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Selection of *Trichoderma* spp. isolates to control the bean white-mold fungus *Sclerotinia sclerotiorum* in winter crops

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Abstract: Bean white mold is a destructive disease on autumn-winter crops in Brazil, when daylight length is short and the temperature vary from 15 to 25ºC. Chemical control is expensive and can be poorly efficient when applied alone. Several *Trichoderma* species are natural antagonists to *S. sclerotiorum* sclerotia in soil. However, in general the development of the isolates applied as biocontrol agents in Brazil are favoured by temperatures above 25ºC. In this case, the use of these isolates on autumn-winter crops can be not efficient. The objective of this work was to select *Trichoderma* spp. isolates able to parasitate the pathogen sclerotia in lower temperatures. In the pot experiment, twenty isolates of *Trichoderma* spp. and one of *Clonostachys rosea* were evaluated. Sclerotia were buried in soil and the following treatments were applied: check; *Trichoderma* spp. isolates (300 L/ha suspension volume at 10^7 conidia/mL); and, fungicide (cerconil, recommended dose). After five days at 20±2ºC, the sclerotia were removed from soil and transferred to carrot slices over water-agar medium. The number of germinated and parasitized sclerotia was accessed after 10 days. The experiment was conduced twice in a completely randomized design with seven replications. In the micro-plots experiment, two isolates of *Trichoderma* spp. and one of *C. rosea* were evaluated. The treatments (antagonists, fluazinan, and water check) were applied weekly from 20 days after plants emerging until the beginning of pods maturation. The experiment was conduced in a randomized block design with three replications. In the pot experiment, the isolates ALF111 and ALF409 consistently inhibited the germination and parasitized more than 80% of the sclerotia. Beside these, the isolates ALF02, ALF57, ALF66, ALF324 and ALF402 were efficient too. The isolate 172H inhibited significantly germination, but it was not capable to parasitize the sclerotia, which suggests that other biocontrol mechanisms, such as antibiosis, are involved. Although there were no visible symptoms of the white mold disease in the micro-plot experiment, the yield in the treatments with *C. rosea*, ALF66 and Trichode were superior to the check plots. The selected isolates are potential biocontrol agents against bean white mold and will be tested in field conditions.

Key words: biological control, *Phaseolus vulgaris*, micoparasitism, common bean

Introduction

White mold, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is one of the most destructive diseases of common bean (*Phaseolus vulgaris* L.) on irrigated autumn-winter crops in Brazil, when daylight length is short and the temperatures vary from 15 to 25ºC (Paula Jr. & Zambolim, 2006). Fungicide sprays to prevent white mold are the standard practice in Brazil, but it is expensive and, frequently, not economically feasible. Beside that, because of environmental considerations, new disease management strategies are needed. The use of

biological control agents to reduce the inoculum of *S. sclerotinia* in soil is a promising alternative (Gerlagh et al., 1999).

Numerous fungal mycoparasites are reported as biological control agent (BCA) against sclerotia of the pathogen in soil, including several *Trichoderma* species (Whipps & Budge, 1990). In warmer times of the year *Trichoderma* spp. are frequently found associated with sclerotia in soil, especially on no-till crops in Brazil (Arancibia et al., 2001). In general the development of these isolates are favoured by temperatures above 25ºC. In this case, the use of these isolates on autumn-winter crops can be not efficient (Bernardes, 2006). The objective of this work was to select *Trichoderma* spp. isolates adapted to winter conditions in Brazil and able to control the bean white-mold in such crops.

Material and methods

Pot experiments

Twenty isolates of *Trichoderma* spp. and one of *Clonostachys rosea* were evaluated. Sclerotia of *S. sclerotiorum* produced in carrots-corn media were buried on soil in 3 l pots and the following treatments were applied: check; *Trichoderma* spp. isolates (300 l ha^{-1} suspension volume at 10^7 conidia ml^{-1}); and, fungicide (cerconil, recommended dose). After five days at 20±2ºC, the sclerotia were removed from soil and transferred to carrot slices over water-agar medium. The number of germinated and parasitized sclerotia was accessed after 10 days. The experiment was conduced twice in a completely randomized design with seven replications.

Micro-plots experiments

Bean (cv. 'Talismã') was grown in one-square meter micro-plots previously infested with *S. sclerotiorum* sclerotia. The treatments consisted of two isolates of *Trichoderma* spp. (Trichode and ALF66), one isolate of *C. rosea*, fungicide sprays (fluazinan) and a check plot. The treatments were applied weekly from 20 days after plants emerging until the beginning of pods maturation. Disease incidence (percentage of diseased plants) was monitored during the complete crop cycle. The final stand and grain yield were evaluated at harvest time. The experiment was conduced in a randomized block design with three replications.

Results and discussion

Significant differences among the *Trichoderma* isolates were found. The isolates ALF111 and ALF409 consistently inhibited the germination and parasitized more than 80% of the sclerotia in the pot experiment (Figs 1 and 2). Beside these, the isolates ALF02, ALF57, ALF66, ALF324 and ALF402 were efficient too. The isolate 172H inhibited significantly germination, but it was not capable to parasitize the sclerotia, which suggests that other biocontrol mechanisms, such as antibiosis, are involved. Isolates ALF69, ALF70, ALF77, ALF1114 and TCNYG were not only ineffective, but stimulated sclerotia germination. Similar results with a *Trichoderma* spp. isolate were reported previously by Gerlagh et al. (1999).

Although there were no visible symptoms of the white-mold disease in the micro-plot experiment, the yield in the treatments with *C. rosea*, ALF66 and Trichode were superior to the check plots (Table 1). No reason for this effect can be advanced. Additional studies to evaluate this effect are warranted. The selected isolates in these preliminary studies are potential biocontrol agents against the bean white-mold. The isolates will be morphologically, biochemically and molecularly characterized and tested in field conditions.

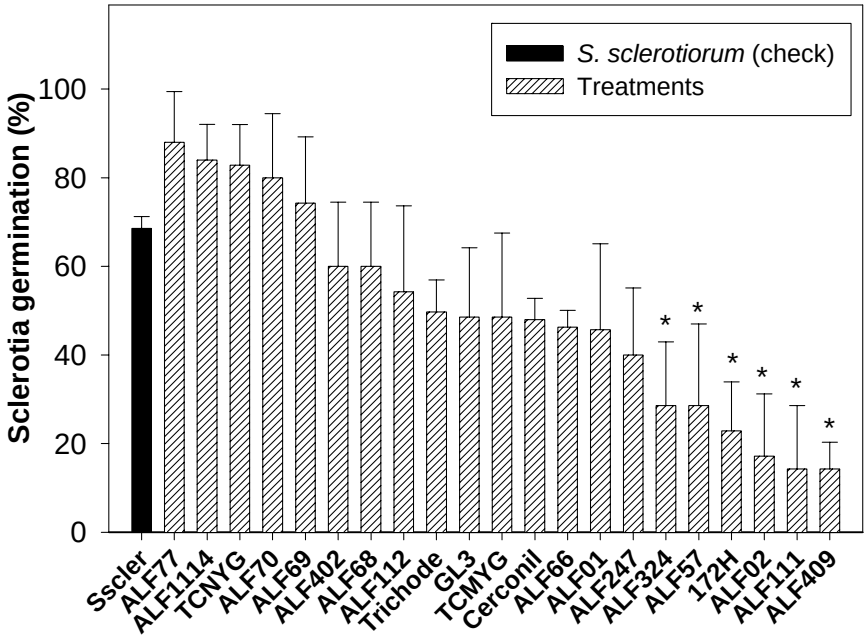

Figure 1. Germination of *Sclerotinia sclerotiorum* sclerotia. The sclerotia were buried in soil (0.5cm) treated with different isolates of *Trichoderma* spp. or fungicide for five days, transferred to carrot slices over water-agar medium and kept at 20±2ºC for 12 days. Bars are means followed by standard error. Bars marked with an (*) are statistically different from check (pLSD; *P*=0.05).

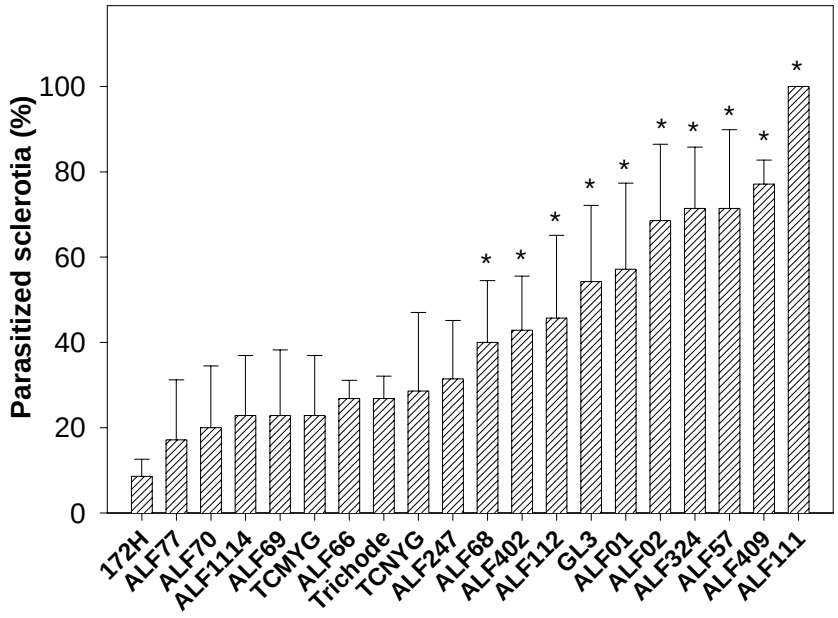

Figure 2. Sclerotia of *Sclerotinia sclerotiorum* parasitized by *Trichoderma* spp. isolates. The sclerotia were buried in soil (0.5 cm) treated with different isolates of *Trichoderma* spp. or fungicide for five days, transferred to carrot slices over water-agar medium and kept at 20±2ºC for 12 days. Bars are means followed by standard error. Bars marked with an (*) are statistically different from check (pLSD; *P*=0.05).

Table 1. Bean yield in micro-plot experiment

Treatment	Nº of plants/m^2	Yield (kg/ha)	Weight of 100 seeds (g)
Check	18.7	2842.92 ab	30.88
S. sclerotiorum	18.7	2624.22 b	31.60
Fluazinam	18.0	2653.45 b	29.95
Trichode	18.7	3175.40 ab	29.32
C. rosea	18.7	3792.80 a	29.97
ALF66	18.0	3022.22 ab	31.81

Means followed by the same letters are not significantly different (pLSD; P=0.05).

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