European Journal of Plant Pathology **100**: 315–336, 1994.

Control of infection and sporulation of *Botrytis cinerea* on bean and tomato by saprophytic bacteria and fungi

Y. ELAD*, J. KÖHL and N. J. FOKKEMA
*DLO Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, The Netherlands; * Permanent address: Department of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250, Israel*

Accepted 12 July 1994

Key words: biological control, *Botrytis cinerea*, grey mould, sporulation

Abstract. Sixty isolates of saprophytic microorganisms were screened for their ability to reduce the severity of grey mould (*Botrytis cinerea*) infection and sporulation. Isolates of the bacteria *Xanthomonas maltophilia*, *Bacillus pumilus*, *Lactobacillus* sp., and *Pseudomonas* sp. and the fungus *Gliocladium catenulatum* reduced germination of conidia of the pathogen and controlled disease on bean and tomato plants. Their activity under growth room conditions was good, consistent, and similar to the activity of the known biocontrol agent, *Trichoderma harzianum* T39 (non-formulated). Although the tested isolates may for nutrients with the germinating conidia of *B. cinerea*, resistance induced in the host by live or dead cells were also found to be involved. Inhibitory compounds were not detected on treated leaves. Sporulation of *B. cinerea* after its establishment on leaves was also reduced by the above mentioned isolates and by *Penicillium* sp., *Arthrinium montagnei*, *Ar. phaeospermum*, *Sesquicillium candelabrum*, *Chaetomium globosum*, *Alternaria alternata*, *Ulocladium atrum*, and *T. viride*. These sporulation-inhibiting fungi did not reduce the infection of leaves by *B. cinerea*. Most of these selected fungi and bacteria were capable of reducing lesion expansion.

Introduction

Saprophytic bacteria and fungi are common inhabitants of plant surfaces [Blakeman, 1982; Dickinson, 1976; Fokkema, 1971]. The importance of nutrient competition in antagonism of necrotrophic fungi has been demonstrated [Brodie and Blakeman, 1975; Dik et al., 1992; Fokkema, 1984; Fokkema et al., 1983]. Bacteria and yeasts are known for their ability to reduce conidial germination by competition for nutrients [Blakeman and Fokkema, 1982]; however, the introduction of antagonists into the phyllosphere to control leaf infections by necrotrophic pathogens has been, in many cases, only moderately effective.

Grey mould, caused by *Botrytis cinerea*, is a serious foliar, fruit and post-harvest disease of various protected and field crops. Current control measures heavily rely on broad spectrum and more specific fungicides. Exploitation of biological control might be rewarding with respect to this pathogen. In an attempt to reduce the chemical input into the environment and the risk of the development of fungicide resistant populations [Elad,

1990]. When greenhouse strawberries were inoculated at late bloom stage with spore suspensions of *Cladosporium herbarum*, *Pullularia pullulans* or *Penicillium* sp., inhibition of grey mould was 42, 31, and 4%, respectively [Bhatt and Vaughan, 1962]. Tronsmo and Dennis [1977], testing five antagonistic isolates of *Trichoderma* spp. in field experiments involving flowering strawberry plants, obtained 14–43% control of pre- and post-harvest spoilage (*B. cinerea* and *Mucor mucedo*) similar to the control achieved by dichlofluanid.

After screening 230 isolates of various microorganisms, Peng and Sutton [1990] found isolates of *Gliocladium roseum* and *T. viride* as effective as captan in reducing fruit rot by 39–71% under field conditions. In Italy, an isolate of *Trichoderma* was tested alone or in alternation with fungicides [Gullino et al., 1990]. Results for *Trichoderma* alone were inconsistent showing only partial control in part of the experiments under field conditions. In alternation with fungicides, the application of *Trichoderma* enabled the reduction of the quantity of chemical treatments required. Sufficient control of *B. cinerea* by isolates of *Trichoderma* and *Gliocladium* sp. has been reported for other crops, such as grapes [Dubos and Bulit, 1981; Dubos, 1987; Gullino and Garibaldi, 1988] and apple [Tronsmo and Raa, 1977] and of *B. aclada* in onion [Köhl et al., 1991]. A preparation of *Trichoderma harzianum* isolate T39 has been suggested for the control of grey mould in greenhouse cucumber and tomato [Elad et al., 1993] and in vineyards [Elad, 1990].

Diseases caused by necrotrophs can also be controlled by bacteria. Some examples are pea infection by *Sclerotinia sclerotiorum* controlled by *Bacillus cereus* [Huang et al., 1992], various cherry diseases including that caused by *B. cinerea* controlled by *Bacillus subtilis* and *Enterobacter aerogenes* [Utkhede and Sholberg, 1986], and blue and grey moulds of apple and pear controlled by *Pseudomonas cepacia* [Janisiewicz and Roitman, 1988]. It was suggested [Fokkema, 1991] that in order to provide effective control, the introduced microorganism has to be well established in the phyllosphere before the arrival of the pathogen. If disease is already present, it may be ineffective, because the antagonistic properties may not be diffusible or even because the antagonist supporting nutrients may stimulate the pathogen. In this situation biocontrol should also aim at suppression of the dissemination of the pathogen [Fokkema, 1993].

Many necrotrophs sporulate abundantly on lesions as well as on dead plant material, and conidia contribute to epidemics in the same season or in the one that follows. *Botrytis* spp. sporulate abundantly on necrotic tissue and crop remains, and the conidia from successive cycles of infection contribute to the development of epidemic within the crop [Sutton, 1990; Yunis et al., 1990]. Recently, Peng and Sutton [1991] working with *B. cinerea* on strawberries, and Fokkema and coworkers [Fokkema et al., 1992; Köhl and Fokkema, 1993; Köhl et al., 1992] working with *Botrytis* spp. on onion, demonstrated that reduction of pathogen sporulation by

means of several isolates of filamentous fungi may minimize the conidial load in the crop. Similarly, *T. harzianum* suppressed sporulation of *Cochliobolus sativus* on excised leaves of wheat seedlings [Biles and Hill, 1988].

Interaction between saprophytes and germinating propagules of pathogens is different from their interaction with the sporulating phase of the pathogens with respect to the length of the interaction time. In addition, the interaction with germination takes place on the undamaged plant surface whereas the interaction with sporulation is in a necrotic leaf tissue.

The purpose of the presented work was to study the biocontrol of grey mould by saprophytic bacteria and fungi with the goal of controlling leaf infection as well as reducing sporulation of *B. cinerea* on established lesions.

Materials and methods

Cultures of saprophytic microorganisms. Sixty isolates of *Xanthomonas*, *Bacillus*, *Lactobacillus* and *Pseudomonas*, *Gliocladium*, *Trichoderma*, *Penicillium*, *Arthrinium*, *Sesquicillium*, *Chaetomium*, *Alternaria* and *Ulocladium* (all from the collection of IPO-DLO, Wageningen, The Netherlands) were used. The isolates originated from potato leaves (provided by G.J. Kessel and P. Jongebloed, IPO-DLO) or from dead parts of onion leaves. The bacteria were grown on tripticase soy agar and the filamentous fungi were grown on oat meal agar. Bacterial cells scraped from the surface of 1-day-old cultures, or conidia from 2–3-week-old cultures, were suspended in water with 0.01% Tween 80. The concentration of bacterial cells was adjusted to 10^7 cells ml^{-1} and the concentration of conidia was adjusted to 10^4–10^6 cells ml^{-1}. If not otherwise specified, 10^7 bacteria and 10^6 fungal conidia ml^{-1} were used. In some experiments, the performance of the selected microorganisms was compared with the performance of the biocontrol agent *Trichoderma harzianum* T39 [Elad, 1993; Elad et al., 1992, 1993], at concentration of 10^6 conidia ml^{-1}.

Pathogens. Botrytis cinerea Pers.: Fr. (isolate 700) was grown on tomato leaf agar [Salinas and Schot, 1987] at 20 °C. Conidia from 10-day-old cultures of the pathogen were suspended in water containing 0.01% Tween 80 to give 10^5–10^6 cells ml^{-1}. If not otherwise specified, 6×10^5 cells ml^{-1} were used.

Host plants. Plants of bean (*Phaseolus vulgaris* L. cv. Groffy) and tomato (*Lycopersicon esculentum* Mill. cv. Money Maker) were planted in 1 L plastic pots (containing potting mixture based on peat) and grown in a greenhouse under conditions of 20–25 °C, vapor pressure deficit (VPD) of 8.06–3.52 mbar (75–85% relative humidity-RH) and 16 h light in a

greenhouse. Leaves were collected from 3–4-week-old bean plants and 4–5-week-old tomato plants. Similar plants were used for whole plant experiments. Detached leaves were incubated in 30 × 45 × 5 cm plastic trays on a plastic grid that was laid over water-soaked filter paper. The cut end of each petiole was inserted into wet floral foam, as described by Leone and Tonneijck (1990), in order to maintain their freshness throughout the course of the experiments. The trays were kept in a transparent polyethylene bag to allow conditions of VPF < 1.05 mbar (high RH) and condensation. Detached flowers from 1-month-old bean plants and fruits from 7-week-old bean plants were incubated under conditions similar to that of the detached leaves. Pots of whole plants were placed on a tray containing water and plants were kept under polyethylene bags, to obtain the same VPD, unless otherwise specified. The plant material was kept in an illuminated (1200 lux) walk-in growth chamber at 18 °C for 5–8 days, unless otherwise specified. When other temperatures were required, plant material was incubated in growth chambers having a similar light intensity.

Treatment of plant material. Plant material was inoculated with a conidial suspension of *B. cinerea* containing 0.02M glucose and 0.02M KH_2PO_4 to promote infection [Leone and Tonneijck, 1990]. The suspension was applied on whole plants by means of an atomizer at a volume of 1.5 ml $plant^{-1}$ (ca 50 µl $leaf^{-1}$), resulting in ca 50–100 conidia cm^{-2}. Suspensions of test saprophytic microorganisms were applied similarly within 10 min after pathogen inoculation, unless a different spraying sequence is indicated. Densities of introduced fungal conidia were 7×10^2–1×10^3 cells cm^{-2} leaf. Densities of bacterial isolates were 10 times higher. Actual densities of microorganisms specified above were measured by washings from leaf surface immediately after application, serial dilutions and plating on the appropriate media mentioned above. Detached leaves were inoculated with 30 µl drops containing mixed suspensions of *B. cinerea* and cells of saprophytes. Up to four treatments (twenty drops) were applied to each leaf spaced of at least 1 cm apart.

Elimination of native surface microflora. In certain experiments, surface microflora was eliminated by rinsing the leaves with 1% active chloride for 1 min and washing in sterile distilled water. The effect of active chloride was verified by pating leaf segments on PDA and tripticase soy agar.

Inhibitory compounds. In order to trace possible inhibitory compounds produced on leaves, drops of water containing the saprophytic micro-organisms and cells of *B. cinerea* were collected with a popette into Ependorff tubes from the treated leaves after 2 days of incubation. The suspensions were centrifuged twice, filtered in order to obtain cell-free liquid, and were replaced on fresh leaves along with a new suspension of pathogen conidia, as mentioned above.

Remote application. In certain experiments, drops of the pathogen were placed on leaves at sites remote (2 cm) from the drops of the antagonist.

The effect of killed cells. The effect of dead cells of selected isolates was tested after application by each of three following methods: i. Gamma irradiation (4.5 mega rad from a cobalt source) to a paste of the cells, ii. Heat treatment at 100 °C for 1 h (Bacilli were autoclaved for 15 min), and iii. Immersion in a solution of 60% ethanol for 10 min. Thereafter all treated cells were washed aseptically 5 times by centrifugation and resuspended in sterile distilled water to the concentration mentioned above for live cells. The death of the cells was verified by plating on agar media.

Germination of conidia. The germination of conidia of *B. cinerea* in water drops on detached leaves was determined microscopically after 20 h of incubation. Leaf pieces bearing a drop of the interacting microorganisms were placed on glass slides, dyed with aniline blue, incubated at room temperature (20 °C) for 5 min, and observed. For each treatment germination of 200 conidia, and the average length of germ tubes of the germinated conidia, were recorded for 5 individual drops. An estimation of total germ tube length produced by the pathogen on leaves was made by multiplying the germination rate by the average germ tube length. As germ tubes lengths were not variable in between replicates of each treatment,this was regarded as a good estimation of the germ tube biomass.

Severity of symptoms induced by the pathogen on leaves. Symptoms of the drop inoculated leaves were evaluated according to a six-score index of intensity of rot under neath the inoculation droplet where 0 = symptomless leaf tissue, 1 = 1–12% rot, 2 = 13–25% rot, 3 = 26–50% rot, 4 = 51–100% rot under neath the droplet and 5 = rot extending about 2 mm around the droplet. Symptoms on whole plants were evaluated according to a severity index of 0–5 where 0 = healthy plant and 5 = completely destroyed plant [Elad et al., 1992].

Established lesions. Leaves on plants or detached leaves inoculated by mycelium plugs (3 mm diam.) from the edges of 3–4-day-old non sporulating cultures of *B. cinerea.* Lesions appeared within 2–3 days. Leaves bearing 10–15 cm^2 non sporulating lesions were sprayed with suspension of the test isolates. The treated plant material was incubated under continuous conditions of high humidity (VPD lower than 1.05 mbar) until sporulation was assessed. In certain cases, the treated plants were exposed to a lower RH – 75–85% (VPD 5.24–3.14 mbar) – during the 2–4 day period after treatment. Sporulation was evaluated by counting the conidiophores of all lesion under a stereo binocular microscope.

Dead plant material. Leaves of bean or tomato and stem segments (3 cm) from the 3rd internode were killed by one of two methods: i. Exposure to microwave irradiation of plant material which was placed over moistened filter paper in a glass Petri plate (fifty leaves at low energy input for 5 min). The plates with killed leaves were rinsed with sterile water five times to wash away excess nutrients released from the plant tissues, and ii. Exposure of the plant material to gamma irradiation (4.5 mega rad from a cobalt source). Each plant material was washed with a 20-fold-volume of water for 1–3 h to remove soluble nutrients, and placed in sterile petri plates over moistened filter paper. The dead plant material was inoculated aseptically with a suspension of 104 conidia of *B. cinerea* ml^{-1} (resulting in 20 conidia cm^{-2}), allowed to become colonized for 2 days, and sprayed aseptically with a suspension of 10^4–10^6 ml^{-1} saprophyte cells (resulting in 2000 cells cm^{-2}) immediately after spraying.

Statistical analysis. Experiments were arranged in completely randomized or randomized block designs and repeated at least twice. Treatments were replicated 6–12 times in experiments with leaves and whole plants and 20 times in experiments with flowers and fruits. Data were transformed (Arcsin), analyzed by analysis of variance and tested for significance using Student-Newman-Keuls's (SNK) Multiple Range Test.

Results

Control of conidial germination of B. cinerea *and grey mould development.* Bacteria and filamentous fungi were screened for their effect on germination and germ tube elongation of *B. cinerea*, on severity of symptoms of infected leaves, and on sporulation of the pathogen on established lesions. The bacterial isolates (with their number) *Xanthomonas maltophilia* (B39), *Bacillus pumilus* (F17) *Lactobacillus* sp. (Lact) and *Pseudomonas* sp. (C155), and the fungus *Gliocladium catenulatum* (162), as well as *T. harzianum* T39, were found more effective than other tested microorganisms (see Table 9 below). The effectiveness of the above mentioned antagonists is demonstrated on bean (Table 1) and tomato (Table 2) leaves. These isolates were used in further experiments. The rate of conidial germination on detached = bean leaves was reduced better by isolates F17, C155, and 162 than by other isolates. Symptoms induced on leaves by drops of conidial suspensions were reduced better by isolates C155, 162, and T39 than by other isolates. Flower, pod, and whole plant infection was reduced by all the selected isolates with no significant difference among their activity (Table 1). On tomato leaves, isolates reduced disease significantly on detached leaves and whole plants but the germination of *B. cinerea* conidia and germ tube biomass on detached leaves was less reduced by F17 than by the other isolates (Table 2).

Table 1. Effect of saprophytic microorganisms[a] on *Botrytis cinerea* infection of bean

Treatment	Germination of conidia of *B. cinerea* (%)	Total germ tube length (µm/100 conidia)[b]	Symptom severity on detached leaves[c]	Flower infection (%)	Pod infection[c] severity[d]	grey mould
Control	86a[e]	26000a	3.3a	100a	4.1a	3.30a
Bacillus pumilus (F17)	7c	210c	0.8bc	0b	0.7b	0.4b
Xanthomonas maltofilia B39	22b	880b	0.3b	17b	1.4b	0.2b
Pseudomonas sp. (C155)	7c	300c	0.02c	21b	0.7b	0.3b
Lactobacillus sp. (Lact)	15bc	950b	1.1b	26b	0.3b	0.9b
Gliocladium catenulatum (162)	0c	0c	0.5c	0b	0.7b	0.35b
Trichoderma harzianum (T39)	8c	900b	0.4c	26b	1.1b	0.00b

[a] Suspensions contained mixtures of conidia of *B. cinerea* (10^5 ml^{-1}) and cells of the antagonistic isolate 10^6 ml^{-1} (fungi) or 10^7 ml^{-1} (bacteria) in solutions of 0.01% tween 80, 0.02M glucose and 0.02M phosphate.

[b] Total length of germ tubes of the germinated conidia from each 100 conidia.

[c] Symptom occurring under drops of inoculum of *B. cinerea* and the antagonists after 8 days of incubation. Severity was indexed on a scale of 0–5 where 0 = no symptoms.

[d] Severity of gray mold of whole plants was indexed on a scale of 0–5 where 0 = non-infected plant and 5 = plant completely covered with grey mould, after 15 days of incubation.

[e] Numbers of each parameter followed by a common letter do not differ significantly at $P \leq 0.05$. Detached leaves and whole plants were incubated at 18 °C.

Table 2. Effect of live saprophytic microorganisms[a] on *Botrytis cinerea* infection of tomato

Treatment	Germination of conidia of *B. cinerea* (%)	total germ tube length (μm/100 conidia)[b]	Symptom severity on detached leaves[c]	grey mould severity[d]
Control	80a[e]	1200a	3.4a	2.30a
Bacillus pumilus (F17)	22b	240b	1.6b	0.60b
Xanthomonas maltofilia B39	0c	0c	0.65b	0.90b
Pseudomonas sp. (C155)	5c	10c	1.22b	1.50b
Lactobacillus sp. (Lact)	6c	24c	1.06b	1.60b
Gliocladium catenulatum (162)	6c	24c	1.05	1.60b
Trichoderma harzianum (T39)	5.2b	41b	1.1b	0.15b

[a] Suspensions contained mixtures of conidia of *B. cinerea* (10^5 ml^{-1}) and cells of the antagonistic isolate 10^6 ml^{-1} (fungi) or 10^7 ml^{-1} (bacteria) in solutions of 0.01% tween 80, 0.02M glucose and 0.02M phosphate.

[b] Total length of germ tubes 100 by the length of germ tubes.

[c] Symptoms occurred under drops of inoculum of *B. cinerea* and the antagonist after 8 days of incubation. Severity of symptoms was indexed on a scale of 0–5 where 0 = no symptoms.

[d] Severity of grey mould of whole plants was indexed on a scale of 0–5 where 0 = healthy plant and 5 = completely destroyed plant, after 15 days of incubation.

[e] For each column, numbers followed by a common letter do not differ significantly at $P \leq 0.05$. Detached leaves and whole plants were incubated at 18 °C.

Temperature effect on activity of introduced saprophytes. Leaves of bean were treated by droplets containing the antagonistic isolates and conidia of *B. cinerea* and incubated at 10 °C and 15 °C. Percentages of germination of conidia on leaves without antagonists at both temperatures were 45 and 95, respectively; average germ tube length of the germinated conidia was 150 and 200 μm, respectively; and symptom severity was 2.6 and 3.2, respectively. All isolates significantly reduced germination of conidia at 15 °C. The rate of germination at 10 °C was significantly ($P \leq 0.05$) reduced by all isolates – it was totally reduced by all isolates except for F17 and T39, that reduced germination by 85% and 80%, respectively. Germ tube length was reduced by all isolates by more than 80% at both temperatures except for T39 that reduced it only by 50% (Significant, $P \leq 0.05$). Disease severity on leaves incubated at 10 °C was reduced significantly ($P \leq 0.05$) by isolates F17, B39, C155, Lact, 162 and T39 by 50, 90, 100 and 60%, respectively. Disease severity on leaves incubated at 15 °C was reduced significantly ($P \leq 0.05$) by 75, 70, 65, 65, 50 and 75%, respectively.

Possible mechanisms. According to the literature and our experience, competition for nutrients seems to be a major mechanism involved in the interaction between antagonists and *B. cinerea* on the plant surface. However, in order to test other possible modes of action, various tests

were carried out on bean leaves. The effect of dead cells of saprophytic microorganisms on *B. cinerea* infection is described in Table 3. Gamma-irradiated cells reduced disease severity significantly on detached bean leaves and on whole plants but in the case of isolate F17 the living cells were more effective on whole plants, and the inhibition obtained by treated conidia of isolate 162 on detached leaves was not significant. Suspensions of heat-killed cells tested on detached bean and tomato leaves (Table 3) controlled the disease similarly to the living cultures except for heat killed isolate 162, that did not reduce disease significantly on bean and tomato leaves, and isolate F17 that did not reduce the disease significantly on tomato leaves (Table 3). Ethanol-treated suspensions were also as effective as the living cell suspensions when tested on bean leaves (Table 3) except for isolate 162. The effect of the bacteria and fungus was not nullified by the presence of an antibiotic and benomyl (respectively). The chemicals did not inhibit the activity of the pathogen (Table 3).

Live cells of the antagonistic microorganisms were applied in drops separated 2 cm from the drops of the pathogen conidia. Disease severity in the control bean leaves was indexed 1.3 and 1.8 after 5 and 7 days, respectively. The remote application of isolate C155 significantly ($P < 0.05$) reduced disease severity to indices 0.25 and 0.75, respectively. Isolate 162 significantly ($P < 0.05$) reduced disease severity to indices 0.25 and 0.3, respectively. The rest of the isolates did not reduce the disease when applied at sites remote from the pathogen.

The possibility that the antagonists produced inhibitory compounds on treated leaves was tested. Water drops from leaves treated with the various isolates were collected, filter-sterilized, and applied to fresh leaves of beans along with conidia of the pathogen. No significant inhibition of the disease was observed.

The involvement of natural populations of microorganisms in the biocontrol achieved by the introduced was tested (Table 4). Natural populations of bacteria and yeast in the drops of water placed on leaves of beans were found to be 90–150 and 36–60 CFU ml^{-1}, respectively. The natural population was eliminated by disinfection with NaOCl. The three bacterial suspensions but not *G. catenulatum* applied on surface-disinfected bean leaves were capable of controlling disease incited by *B. cinerea* (Table 4).

Sporulation of Botrytis cinerea *on dead plant material.* The above mentioned microorganisms and 55 other isolates of filamentous fungi were tested for their ability to control the sporulation of *B. cinerea* already established on dead or live plant material. The following isolates were chosen for further study: *G. catenulatum* (017), *Penicillium sp.* (023), *Arthrinium montagnei* (242), *Ar. phaeospermum* (243), *Sesquicillium candelabrum* (249), *Chaetomium globosum* (256), *Alternaria alternata* (317, 319), *Ulocladium atrum* (385), and *T. viride* (T048).

Table 3. Effect of dead cells of saprophytic microorganisms[a] on infection by *Botrytis cinerea*

Treatment	Detached bean leaves[b]								Detached tomato leaves[b]		Whole bean plants[d]	
	Gamma-irradiated cells		Heat-killed cells		Ethanol-killed cells		Cells incubated with an inhibition[c]		Heat-killed cells		Gamma-irradiated cells	
	–[e]	+	–	+	–	+	–	+	–	+	–	+
Control	3.82a[f]		2.90a		2.60a		3.18a	3.05a	3.70a		4.10a	
Bacillus pumilus (F17)	0.70c	0.85c	1.00b	0.8b	1.05bc	0.80c	0.40b	1.40b	1.12b	2.20ab	0.50c	2.90b
Xanthomonas maltofilia B39	1.00bc	1.70b	1.00b	0.70b	0.90bc	0.70b	0.97b	1.50b	1.05b	1.00b	1.40bc	2.95b
Pseudomonas sp. (C155)	0.35c	0.62c	0.75b	0.80b	0.75c	0.60c	0.10b	0.60b	1.50b	1.30b	2.50b	3.00b
Gliocladium catenulatum (162)	1.25b	2.35ab	0.70b	1.70ab	0.75c	1.40b	0.90b	0.70b	1.42b	2.60ab	2.55b	2.60b

[a] Suspensions contained mixtures of conidia of *B. cinerea* (10^5 ml^{-1}) and cells of the antagonistic isolate 10^6 ml^{-1} (fungi) or 10^7 ml^{-1} (bacteria) in solutions of 0.01% Tween 80 with 0.02M glucose and 0.02M phosphate.

[b] Severity of symptoms underneath the drop of inoculum of *B. cinerea* with antagonist cells was rated on a scale of 0–5 where 0 = noninfected leaves.

[c] Tetracycline hydrochloride (15 μg ml^{-1}) was added to the suspension drop of bacteria, and benomyl was added to the suspension drop of *G. catenulatum* (162).

[d] Severity of diseases was rated on a scale of 0–5 where 0 = healthy plant and 5 = completely destroyed plant.

[e] – = Plant material treated with lives cells of the antagonist, + = Treatments with dead cells.

[f] Numbers of treatments of the same plant material followed by a common letter do not differ significantly at $P \leq 0.05$.

Table 4. Effect of surface disinfection[a] of bean leaves on the ability of the antagonistic microorganisms[b] to reduce severity of grey mould symptoms[c]

Treatment	Incubation time (days)			
	5		7	
	Surface disinfection[a]			
	–	+	–	+
Control	1.20b[d]	1.82aa	2.05a	2.20a
Bacillus pumilus (F17)	0.15c	0.76bc	0.55c	0.80c
Xanthomonas maltofilia (B39)	0.40c	0.60c	0.63c	0.80c
Pseudomonas sp. (C155)	0.45c	1.50b	0.45c	1.50b
Gliocladium catenulatum (162)	0.25c	2.10a	0.45c	2.40a

[a] Surface disinfection (+) was carried out with 1% active chloride for 1 min followed by washing with sterile distilled water (– = no disinfection).
[b] Suspensions contained mixtures of conidia of *B. cinerea* (10^5 ml^{-1}) and cells of the antagonistic isolate 10^6 ml^{-1} (fungi) or 10^7 ml^{-1} (bacteria) in solutions of 0.01% Tween 80, 0.02M glucose and 0.02M phosphate.
[c] Severity of symptoms underneath the drop of inoculum of *B. cinerea* with antagonist cells was rated on a scale of 0–5 where 0 = symptomless leaf.
[d] Numbers at each sampling date followed by a common letter do not differ significantly at $P \leq 0.05$.

Tomato or bean leaves and stem segments were killed by microwave or gamma irradiation. The saprophytic microorganisms were applied after establishment of the pathogen (2 days after its inoculation) on the dead plant material (Tables 5–6). Sporulation was evident 2 days later. The ability of the microorganisms to reduce sporulation on microwaved plant material varied with the test crop. On bean plant parts, 4–7 isolates failed to reduce sporulation, whereas all isolates were capable of reducing sporulation on tomato plant parts (Table 5). On gamma-irradiated plant parts, a significant reduction in sporulation was obtained by all isolates on bean leaves, and by most of them on tomato stem segments or leaves (Table 6). The concentration of the various saprophytic fungi tested differed because production of their conidia in plates was variable. However, even at low inoculum level of 10^4 ml^{-1} some of them significantly reduced sporulation of *B. cinerea.*

The ability of the various isolates to reduce sporulation under three temperature conditions was tested for gamma-irradiated tomato segments (Table 7). Sporulation was reduced significantly by most isolates at 10–18 °C. At 5 °C, sporulation was reduced significantly by isolates B39, C155,)17, 023, 242, 243, and 317. Isolates 242 and 317 were more effective at the low temperature, as compared with their activity at the warmer emperatures (Table 7).

Table 5. Sporulation[a] of *Botrytis cinerea* on microwave-treated plant material with suspensions of microorganisms[b]

Treatment		Bean stem		Tomato stem		Bean leaf		Tomato leaf	
		Days of incubation after saprophyte application							
	Conc[b]	2	4	2	4	2	4	2	4
Control	–	35b[c]	110a	21a	105a	52a	107ab	85a	110a
Bacillus pumilus (F17)	10^7	0f	51bc	0d	5f	3ef	25ef	0d	5e
Xanthomonas maltofilia (B39)	10^7	5def	38cd	0d	12f	1f	13f	0d	4e
Pseudomonas sp. (C155)	10^7	6def	70b	0d	7f	6cdef	12f	0d	3e
Lactobacillus sp.	10^7	0f	10e	0d	22def	0f	22f	0d	6e
Gliocladium catenulatum (162)	10^6	6def	16de	0d	18ef	0f	23ef	0d	9e
Trichoderma harzianum (T39)	10^6	9def	37cd	5cd	49bc	0f	78bcd	0d	21de
G. catenulatum (017)	10^6	49a	110a	3cd	62b	62a	105ab	5d	45c
Penicillium sp. (023)	10^6	8def	73b	5cd	47bc	21bc	53de	8cd	15e
Arthrinium montagnei (242)	10^4	0f	103a	0d	45bc	0f	102ab	0d	35d
Ar. phaeospermum (243)	10^4	0f	113a	6bc	42bcd	12bcdef	110a	49b	72b
Sesquicillium candelabrum (249)	10^4	11cde	108a	6bc	50bc	4def	111a	2d	75b
Chaetomium globosum (256)	10^4	8def	52bc	10b	35cde	10bcdef	85abc	6d	35cd
Alternaria alternata (317)	10^5	15cd	65b	7bc	46bc	12bcdef	96abc	9cd	42c
Al. alternata (319)	10^5	14cde	52bc	10b	52bc	25bc	82abcd	16c	35cd
Ulocladium atrum (385)	10^4	22c	65b	6bc	43bc	14bcdef	67cde	12c	65bc
T. viride (T048)	10^6	11cde	69b	7bc	52bc	29b	71cde	11c	49c

[a] Conidiophores/cm^2.

[b] Microwave-treated plant material was inoculated suspensions of *B. cinerea* (10^4 ml^{-1}), incubated for two days at 20 °C, and treated with cell suspensions of the bacteria (10^7 ml^{-1}) or of fungi (10^4–10^6 ml^{-1}) and further incubated at 20 °C. The actual concentration per ml of cells of each of the saprophytes is indicated in the "conc" column.

[c] Numbers of conidiophores on each plant organ followed by a common letter do not differ significantly at $P \leq 0.05$.

Table 6. Sporulation[a] of *Botrytis cinerea* on gamma-irradiated plant material treated with suspensions of microorganisms[b]

Treatment	Bean leaves		Tomato stem		Tomato leaf	
	Days of incubation after saprophyte application					
	2	4	2	4	2	4
Control	18a[c]	98a	24abc	110a	12a	52a
Bacillus pumilus (F17)	18a	52b	9de	65bcdef	18a	53a
Xanthomonas maltofilia (B39)	0c	0g	25ab	65bcdef	0b	0e
Pseudomonas sp. (C155)	12ab	31c	32a	64bcdef	8ab	32bc
Lactobacillus sp.	5bc	33c	16cd	45efgh	11ab	36ab
Gliocladium catenulatum (162)	6bc	12defg	25ab	76bcd	5b	13cde
Trichoderma harzianum (T39)	4bc	13defg	22bc	76bcd	6b	12cde
G. catenulatum (017)	13ab	25cde	23abc	55cdef	6d	15cde
Penicillium sp. (023)	4bc	9defg	21bc	52defg	4b	12cde
Arthrinium montagnei (242)	4bc	7efg	16cd	59cdef	2b	6de
Ar. phaeospermum (243)	15ab	22cdef	15cd	39fgh	8ab	25cd
Sesquicillium candelabrum (249)	2bc	6fg	27ab	72bcde	1b	7de
Chaetomium globosum (256)	7bc	26cd	11de	45efgh	12ab	25cd
Alternaria alternata (317)	6bc	19cdef	32a	82abcd	2b	6de
Al. alternata (319)	5bc	17defg	24abc	53defg	6b	14cde
Ulocladium atrum (385)	4bc	19cdef	9de	23gh	3b	6de
T. viride (T048)	9abc	22cdef	5e	16h	7b	13cde

[a] Conidiophores/cm^2.

[b] Gamma-irradiated plant material was inoculated with conidial suspensions of *B. cinerea* (10^4 ml^{-1}), incubated for two days at 20 °C, and treated with cell suspensions of the bacteria (10^7 ml^{-1}) or of fungi (10^4–10^6 ml^{-1}), and further incubated at 20 °C. Actual concentration of the antagonists is detailed in Table 5.

[c] Numbers of conidiophores on each plant organ followed by a common letter do not differ significantly at $P \leq 0.05$.

Sporulation of Botrytis cinerea *on living plant material colonized by the fungus.* The selected isolates were also tested on detached bean flowers, and on detached leaves of tomato and bean. The antagonists were applied on the plant material after infection was established by the pathogen (Table 8). Only isolates 162, 017, 256, and 317 reduced sporulation significantly on bean flowers, 5 days after inoculation. Most of the isolates reduced sporulation on the tested leaves, this reduction being significant for leaves of tomato (Table 8).

All isolates of the filamentous fungi were tested for their ability to reduce infection of bean leaves by *B. cinerea* (Table 9). Six of the isolates reduced disease severity significantly after 2 days of incubation. However, all the isolates selected for their ability to reduce sporulation failed to reduce disease severity according to the evaluation two days later. Isolates 162 and T39 controlled the disease effectively at both sampling dates (Table 9).

Table 7. Effect of temperature and saprophytic microorganisms[a] on sporulation of *Botrytis cinerea* on gamma-irradiated tomato stem segments

Treatment	Temperature (°C)		
	5[b]	10	18
Control	48ab[c]	75ab	112a
Bacillus pumilus (F17)	52ab	72abc	76cde
Xanthomonas maltofilia (B39)	28c	35e	77cde
Pseudomonas sp. (C155)	18c	53cde	55e
Lactobacillus sp.	32abc	49de	76cde
Gliocladium catenulatum (162)	62a	51cde	72cde
Trichoderma harzianum (T39)	53ab	42de	62de
G. catenulatum (017)	*22c*	*62bcd*	*64de*
Penicillium sp. (023)	23c	52cde	81bcd
Arthrinium montagnei (242)	22c	61bcd	75cde
Ar. phaeospermum (243)	13c	41de	73cde
Chaetomium globosum (256)	51ab	41de	69dc
Alternaria alternata (317)	23c	62bcd	85abcd
Ulocladium atrum (385)	50ab	40e	60de
T. viride (T048)	63a	85a	69de

[a] Tomato stem segments were inoculated with conidial suspensions of *B. cinerea* (10^4 ml^{-1}), incubated for two days at 20 °C, treated with cell suspensions of the bacteria (10^7 ml^{-1}) or of fungi (10^4–10^6 ml^{-1}) and further incubated at 20 °C. Actual concentration of the antagonists is detailed in Table 5.

[b] Sporulation was evaluated 4, 5, and 8 days after yeast application and incubation at 18, 10, and 5 °C, respectively (conidiophores/cm^2).

[c] Numbers of conidiophores at each temperature followed by a common letter do not differ significantly at $P \leq 0.05$.

The effect of dryness, as compared with uninterrupted high humidity conditions, on the ability of the microorganisms to reduce sporulation was tested on leaves of beans attached to plants. The results are exemplified with one bacterial isolate and three fungal isolates (Fig. 1). The bacterium (C155) reduced sporulation better when the dry period did not interrupt the high humidity conditions of incubation. Isolates 023, 242, and 249 reduced sporulation under both conditions of incubation.

The effect of the tested isolates on lesion expansion and on sporulation on the lesions observed on tomato and bean leaves was monitored for 9 days after treatment (Fig. 2). Expansion of the lesion caused by *B. cinerea* on tomato leaves was reduced significantly by isolates 162, 017, 023, and 385 after 9 days. All isolates reduced sporulation at this sampling date, but B39, 242, and 243 were less effective compared with the rest of the isolates applied on tomato leaves (Fig. 2a,b). On bean leaves, isolates T39 and 242 did not reduce the expansion of the lesion after 9 days, however all isolates except 242 reduced sporulation on bean leaves significantly.

Table 8. Sporulation[a] of *Botrytis cinerea* on bean flowers or leaves of bean or tomato, treated with suspensions of microorganisms infected previously by the pathogen[b]

Treatment	Bean Flowers		Bean leaves		Tomato leaves	
	Days of incubation after saprophyte application					
	3	5	3	5	3	5
Control	30ab[c]	75bc	19efgh	95a	24bcde	55ab
Bacillus pumilus (F17)	11ef	84ab	45a	85ab	21cde	52abc
Xanthomonas maltofilia (B39)	28bc	65bcde	37abc	88ab	15de	32def
Pseudomonas sp. (C155)	17cde	64bcde	28bcde	23f	39ab	62a
Lactobacillus sp.	24bcd	74bc	7gh	65bcde	38ab	25def
Gliocladium catenulatum (162)	11ef	32fg	25cdef	75abc	42a	53ab
Trichoderma harzianum (T39)	19bcde	67bcd	5h	45ef	34abc	24def
G. catenulatum (017)	0f	22g	12fgh	65bcde	17de	43abcd
Penicillium sp. (023)	13de	74bc	35abcd	64bcde	38ab	29def
Arthrinium montagnei (242)	42a	97a	31abcde	44ef	21cde	23ef
Ar. phaeospermum (243)	10ef	85ab	24cdef	49de	15de	12f
Sesquicillium candelabrum (249)	17cde	63cde	21def	65bcde	12e	33cdef
Chaetomium globosum (256)	12e	53def	27bcdef	51cde	33abc	23ef
Alternaria alternata (317)	9ef	44ef	26cdef	69bcd	24bcde	32def
A. alternata (319)	8ef	63cde	20defgh	54cde	28abcd	12f
Ulocladium atrum (385)	16de	84ab	22cdefg	49de	43a	39bcde
T. viride (T048)	14de	76bc	42ab	73abcd	22cde	24def

[a] Conidiophores/flower.

[b] Detached flowers or were inoculated with conidial suspensions of *B. cinerea* (10^4 ml^{-1}), incubated for two days at 20 °C, treated with cell suspensions of the bacteria (10^7 ml^{-1}) or of fungi (10^4–10^6 ml^{-1}), and further incubated at 20 °C (actual concentration of the antagonists is detailed in Table 5). Leaves were infected with mycelial disks of *B. cinerea* and four days later with the suspensions of microorganisms. Plant material was incubated under conditions of low VPD (high humidity) for 24 h, followed by incubation under high VPD for 24 h and another 24h at low VPD.

[c] Numbers of conidiophores on each plant organ followed by a common letter do not differ significantly at $P \leq 0.05$.

Total number of conidiophores produced by the pathogen per lesion, at the 9 days sampling time was calculated by multiplying the lesion area by the density of sporulation presented in Fig. 2. The total quantities of conidiophores produced on the control leaves were 19570 and 23900 for tomato and bean leaves, respectively. On tomato leaves sprayed with isolates B39, C155, Lact, 162, T39, 023, 242, 243, 385, 017 and 256 the respective amounts of conidiophores produced were 14300, 11350, 4460, 5000, 8500, 5800, 15400, 15500, 3400, 4500 and 8450. The respective amounts of conidiophores produced on bean leaves were 4200, 6450, 250, 2750, 11300, 3450, 300, 24100, 5150, 11500 and 7400.

Table 9. Effect of saprophytic microorganisms[a], selected for suppression of sporulation, on severity of symptoms caused on bean leaves by *Botrytis cinerea*[b]

Treatment	Days of incubation	
	2	4
Control	1.70ab[c]	2.35ab
Gliocladium catenulatum (162)	0.42de	0.70de
Trichoderma harzianum (T39)	0.22e	0.52e
G. catenulatum (017)	0.53cde	1.50bcde
Penicillium sp. (023)	0.85cd	1.70abcd
Arthrinium montagnei (242)	0.95bcd	1.72abcd
Ar. phaeospermum (243)	2.03a	2.35ab
Sesquicillium candelabrum (249)	1.05bcd	2.05abc
Chaetomium globosum (256)	1.27abc	1.64bcd
Alternaria alternata (317)	0.34de	1.65bcd
A. alternata (319)	1.58abc	2.66a
Ulocladium atrum (385)	0.74cde	1.45bcde

[a] Suspensions contained mixtures of conidia of *B. cinerea* (10^5 ml^{-1}) and conidia of the antagonistic isolate 10^4–10^6 ml^{-1} in solutions of 0.01% Tween 80, 0.02M glucose and 0.02M phosphate.

[b] Symptoms occurred under drops of inoculum of *B. cinerea* and the antagonist. Severity of symptoms was indexed on a scale of 0–5 where 0 = no symptoms.

[c] For each column, numbers of conidiophores followed by a common letter do not differ significantly at $P \leq 0.05$.

Discussion

The bacterial isolates and isolate 162 of *Gliocladium catenulatum*, selected at the beginning the present work for biological control of infection by *Botrytis cinerea*, effectively reduced the germination of conidia of the pathogen and severity of disease on leaves, flowers and pods of bean, and on leaves of tomato (Tables 1 and 2). They were just as effective as the known biocontrol agent *Trichoderma harzianum* T39 [Elad, 1993; Elad et al., 1992; Elad et al., 1993], that was applied during this study as a conidial suspension. Some of the isolates were even effective at low temperature. This may be of advantage since grey mould develops at a wide range of temperatures, including low ones. Control of grey mould by several microorganisms has been reported for several crops [Bhatt and Vaughan, 1962; Dubos and Bulit, 1981; Gullino et al., 1990; McLaughlin et al., 1992; Redmond et al., 1987; Roberts, 1990; Tronsmo and Dennis, 1977; Tronsmo and Raa, 1977]. However, the effect of saprophytic microorganisms on sporulation of the pathogen on plant material was studied only in the case of strawberry leaves [Peng and Sutton, 1990; Sutton and Peng, 1993] and in the case of dead onion leaves [Köhl et al., 1992, 1993].

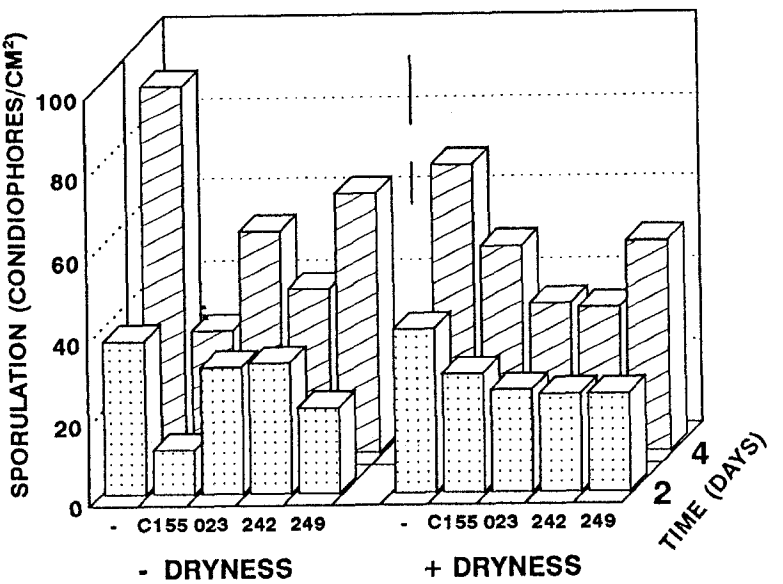
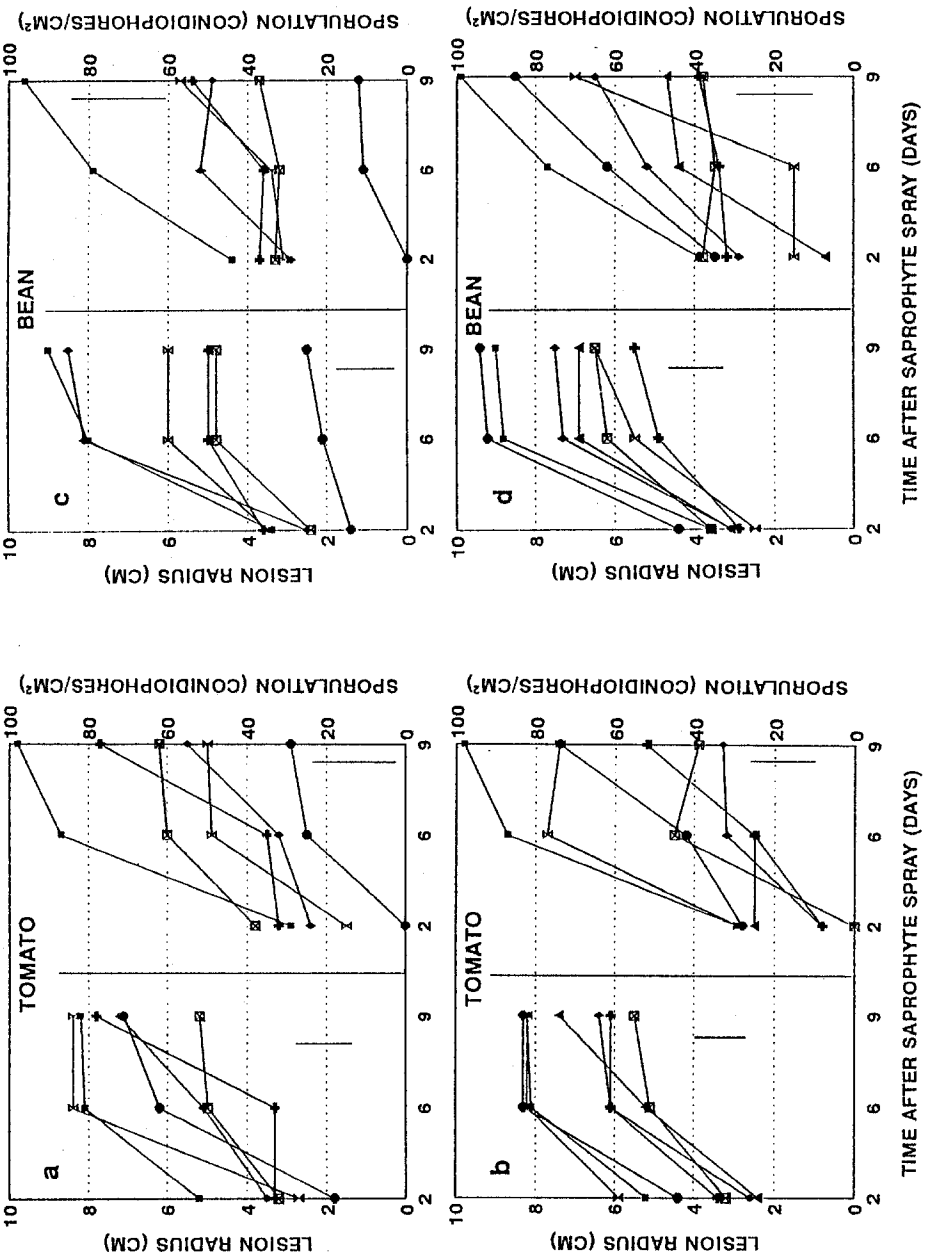


Fig. 1. Effect of saprophytic microorganisms *Pseudomonas* sp. (C155), *Penicillium* sp. (023), *Arthrinium montagnei* (242), and *Sesquicillium candelabrum* (249) on sporulation of *Botrytis cinerea* on bean leaves. Leaves on plants were infected by mycelium diks of *Botrytis cinerea* and maintained at VPD of 1.05 mbar and 20 °C until lesions of 2–3 diam. were observed and saprophyte suspensions were applied on them. Plants after saprophyte application were exposed to a continuous VPD lower than 1.05 mbar (– dryness) or to an atmosphere of 1.05 mbar VPD for 1 day, 3.14 mbar VPD for 1 day, and then back to a VPD of 1.05 mbar (= + dryness). Bars = LSD ($P \leq 0.05$) of data obtained after 2 and 4 days of incubation, at 8

Sporulation of *B. cinerea* was affected significantly by some of the bacterial and fungal isolates on various dead plant tissues precolonized by *B. cinerea*, even at temperature as low as 5 °C (Tables 5–7) and on plants exposed to low VPD interrupted by higher VPD (Fig. 1). However, the intensity of inhibition obtained by a certain isolate varied probably due to their interaction with the varied nature of nutrients available on the test plant material.

Antagonism of microorganisms to pathogens is expressed in a variety of ways [Elad, 1990]. Tronsmo and Dennis [1977] found that *Trichoderma* controls *Botrytis* rot of strawberry, presumably by direct parasitism and antibiotic production. Gueldner et al. [1988] found that *Bacillus subtilis* expressed its antagonism to *Monilinia fructicola* by the production of an iturin antibiotic. Janisiewicz and Roitman [1988] found that an isolate of *Pseudomonas cepacia* was effective against *Botrytis*, *Penicillium*, and *Mucor* rots of apples and pears by producing the powerful antibiotic, pyrrolnitrin. Induced resistance is also being recognized as an important mode of action of biocontrol in vegetative tissues [Kúc, 1987; Sequeira, 1983]. Wilson [1989] suggested that part of the mode of action of yeasts


TOMATO
BEAN
a
b
c
d
LESION RADIUS (CM)
SPORULATION (CONIDIOPHORES/CM²)
TIME AFTER SAPROPHYTE SPRAY (DAYS)

against certain citrus fruit rots may be through induced resistance in the fruit.

The mechanism underlying the activity of the tested saprophytes may be complex. Germinating *Botrytis* conidia are volunerable to competition for nutrients [Brodie and Blakeman, 1975]. In order to induce germination of conidia and infection of leaves, the applied suspensions were supplemented by a low concentration of glucose and phosphate which imitate natural exogenous nutrients such as pollen grains and dead flower petals. The introduced saprophytic microorganisms probably competed with the germinating conidia for the available nutrients. However, dead cells of the antagonists were also capable of reducing grey mould. This effect was significant (Table 3). Moreover, *Pseudomonas* sp. (C155) and *G. catenulatum* induced resistance when applied a short distance away from = the pathogen. On the other hand, the tested microorganisms did not produce detectable inhibitory compounds. It was suggested recently [Wisniewski et al., 1991] that the mode of action of the post-harvest biocontrol yeast, *Pichia guilliermondii*, is tenacious attachment, along with secretion of cell wall degrading enzymes. Our observation did reveal degradation of host cell wall (results not presented). It may be concluded that the activity of the tested microorganisms is associated with their cell or cell walls and partial activity is not associated with live cells. Thus, locally-induced resistance, along with competition for nutrients results in the inhibition of grey mould on bean and tomato.

The epidemiological implications of pre- and post-infection biological control in the field is reduced disease spread. Reduction in inoculum production followed by a suppression of its ability to infect would create an accumulative effect over several disease cycles [Jarvis et al., 1980; Köhl et al., 1992] in greenhouses where the population of *Botrytis* is developing independently of exogenously-contributing inoculum [Jarvis, 1980; Yunis et al., 1990] and in the open field [Köhl et al., 1993; Sutton, 1990; Sutton and Peng, 1993]. As most of the microorganisms tested throughout this study were incapable of both prevention of infection and reduction in sporulation, a concurrent application of more than one isolate may be needed for the control of these two important features of grey mould disease.

←

Fig. 2. Effect of introduced populations of the microorganisms *Xanthomonas maltophilia* (B39 +), *Lactobacillus* sp. (Lact •), and *Pseudomonas* sp. (C155 ⧖), *Gliocladium catenulatum* (162 ⊠), and *T. harzianum* (T39 ♦) (a and c), and *G. catenulatum* (017 ♦), *Penicillium* sp. (023 +), *Arthrinium montagnei* (242 ⧖), *Ar. phaeospermum* (243 •), *Chaetomium globosum* (256 ▲), and *Ulocladium atrum* (385 ⊠) (b and d) on lesion size (left) and on sporulation of *Botrytis cinerea* (right) on leaves of tomato (a,b) and on leaves of bean (c,d). Bars = LSD ($P \leq 0.05$) of data obtained after 2 and 4 days of incubation, at 8 df. Control in both cases is indicated as •.

Acknowledgements

We wish to thank W. M. L. Molhoek, G. J. Kessel, P. Jongebloed, and C. H. van der Plas of IPO-DLO and B. Kirshner and N. Gagulashvily of the Volcani Center for their help. Work was conducted during a sabbatical leave of the first author at IPO-DLO. The financial support of the International Agricultural Center (IAC) at Wageningen and of The European Environmental Research Organization (EERO) at Wageningen to one of us (Y. E.) is gratefully acknowledged.

References

Bhatt DD and Vaughan EK (1962) Preliminary investigations on biological control of gray mold (*Botrytis cinerea*) of strawberries. Plant Dis Reptr 46: 342–345

Biles CL and Hill JP (1988) Effect of *Trichoderma harzianum* on sporulation of *Cochliobolus sativus* on excised wheat seedling leaves. Phytopathology 78: 656–659

Blakeman JP (1982) Phylloplane interactions. In: Mount, MS, Lacey GH, eds, Phytopathogenic Procaryots (Vol. I), pp 308–333. Academic Press, London

Blakeman JP and Fokkema NJ (1982) Potential for biological control of plant diseases on the phylloplane. Annu Rev Phytopathol 20: 167–192

Brodie IDS and Blakeman JP (1975) Competition for carbon compounds by a leaf surface bacterium and conidia of *Botrytis cinerea*. Physiol Plant Pathol 6: 125–136

Dickinson CH (1976) Fungi on aerial surfaces of higher plants. In: Dickinson CH, Preece TF, eds, Microbiology of Aerial Plant Surfaces, pp 293–324. Academic Press, London

Dik DJ, Fokkema NJ and Van Pelt JA (1992) Influence of climate and nutritional factors on yeast population dynamics in the phyllosphere of wheat. Microb Ecol 23: 41–52

Dubos B (1987) Fungal antagonism in aerial agrobioceoses. In: Chet I, ed, Innovative Approaches to Plant Disease Control, pp 107–135. John Wiley & Sons NY

Dubos B and Bulit J (1981) Filamentous fungi as biocontrol agents on aerial plant surfaces. In: Blakeman JP, ed, Microbial Ecology of the Phylloplane, pp 353–367. Academic Press, London

Elad Y (1990) Reasons for the delay in development of biological control of foliar pathogens. Phytoparasitica 18: 99–105

Elad Y (1993) Biological control of grape grey mould by means of *Trichoderma harzianum*. Crop Prot 13: 35–38

Elad Y, Kirshner B and Gotlib Y (1992) Attempts to control *Botrytis cinerea* of rose by pre- and post-harvest treatments with biological and chemical agents. Crop Prot 12: 69–73

Elad Y, Zimand G, Zaqs Y, Zuriel S and Chet I (1993) Biological and integrated control of cucumber grey mould (*Botrytis cinerea*) under commercial greenhouse conditions. Plant Pathology 42: 324–332

Fokkema NJ (1971) The effect of pollen in the phyllosphere of rye on colonization by saprophytic fungi and on infection by *Helminthosporium sativum* and other leaf pathogens. Neth J Plant Pathol 77(Suppl 1): 60 pp

Fokkema NJ (1984) Competition for endogenous and exogenous nutrients between *Sporobolomyces reseus* and *Cochliobolus sativus*. Can J Bot 62: 2463–2468

Fokkema NJ (1991) The phyllosphere as an ecologically neglected milieu: a plant pathologist's point of view. In: Andrews JH, Hirano SS, eds, Microbial Ecology of Leaves, pp 3–18. Springer Verlag, NY

Fokkema NJ, Riphagen I, Poot RJ and De Jong C (1983) Aphid honeydew, a potent stimulant of *Cochliobolus sativus* and *Septoria nodorum* and the competitive role of saprophytic microflora. Trans Brit mycol Soc 81: 355–363

Fokkema NJ (1993) Opportunities and problems of control of foliar pathogens with micro-organisms. Pest Sci 37: 411–416

Fokkema NJ, Gerlagh M and Köhl J (1992) Biological control of *Sclerotinia sclerotiorum* and *Botrytis* spp. In: ES Tjamos, G Papavizas, RJ Cook, eds, Biological Control of Plant Diseases, pp 267–271. Plenum Press, NY

Gueldner RC, Reilly CC, Pusey PL, Costello CE, Arrendalle RF, Cox RH, Himmelsbach OS, Crumley FG and Cutler HG (1988) Isolation and identification of iturins as antifungal peptides in biological control of peach brown rot with *Bacillus subtilis*. J Agric Food Chem 36: 366–370

Gullino ML, Aloi C and Garibaldi A (1990) Chemical and biological control of grey mould of strawberry. Meded Facult Landbouwwetenschappen Rijksuniversiiteit Gent 55: 967–970

Gullino ML and Garibaldi A (1988) Biological and integrated control of grey mold of grapevine: Results in Italy. EPPO Bull 18: 9–12

Huang H C, Kokko EG, Yanke LJ and Phillippe RC (1992) Bacterial suppression of basal pod rot and end rot of dry peas caused by *Sclerotinia sclerotiorum*. Can J Microbiol 39: 227–233

Janisiewicz WJ and Roitman J (1988) Biological control of blue mold and grey mould of apple and pear with *Pseudomonas cepacia*. Phytopathology 78: 1697–1700

Jarvis WR (1980) Epidemiology. In: JR Coley-Smith, K Verhoeff, VR Jarvis, eds, The Biology of Botrytis, pp 219–250. Academic Press, London

Köhl J, Molhoek WML and Fokkema NJ (1991) Biological control of onion neck rot (*Botrytis aclada*): Protection of wounds made by leaf topping. Biocontrol Sci Technol 1: 261–269

Köhl J and Fokkema NJ (1993) Fungal interactions on living and necrotic leaves. In: Blakeman JP, Williamson B, eds, Ecology of Plant Pathogens. CABI (in press)

Köhl J, Molhoek WML, Van Der Plas CH and Fokkema NJ (1993) Drought resistance as a major selection criterium for antagonists of *Botrytis* spp. WPRS Bull 16: 169–172

Köhl J, Molhoek WML, Van Der Plas CH and Fokkema NJ (1993) Biological control of Botrytis leaf blight in onions. WPRS Bull 16: 53–56

Köhl J, Molhoek WML, Van Der Plas CH, Kessel GJT and Fokkema NJ (1992) Biological control of Botrytis leaf blight in onions: Significance of sporulation suppression. In: Verhoeff K. Malathrakis NE, Williamson, B, eds, Recent Advances in Botrytis Research., pp 192–196 Pudoc Scientific Publishers, Wageningen, The Netherlands

Kúc J (1987) Plant immunization and its applicability for disease control. In: Chet I, ed, Innovative Approaches to Plant Disease Control, pp 255–274. John Wiley & Sons, NY

Leone G and Tonneijck AEG (1990) A rapid procedure for screening the resistance of bean cultivars (*Phaseolus vulgaris* L.) to *Botrytis cinerea* and *Sclerotinia sclerotiorum*. Euphytica 48: 87–90

McLaughlin RJ, Wilson CL, Drobi S, Ben-Arie R and Chalutz E (1992) Biological control of post-harvest diseases of grape, peach and apple with the yeast *Kloeckera apiculata* and *Candida guilliermondii*. Plant Dis 76: 470–473

Peng G and Sutton JC (1990) Biological methods to control grey mould of strawberry. Brighton Crop Protection Conference – Pests and Diseases 3C: 233–240

Peng G and Sutton JC (1991) Evaluation of microorganisms for biocontrol of *Botrytis cinerea* in strawberry. Can J Plant Pathol 13: 247–257

Redmond JC, Marois JJ and MacDonald JD (1987) Biological control of *Botrytis cinerea* with epiphytic microorganisms. Plant Dis 71: 799–802

Roberts RG (1990) Postharvest biological control of grey mould of apple by *Cryptococcus laurentii*. Phytopathology 80: 526–530

Salinas J and Schot CP (1987) Morphological and physiological aspects of *Botrytis cinerea*. Meded Facult Landbouwwetenschappen Rijksuniv Gent 52: 771–776

Sequeira L (1983) Mechanisms of induced resistance in plants. Annu Rev Microbiol 37: 51–79

Sutton JC (1990) Epidemiology and management of Botrytis leaf blight of onion and grey mould of strawberry: a comparative analysis. Can J Plant Pathol 12: 100–110

Sutton JC and Peng G (1993) Biocontrol of Botrytis cinerea in strawberry leaves. Phytopathology 83: 615–621

Tronsmo A and Dennis C (1977) The use of *Trichoderma* species to control strawberry fruit rots Neth J Plant Pathol 83: 449–455

Tronsmo A and Raa J (1977) Antagonistic action of *Trichoderma pseudokoningii* against the apple pathogen *Botrytis cinerea*. Phytopathologische Z 89: 216–220

Utkhede RS and Sholberg PL (1986) *In vitro* inhibition of plant pathogens by *Bacillus subtilis* and *Enterobacter aerogenous* and *in vivo* control of two postharvest cherry diseases. Can J of Microbiol 32: 963–967

Wilson CL (1989) Managing the microflora of harvested fruits and vegetables to enhance resistance. Phytopathology 79: 1387–1390

Wisniewski M, Biles C, Droby S, McLaughlin Wilson C and Chalutz E (1991) Mode of action of postharvest biocontrol yeast, *Pichia guilliermondii*. I. Characterization of attachment to *Botrytis cinerea*. Physiol Mol Plant Pathol 39: 245–258

Yunis H, Elad Y and Mahrer Y (1990) The effect of air temperature, relative humidity and canopy wetness on grey mould of cucumbers in unheated greenhouses. Phytoparasitica 18: 203–215