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Nicot PC, Decognet V, Fruit L, Bardin M, Trottin Y, 2002. Combined effectif microclimate and dose of application on the efficacy of biocontrol agents for the protection of pruning wounds on tomatoes against Botrytis cinerea. *IOBC WPRS Bulletin* **25**, 73-76.

Combined effect of microclimate and dose of application on the efficacy of biocontrol agents for the protection of pruning wounds on tomatoes against *Botrytis cinereal*

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Abstract: The efficacy of two fungi shown in earlier studies to provide high levels of protection on pruning wounds, *Ulocladium atrum* and *Microdochium dimerum*, was evaluated in precisely regulated growth chambers over a range of conditions known to occur in commercial greenhouses in southern France. The effect of microclimate was not identical for all components of biocontrol efficacy. It was most pronounced on the ability of the biocontrol agents (BCAs) to prevent or reduce the incidence of wound infection by *B. cinerea*. The efficacy of protection was mostly influenced by temperature rather than by relative humidity. Wound protection was reduced at 10°C and increased significantly with increasing temperatures for both BCAs. Other components of biocontrol efficacy, such as the ability to delay the outbreak of wound infection and the ability to slow down lesion expansion, were little influenced by climate.

The observation of microclimatic effects on efficacy of biocontrol was highly influenced by the dose of BCA applied to the plants. Effects such as described above were strongly attenuated or completely masked when BCA spores outnumbered the pathogen's 10 to 1 after inoculation. In this situation, wound protection was uniformly high over most climate regimes, suggesting that doses may be adjusted to provide satisfactory levels of efficacy in conditions unfavourable for biocontrol.

This study demonstrated that *U. atrum* and *M. dimerum* may be useful for wound protection of greenhouse tomatoes in a wide variety of microclimatic conditions, including those occurring in minimally heated structures. Those results concur with several years of trials at crop level. Combined with estimates of naturally occurring inoculum levels of *B. cinerea* and data on the dynamics of *B. cinerea* and the BCA in the wounds, these results should provide useful information to adapt inoculum doses to the environmental conditions for achievement of optimal disease control.

Key words: Botrytis, grey mould, tomato, biological control, microclimate, dose

Introduction

Infection of pruning wounds and stem wounds by *Botrytis cinerea* in protected tomato production are found in France both in minimally heated tunnels and in heated glasshouses (with sophisticated microclimatic regulation) where they constitute the most damaging Botrytis symptom. They can occur in a wide range of microclimatic situations, some of which may not be favourable to the efficacy of biocontrol agents (BCAs). The main purpose of the present study was to evaluate the effect of temperature and relative humidity on the efficacy of *Ulocladium atrum* and *Microdochium dimerum,* two fungi shown to provide high levels of protection on pruning wounds (Decognet *et al*., 1999; Fruit and Nicot, 1999). It was further hypothesised that the dose of application of the BCAs might influence the impact of microclimate on efficacy

Materials and methods

The same fungal isolates were used throughout this study: monoconidial tomato isolate BC1 of *B. cinerea* (Nicot *et al*., 1996), isolate Ua385 of *U. atrum* (kindly provided by J. Köhl) and isolate L13 of *M. dimerum* (obtained from a healthy pruning wound in a tomato greenhouse with particularly high incidence of grey mould).

We used potted tomato plants cv Raissa and cv Felicia. The plants were produced in a heated greenhouse and used when they had 7-9 fully expanded leaves. The plants were watered daily with a nutrient solution. Watering was interrupted 8-24 hours before the plants were used in experiments to avoid guttation following leaf removal and to facilitate the absorption of inoculum into the wounds.

The effect of microclimate on efficacy of biocontrol was evaluated in multifactorial studies conducted in precisely regulated growth chambers (Boulard *et al*., 1996). Eighteen regimes were tested, combining a temperature of 10°C, 15°C, 20°C, 25°C, 30°C or 35°C with a relative humidity (RH) of 50%, 70% or 90%. Separate tests with different inoculum concentrations and methods of inoculation were designed to evaluate different components of biocontrol efficacy.

For each climate regime, three critical steps of the disease cycle were considered: (1) infection of pruning wounds; (2) lesion expansion and (3) sporulation on diseased tissue. Five plants were used for each climate regime and each type of wound treatment under study and the whole set of experiments was repeated at least 3 times in 1998-2001 for each BCA (up to 5 times). Pruning wounds were inoculated with *B. cinerea* alone or together with either BCA. The number of infected pruning wounds per plant and if applicable, the length (in mm) of lesions that developed on the petiole stubs and on the stems were recorded daily between the third and seventh days after inoculation. Spore production was quantified on stem lesions seven days after inoculation in all tests with *U. atrum*.

To evaluate the combined effect of climate and dose of BCA application, varying inoculum concentrations were used in some of the replicated studies, to obtain different BCA-pathogen ratios in the initial microbial populations.

Results

Effect of microclimate on efficacy of wound protection

Infection of pruning wounds on plants inoculated with *B. cinerea* alone was observed in all conditions tested except those with a temperature of 35°C. The highest rates of disease incidence were recorded at 90% relative humidity and the lowest at 50% (Figure 1). They tended to be lower at higher temperatures.

Treatment of wounds with either *U. atrum* or *M. dimerum* immediately after inoculation with *B. cinerea* significantly ($p<0.01$) reduced the incidence of wound infection (Figure 1). However, the effect of the biocontrol agents was not identical over the different climate regimes. Infection levels on BCA-treated plants were disticntly lower at higher temperatures and also somewhat lower at 90% and 70% RH than at 50%.

To distinghuish the effect of microclimate on biocontrol from that on the infection process by *B. cinerea* alone, a protection index was computed as:

$$\text{Protection Index} = 100 \times (\text{Disease}_{\text{control}} - \text{Disease}_{\text{BCA}}) / \text{Disease}_{\text{control}};$$

where "Disease" represented the area under the progress curve over 7 days after inoculation for plants inoculated with *B. cinerea* alone ("control") or together with a BCA ("BCA"). Analysis of variance on these data (Figures 2 A and C) indicated a significant effect of temperature but not of relative humidity (for example, p values of 0.001, 0.17 and 0.81 for T, RH and T*RH interaction factors, respectively, for *M. dimerum*). Efficacy of both BCA's was distinctly lower at 10°C and increased with increasing temperatures.

Effect of microclimate on other components of biocontrol efficacy

In cases when wound infection was not prevented by treatment with the BCAs, their ability to delay the outbreak of lesion development was monitored. Both BCAs had a significant effect (average delay of 0.7 day; $p<0.001$ for *M. dimerum* for example), but a high level of variability was recorded over the different climate regimes and no significant temperature or RH effects were observed (for example, $p>0.21$ and 0.38, respectively, for *M. dimerum*).

Similarly, the ability to slow down lesion expansion and to inhibit sporulation of *B. cinerea* was variable and little influenced by climate.

Combined effect of dose and microclimate on efficacy of biocontrol

When the dose of BCA applied to the wounds was ten times that of the inoculum of *B. cinerea*, the protective effects of *U. atrum* and *M. dimerum* were strongly increased (Figures 2 B and D) and temperature no longer influenced significantly their efficacy ($p>0.08$ for *M. dimerum*, for example).

Other components of efficacy were also significantly strengthened in the rare cases when lesions developed (*eg*, average delay of 1.2 days in the outbreak of lesions and reduction of lesion expansion from 6.6 mm/day for control plants to 3.3 with *M. dimerum*). No sporulation of *B. cinerea* was observed on any of the wounds treated with the "high" dose of *U. atrum*.

Discussion-conclusions

This study demonstrated that *U. atrum* and *M. dimerum* may be useful for wound protection of greenhouse tomatoes in a wide variety of microclimatic conditions. Those results concur with several years of trials at crop level. The generally limited effect of microclimate (particularly that of relative humidity) on efficacy of wound protection merits further attention, as both temperature and relative humidity are known to exert a high influence on the epidemiology of diseases caused by *B. cinerea*.

Combined with estimates of naturally occurring inoculum levels of *B. cinerea* and data on the dynamics of *B. cinerea* and the BCA in the wounds, these results should provide useful information to adapt inoculum doses to the environmental conditions for achievement of optimal disease control.

References

Boulard, T., Mermier, M., Fauvel, C., Nicot, P., & Lecomte, A. 1996: Development of a controlled-environment chamber for studying the epidemiology of fungal pathogens. J. Agric. Eng. Res. 65:51-61.

Decognet V., Trottin-Caudal Y., Fournier C., Leyre J.M. & Nicot P.C. 1999: Protection of stem wounds against *Botrytis cinerea* in heated tomato greenhouses with a strain of *Fusarium* sp. IOBC WPRS Bulletin 22:53-56.

Fruit L. & Nicot P.C. 1999: Biological control of *Botrytis cinerea* on tomato stem wounds with *Ulocladium atrum*. IOBC WPRS Bulletin 22:81-84.

Nicot, P. C., Mermier, M., Vaissière, B. E., & Lagier, J. 1996: Differential spore production by *Botrytis cinerea* on agar medium and plant tissue under near-ultra-violet light-absorbing polyethylene film. Plant Disease. 80:555-558.

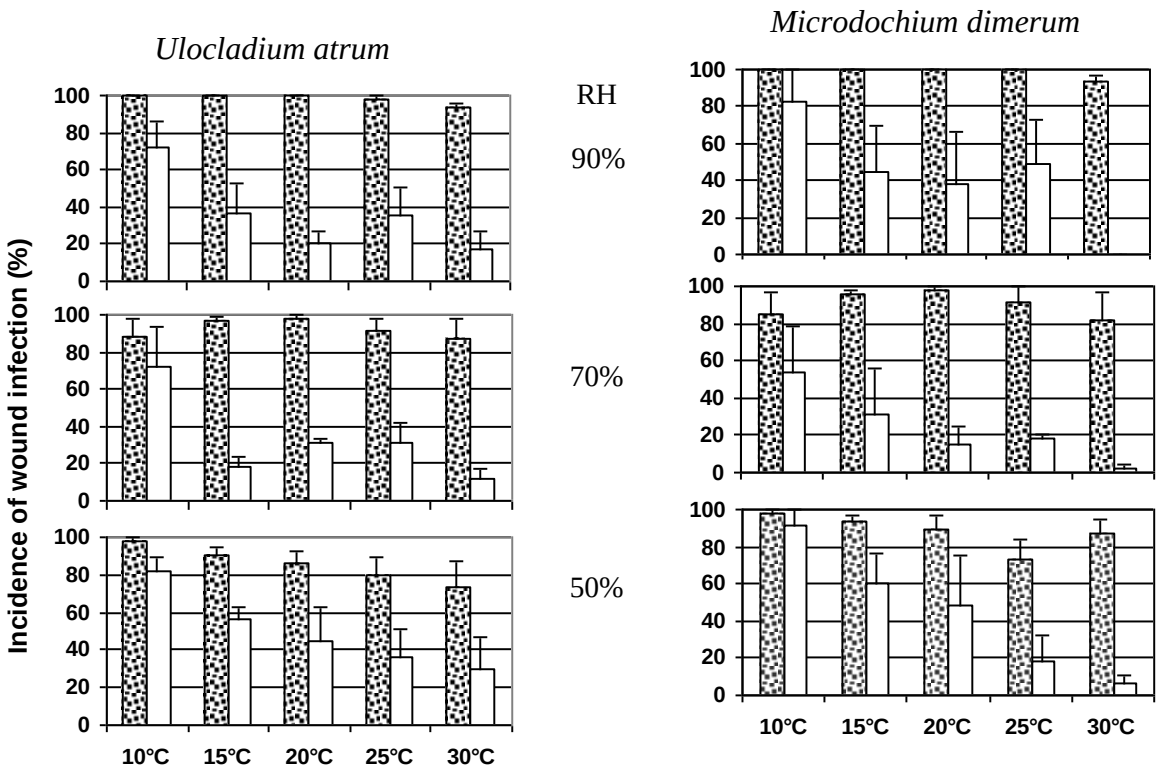

Figure 1. Effect of temperature (°C) and relative humidity (RH) on the incidence of wound infection 7 days after inoculation with *Botrytis cinerea* alone (■) or together with a biocontrol agent (□). Both the pathogen and the biocontrol agents (*U. atrum* and *M. dimerum*) were inoculated at 10^7 spores/m.l. Error bars indicate the standard errors of the means.

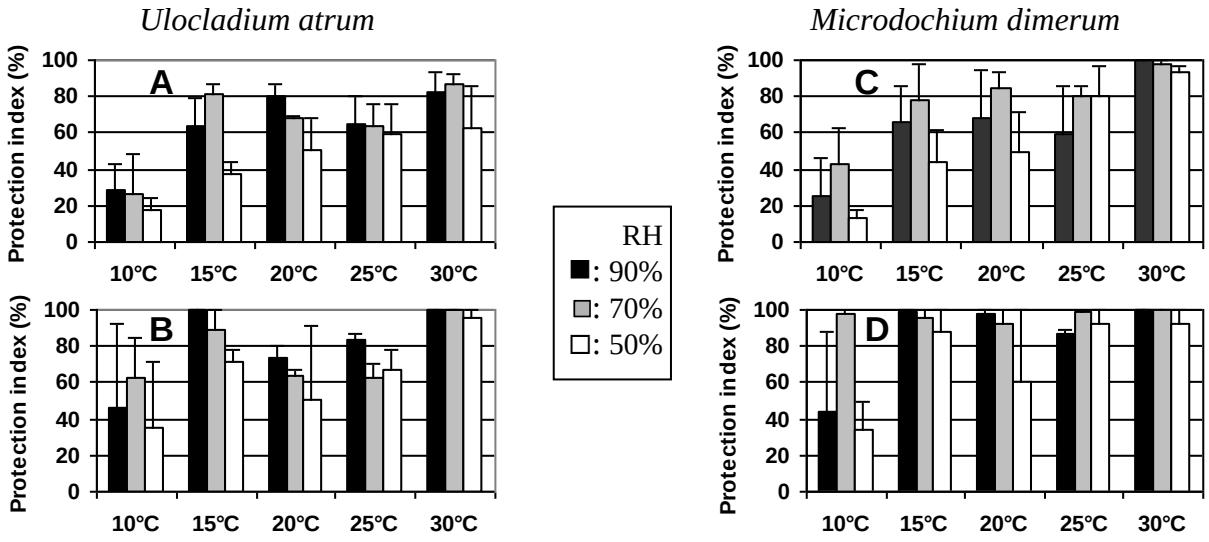

Figure 2. Effect of temperature (°C) and relative humidity (RH) on protection of tomato pruning wounds by *U. atrum* and *M. dimerum* (applied at 10^7 spores/ml) against infection by *Botrytis cinerea*. Protection indices were computed on the basis of percent infected petiole stubs 7 days after inoculation with *B. cinerea* at 10^7 (A, C) and 10^6 (B, D) spores/ml. Error bars indicate the standard errors of the means.