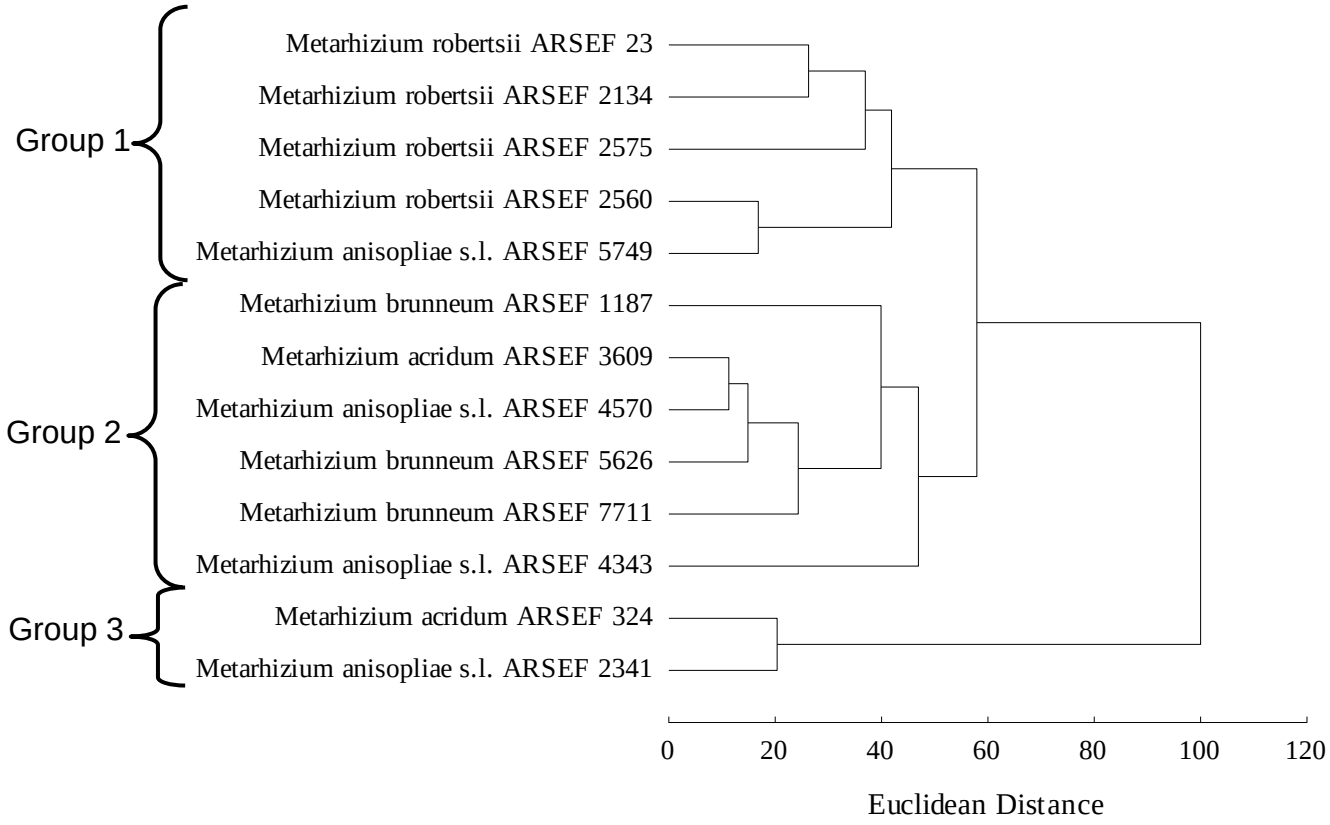
Group 1 – Low tolerance

Group 2 – Moderate tolerance

Group 3 – High tolerance

Figure 12 – Dendrograms of the hierarchical cluster analysis performed by calculating the Euclidean distance between many variables and using Ward's algorithm to obtain similar access groups. The analysis was processed with the software STATISTICA version 10 (StatSoft, 2010).

A similar analysis of hierarchical cluster was performed only for the *Metarhizium* species by calculating the Euclidean distance using the growth on the 7th day and the colony sizes at the concentrations 50, 100, 300, and 600 μg/ml. The tolerances of the *Metarhizium* isolates were classified into three categories as follow: Group 1 – High tolerance, Group 2 – Moderate tolerance, and Group 3 – Low tolerance (Figure 13).



Group 1 – High tolerance

Group 2 – Moderate tolerance

Group 3 – Low tolerance

Figure 13 – Dendrogram of the hierarchical cluster analysis performed calculating the Euclidean distance between many variables and using Ward's algorithm to obtain similar access groups. The analysis was processed with the software STATISTICA version 10 (StatSoft, 2010).

Hierarchical clustering provides excellent visual representations because they facilitate rapid and simple comparisons of two of more datasets. Hierarchical clustering has been used in many approaches such as organize genes into hierarchical dendograms on the basis of their expression. The Euclidean diversity coefficient allows the diversity in a set of species to be measured beyond their relative abundances using biological information about the dissimilarity between the species. It also involves geometrical interpretations

and graphical representations. Moreover, several populations can be compared using a Euclidean dissimilarity coefficient derived from the Euclidean diversity coefficient (Champely and Chessel, 2002).

We previously illustrate that microbial ecology/niche is a determinant of fungal osmotolerance (Araújo et al., 2020). Accordingly, saprotrophs fungi are the most tolerant to osmotic stress (caused by KCl) than plant pathogens, in its turn plant pathogens are more tolerant to osmotic stress than insect pathogens (Araújo et al., 2020). In this study the fungal species were grouped in the four niches: insect pathogens, plant pathogens, saprotrophs, and mycoparasitics. According to the growth of 29 fungal isolates of 19 species under CR a dendrogram was built and separate all fungal species in three main groups. All insect-pathogenic fungi, that are used for biological control of insects (Li et al., 2010), were separated in group 1 with the lowest tolerance, interestingly, the highly osmotolerant *Aspergillus pseudoglaucus* (JH06JPD) (Araújo et al., 2020), a saprotrophic fungus was the only exception and it was placed in group 1 (Figure 12). The plant-pathogenic fungi including *Fusarium* species, the saprotrophs *Cladosporium herbarium* and *Aspergillus nidulans*, as well as the mycoparasite *Clonostachys rosea* were separated in group 2 with moderate tolerance. Finally, in group 3 the saprotrophs *Aspergillus niger* and *Trichoderma atroviridae* (that is also a mycoparasite) stood out as the most resistant species to cell wall stress (Figures 12 and 16). Therefore, the microbial ecology/niche also determines the tolerance to cell wall integrity stress caused by Congo red.

The group 1, the least tolerant to CR, was subdivided by the dendogram in three smaller groups (Figure 14). Group 1A was the most tolerant to CR in the group 1. In this group includes the fungus *Trichothecium roseum* that was the most tolerant species to CR among the group 1 of entomopathogenic fungi (Figures 12 and 15). This species has also shown higher tolerances to UV radiation (Dias et al., 2018), to osmotic stress (Araújo et al., 2020), and to the mutagen 4-nitroquinoline 1-oxide (4-NQO) (Araújo et al., 2018). The second most tolerant in group 1A was *M. robertsii* ARSEF 2575. The least tolerant in group 1A was *M. robertsii* ARSEF 2134. The fungus *M. robertsii* ARSEF 2575 (Figures 12 and 14) is among the species of *Metarhizium* very tolerant to many stress conditions, such as osmotic stress (Araújo et al., 2020), to UV radiation (Dias et al., 2018), to heat (Rangel et al., 2005), to menadione (Azevedo et al.,

2014), and to agricultural fungicide (Rangel et al., 2010a), therefore, due to its higher stress tolerance, the isolate ARSEF 2575 has been considered to be used for biological control of insects (Alston et al., 2005; Keyser et al., 2017; Roberts et al., 2007).

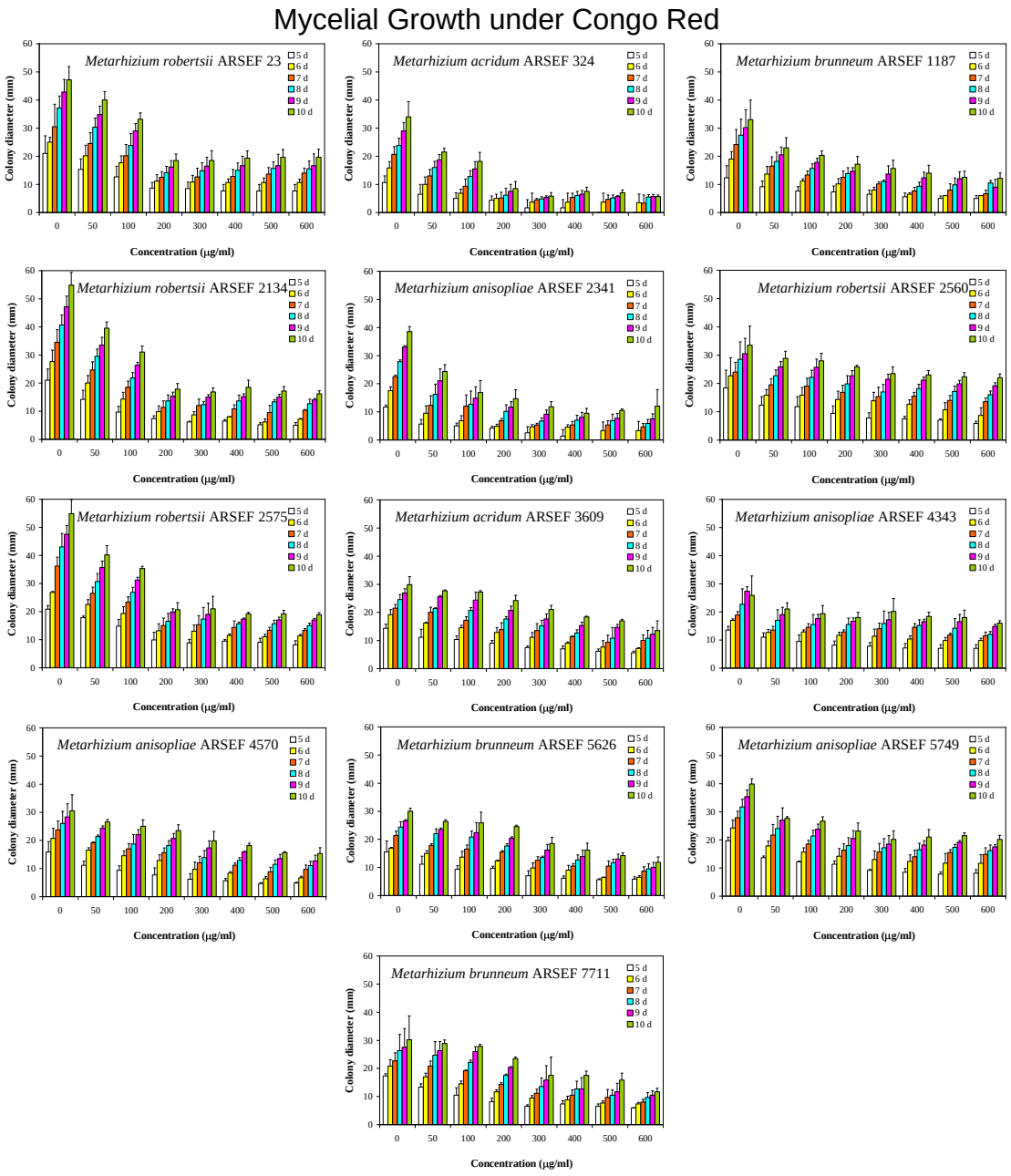


Figure14 – Effect of Congo Red on mycelial growth of 13 species of *Metarhizium.*

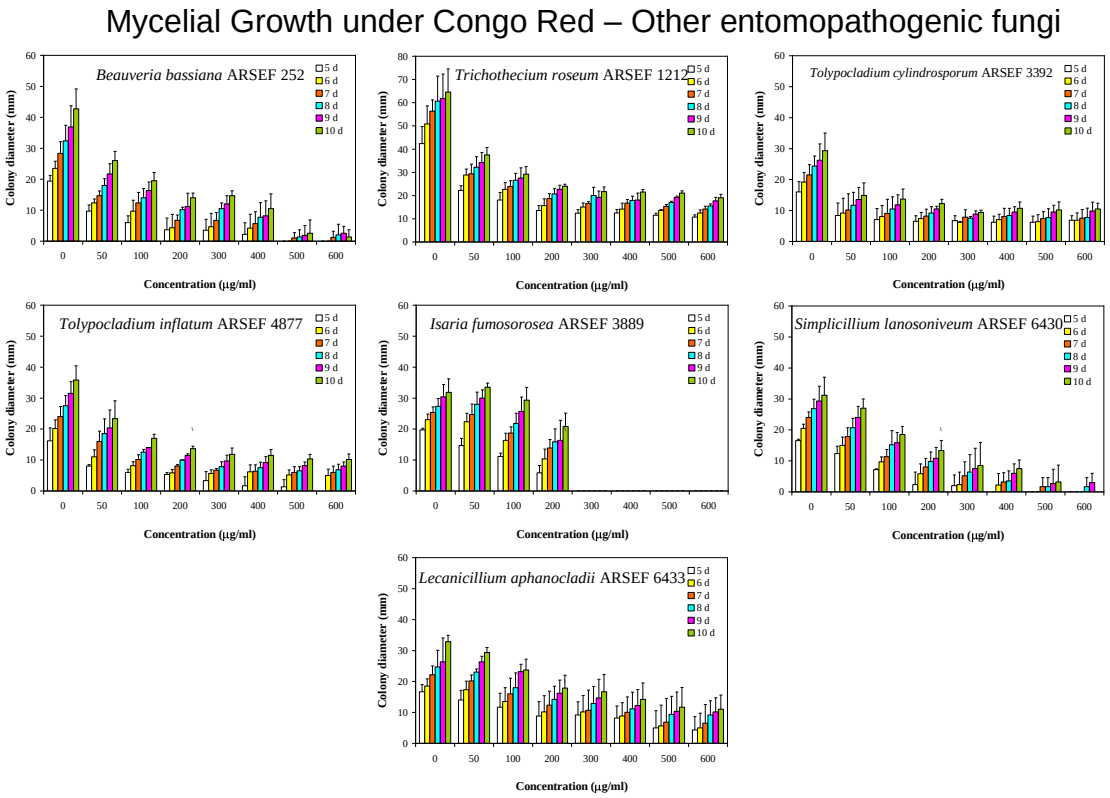


Figure 15 – Effect of Congo on mycelial growth of 7 entomopathogenic fungi.

Group 1B with moderate tolerance include *M. acridum* ARSEF 3609 as the most tolerant in group 1B and *M. brunneum* ARSEF 1187 as the least tolerant in group 1B. The *M. acridum* are frequently isolated from deserts (Rangel et al., 2005) and, therefore, due to natural selection by living in their harsh environments. *M. acridum* (ARSEF 3609) are the very tolerant to heat (Rangel et al., 2005; Rangel et al., 2010b) and UV radiation (Braga et al., 2001), but it is very susceptible to chemicals such as 4-NQO (Araújo et al., 2018), KCl (Araújo et al., 2020), and agricultural fungicides (Rangel et al., 2010a). The fungi *M. anisopliae* (ARSEF 4343 and ARSEF 4570) and *M. brunneum* (ARSEF 5626), both from the cold Antarctic and Arctic habitats, respectively (Table 2) they are also very susceptible to heat (Rangel et al., 2005) and UV radiation (Braga et al., 2001). ARSEF 4343, from Antarctic, is also very susceptible to KCl (Araújo et al., 2020) but with higher tolerance to 4-NQO (Araújo et al., 2018). ARSEF 5626, from Arctic, is also very susceptible to KCl (Araujo et al., 1989) and 4-NQO (Araújo et al., 2018), but very tolerant to menadione (Azevedo et al., 2014). ARSEF 4570 is also very susceptible to KCl (Araújo et

al., 2020) and 4-NQO (Araújo et al., 2018). The fungus *L. aphanocladii* ARSEF 6433 is also very susceptible to heat (Souza et al., 2014) and UV radiation (Braga et al., 2002; Dias et al., 2018), but with moderate osmotolerance (Araújo et al., 2020), very high tolerance to 4-NQO (Araújo et al., 2018), and high tolerance to menadione (Azevedo et al., 2014).

Group 1C with the lowest tolerance include *I. fumosorosea* ARSEF 3889 as the most tolerant of this group and *M. acridum* ARSEF 324 as the least tolerant of group 1C. *I. fumosorosea* ARSEF 3889 is very susceptible to heat (Souza et al., 2014), but show moderate tolerance to UV radiation (Dias et al., 2018), menadione (Azevedo et al., 2014), and KCl (Araújo et al., 2020). ARSEF 3889 show higher tolerance to 4-NQO (Araújo et al., 2018). *B. bassiana* (ARSEF 252) is very susceptible to UV radiation (Dias et al., 2018) and KCl (Araújo et al., 2020), with moderate tolerance to heat (Souza et al., 2014), menadione (Azevedo et al., 2014), and 4-NQO (Araújo et al., 2018). *Tolypocladium* species are known to be very susceptible to toxic chemicals such as, menadione (Azevedo et al., 2014), fungicide dodine (Rangel et al., 2010a), and potassium chloride (Araújo et al., 2020), as well as very susceptible to UV-B radiation (Dias et al., 2018; Santos et al., 2011) and heat (Santos et al., 2011), but these species showed moderated tolerance to 4-NQO (Araújo et al., 2018). Although the isolate ARSEF 324 of *M. acridum* is the most tolerant to UV-B radiation (Dias et al., 2018; Rangel et al., 2006; Rangel and Roberts, 2018) and heat (Rangel et al., 2005; Rangel et al., 2006; Rangel and Roberts, 2018) than all other entomopathogenic fungal species, it was the least tolerant to CR and also it is the least tolerant to chemicals such as menadione (Azevedo et al., 2014), 4NQO (Araújo et al., 2018), potassium chloride (Araújo et al., 2020), the agricultural fungicide dodine (Rangel et al., 2010a), and clinical antifungal agents (Brancini et al., 2018).

In a separate dendrogram only the *Metarhizium* species were studied and the growth under CR divided all *Metarhizium* species in also three groups (Figures 13 and 14). All *Metarhizium* species studied are part of the *Metarhizium anisopliae* species complex (Kepler et al., 2014). All four *Metarhizium robertsii* were place in group 1 with the highest tolerance (Figure 13). All three *Metarhizium brunneum* were placed in group 2 with moderate tolerance to CR (Figure 14). *Metarhizium anisopliae* are those fungal species

that the taxonomy have not been molecular confirmed and, therefore, they are placed in separate listings for these species in their broad senses (*sensu lato*). Due to this *Metarhizium anisopliae* were placed in all three different groups with low, moderate, and high tolerances because we do not really know which species they are (Figure 13). *Metarhizum acridum* were placed in group 2 and group 3, with moderate (ARSEF 3609) and low tolerance (ARSEF 324) (Figure 13).

The group 2, set the fungi with moderate tolerance to CR (Figure 12). The most tolerant fungal species from this group was the two plant pathogenic fungi *F. fujikuroi* FKMC 1995, followed by *F. verticillioides* FGSC 7600 (Figure 12 and 16). Although the least tolerant of this group was another plant pathogenic fungi *F. fujikuroi* SF 116, however, this strain is a mutation from FKMC 1995. It is also interesting that both *F. fujikuroi* FKMC 1995 and *F. verticillioides* FGSC 7600 had moderate osmotolerance, but SF 116 is osmo-sensitive (Araújo et al., 2020), therefore, the mutation might influenced and reduced its tolerance to CR and KCl. *A. nidulans* ATCC 10074 was the third most tolerant fungus to CR from group 2 (Figure 12) and it is also highly osmotolerant (Araújo et al., 2020). *C. herbarum* ARSEF 4361 is also highly osmotolerant (Araújo et al., 2020) and highly UV tolerant (Dias et al., 2018). *Cladosporium* species are highly melanized and they are found in hypersaline waters (Cantrell et al., 2011), in the stratosphere (Pederson Jr., 1968), and in the damaged Chernobyl nuclear reactor (Zhdanova et al., 2000). C. rosea ARSEF 7200 was also put in the group with moderate tolerances to CR.

The *Aspergillus* species examined here, except *A. pseudoglaucus* JH06JPD, were relatively resistant to the cell wall stress caused by CR (Figures 12 and 16) and displayed similar responses with high resistance to osmotic stress (KCl) (Araújo et al., 2020). Congo red induced fungal hormoligosis [= hormosis = reproductive or growth stimulation by sublethal doses of chemicals (Rangel et al., 2010a)], especially with *Aspergillus niger* F111 that responded to CR at higher concentrations with better growth than the controls without CR (Figure 16). The fungus *A. pseudoglaucus* JH06JPD was strangely put in the group 1 with the least tolerant to CR, a fungus extremely tolerant to osmotic stress (Araújo et al., 2020; Stevenson et al., 2015). *A. nidulans* was placed in group 2 with moderate tolerance. *A. niger* was placed in group 3, which is the

group with the most resistant species to CR (Figures 12 and 16). These three fungal species were placed together in the group highly osmotolerant or mildly osmophilic (Araújo et al., 2020).

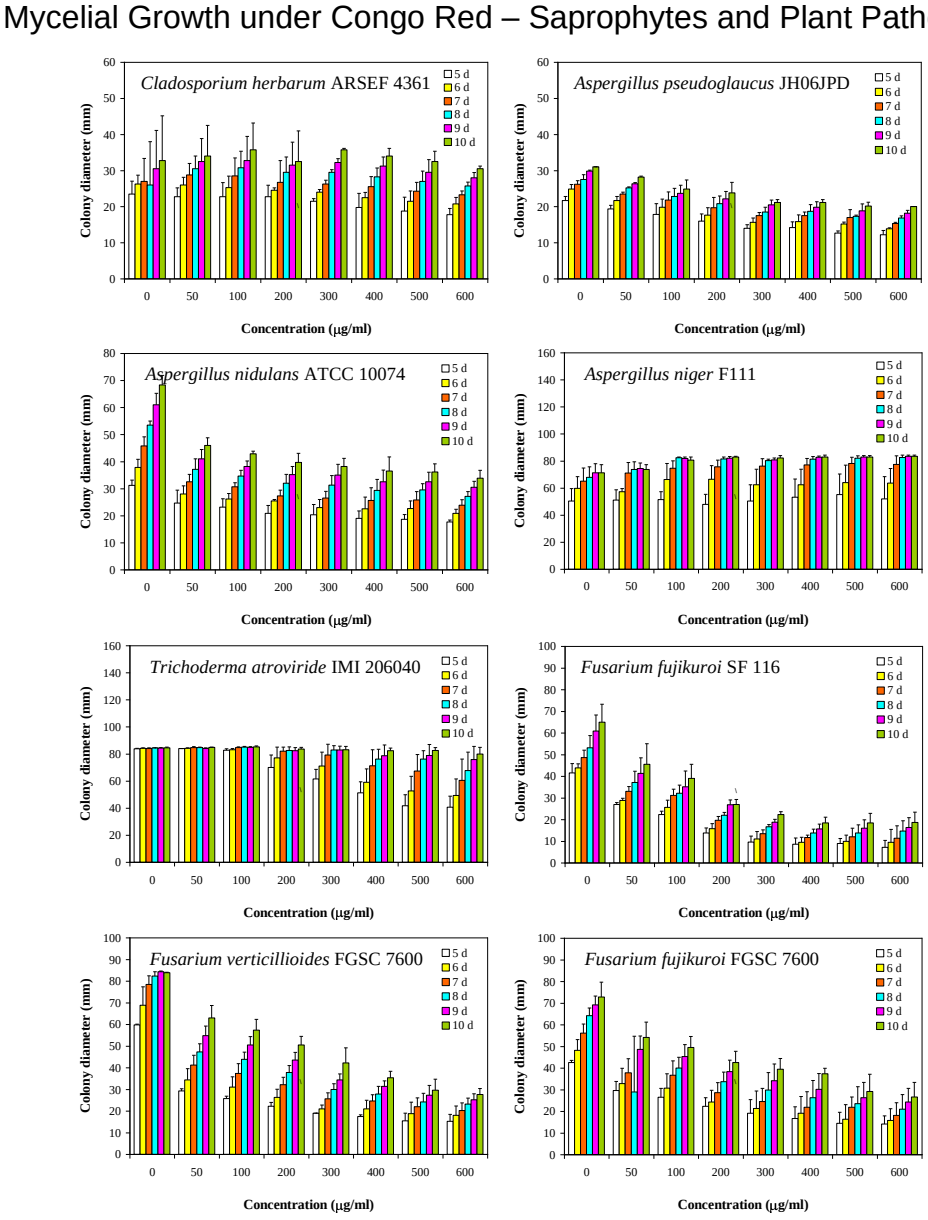


Figure 16 – Effect of Congo on mycelial growth of 8 saprotrophic and mycoparasitic fungi.

Group 3 grouped only the fungi *A. niger* and *T. atroviride* as the fungal species with the highest tolerances to CR. Although *A. niger* is highly osmotolerant but *T. atroviride* was placed in the group with the osmo-sensitive (Araújo et al., 2020). Although both are well known to be saprotrophs, but *T. atroviride* is also known to be a mycoparasite (Medina et al., 2020). Aspergillus niger is an important fungus that solubilizes rock phosphate (Nahas, 1996)

Congo red degradation halos has been observed with *A. niger* in the Fungal Stress Database (http://www.fung-stress.org) (e.g. http://www.fung-stress.org/Files/Aspergillus_niger_n402.25oc10d.pdf) as well as by other authors (Asses et al., 2018). We found CR degradation halo only for the isolates: from the smallest to the largest halo of degradation: *M. anisopliae* ARSEF 4343 (degradation halo with 3 mm), *M. acridum* ARSEF 3609 (degradation halo with 4 mm), *A. niger* F111 (degradation halo with 6 mm), *A. pseudoglaucus* JH06JPD (degradation halo with 7 mm), *M. anisopliae* ARSEF 4570 (degradation halo with 8 mm), and *M. robertsii* ARSEF 2560 (degradation halo with 9 mm). All halo measurements were done in the day 9.

Only *Isaria fumosorosea* was found to have a minimum inhibitory concentration (MIC) for CR, but all other fungal species did not die even at the highest CR concentration. Because of the unusual fungal behavior under the stress of CR, we could not extract so much information from the data as with other stress generating agents. Usually when fungi are submitted to stress conditions the minimum inhibitory concentration (MIC) will often occur at the highest doses or concentrations of the stress. This occur for stress caused by UV-radiation (Braga et al., 2001; Dias et al., 2018; Santos et al., 2011), heat (Rangel et al., 2010b; Souza et al., 2014), the mutagen 4-nitroquinoline 1-oxide (Araújo et al., 2018), menadione (Azevedo et al., 2014), the fungicide dodine (Rangel et al., 2010a), and potassium chloride (Araújo et al., 2020). However we did not see this trend for CR (Figures 14, 15, and 16). There were some growth reduction after the concentration of 200 micrograms/ml, but after this concentration, the growth remained constant until the concentration of 600 μg/ml. This facet of Congo red fungal tolerance has been seen by many scientists (Esquivel-Naranjo et al., 2016; Nikolaou et al., 2009). For example, Nikolaou et al. (2009) found this same performance in the fungi *Ashbya gossypii*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Candida glabrata*,

Kluyveromyces lactis, *Magnaporthe grisea*, and *Schizosaccharomyces pombe*. According from Nikolaou et al. (2009) only three fungal species i.e. *Debaryomyces hansenii*, Yarrowia lipolytica, and *Ustilago maydis* have a MIC from 14 fungal species studied. In our study only *Isaria fumosorosea* had the MIC, none of the other 28 isolates were completely inhibited at the highest CR concentration.

Conclusion

Our comparison of the stress resistance of diverse fungal species and ecological niches has revealed a high degree of variation in their resistance to cell wall stress caused by CR. Similar fungal species in phylogenetic terms, e.g. all isolates of *Metarhizium robertsii* or all isolates of *Metarhizium brunneum* displayed similar levels of stress resistance. Saprotrophs were more tolerant to CR (this study) and osmotic stress caused by KCl (Araújo et al., 2020) than other fungal niches. Saprotroph fungi are important regulators of global biogeochemical cycling, responsible for mineralizing organic nutrients and governing the exchanges of carbon and nutrients between the biosphere and atmosphere (Crowther et al., 2012; Crowther et al., 2014; Ferreira et al., 2018). Saprotrophs may be, consequently, more prone to be under strenuous stress conditions than plant pathogens and insect pathogens; therefore, natural selection must improved these fungi to survive under extreme stress conditions.

Acknowledgments

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4. FINAL CONSIDERATIONS

This work aimed to evaluate the tolerance of insect pathogenic fungi and plant pathogenic fungi to the conditions of osmotic stress, UV radiation stress, cell wall stress, and thermal stress. As discussed in chapter 1, we found that *Trichothecium roseum* is more tolerant than *M. robertsii* and *M. acridum*, when in situations of osmotic stress due to KCl, stress due to the 4-NQO, and stress from Congo Red. However, under conditions of UV radiation stress, *Metarhizium* species were more tolerant. In the thermal stress study, our results were different from others published, suggesting new experiments are needed. The data on osmotic and cell wall stress suggest that *T. roseum* is a promising agent for the biological control of insects. In chapter 2, *C. acutatum* conidia were at least 30% more tolerant to ultraviolet radiation when produced under white light than under blue and green light and at least 20% more tolerant than conidia produced in the dark. *C. acutatum* conidia produced under red light were the least tolerant. *F. fujikuroi* conidia produced under white and blue light were at least 30% more tolerant to ultraviolet rays than conidia produced in the dark or under green and red light. In conclusion, no differences were found for germination and growth for both fungi under different light and dark regimes; however, significant differences occurred in both production and UV radiation of conidia. In chapter 3, the Congo red tolerances and sensitivities of a varied collection of fungal species grouped in ecological niches of insect pathogens, phytopathogens, saprotrophic and mycoparasitic, revealed the saprotrophic *Aspergillus niger* and the mycoparasite *Trichoderma atroviride* as the species most resistant to cell wall stress caused by Congo red, followed by phytopathogenic and other saprotrophic fungi. Insect pathogens had low or moderate tolerance to Congo red, and the fungi *Metarhizium acridum* and *Isaria fumosorosea* were the most sensitive to Congo red.

In view of these observations, further research is suggested involving the fungus *Trichothecium roseum*, which has still been scarcely researched. Its antagonistic activity can be worked as a biopesticide to fight other pathogenic fungi and insects.

REFERENCES

AGRIOS, G. N. (2005). ***Plant Pathology*** (5ed.). San Diego: Elsevier Academic Press.

ARAÚJO, C.A.S., FERREIRA, P.C., PUPIN, B., DIAS, L.P., AVALOS, J., EDWARDS, J., HALLSWORTH, J.E., RANGEL, D.E.N., 2019. Osmotolerance as a determinant of microbial ecology: A study of phylogenetically diverse fungi. **Fungal Biology**. https://doi.org/10.1016/j.funbio.2019.09.001.

ARAÚJO, C.A.S., DIAS, L.P., FERREIRA, P.C., MITTMANN, J., PUPIN, B., BRANCINI, G.T.P., BRAGA, G.Ú.L., RANGEL, D.E.N., 2018. Responses of entomopathogenic fungi to the mutagen 4-nitroquinoline 1-oxide. **Fungal Biology**. 122, 621-628. https://doi.org/10.1016/j.funbio.2018.03.007.

ATOUI, A., KASTNER, C., LAREY, C. M., THOKALA, R., ETXEBESTE, O., ESPESO, E. A., et al. (2010). Cross-talk between light and glucose regulation controls toxin production and morphogenesis in *Aspergillus nidulans*. *Fungal Genetics and Biology, 47*(12), 962-972, doi:https://doi.org/10.1016/j.fgb.2010.08.007.

AVALOS, J., CASADESÚS, J., CERDÁ-OLMEDO, E. (1985). *Gibberella fujikuroi* mutants obtained with UV radiation and N-methyl-N'-nitro-N-nitrosoguanidine. ***Applied and Environmental Microbiology****, 49*(1), 187-191.

AVALOS, J., ESTRADA, A. F. (2010). Regulation by light in *Fusarium*. ***Fungal Genetics and Biology****, 47*(11), 930-938, doi:10.1016/j.fgb.2010.05.001.

AVER'YANOV, A. A., LAPIKOVA, V. P., PASECHNIK, T. D., ABRAMOVA, O. S., GAIVORONSKAYA, L. M., KUZNETSOV, V. V., et al. (2014). Pre-illumination of rice blast conidia induces tolerance to subsequent oxidative stress. ***Fungal Biol,*** *118*(8), 743-753, doi:10.1016/j.funbio.2014.06.003.

AZEVEDO, R.F.F., SOUZA, R.K.F., BRAGA, G.U.L., RANGEL, D.E.N., (2014). Responsiveness of entomopathogenic fungi to menadione-induced oxidative stress. **Fungal Biol**-Uk 118, 990-995. 10.1016/j.funbio.2014.09.003.

BARNETT, H.L. AND HUNTER, B.B. (1998) **Illustrated Genera of Imperfect Fungi** (4th edn), 218 pp. St. Paul, MN: APS Press.

BATTA, Y. Entomopathogenic effect of *Trichothecium roseum* (Pers.) Link (Hypocreales: Ascomycota) against *Pauropsylla buxtoni* (Psylloidea: Hemiptera) infesting *Ficus carica* leaves and its potential use as biocontrol agent of the insect. **Journal of Applied Microbiology** ISSN 1364-5072. https://doi.org/10.1111/jam.14613.

BLEACKLEY, M.R, DAWSON, C.S, PAYNE, J.A.E, HARVEY, P.J, ROSENGREN, K.J, QUIMBAR, P.,… ANDERSON, M.A (2019). The interaction with fungal cell wall polysaccharides determines the salt tolerance of antifungal plant defensins. **The Cell Surface**, 5, 100026. doi: 10.1016 / j.tcsw.2019.100026.

BLOMBERG, A., ADLER, L. (1992). Physiology of Osmotolerance in Fungi. **Advances in Microbial Physiology**, 145–212. doi:10.1016/s0065-2911(08)60217-9.

BOJKE A, TKACZUK C, STEPNOWSKI P, GOŁĘBIOWSKI M. Comparison of volatile compounds released by entomopathogenic fungi. **Microbiol Res**. 2018 Sep;214:129-136. doi: 10.1016/j.micres.2018.06.011. Epub 2018 Jun 24. PMID: 30031475.

BORUTA, T. (2018) Uncovering the repertoire of fungal secondary metabolites: From Fleming's laboratory to the International Space Station, **Bioengineered,** 9:1, 12-16, DOI: 10.1080/21655979.2017.1341022.

BOWMAN, S.M., FREE, S.J. (2006). The structure and synthesis of the fungal cell wall. **BioEssays**, 28 (8), 799–808. doi: 10.1002 / bies.2044.

BRAGA, G. U. L., RANGEL, D. E. N., FLINT, S. D., MILLER, C. D., ANDERSON, A. J., ROBERTS, D.W. (2002). Damage and recovery from UV-B exposure in conidia of the entomopathogens *Verticillium lecanii* and *Aphanocladium album*. ***Mycologia**, 94*,912-920, doi:http://www.jstor.org/stable/3761859.

BRANCINI, G. T. P., FERREIRA, M. E. S., RANGEL, D. E. N., & BRAGA, G. Ú. L. (2019). Combining transcriptomics and proteomics reveals potential post-transcriptional control of gene expression after light exposure in *Metarhizium acridum*. *G3: **Genes|Genomes|Genetics**, 9*, 2951-2961, doi:10.1534/g3.119.400430.

BRANCINI, GUILHERME T.P et al. Species of the *Metarhizium anisopliae* complex with diverse ecological niches display different susceptibilities to antifungal agents. **Fungal biology**, v.122, n. 6, p. 563-569, 2018.

BREITENBACH, M .; WEBER, M .; RINNERTHALER, M .; KARL, T .; BREITENBACH-KOLLER, L. Oxidative Stress in Fungi: Its Function in Signal Transduction,Interaction with Plant Hosts, and Lignocellulose Degradation **Biomolecules** 2015 , 5 , 318-342.

BROWN A.J.P., COWEN L. E, DI PIETRO A., QUINN J. Stress Adaptation. Microbiol Spectr. 2017 Jul;5(4):10.1128/microbiolspec.FUNK-0048-2016. doi: 10.1128/**microbiolspec.FUNK**-0048-2016. PMID: 28721857; PMCID: PMC5701650.

BROWN, AJP, LARCOMBE, DE, & PRADHAN, A. (2020). Thoughts on the evolution of Core Environmental Responses in yeasts***. Fungal Biology****.* doi: 10.1016 / j.funbio.2020.01.003.

CADET, J.; WAGNER, R. DNA Base Damage by Reactive Oxygen Species, Oxidizing Agents, and UV Radiation. **Cold Spring Harb Perspect Biol** 2013;5:a012559.

CANESSA, P., SCHUMACHER, J., HEVIA, M. A., TUDZYNSKI, P., & LARRONDO, L. F. (2013). Assessing the effects of light on differentiation and virulence of the plant pathogen *Botrytis cinerea*: characterization of the white collar complex. [Article]. ***PLoS One****, 8*(12), doi:10.1371/journal.pone.0084223.

CHANDLER D, BAILEY AS, TATCHELL GM, DAVIDSON G, GREAVES J, GRANT WP. The development, regulation and use of biopesticides for integrated pest management. **Philos Trans R Soc Lond B Biol Sei**. 12 de julho de 2011; 366 (1573): 1987-98. doi: 10.1098 / rstb.2010.0390. PMID: 21624919; PMCID: PMC3130386.

CHANDRASEKARAN, M., THANGAVELU, B., CHUN, SC et al. Proteases de fungos fitopatogênicos e sua importância na fitopatogenicidade. **J Gen Plant Pathol** 82, 233–239 (2016). https://doi.org/10.1007/s10327-016-0672-9.

CHASSEUR, C., SUETENS, C., MICHEL, V., MATHIEU, F., BEGAUX, F., NOLARD, N., HAUBRUGE, E. A 4-year study of the mycological aspects of Kashin-Beck disease in Tibet (2001). **International Orthopaedics** (SICOT) (2001) 25:154–158. DOI 10.1007/s002640000218.

CHEN, C.-H., RINGELBERG, C. S., GROSS, R. H., DUNLAP, J. C., & LOROS, J. J. (2009). Genome wide analysis of light-inducible responses reveals hierarchical light signalling in *Neurospora*. ***The EMBO Journal****, 28*(8), 1029-1042, doi:10.1038/emboj.2009.54.

COCHRANE, V. W. (1958). ***Physiology of Fungi***. New York: John Wiley & Sons, Inc.

DANNON, H.F., DANNON, A.E., DOURO-KPINDOU, O.K. et al. Toward the efficient use of *Beauveria bassiana* in integrated cotton insect pest management. **J Cotton Res** 3, 24 (2020). https://doi.org/10.1186/s42397-020-00061-5.

DARA S. K. Non-Entomopathogenic Roles of Entomopathogenic Fungi in Promoting Plant Health and Growth. **Insects.** 2019 Sep 1;10(9):277. doi: 10.3390/insects10090277. PMID: 31480565; PMCID: PMC6780571.

DASGUPTA, A., CHEN, C.-H., LEE, C., GLADFELTER, A. S., DUNLAP, J. C., & LOROS, J. J. (2015). Biological significance of photoreceptor photocycle length: VIVID photocycle governs the dynamic VIVID-White Collar Complex pool mediating photo adaptation and response to changes in light intensity. ***Plos Genetics****, 11*(5),e1005215, doi:10.1371/journal.pgen.1005215.

DAY A.M, QUINN J. Stress-Activated Protein Kinases in Human Fungal Pathogens. **Front Cell Infect Microbiol.** 2019 Jul 17;9:261. doi: 10.3389/fcimb.2019.00261. PMID: 31380304; PMCID: PMC6652806.

DAY A. M, MCNIFF M. M, DA SILVA DANTAS A, GOW NAR, QUINN J. Hog1 Regulates Stress Tolerance and Virulence in the Emerging Fungal Pathogen Candida auris. **mSphere**. 2018 Oct 24;3(5):e00506-18. doi: 10.1128/mSphere.00506-18. PMID: 30355673; PMCID: PMC6200985.

DE GROOT, P.,W.,J, RAM, A.F. KLIS, F.M (2005). Features and functions of covalently linked proteins in fungal cell walls. **Fungal Genetics and Biology**, 42 (8), 657-675. doi: 10.1016 / j.fgb.2005.04.002.

DE MENEZES, H. D., MASSOLA, N. S., FLINT, S. D., SILVA, G. J., BACHMANN, L., RANGEL, D. E. N., et al. (2015). Growth under visible light increases conidia and mucilage production and tolerance to UV-B radiation in the plant-pathogenic fungus *Colletotrichum acutatum*. ***Photochemistry and Photobiology****, 91*(2), 397-402,
doi:10.1111/php.12410.

DIAS, L. P. (2018). **Luz visível e limitação de oxigênio durante o crescimento micelial de fungos entomopatogênicos alteram expressão gênica e tolerância de conídios a condições de estresse.** Tese de Doutorado, Escola de Engenharia de Lorena, Universidade de São Paulo, Lorena. doi:10.11606/T.97.2018.tde-03122018-184427. Recuperado em 2020-10-23, de www.teses.usp.br.

DIAS, L.P., ARAÚJO, C.A.S., PUPIN, B., FERREIRA, P.C., BRAGA, G.Ú.L., RANGEL, D.E.N., 2018. The Xenon Test Chamber Q-SUN® for testing realistic tolerances of fungi exposed to simulated full spectrum solar radiation. **Fungal Biology**-Uk 122, 592-601. https://doi.org/10.1016/j.funbio.2018.01.003.

DIAS, L. P., PEDRINI, N., BRAGA, G. U. L., FERREIRA, P. C., PUPIN, B., ARAÚJO, C. A. S., et al. (2019). Outcome of blue, green, red, and white light on *Metarhizium robertsii* during mycelial growth on conidial stress tolerance and gene expression. ***Fungal Biology***, doi:https://doi.org/10.1016/j.funbio.2019.04.007.

DURAN, R .; CARY, JW; CALVO, AM Role of the Osmotic Stress Regulatory Pathway in Morphogenesis and Secondary Metabolism in Filamentous Fungi. **Toxins** 2010 , 2 , 367-381.

ESTRADA, A. F., & AVALOS, J. (2008). The White Collar protein WcoA of *Fusarium fujikuroi* is not essential for photocarotenogenesis, but is involved in the regulation of secondary metabolism and conidiation. ***Fungal Genetics and Biology****, 45*(5), 705- 718, doi:https://doi.org/10.1016/j.fgb.2007.12.003.

ESTRADA, A. F., & AVALOS, J. (2009). Regulation and targeted mutation of opsA, coding for the NOP-1 opsin orthologue in *Fusarium fujikuroi*. ***Journal of Molecular Biology****,*
387(1), 59-73, doi:https://doi.org/10.1016/j.jmb.2009.01.057.

FANELLI, F., SCHMIDT-391 HEYDT, M., HAIDUKOWSKI, M., SUSCA, A., GEISEN, R., LOGRIECO, A., et al. (2012). Influence of light on growth, conidiation and fumonisin production by Fusarium verticillioides. **Fungal Biol**, 116(2), 241-248, doi:10.1016/j.funbio.2011.11.007.

FERNANDES, É. KK, RANGEL, DEN, MORAES, Á. ML, BITTENCOURT, VREP E ROBERTS, DW (2007). Variabilidade na tolerância à radiação UV-B entre Beauveria spp. isolados. **Journal of Invertebrate Pathology**, 96 (3), 237–243. doi: 10.1016 / j.jip.2007.05.007.

FERNANDES, É.KK, RANGEL, DEN, BRAGA, GUL *et al.* Tolerance of entomopathogenic fungi to ultraviolet radiation: a review of strain screening and its formulation. ***Curr Genet*** 61**,** 427–440 (2015). https://doi.org/10.1007/s00294-015-0492-z

FERREIRA, D. F. (1999). SISVAR 4.3. **Sistema de análises estatísticas**. (CD-ROM ed.). Lavras, MG, Brazil: Universidade Federal de Lavras, UFLA.

FERREIRA, D. F. (2011). Sisvar: a computer statistical analysis system. ***Ciência e Agrotecnologia**, 35*, 1039-1042.

FREEMAN, G., MORRISON, R. Trichothecin : an Antifungal Metabolic Product of *Trichothecium roseum* Link. ***Nature*** 162, 30 (1948). https://doi.org/10.1038/162030a0.

FUCHS, B.B. MYLONAKIS, E. (2009). Our Paths Might Cross: the Role of the Fungal Cell Wall Integrity Pathway in Stress Response and Cross Talk with Other Stress Response Pathways. **Eukaryotic Cell**, v.8, Nov. 2009, p. 1616–1625.

FUCHS, B.B; MYLONAKIS, E. Our Paths Might Cross: the Role of the Fungal Cell WallIntegrity Pathway in Stress Response and Cross Talk with Other Stress Response Pathways. **Eukaryotic Cell**, Nov. 2009, p. 1616–1625 Vol. 8, No. 11 1535-9778/09/$12.00 doi:10.1128/EC.00193-09

FULLER K.K, LOROS J.J, DUNLAP J.C. Fungal photobiology: visible light as a signal for stress, space and time. **Curr Genet.** 2015 Aug;61(3):275-88. doi: 10.1007/s00294-014-0451-0. Epub 2014 Oct 17. PMID: 25323429; PMCID: PMC4401583.

FULLER, K. K., CRAMER, R. A., ZEGANS, M. E., DUNLAP, J. C., & LOROS, J. J. (2016). *Aspergillus fumigatus* photobiology illuminates the marked heterogeneity between isolates. ***MBio**, 7*(5), doi:10.1128/mBio.01517-16.

FULLER, K. K., RINGELBERG, C. S., LOROS, J. J., & DUNLAP, J. C. (2013). The fungal pathogen *Aspergillus fumigatus* regulates growth, metabolism, and stress resistance in response to light. ***MBio**, 4*(2), doi:10.1128/mBio.00142-13.

GARCIA-RUBIO, R.; OLIVEIRA, H. C.; RIVERA, J.; TREVIJANO-CONTADOR, N. The Fungal Cell Wall: Candida, Cryptococcus, and Aspergillus Species. **Frontiers in Microbiology** , (10) January (2020).doi: 10.3389/fmicb.2019.02993

GOTTLIEB, D. (1950). The Physiology of Spore Germination in Fungi. ***Botanical Review**, 16*(5), 229-257.

GOW, N. LATGE J., MUNRO, C. 2017. The Fungal Cell Wall: Structure, Biosynthesis, and Function, p 267-292. Em Heitman J, Howlett B, Crous P, Stukenbrock E, James T, Gow N (ed), **The Fungal Kingdom** . ASM Press, Washington, DC. doi: 10.1128 / microbiolspec.FUNK-0035-2016.

GRIFFITHS, H.R., MISTRY, P., HERBERT, K.E., LUNEC, J. Molecular and cellular effects of ultraviolet light-induced genotoxicity. **Crit. Rev. Clin. Lab. Sci.** v. 35, p. 189-237, 1998.

HARDING D.P, RAIZADA M.N. Controlling weeds with fungi, bacteria and viruses: a review. **Front Plant Sci**. 28 de agosto de 2015; 6: 659. doi: 10.3389 / fpls.2015.00659. PMID: 26379687; PMCID: PMC4551831.

HONG S.Y, ROZE L.V, LINZ J.E. Oxidative stress-related transcription factors in the regulation of secondary metabolism. **Toxins** (Basel). 2013 Apr 18;5(4):683-702. doi: 10.3390/toxins5040683. PMID: 23598564; PMCID: PMC3705287.

HOPKE, A., BROWN, A.J.P., HALL, R.A., WHEELER, R.T. (2018). Dynamic Fungal Cell Wall Architecture in Stress Adaptation and Immune Evasion. **Trends in Microbiology**, 26 (4), 284–295. doi: 10.1016 / j.tim.2018.01.007.

HOSSAIN, M. T., KHAN, A., CHUNG, E. J., RASHID, M. H.-O., & CHUNG, Y. R. (2016). Biological control of rice bakanae by an endophytic *Bacillus oryzicola* YC7007.***The plant pathology journal****, 32*(3), 228-241, doi:10.5423/ppj.oa.10.2015.0218.

HU, Z., TAO, Y., TAO, X., SU, Q., CAI, J., QIN, C., ... LI, C. (2019). Sesquiterpenes with Phytopathogenic Fungi Inhibitory Activities from Fungus Trichoderma virens from Litchi chinensis Sonn. **Journal of Agricultural and Food Chemistry**. doi: 10.1021 / acs.jafc.9b04053.

IDNURM, A., & HEITMAN, J. (2005). Light controls growth and development via a conserved pathway in the fungal kingdom. [Article]. ***Plos Biology****, 3*(4), 615-626, doi:10.1371/journal.pbio.0030095.

IDNURM, A., VERMA, S., & CORROCHANO, L. M. (2010). A glimpse into the basis of vision in the kingdom Mycota. ***Fungal Genetics and Biology****, 47*(11), 881-892, doi:10.1016/j.fgb.2010.04.009.

Iglesias, M., Ballesta, J.P.G. Mechanism of resistance to the antibiotic trichothecin in the producing fungi. **Eur. J. Biochem**. 223,447-453 (1994).

IGBALAJOBI, O., YU, Z., & FISCHER, R. (2019). Red- and blue-light sensing in the plant pathogen *Alternaria alternata* depends on phytochrome and the white-collar proteinLreA. ***MBio****, 10*(2), e00371-00319, doi:10.1128/mBio.00371-19.

IKEDA, K., PARK, P., NAKAYASHIKI, H. (2019). Cell biology in phytopathogenic fungi during host infection: commonalities and differences. **Journal of General Plant Pathology.** doi: 10.1007 / s10327-019-00846-w.

ISHII, K., KOBAYASHI, J., UENO, Y., ICHINOE, M. Occurrence of Trichothecin in Wheat. **Applied and Environmental Microbiology**, Aug. 1986, Vol. 52, p. 331-333.

KAISER D, BACHER S, MÈNE-SAFFRANÉ L, GRABENWEGER G. Efficiency of natural substances to protect Beauveria bassiana conidia from UV radiation. **Pest Manag** Sci. 2019 Feb;75(2):556-563. doi: 10.1002/ps.5209. Epub 2018 Oct 25. PMID: 30221461; PMCID: PMC6587961.

KARÁNYI Z, HOLB I, HORNOK L, PÓCSI I, MISKEI M. FSRD: fungal stress response database. **Database** (Oxford). 2013 Jun 11;2013:bat037. doi: 10.1093/database/bat037. PMID: 23757396; PMCID: PMC3678302.

KENDRICK, W.B., COLE, G.T. Conidium ontogeny in hyphomycetes . *Trichothecium roseum* and its meristem arthrospores(1969). **Canadian Journal Botany**, 47: 345-350.

KIM, J.S., KASSA, A., SKINNER, M., HATA, T., PARKER, B.L. (2010). Production of thermotolerant entomopathogenic fungal conidia on millet grain. **Journal of Industrial Microbiology & Biotechnology**, 38 (6), 697-704. doi: 10.1007 / s10295-010-0850-2.

KOPECKÁ, MARIE; GABRIEL, MIROSLAV. The influence of Congo red on the cell wall and(1→ 3)-β-d-glucan microfibril biogenesis in Saccharomyces cerevisiae. **Archives of Microbiology,** v. 158, n. 2, p. 115-126, 1992.

KUMAR. J. Biopesticides: A Need for Food and Environmental Safety. **Biofertil Biopestici** (2012), 3:4http://dx.doi.org/10.4172/2155-6202.1000e107.

KUMAR, S. SINGH, A. Biopesticides: Present Status and the Future Prospects. **J Fertil Pestic** (2015), 6:2. http://dx.doi.org/10.4172/jbfbp.1000e129.

KUNZ, B.A., CAHILL, D.M., MOHR, P.G., OSMOND, M.J., VONARX, E.J. Plant responses to UV radiation and links to pathogen resistance. **International Rev. Cytol**. v. 255, p. 1-40, 2007.

LATGÉ, J.P. The cell wall: a carbohydrate armour for the fungal cell. **Molecular Microbiology** (2007) *66 (2), 279–290.* doi: 10.1111 / j.1365-2958.2007.05872.x.

LAUTER, F.-R., YAMASHIRO, C. T., & YANOFSKY, C. (1997). Light stimulation of conidiation in *Neurospora crassa*: Studies with the wild-type strain and mutants wc-1, wc-2 and acon-2. ***Journal of Photochemistry and Photobiology B: Biology**, 37*(3), 203-211, doi:https://doi.org/10.1016/S1011-1344(96)07405-2.

LEACH, M.D, COWEN, L.E. (2013) Surviving the Heat of the Moment: A Fungal Pathogens Perspective. **PLoS Pathog** 9 (3): e1003163. https://doi.org/10.1371/journal.ppat.1003163

LEE, K., SINGH, P., CHUNG, W.-C., ASH, J., KIM, T. S., HANG, L., PARK, S., 2006. Light regulation of asexual development in the rice blast fungus, Magnaporthe oryzae. **Fungal Genetics and Biology.** 43, 694-706.

LI Q, HARVEY L. M, MCNEIL, B. (2009) Oxidative stress in industrial fungi, Critical Reviews in Biotechnology, 29: 3, 199-213, DOI: 10.1080 / 07388550903004795.

LILLY, V. G., & BARNETT, H. L.(1951). ***Physiology of the Fungi***. New York: McGraw-Hill Book Company.

MAHLO S. M, CHAUKE H.R, MCGAW L, ELOFF J. Antioxidant and antifungal activity of selected medicinal plant extracts against phytopathogenic fungi. **Afr J Tradit Complement Altern** Med. 3 de julho de 2016; 13 (4): 216-222. doi: 10.21010 / ajtcam.v13i4.28. PMID: 28852739; PMCID: PMC5566147.

MARCANO, D. HASEGAWA, M. **Fitoquímica orgánica/Deanna Marcano. Consejo de Desarolho Cientifico y Humanístico**. Universidad Central de Venezuela. Caracas, 2002, degunda edición. ISBN: 980-00-2066-7. 309 p.

MAY-DE MIO, L.L., NEGRI, G.,MICHAILIDES, T.J. (2014) Effect of Trichothecium roseum, lime sulphur and phosphites to control blossom blight and brown rot on peach. **Canadian Journal of Plant Pathology**, 36: 4, 428-437 , DOI: 10.1080 / 07060661.2014.964778.

MILNER, R. J., HUPPATZ, R. J., & SWARIS, S. C. (1991). A new method for assessment of germination of *Metarhizium* conidia. [Milner, R.J., Huppatz, R.J., Swaris, S.C. 1991. A new method for assessment of germination of *Metarhizium* conidia. J. Invertebr. Pathol. 57, 121-123.]. ***J. Invertebr. Pathol**., 57*, 121-123.

NEGRI, G., MAY-DE MIO, L.L., WORDELL FILHO, J. A. Production and storage of *Trichothecium roseum* to use as biopesticide. **Scientia Agraria**, Curitiba, v.11, n.3, p.247-254, May/Jun. 2010.

NOBEL, H.; ENDE, H.; KLIS, F. M. Cell wall maintenance in fungi (2000). Comment. **Trends in Microbiology** 344 Vol. 8 No. 8 August 2000.

OGAWA, KOZO; DOHMARU, TAKAAKI; YUI, TOSHIFUMI. Dependence of complex formation of (1→ 3)-β-D-glucan with Congo Red on temperature in alkaline solutions. **Bioscience, biotechnology, and biochemistry**, v. 58, n. 10, p. 1870-1872, 1994.

OLIVEIRA, A. S., BRAGA, G. U. L., & RANGEL, D. E. N. (2018). *Metarhizium robertsii* illuminated during mycelial growth produces conidia with increased germination speed and virulence. ***Fungal Biology**, 122*(6), 555-562, doi:https://doi.org/10.1016/j.funbio.2017.12.009.

OROSZ E, VAN DE WIELE N, EMRI T, ZHOU M, ROBERT V, DE VRIES RP, PÓCSI I. Fungal Stress Database (FSD)--a repository of fungal stress physiological data. **Database** (Oxford). 2018 Jan 1;2018:bay009. doi: 10.1093/database/bay009. PMID: 29688353; PMCID: PMC5810435.

ORTIZ-URQUIZA, A.; KEYHANI, N. Action on the Surface: Entomopathogenic Fungi versus the Insect Cuticle (2013). ***Insects*** 2013, *4*, 357-374; doi:10.3390/insects4030357

PANCALDI, SIMONETTA et al. Morphological anomalies induced by Congo red in *Aspergillus niger*. **Archives of microbiology**, v. 137, n. 3, p. 185-187, 1984.

PAN, H., WEI Y., XIN, F., ZHOU, M., ZHANG S. Characterization and biocontrol ability of fusion chitinase in Escherichia coli carrying chitinase cDNA from *Trichothecium roseum.* **Z Naturforsch C J Biosci**. 2006 May-Jun;61(5-6):397-404. doi: 10.1515/znc-2006-5-616. PMID: 16869499.

PAPAGIANNI, M. (2004). Fungal morphology and metabolite production in submerged mycelial processes. **Biotechnology Advances**, 22 (3), 189–259. doi: 10.1016 /j.biotechadv.2003.09.005.

PEREZ-NADALES, E.; NOGUEIRA, M.F.A.; BALDIN, C. et al. Fungal model systems and the elucidation of pathogenicity determinants. **Fungal Genetics and Biology** 70 (2014) 42-67.

PIERISTÈ, M., FOREY, E., LOUNÈS-HADJ SAHRAOUI, A., MEGLOULI, H., LARUELLE, F., DELPORTE, P., et al. (2020). Spectral composition of sunlight affects the microbial functional structure of beech leaf litter during the initial phase of decomposition. ***Plant and Soil*****,** doi:10.1007/s11104-020-04557-6.

PRADO, M. M., PRADO-CABRERO, A., FERNÁNDEZ-MARTÍN, R., & AVALOS, J. (2004). A gene of the opsin family in the carotenoid gene cluster of *Fusarium fujikuroi*. ***Current Genetics**, 46*(1), 47-58, doi:10.1007/s00294-004-0508-6.

QUAITE, F. E., SUTHERLAND, B. M., & SUTHERLAND, J. C. (1992). Action spectrum for DNA damage in alfalfa lowers predicted impact of ozone depletion. [Quaite, F.E, Sutherland, B.M., Sutherland, J.C., 1992. Action spectrum for DNA damage in alfalfa lowers predicted impact of ozone depletion. Nature 358, 576-578.]. ***Nature**, 358*, 576-578.

RAM, ARTHUR FJ; KLIS, FRANS M. Identification of fungal cell wall mutants using susceptibility assays based on Calcofluor white and Congo red. **Nature protocols**, v. 1, n. 5, p. 2253, 2006.

RAMIREZ, J., L., DUNLAP, C., A., MUTURI, E.J., BARLETTA, A.B. AND ROONEY, A.P. (2018) Entomo-pathogenic fungal infection leads to temporospatial modulation of the mosquito immune system. **PLoS Negl Trop Dis** 12, e0006433. https://doi.org/10.1371/journal.pntd.0006433.

RANGEL, D.E.N., ANDERSON, A.J., ROBERTS, D.W., 2008. Evaluating physical and nutritional stress during mycelial growth as inducers of tolerance to heat and UV-B radiation in *Metarhizium anisopliae* conidia. **Mycol. Res**. 112, 1362e1372. https://doi.org/10.1016/j.mycres.2008.04.013.

RANGEL, D.E.N., BRAGA, G.U.L., FERNANDES, É.K.K., KEYSER, C.A., HALLSWORTH, J.E., ROBERTS, D.W., 2015. Stress tolerance and virulence of insect-pathogenic fungi are determined by environmental conditions during conidial formation. **Current Genetics**. 61, 383e404. https://doi.org/10.1007/s00294-015-0477-y.

RANGEL, D.E.N. Stresse induced cross-protectin against environmental challenges on prokaryotic and eukaryotic microbes. **World Journal of Microbiology and Biotechnology, v.27**, n. 6, p. 1281 – 1296 ,16 jun. 2011.

RANGEL, D. E. N., BRAGA, G. U. L., FLINT, S. D., ANDERSON, A. J., & ROBERTS, D. W. (2004). Variations in UV-B tolerance and germination speed of *Metarhizium anisopliae* conidia produced on artificial and natural substrates. ***Journal of Invertebrate Pathology**, 87*, 77-83, doi:https://doi.org/10.1016/j.jip.2004.06.007.

RANGEL, D. E. N., FERNANDES, E. K. K., BRAGA, G. U. L., & ROBERTS, D. W. (2011). Visible light during mycelial growth and conidiation of *Metarhizium*

robertsii produces conidia with increased stress tolerance. ***FEMS Microbiol**. Lett., 315*(2), 81-86, doi:10.1111/j.1574-6968.2010.02168.x.

ROBERTSON, R., SMITHIES,W.R.,TITTENSO, E. The Chemistry of Fungi. Part VI. Rosenonolactone fyom *Trichothecium roseum* Link. Published on 01 January 1949. **Journal Chemtry Society**, 1949, 879-884 Downloaded by University of Reading on 03/01/2018 03:34:43.

SANCHEZ-MURILLO, R. I., DE LA TORRE-MARTINEZ, M., AGUIRRE-457 LINARES, J., & HERRERA-ESTRELLA, A. (2004). Light-regulated asexual reproduction in *Paecilomyces fumosoroseus*. ***Microbiology,** 150*(Pt 2), 311-319.

SHAMSI, S., SULTANA, R. *Trichothecium roseum* Link - a new record of hyphomycetous fungus for Bangladesh. **Bangladesh Journal. Plant Taxonomy**. 15(1): 77-80, 2008 (June).

SPANU, P. D. PANSTRUGA, R. (2017). Editorial: Biotrophic Plant-Microbe Interactions. **Frontiers in Plant Science**. doi: 10.3389/fpls.2017.00192

TIAGO, P. V.; OLIVEIRA, N.T.; LIMA, E.A.L.A. Controle biológico de insetos com Metarhizium anisopliae: aspectos morfológicos, moleculares e ecológicos. **Cienc. Rural** , Santa Maria, v. 44, n. 4, pág. 645-651, abril de 2014. doi.org/10.1590/S0103-84782014000400012.

TISCH, D., & SCHMOLL, M. (2010). Light regulation of metabolic pathways in fungi. *Appl **Microbiol Biotechnol**, 85*(5), 1259-1277, doi:10.1007/s00253-009-2320-1.

TIWARI, S., THAKUR, R., & SHANKAR, J. (2015). Role of Heat-Shock Proteins in Cellular Function and in the Biology of Fungi. **Biotechnology research international**, 2015, 132635. https://doi.org/10.1155/2015/13263.

TSUGE T, HARIMOTO Y, AKIMITSU K, OHTANI K, KODAMA M, AKAGI Y, EGUSA M, YAMAMOTO M, OTANI H. Host-selective toxins produced by the plant pathogenic fungus Alternaria alternata. FEMS Microbiol Rev. 2013 Jan;37(1):44-66. doi: 10.1111/j.1574-6976.2012.00350.x. **Epub** 2012 Aug 24. PMID: 22846083.

VALIANTE V. (2017). The Cell Wall Integrity Signaling Pathway and Its Involvement in Secondary Metabolite Production. **Journal of fungi** (Basel, Suíça) , 3 (4), 68. https://doi.org/10.3390/jof3040068.

VANNINI, GIAN LUIGI et al. Effects of Congo red on wall synthesis and morphogenesis in *Saccharomyces cerevisiae*. **Plant science letters**, v. 31, n. 1, p. 9-17, 1983.

VINNERE PETTERSSON, O.; LEONG, S. Fungal Xerophiles (Osmophiles) *. In: eLS. John Wiley & Sons,* ***Ltd: Chichester****. DOI: 10.1002/9780470015902.a0000376.pub2*

WANG, C., WANG, S. (2017). Insect Pathogenic Fungi: Genomics, Molecular Interactions, and Genetic Improvements. **Annual Review of Entomology**, 62 (1), 73–90. doi: 10.1146 / annurev-ento-031616-035509.

WAKIL, W., BRUST, G. E., PERRING, T.M. Susteainable management of arthropod pests of tomato. **Academic Press**. ISBN: 9780128135082.

WARRIS, A. BALLOU, E., R. Oxidative responses and fungal infection biolgy. **Seminars in Cell & Developmental Biology**, 89, mai, 2019 34-46. https://doi.org/10.1016/j.semcdb.2018.03.004.

WEI, M., GE, Y., LI, C. CHEN Y., WANG, W. DUAN, B., LI, X. Antifungal activity of ε-poly-L-lysine on *Trichothecium roseum* in vitro and its mechanisms. **Physiological and Molecular Plant Pathology** (2018), doi: 10.1016/j.pmpp.2018.04.005.

WU, C., YANG, F., SMITH, K. M., PETERSON, M., DEKHANG, R., ZHANG, Y., et al. (2014). Genome-wide characterization of light-regulated genes in *Neurospora crassa*. *G3-* ***Genes Genomes Genetics****, 4*(9), 1731-1745, doi:10.1534/g3.114.012617.

XIAO, G.; YING, S.H.; ZHENG, P.; WANG, Z.L.; ZHANG, S.; XIE, X.Q.; SHANG, Y.; ST LEGER, R.J.; ZHAO, G.P.; WANG, C.; *et al.* Genomic perspectives on the evolution of fungal entomopathogenicity in *Beauveria bassiana*. ***Sci. Rep****.* 2012, *2*, 483.

XIAN, H., LI, J., ZHANG, L., LI, D. Cloning and functional analysis of a novel chitinase gene *Trchi1* from *Trichothecium roseum.* 2012. **Biotechnol Lett** (2012) 34:1921–1928. DOI 10.1007/s10529-012-0989-1.

YU, S. M., RAMKUMAR, G., & LEE, Y. H. (2013). Light quality influences the virulence and physiological responses of *Colletotrichum acutatum* causing anthracnose in pepper plants. ***Journal of Applied Microbiology****, 115*(2), 509-516, doi:10.1111/jam.12252.

YU, Z., & FISCHER, R. (2019). Light sensing and responses in fungi. ***Nat. Rev. Microbiol.,*** *17*(1), 25-36, doi:10.1038/s41579-018-0109-x.

ZACCARIA M, LUDOVICI M, SANZANI SM, IPPOLITO A, CIGLIANO RA, SANSEVERINO W, SCARPARI M, SCALA V, FANELLI C, REVERBERI M. Menadione-Induced Oxidative Stress Re-Shapes the Oxylipin Profile of Aspergillus flavus and Its Lifestyle. **Toxins** (Basel). 2015 Oct 23;7(10):4315-29. doi: 10.3390/toxins7104315. PMID: 26512693; PMCID: PMC4626736.

ŽABKA, M., DRASTICHOVÁ, K., JEGOROV, A. et al. Direct evidence of plant-pathogenic activity of fungal metabolites of Trichothecium roseum on apple. **Mycopathologia** 162, 65–68 (2006). https://doi.org/10.1007/s11046-006-0030-0.

ZHANG, X., LI, G., MA, J., ZENG, Y., MA, W. AND ZHAO, P. (2010) Endophytic fungus *Trichothecium roseum* LZ93 antagonizing pathogenic fungi in vitro and its secondary metabolites. **Journal Microbiology** 48, 784–790. https://doi.org/10.1007/s12275-010-0173-z.

ZHANG, Y.-J., LI, Z.-H., LUO, Z.-B., ZHANG, J.-Q., FAN, Y.-H., & PEI, Y. (2009). Light stimulates conidiation of the entomopathogenic fungus *Beauveria bassiana*. ***Biocontrol Sci. and Technol.,*** *19*(1), 91 - 101, doi:10.1080/09583150802588516

ZHENG, P.; XIA, Y.L.; XIAO, G.H.; XIONG, C.H.; HU, X.; ZHANG, S.W.; ZHENG, H.J.; HUANG, Y.; ZHOU, Y.; WANG, S.Y.; *et al.* Genome sequence of the insect pathogenic fungus *Cordyceps militaris*, a valued traditional chinese medicine. ***Genome Biol****.* 2011, *12*, doi:10.1186/gb-2011-12-11-r116.

ATTACHMENT A - Published article

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Different wavelengths of visible light influence the conidial production and tolerance to ultra-violet radiation of the plant pathogens *Colletotrichum acutatum* and *Fusarium fujikuroi*

Tacyana P. C. Costa · Eliane M. Rodrigues · Luciana P. Dias · Breno Pupin · Paulo C. Ferreira · Drauzio E. N. Rangel

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Abstract The effects of the visible light wavelengths on germination, mycelial radial growth, and conidial production of the plant pathogens *Colletotrichum acutatum* and *Fusarium fujikuroi* were studied. Both fungi were grown on potato dextrose agar medium (PDA) in the dark (control) or on PDA under continuous white, blue, green or red light. In addition, the conidia from each treatment were exposed to UV radiation. The germination and growth of both plant pathogenic fungi were not affected by any of the treatments. *C. acutatum* produced more conidia when the fungus grew under white and red light. *F. fujikuroi* produced more conidia in the dark. The tolerances to UV radiation of conidia produced on different light and dark treatments differed for both *C. acutatum* and *F. fujikuroi*. Conidia of *C. acutatum* were at least 30% more tolerant to UV radiation when they were produced under white light than under blue and green light and at least 20% more tolerant than conidia produced in the dark. Conidia of *C. acutatum* produced under red light were the least tolerant. Conidia of *F. fujikuroi* produced under white and blue light were at least 30% more UV tolerant than conidia produced in the dark, green, and red light. In conclusion, no differences were found for germination and growth for both fungi under different light regimes and dark; however, significant differences occurred both in production and UV radiation of conidia.

Keywords Photobiology · Stress tolerance · UV radiation · Germination · Mycelial growth

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Introduction

Most organisms on Earth sense visible light (380 to 740 nm) through the use of photoreceptive proteins specifically adapted to respond to it (Dasgupta et al. 2015). The response of fungi are reflected in different physiological responses such as conidial production (Yu et al. 2013; de Menezes et al. 2015), conidial stress tolerance (Idnurm and Heitman 2005; Rangel et al. 2011; Fuller et al. 2013; Aver'yanov et al. 2014; de Menezes et al. 2015; Rangel et al. 2015; Dias et al. 2020), conidial pigmentation (Fuller et al. 2013; Yu et al. 2013), virulence (Yu et al. 2013; Aver'yanov et al. 2014; Oliveira et al. 2018), germination speed (Fuller et al. 2013; Oliveira et al. 2018), and secondary metabolism (Tisch and Schmoll 2010; Fanelli et al. 2012). Fungal molecular biology studies have identified

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AVALIAÇÃO DO Trichothecium roseum AO 4-NITROQUINOLINA-1-OXIDO (4-NQO)

Tacyana P.C. Costa*[1]*, Alcides P. Brito [2], Eliane M. Rodrigues[3], Eliezer P. Andrade [4], Drauzio. E. N. Rangel [5]

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Resumo: Foi avaliada a germinação dos fungos entomopatogênicos *Trichothecium roseum, Metarhizium robertsii* e *Metarhizium acridum* sob condições de estresse ao 4-nitroquinolina-1-óxido (4-NQO). Todos os fungos foram cultivados em meio de ágar batata dextrose (PDA). As germinações foram observadas 24 h após a inoculação das suspensões de conídios no meio e mantidas a 26 ºC no escuro. Foram avaliados pelo menos 300 conídios por placa. Ao confrontarmos o *T. roseum*, o *M. robertsii* e o *M. acridum*, sob condições de estresses, constamos que o *T. roseum* é mais tolerante em relação aos outros quando em situação de estresse ao 4-NQO.

Palavras-chave: *Trichothecium roseum*, fungos entomopatogênicos, estresse genotóxico.

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