Effects of UV-B radiation on the antagonistic ability of Clonostachys rosea to Botrytis cinerea on strawberry leaves

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Abstract

Clonostachys rosea is effective to control of Botrytis cinerea on strawberry, although is highly susceptible to ultraviolet radiation and has reduced ability to antagonize a pathogen in solar radiation conditions. The objective of this work was to evaluate the ability of an isolate of C. rosea, previously selected for its tolerant to UV-B radiation, to control B. cinerea on strawberry leaves in controlled experiments. Leaf discs of 1 cm diameter were placed on Petri dishes and each received 20 µL of a C. rosea LQC 62 concentrations (10^4, 10^5, and 10^6 conidia mL^-1). They were then exposed to UV-B irradiance 600 mW m^-2 (0, 2.1, 4.2, and 6.3 kJ m^-2), and after radiation, half of the discs were inoculated with an aliquot of 10 µL B. cinerea (10^5 conidia mL^-1). The colonization of fungi on the leaf disc was measured with diagrammatic scale formation of conidiophores. The presence and sporulation of C. rosea on leaf disc was influenced by the dose of UV-B radiation and the conidial concentration of antagonist. The incidence and severity of B. cinerea on leaf discs were inversely correlated to presence and sporulation of C. rosea. The growth of the pathogen was higher in the lower C. rosea concentration. The highest concentration of C. rosea (10^6 conidia mL^-1) reduced the incidence and severity by 91% and 98% of B. cinerea on strawberry leaf discs. The UV-B radiation reduced the ability of C. rosea to control B. cinerea. The higher dose of UV-B reduced the presence and sporulation of C. rosea by 20% and 42%, respectively. Consequently, the incidence of B. cinerea increased twice and the severity was three-folder higher. Taken together this data means that, for the development of biological control agents based products, the effect of UV-B should be considered on the efficacy studies.

Keywords:
Climate change
Gliocladium roseum
Ultraviolet - B
Gray mold
Clonostachys rosea
1. Introduction

The gray mold fungus *Botrytis cinerea* Pers. ex Fr., is a cosmopolitan necrotrophic pathogen, able to attack more than 200 genera of plants (Jarvis, 1989), and is severely destructive in protected cultivation systems. Crops such as strawberry are highly affected when produced in large scale. The fungus can cause losses of up to 50% in a strawberry field (Blanco et al., 2006), and is capable of attacking the crop in the field, during harvest, transportation, and commercialization (Card et al., 2009; Timudo-Torreilla et al., 2005). All parts of strawberry plants, including stems, leaves, flowers, and fruits, are susceptible to the fungus (Sutton, 1990).

The chief inoculum sources of *B. cinerea* are the conidio produced in senescent foliage, petals and fruit, which accumulate on the soil surface (Guetsky et al., 2001). The abundant *B. cinerea* sporulation on plant tissues contributes to the development and maintenance of an epidemic within a crop (Blanco et al., 2006). The suppression of pathogen sporulation on crop debris was proposed as a potential strategy of biological control (Morandi et al., 2003; Cota et al., 2009).

For many years, the control of gray mold was successfully performed exclusively by fungicides during the flowering of the cultures (Mertely et al., 2002). However, there have been many reports of populations resistant to the main fungicides used against *B. cinerea* (Bardas et al., 2008; Timudo-Torreilla et al., 2005). This has led to an increasingly smaller application intervals, with higher concentrations of active ingredients, and the mix of active principles (Card et al., 2009), which increases pesticide residues on fruits for human consumption (Rabolle et al., 2006). Resistant cultivars have not been effective, due to the great genetic variability and the necrotrophic habit of the pathogen (Williamson et al., 2007).

Biological control has been shown to be a promising strategy against the pathogen, achieving good results in a variety of crops (Boff et al., 2002; Card et al., 2009; Cota et al., 2009; Sutton et al., 1997; Swadling and Jeffries, 1996).

*Clonostachys rosea* (Link: Fr.) Schroers, Samuels, Seifert & W. Gams ([sin. Gloecladium roseum: teleomorph Bionectria ochroleuca] (Schroers et al., 1999)) is found commonly as saprophyte fungus in soil and crop residues, with cosmopolitan distribution (Schroers, 2001). The competition for nutrients and substrate are the major mechanism that *C. rosea* uses against pathogens such as *B. cinerea*, which requires an external source of sugars to cause infection (Sutton et al., 1997).

Although effective against gray mold, *C. rosea* is highly susceptible to ultraviolet radiation and has reduced ability to antagonize a pathogen in solar radiation conditions (Costa et al., 2012; Morandi et al., 2008). Arguably, solar radiation is an important factor that interferes with application of biological control agents in the field (Card et al., 2008; Li and Feng, 2009; Morandi et al., 2006). UV-A and UV-B can inactivate propagules of the agents in a few hours, due to genetic and morphological changes (Rangel et al., 2006b; Rotem et al., 1985; Santos et al., 2011). UV-B and UV-A cause protein denaturation, inactivation of respiratory metabolism, oxidative stress, and damage DNA, RNA, and ribosomes. The accumulation of cyclobutane pyrimidine dimer and pyrimidine bases inside the cells prevent the duplication of genetic material (Griffiths et al., 1998).

Few works have studied the effect of the UV-B radiation on biocontrol agents of plant pathogens, like *C. rosea* (Costa et al., 2012; Morandi et al., 2008). Considering the growing market of this biopesticides in Brazil and other countries, the objective of this work was to evaluate the ability of an isolate of *C. rosea*, previously selected for its tolerant to UV-B radiation, to control *B. cinerea* on strawberry leaves in controlled experiments.

2. Materials and methods

2.1. Isolates and inocula preparation

*C. rosea* LQC 62, previously selected as a tolerant isolate to UV-B radiation (Costa et al., 2012), and *B. cinerea* LQC 126 isolated from strawberry crops at São Paulo state, Brazil (altitude: 925 m; latitude: 22°36' South; longitude: 46°42' West) were used in the studies. The isolates are deposited in Embrapa Environment’s collection of microorganisms.

The isolates were grown in 20 mL of Potato-Dextrose-Agar (PDA) (Acumedica Manufacturers, Michigan) in plates (polystyrene, 90 × 10 mm, Pleion) and incubated at 25 ± 2 °C and 12 h light/12 h dark for 21 days. The conidia were suspended in a Tween 80 solution with distilled water (0.01% v/v), vigorously shaken using a vortex and filtered through a polycarbonate membrane (80 mm diameter, 8 μm pore size, Whatman Nucleopore, Clifton, NJ, USA) to remove spore aggregates. Conidial concentrations were estimated by hemocytometer counts and dilutions were made with sterile Tween 80 solution (0.01% v/v) for immediate use in the irradiation studies.

2.2. Irradiation chambers, lamps, and filters

Irradiation experiments were conducted in a temperature-controlled chamber with four UV-B 313EL lamps (Q-lab Cleveland, OH). The lamps were aged prior to the start the experiments, resulting in a stable level of irradiation. Each lamp was covered with a 0.13 mm–thick cellulose diacetate film (Málagu 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). The temperature inside the controlled room was adjusted to 25 ± 2 °C and was verified periodically throughout the sequence of experiments.

The DNA damage 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). This spectral weighting was selected based on the spectral characteristics of nine fungal responses reviewed by Paul et al. (1997), which concluded that this DNA damage spectrum is closely approximated to the fungal responses. All the light measurements were made with a spectroradiometer (Ocean Optics model USB2000 + rad) connected to a portable computer.

2.3. Effects UV-B radiation on *C. rosea* applied on strawberry leaf discs at different conidial concentrations

*C. rosea* growth on strawberry leaf discs was evaluated indirectly by quantifying the potential of the fungus to cover the surface of leaf discs and sporulate (Morandi et al., 2000). One-cm-diameter leaf discs of 30–60 day old strawberry plants (cv. Camarosa) were surface sterilized in 70% ethanol (1 min) followed by 2% sodium hypochlorite (1 min) and rinsed three times in sterile distilled water. After that, the leaf discs were placed in disposable plates (90 × 10 mm, Pleion) on humidified sterile absorbent paper (5 mL of sterilized water). Subsequently, each disc received an aliquot of 20 μL of *C. rosea* conidial suspension at 10⁴, 10⁵, or 10⁶ conidia mL⁻¹ and the material was exposed to UV-B radiation (irradiance 600 mW m⁻²) (Costa et al., 2012) for 0, 1, 2, or 3 h, corresponding to doses 0 (control), 2.1, 4.2, and 6.3 kJ m⁻². Control plates were placed inside each of the chambers and wrapped with aluminum foil to physically protect them from radiation. The Petri plates were randomized in intervals of 30 min to homogenize the received doses of UV-B radiation. The discs were then transferred to parafilm chloramphenicol agar medium (PCA) in Petri dishes.
(Peng and Sutton, 1991), at a number of 10 discs per plate. Sporulation of C. rosea was estimated after the tissues were incubated at 25 ± 2 °C (12 h light/12 h dark) for 3, 7, and 10 days. The evaluation was accomplished following a scale of notes for the area of the discs covered with conidiophores of the C. rosea, as follow: 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). All the evaluations were made with a stereomicroscope.

2.4. Reduction of B. cinerea sporulation on leaf discs treated with different concentrations of C. rosea conidia, irradiated with UV-B and challenged by the pathogen

Half of the discs treated with C. rosea and irradiated with UV-B radiation received an aliquot of B. cinerea (10 μL–106 conidia mL–1). The growth and sporulation of the pathogen were estimated after the tissues had been incubated at 25 ± 2 °C (12 h light/12 h dark) for 3, 7, and 10 days. The control treatment was B. cinerea inoculated on leaf discs without C. rosea and not treated with UV-B radiation. The evaluation was accomplished following a scale of notes for the area of the discs covered with conidiophores of B. cinerea, as follow: 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 and Sutton, 1991).

2.5. Experimental design and data analysis

Each experiment was conducted with a completely randomized design and repeated three times. For evaluations on leaf discs there were three replication plates each containing 10 discs. The data from the three experimental repetitions invariably resulted in treatment effects in the same significance classes; therefore, the data were grouped for analyses. Statistical computations were performed using the Statistical Analysis Systems (SAS Institute Inc., Cary, NC). Data for fungal sporulation were transformed when necessary and examined using analysis of variance (ANOVA). The Area Under Presence Curve (AUPC) and Area Under Sporulation Curve (AUSC) were calculated for C. rosea and B. cinerea growth on leaf discs and treatment means were compared by Tukey key test. A factorial arrangement was used to evaluate the interactions between length of exposure to UV-B radiation and inoculums concentration of C. rosea. When there were no significant interactions among factors, the data were grouped for analysis.

3. Results

3.1. Effects UV-B radiation on C. rosea applied on strawberry leaf discs at different conidial concentrations

The presence and sporulation of C. rosea on leaf disc surface was influenced by the dose of UV-B radiation and the conidial concentration of antagonist, but there were no significant interactions between the factors (P > 0.05; Table 1). The higher conidial concentration greater the presence and sporulation of the antagonist, regardless of the dose of UV-B radiation (Fig. 1 A and B). Similarly, increases in the UV-B dose reduced the presence and sporulation of the antagonist, regardless of conidial concentration (Fig. 1 C and D).

The deleterious effects of UV-B radiation on C. rosea growth were more pronounced at lower spore concentrations. At 104 conidia mL–1 the AUPC and AUSC of C. rosea was reduced by 40% and 60%, respectively, when irradiated at 0 (control) and 6.3 (kJ m–2). At 106 conidia mL–1, the reductions were 3% and 40% for AUPC and AUSC C. rosea, respectively, at the same doses of UV-B radiation.

3.2. Reduction of B. cinerea sporulation on leaf discs treated with different concentrations of C. rosea conidia, irradiated with UV-B and challenged by the pathogen

The incidence and severity of B. cinerea on leaf discs were inversely correlated to presence and sporulation of C. rosea (Pearson correlation coefficients of p = –0.8184 and p = –0.5962, respectively, for B. cinerea incidence and severity).

The growth of the pathogen was higher in the lower C. rosea concentration. The application of 100 conidia mL–1 of the biocontrol agent regardless of the dose of UV-B reduced the average incidence and severity of the pathogen by 40% and 80%, respectively. The highest concentration of C. rosea (106 conidia mL–1) reduced the incidence and severity by 91% and 98% of B. cinerea on strawberry leaf discs after 10 days after inoculation (Fig. 2).

The UV-B radiation reduced the ability of C. rosea to control B. cinerea. The higher dose of UV-B reduced the presence and sporulation of C. rosea by 20% and 42%, respectively. Consequently, the incidence of B. cinerea increased twice and the severity was three-folder higher after 10 days after inoculation (Fig. 3).

4. Discussion

The ability of C. rosea to establish on leaf tissues is a critical factor for the effectiveness of biocontrol against B. cinerea. In studies involving dose-response to C. rosea–B. cinerea interactions performed on leaves and flowers of begonia, cyclamen, and raspberry under controlled conditions (Li et al., 1996), significant control was achieved only when the inoculum of C. rosea was 10–100 times that of B. cinerea (Yu and Sutton, 1998). Variables such as phenological stage of the host or organ to be colonized, concentration of bioagent inoculum, and environmental factors (temperature, humidity and UV-B radiation) can affect the viability of the conidia and the establishment of C. rosea and, consequently, the level of B. cinerea control (Sutton et al., 1997).

UV-B and UV-A, naturally present in solar radiation, reduces the longevity of fungal spores and their establishment on the host tissue (Ragaei, 1999; Rotem et al., 1985). However, few studies on the effects of radiation on C. rosea are available (Morandi et al., 2008). In a previous study (Costa et al., 2012), comparing the effects of UV-B radiation on C. rosea conidia on agar or bean leaves surfaces, found that in lower concentrations of conidia, C. rosea were more sensitive to UV-B radiation and that the effects were more pronounced in agar medium compared to leaf surface. The pigmentation on leaf tissues and the physical protection to the conidia due to the presence of trichomes, grooves, and bumps were suggested as hypothesis to explain that results.

The present work found that UV-B radiation can affect the inoculum viability of C. rosea and reduce its ability to control B. cinerea on strawberry leaves. Increases in UV-B radiation dose reduced the viability of the antagonist, and the effects were most pronounced at lower spore concentrations. At 106 conidia mL–1 the viability of the conidia was reduced to 40%, while at 104 conidia mL–1, the reduction was up to 60%. Consequently, the incidence of B. cinerea increased up to twice and the severity was up to three-folder higher. Although not evaluated in this study, it is possible that the increased UV-B radiation may not be as detrimental to the pathogen as it is for C. rosea.

Our results indicate that the applied inoculum of the biocontrol agent must be higher than the effective dose to control the pathogen. Part of this problem could be overcome by more effective methods of multiplication (Rangel et al., 2006a, 2004) and use of
additives in the formulation to increase resistance and/or reduce exposure of the conidia to UV-B radiation (Moore et al., 1993; Reddy et al., 2008).

In greenhouses, this problem can be partially solved by the plastic covering materials such as polyethylene (Costa et al., 2001). Most greenhouse polyethylene plastic films contain UV light-blocking components to prolong the life of the plastic covering while maintaining transmission of photosynthetically active radiation. The majority of the greenhouse plastics on the market block transmission of UV light with wavelengths of 360 nm or less. Another well-known effect of polyethylene films that trap the radiation in the wavelength 300–400 nm is the decrease on $B.\ cinerea$ sporulation (Elad, 1997). Therefore, the effectiveness of biological control in greenhouses is expected to be higher not only by the increase in the viability of conidia of $C.\ rosea$ but also due to the reduced sporulation of the pathogen.

The knowledge of the ecological behavior of the pathogens and the antagonists is essential for the development of successful

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**Table 1**

$C.\ rosea$ development to differences conidia concentrations ($10^4$, $10^5$ and $10^6$ conidia mL$^{-1}$) and irradiated with 0, 2.1; 4.2 and 6.3 kJ m$^{-2}$ of UV-B radiation doses (Quaite weighted irradiance of 600 mW m$^{-2}$ at a different weighted dose) at evaluation.

<table>
<thead>
<tr>
<th>Area under presence curve</th>
<th>UV-B dose (kJ m$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>$10^4$</td>
<td>393</td>
</tr>
<tr>
<td>$10^5$</td>
<td>438</td>
</tr>
<tr>
<td>$10^6$</td>
<td>433</td>
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</tbody>
</table>

$P = 0.12$

<table>
<thead>
<tr>
<th>Area under growth curve</th>
<th>UV-B dose (kJ m$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>$10^4$</td>
<td>37.9</td>
</tr>
<tr>
<td>$10^5$</td>
<td>54.5</td>
</tr>
<tr>
<td>$10^6$</td>
<td>55.0</td>
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$P = 0.93$

$P > 0.05$ there were significantly different according analysis of variance.

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**Fig. 1.** Effect of concentrations of $Clonostachys\ rosea$ conidia and doses of UV-B radiation on leaf disc of strawberry and challenged to $Botrytis\ cinerea$. Area Under Presence Curve – AUPC (A) and Area Under Sporulation Curve – AUSC (B) of $C.\ rosea$ (–) and challenged of $B.\ cinerea$ (–) on leaf discs in different concentrations of $C.\ rosea$ 0–$10^6$ conidia mL$^{-1}$ and $B.\ cinerea$; Area Under Presence Curve – AUPC (C) and Area Under Sporulation Curve – AUSC (D) of $C.\ rosea$ (–) and challenged of $B.\ cinerea$ (–) on leaf discs with only $C.\ rosea$ were irradiated with UV-B radiation doses 0–6.3 kJ m$^{-2}$ (Quaite weighted irradiance of 600 mW m$^{-2}$ at a different weighted dose). Curves show mean values plus standard error bars of three independent experiments. $P = values$ from the analyses of variance and respective level of significance.
biopesticides (Köhl et al., 2011). In addition to studies on tolerance to UV radiation, further studies are needed involving other environmental factors in the development of C. rosea in the future because the scenarios of global climate change project not only an alteration in background radiation, but also in global temperature and CO₂ concentration.

According to our results, in addition to showing less growth under UV-B, conidia of C. rosea had lower antagonistic ability. Further studies are needed to observe the tolerance of B. cinerea conidia to UV-B radiation and thereby prove that an environment with increased UV-B radiation may be favoring the pathogen due to a lower ability of C. rosea to control the pathogen B. cinerea in conditions of increased UV-B.

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References


