Tropical Plant Pathology, vol. 39(5):392-400, 2014

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RESEARCH ARTICLE

Effects of UV-B radiation on *Lecanicillium* spp., biological control agents of the coffee leaf rust pathogen

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ABSTRACT

Coffee leaf rust is the main disease of coffee and its causal agent is naturally hyperparasited by *Lecanicillium lecanii*, indicating its potential for biocontrol. Ultraviolet-B (UV-B) radiation is an important factor that interferes on application of biocontrol agents, and *Lecanicillium* can be affected by UV-B. The objective of this work was to evaluate the effects of UV-B on *Lecanicillium* isolates and on its capacity to colonize rust lesions. There were variations among *Lecanicillium* strains in sensitivity to UV-B radiation, causing inactivation and delayed spore germination. The most tolerant strain (CCMA-1143) had LD_{50}=1.63 kJ/m^2 of UV-B. The incidence and colonization of *Lecanicillium* on coffee leaf rust lesions were influenced by the dose of UV-B radiation, and were increased when the isolate CCMA-1143 was sprayed on rust lesions. The effects of UV-B should be considered on efficacy studies for the development of biopesticides.
Key words: Climate change, UV-B, *Hemileia vastatrix*, biopesticide.

INTRODUCTION

Coffee has great importance to the Brazilian economy. Brazil is the largest producer and exporter of coffee, with a planted area of 2.3 million ha. In 2012, the production was 50.8 million bags of 60 kg (CONAB, 2014). Coffee leaf rust, the most important disease to this crop, caused by *Hemileia vastatrix*, is controlled with the application of fungicides (Maffia et al., 2009). However, the intense use of fungicides negatively impacted the environment (Pal & Gardener, 2006; Lopes, 2009) leading to an urgent need to develop alternative methods to control the disease. According to Haddad et al. (2009), the use of biological control agents is one alternative. *Lecanicillium lecanii* (sin. *Verticillium lecanii*, *Verticillium hemileiae*), which can be found naturally hyperparasiting uredinospores of *H. vastatrix* in coffee rust lesions (Shaw, 1988; Vandermeer et al., 2009) among others (Spencer & Atkey, 1981; Leinhos & Buchenauer, 1992), may be considered a biological control agent. The role of this antagonist in the reduction of coffee leaf rust was also observed by Jackson et al. (2012).

Lecanicillium lecanii is a biocontrol agent of rust diseases and insects. However, may be less its efficiency may be reduced in the field or greenhouse due to solar UV radiation, which is harmful to several biological control agents. Ultraviolet radiation can be classified in UV-C (100-280 nm), UV-B (280-315 nm) and UV-A (315-400 nm). UV-C radiation is completely filtered by the ozone layer and absorbed by other atmospheric gases (Kuluncsics et al. 1999). UV-B and UV-A radiations can cause cellular membrane disorganization, protein denaturation, oxidative stress, and damage to DNA, RNA, and ribosomes (Griffiths et al., 1998), and possibly cause damage to several organisms, such as plant pathogens, insects pests, plants and beneficial organisms, impacting the agroecosystems (Caldwell et al., 2003). UV-A and UV-B radiation can inactivate the structures of biological control agents due to genetic and morphological changes, resulting in lower efficacy of the biocontrol agent (Braga et al., 2001c), and is considered one of the main limitations for applying these organisms in the field (Braga et al., 2001b; Costa et al., 2012; Costa et al., 2013).

The effects of UV-B radiation on biocontrol agents have been studied for *Clonostachys rosea,* biocontrol agent of *Botrytis cinerea* (Costa et al., 2012; Costa et al. 2013), the causal agent of gray mold; and *Metarhizium anisopliae*, which is used to control insect pests in several crops (Braga et al., 2001bd; Rangel et al., 2005). Costa et al. (2012) observed that *C. rosea* isolates showed different sensitivities to UV-B radiation. Braga et al. (2001d), Rangel et al. (2004) and Rangel et al. (2006) observed high phenotypic plasticity of *M. anisopliae* to UV-B radiation, according to the growth media and geographical origin of the isolate.

UV-B radiation has harmful effects to host plants, pathogenic organisms, and on plant-pathogen interactions (Ghini et al., 2012). UV-B radiation reduced the ability of *C. rosea* to control *B. cinerea*, and the presence and sporulation of *C. rosea* on strawberry leaf discs was influenced by the dose of UV-B radiation (Costa et al., 2013).

Little is known on the effect of UV-B radiation on biocontrol agents and considering the importance of this factor on the efficacy of antagonistic and the growing market of the biopesticides in Brazil (Bettiol, 2011) and other

countries, the objectives of this work was to evaluate the effects of UV-B radiation on several isolates of *Lecanicillium,* including their capacity to colonize coffee leaf rust lesions.

MATERIAL AND METHODS

Isolates and inocula preparation

Nine isolates of *L. lecanii* and one isolate of *Lecanicillium longisporum* obtained from different regions in Brazil were used in this study (Table 1). The isolates were deposited in the collection of microorganisms of Embrapa Meio-Ambiente. Isolates were grown on potato-dextrose-agar (PDA) (Acumedia) + 1 g/L of streptomycin sulfate (Sigma) in plates of polystyrene (50×10 mm) and incubated at 22±1ºC and 12 h light/12 h dark for 7 to 10 days. In the preliminary tests to establish the appropriate irradiance and incubation period for conidia germination evaluation, the strain CCMA-1144 of *L. longisporum* was used. Conidia were suspended in distilled water with tween 80 (0.01% v/v) and suspensions were filtered through a triple layer of sterilized cheesecloth to remove hyphal fragments and spore aggregates. The concentrations of conidia suspension were estimated by hemocytometer counts for immediate use in the irradiation and germination studies.

UV-B chambers

The irradiation experiments were conducted in chambers with four UV-B 313EL lamps (Q-lab). Twenty minutes prior to the experiments lamps were turned on resulting in a stable level of irradiation. The lamps were covered with a 0.13 mm–thick cellulose diacetate film (Málaga Ltda.), which had a cutoff point at 290 nm. This permitted the passage of most UV-B and UV-A (290-400 nm), but prevented exposure to UV-C (280 nm) and short-wavelength UV-B (290 nm). This film was changed every experiment. Control-plates were physically protected from radiation with aluminum foil. The temperature where the bioassays were conducted was adjusted to 22±2ºC.

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 the weighted UV irradiances (mW/m^2). We selected this spectral weighting function based on the fact that Paul *et al.* (1997) reviewed the spectral characteristics of nine fungal responses and concluded that this DNA damage spectrum closely approximated the fungal responses. All the light measurements were done with a spectroradiometer (Ocean Optics model USB2000 + rad) connected to a portable computer.

Conidial germination

Conidial suspension of *Lecanicillium* (20 µl, 10^5 conidia/mL) was placed on 7 mL-agar medium (PDA + 1 g/L of streptomycin sulfate) in polystyrene plates (50×10 mm) and immediately exposed to the UV-B radiation for evaluation of germination. Lactofenol + 0.05% tripan blue was used to interrupt conidial germination and growth of the germ tube. Observations were done in an optical microscope at 250×. A total of 300 conidia per plate were evaluated and considered germinated when the germ tube was longer than the diameter of the conidia. Relative percent germination was calculated according to Braga et al. (2001a), by the following equation: relative germination (%) = (Wt/Wc) x 100, where Wt is the number of germlings at exposure time t and Wc is the number of germlings of the control plate. The experiment was repeated three times.

Irradiance and incubation period to evaluate spore germination

Conidia of strain CCMA-1144 of *L. longisporum* were produced and placed on PDA + 1 g/L of streptomycin sulfate in polystyrene plates as described and exposed to 649, 452 and 378 mW/m^2 of UV-B radiation. For this, the plates were positioned at 18, 33, and 48 cm from the lamps, respectively. After 2 h exposure, doses corresponded to 4.68, 3.26 and 2.70 kJ/m^2, respectively. After irradiated, Petri dishes were incubated at 22±1ºC, in the dark. Germination of conidia was evaluated at 12, 24 and 36 h after exposure.

Previous trials with irradiance of 452 mW/m^2 (33 cm from the lamp) were done to establish the best incuba-

TABLE 1 - Origin of the *Lecanicillum* spp. strains used in this study.

Isolate	Origin	Local	Elevation (m)
CCMA-1144 (*L. longisporum*)	U	Vertirril (Itaforte Ltda.)	Ni
CCMA-1140 (*L. lecanii)*	U	UFLA/Lavras, MG	Ni
CCMA-1139 (*L. lecanii*)	U	UFV/Viçosa, MG	Ni
OTC-1 (*L. lecanii*)	Coffee	Jaguariúna, SP	580
OTC-2 (*L. lecanii*)	Coffee	Jaguariúna, SP	580
CCMA-1142 (*L. lecanii*)	Coffee	Jaguariúna, SP	580
CCMA-1138 (*L. lecanii*)	Coffee	Jaguariúna, SP	580
CCMA-1141 *L*(*L. lecanii*)	Coffee	Pedreira, SP	900
CCMA-1143 (*L. lecanii*)	Coffee	Serra Negra, SP	1050
SGI-01 (*L. lecanii*)	Coffee	Jaguariúna, SP	580

U = Unknown

tion period after exposure to UV-B radiation and controls. A conidial suspension of strain CCMA-1144 was transferred to PDA + 1 g/L of streptomycin sulfate and exposed at 0, 15, 30, 60, 90 and 120 min (corresponding to 0, 0.41, 0.82, 1.63, 2.45 and 3.26 kJ/m^2, respectively) of UV-B radiation and then incubated at 22±1ºC in the dark. The determination of conidia germination was done at 12, 16, 20, 24 and 36 h after exposure. The experiments were repeated three times.

Effects of UV-B radiation on spore germination and survival curve

Once the appropriate period of incubation for germination evaluation was established, ten *Lecanicillium* isolates (Table 1) were compared for their tolerance to UV-B radiation. The conidial suspensions of these isolates were exposed to UV-B radiation (irradiance of 452 mW/m^2) for 0, 40 and 60 min, corresponding to 0, 1.09 and 1.63 kJ/m^2, respectively. After exposure to UV-B, the conidia were kept at 22±1ºC in the dark for 20 h and the germination was evaluated. The experiment was repeated three times.

To establish the survival curve, conidial suspensions of *Lecanicillium* (strains CCMA-1138, CCMA-1139, CCMA-1140, CCMA-1141, CCMA-1142 and CCMA-1143) were placed in plates containing PDA + 1 g/L of streptomycin sulfate and were exposed to UV-B radiation (irradiance 452 mW/m^2) for 0, 15, 30, 45, 60, 75 and 90 min, corresponding to 0, 0.41, 0.82, 1.23, 1.63, 2.04 and 2.45 kJ/m^2, respectively. After irradiation, the plates were incubated at 22±1ºC in the dark for 20 h until the evaluation of germination. After this period lactophenol + 0.05% tripan blue was applied to evaluate germination under a microscope. The experiment was repeated three times.

To establish the most sensitive period prior to germination, conidia of strain CCMA-1143 were placed on PDA + 1 g/L of streptomycin sulfate and incubated for 0, 3, 6, 9, 12, 15, 18 and 21 h before exposure to 1.74 kJ/m^2 (LD_{50}), corresponding to 64 min under UV-B radiation (irradiance 452 mW/m^2). After irradiation, plates were kept at 22±1ºC in the dark for 20 h until the evaluation of germination. The experiment was repeated three times.

Effects of UV-B radiation on the colonization of rust lesions

Leaf discs of *Coffea arabica* L cv. Mundo Novo with young sporulated lesions of coffee leaf rust were removed with a 1.5 cm diameter cork punch and placed into plastic boxes (30×40×5 cm). The discs were placed on a layer of 1 cm foam saturated with water. Using a 20 mL spray dispenser, 3.0 µl/cm^2 of the spore suspension (10^7 spores /mL) of strain CCMA-1143 were or were not sprayed on the leaf discs and the discs were exposed to UV-B radiation (irradiance 452 mW/m^2) for 0, 64 and 128 min, corresponding to doses 0, 1.74 (LD_{50}) and 3.48 kJ/m^2. Control discs were placed on the foam and wrapped with aluminum foil. After exposure to radiation, the boxes were covered with glass plates and maintained under 12 h photoperiod, 1000 lux, 26±2ºC, and approximately 100% relative humidity, for 5 days. The experiment was arranged in a randomized block design (n=4), with 50 leaf discs for each replication. The incidence of *L. lecanii* on lesions and percentage of the colonized area of lesions were evaluated 3, 4, and 5 days after irradiation. The Area Under Incidence of the Antagonist Progress Curve (AUIAPC) and Area Under Colonization of Antagonist Progress Curve (AUCAPC) were calculated. The experiment was repeated three times.

Experimental design and data analysis

The experiment design was completely randomized. For experiments on PDA media, there were two plates as replicates for each treatment. The data from three experimental repetitions invariably resulted in treatment effects in the same significance classes; therefore, the data were grouped for analyses. Statistical Analysis Systems (SAS Institute Inc.) was used to statistical analyses. Data for conidial germination were examined using analysis of variance (ANOVA) and treatment means were compared by Tukey test (α=0.05). Quantitative relationships between germination of *L. lecanii* conidia and hours of exposure after inoculation were examined by regression models. For evaluations on leaf discs, there were three replication plates each contained 50 disks. The data were analyzed using analysis of variance (ANOVA), and differences among treatments were compared by Tukey test (α=0.05), using SAS.

RESULTS

Determination of the appropriate exposition periods and doses of radiation

The germination of *L. longisporum* CCMA-1144 conidia in the control treatment was 62% after 12 h, and >90% after 24 and 36 h incubation. After 36 h incubation, it was impossible to count conidial germination in the control because the germ tubes were too long. For the conidia exposed to UV-B radiation 18 cm distant from the lamps, corresponding to 4.68 kJ/m^2 (irradiance 649 mW/m^2) the germination was zero for all incubation periods. Conidia submitted to UV-B 48 cm distant from the lamps, corresponding to 2.70 kJ/m^2 (irradiance 378 mW/m^2), the germination was 75% and 87% after incubation for 24 and 36 h, respectively. The medium irradiance (33 cm distant from the lamps - 452 mW/m^2) which corresponds to a final dose of 1.63 kJ/m^2 provided the medium lethal dose (conidia germination 6% and 40% after incubation for 24 and 36, respectively) and this dose was selected for the next experiment.

The germination of *L. longisporum* conidia was inversely proportional to the UV-B (irradiance 452 mW/m^2) (Table 2), i.e. low conidial germination was found in the dose >1.63 kJ/m^2 (>60 min) and higher conidial germination was found in the dose <0.41 kJ/m^2 (<15 min) (Table 2). The exposure of 120 min (3.26 kJ/m^2) was lethal for the

TABLE 2 - Germination of *Lecanicillium longisporum* conidia exposed to UV-B radiation (irradiance 452 mW/m^2).

Incubation period	UV-B radiation (kJ/m^2 and exposition time)					
	0 (0 min)	0.41 (15 min)	0.82 (30 min)	1.63 (60 min)	2.45 (90 min)	3.26 (120 min)
Germination (%)						
12 h	53.8aB	0.0bC	0.0bB	0.0bB	0.0bA	0.0bA
16 h	77.0aAB	50.8abB	41.2bA	0.7cB	4.8cA	0.0cA
20 h	80.2aAB	71.3abAB	44.5bA	13.7cAB	1.3cA	0.0cA
24 h	84.0aA	70.7aAB	65.8aA	8.8bB	1.7bA	0.0bA
36 h	99.7aA	85.0abA	62.7bcA	39.1cdA	13.9deA	0.0eA

Means followed by the same lowercase letters in the line and the same uppercase letters in the columns do not differ significantly (Tukey test, α=0.05).

conidia. The exposure of 30 min (0.82 kJ/m^2) provided the medium lethal dose and was selected for next the experiments. The most appropriate incubation period to evaluate conidia germination was between 12 to 20 h for the control and the irradiated treatments, respectively, and the most appropriate irradiation dose was between 0.41 kJ/m^2 (15 min) and 1.63 kJ/m^2 (60 min) (Table 2).

Effects of UV-B radiation on spore germination and survival

There were variations among *Lecanicillium* strains in sensitivity to UV-B radiation. Strains CCMA-1138, CCMA-1143, CCMA-1141 and CCMA-1140 were the most tolerant, while strains CCMA-1142, OTC-2 and CCMA-1144 were the most sensitive isolates to UV-B radiation. The other isolates presented an intermediate behavior (Figure 1).

Survival curves showed differences in sensitivity to UV-B radiation among the six *Lecanicillium* isolates tested. LD_{50} for the isolates, obtained by regression, ranged from 0.41 kJ/m^2 for strain CCMA-1142 to 1.74 kJ/m^2 for CCMA-1143 kJ/m^2 (Figure 2A).

In the bioassay to determine the most sensitive period of germination prior to exposure to UV-B radiation, conidia germination in the control treatment (without radiation) was approximately 100% in all incubation periods. The germination of conidia not incubated and irradiated (time 0) was approximately 50% according to expected for isolate CCMA-1143, exposed to a dose of 1.74 kJ/m^2 (LD_{50}). Incubation for 3 to 9 h prior to irradiation caused reduction in *Lecanicillium* conidia germination. When incubated for 6 h before irradiation nearly 100% inhibition of conidia germination was observed. After 12 h or more of the incubation prior to UV-B radiation exposure, no change on germination was observed (Figure 2B).

Effects of UV-B radiation on colonization of rust lesions

The first signs of *L. lecanii* colonizing rust lesions were observed approximately 72 h after spraying the antagonist and exposure to UV-B radiation, in all treatments. The AUIAPCs for UV-B radiation doses 1.74 kJ/m^2 (LD_{50}) and 3.48 kJ/m^2 differed significantly among the treatments with and without *L. lecanii* inoculation. The AUIAPC and AUCAPC increased when *Lecanicillium* isolate CCMA-1143 was sprayed in coffee leaf rust lesions (Figures 3AB). For the two UV-B radiation doses (1.74 kJ/m^2 and 3.48 kJ/m^2) we did not observed inhibition of *Lecanicillium* grown on the rust. Lesions of coffee rust exposed or not to UV-B, without inoculation with *Lecanicillium*, showed a low AUIAPC and AUCAPC (Figure 3AB).

DISCUSSION

The harmful effects of UV-B radiation on biocontrol fungi were observed for *Metarhizium anisopliae*, *Lecanicillium lecanii* and *Clonostachys rosea* (Braga et al., 2001b; Braga et al., 2002; Costa et al., 2012). This radiation is biologically active against plants (Jaakola & Hohtola, 2010), bacteria and viruses (Paul & Gwynn-Jones, 2003), affecting the organisms and their interactions (Paul, 2000). We showed different biological effects of UV-B radiation on conidia of *Lecanicillum* isolates, including inactivation and delayed in germination (Table 2, and Figures 1-3). According to Braga et al. (2002), a strong negative effect of UV-B radiation on conidial culturability was observed with *V. lecanii*.

Cabrera et al. (1995) and Piazena (1996) observed higher UV-B intensity in higher altitudes. Braga et al. (2001c) discuss the differential tolerance to UV-B radiation among *Metharizium* isolates from different geographical areas and altitudes. Although our sampling was very limited, there is an indication that isolates obtained from higher altitudes may be more tolerant to UV-B radiation (Figures 1 and 2). Further studies are necessary to confirm this hypothesis.

UV-B radiation chamber used in the experiments was similar to the one described by Costa (2011), who observed inactivation of *Clonostachys rosea* and *Trichoderma* spp. conidia by UV-B radiation. The DNA and other macromolecules of conidia exposed to UV-B radiation may suffer damage (Kunz et al., 2006), resulting in loss of biological functions (Gerhardt et al., 1999) and delayed recovery from the damages (Braga et al., 2002).

The levels of sensitivity to UV-B radiation were different for the 10 *Lecanicillium* strains studied. The causes

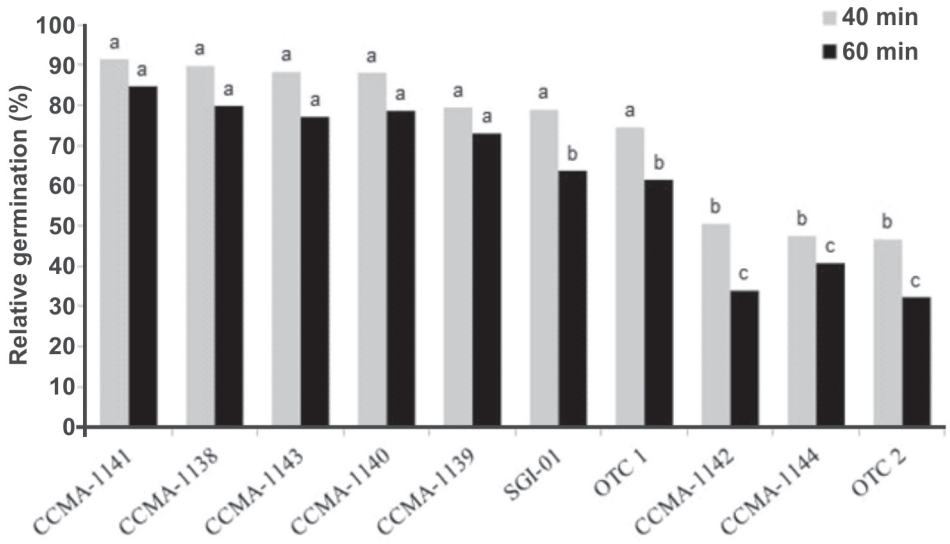

FIGURE 1 - Relative germination of *Lecanicillium* conidia of ten strains after exposure for 40 and 60 min to UV-B radiation (irradiance 452 mW/m^2 at a dose of 1.09 kJ/m^2 and 1.63 KJ/m^2, respectively). After exposure to UV-B, the conidia were kept at 22±1ºC in the dark for 20 h and the germination was evaluated. Relative germination was calculated in relation to control plates. Means followed by the same letter do not differ from each other (Scott-Knott α =0.05), within the same exposure time.

for this effect may be the adaptation of the isolates to the geographic origin, with different incidences of UV-B radiation (Piazena, 1996), genetic variability (Fernandes et al., 2007) and genotypic plasticity (Braga et al. 2001a, Rangel et al. 2004; Rangel et al., 2005; Rangel et al., 2006). Fernandes et al. (2007) tested the sensitivity of 60 isolates of *Beauveria* spp. to UV-B radiation and the conidia germination ranged between 0 and 80%. Costa (2011) exposed conidia of *Trichoderma* isolates to the same radiation and did not observe differences in germination among them. These results indicate that fungal species and isolates within each species have different sensitivity to UV-B radiation.

Costa et al. (2012) and Costa (2011), using approximately the same radiation of this study, found that the LD_{50} for *Trichoderma asperellum* LQC-96 and *C. rosea* LQC-62 were 4.9 kJ/m^2 and 4.1 kJ/m^2, respectively. We have found that for *Lecanillium* CCMA-1143 the LD_{50} was 1.63 kJ/m^2, indicating that it is more sensitive than the other two-biocontrol agents. *Lecanicillium* CCMA-1143 was also more sensitive than *M. anisopliae* strain ARSEF 2575 (Braga et al., 2001a). In general, *M. anisopliae* mutants with white conidia were more sensitive to simulated solar UV radiation than mutants with purple conidia, which were more sensitive than mutants with yellow conidia, which in turn were more sensitive than the green wild strain (Braga et al., 2006). Therefore, greater sensitivity of *Lecanicillium* to UV-B radiation is linked to the colour of their spores.

These results indicate that it is important to select strains of biocontrol agents which are more tolerant to UV-B radiation, as well as prepare formulations with capacity to increase the bioagent tolerance to radiation. Alves et al. (1998) observed that oil-based formulations for bioagents in general provide higher protection against radiation than water-based formulations.

The sensitivity of conidia germination of *Lecanillium* to UV-B radiation depends on the germination stage in observed the same trend they are (Figure 2B). Costa et al. (2012) obtained the same tendency for the biocontrol agent *C. rosea*. Braga et al. (2001a) found that UV-B distinctly affects various stages of germination of *Metarhizium*. This result suggests that the effect of UV-B was on the germination of conidia of *Lecanicillium* not on the germtube and micelial growth.

The low AUIAPC and AUCAPC observed in lesions of coffee rust exposed or not to UV-B, without inoculation with *Lecanicillium*, are due to natural contamination of lesions. This antagonist is found naturally hyperparasiting urediniospores of *H. vastatrix* in coffee rust lesions (Shaw, 1988; Vandermeer et al., 2009). There is also need to consider that *Lecanicillium* occurs naturally parasitizing other pathogenic fungi, as well as several species of insects and nematodes (Askary et al., 1998, Goettel et al., 2008; Park & Kim, 2010).

The use of *Lecanicillium* isolate CCMA-1143 (the most tolerant strain selected in this study), possibly favoured the establishment of the antagonist in rust lesions when the leaf was exposed to radiation (Figure 3). However, it is important to evaluate the effects on the colonization of coffee rust lesions when using a sensitive *Lecanicillium* strain. Another important aspect to be considered is the sensitivity of *H. vastatrix* urediniospores to doses of UV-B radiation, because Lazzaretti et al. (2014) observed that the urediniospores germination is reduced when these are exposed to UV-B.

The UV-B radiation may have altered the communities of phylloplane microorganisms and have a regulatory role on microorganism communities associated with natural biological control of coffee leaf rust. Despite the fact that this bioagent is not yet being used commercially for

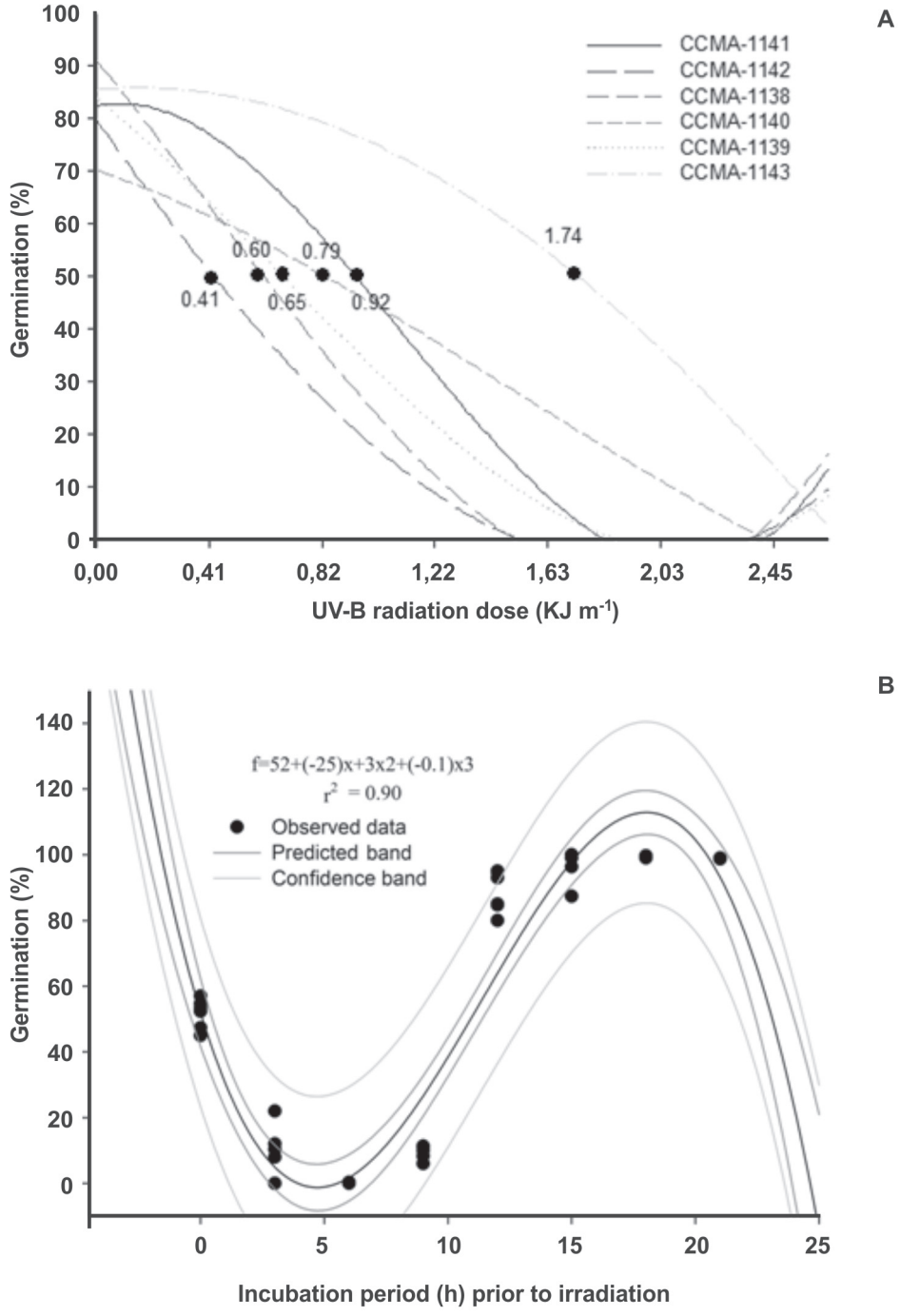

FIGURE 2 - A. Survival curve and LD_{50} of *Lecanicillium lecanii* conidia exposed to UV-B radiation on agar media to doses of UV-B radiation (irradiance of 452 mW/m^2). **B.** Effect of incubation (22±1ºC in the dark) period for 0, 3, 6, 9, 12, 15, 18 and 21 h of strain CCMA-1143 conidia prior to irradiation of UV-B radiation [1.74 kJ/m^2 (LD_{50}), corresponding to 64 min] and kept at 22±1ºC in the dark for 20 h until the evaluation of germination.

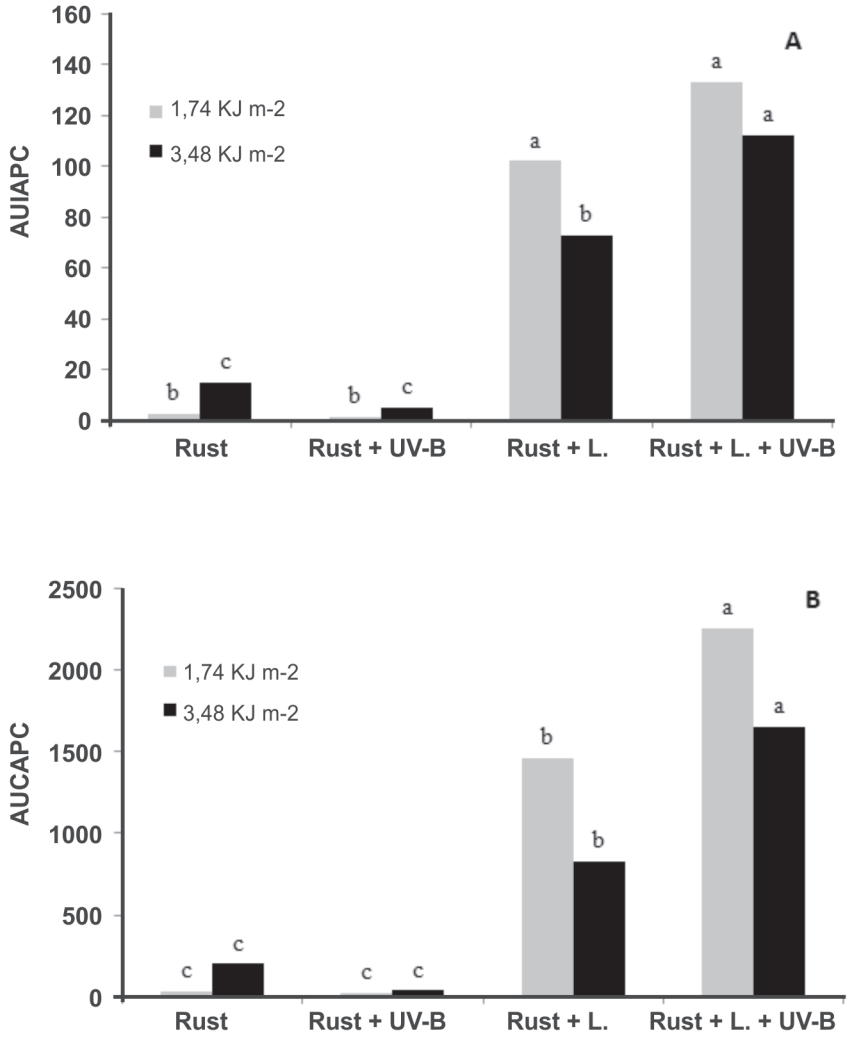

FIGURE 3 - Effect of doses 1.74 (64 min) and 3.48 kJ/m^2 (128 min) of UV-B radiation in the Area Under Incidence of the Antagonist Progress Curve (AUIAPC) **(A)** and Area Under Colonization of the Antagonist Progress Curve (AUCAPC) **(B)** of strain CCMA-1143 of *Lecanicillium lecanii* on coffee leaf discs with rust lesions. Treatments: rust=leaf disc of young sporulated lesion of rust not submitted to UV-B radiation; rust+UV-B= leaf disc of young sporulated lesion of rust submitted to UV-B radiation; rust+L= rust + sprayed with conidia suspension of *Lecanicillium* not submitted to UV-B radiation; rust+L+UV-B= rust + sprayed with conidia suspension of *Lecanicillium* submitted to UV-B radiation.

the control of coffee leaf rust, it is important to study all aspects of its bioecology, because with the possible changes in coffee and possibly other cropping systems, this antagonist may have its use expanded in the near future.

ACKNOWLEDGEMENT

The senior author acknowledges Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq for

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TPP-2013-0154
Submitted: 5 September 2013
Revisions requested: 15 November 2013
Accepted: 7 June 2014
Section Editor: Jorge Teodoro de Souza