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Protection of stem wounds against *Botrytis cinerea* in heated tomato greenhouses with a strain of *Fusarium* sp.

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Abstract: An antagonistic strain of *Fusarium* sp. significantly reduced the incidence of stem lesions on tomatoes in 16 greenhouse trials conducted between 1996 and 1998 in southern France. Its efficiency in protecting pruning wounds was established for two tomato cultivars, and over a variety of fluctuating environmental conditions and levels of disease pressure. Delivery of this biological agent with a sprayers or with pruning shears bearing a small spray device was evaluated. Delivery of the antagonist via pruning shears did not seem to reduce the efficacy of wound protection. This result offers an interesting potential as the pruning-spraying shears allow simultaneous leaf removal and preventive wound protection. They present the additional advantage of delivering minute amounts of active ingredient in an optimally targeted fashion, reducing potential impact on the environment, including pollinators and other beneficial organisms.

Key words: grey mould, pruning shear with spray device, integrated and chemical controls

Introduction

Stem lesions, resulting mainly from infection of pruning wounds by *B. cinerea*, are one of the most devastating symptoms in heated tomato greenhouses. The incidence of the disease can be reduced by cultural practices, by physiological and climate controls and by the application of fungicides (Elad & Shtienberg, 1995; Nicot & Baille, 1996). Biological control could provide an alternative control measure. Recently, we selected from the epiphytic microflora of greenhouse tomatoes a fungal antagonist, provisionally identified as *Fusarium* sp., for its efficiency to protect pruning wounds on tomato plants (Nicot *et al*., 1996; Decognet *et al*., 1997).

This paper presents the results of greenhouse trials conducted in conditions close to a commercial situation to evaluate the efficiency of the antagonist to protect pruning wounds against *Botrytis*. Three means of delivering the antagonist were tested.

Material 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.

Experimental design

Three tests were conducted between 1994 and 1996 on plants (cv. "*Rondello* ") approximately 5 months old in a glasshouse at INRA. Treatments were arranged in a block design with four replicates (10 plants per replicate, 5 leaves per plant). Similar tests were then conducted (1996 to 1998) in a glasshouse at CTIFL on

3-month old plants (cv "*Trust*"). The treatments were distributed randomly in a block design. The cultural practices in all trials were kept as close as possible to a commercial situation.

Inoculations and disease rating

Five leaves were removed from each plant and spores of *B. cinerea* were applied on pruning wounds as a suspension in water (most experiments- see Table 1) or as dry airborne inoculum. Different inoculum levels were used to obtain different conditions of disease severity (Table 1). Spores of *Fusarium* sp. were applied on pruning wounds as a suspension in water ($5x10^5$-$5x10^6$ CFU/ml). The efficiency of the strain of *Fusarium* sp. was compared to that of Sumico L and water was applied on the untreated control plants.

Stem infection was monitored twice weekly for 10-12 weeks after inoculation. The efficiency of a control method was estimated by the reduction in percentage of pruning wounds developing stem lesions for treated plants relative to the untreated control plants. The statistical analyses were performed on these figures.

Three means of application of the antagonist and the fungicide treatments were tested:
(i) by localized spray with a hand-held sprayer to ensure that the treatment was evenly distributed on all wound surfaces, (ii) application on the whole deleafed area of the plants with a back-pack sprayer and (iii) with pruning shears equipped with a small spray device.

Results and discussion

In all the trials, substantial disease development was observed on the plants inoculated with *B. cinerea* alone, and varied according to the level of inoculum applied to the pruning wounds (Table 1). Symptoms were first detected within 1-2 weeks after inoculation and the incidence of stem lesions progressed over the growing season (Fig. 1-A).

Efficacy of the antagonist delivered as a spray treatment

On wounds sprayed with the fungicide or with *Fusarium* sp., the incidence of stem lesions increased slowly until 40-50 days after inoculation and remained very low throughout the trials (Fig. 1-A). The antagonist provided significant, long lasting protection of the pruning wounds, despite wide differences in environmental conditions and severity of inoculum pressure among the different trials. The protection level, often close to that afforded by the fungicide, did not appear to be affected by the tomato variety (Table 1-A-B).

Efficacy of the antagonist applied with pruning shears bearing a small spray device

On wounds inoculated with *B. cinerea* alone, disease development was similar to the previous trials (Fig. 1-B), although maximum disease incidence appeared to be overall lower on trials where the pruning shears were used (Table 1-C). In all trials, the antagonist delivered with the pruning shears provided highly significant, long lasting protection of the pruning wounds.

The antagonistic strain of *Fusarium* sp. isolated from the epiphytic microflora of greenhouse tomatoes significantly reduced the incidence of stem lesions on tomatoes in 16 greenhouse trials conducted between 1996 and 1998 in southern France. Its efficiency in protecting pruning wounds was established for two tomato cultivars, and over a variety of fluctuating environmental conditions and levels of disease pressure. 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.

Delivery of the antagonist via pruning shears bearing a small spray device did not seem to reduce the efficacy of wound protection. This offers an interesting potential for tomato growers. Such pruning tools are generating increasing interest among greenhouse tomato growers in southern France as they allow simultaneous leaf removal and preventive wound protection. They present the additional advantage of delivering minute amounts of active ingredient in an optimally targeted fashion, reducing potential impact on the environment, including pollinators and other beneficial organisms.

Work is in progress to further evaluate the potential of this micro-organism as a biocontrol agent on other host-parasite systems.

References

Decognet, V., Nicot, P., Trottin-Caudal, Y. & Fournier, C. 1997: Biocontrol of *Botrytis cinerea* stem infection on greenhouse tomatoes with an antagonistic strain of *Fusarium*. *10th congress of the Mediterranean Phytopathological Union,* Montpellier, France: pp 695-699.

Elad, Y. & Shtienberg, D. 1995: *Botrytis cinerea* in greenhouse vegetables: chemical, cultural, physiological and biological controls and their integration. Integrated Pest Management Reviews 1:15-29.

Nicot, P. C. & Baille, A. 1996: Integrated control of *Botrytis cinerea* on greenhouse tomatoes. In Aerial plant surface microbiology, Eds Morris, Nicot & Nguyen-the, Plenum Press, New York: pp. 169-189.

Nicot, P. C., Morison, N. & Decognet, V. 1996: Protection of pruning wounds on greenhouse tomatoes by saprophytic microorganisms against infection by *Botrytis cinerea*. *International Botrytis Symposium,* Wageningen, Netherlands: p 70.

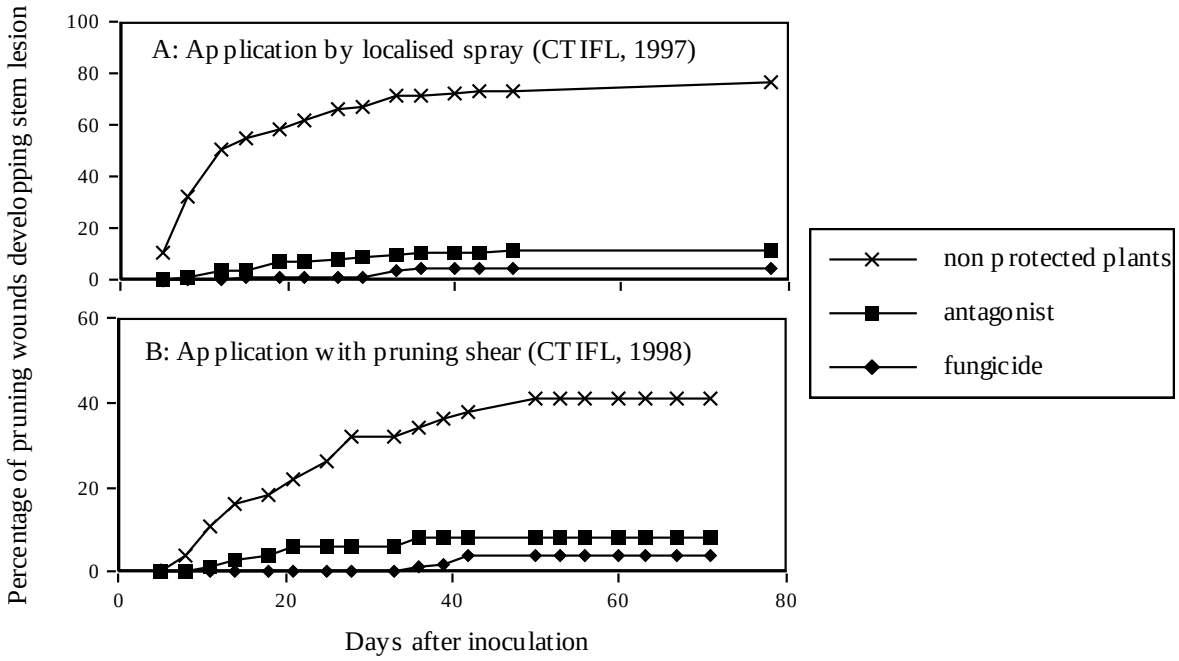

Figure 1: Protection of pruning wounds on tomato plants against *B. cinerea* in greenhouse trials in 1997 and in 1998. The treatments, water on non protected plants (□), the antagonist (□) and the fungicide (□) were applied by localised spray (A) or with pruning shears with spray device (B).

Table 1: Efficiency of *Fusarium* sp. to protect the pruning wounds in heated tomato greenhouses. The experiments were conducted in experimental greenhouses with cultural practices close to a commercial situation.

Year	*B. cinerea* inoculum[1]	% pruning wounds developing stem lesions on control plants	% protection[2] *Fusarium* sp.[3]	% protection[2] Fungicide[4]
A: Application of the antagonist and fungicide by localized spray at INRA (i) and CTIFL(c)				
1994-1995 (i)	500	78,5	48*[2]	70*
1994-1995 (i)	airborne	26,5	30	77*
1995-1996 (i)	50	56,0	47*	87*
1996 (i)	airborne	43,3	62*	82*
1996 (c)	50	42,3	79*	100*
1997 (c)	5000	50,6	95*	100*
1997 (c)	5000	73,4	82*	96*
1997 (c)	5000	76,1	85*	94*
B: Application of the antagonist and fungicide with a back-pack sprayer at CTIFL				
1998	5000	15.0	80	93
1998	5000	71.0	82*	97*
1998	5000	78.0	78*	99*
1998	aerial	65.7	83*	100*
C: Application of the antagonist and fungicide with pruning-spraying shears at CTIFL				
1997	5000	58,0	62*	99*
1998	5000	37,2	97*	100*
1998	5000	45,5	74*	100*
1998	5000	41,0	81*	90*
1998	aerial	60,6	82*	99*

[1]: number of spores of *B. cinerea* applied on the pruning wounds; airborne: no spray inoculation of *B. cinerea;* aerial: spores of *B. cinerea* were blown on pruning wounds

[2]: % reduction in the number of stem lesions at the end of the growing season on treated plants compared with the untreated control plants; An asterisk (*) indicates that the treatment was significantly different at the 5% level from the control untreated plants

[3]: 5 10^5 to 5 10^6 spores of *Fusarium* sp. applied per pruning wound

[4]: Sumico L applied at 2 l/hl (tests A and C) or 0.2 l/hl (test B)