European Journal of Plant Pathology **105:** 115–122, 1999.

Evaluation of microbial antagonists for biological control of *Botrytis cinerea* stem infection in cucumber and tomato

A.J. Dik[1], G. Koning[2] and J. Köhl[2]
[1]*Research Station for Floriculture and Glasshouse Vegetables (PBG), P.O. Box 8, 2670 AA Naaldwijk, The Netherlands (Fax: 31-174-636835; E-mail: A.J.Dik@PBG.AGRO.NL);* [2]*DLO Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, The Netherlands*

Accepted 2 November 1998

Key words: antagonism, *Aureobasidium pullulans*, *Cryptococcus albidus*, *Trichoderma harzianum*, grey mould

Abstract

Of fifteen isolates of yeasts, filamentous fungi and bacteria and a commercial product, tested in a bioassay with stem segments, eleven isolates consistently reduced incidence of disease and sporulation of *Botrytis cinerea* Pers; Fr in tomato and seven isolates in cucumber. Several isolates reduced disease by more than 75% in all experiments. Six antagonists that performed well in the bioassays and that were fairly easy to produce *in vitro*, were selected for further testing in two glasshouse experiments with cucumbers. After application of spores of *B. cinerea* and the antagonists or the fungicide tolylfluanid to pruning wounds, disease incidence was reduced by 50–100% by all antagonists in both experiments and only in one experiment by tolylfluanid.

For *Trichoderma harzianum* T39, *Aureobasidium pullulans* and *Cryptococcus albidus*, biological control efficacy in bioassays with cucumber stem segments was not strongly influenced by temperatures in the range between 18 and 30 °C, but at 24 °C the efficacy of the three antagonists strongly decreased at relative humidities of 90% and 80% (vapour pressure deficits 0.299 and 0.598 kPa, respectively) compared to 100%.

Introduction

Botrytis cinerea Pers; Fr. is an important pathogen in many glasshouse vegetables. At high humidity or when free moisture is present on the plant surface, the pathogen can infect flowers, fruits, leaves and stems. In modern, climate-controlled glasshouses, grey mould on flowers, fruits and leaves can be minimized by preventing conducive conditions for the pathogen (Wilson, 1963; Winspear et al., 1970; Bravenboer and Strijbosch, 1975; Morgan, 1984), but stem lesions on pruning or harvesting wounds remain an important yield-reducing factor, especially in tomato and cucumber. The wound tissue apparently provides sufficient moisture and nutrients for conidial germination and infection by the pathogen.

B. cinerea frequently becomes resistant to chemical fungicides (Katan, 1982; Katan et al., 1989; Steekelenburg, 1987). Furthermore, for environmental reasons research is aimed at limiting the input of fungicides. Biological control is an alternative option to reduce *Botrytis* infection and has been shown to be effective in many crops (Elad et al., 1996). However, commercialization of biocontrol agents is thus far limited to one product based on *Trichoderma harzianum* T39 (Trichodex, Makhteshim Agan Chemical Works LTD., Be'er Sheva, Israel), registered in Israel, Greece and other countries. This product is used mainly against infection of leaves and subsequent growth of the fungus into the stem. In Dutch glasshouses, biological control should aim chiefly at prevention of infection of stem wounds. The objective of our study was to evaluate the ability of microbial antagonists including *T. harzianum* T39 to protect stem wounds of cucumber and tomato against *B. cinerea*. After initial studies in a bioassay, the most promising isolates were tested on wounds in

glasshouse-grown cucumbers. Subsequently, for three selected isolates the influence of climatic conditions on biological control efficacy was tested under controlled conditions.

Material and methods

Fungi and bacteria. The following isolates were used in biocontrol studies: *Botrytis cinerea* Pers.; Fr. (Bc700) from a gerbera flower; *Aureobasidium pullulans* (de Bary) Arnaud, *Cryptococcus luteus* (Saito) Skinner and *C. laurentii* var. *flavescens* Lodder et v. Rij from rye leaves (Fokkema, 1973); *C. albidus* from a potato leaf; *Gliocladium catenulatum* Gil. & Abbott from roots of red clover (kindly provided by P. Lüth, Prophyta Biologischer Pflanzenschutz GmbH, Malchow, Germany); *G. roseum* Bain. from potato peel; *Trichoderma harzianum* Rifai aggr. (T39) from cucumber fruit (Elad et al., 1993); *T. hamatum* (Bon.) Bain aggr. and *T. viride* Pers. ex S.F. Gray aggr. from soil and bark, respectively (Köhl, 1989); *Chaetomium globosum* Kunze ex Fr. and *Ulocladium atrum* Preuss from necrotic onion leaf tips (Köhl et al., 1995b); *Bacillus pumilus*, *Bacillus* sp., *Pseudomonas* spp. isolates 1 and 2 from potato leaves. The commercial product Trichodex, containing 10^{10} conidia g^{-1} of *T. harzianum* T39, was provided by Makhteshim Agan Chemical Works LTD., Be'er Sheva, Israel.

Inoculum production. *B. cinerea* was grown for 15–20 days and other filamentous fungi were grown for 20–35 days, all on oatmeal agar under black light at 18 °C. Yeasts were grown on basal yeast agar (10 g bacteriological peptone (Difco) l^{-1}, 1 g yeast extract (Difco) l^{-1}, 20 g glucose (Difco) l^{-1}, 20 g agar (Difco) l^{-1}) for 7 days and bacteria for 2 days on tryptic soy agar (Difco) at 20 °C in the dark. Spores or cells were washed from the agar with tap water containing 0.01% Tween-80 to prepare inoculum suspensions. Inoculum concentration was 1×10^5 conidia ml^{-1} for *B. cinerea*, 1×10^6 spores ml^{-1} for other filamentous fungi, 1×10^7 cells ml^{-1} for yeasts (including *A. pullulans*) and 1×10^8 cells ml^{-1} for bacteria. The product Trichodex was applied using the same concentration of conidia as the treatment with unformulated *T. harzianum* T39 in the bioassays and at a rate of 2.67 g l^{-1} in the glasshouse experiment.

Bioassays with tomato and cucumber stem segments. Tomato plants (*Lycopersicum esculentum* Mill.) cultivar 'Moneymaker' and cucumber plants (*Cucumis sativus* L.) cultivar 'Jessica' were grown in a glasshouse at 20 °C for 6–8 weeks. Stems of tomato and cucumber were cut into segments approximately 3 cm in length with a knife or blunt scissors, respectively (simulating wound structure in glasshouse crops). The stem segments were positioned vertically on a PVC strip using drawing pins (Koning and Köhl, 1995). The experimental design was a randomized block with four replicates, each with ten stem segments. PVC strips with stem segments of each treatment within a block were placed on two layers of wet filter paper in the same polycarbonate box ($46 \times 29 \times 7$ cm). Freshly cut wounds on stem segments were sprayed first with suspensions of antagonists or sterile tap water containing 0.01% Tween-80 by means of sterile atomizers and immediately thereafter with the conidial suspension of *B. cinerea* or tap water containing 0.01% Tween-80. The following treatments were included in each of eight experiments with tomato stem segments and 11 with cucumber stem segments: water–water, water–*B. cinerea*, antagonist–water, and antagonist–*B. cinerea*. Four to six antagonists were evaluated in each experiment, and each antagonist was tested twice. Stem segments were incubated in growth chambers at 18 °C with 16-h day length. Incidence and severity of infection and sporulation of *B. cinerea* were recorded after 8–11 days. Severity of symptoms and of sporulation were recorded separately using 5 classes: 0 = treated surface of stem segment green, no sporulation; 1 = only treated surface of stem segment affected; 2 = up to half of the length of the stem segment below the treated surface affected; 3 = 1/2 to 3/4 of stem segment affected; and 4 = > 3/4 of stem segment affected. Incidence of disease was defined as the percentage of stem segments with symptom severity >1. Segments in which only the inoculated surface was brownish (symptom severity = 1) were excluded because the symptoms lacked specificity for *Botrytis*. Incidence of sporulation of *B. cinerea* was defined as percentage of stem segments with sporulation value > 0. A disease severity index was calculated from the number of stem segments (n_x) belonging to class x for stem segments with incidence of symptoms (i_d) and sporulation (i_s), respectively, according to formula (1) and (2):

$$i_d = \frac{2n_2 + 3n_3 + 4n_4}{n_2 + n_3 + n_4}, \quad (1)$$

$$i_s = \frac{1n_1 + 2n_2 + 3n_3 + 4n_4}{n_1 + n_2 + n_3 + n_4}. \quad (2)$$

Glasshouse experiments with cucumber. Cucumber plants cv. Jessica and cv. Flamingo, of similar susceptibility to *B. cinerea*, were planted on June 2 in two glasshouses of 150 m^2. Half of each glasshouse was planted with each cultivar (96 plants of each cultivar per glasshouse). The plants were grown on rockwool slabs in recirculating nutrients according to normal Dutch practice.

Six antagonists were tested, i.e. *A. pullulans*, *C. albidus*, *G. roseum*, *C. globosum*, *Pseudomonas* sp. isolate 1 and the commercial product Trichodex. The controls consisted of a treatment with *B. cinerea* only and a treatment with the standard fungicide tolylfluanid (Eupareen M, Bayer B.V., Mijdrecht, The Netherlands, 50% active ingredient, dosage 1.5 g l^{-1}). Tolylfluanid was chosen because there is no risk of resistance against this fungicide. An extra control with water to which no *B. cinerea* was applied was added to assess natural infection.

Two experiments were run, one in each glasshouse. For each treatment there were two replicates, one in cv. Jessica and one in cv. Flamingo, and five arbitrarily selected plants per replicate. On each plant, two wounds were made by removing leaves 10 and 14, positioned respectively approximately 1.2 and 1.7 m above the ground. Within 5 min of removing the leaves, the fresh deleafing wounds were sprayed with a suspension of a biocontrol agent, sterile water or fungicide. Immediately after treatment, wounds were sprayed with inoculum of *B. cinerea*. Sterile glass reagent sprayers were used to apply the antagonists and pathogen.

In the first experiment, started on August 1, treatments were applied in the evening, because it was a warm and sunny day which is not conducive for *B. cinerea*. Afterwards, the floor was wetted and screens were closed to increase the humidity in the glasshouse and enhance infection. The second experiment was started on August 5, which was humid and cloudy. Treatments were applied in the morning.

In each experiment, RH and temperature were measured in the crop at a height of 1.5 m at 1-min intervals, averaged for 30-min intervals and stored in a VAX mainframe computer (Digital, Utrecht, The Netherlands). Vapour pressure deficit (VPD) was calculated.

Disease was assessed on days 7, 14 and 17 in exp. 1 and on days 10 and day 13 in exp. 2. The length of the lesion associated with each wound was measured and the percentage of deleafing wounds with lesions was calculated.

Effect of climatic conditions on biological control efficacy on cucumber stem segments. For *A. pullulans*, *C. albidus* and *T. harzianum* T39 (Trichodex), the effect of temperature and RH on their efficacy in controlling *B. cinerea* was tested in the bioassay with cucumber stem segments, as described above. Each bioassay was done in the dark at temperatures of 18, 24, 28 and 30 °C at approximately 100% RH (VPD = 0 kPa), which was achieved by closing the boxes, and at 24 °C at 90% (VPD = 0.299 kPa) and 80% (VPD = 0.598 kPa) RH in open boxes in a climate cabinet (Fitotron, Sanyo Gallenkamp PLC, Loughborough, England). Each combination of temperature and VPD was tested three times. In addition to assessment of incidence and intensity of disease and sporulation after one and two weeks, germination and germ tube length of *B. cinerea* conidia after 24 h were assessed in each treatment. Prints were made of four additional stem segments per treatment on cellulose tape (Sellotape), placed on microscopic slides and stained with lactophenol cotton blue. Germination and germ tube growth were assessed for 50 conidia per sample with a microscope and a semi-automatic image analyser (Videoplan, Carl Zeiss B.V., Weesp, The Netherlands). Germination incidence and average length of germ tube of germinated conidia were calculated.

Statistics. For the bioassays, incidence of symptoms and incidence of sporulation of *B. cinerea* on stem segments were analysed by means of the 'generalized linear model' (GLM). Data were examined by analysis of variance and treatment means were compared by an approximate Student's *t*-test ($P < 0.05$). Data on disease severity, represented by the i_d, were not statistically analysed because data frequently were based on only a few observations on infected stem segments when incidence of disease or sporulation had been reduced by efficient antagonists.

For the glasshouse experiments, angular transformed data on the percentage infected wounds and lesion size per infected wound were analysed with analysis of variance. The treatment means were compared by Fisher's protected LSD test ($P < 0.05$).

In the bioassays in which three antagonists were evaluated under six different sets of climatic conditions, the percentage inhibition was analysed rather than the absolute levels of disease since germination, disease and sporulation were variable in the controls. The percentage inhibition of germination after 24 h, of

incidence of disease after two weeks and of sporulation after two weeks were analysed as a split-plot experiment with six climates, three treatments and three replicates, in which the repetition of the experiment counted as replicate. Analysis of variance was followed by Fisher's protected LSD test ($P < 0.05$). All analyses were done with Genstat (Genstat 5 Committee, 1992).

Results

Bio-assays for antagonist screening. Grey mould incidence on stem segments treated only with the pathogen ranged between 74 and 100% in the eight experiments conducted with tomato stem segments and between 84 and 100% in the 11 experiments conducted with cucumber stem segments. *B. cinerea* sporulated on most infected stem segments. Disease severity and sporulation ranged between 3.4 and 4.0 for tomato 3.0 and 4.0 for cucumber (results not presented). Stem segments not treated with *B. cinerea* were not infected by the pathogen.

On tomato stem segments, all microorganisms tested except *T. viride*, *T. harzianum* T39, *Bacillus* sp., *B. pumilus* and Trichodex significantly reduced *B. cinerea* symptoms in both experiments (Table 1). The same isolates and *T. viride* significantly reduced sporulation. Three isolates, *A. pullulans*, *G. catenulatum* and *G. roseum* reduced disease symptoms and sporulation by more than 75% in both experiments.

Observations for stem segments of cucumber varied widely between experiments (Table 2). Some isolates, i.e. *C. luteus*, *C. albidus*, *C. laurentii* and *G. roseum*, failed to suppress *Botrytis* symptoms but inhibited sporulation in one experiment, and reduced both disease symptoms and sporulation in the second experiment. Several bacterial strains failed to reduce disease and sporulation in the two experiments. All other isolates showed effective control of disease and sporulation. Three isolates, *A. pullulans*, *G. catenulatum* and *C. globosum* reduced disease and sporulation by more than 75% in both experiments.

Once infection had been established, none of the antagonists tended to cause a large reduction in severity of disease or sporulation on stem segments of both

Table 1. Control efficacy of antagonists against disease development and sporulation of *Botrytis cinerea* in wounds of stem segments of tomato

Antagonist	Experiment I		Experiment II	
	Inhibition of disease[a] (%)	Inhibition of sporulation (%)	Inhibition of disease (%)	Inhibition of sporulation (%)
Aureobasidium pullulans	91	96	97	97
Cryptococcus luteus	39	50	44	55
C. albidus	30	37	49	59
C. laurentii var. *flavescens*	40	42	90	92
Gliocladium catenulatum	89	100	97	97
G. roseum	78	78	93	93
Trichoderma hamatum	38	67	34	51
T. harzianum (T39)	6 ns[b]	11 ns	12 ns	22 ns
T. viride	16 ns	44	39	41
Trichodex	17 ns	27 ns	10 ns	10 ns
Chaetomium globosum	66	68	97	97
Ulocladium atrum	68	75	80	100
Bacillus pumilus	15 ns	15 ns	26	36
Bacillus sp.	13 ns	17 ns	6 ns	38
Pseudomonas sp. isolate 1	55	58	78	79
Pseudomonas sp. isolate 2	57	70	77	85

[a]The efficacies of the antagonists were calculated compared to the control. The incidence of disease and sporulation in the control treatments ranged from 74 to 100 and 72 to 100%, respectively.

[b]Not significantly different from control treatment (Student's *t*-test, $P < 0.05$).

Table 2. Control efficacy of antagonists against disease development and sporulation of *Botrytis cinerea* in wounds of stem segments of cucumber

Antagonist	Experiment I		Experiment II	
	Inhibition of disease[a] (%)	Inhibition of sporulation (%)	Inhibition of disease (%)	Inhibition of sporulation (%)
Aureobasidium pullulans	85	95	95	97
Cryptococcus luteus	63	86	10 ns[b]	27
C. albidus	63	83	10 ns	47
C. laurentii var. *flavescens*	39	61	7 ns	25
Gliocladium catenulatum	77	95	95	100
G. roseum	−5 ns	96	74	97
Trichoderma hamatum	96	96	49	95
T. harzianum (T39)	41	55	82	100
T. viride	37	77	60	95
Trichodex	26	42	42	67
Chaetomium globosum	80	100	90	100
Ulocladium atrum	−15 ns	100	−5ns	100
Bacillus pumilus	27	33	2 ns	3 ns
Bacillus sp.	32	41	5 ns	5 ns
Pseudomonas sp.isolate 1	27	50	12 ns	54
Pseudomonas sp.isolate 2	10 ns	23	2 ns	32

[a]The efficacies of the antagonists were calculated compared to the control. The incidence of disease and sporulation in the control treatments ranged from 84 to 100 and 69 to 100%, respectively.
[b]Not significantly different from control treatment (Student's *t*-test, $P < 0.05$).

tomato and cucumber. Antagonist applications alone did not result in symptoms on the tomato stem segments except for *A. pullulans*, causing a number of small brown spots on the stem surface, and for *G. roseum* and *U. atrum* that induced browning of cucumber stem segments.

Selection of isolates for further testing. Six isolates selected for further testing in glasshouse experiments were *A. pullulans*, *C. albidus*, *G. roseum*, *C. globosum*, *Pseudomonas* sp. isolate 1 and the commercial product Trichodex. The selection was based on both the results in the bioassays and on growth on artificial media.

Glasshouse experiments. Values for temperature and VPD, averaged for 30-min intervals during the first 12 h after inoculation, were lower in exp. 1 in which inoculation took place late in the afternoon and extra moisture was provided by wetting the floors, than in exp. 2 in which plants were inoculated in the morning (Figure 1).

In exp. 1, no disease was observed on day 7. On day 14, disease had developed in all treatments except Trichodex and *Pseudomonas* sp. (Figure 2). The reduction in disease compared to the treatment with only *B. cinerea* was significant for Trichodex, *Pseudomonas* sp. and *G. roseum*. The fungicide treatment was not effective. On day 17, disease incidence had increased in the treatment with only *B. cinerea* and in the treatment with tolylfluanid and to a lesser extent in the Trichodex treatment. On this assessment date, all antagonists significantly reduced disease incidence, whereas tolylfluanid had no effect. In exp. 2, disease incidence in the control was higher on day 10 than on day 14 in exp. 1, and the increase between the first and second assessment date was smaller. On both assessment dates, all antagonists and tolylfluanid significantly reduced incidence of *B. cinerea*. Both *A. pullulans* and *G. roseum* completely prevented disease on both assessment dates (Figure 3). The control treatment with water without *B. cinerea* showed no grey mould symptoms in exp. 1 and a low disease level in exp. 2, indicating that the background level of infection was low. The average size of the lesions on infected wounds was similar in all treatments. In general, lesions were more frequent on leaf layer 10 than on leaf layer 14.

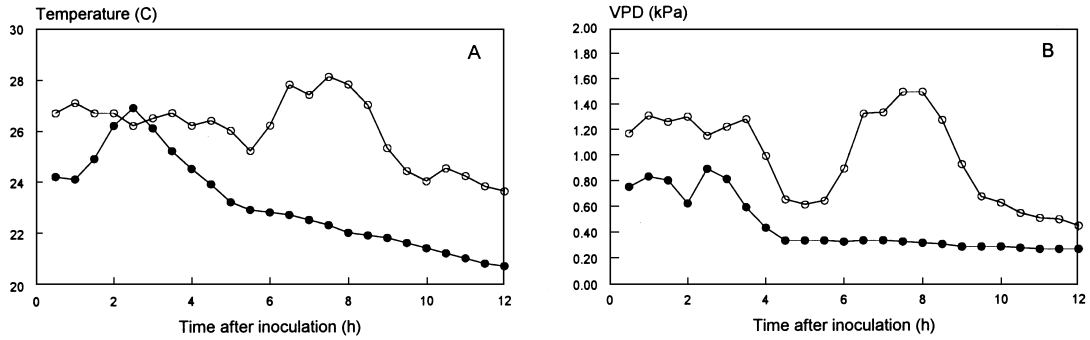

Figure 1. Temperature (A) and vapour pressure deficit (B) during the first twelve hours after inoculation in glasshouse experiments 1 (●) and 2 (○). Parameters were measured at 1-min intervals and averaged per 30 min.

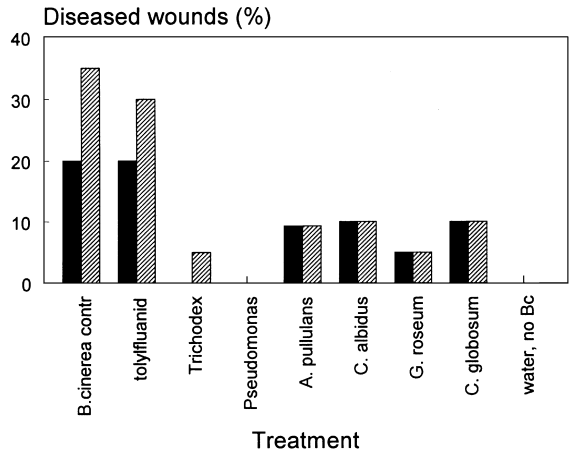

Figure 2. Effect of six biocontrol agents and tolylfluanid on the incidence of *B. cinerea* lesions on stem wounds in glasshouse-grown cucumber on day 14 (closed bar) and day 17 (open bar) in exp. 1. See text for statistical analysis.

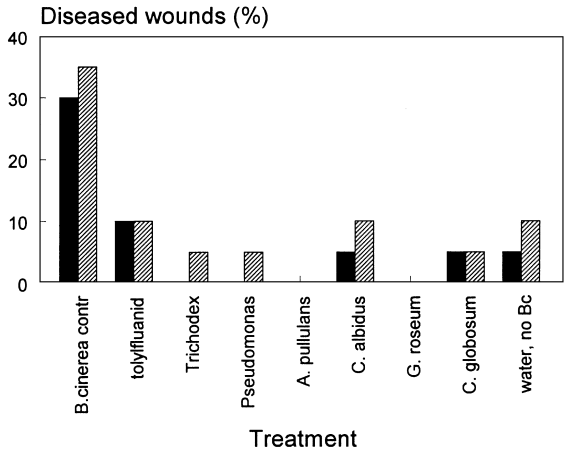

Figure 3. Effect of six biocontrol agents and tolylfluanid on the incidence of *B. cinerea* lesions on stem wounds in glasshouse-grown cucumber on day 10 (closed bar) and day 13 (open bar) in exp. 2. See text for statistical analysis.

Effect of climatic conditions on biological control efficacy on cucumber stem segments. Germination of *B. cinerea* conidia after 24 h in the controls ranged from 33 to 96% at 100% RH (all temperatures), but was below 10% at 80 and 90% RH at 24 °C. In most experiments, disease and sporulation were already visible after one week. After two weeks, incidence of disease and sporulation in the control treatment at 100% RH was between 30 and 100%. No effect of temperature was found on germination, incidence of disease or of sporulation in the control treatment.

Germination after 24 h was not significantly affected by treatments or climatic conditions (Table 3). Inhibition of disease after 2 weeks was higher than inhibition of germination after 24 h. The average germ tube length per germinated spore was not reduced by any of the antagonists. The efficacy of the three antagonists was not affected by temperature at 100% RH, but it declined with decreasing RH at 24 °C (Table 3).

Discussion

The bioassay, developed by Koning and Köhl (1995), has already been successfully used (O'Neill et al., 1996) and was in our work effective for selecting antagonists that protected wounds in stem segments of both tomato and cucumber against *B. cinerea*. From 15 antagonists and a commercial product tested, disease and sporulation were consistently reduced by 11 isolates in tomato and by seven isolates in cucumber. Eden et al. (1996) found effects similar to the results presented here for a range of filamentous fungi tested as antagonists of *B. cinerea* in tomato. Compared with

Table 3. Effectiveness of three antagonists against germination, disease development and sporulation of *B. cinerea* in cucumber stem segments under various climatic conditions

Climate	Treatment	Inhibition (%) of percentage germination	Inhibition (%) of disease incidence after two weeks	Inhibition (%) of incidence of sporulation after two weeks
18°C	*T. harzianum*	18.3[a]	68.3	64.7
100% RH	*A. pullulans*	10.3	68.3	52.0
(VPD = 0 kPa)	*C. albidum*	23.5	52.3	33.0
24°C	*T. harzianum*	24.5	60.0	48.0
100% RH	*A. pullulans*	20.1	76.7	67.0
(VPD = 0 kPa)	*C. albidus*	11.3	83.3	63.3
28°C	*T. harzianum*	23.9	90.0	71.4
100% RH	*A. pullulans*	7.8	95.4	32.4
(VPD = 0 kPa)	*C. albidus*	10.5	53.9	27.4
30°	*T. harzianum*	50.1	100.0	100.0
100% RH	*A. pullulans*	25.9	97.9	88.4
(VPD = 0 kPa)	*C. albidus*	28.1	41.4	15.9
24°C, 90% RH	*T. harzianum*	26.7	11.0	0.0
(VPD = 0.299 kPa)	*A. pullulans*	32.9	66.7	6.7
	C. albidus	32.9	0.0	0.0
24°C, 80% RH	*T. harzianum*	41.3	0.0	0.0
(VPD=0.598 kPa)	*A. pullulans*	41.3	0.0	0.0
	C. albidus	42.9	0.0	0.0
LSD[b] for climate		n.s.[c]	31.5	27.1
LSD for treatment		n.s.	22.3	19.2
LSD for treatment within the same		n.s.	54.6	47.0

[a]Data are averages of three replicate experiments.
[b]LSD = Least Significant Difference at $P = 0.05$.
[c]n.s. = not significant at $P = 0.05$.

the bioassays, all isolates performed at least as well on whole cucumber plants in the glasshouse. The most pronounced difference occurred in the performance of Trichodex in the bioassays in which a concentration of 1.0×10^6 CFU ml^{-1} was used and in the glasshouse experiments in which the recommended dose was used. It has been shown by Eden et al. (1996), that for filamentous fungi biocontrol activity was better at concentrations of 10^8 CFU ml^{-1} than at 10^7, 10^6 or 10^5 CFU ml^{-1} and that efficacy decreased with increasing inoculum density of the pathogen. Since the inoculum density of the pathogen in glasshouses will be much lower than what we used in our tests, the minimum effective inoculum density of the antagonists has to be further investigated in glasshouse experiments with natural infection of *B. cinerea*.

In the bioassay, the antagonists protected the wound surface from the development of symptoms but did not reduce severity of disease or sporulation of *B. cinerea* once lesions had been formed. The fact that the size of established lesions in the glasshouse experiments was not decreased by the antagonists is in accordance with results found in the bioassays. *U. atrum* was less efficient in wound protection but did suppress sporulation of *B. cinerea* effectively on cucumber and tomato, as was found also on dead onion and cyclamen leaves (Köhl et al., 1995a,b, 1998). In tomato and cucumber where *B. cinerea* causes yield losses by causing death of plants after stem girdling, prevention of infection of stem wounds as achieved by the selected antagonists is an effective control strategy. Microbial suppression of sporulation on dead tissue leading to lower inoculum densities may be an additional means to lower the risk of new wound infection.

Of the isolates tested in the glasshouse, *Gliocladium* spp. and *Trichoderma* spp. were very susceptible to desiccation in onion (Köhl et al., 1995b), but this is apparently not relevant in wound protection on whole plants since both isolates were very effective. In our bioassays, the yeasts *A. pullulans* and *C. albidus* but also *T. harzianum* T39 inhibited germination, disease and sporulation under a range of temperatures, but biocontrol efficacy was inhibited by increasing VPD at 24 °C. The inhibitory effect of VPD on biocontrol efficacy

was stronger than reported by O'Neill et al. (1996), who found that in tomato stem segments at 20 °C, the effect of *T. harzianum* T39 on infection was only negatively affected at a VPD above 1.3 kPa. However, in the glasshouse experiments all antagonists were effective even at the high temperature and VPD in exp. 2. For tomato it was shown that wounds remain susceptible to *B. cinerea* much longer on whole plants than on stem segments (O'Neill et al., 1997), indicating that wounds remain open longer on whole plants. This means that VPD will probably have less effect on biocontrol efficacy when the antagonists are used as whole-crop sprays. Furthermore, VPD in glasshouses is more variable than in our experiments and therefore, efficacy has to be assessed in larger scale experiments under conditions reflecting the normal glasshouse situation.

In conclusion, several isolates showed good control in our experiments and the next step is to test some of the successful isolates for their efficacy when applied as whole-crop sprays, both in tomato and cucumber. The experiments will compare the effect of these strains to chemical fungicides on natural *B. cinerea* infection under different climatic conditions to test the robustness of the isolates. If the antagonists will provide good control of *B. cinerea* in glasshouse trials on a semi-commercial scale, biocontrol may become an effective alternative control method for the Dutch glasshouse growers.

Acknowledgements

This research was funded by the Dutch-Israeli Agricultural Research Program (DIARP), Grant 93/32, in a collaborative project between A.J. Dik (PBG, Naaldwijk, The Netherlands), Y. Elad and D. Shtienberg (ARO, Volcani Center, Bet-Dagan, Israel) and N.J. Fokkema and J. Köhl (IPO-DLO, Wageningen, The Netherlands). The technical assistance of W. Molhoek (IPO-DLO) and E. Jansen (PBG) is gratefully appreciated.

References

Bravenboer L and Strijbosch T (1975) Humidity conditions and the development of fungus diseases in glasshouses. Acta Hort 51: 333–335

Eden MA, Hill RA and Stewart A (1996) Biological control of *Botrytis* infection of greenhouse tomatoes. Plant Pathol 45: 276–284

Elad Y, Malathrakis NE and Dik AJ (1996) Biological control of *Botrytis*-incited diseases and powdery mildews in greenhouse crops. Crop Prot 15: 229–240

Elad Y, Zimand G, Zaqs Y, Zuriel S and Chet I (1993) Use of *Trichoderma harzianum* in combination or alternation with fungicides to control cucumber grey mould (*Botrytis cinerea*) under commercial greenhouse conditions. Plant Pathol 42: 324–332

Fokkema NJ (1973) The role of saprophytic fungi in antagonism against *Drechslera sorokiniana* (*Helminthosporium sativum*) on agar plates and on rye leaves with pollen. Physiol Plant Pathol 3: 195–205

Genstat 5 Committee (1992) Genstat 5 Release 3 Reference Manual. Oxford University Press, Oxford, UK

Katan T (1982) Resistance to 3,5-dichlorophenyl-*N*-cyclicimide (dicarboximide) fungicides in the grey mould pathogen *Botrytis cinerea* in protected crops. Plant Pathol 31: 133–141

Katan T, Elad Y and Yunis H (1989) Resistance to diethofencarb (NPC) in benomyl-resistant field isolates of *Botrytis cinerea*. Plant Pathol 38: 86–92

Köhl J (1989) Eignung von Stämmen aus der Gattung *Trichoderma* für die biologische Bekämpfung phytopathogener Pilze. Dissertation, University Giessen, Germany

Köhl J, Molhoek WML, van der Plas CH and Fokkema NJ (1995a) Effect of *Ulocladium atrum* and other antagonists on sporulation of *Botrytis cinerea* on dead lily leaves exposed to field conditions. Phytopathology 85: 393–401

Köhl J, van der Plas CH, Molhoek WML and Fokkema NJ (1995b) Effect of interrupted leaf wetness periods on suppression of sporulation of *Botrytis allii* and *B. cinerea* by antagonists on dead onion leaves. Eur J Plant Pathol 101: 627–637

Köhl J, Gerlagh M, de Haas BH and Krijger MC (1998) Biological control of *Botrytis cinerea* in cyclamen with *Ulocladium atrum* and *Gliocladium roseum* under commercial growing conditions. Phytopathology 88: 568–575

Koning GP and Köhl J (1995) Wound protection by antagonists against *Botrytis* stem rot in cucumber and tomato. In: M. Manka (ed.) Environmental Biotic Factors in Integrated Plant Disease Control, 313–316, The Polish Phytopathological Society, Poznan.

Morgan WM (1984) The effect of night temperature and glasshouse ventilation on the incidence of *Botrytis cinerea* in a late planted tomato crop. Crop Prot 3: 243–251

O'Neill TM, Niv A, Elad Y and Shtienberg D (1996) Biological control of *Botrytis cinerea* on tomato stem wounds with *Trichoderma harzianum*. Eur J Plant Pathol 102: 635–643

O'Neill TM, Shtienberg D and Elad Y (1997) Effect of some host and microclimate factors on infection of tomato stems by *Botrytis cinerea*. Plant Dis 81: 36–40

Steekelenburg NAM van (1987) Resistance to benzimidazole and dicarboximide fungicides in *Botrytis cinerea* and *Didymella bryoniae* in cucumbers in the Netherlands. Med Fac Landbouww Univ Gent 52(3a): 875–880

Wilson AR (1963) Some observations on the infection of tomato stems by *Botrytis cinerea* Fr. Ann Appl Biol 51: 171

Winspear KW, Postlethwaite JD and Cotton RF (1970) The restriction of *Cladosporium fulvum* and *Botrytis cinerea*, attacking greenhouse tomatoes, by automatic humidity control. Ann Appl Biol 65: 75–83