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RESEARCH ARTICLE

UV-B radiation reduces biocontrol ability of *Clonostachys rosea* against *Botrytis cinerea*

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ABSTRACT

There is a lack of information comparing UV-B radiation conidial sensitivity of the biocontrol agent *Clonostachys rosea* (Cr) and its target pathogen, *Botrytis cinerea* (Bc). We investigated the interactions *in vitro* and on strawberry leaf discs between previously selected Cr and Bc strains tolerant to UV-B radiation. Strawberry leaf discs inoculated with Bc, Cr, or combinations of both fungi were exposed to UV-B doses (2.9, 5.9, and 8.9 kJ m⁻²). Incidence and sporulation of both fungi were evaluated, and the Area Under Incidence Progress Curve (AUIPC) and Area Under Sporulation Progress Curve (AUSPC) were calculated. AUIPC and AUSPC of Cr on leaf discs were negatively correlated to increased UV-B. When inoculated alone on leaf discs, Bc was not affected by UV-B, but when inoculated with Cr the incidence and sporulation of Bc were positively correlated to UV-B radiation dose. In the absence of UV-B, Cr reduced incidence and sporulation of Bc. However, the ability of Cr to control Bc was reduced by 20% to 50% with increasing UV-B radiation. Increasing the applied concentration of Cr conidia 10-fold partially overcame the deleterious effects of UV-B on the ability of the biocontrol agent to reduce Bc sporulation in strawberry leaves. The selection of antagonists must fulfil many requirements; besides being active against the specific targeted plant pathogens, they must be cost-effective and have ecological characteristics suitable for the desired use conditions. We suggest that UV-B exposure must be taken into account during the development of bio-fungicides based on Cr.

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Climate change; ultraviolet radiation; UV-B radiation tolerance; grey mould

1. Introduction

Botrytis cinerea Pers. ex Fries is the causal agent of grey mould disease. It is a necrotrophic cosmopolitan fungus, which can infect more than 200 plant genera (Jarvis, 1989). This pathogen can cause yield losses up to 50% in strawberry crops (Blanco, Santos, & Romero, 2006) as well as significant postharvest losses (Card, Walter, Jaspers, Aztejnberg,

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& Stewart, 2009). All parts of the strawberry plant are susceptible to the pathogen including senescent fruits and leaves, which serve as the primary inoculum source for epidemic development (Guetsky, Shtienberg, Elad, & Dinooor, 2001; Sutton, 1990). The pathogen can also infect young leaves, and stay in a quiescent stage until the leaves senesce and die (Sutton, 1990).

Biological control is a promising approach to control grey mould in several crops (Boff et al., 2002; Card et al., 2009; Cota, Maffia, & Mizubuti, 2008; Cota, Maffia, Mizubuti, Macedo, & Antunes, 2008; Peng & Sutton, 1991; Swadling & Jeffries, 1996; Tronsmo & Dennis, 1977). The use of biocontrol agents that compete for space and nutrients is a well-known strategy to suppress the saprophytic growth and sporulation of necrotrophic pathogens, such as *B. cinerea* (Köhl, Molhoek, Vanderplas, & Fokkema, 1995).

Clonostachys rosea Schroers can colonise leaves of several plant species including strawberry without expression of disease symptoms (Sutton et al., 1977). The fungus *C. rosea* can be to be highly effective at decreasing grey mould on strawberry plants (Cota, Maffia, & Mizubuti, 2008; Cota, Maffia, Mizubuti, et al., 2008). *C. rosea* works as a biocontrol agent by several mechanisms, which include mycoparasitism, antibiosis, induced resistance, suppression of pathogen sporulation due to competition for space and nutrients (Morandi, Maffia, Mizubuti, Alfenas, & Barbosa, 2003; Sun, Sun, & Li, 2015; Sutton et al., 1997) and growth promotion (Ravnskov et al., 2006; Saraiva, Czymmek, Borges, Caires, & Maffia, 2014).

Sensitivity to solar radiation is one limitation of applying biocontrol agents in the field (Braga, Flint, Messias, Anderson, & Roberts, 2001a; Li & Feng, 2009). Solar ultraviolet (UV) radiation is conventionally classified by wavelength. UV-C radiation (100–280 nm) is completely filtered by the ozone layer and absorbed by other atmospheric gases (Kuluncsics, Perdiz, Brulay, Muel, & Sage, 1999). UV-B radiation (280–315 nm) is partially filtered by the ozone layer, while UV-A radiation (315–400 nm) is not absorbed by the ozone layer and directly reaches the Earth (Madronich, McKenzie, Bjorn, & Caldwell, 1998). Since the ozone depletion of the 1990s environmental UV-B radiation intensity has increased and therefore it is necessary to study the effects of such radiation on different biological systems (Godin-Beekmann, 2010). These studies are important in tropical regions where the incident UV radiation at Earth's surface is expected to increase between 3% and 8% by 2050, due to decreases in clouds and ozone, caused by increased greenhouse gas concentrations in the atmosphere (Williamson et al., 2014).

The effect of UV-B radiation on biocontrol agents has been studied for only a few fungi including *Metarhizium anisopliae* (Metch.) Sorok, *Beauveria bassiana* (Bals.-Criv.) Vuill, *C. rosea*, and *Lecanicillium lecanii* Zare & Gams (Braga et al., 2001a; Costa, Robb, & Wilen, 2001; Costa, Rangel, Morandi, & Bettiol, 2012, 2013; Galvão & Bettiol, 2014). In general, short periods of exposure to UV-B radiation can inactivate propagules of fungi due to genetic and morphological changes, resulting in decreased viability of propagules (Braga et al., 2001a).

UV-B radiation reduces conidial germination, mycelial growth and sporulation of *C. rosea* on strawberry leaf discs (Costa et al., 2012, 2013). The growth of the fungus is influenced by the UV-B radiation dose and the inoculation conidia concentration. The reduction of *C. rosea* colonisation due to UV-B radiation can cause a loss of the competitive ability against *B. cinerea* on plant tissues. Costa et al. (2012) observed variations in sensitivity of *C. rosea* isolates to UV-B radiation and selected the most tolerant strain

(LQC 62), which exhibited a relative germination of approximately 60% after irradiation of 4.2 kJ m^{-2} .

On the other hand, the light requirement for sporulation and viability of fungal pathogen spores such as *B. cinerea* is recognised. Studies have demonstrated that control methods for pathogenic fungi may change the light quality and quantity that reaches the plants in the greenhouse (Honda, Toki, & Yunoki, 1977; Reuveni, Raviv, & Bar, 1989). However, more research is required to determine the tolerance of *B. cinerea* conidia to UV-B radiation, as well as the interaction between isolates of the pathogen which are more tolerant to UV-B radiation with biocontrol agents.

The purpose of our current study was to evaluate the effect of UV-B radiation on germination and sporulation of different *B. cinerea* strains and to investigate the interactions of the most UV-B tolerant strain with a previously selected UV-B tolerant *C. rosea* LQC 62 strain in conditions of increased UV-B radiation *in vitro* and on strawberry leaf discs. The increase in UV-B radiation may affect the plant pathogen as well as the biocontrol agent's effectiveness. The assurance of *C. rosea* efficiency in grey mould control is of great importance for the strawberry crop in Brazil since it is an alternative to the heavy fungicide applications which result in fruit contamination and lack of control efficacy due to resistance selection.

2. Materials and methods

2.1. Fungal isolates

Thirteen *B. cinerea* isolates were collected from strawberry plants in production areas of São Paulo State, Brazil (strains LQC 150–160 from Serra Negra, LQC 161 from Atibaia and LQC 162 from Jaguariúna) and deposited at the Embrapa Environment Collection of Microorganisms. The *C. rosea* strain LQC 62 was collected from a rose field in Viçosa, Minas Gerais, Brazil. This strain was determined to be tolerant to UV-B radiation compared to other isolates in previous work (Costa et al., 2012).

The *B. cinerea* isolates and the *C. rosea* strain LQC 62 were grown on potato dextrose agar media (PDA, Acumedia Manufacturers, Michigan) in Petri dishes (polystyrene, $90 \times 10 \text{ mm}$, Pleion) at $22 \pm 2^\circ\text{C}$ with 12 h light/12 h dark for 21 days, lighting was provided by fluorescent bulbs with no additional UV-B radiation and plates were covered with polystyrene lids to further reduce UV-B radiation exposure. Conidial suspensions were prepared in Tween 80 solution with distilled water (0.01%, v/v), vigorously shaken in a vortex and filtered through a triple layer of sterilised cheesecloth to remove hyphal fragments and spore aggregates. Conidial concentrations were estimated using hemocytometer counts and dilutions were made with Tween 80 solution (0.01%, v/v) for immediate use in the irradiation and germination studies.

2.2. Irradiation chambers, lamps, and filters

Irradiation experiments were conducted in a temperature-controlled chamber (adjusted to $22 \pm 2^\circ\text{C}$) with four UV-B 313EL lamps (Q-lab Cleveland, OH). The lamps were aged prior to the start of the experiments, resulting in a stable level of irradiation. Each lamp was covered with a 0.13 mm-thick cellulose diacetate film (Málaga Ltda), which had a cut-

off point of 290 nm. This permitted the passage of most UV-B and UV-A radiation (290–400 nm), but prevented exposure to UV-C radiation (<280 nm) and short-wavelength UV-B radiation (<290 nm).

The DNA damage action spectrum developed by Quaite, Sutherland, and Sutherland (1992) and normalised to unity at 300 nm was used to calculate the weighted UV irradiances (mW m^{-2}). This spectral weighting was selected based on the spectral characteristics of nine fungal responses, reviewed by Paul, Rasanayagam, Moody, Hatcher, and Ayres (1997), which concluded that this DNA damage spectrum is closely similar to the fungal responses. All of the light measurements were made with a spectroradiometer (Ocean Optics model USB2000 + rad) connected to a portable computer. An average of the spectral irradiances was made of the lamp setups used for the UV treatments (Figure 1).

2.3. Evaluation of *B. cinerea* conidial germination on agar media under UV-B radiation

To assess germination of *B. cinerea* *in vitro*, a conidial suspension ($20 \mu\text{L}$, 10^5 conidia mL^{-1}) was spread over 7 mL PDA + benomyl (0.05 mg L^{-1}) (Hi-yield Chemical, Bonham, TX) in Petri dishes (polystyrene, $50 \times 10 \text{ mm}$, Pleion). Benomyl was added to reduce the growth rate of the germ tube, preventing the superposition of hypha, which allowed the monitoring of the germination for a longer period of time (Milner, Huppatz, & Swaris, 1991). The conidia were exposed to irradiance of 823 mW m^{-2} of UV-B radiation (Figure 1). According to Melendez (2002), a UV-B dose radiation of 5.61 kJ m^{-2} represents approximately two hours and 39 minutes of natural sunlight.

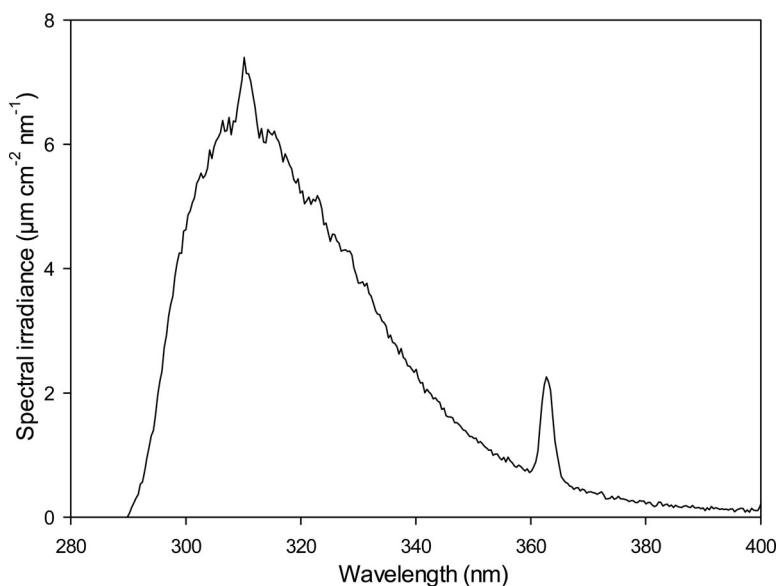


Figure 1. Average spectral irradiance of the lamp setups used for the UV-B treatments. The lamps provided irradiation of 823 mW m^{-2} , Quaite weighted dose of 2.96 kJ m^{-2} of UV-B radiation per hour. The lamps were covered with 0.1 mm thick cellulose acetate which blocked radiation <290 nm.

The Petri dishes were positioned 18 cm from the lamps and exposed to UV-B radiation for 2, 3, or 4 h, which corresponds to doses of 5.9, 8.9, and 11.9 kJ m⁻², respectively. After being irradiated, the Petri dishes were incubated at 22 ± 2°C in the dark and the conidial germination was evaluated at 12, 24, and 36 h post-treatment.

Afterwards the incubation time the fungal growth was interrupted by the addition of lactofenol + 0.05% trypan blue. Germination of 300 conidia per treatment was evaluated in an optical microscope at 400× magnification. Conidia with a germ tube longer than the length of the conidia were considered germinated. Relative percentage of germination after each period of incubation was calculated by the equation: Relative germination (%) = (Wt/Wc) × 100; where Wt is the number of germlings at exposure time *t* and Wc is the number of germlings on the control plate (Braga, Flint, Messias, Anderson, & Roberts, 2001b). Once the appropriate period of incubation for germination evaluation was established (using *B. cinerea* LQC 162 as a model), the tolerance to UV-B radiation of all thirteen *B. cinerea* strains was compared.

To draw a survival curve, a conidial suspension of *B. cinerea* strain LQC 150 was placed in Petri dishes containing PDA + benomyl and exposed to UV-B radiation (irradiance 823 mW m⁻²) for 0, 1, 2, 3, 4, 5, and 6 h, corresponding to time-weighted exposure values of 0, 2.9, 5.9, 8.9, 11.9, 14.9, and 17.9 kJ m⁻², respectively. After irradiation, the Petri dishes were incubated at 22 ± 2°C in the dark until the evaluation of germination 12 h post-treatment for control plates and 24 h post-treatment for UV-B radiation treated plates.

2.4. Interaction of *C. rosea* and *B. cinerea* under UV-B radiation in strawberry leaf discs

To evaluate the sporulation of the fungi on leaf discs, the experiments were conducted as described by Peng and Sutton (1991) and Morandi, Sutton, and Maffia (2000). One-centimetre leaf discs of 30–60-day-old strawberry leaves, cultivar ‘Oso-grande’, were surface sterilised in 70% ethanol (1 min) followed by 2% sodium hypochlorite (1 min) and rinsed three times in sterile distilled water. Ten leaf discs were then placed in disposable Petri dishes (polystyrene, 90 × 10 mm, Pleion) over humidified sterile absorbent paper (5 mL of sterilised water). Each disc was treated with 20 µL of conidial suspension of *C. rosea* strain LQC 62 (previously selected by Costa et al. (2012)) or *B. cinerea* strain LQC 150 (selected from this study) or the combination of both fungi on the same disc. The treatments were as follows: *B. cinerea* only at 10⁵ conidia mL⁻¹ (*Bc* 10⁵), *C. rosea* only at 10⁶ conidia mL⁻¹ (*Cr* 10⁶), *C. rosea* only at 10⁷ conidia mL⁻¹ (*Cr* 10⁷), *B. cinerea* at 10⁵ conidia mL⁻¹ plus *C. rosea* at 10⁶ conidia mL⁻¹ (*Bc* 10⁵ + *Cr* 10⁶) or *B. cinerea* at 10⁵ conidia mL⁻¹ plus *C. rosea* at 10⁷ conidia mL⁻¹ (*Bc* 10⁵ + *Cr* 10⁷).

After inoculation, the discs were immediately exposed to UV-B radiation (irradiance 823 mW m⁻²) for 0, 1, 2, or 3 h, corresponding to time-weighted doses of 0, 2.9, 5.9, and 8.9 kJ m⁻², respectively. Ten discs per Petri dish were then transferred to paraquat chloramphenicol agar medium in Petri dishes (Peng & Sutton, 1991). Incidence (number of discs with fungal growth in relation to the total number of units examined) and sporulation amount of *B. cinerea* and *C. rosea* were estimated after the tissues were incubated at 22 ± 2°C (12 h light/12 h dark) for 7 and 10 days. The *B. cinerea* sporulation was assessed using the following rating scale (per cent area of the disc covered with

conidiophores of the fungus): 0 = 0% (0%), 1 = 2% (1–3%), 2 = 5% (4–6%), 3 = 10% (7–12%), 4 = 20% (13–26%), 5 = 40% (27–53%), 6 = 65% (54–76%), and 7 = 90% (77–100%) (Peng & Sutton, 1991). The evaluation of *C. rosea* sporulation was estimated using the following rating scale (per cent area of the discs covered with conidiophores of the fungus): 0 = 0% (0%), 1 = 2% (1–3%), 2 = 5% (4–6%), 3 = 10% (7–13%), 4 = 20% (14–27%), 5 = 40% (28–52%), 6 = 70% (53–87%), and 7 = 94% (88–100%) (Morandi et al., 2000).

The Area Under Incidence Progress Curve (AUIPC) and Area Under Sporulation Progress Curve (AUSPC) were calculated for both fungi. The AUIPC and AUSPC data were used to calculate the biological control ability (%) as follows: Biological control ability = $100\% - (\text{AUIPC of } B. \text{ cinerea alone at 0 UVB} - \text{AUIPC of } B. \text{ cinerea with } C. \text{ rosea at the UVB dosage}) / \text{AUIPC of } B. \text{ cinerea alone at 0 UVB} \times 100\%$, for AUIPC. A similar formula was used for AUSPC.

2.5. Experimental design and data analysis

Each experiment was conducted with a completely randomised design and repeated three times. For the experiments on agar media, there were two replicates for each treatment. For the evaluations on leaf discs, there were three replicate plates, each containing 10 leaf discs. The data from the three experiment repetitions resulted in treatment effects in the same significance class, therefore the data were grouped for analysis. Statistical computations were performed using the Statistical Analysis Systems (SAS 9.0, SAS Institute Inc., Cary, NC). Data for fungal germination and sporulation were examined using analysis of variance (ANOVA). AUIPC and AUSPC were calculated for *C. rosea* and *B. cinerea* on leaf discs, and the average of the treatments was compared using Tukey's test ($p = .05$).

Regression models were used to examine quantitative relationships between germination of *B. cinerea* conidia and hours of exposure after inoculation. A factorial arrangement was used to evaluate the interactions between the length of exposure, inoculum concentration of *C. rosea*, and the application of *B. cinerea*. When there were no significant interactions among factors, the data were grouped for analysis.

3. Results

3.1. *B. cinerea* conidial germination on agar media under UV-B radiation

The conidial germination of *B. cinerea* strains on PDA + benomyl plates was inversely proportional to UV-B radiation dose at 24 and 36 h with $R^2 = 0.92$ ($y = -8.834x + 104.85$) and $R^2 = 0.89$ ($y = -8.682 + 108$), respectively. The average germination of *B. cinerea* LQC 162 strain at 5.9 kJ m^{-2} of UV-B radiation after 24 and 36 h of incubation was 70.4% and 79.6%, respectively. Under 8.9 kJ m^{-2} the germination rate decreased to 14% and 21.2% after 24 and 36 h of incubation, respectively. At the maximum UV-B radiation dosage, the germination of *B. cinerea* was below 1% (Table 1). The most appropriate incubation period for conidial evaluation was determined to be 24 h. After 36 h of incubation, it was difficult to count germination because the germ tubes were long, intermingled and not easily distinguishable.

Table 1. *B. cinerea* strain LQC 162 germination on potato dextrose agar media exposed to different UV-B radiation doses.

Incubation time (h)	UV-B radiation dose (kJ m ⁻²) ^a			
	0	5.9	8.9	11.9
12	97.0 ± 1.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
24	99.0 ± 0.7	70.4 ± 3.0	14.0 ± 6.14	0.0 ± 0.0
36	98.9 ± 0.6	79.6 ± 2.8	21.2 ± 6.8	0.5 ± 0.3

Note: Data show the average percentage ± standard deviation of three independent experiments.
^aSpores were exposed for 0, 2, 3, and 4 h to UV-B irradiation which corresponds to irradiance of 823 mW m⁻² at Quaité weighted doses of 0, 5.9, 8.9, and 11.9 kJ m⁻². Spores were incubated for 12, 24, and 36 h before evaluation.

The relative germination rate of eleven *B. cinerea* strains (LQC 162, LQC 150, LQC 159, LQC 157, LQC 156, LQC 161, LQC 151, LQC 163, LQC 158, LQC 160, and LQC 155) ranged from 70% to 90%, with no significant difference between strains under a UV-B radiation dose of 6.4 kJ m⁻² (Figure 2). The germination rate of strains LQC 154 and LQC 153 was below 20% under the same conditions.

The lethal dose of 50% of the *B. cinerea* LQC 150 conidia (LD₅₀) was 6.2 kJ m⁻² and the lethal dose of 100% (LD₁₀₀) was 12.52 kJ m⁻² (Figure 3). Strain LQC 150 of *B. cinerea* was selected for competition studies with the biological control agent *C. rosea*. Strain LQC 62 of *C. rosea* was chosen for this experiment since it was among the most tolerant to UV-B radiation and reduces sporulation of *Botrytis* on strawberry leaf tissues (Costa et al., 2012).

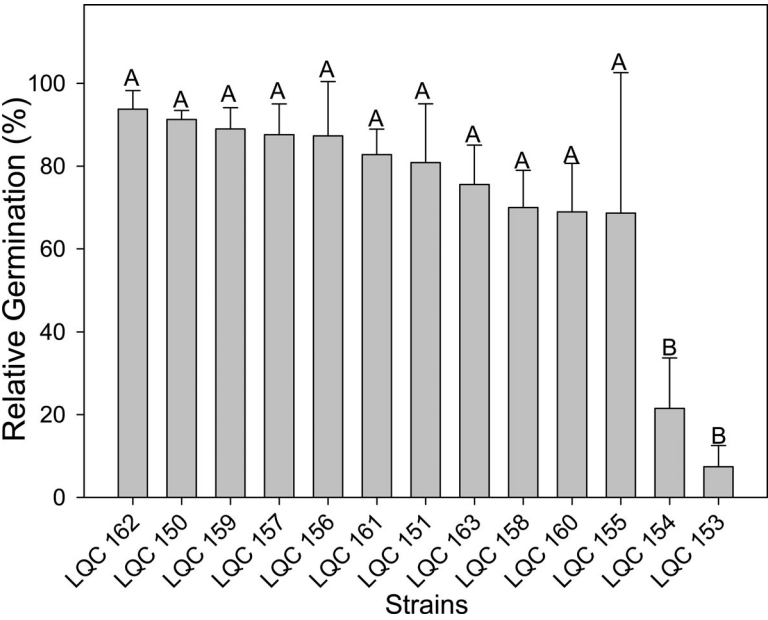


Figure 2. Relative conidial germination of 13 *B. cinerea* isolates exposed to irradiation of 823 mW m⁻² at a Quaité weighted dose of 6.4 kJ m⁻². Relative germination was calculated in relation to control plates. Bars with a letter in common are not significantly different (Tukey test, *p* < .001). Error bars show the standard deviation of three replicates.

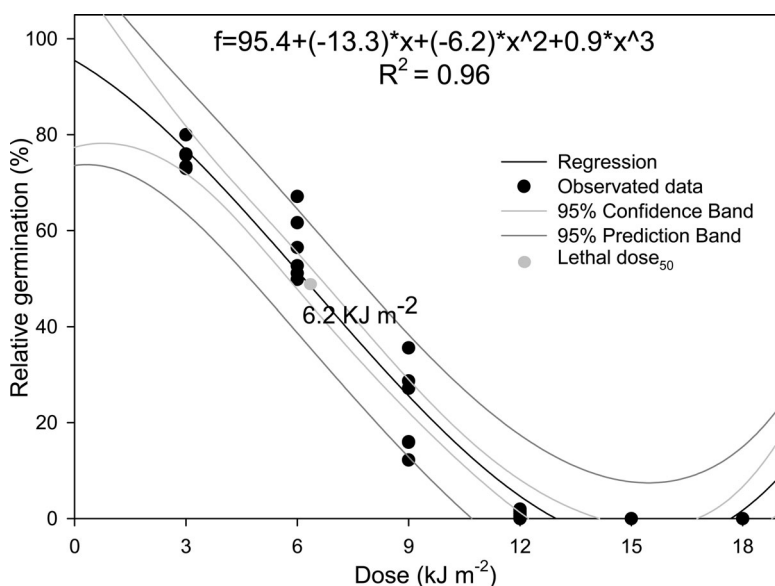


Figure 3. Survival curve of *B. cinerea* strain LQC 150 conidia on agar medium exposed to different time-weighted doses of UV-B radiation (Quaite weighted irradiance of 823 mW m⁻²).

3.2. Interaction of *C. rosea* and *B. cinerea* under UV-B radiation on strawberry leaf discs

The incidence (AUIPC) of *C. rosea* was reduced with increasing UV-B radiation dose, independently of the conidial concentration or the presence of *B. cinerea*. The incidence was reduced by 25% in the *Cr* (10⁷) treatment (Pearson correlation coefficient $r = -0.68$, $p < .001$) and 36% in the *Cr* (10⁶) treatment (Pearson correlation coefficient $r = -0.52$, $p < .001$), at 8.9 kJ m⁻² UV-B radiation dose compared to non-irradiated plates (Table 2A). The incidence of *B. cinerea* was not significantly affected by UV-B radiation dose when the fungus was applied alone (Pearson correlation coefficient $r = -0.15$, $p < .38$). When *B. cinerea* was applied with *C. rosea*, the incidence of the pathogen on non-irradiated plates was reduced by two- and threefold for *Cr* (10⁶) and *Cr* (10⁷) treatments, respectively (Table 2A). However, under the 8.9 kJ m⁻² UV-B radiation dose, there was an increase of the AUIPC of *B. cinerea* when applied with *C. rosea* at 10⁶ conidia mL⁻¹, reaching the same level as the pathogen alone. In the treatment with *C. rosea* at 10⁷ conidia mL⁻¹, the same pattern was observed, although the final incidence of *B. cinerea* was half that of the *B. cinerea* alone treatment (Table 2A). The *C. rosea* biological control ability was reduced by UV-B radiation and the 10⁶ conidia mL⁻¹ treatment had lower average biological control ability compared to 10⁷ conidia mL⁻¹ (Figure 4(a)).

A similar pattern was observed for the sporulation of both fungi. The AUSPC of *C. rosea* was reduced with increased UV-B radiation, independent of the conidia concentration or the presence of *B. cinerea*. The sporulation of *C. rosea* was reduced by 45% in the *Cr* (10⁷) treatment (Pearson correlations coefficient $r = -0.57$, $p < .001$), and by 59% in the *Cr* (10⁶) treatment (Pearson correlations coefficient $r = -0.62$, $p < .001$) when treated with a 8.9 kJ m⁻² UV-B radiation dose compared to non-irradiated plates. The ability of

Table 2. Incidence (A) and sporulation intensity (B) of *Clonostachys rosea* (Cr) and *Botrytis cinerea* (Bc), applied isolated or in combination on strawberry leaf discs and exposed to different doses of UV-B radiation.

		UV-B radiation dose (kJ m ⁻²)			
		0	2.9	5.9	8.9
A					
<i>Clonostachys</i> (AUIPC) Incidence	Cr (10 ⁷)	290 ± 3aA	260 ± 4abA	242 ± 6bcA	218 ± 18cA
	Cr (10 ⁷) + Bc (10 ⁵)	290 ± 5aA	260 ± 8aA	253 ± 9aA	185 ± 21bA
	Cr (10 ⁶)	280 ± 5aA	248 ± 14aA	242 ± 16abA	195 ± 18bA
	Cr (10 ⁶) + Bc (10 ⁵)	240 ± 16aB	225 ± 17abA	162 ± 26bcB	145 ± 20cA
<i>Botrytis</i> (AUIPC) Incidence	Bc (10 ⁵)	300 ± 0aA	275 ± 10aA	285 ± 8aA	287 ± 7aA
	Bc (10 ⁵) + Cr (10 ⁶)	168 ± 28aB	215 ± 18abA	240 ± 23abA	267 ± 7bA
	Bc (10 ⁵) + Cr (10 ⁷)	85 ± 19aC	120 ± 24aB	152 ± 18aB	155 ± 27aB
B					
<i>Clonostachys</i> (AUSPC) Sporulation	Cr (10 ⁷)	36 ± 2aA	24 ± 1bA	20 ± 2bAB	21 ± 3bA
	Cr (10 ⁷) + Bc (10 ⁵)	34 ± 2aA	27 ± 3abA	24 ± 2abA	19 ± 4bA
	Cr (10 ⁶)	36 ± 3aA	24 ± 3bA	25 ± 2bA	17 ± 2bA
	Cr (10 ⁶) + Bc (10 ⁵)	31 ± 3aA	20 ± 2bA	14 ± 2bB	13 ± 3bA
<i>Botrytis</i> (AUSPC) Sporulation	Bc (10 ⁵)	134 ± 7aA	117 ± 13aA	113 ± 11aA	142 ± 7aA
	Bc (10 ⁵) + Cr (10 ⁶)	32 ± 6aB	50 ± 6abB	56 ± 7abB	71 ± 7bB
	Bc (10 ⁵) + Cr (10 ⁷)	12 ± 4abC	10 ± 2aC	27 ± 6abB	34 ± 9bC

Notes: Data show the AUIPC or AUSPC ± standard deviation of three independent experiments. Cr was applied at 10⁶ or 10⁷ conidia mL⁻¹ and Bc was applied at 10⁵ conidia mL⁻¹. Both fungi were applied alone or in combination on the same leaf disc. The discs were exposed to UV-B radiation at weighted doses of 0, 2.9, 5.9, and 8.9 kJ m⁻² (irradiance of 823 mW m⁻²). Small letters compare averages in row and capital letters compare averages in columns. Averages with the same letters are not significantly different (Tukey test, $p < .05$).

C. rosea to reduce the sporulation of the pathogen was decreased by UV-B radiation and the Cr (10⁶) treatment had lower average biological control ability compared to the Cr (10⁷) treatment (Table 2B and Figure 4(b)).

4. Discussion

According to the author's knowledge, this study is the first investigation conducted to evaluate the interaction of UV-B tolerant selected strains of *B. cinerea* and *C. rosea* under increased UV-B radiation.

The climatic conditions during application of the biocontrol agent and the subsequent hours are critical to the establishment of *C. rosea* (Morandi, Mattos, Santos, & Bonugli, 2008; Sutton et al., 1997). For an effective colonisation of the host tissues, *C. rosea* requires favourable environmental conditions on the leaf surface. Weather conditions that affect the establishment of *C. rosea* on host tissues, such as humidity and temperature, have been widely studied (Sutton et al., 1997; Yu & Sutton, 1998). We suggest that other environmental conditions, such as ultraviolet radiation, must also be observed since *C. rosea* conidia can be damaged within a few hours of exposure (Costa et al., 2012; Morandi et al., 2008), which can reduce the antagonistic ability of the fungus (Table 2 and Figure 4).

The UV-B radiation intensity is an important limiting factor for the efficiency of biocontrol agents, as confirmed in this study (Table 2). The efficacy of *C. rosea* against *B. cinerea* is directly related to the ability of the antagonist to colonise tissues of the host faster than the pathogen (Morandi et al., 2008). We found that the *Clonostachys* ability to colonise the strawberry tissues was negatively correlated with increased UV-B radiation, allowing for the colonisation of tissues by *B. cinerea* (Figure 4). The dosage

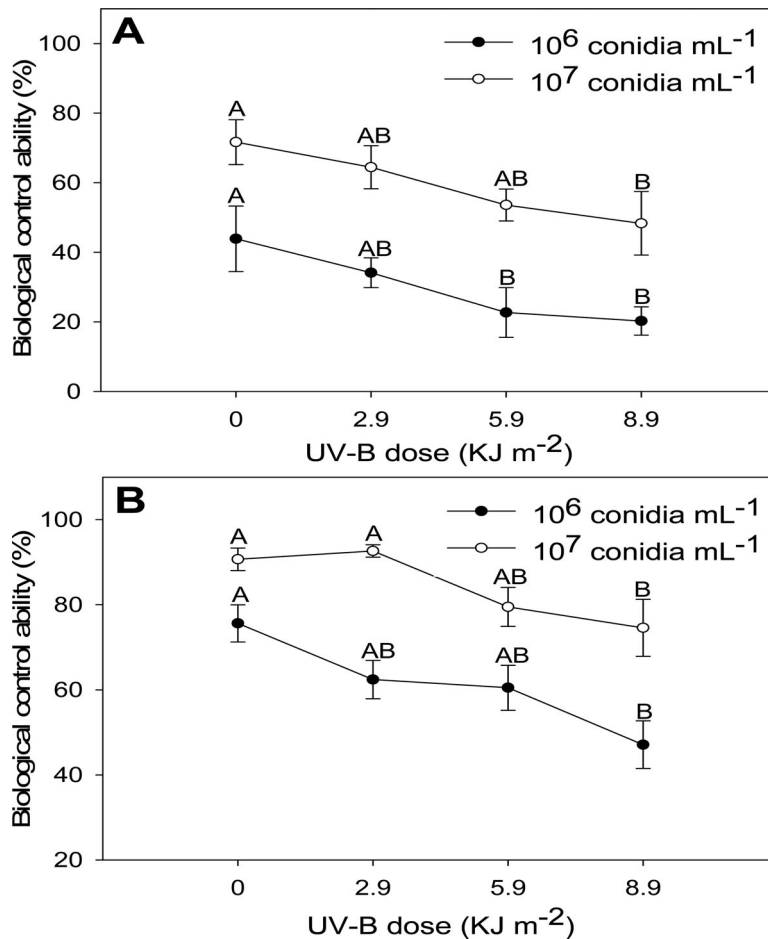


Figure 4. Biological control of *B. cinerea* (LQC 150) by *C. rosea* (LQC 62) on strawberry leaf discs under UV-B radiation doses of 0, 2.9, 5.9, and 8.9 kJ m⁻² (Quaite weighted irradiance of 823 mW m⁻²). *C. rosea* was applied at 10⁷ conidia mL⁻¹ (white circles) and at 10⁶ conidia mL⁻¹ (black circles). (a) Per cent suppression of *B. cinerea* incidence on leaf discs. (b) Per cent suppression of *B. cinerea* sporulation on leaf discs. Bars with a letter in common are not significantly different (Tukey test, $p < .001$). Error bars show the standard deviation of three replicates.

of UV-B radiation in this study corresponds to exposures that biocontrol agents and plant pathogens frequently encounter in nature (Melendez, 2002). UV radiation can interfere with the induced host resistance against plant pathogens, as well damaging the structures of the pathogen and biocontrol agents (Ghini, Hamada, Angelotti, Costa, & Bettiol, 2012; Paul, Jacobson, Taylor, Wargent, & Moore, 2005), it can also interfere in aphid population (Paul et al., 2012). In our study, we verified that *B. cinerea* conidia are more tolerant to UV-B radiation than those of *C. rosea*. When comparing the most UV-B-tolerant strains of *C. rosea* (LQC 62) and *B. cinerea* (LQC 150), conidia of the pathogen can withstand a 50% higher dosage of UV-B radiation (data not presented). Compared with other biological control agents, *C. rosea* exhibits intermediate tolerance to UV-B radiation. Paul et al. (2005) calculated the LD₉₀ for a *Trichoderma harzianum* strain to be 8.8 kJ m⁻². On

the other hand, Galvão and Bettiol (2014) observed the most tolerant strain of *L. lecanii*, found naturally hyperparasitising urediniospores of *Hemileia vastatrix* in coffee leaf rust lesions, had an LD₅₀ of 1.63 kJ m⁻² for UV-B radiation exposure, and is therefore more sensitive than *C. rosea*.

Our work corroborates the hypothesis raised by Ignoffo and Garcia (1992) that the colour of conidia is correlated with tolerance to solar radiation. *B. cinerea* has grey conidia and thus greater tolerance to UV-B radiation than the *C. rosea* hyaline conidia. *C. rosea* exhibits an LD₅₀ of 4.3 kJ m⁻² (Costa et al., 2012) and *B. cinerea* LQC 150 exhibits an LD₅₀ of 6.2 kJ m⁻² (Figure 3). This implies that *C. rosea* has a natural disadvantage to *B. cinerea* when exposed to solar radiation. UV-B radiation may stimulate the transcription and expression of the melanin gene and thus produce more melanin to protect plant pathogens from UV-B radiation (Cheng et al., 2014; Raviv & Antignus, 2004).

Ultraviolet radiation inhibits the conidial germination of other beneficial organisms, such as *Metarhizium*, *Lecanicillium* and *C. rosea* on the phylloplane (Braga et al., 2001a; Costa et al., 2001, 2012, 2013; Galvão & Bettiol, 2014). Competition is the most important mechanism of *C. rosea* to control *B. cinerea* in strawberry plants, and the applied concentration of the biocontrol agent conidia seems to have a crucial role. We found that increasing the applied concentration of *C. rosea* conidia 10-fold (from 10⁶ to 10⁷ conidia mL⁻¹) allowed to partially overcoming the deleterious effects of UV-B radiation on the ability of the biological control agent to reduce sporulation of *B. cinerea* in strawberry leaves.

This research will be useful to provide recommendations on improvements to biocontrol products containing *C. rosea* and to implement management practices to insure favourable conditions for the biological control agent, thus guaranteeing the viability of conidia and successful colonisation. For instance, the development of formulations of the fungus a higher concentration of conidia may be required. The formulation of *C. rosea* conidia in a UV-B protection matrix could be a feasible strategy to overcome the natural disadvantage of the antagonist in the field (Reddy, Khan, Devi, Victor, & Sharma, 2008), which may therefore increase the cost of mass production and formulation of conidia. An additional management practice that could be adopted is the application of the antagonist at the end of the day, during periods of low sunlight and high humidity, in order to maximise pathogen suppression as stated by Morandi et al. (2008) for grey mould control on roses.

Another strategy, specifically for greenhouse crops, could be the exclusion of certain wavelengths, including ultraviolet radiation that can reduce the sporulation of some pathogens such as *B. cinerea* (Elad, 1997; Nicot, Mermier, Vaissiere, & Lagier, 1996). Additionally, plastic that filters ultraviolet radiation can increase conidial viability of biocontrol agents. Costa et al. (2001) verified that using plastic to filter the radiation at a wavelength below 320 nm resulted in an increase in the conidial viability of *B. bassiana*. In greenhouses covered with polyethylene films that reduced the exposure to wavelengths of 280–320 nm, the sporulation of *Botrytis* was significantly decreased (Nicot et al., 1996). In Brazil, the cultivation of strawberries under plastic cover becoming more common (Antunes, Campos, Duarte Filho, Medeiros, & Santos, 2005). In these conditions, the *C. rosea* efficiency can be increased and sporulation of *B. cinerea* reduced. Paul et al. (2005) observed similar effects; greenhouses covered with standard plastic or UV-opaque film that filters UV radiation improved the efficacy of *Trichoderma* biocontrol agents against *B. cinerea*.

Our work suggests that the UV-B exposure should be taken into account during the development of biocontrol products based on *C. rosea*, and other biocontrol agents. According to our results, even the *C. rosea* strain most tolerant to UV-B radiation was more sensitive than the tolerant strain of *Botrytis*. To improve the antagonistic action of *C. rosea* under UV-B radiation, the applied concentration of conidia was increased 10-fold. These data reinforce the suggestions of Köhl, Postma, Nicot, Ruocco, and Blum (2011) in the stepwise screening of microorganisms for development of commercial products for the biological control of plant disease.

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Disclosure statement

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