Potential for including Microdochium dimerum, a biocontrol agent against Botrytis cinerea, into an integrated protection scheme of greenhouse tomatoes

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Abstract: Strain L13 of *Microdochium dimerum* was shown in earlier work to be efficient as a protectant of tomato pruning wounds against *Botrytis cinerea* at crop level in a wide range of environmental conditions. In a study implicating seven independent trials at crop level in 1999-2001 with different doses and application schedules, the biocontrol agent provided efficient protection of foliage against *B. cinerea*. It had no effect against powdery mildew at the doses tested. In these trials, it displayed complete compatibility with extracts of *Reynoutria sachalinensis* (Milsana), a biocontrol preparation known to control powdery mildew. The efficacy of *M. dimerum* against *B. cinerea* was not altered by co-application on foliage with recommended doses of Milsana, and the efficacy of Milsana for powdery mildew control was not altered by co-application with *M. dimerum*. Utilisation in combination or alternation with chemical control is also promising, as several insecticides and fungicides used on tomatoes against *B. cinerea*, powdery mildew and other pathogens, show no toxicity *in vitro* against *M. dimerum*.

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

Résumé: Des travaux précédents ont mis en évidence l'efficacité de la souche L13 de *Microdochium dimerum* pour la protection des plaies d'effeuillage de la tomate sous abri contre *Botrytis cinerea*. Sept essais indépendants réalisés en 1999-2001 en conditions proches de la production commerciale ont montré l'efficacité de cet agent de lutte biologique pour la protection du feuillage contre *B. cinerea*. Aucune protection contre l'oïdium n'a été constatée aux doses testées. Une compatibilité complète a été observée entre *M. dimerum* et un extrait de *Reynoutria sachalinensis* (Milsana), l'efficacité de l'un (*M. dimerum* contre *B. cinerea* et Milsana contre l'oïdium) n'étant pas altérée par une co-application avec l'autre sur le feuillage. L'utilisation de *M. dimerum* en combinaison ou alternance avec la lutte chimique est aussi envisageable, du fait de l'absence de toxicité *in vitro* de plusieurs insecticides et fongicides utilisés sur la tomate contre *B. cinerea* et d'autres champignons phytopathogènes.

Mots-clés : Botrytis, pourriture grise, tomate, lutte biologique, microclimat, dose

Introduction

Strain L13 of *Microdochium dimerum* was shown to be efficient at crop level as a protectant of pruning wounds against *Botrytis cinerea*, a recurrent and important nuisance in tomato glasshouses (Decognet *et al*., 1999). Its use in an integrated protection scheme, however, requires compatibility with other plant health management interventions. One objective of the present study was to assess its protective effect on foliage against two major aerial diseases of greenhouse tomato, *Botrytis* leaf blight, and powdery mildew caused by *Oidium neolycopersici*. The other objective was to investigate the compatibility of using *M. dimerum* with chemical protectants and with biocontrol agents used on tomatoes.

Materials and methods

Trials at crop level.
Tomatoes were grown hydroponically in seven 60-m² glasshouse compartments at the Balandran experimental station (Ctifl) in conditions similar to those of commercial production. Four compartments were used for the assessment of powdery mildew control and three for *Botrytis*. Different spray schedules, doses and combinations of protectants were tested between 1999 and 2001 in seven independent trials. Large sheets of plastic were used to isolate plots during the treatments to prevent cross-contaminations. The plants were artificially inoculated with either *Oidium neolycopersici* (10^4 spores/ml) or *B. cinerea* (dry spores) and conditions favourable to disease development were maintained (increased humidity). Disease incidence and severity were recorded at weekly intervals on at least 10 contiguous plants per compartment (8 in the powdery mildew compartments).
Effect of chemical protectants* in vitro *on* Microdochium dimerum *L13.
Commercial preparations of fungicides and insecticides were incorporated into PDA medium to obtain active ingredient concentrations of 0.1, 1, 10 and 100 mg/L. For each compound the germination rate of *M. dimerum* spores after 24 hrs at 21°C was compared to that on unamended medium (3 x 100 spores examined for each concentration) and the concentration responsible for 50% or 100% reduction in germination was estimated (IC_{50} and IC_{100}). Similarly, the IC_{50} and IC_{100} were assessed for germ tube elongation after 24 hrs (35 germ tubes measured per concentration) and for mycelial growth after 7 days (3 plates per concentration).

Results

Protection of foliage by* M. dimerum *L13 against* Botrytis *blight and powdery mildew
In all crop level trials, incidence of leaf blight in the *Botrytis*-infested compartments remained lower on plants treated weekly with $5x10^7$ sp/ml *M. dimerum* than on control plants. The protection relative to control plants was statistically significant (and statistically not different from the chemical reference) in 5 of 7 independent trials (data not shown). Disease severity, assessed at the end of the trials, was also noticeably reduced (Table 1). However, no protective effect of the BCA was observed against powdery mildew.

Compatibility of* M. dimerum *and Milsana for the control of* Botrytis *and powdery mildew
The adjunction of *Reynoutria sachalinensis* extract (Milsana-VP99) did not reduce the efficacy of *M. dimerum* treatments against Botrytis leaf blight (Table 1). Similarly, the adjunction of *M. dimerum* did not reduce the efficacy of Milsana against powdery mildew (Table2).

Sensitivity of* Microdochium dimerum *L13 to chemical protectants of Tomato
The compounds tested varied in their effect on spore germination and mycelial growth of *M. dimerum* L13 *in vitro* (Table 2). The BCA showed no sensitivity to several botryticides. It was also unaffected by several compounds against powdery mildew (including sulphur, and the plant extract Milsana), or other airborne or soilborne pathogens. In contrast, it was highly sensitive to compounds registered against late blight, with the exception of copper. None of the insecticides included in the tests was toxic to *M. dimerum* (data not shown).
Validation of these results on plants will be necessary, as it was shown in an earlier study (Decognet & Nicot, 1999) that fungicides which reduced germination or/and mycelial growth *in vitro* did not necessarily disrupt the efficacy of wound protection by the biocontrol agent.

Table 1: Efficacy of *Microdochium dimerum* L13, alone or in combination with Milsana-VP99, for the protection of foliage against *Botrytis* blight at crop level.

Trial	% necrotic leaf area on control plants	Protection (%) relative to control plants		
		Md-L13 (5.10^7 sp./ml)	Md-L13 (5.10^7 sp./ml) + Milsana 0,25%	Sumico L 2 L/ha
2	74.4 ± 2.5[a]	83.0 ± 3.3	-	99.1 ± 0.3
3	20.2 ± 0.6	91.3 ± 1.2	-	94.4 ± 0.7
4	27.9 ± 11.5	43.8 ± 18.9	68.6 ± 9.8	82.8 ± 3.6
5	50.2 ± 13.4	69.0 ± 10.8	77.7 ± 10.9	83.6 ± 8.7
6	48.8 ± 2.1	70.0 ± 3.4		71.2 ± 14.2
7	21.8 ± 9.9	59.3 ± 24.8		87.7 ± 5.4

[a] standard error of the mean

Table 2: Compatibility of *Microdochium dimerum* L13 with *Reynoutria sachalinensis* extract Milsana-VP99, for the protection of foliage against powdery mildew at crop level,.

Trial	% mildewed leaf area on control plants	Protection (%) relative to control plants		
		Milsana 0,25%	Milsana 0,25% + Md-L13 (5.10^7 sp./ml)	Anvil 0.6 L/ha
4	31.7 ± 15.0 [a]	94.8 ± 1.9	91.0 ± 2.6	100
5	94.1 ± 3.7	93.3 ± 0.9	92.8 ± 1.3	100

[a] standard error of the mean

Table 3: Effect of chemical protectants of tomato on germination and growth of *Microdochium dimerum* L13 *in vitro*.

Compound (Registered use in France[a])	50% Inhibitory concentration (mg / L) for		
	germination	germ tube elongation	mycelial growth
bupirimate (PM); carbendazime (F, CR, V); carbendazime + diethofencarb (GM); copper (B, LB); Milsana (PM- not registered); pyrimethanil (GM); sulphur (PM, mites)	> 100	> 100	> 100
procymidone (GM); vinchlozolin[b] (GM)	> 100	1-10[c]	> 100
iprodione (GM, AL); hexaconazole (PM)	> 100	1-10[c]	10-100[c]
triforin (PM, C)	10-100	10-100	10-100
difenoconazole (AL, FR)	10-100[c]	0,1-1[e]	0,1-1[c]
chlorothalonil (AL, BF; C, LB, FR)	1-10[d]	<0,1[d]	> 100
mancozeb (AL, AN, C, LB, BF)	1-10	1-10	> 100
dichlofluanid (GM, LB)	1-10	1-10	1-10[c]
azoxystrobin (AL, LB, PM)	1-10	0,1-1[e]	1-10[c]

[a] source: Index Phytosanitaire 2003 , ACTA, France. AL: Alternaria; AN: Anthracnose; B: bacteria; BF: black foot; C: Cladosporium; CR: corky root; FR: fruit rot; F: Fusarium; GM: Gray mould; LB: late blight; PM: powdery mildew; V: Verticillium

[b] no more registered on tomato in 2003

[c] IC_{100} > 100

[d] IC_{100}: 10-100

[e] IC_{100}: 1-10

Discussion-conclusions

Efficient biological control agents (BCAs), including strain L13 of *M. dimerum*, have been selected to protect tomato pruning wounds against *B. cinerea*, a recurrent and important nuisance in greenhouse production. However, their use in commercial conditions must be considered in a context of integrated protection. This requires that their efficiency is not altered when applied in conjunction with other control methods against *B. cinerea*, and when growers intervene against other plant pathogens and pests that may also occur in the greenhouse.

Combined with findings from earlier work, the results obtained in the present study suggest that *M. dimerum* has potential as a component of an integrated protection scheme in the tomato greenhouse. It retains efficacy as a wound protectant over a wide range of temperature and relative humidity levels, including humid, disease-enhancing conditions, and dry conditions sought by growers to limit the epidemics of *Botrytis* blight (Nicot *et al.* 2002).

The spectrum of tolerance of *M. dimerum* to chemical protectants suggests that its application could be performed in alternation or in combination with several of the pesticides registered for use in France in tomato greenhouses. Its compatibility with copper, sulphur and Milsana also makes it a possible candidate for use in organic production.

Further work will include assessing the compatibility of *M. dimerum* with biocontrol agents against whiteflies, an increasingly important pest in the tomato greenhouse, and the impact of airborne inoculum, and variability of natural populations of *B. cinerea* on epidemics and the durability of non-chemical control methods.

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