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Influence of application time on survival, establishment and ability of *Clonostachys rosea* to control *Botrytis cinerea* conidiation on rose debris

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Abstract: The influence of application time (9, 12, 15 and 18 h) and the length of exposure to natural sunlight (0, 0.5, 1, 2, 4 and 8 h) on survival, establishment, and ability of *Clonostachys rosea* to suppress *Botrytis cinerea* conidiation on senescing rose leaves were investigated. The experiments were carried out in a climate-controlled greenhouse (Exp. 1) and in a plastic-covered greenhouse (Exp. 2). The conidia germination was significantly increased in the treatment kept on shadow and negatively correlated with application time from 9 to 18 h. The recovery of viable conidia from leaves reduced exponentially with length of exposure to sunlight. However, germination incidence was inversely proportional to the application time, independently of exposure to sunlight. These findings indicate that other factors beside solar radiation influenced germination. The relative humidity (RH) in the hours following inoculation correlated positively with germination, independently of sunlight exposure. Colonization of tissues by *C. rosea* was significantly reduced (40 to 50%) as exposure to sunlight increased. Despite the drastic effects of exposure to sunlight on *C. rosea*, the suppression of *B. cinerea* conidiation was only marginally affected (suppression of 94.5 to 100% and 65 to 93% at exp. 1 and exp. 2, respectively). Exposure of conidia to sunlight on the phylloplane for several hours reduced the efficiency *C. rosea* in colonizing tissues, but only partially affects its ability to suppress *B. cinerea*. These results show the ability of *C. rosea* to withstand adverse environmental conditions and still provide suppression of *B. cinerea* conidiation on rose debris. Although biocontrol was still effective, we recommend that the application of the antagonist to crops should be done preferentially during periods of low sunlight and high RH, in order to maximize the pathogen suppression.

Key words: biological control, *Gliocladium roseum*, grey mould, *Rosa* sp., UV radiation

Introduction

Clonostachys rosea (Link: Fr.) Schroers, Samuels, Siefert and W. Gams (ex. *Gliocladium roseum* Bainier) was selected as an efficient antagonist against the grey mould pathogen *Botrytis cinerea* on roses (Morandi et al., 2003). The effectiveness of *C. rosea* is attributed to its ability in colonizing senescing and dead tissues faster than the pathogen and suppressing its conidiation. This ability, although inherent, is affected by several factors, including developing stage of host organs, inoculum concentration, time of application, and microclimatic conditions (Sutton et al., 1997). Earlier studies indicated that *C. rosea* is highly flexible regarding microclimatic conditions required by the fungus for colonizing rose debris and reducing conidiation of *B. cinerea* (Morandi et al., 2003, 2006).

Another factor influencing the performance of the biocontrol agent is the susceptibility to ultraviolet (UV) radiation on leaf surfaces. It is known that the long wavelength UV, especially the spectral portions of UV-B (280-310 nm) and UV-A (320-400 nm) present in natural sunlight can reduce both longevity of fungal spores and phylloplane colonization

(Rotem et al., 1985). As a result, the reduction in viability of the biocontrol agent increases the cost of the process due to the need for frequent applications and the use of protective additives in formulations (Ragaei, 1999).

Although *C. rosea* has been reported to be an effective antagonist against *B. cinerea* and other pathogens in many crops, the ecology of the fungus on the aerial plant parts, especially its tolerance to sunlight, has received little attention (Sutton et al., 1997; Hoopen et al., 2003). Therefore we evaluated the influence of application time and the exposure to natural sunlight on survival, establishment, and ability of *C. rosea* to suppress *B. cinerea* conidiation on senescing rose leaves.

Material and methods

Two experiments were conducted in two different locations. The first was conducted in a climate-controlled greenhouse at the Environmental Biology Department of University of Guelph, Ontario, Canada, whereas the second was in a plastic-covered greenhouse without climatic control at Embrapa Environment, Jaguariúna, São Paulo, Brazil. The air temperature, relative humidity (RH), and global solar irradiance were monitored during the experiments. Green fully expanded leaves of 'Sonia' and 'Nivea' plants were used in the first and second experiment, respectively. The leaves were detached to simulate senescing, washed on tap water, and superficially disinfested.

In the first experiment, each leaflet was inoculated by placing a 10 µl droplet of *C. rosea* inoculum (10^7 conidia ml^{-1}) on five sites that were approximately equidistant from each other and from the leaflet margin at each application time (9, 12, 15 and 18 h). Half of the leaves were kept in a black plastic covered area and the other half was exposed to sunlight. In the second experiment, a suspension of *C. rosea* (10^7 conidia ml^{-1}) was applied to entire leaves by means of an air-pressurized hand sprayer. The inoculated leaves were kept exposed to sunlight inside the greenhouse for 0 (check), 0.5, 1, 2, 4 and 8 h from 8 am to 4 pm. The inoculated leaves were kept inside the greenhouses until the next morning.

To estimate germination incidence of *C. rosea* (Exp. 1), 1-cm-diameter leaf disks (25 disks per treatment) were mounted in lactophenol plus 0.05% trypan blue on microscope slides and examined on a compound microscope. Germination was estimated on 100 conidia on each disk. A conidium was considered germinated when length of the germ tube exceeded the greatest diameter of the conidium. In the second experiment, the leaflets were shaken for 10 min in 50 ml water plus Tween 80 (0.05% v/v). From each suspension, one aliquot of 10 µl was placed on the center of a PDA plate and distributed over the surfacing using a bent glass rod. After four days at 25°C, the number of colony forming units (cfu) of *C. rosea* was counted.

To estimate *C. rosea* conidiation, 1-cm diameter leaf disks were removed from the inoculated sites at each leaflet (Exp. 1) or from the entire leaflets (Exp. 2) and transferred to paraquat-chloramphenicol agar (PCA) medium. There were five replicate plates with 10 disks per treatment. Conidiation was estimated after incubation at 25°C for 10 days through an eight-category scale (Morandi et al., 2001). To estimate suppression of *B. cinerea* conidiation, the disks were challenged with the pathogen (one 10 µl droplet of 10^5 conidia ml^{-1}) before placed in PCA. The control consisted in leaf disks inoculation with *B. cinerea* but not with *C. rosea*. Conidiation of *B. cinerea* was estimated after incubation at 25°C for 10 days through an eight-category scale (Peng & Sutton, 1991). Conidiation suppression (%) was calculated in relation to the check. Each experiment was set in completely randomized design and repeated once. Analysis of data of the two experimental repetitions invariably resulted in treatment effects in the same significance classes. Accordingly, data of one repetition are presented.

Results and discussion

C. rosea conidia germinated significantly more on treatments kept under shadow than under sunlight on all application times (Exp. 1). However, the germination incidence was inversely proportional to the application time, independently of exposure to sunlight, indicating that other factors beside solar radiation influenced conidia germination ability. The number of CFU recovered from inoculated leaves (Exp. 2) was exponentially reduced with the increase of exposure time (CFU=42.46*exp[-0.2224*h]; R^2=0,88). On treatments exposed to sunlight, the radiation correlated negatively with germination (r=-0.94). The RH was positively correlated with germination independently of exposure to sunlight (r=0.95 and r=0.97, for shadow and sun, respectively). This result is in accordance with previous finding that RH is a main factor on the establishment of *C. rosea* on rose debris (Morandi et al., 2006).

The exposure of the conidia of C. rosea on the phylloplane for several hours to a direct sunlight reduced their viability and efficiency in colonizes tissues. On both experiments, the area of the leaf disks colonized by *C. rosea* was significantly reduced with the increase of exposure time to sunlight after inoculation (Fig. 1A and C). However, in the first experiment the percentage colonized area values were higher than in the second experiment.

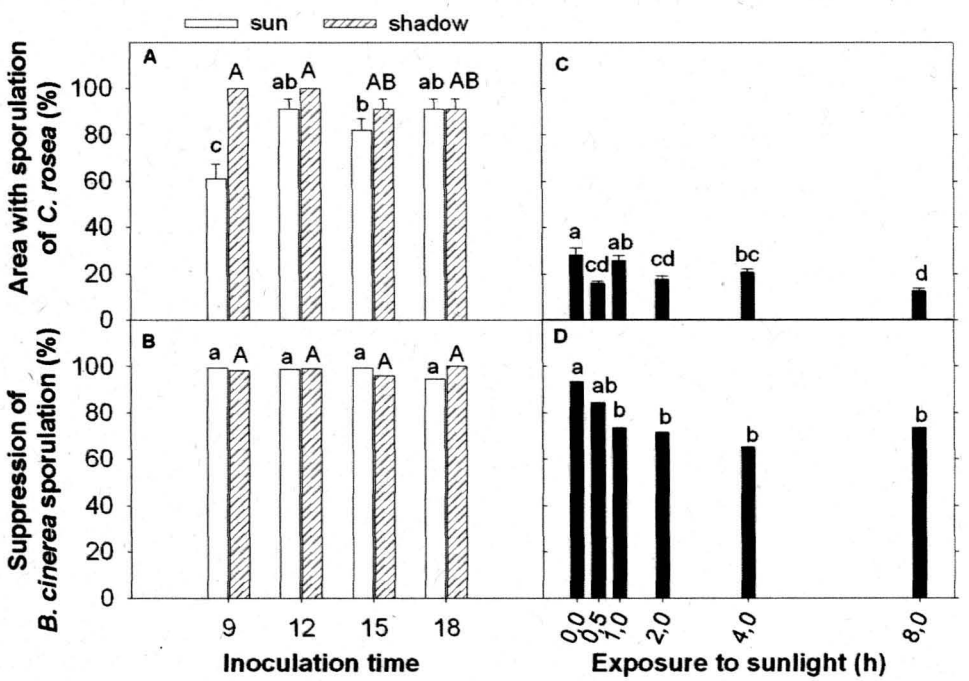

Figure 1. *C. rosea* conidiation and suppression of *B. cinerea* conidiation on rose leaves inoculated with the antagonist at different times of the day (9, 12, 15 and 18 h) inside a climate-controlled greenhouse (A and B) or exposed to sunlight for 0 (control), 0.5, 1, 2, 4 and 8 hours inside a plastic-covered greenhouse (C and D). Evaluations were carried out after 10 days of incubation on PCA. Means followed by the same small letters or capital letters are not significantly different using Tukey test at 5%.

Despite reductions in *C. rosea* conidiation, there was no effect of application time on suppression of *B. cinerea* in experiment 1 (Fig. 1B). Although there was some reduction in

suppression with initial exposure to sunlight in experiment 2 (from 93 to 65% from no sunlight to 1 h sunlight), there was no reduction in suppression with additional sunlight, since no changes from 1 to 8 h, and only small, no significant change from 0.5 to 1 h sunlight was observed (Fig. 1D). Suppression of *B. cinerea* was still quite effective through all levels of exposure to sunlight (65-84% suppression from 0.5-8 h sunlight). These results show the ability of *C. rosea* to withstand adverse environmental conditions and still provide suppression of *B. cinerea* conidiation on rose debris.

Although biocontrol was still effective, we recommend that the application of the antagonist to crops should be performed preferentially during periods of low sunlight and high humidity, in order to maximize the pathogen suppression. In conclusion, these findings support the importance in studying ecological attributes of biocontrol agents to avoid failure and maximize their efficiency in the field (Köhl & Fokkema, 1998; Sutton et al., 1997).

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