Biocontrol of Botrytis cinerea stem infection on greenhouse tomatoes with an antagonistic strain of Fusarium

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BIOCONTROL OF *BOTRYTIS CINEREA* STEM INFECTION ON GREENHOUSE TOMATOES WITH AN ANTAGONISTIC STRAIN OF *FUSARIUM*

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INTRODUCTION

In heated greenhouses of southern France, the most devastating gray mold symptom on tomato is the development of stem lesions resulting from infection of pruning wounds by *Botrytis cinerea*. Stem infections are very difficult to avoid although the incidence of the disease can be reduced by several cultural practices, such as cutting the leaves close to the stem (Martin *et al*., 1994), removing the leaves on bright and sunny days and irrigating the plants early in the day rather than late (Jarvis, 1992), by avoiding excess humidity in the greenhouse, or by the use of fungicides (Nicot and Baille, 1996).

With the objective of developing a biological control method, 175 isolates were collected from the microflora of pruning wounds on tomato plants (Nicot *et al*., 1996). In a screening procedure, two bacteria (a pseudomonad and an enteric bacterium) and a fungus (provisionally identified as *Fusarium* sp.) were selected for their efficiency in protecting the pruning wounds on tomato plants. This paper presents the results of greenhouse trials with the fungal antagonist and the evaluation of several factors of its efficiency.

MATERIALS AND METHODS

Inoculum production: Shake cultures of the fungal antagonist were prepared in yeast-malt extract broth. Prior to utilization, the cell suspensions were filtered to remove mycelium fragments, centrifuged and resuspended in water. *B. cinerea* (BC1 strain) was grown on Potato Dextrose Agar. After 10 days of incubation, spores were obtained by washing the cultures with sterile water. Spore concentrations were determined with a haemocytometer and adjusted as desired.

Tests in growth chamber: The effect of spore density on the efficacy of the antagonist was tested on 9-week old tomato plants, cv "*Rondello*". *B. cinerea* and the antagonist were applied simultaneously on fresh pruning wounds. Leaf removal was conducted either by cutting the petioles flush to the stems or by leaving 5-cm long stubs attached to the stems.

The progression of lesions on the petiole stubs or on the stems was measured daily. The areas under the disease progress curves (AUDPC) were used to calculate an index of antagonism (Nicot *et al*., 1993).

Tests in experimental greenhouses: Three tests were conducted between 1994 and 1996 on plants (cv. "*Rondello* ") approximately 5 months old in a glasshouse at INRA. The efficiency of the strain of *Fusarium* sp. was compared to that of a fungicide (carbendazime + diethofencarb). Treatments were arranged in a block design with four replicates (10 plants per replicate, 5 leaves per plant).

A similar test was conducted in 1996 in a glasshouse at CTIFL (cv "*Trust*") on plants 3 months old. The treatments were distributed randomly in a block design with three replicates (10 plants per replicate, 5 leaves per plant).

The cultural practices were kept as close as possible to a commercial situation. The antagonist, the fungicide and *B. cinerea* were applied once, immediately after leaf removal on petioles cut at 5 cm from the

stem. They were sprayed in a localized fashion to ensure that the treatment was evenly distributed on all wound surfaces. Different levels of inoculum of *B. cinerea* were used in the different trials to obtain different conditions of disease severity. The antagonist was applied as a suspension containing 3-8 x 10^5 spores per ml and the fungicide was dosed at 20 ml commercial product (Sumico) per liter. Stem infection was monitored weekly after inoculation until the end of the growing season. The results were expressed as percentages of pruning wounds developing stem lesions and the statistical analyses were performed on the AUDPC's. The efficiency of the control methods was estimated by the reduction in AUDPC for treated plants relative to the AUDPC of the untreated plants.

Survival of *Fusarium* sp. on plant tissue: During one of the experiments conducted in the glasshouse at INRA, petiole stubs were inoculated with the antagonist to study its survival in greenhouse conditions. Petiole stubs were collected weekly and ground in phosphate buffer. Serial dilutions were then plated on synthetic agar medium amended with streptomycin sulfate (50 mg/l) and the results were expressed as colony forming units per gram of fresh plant tissue.

RESULTS

Factors of efficacy of the antagonist on young potted plants: The incidence of infections on petiole stubs was reduced significantly when 10^3 and 10^4 spores of *Fusarium* sp. were applied on the pruning wounds simultaneously with 10^5 spores of *B. cinerea* (Table 1a). Approximately, 90-95% protection was provided by the antagonist. Efficiency rates were reduced when only 100 or 10 spores were applied on the pruning wounds. High rates of protection were observed when the antagonists were applied on pruning wounds from leaves removed flush to the stem (Table 1b).

Efficacy of the antagonist in greenhouse trials: In the experiment conducted at CTIFL, the first stem lesions were recorded 14 days after inoculation (DAI) on the plants not protected by the antagonist or the fungicide (Figure 1). During the following 2 weeks the incidence of stem lesions increased sharply on these plants, then stabilized at approximately 40 %. For the plants protected by the antagonist, disease incidence increased slowly during 40 days and then stabilized at approximately 9%. No stem lesions were observed on the fungicide treated plant.

The evolution of the disease was comparable in the trials conducted at INRA (see Fig. 1; data shown for 1996). The incidence of stem lesions was very high in all trials on the plants inoculated with *B. cinerea* alone, and it was variable according to the level of inoculum of the pathogen (Table 2-a). In all trials, both the antagonist and the fungicide significantly limited disease development (Table 2-b). The efficacy of the antagonist was slightly less affected by the overall severity of the tests than that of the fungicide (Table 2).

Survival of the antagonist in wounds on tomato plants: Two tests were conducted in greenhouse conditions and the population of *Fusarium* sp. was monitored for 3 weeks after a spore suspension was applied on petiole stubs. Only slight changes in population size were observed for the duration of the experiment (Figure 2), indicating a steady survival of the fungus within the pruning wounds.

DISCUSSION

The antagonistic strain of *Fusarium* sp. isolated from the epiphytic microflora of greenhouse tomatoes significantly reduced the incidence of stem lesions on tomatoes in four greenhouse trials conducted between 1994 and 1996. Its efficiency in protecting pruning wounds was consistent for two cultivars of tomatoes, and over a variety of fluctuating environmental conditions and levels of disease pressure.

The antagonist survived well in the pruning wounds, with population densities remaining close to those introduced. It appeared to retain efficiency over several months even in the most severe of the tests, where disease incidence reached nearly 80% on the control plants. Work is in progress to further evaluate the potential of this micro-organism as a biocontrol agent.

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Table 1: Efficiency of a strain of *Fusarium* sp. to protect pruning wounds on 9-week old tomato plants against infection by *Botrytis cinerea*.

A: Pruning by cutting leaves 5 cm from the stem

Treatment (Number of spores/wound)	AUDPC * (7 days)		Percentage of protection
B. cinerea (10^5)	143.8	a	
B. cinerea (10^5) + antagonist (10^4)	13.7	b	90
B. cinerea (10^5) + antagonist (10^3)	6.9	b	95
B. cinerea (10^5) + antagonist (10^2)	108.1	a	25
B. cinerea (10^5) + antagonist (10)	123.1	a	14

B: Pruning by cutting leaves flush to the stem

Treatment (Number of spores/wound)	AUDPC * (13 days)		Percentage of protection
B. cinerea (10^4)	294.6	a	
B. cinerea (10^4) + antagonist (10^6)	15.0	b	95
B. cinerea (10^4) + antagonist (10^5)	32.1	a	89

*: AUDPC: « area under the disease progress curve » over 7 days or 13 days after inoculation; the values followed by different letters differ significantly at 5% level (Newman and Keuls test).

Table 2: Efficacy of a strain of *Fusarium* sp. to protect pruning wounds of tomato against *Botrytis cinerea* in greenhouse conditions.

Year	1994-1995	1995-1996	1996	1996 (CTIFL)
B. cinerea inoculum [1]	500	50	airborne	50

A. Percentage of pruning wounds developping stem lesions at the end of the growing season.

Botrytis cinerea	78,5	a[2]	56,0	a	43,3	a	42,3	a
+ *Fusarium* sp.	41,2	b	30,0	b	16,8	b	9,0	b
+ Sumico	23,8	c	7,0	c	8,0	b	0	c

B. Rate of protection of the pruning wounds (%), computed on the basis of the AUDPC's over the growing season

Botrytis cinerea		a[2]		a		a		a
+ *Fusarium* sp.	69	b	64	b	69	b	78	b
+ Sumico	91	c	94	c	83	b	100	c

1: number of spores of *B. cinerea* applied on the petiole stub; airborne = no spray-inoculation of *B. cinerea*
2: numbers followed by the same letters are not significantly different at the 5% level; tests of Newman and Keuls performed on percent disease incidence (A) or AUDPC values (B)

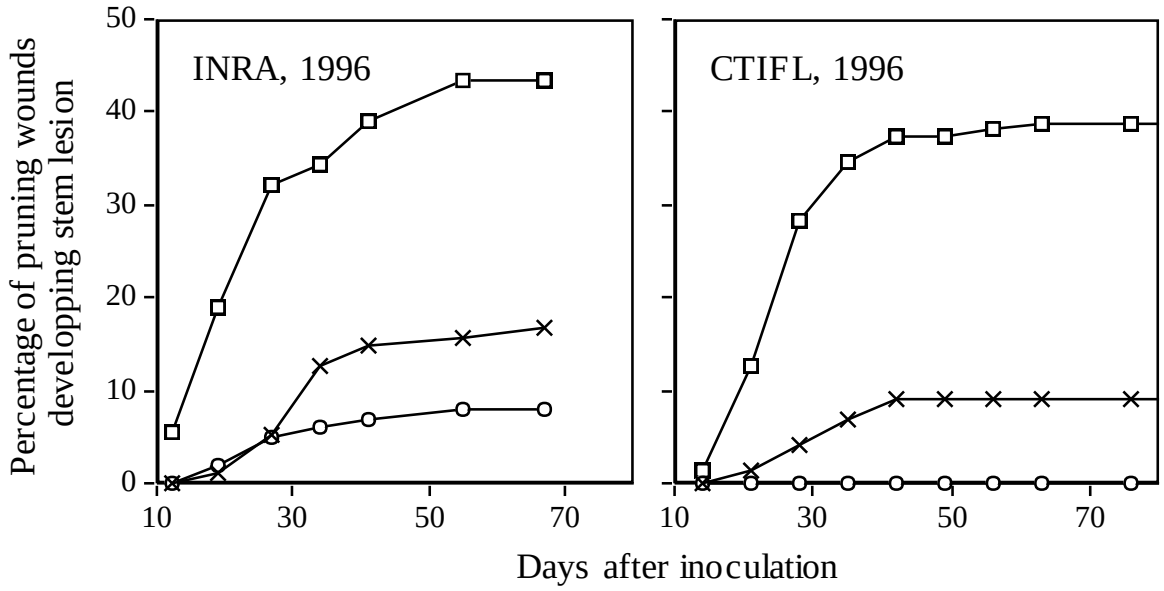

Figure 1: Protection of pruning wounds on tomatoes against *Botrytis cinerea* in greenhouse trials in 1996: (□) non protected control; (❍) fungicide; (▯) antagonist.

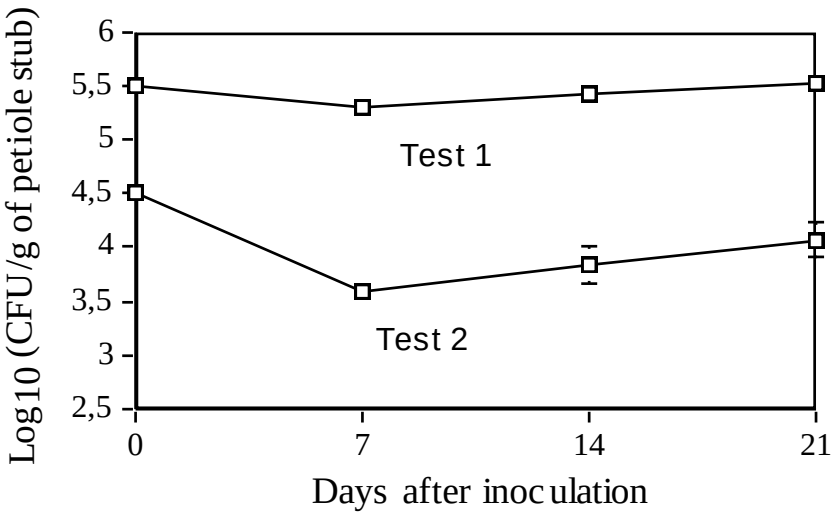

Figure 2: Survival of the antagonist *Fusarium* sp. in pruning wounds on tomatoes. Error bars indicate the standard error of the mean.