Biological control of fungal and bacterial plant pathogens
IOBC/wprs Bulletin Vol. 43, 2009
pp. 243-246

Biological control of Sclerotinia sclerotiorum on beans in field by *Trichoderma asperellum* and *Clonostachys rosea*

Marcelo A.B. Morandi, Lucio B. Costa
Embrapa Environment, CP 69, 13820-000, Jaguariúna, SP, Brazil,
E-mail: mmorandi@cnpma.embrapa.br

Abstract: White mold (*Sclerotinia slerotiorum*) is a destructive disease of bean crops in winter in Brazil. Biocontrol agents (BCAs) are being tested against the pathogen, including species of *Trichoderma* and *Clonostachys rosea*. The objective of this work was to evaluate the effectiveness of one isolate of *T. asperellum* and one of *C. rosea* previously selected in controlled conditions against the white mold in an irrigated field during a winter crop. The experiment was composed of 36 micro-plots (1 m^2 each) severely infested with sclerotia in a previous bean crop. There were six treatments: check (no sclerotia), infested check, fungicide (Fluazinan), *T. asperellum*, *C. rosea* I62, and Tricho-dermil (commercial product). We observed a significant reduction in apothecium emergence in all plots treated with BCAs and with fungicide. The incidence and severity of the disease were only marginally reduced in the biocontrol treatments. Although the intensity of the disease was significantly reduced in the fungicide plots, no differences were observed in the yield among treatments. These results are probably due to the high level of ascospores produced in the check and disseminated to the other plots. The observed reduction in apothecium counts on treated plots indicates the potential of the BCAs to reduce the survival and multiplication of the pathogen in field along time.

Key words: biological control, *Phaseolus vulgaris*, white-mold, common bean

Introduction

White mold, (*Sclerotinia sclerotiorum* (Lib.) de Bary) is a destructive disease of common bean (*Phaseolus vulgaris* L.) on irrigated autumn-winter crops in Brazil. The disease is severe when daylight length is short associated with temperatures varying from 15 to 25°C (Paula Jr. & Zambolim, 2006). Fungicide sprays to prevent white mold are the standard practice in Brazil, but, because of environmental and cost concerns, new disease management strategies are needed. Several studies around the word have demonstrated that 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 *Trichoderma* species and *Clonostachys rosea* (Whipps & Budge 1990; Zhang et al., 2004). *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 is favoured by temperatures above 25°C, wich restrict their efficiency on autumn-winter crops (Bernardes, 2006; Paula Jr. et al., 2006).

We selected one *T. asperellum* isolate and one *C. rosea* isolate that both are effective against *S. sclerotiorum* in laboratory and greenhouse conditions (Morandi et al., 2007). The objective of this work was to evaluate the effectiveness of the selected *T. asperellum* and *C. rosea* isolates against white mold in irrigated field microplots during a winter crop.

Material and methods

The experiment was composed of 36 micro-plots (1 m^2 each, 30 plants, cv. Talismã) severely infested with sclerotia in a previous bean crop. There were six treatments, distributed in six blocks: check (no sclerotia), infested check, fungicide (Fluazinan), *T. asperellum*, *C. rosea* I62, and Trichodermil (commercial product). The treatments were applied on stages V4 (third trifoliolate leaf is unfolded), R6 (mid to full flower period) and R8 (one pod with fully developed seeds). The fungicide was applied following the recommended dose. The BCA suspensions were adjusted to 10^7 conidia ml^{-1} and sprayed at a ratio of 200 l ha^{-1}.

Apothecium emergence (number of apothecia/m^2) was evaluated weekly from stage R5 (one open flower per plant) until R8. The white mold incidence (number of plants diseased) and severity (five-category scale: 0, 1-25, 26-50, 51-75, and 76-100% of stems infected; Hall & Phillips, 1996) were evaluated weekly starting at R7 (one pod at maximum length). The number of pods per square meter and the final yield (kg/ha) were evaluated after harvest.

Data analyses were performed by the GLM Procedures (SAS Institute Inc., Cary, NC). The treatment means were compared by the protected least significant difference test (pLSD; Snedecor & Cochran, 1989).

Results and discussion

A significant reduction of apothecium emergence in all plots treated with BCAs was observed (Figs 1A and 1B). The inhibition of apothecium emergence was greater in the fungicide plots than in the BCAs treatments. In some BCA plots, apothecia were parasitized at frequency <1%.

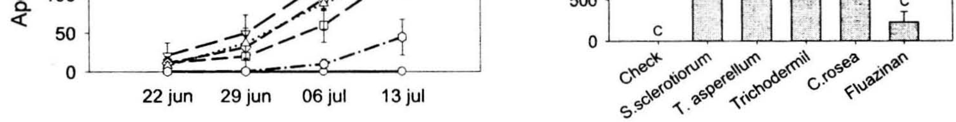

Figure 1. Emergence of *Sclerotinia sclerotiorum* apothecia in the field. (A) Number of apothecia per square meter during bean flowering period (cv. Talismã) in a winter crop. (B) Area Under Curve of Apothecia Emergence (AUCAE). Means (with standard errors) designated by bars followed by the same letter are not significantly different (pLSD; P=0.05).

The incidence and severity of the disease were only marginally reduced in the biocontrol treatments along the evaluation dates (Fig. 2A), and there were no differences in the area under disease curves (Fig. 2B). These results are probably due to the high level of ascospores produced in the inoculated check (*S. sclerotiorum* treatment) and disseminated to the other plots. This hypothesis is supported by the observation that even though there was no apothecia emergence on the non-inoculated check, the incidence and severity of the disease were as high as in the inoculated check. The intensity of the disease was significantly reduced in the fungicide plots (Figs 2A and 2B), but no differences were observed in the yield among treatments (Table 1).

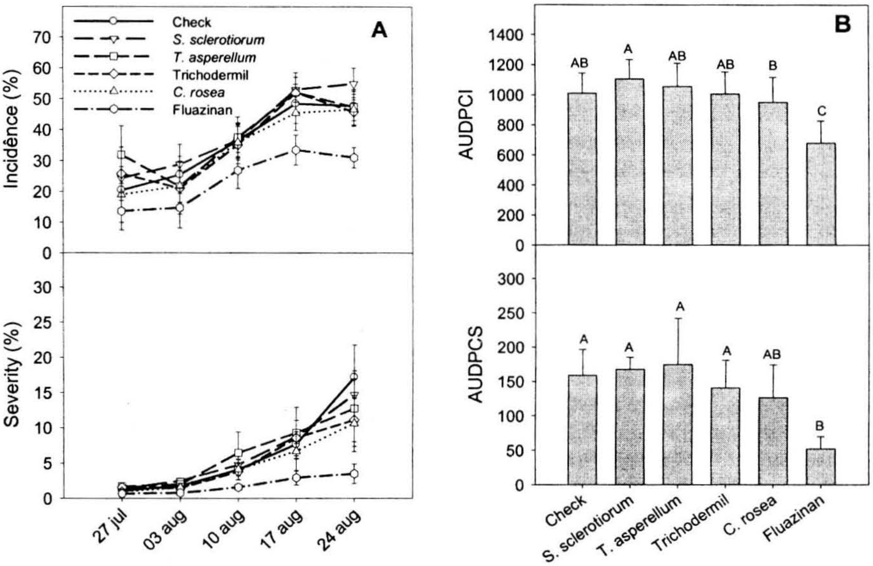

Figure 2. White mold progress in a winter crop of beans (cv. Talismã) treated with different BCAs or fungicide. (A) Incidence and severity progress curves; (B) Area Under Disease Progress Curve of Incidence (AUDPCI) and Severity (AUDPCS). Bars followed by the same letter are not significantly different (pLSD; *P*=0.05).

Table 1. Yield in a winter crop of beans (cv. Talismã) treated with different BCAs or fungicide.

Treatment	N° of pods/m^2	Yield (kg/ha)
Check	222.2 ± 11.2	2214.6 ± 182.6
Sclerotinia sclerotiorum	240.0 ± 9.0	2658.0 ± 101.9
Trichoderma asperellum	227.5 ± 10.5	2399.4 ± 160.3
Trichodermil	220.0 ± 12.1	2311.9 ± 97.9
Clonostachys rosea	235.3 ± 16.7	2504.1 ± 243.7
Fluazinan	217.0 ± 18.1	2370.6 ± 354.3

Values are means ± SE. There was no significant difference among treatments (pLSD; *P*=0.05).

The decrease in apothecium counts on treated plots indicates the potential of the BCAs to reduce the survival and multiplication of the pathogen in field along time. A long-term biosanitation by application of *Coniothyrium minitans* on *S. sclerotiorum*-infected crops was reported by Gerlagh et al. (1999). In the same work, in a temperate climatic area, the authors had no success with a *Trichoderma* isolate. However, in tropical areas, *Trichoderma* spp. and *C. rosea* are frequently found associated with sclerotia in soil, which indicates the potential of these antagonists to be used in an integrated mangement program to reduce the multiplication of the pathogen in highly insfested areas. This experiment will be repeated in the same plots to check this hypothesis.

Acknowledgements

FAPESP ('Fundação de Amparo à Pesquisa do Estado de São Paulo').

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