Effect of *Ulocladium atrum* and Other Antagonists on Sporulation of *Botrytis cinerea* on Dead Lily Leaves Exposed to Field Conditions

J. Köhl, W. M. L. Molhoek, C. H. van der Plas, and N. J. Fokkema

DLO Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, the Netherlands.
We thank I. Koomen for critically reading the manuscript and V. Karoumpa for practical assistance.
Accepted for publication 24 November 1994.

ABSTRACT

Köhl, J., Molhoek, W. M. L., van der Plas, C. H., and Fokkema, N. J. 1995. Effect of *Ulocladium atrum* and other antagonists on sporulation of *Botrytis cinerea* on dead lily leaves exposed to field conditions. Phytopathology 85:393-401.

The potential of the antagonistic fungi *Aureobasidium pullulans, Chaetomium globosum, Gliocladium catenulatum,* and *Ulocladium atrum* to suppress sporulation of *Botrytis cinerea* was tested in nine experiments on dead lily leaves exposed to varying microclimatic conditions in the field. *U. atrum* competed successfully with naturally occurring saprophytes, mainly *Cladosporium* spp., colonized the dead lily leaves, survived dry periods, and consistently reduced sporulation of naturally occurring *B. cinerea. U. atrum* reduced the area of the leaf surface covered with conidiophores of *B. cinerea* by 80–96% compared to the control treated only with water. Germination rates of conidia of *U. atrum*, determined 18 h after field application, varied between 0 and 99%, depending on duration of leaf wetness periods, which ranged from 0 to 18 h, and on temperatures during leaf wetness periods. Germ tube length, determined 5–6 days after application, increased with total leaf wetness duration unless individual wetness periods were short. *C. globosum* reduced sporulation of *B. cinerea* only in three of nine experiments. *A. pullulans, G. catenulatum,* and a mixture of the fungicides chlorothalonil and maneb did not suppress sporulation of *B. cinerea.* The differential effect of the antagonists may mainly be caused by differences in response to the microclimatic conditions. The high saprophytic competitive ability of *U. atrum* under various microclimatic conditions makes this fungus an attractive candidate for the development of a biological control product aimed at suppression of sporulation of *Botrytis* spp. on necrotic leaf tissue. To our knowledge, *U. atrum* has not been described as an antagonist of *Botrytis* spp. or other fungal plant pathogens.

Additional keywords: inoculum production, microbial ecology.

Botrytis spp. incite economically important diseases in numerous protected and field crops such as grapevine, strawberry, onion, tomato, and bulb flowers. In lilies, *B. elliptica* can cause destructive leaf infections (leaf fire) resulting in serious yield losses (13). The control of *Botrytis* diseases depends primarily on the frequent use of fungicides. *Botrytis* spp. have a great potential to develop fungicide resistance (23). The threat of fungicide resistance and governmental restrictions on the use of pesticides raises the need to find alternative control methods. Biological control may offer an environmentally friendly supplement or alternative to chemical control.

For the development of a valid biocontrol strategy, ecological characteristics of both the pathogen and potential antagonists must be considered to identify developmental stages of the pathogen in which interference by an antagonist is effective. In principle, antagonists may be aimed at 1) the prevention of infection of healthy tissue, 2) the reduction of sporulation, or 3) the reduction of survival structures such as sclerotia of the pathogen. Biocontrol based on interactions between saprophytic fungi and necrotrophic pathogens, such as *Botrytis* spp. in necrotic plant tissue, affects the sporulation capacity of the pathogen (7,24,33,36). The advantage of such an approach is the long interaction time of at least several days between the saprophytically growing mycelium of the antagonist and of the pathogen compared to antagonism on healthy leaves where the pathogen may escape by rapid penetration of the leaf allowing an average interaction time of shorter than 12 h (19,26).

Necrotrophic pathogens such as *Botrytis* spp. can colonize plant tissue killed either by the pathogen or by other factors. The development of an epidemic in the field largely depends on the presence of necrotic tissue available for colonization by the pathogen, because sporulation occurs exclusively on necrotic tissue. Depending on the crop, different plant parts may serve as main substrates for sporulation of *Botrytis* spp. In strawberries, overwintering leaves are the main source of conidium inoculum during the spring for flower infections (4). In grapevine, senescent remains of flowers (15) colonized by *B. cinerea* Pers.:Fr. are important inoculum sources. *B. elliptica* in lilies can sporulate abundantly on necrotic leaves. In onions, *B. squamosa* the majority of the inoculum on necrotic tissue of dead leaf tips (35). Conidia of *Botrytis* spp. produced inside a crop such as onion or strawberries form a much more important source of inoculum during the polycyclic epidemic than do conidia produced outside the crop (27,30).

The successful use of antagonists against necrotrophic pathogens to reduce sporulation on necrotic tissue has been reported for several plant-pathogen relationships. The production of ascospores of *Venturia inaequalis* on dead apples leaves was reduced substantially by spraying spores of *Athelia bombacina* or *Chaetomium globosum* Kunze:Fr. on apple leaf litter in the orchard (24,29). Pfender et al (33) found that after application of the antagonist *Limonomyces roseipellis* the development of ascospores of *Pyrenophora tritici-repentis* on wheat straw was reduced by 60–80% under field conditions. Spraying *Gliocladium roseum* controlled sporulation of *B. cinerea* on dead leaves of field grown strawberries (36). Spore production of *Cochliobolus sativus* in lesions of wheat leaves was reduced under controlled climatic conditions with applications of *Trichoderma harzianum* (2).

In a previous screening under laboratory conditions, 41 fungal isolates belonging to 22 species were tested for suppression of sporulation of *Botrytis* spp. on dead leaf tissue (26). Under continuous wet conditions, *G. catenulatum* Gilman & E. Abbott, *C. globosum* and *Ulocladium atrum* G. Preuss inhibited sporulation of *Botrytis* spp. completely, whereas *Aureobasidium pullulans* (de Bary) G. Arnaud was less efficient. In bioassays with interrupted wetness periods, *C. globosum, U. atrum,* and *A. pullulans* suppressed sporulation of *Botrytis* almost completely, but *G. catenulatum* was distinctly less effective. These four isolates were tested in the present investigation under field conditions.

© 1995 The American Phytopathological Society

Our main objective was to compare the ability of the antagonists *A. pullulans, C. globosum, G. catenulatum, and U. atrum* to colonize dead lily leaf tissue and to protect the substrate from colonization by naturally occurring airborne inoculum of *Botrytis* spp. under field conditions. A sequence of nine experiments was performed under a variety of natural microclimatic conditions. The performance of antagonists was quantified by determining the sporulation potential of *Botrytis* spp. after incubation of the necrotic leaves in moist chambers after 5–6 days of exposure to field conditions. Determination of germination of spores of *C. globosum* and *U. atrum* and hyphal growth of *U. atrum* on dead lily leaves under field conditions provided additional information on the colonization potential of the antagonists.

MATERIALS AND METHODS

Antagonists. The antagonists (with isolate number) *A. pullulans* (490), *C. globosum* (256), and *U. atrum* (385) were isolated from necrotic leaf tips of field-grown onions. *G. catenulatum* (162), originating from red clover root was provided by P. Lüth, Prophyta Biologischer Pflanzenschutz GmbH, Malchow, Germany.

Lily leaves. Asiatic hybrid lilies (*Lilium*) cv. Mont Blanc were grown in pots during spring in the greenhouse at 20 C with 16 h of light per day. Flower buds were removed as usual in bulb production and to prevent pollen deposition on leaves, which is known to enhance infection by *B. cinerea* (6). No infections by *Botrytis* spp. or other diseases occurred on the leaves. Leaves, approximately 8 cm long from 3-mo-old plants, were cut from the stems, dried at 60 C for 48 h, and stored at room temperature in sealed plastic bags.

Leaves were placed in tap water for 30 min to allow water absorption before they were used for the experiments. Subsequently, they were washed thoroughly three times for 30 min in water to remove soluble nutrients, using 1-L bottles with approximately 100 leaves in 500 ml of tap water on a flask shaker (150 strokes per minute). Washed leaves were placed on dry filter paper to remove most of the water.

Leaf holders. Holders were made of iron wire coated with plastic. Fifteen washed leaves per holder were vertically suspended with clear adhesive tape at a distance of approximately 1 cm between the leaves. The upper leaf ends, covered by the tape for approximately 1 cm, were discarded later during sampling. Possible effects of the tape used to suspend the leaves on spore germination or mycelial growth of fungi involved in our study were checked on agar plates. The tape did not affect fungal development. Holders with lily leaves were placed in a sugar beet field 1 day before antagonist or fungicide application to allow equilibration of their water content to field conditions before antagonists reached the leaves. The upper end of the leaves was at 30 cm above ground. Leaves had no contact with leaves of surrounding plants.

Experimental field design. Leaf holders were placed in a sugar beet field, each holder in one microplot of 0.3 × 0.3 m from which sugar beets had been removed. The distance between microplots was 5 m in each direction. Each experiment was carried out according to a randomized block design with seven treatments assigned to plots within blocks. The seven treatments involved spraying spore suspensions of each of the four antagonists or a mixture of two of the antagonists, the fungicide Daconil M (chlorothalonil and maneb at 250 and 500 g a.i./kg, respectively, Schering AG, Berlin), or water as control. All experiments were performed between 26 May and 28 July. The first four experiments had two replications (blocks), and the last five experiments had four replications. Dates and times of field sprays are given in Table 1.

Sugar beet was chosen as the buffer crop because of the dense but low growth (approximately 0.5 m high), comparable to that of a lily crop. *Botrytis* spp. cause no leaf diseases in sugar beet, no pollen is produced that could stimulate *Botrytis* spp. or naturally occurring and applied antagonists (18). Weeds were efficiently controlled by pre- and postemergence herbicides and mechanically later in the growing season. Sporulation of *B. cinerea* occurred occasionally on dead sugar beet leaves and a few dead weeds during the growing season.

Monitoring of microclimate. Temperature and relative humidity (RH) of the air were measured with an electronic sensor (airprobe YA-100-hygromer, Rontronic AG, Bassersdorf, Switzerland) with an accuracy of <±1% in the range between 80 and 100% RH at 25 C. This sensor was positioned at a height of 35 cm in the same sugar beet field, approximately 10 m away from the lily-leaf holders. Three leaf wetness sensors were used to monitor leaf wetness of dead lily leaves fixed at leaf holders in an additional microplot. Electronic sensors had been developed at our institute for recording leaf wetness of necrotic leaf tissue based on measuring the capacity between two pins inserted into the substrate (J. Köhl, A. J. A. van der Zalm, and H. W. Roelofsen, *unpublished data*). All data were recorded at 30-min intervals and stored in a data-logger (Delta-T Logger, Delta-T Devices Ltd, Burwell, Cambridge). No data on leaf wetness are available for experiment 1. Rainfall per hour and rainfall duration were recorded with a rain gauge at the meteorological station of the Department of Meteorology, Wageningen Agricultural University, Wageningen, the Netherlands, at a distance of 500 m from the experimental field.

Treatments. *C. globosum* and *G. catenulatum* were grown in petri dishes on oatmeal agar (20 g of milled oat, 15 g of agar, and 1 L of tap water) for 28 and 14 days, respectively. *U. atrum*

TABLE 1. Percent germination of spores of *Chaetomium globosum* and *Ulocladium atrum* on dead lily leaves exposed in the field for 18 h in relation to microclimatic conditions

Experiment	Spray date and time	Germination (%)[u]				Duration of leaf wetness period (h)[v]		
		C. globosum		*U. atrum*				
		Alone[x]	Mixture[y]	Alone[x]	Mixture[y]	Total	Temperature >10 C	Temperature range[w]
1	26 May; 1800	2 ± 1	<1	36 ± 14	12 ± 12	...[z]	...	...
2	2 June; 1900	70 ± 7	50 ± 9	95 ± 5	95 ± 1	18.0	18.0	12.0–17.1
3	9 June; 2000	0	0	0	0	0.0	0.0	...
4	16 June; 1900	27 ± 23	18 ± 18	71 ± 9	77 ± 13	15.0	11.5	9.4–15.2
5	23 June; 1900	0	0	11 ± 2	16 ± 15	18.0	8.5	5.4–16.5
6	1 July; 2000	0	0	<1	<1	8.5	8.5	10.5–23.9
7	7 July; 1700	0	0	2 ± 2	0	9.0	3.5	4.4–19.7
8	15 July; 1600	87 ± 13	67 ± 27	99 ± 1	90.0 ± 7	18.0	18.0	15.5–16.7
9	22 July; 1800	2 ± 2	0	16 ± 11	24 ± 22	4.0	4.0	11.6–11.9

[u] Mean ± standard deviation of two replications with two leaves each (experiments 1–4) or four replications with one leaf each (experiments 5–9).
[v] During 18 h after spraying.
[w] During leaf wetness period.
[x] 2×10^6 spores per milliliter were sprayed.
[y] *C. globosum* and *U. atrum* were sprayed at 1×10^6 spores per milliliter each.
[z] Not recorded.

was grown for 28 days on sterilized oat grains in 250-ml Erlenmeyer flasks containing 30 g of oat (dry weight) saturated with water. *A. pullulans* was grown on basal yeast agar (10 g of bacteriological peptone, 20 g of sucrose, 1 g of yeast extract, 20 g of agar, and 1 L of tap water) for 5 days. All fungi were incubated at 18 C in the dark. Blastospores of *A. pullulans* or spores of the other antagonists were removed from agar or oat by flooding the substrate with tap water containing 0.01% Tween 80. After filtration through a plastic grid of 200-μm mesh, concentrations of suspensions were determined with a haemocytometer.

Suspensions applied to the field contained 2×10^6 blastospores of *A. pullulans*, ascospores of *C. globosum*, or conidia of the other antagonists per milliliter of tap water containing 0.01% Tween 80. When applied in mixture, spore suspensions of *C. globosum* and *U. atrum* were mixed just before spraying so the final concentration of each antagonist was 1×10^6 spores per milliliter. Suspensions were kept in ice water before spraying. Control plots were sprayed with tap water containing 0.01% Tween 80 or with the fungicide Daconil M applied in a dose of 4 mg/ml as recommended for *Botrytis* control in lily bulb production. All applications were carried out in the late afternoon with a propane-operated field-plot sprayer (AZO, Ede, the Netherlands) with a pressure of 250 kPa. The application rate was 50 ml of suspension per square meter, equivalent to 500 L/ha.

Sampling. Two leaves per holder in the first four experiments (in total four leaves per treatment) and one leaf per holder in the last five experiments (in total four leaves per treatment) were sampled 18 h after spraying to determine spore germination of *C. globosum* and *U. atrum*. After sampling, fungal growth was stopped by exposing leaves to formalin vapor for 30 min. Five leaves per holder (in total 10 leaves in experiments 1–4 and 20 leaves in experiments 5–9) were sampled after 5 (experiments 3, 4, 6, 8, and 9) or 6 days (experiments 1, 2, 5, and 7) to determine sporulation potential of *Botrytis* spp. The remaining leaves were sampled at the same day and stored at −20 C to measure mycelial growth of antagonists later (discussed below).

Spore germination. Eighteen hours after application of antagonists, the germination rates of spores of *C. globosum* and *U. atrum*, sprayed alone or in combination, were determined on four leaves per treatment. Spores of these antagonists were easy to distinguish from most of the spores of other fungi naturally deposited on the leaf surface. Fungi were stained with cotton blue in lactic acid (2 ml of lactic acid, 4 ml of glycerine, 3.5 ml of distilled water, and 15 mg of cotton blue) and examined under a microscope with 200× magnification. Fifty spores were randomly selected per leaf. They were considered germinated when germ tube lengths were at least half the diameter of a conidium of *U. atrum* or equal to the diameter of an ascospore of *C. globosum*.

The number of spores of *C. globosum* and *U. atrum* per square centimeter of leaf surface was determined in all experiments 18 h and 5 (experiments 3, 4, 6, 8, and 9) or 6 days (experiments 1, 2, 5, and 7) after spraying. The number of spores within a grid of 0.25 mm^2 at 10 randomly chosen parts of each of four leaves was counted using a microscope at 200× magnification. In all experiments, leaves from the control treatment were examined in the same way to monitor a possible background in the field of naturally occurring spores of *C. globosum, U. atrum,* or other fungi not distinguishable from those of the two antagonists. On average, less than 20 of such spores per square centimeter were found in the control treatment compared to an average of approximately 1,000 spores per square centimeter of leaf surface on treated leaves.

Mycelial growth of *U. atrum*. The mycelial development of *U. atrum* on dead lily leaves was determined after a field exposure period of 5 (experiments 3, 4, 6, 8, and 9) or 6 days (experiments 1, 2, 5, and 7) on four leaves per treatment. Fungi were stained with cotton blue in lactic acid. The percent germination of 50 randomly chosen conidia was determined as described above. From 20 randomly chosen germinated conidia, the number of germ tubes and the number of hyphal tips were counted. From the same conidia, the total mycelial length produced per conidium was measured with an interactive digitizer (Minimop, Kontron, Oberkochen, Germany).

Sporulation potential of *Botrytis* spp. Five leaves per holder sampled 5 or 6 days after field treatments were placed into moist chambers (210- × 160- × 50-mm polycarbonate chambers with two layers of sterile wet filter paper, enclosed in polyethylene bags). Leaves were incubated at 18 C for 8 days in the dark. Thereafter, percent leaf area covered with conidiophores of *Botrytis* spp. was visually estimated in classes (i.e., 0, 1, 5, 10, 20,. . .80, 90, 95, 99, 100% coverage of leaf area) using a stereomicroscope with 10–40× magnification. The leaf area covered with conidiophores of the two dominating groups of saprophytes, *Cladosporium*- and *Alternaria*-like fungi (including *Ulocladium* and *Stemphylium*), also was recorded.

Data analysis. Statistical analysis was performed by the statistical package Genstat 5 (21). Percentage data from the nine experiments were subjected to a variance stabilizing angular transformation and simultaneously analyzed by analysis of variance (ANOVA). As an obvious consequence of the experimental design used, possible differences between microclimatic conditions could not be inferred. However, interaction of climatic conditions with treatments, a main interest of this investigation, could be judged from the differences between plots within blocks. The leaf area covered with conidiophores was analyzed separately for each fungal group. LSD values were calculated for comparing means of different treatments within the same experiment.

RESULTS

Microclimatic conditions. The microclimatic conditions during the nine experiments are shown in Figure 1. Frequency and duration of leaf wetness periods of dead lily leaves, which seem to have a major effect on spore germination and colonization, varied considerably between the experiments. In experiment 3, leaf wetness periods longer than 1 h occurred only during two nights. In experiments 8 and 9, long leaf wetness periods after frequent and heavy rainfalls occurred on each day, with wetness periods sometimes lasting longer than 24 h. In experiment 7, the dew periods were short, but leaf wetness was frequently caused by slight rainfalls. A total of 11 leaf wetness periods were recorded, most of them lasting not longer than 2–3 h. Temperatures ranged between 2 and 31 C during the nine experiments. Temperatures below 10 C during leaf wetness periods were recorded during experiments 2, 3, and 7. Temperatures exceeded 30 C during experiment 6.

Germination of spores of *C. globosum* and *U. atrum* 18 h after application. Spores of *C. globosum* and *U. atrum* reached a germination of almost 100% during warm, wet nights, with wetness periods of dead leaves of 18 h and temperatures above 10 C in experiments 2 and 8 (Table 1; Fig. 1). Low temperatures during the leaf wetness periods in experiments 5 and 7 seemed to hamper spore germination of both antagonists. Germination of both antagonists was low in experiment 6, although the wetness period lasted 8.5 h and temperatures were moderate. The germination of spores of *U. atrum* was consistently higher compared to those of *C. globosum*. The average germination of *U. atrum* (61%) was significantly higher compared to spores of *C. globosum* (32%; $P < 0.001$; dates with no germination of one or both antagonists were excluded from ANOVA). No germination of *U. atrum* occurred in experiment 3 when leaves again dried rapidly after field application of antagonists, and leaves were dry during the following night. Standard deviations for percent germination on single leaves were high unless conditions for germination were optimal as in experiments 2 and 8. As germination depends on microclimatic conditions, the high variance between leaves probably reflects different wetness periods of single leaves even within an experimental plot.

Substrate colonization by *U. atrum* and *C. globosum*. After 5 (experiments 3, 4, 6, 8, and 9) or 6 days (experiments 1, 2, 5, and 7) of exposure to field conditions, on average approximately 800 conidia of *U. atrum* per square centimeter of leaf surface could be recovered. More than 50% of the conidia of *U. atrum* recovered on dead lily leaves germinated in all experiments, except

Experiment 1

Treatment[x]	Percentage of leaf area covered with conidiophores[y]		
	B. cinerea	*Cladosporium*-like	*Alternaria*-like
Water	15 a[z]	49 ab	23 b
Daconil M	16 a	45 ab	32 b
A. pullulans	25 a	62 a	26 b
C. globosum	5 bc	42 ab	32 b
G. catenulatum	14 ab	39 ab	22 b
U. atrum	2 c	31 b	91 a
C. globosum/ *U. atrum* in mixture	6 bc	34 b	75 a

Experiment 2

Water	24 b	19 bc	12 b
Daconil M	31 ab	29 ab	22 b
A. pullulans	32 ab	22 bc	18 b
C. globosum	31 ab	48 a	25 b
G. catenulatum	45 a	10 c	9 b
U. atrum	1 c	0 d	82 a
C. globosum/ *U. atrum* in mixture	2 c	0 d	80 a

Experiment 3

Water	19 a	52 a	20 c
Daconil M	13 a	53 a	36 bc
A. pullulans	14 a	50 a	32 bc
C. globosum	15 a	5 b	19 c
G. catenulatum	17 a	49 a	29 bc
U. atrum	1 b	7 b	49 ab
C. globosum/ *U. atrum* in mixture	17 a	33 a	59 a

Experiment 4

Water	30 a	61 a	30 c
Daconil M	26 ab	36 bc	28 c
A. pullulans	26 ab	38 a–c	30 c
C. globosum	34 a	43 ab	26 c
G. catenulatum	15 bc	61 a	40 bc
U. atrum	3 d	26 bc	97 a
C. globosum/ *U. atrum* in mixture	6 cd	21 c	60 b

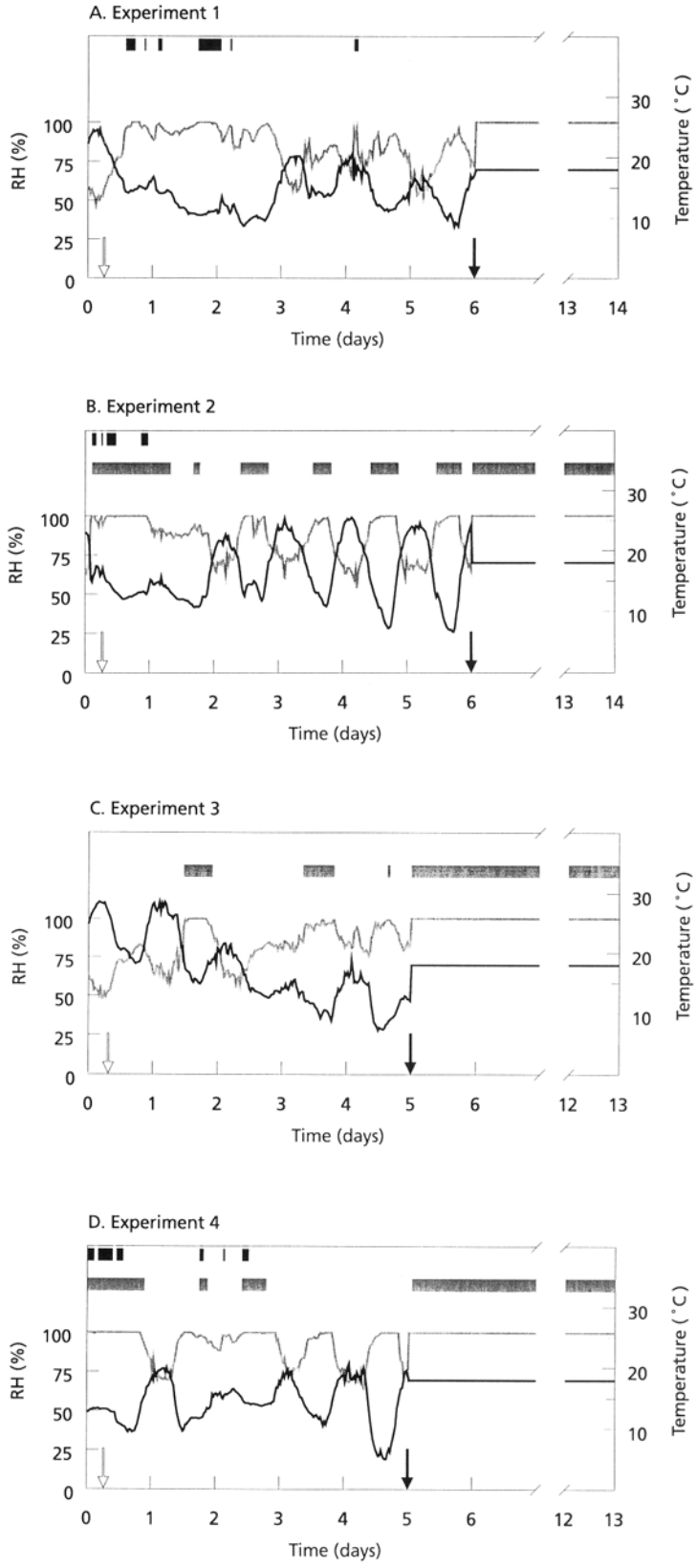


Fig. 1. *continued on next page*

Fig. 1A–I. Column 1 shows the effect of antagonists and Daconil M (chlorothalonil and maneb) on sporulation of *Botrytis cinerea* and *Cladosporium*- and *Alternaria*-like fungi (including *Ulocladium* and *Stemphylium*) on lily leaves. [x]2 × 10^6 spores per milliliter were sprayed. When sprayed in mixture, *C. globosum* and *U. atrum* were sprayed with 1 × 10^6 spores per milliliter each. [y]Mean of two replications (experiments 1–4) or four replications (experiments 5–9) with five leaves each. Total percentage of leaf area can be more than 100 when fungal colonies overlapped. [z]For each experiment, numbers within a column with a common letter did not differ significantly (LSD, $P < 0.05$). Column 2 shows microclimatic conditions for dead lily leaves during field exposure for 5 (experiments 3, 4, 6, 8, and 9) or 6 days (experiments 1, 2, 5, and 7) and subsequent incubation in moist chamber at 18 C for 8 days. Zero time is 12:00 of the day when leaves were sprayed in the field. Time of spraying (⇛); beginning of incubation in moist chamber (⟶); leaf wetness duration (▒); rainfall duration (▮); relative humidity (▒); and air temperature (—). No data on leaf wetness duration are available for experiment 1.

Fig. 1. *continued from previous page*

Experiment 5

Treatment[x]	Percentage of leaf area covered with conidiophores[y]		
	B. cinerea	*Cladosporium*-like	*Alternaria*-like
Water	23 a	32 bc	53 bc
Daconil M	16 ab	25 b–d	66 b
A. pullulans	17 ab	51 a	47 c
C. globosum	10 b	23 cd	49 c
G. catenulatum	24 a	38 ab	63 bc
U. atrum	2 c	19 cd	97 a
C. globosum/ *U. atrum* in mixture	2 c	16 d	92 a

Experiment 6

Water	5 a	29 a	57 b
Daconil M	2 ab	9 c	49 bc
A. pullulans	2 ab	12 bc	38 c
C. globosum	6 a	37 a	65 b
G. catenulatum	4 ab	23 ab	65 b
U. atrum	1 b	11 bc	87 a
C. globosum/ *U. atrum* in mixture	2 ab	12 bc	85 a

Experiment 7

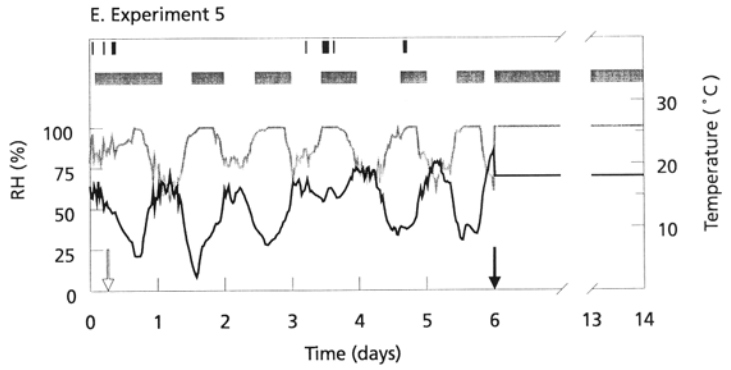
Water	14 a	48 ab	67 b
Daconil M	11 ab	28 cd	55 bc
A. pullulans	11 ab	33 bc	46 c
C. globosum	5 bc	24 cd	53 bc
G. catenulatum	14 a	50 a	59 bc
U. atrum	2 c	21 cd	96 a
C. globosum/ *U. atrum* in mixture	2 c	16 d	96 a

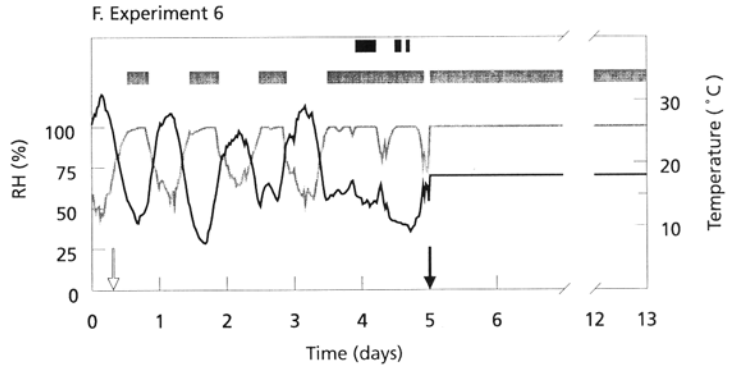
Experiment 8

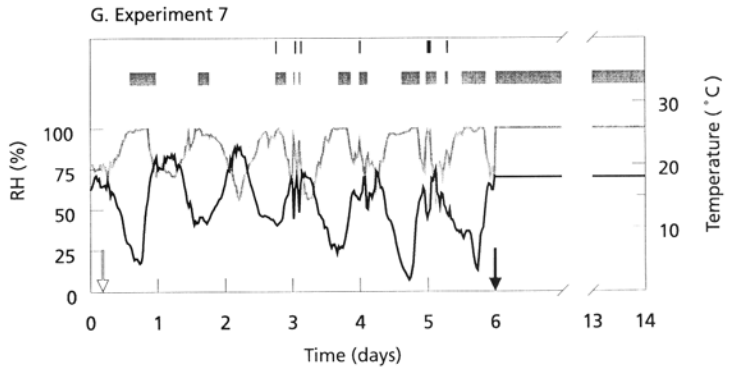
Water	6 ab	6 a	31 b
Daconil M	5 ab	11 a	29 b
A. pullulans	1 bc	9 a	19 b
C. globosum	7 a	6 a	24 b
G. catenulatum	6 a	7 a	30 b
U. atrum	1 c	5 a	51 a
C. globosum/ *U. atrum* in mixture	3 a–c	11 a	50 a

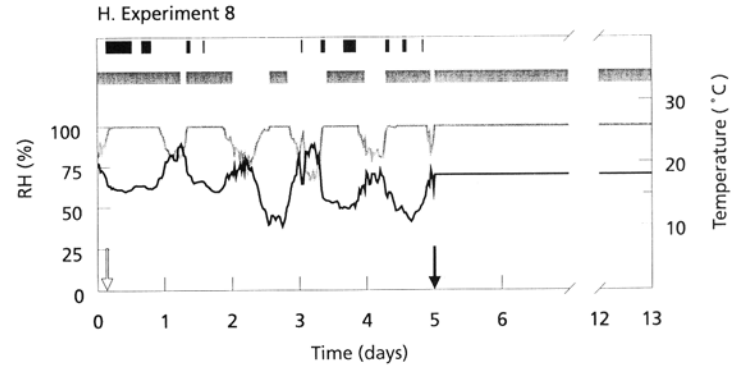
Experiment 9

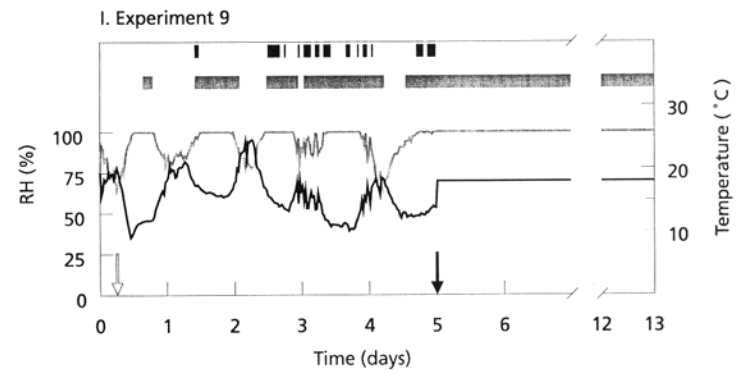
Water	5 ab	7 a	24 bc
Daconil M	3 ab	3 a	23 bc
A. pullulans	3 ab	7 a	13 c
C. globosum	4 ab	5 a	25 bc
G. catenulatum	5 a	5 a	29 b
U. atrum	1 b	5 a	49 a
C. globosum/ *U. atrum* in mixture	3 ab	5 a	47 a











experiment 3 (Table 2). With increasing germination, the number of germ tubes produced per germinated conidium increased from 1.4 (experiment 3) to 2.6 (experiment 9). In all experiments, except in experiments 1, 3, and 7, germ tubes branched and up to 4.6 hyphal tips (experiment 8) were produced per germinated conidium. The overall average of mycelial length produced per germinated conidium was 108 μm. In general, mycelial growth appeared to depend on the total duration of leaf wetness, e.g., mycelium length per germinated conidium was 19 μm in experiment 3 with 24.5 h of total leaf wetness duration and was 239 μm in experiment 9 with 73.0 h of total leaf wetness duration. However, only 28 μm per germinated conidium was produced during 51.5 h of total leaf wetness duration in experiment 7, when frequent but short leaf wetness periods occurred.

Recovery of ascospores of *C. globosum* after 5–6 days was distinctly lower than for *U. atrum*. On average, less than 200 ascospores per square centimeter of leaf surface were found, except in experiment 7, in which approximately 1,000 ascospores per square centimeter of leaf surface were recovered. Spores had not germinated in experiments 1, 3, and 7, which was similar to observations after 18 h. Germination less than 10% was found in experiments 5, 6, and 9 (as after 18 h) but also in experiments 2, 4, and 8, in which germination rates of 20–87% were found after 18 h. On average, 1.2 germ tubes per germinated ascospore with a length of approximately 60 μm were formed. Branching of germ tubes did not occur.

Sporulation potential of *Botrytis* spp. In all cases in which *Botrytis* was found sporulating on dead lily leaves after field exposure, *B. cinerea* was identified. *B. elliptica*, which was heavily sporulating on lily cv. Mont Blanc in an experimental field approximately 50 m away, and *B. squamosa*, which may have been produced on onions in a field experiment approximately 10 m away from our experiments, were never found on dead lily leaves fixed on leaf holders.

Sporulating conidiophores of *B. cinerea* (sporulation potential) in the water control treatment covered between 5 and 30% of the leaf area in the nine experiments (Fig. 1, second column). This may be explained by the differential effect of varying microclimatic conditions on colonization and the naturally occurring inoculum load of *B. cinerea*.

U. atrum consistently reduced the sporulation potential of *B. cinerea*. The reduction of sporulation of *B. cinerea* by *U. atrum* was significant compared to the control treatment in eight of nine experiments ($P < 0.05$), which were carried out under markedly different microclimatic conditions and with probably varying inoculum pressure of the pathogen. Even at the highest level of sporulation of *B. cinerea* in experiment 4, *U. atrum* caused a 90% reduction in sporulation potential of the pathogen. *C. globosum* significantly reduced the sporulation potential of *B. cinerea* in three experiments. The mixture of *C. globosum* and *U. atrum*, both antagonists applied in half the concentration of that when sprayed alone, reduced sporulation potential of *B. cinerea* (significantly in five of nine experiments), except in experiment 3. However, effects were generally less marked compared to those of *U. atrum* when sprayed alone. *G. catenulatum* reduced *Botrytis* sporulation only in experiment 4. *A. pullulans* never reduced sporulation of *Botrytis*. Daconil M showed no fungicidal activity against *B. cinerea* on necrotic tissue of lily leaves.

Sporulation potential of *Cladosporium*- and *Alternaria*-like fungi. *Cladosporium*- and *Alternaria*-like fungi were the dominating saprophytic fungi sporulating on leaves. *U. atrum* reduced the leaf area covered with *Cladosporium*-like fungi significantly ($P < 0.05$) compared to the control treatment in five experiments. No effect of *U. atrum* was found in the last two experiments when *Cladosporium*-like fungi covered less than 10% of the leaf surface. *C. globosum* gave varying results. In two experiments *Cladosporium*-like fungi were significantly ($P < 0.05$) reduced; in six experiments no effect on *Cladosporium*-like fungi was found, and in one experiment *Cladosporium*-like fungi were stimulated significantly ($P < 0.05$) by *C. globosum*. *C. globosum* and *U. atrum* sprayed in mixture affected sporulation of *Cladosporium*-like fungi in five experiments. *G. catenulatum* and *A. pullulans* had no effect on the occurrence of *Cladosporium*-like fungi, except in experiment 5, in which *A. pullulans* stimulated *Cladosporium*-like fungi. Daconil M significantly ($P < 0.05$) reduced the occurrence of *Cladosporium*-like fungi in three experiments but not the sporulation of *B. cinerea*.

Treatments with *U. atrum*, sprayed alone or in mixture with *C. globosum*, significantly ($P < 0.05$) increased the occurrence of *Alternaria*-like fungi (including *Ulocladium* and *Stemphylium*) in all nine experiments. Up to 97% of the surface of dead lily leaves was covered by conidiophores of this fungal group. *Alternaria*-like fungi on leaves sprayed with *U. atrum* had the same color and conidial characteristics as the applied isolate of *U. atrum*, unlike leaves not treated with this antagonist. Treatments with the other antagonists or the fungicides did not affect sporulation of *Alternaria*-like fungi.

Fungal sporulation on dead lily leaves under field conditions. Additional leaf holders were placed in microplots at 22 June and were sprayed weekly from 23 June onward with the antagonists or the fungicide. At 16 July, after 24 days of exposure to field conditions, almost no conidiophores of *B. cinerea* were found. Two groups of saprophytes dominated on such leaves (Table 3). *Cladosporium*-like fungi covered approximately 31–46% of the surfaces of leaves of the control or treated with *A. pullulans*, *C. globosum*, or *G. catenulatum*. On leaves treated with Daconil M or *U. atrum*, significantly less sporulation of *Cladosporium* spp. was found, with 14 and 13%, respectively. On the other hand, the leaf area covered with conidiophores of *Alternaria*-like fungi,

TABLE 2. Germination and germ tube growth of conidia of *Ulocladium atrum*[w] after 5 (experiments 3, 4, 6, 8, and 9) or 6 days (experiments 1, 2, 5, and 7) on dead lily leaves exposed to field conditions

Experiment	Germination[x] (%)	No. of germ tubes[y,z]	No. of hyphal tips[y,z]	Total mycelial length (μm)[y,z]
1	64 ± 12	1.7 ± 0.1	1.8 ± 0.2	30 ± 5
2	93 ± 1	2.0 ± 0.2	2.3 ± 0.2	82 ± 18
3	20 ± 8	1.4 ± 0.2	1.4 ± 0.2	19 ± 3
4	83 ± 12	2.5 ± 0.2	2.9 ± 0.4	85 ± 20
5	63 ± 24	1.8 ± 0.3	2.2 ± 0.4	79 ± 19
6	97 ± 2	2.3 ± 0.4	3.1 ± 0.4	157 ± 32
7	64 ± 4	1.7 ± 0.1	1.7 ± 0.1	28 ± 4
8	84 ± 7	2.2 ± 0.1	4.6 ± 1.8	224 ± 134
9	89 ± 7	2.6 ± 0.4	3.9 ± 0.9	239 ± 85

[w] Conidia of *U. atrum* were sprayed at 2×10^6 conidia per milliliter.
[x] Mean ± standard deviation of two replications with two leaves each (experiments 1–4) or four replications with one leaf each (experiments 5–9). Fifty spores were examined on each leaf.
[y] Per germinated conidium.
[z] Mean ± standard deviation of two replications with two leaves each (experiments 1–4) or four replications with one leaf each (experiments 5–9). Twenty spores were examined on each leaf.

TABLE 3. Effect of antagonists and Daconil M (chlorothalonil and maneb) on the percent area of dead lily leaves covered with conidiophores of *Botrytis cinerea* and *Alternaria*- and *Cladosporium*-like saprophytes after 24 days of field exposure[x]

Treatment	Percent leaf area covered with conidiophores		
	B. cinerea	*Cladosporium*-like	*Alternaria*-like[y]
Water	0	31 ab[z]	10 b
Daconil M	0	14 c	7 b
Aureobasidium pullulans	0	37 ab	7 b
Chaetomium globosum	0	41 a	10 b
Gliocladium catenulatum	0	46 a	11 b
Ulocladium atrum	0	13 c	44 a
C. globosum/ *U. atrum* in mixture	0	24 bc	48 a

[x] Leaves were treated in four replications with five leaves each with antagonists or fungicide at day 1, 9, 15, and 23 of field exposure.
[y] Including *Ulocladium* and *Stemphylium*.
[z] Numbers within a column with a common letter do not differ significantly (LSD test: $P < 0.05$).

including *Ulocladium* and *Stemphylium*, increased from 10% in leaves not treated with *U. atrum* to above 40% when *U. atrum* had been sprayed on leaves. *Alternaria*-like fungi on leaves sprayed with *U. atrum* had the same color and conidial characteristics as the applied isolate of *U. atrum*, unlike leaves not treated with this antagonist.

DISCUSSION

Antagonists used in biological control aimed at the reduction of sporulation by *Botrytis* spp. or other necrotrophic pathogens on necrotic leaf tissue should colonize the substrate rapidly under field conditions, survive in the substrate during unfavorable conditions, and compete successfully with the pathogen and other saprophytes when conditions become favorable for fungal growth. A high competitive ability is a prerequisite for saprophytic antagonists introduced to senescing or necrotic leaf tissue under field conditions. Only strong competitors have the ability to colonize necrotic tissue of lesions induced and already partly colonized by the pathogen or to protect necrotic tissue from external colonization by *Botrytis* spp. Besides competition with the pathogen, the introduced antagonist has to compete with naturally occurring saprophytes. A strong saprophytic competitive ability could be based on two parameters, 1) high enzymatic activity for substrate utilization and 2) adaptation to harsh microclimatic conditions in the phyllosphere. The ability of the antagonist to survive during dry periods and to colonize the substrate rapidly during limited hours of leaf wetness per day is characteristic of an antagonist adapted to the niche of necrotic leaf tissue. Especially during the early stages of substrate colonization, antagonists may differ in their susceptibility to unfavorable environmental conditions. Germinating spores, germ tubes, or hyphal tips of several fungi such as *Botryosphaeria obtusa* (1), *Aspergillus* sp., *Colletotrichum graminicola* (10), *G. roseum*, *Penicillium* spp., and *T. harzianum* (32) are reported to be very sensitive to interrupted wetness periods. Only structures such as hyphal cells, conidia, or chlamydospores remained viable after dry periods but needed a long lag time for regrowth (32). On the other hand, germ tubes or hyphal tips of *Alternaria* spp., *A. pullulans, B. cinerea, Cladosporium cladosporioides*, *Epicoccum purpurascens*, or *Monilinia fructicola* withstand dry periods without losing their capacity to renew their growth rapidly after rewetting of the substrate (9,10, 22,32). For *Alternaria alternata, A. pullulans,* and *B. cinerea* lag times of 60 min for regrowth of hyphal tips were reported (32).

Spores sprayed on necrotic leaf tissue are exposed to UV radiation and possibly to high temperatures during the day. UV radiation is a major factor for mortality of spores on leaf surfaces (34). In general, hyaline spores, such as conidia of *G. catenulatum*, are sensitive to UV radiation, whereas pigments in dematiaceous species protect spores from UV radiation (10,16). However, the intensively pigmented conidia of *Alternaria solani*, closely related to *U. atrum*, are killed by UV radiation of direct sunlight after an exposure to 8 h of sunlight per day during five successive days (34). In our experiments, the pigmented ascospores of *C. globosum* and the black conidia of *U. atrum* may have survived better during field exposure of 5–6 days than did the hyaline conidia of *G. catenulatum* and the hyaline blastospores of *A. pullulans*.

In our experiments, *U. atrum* germinated rapidly on dead lily leaves during the night after spraying, provided temperatures during wetness periods were not below 10 C. Germination rates of almost 20% were reached within 4 h, and germination rates above 90% were found after wetness periods of 18 h. Germination of ascospores of *C. globosum* were also high 18 h after spraying when leaf wetness periods lasted 15 h or longer. Almost no germination occurred when wetness periods were 9 h or shorter. Germination rates of spores of both antagonists also were recorded during an experiment with onions at the same location (data not presented). On naturally senescent leaf tips of field-grown onions, germination of spores of *U. atrum* and *C. globosum* were comparable to those found on the dead lily leaves fixed onto holders; evidently they also were highly dependent on favorable microclimatic conditions. Recovery of conidia of *U. atrum* was high after field exposure of 5–6 days. Apparently conidia of this antagonist were not washed off the leaves during rainfall that occurred during all experiments, except during experiment 3. Germination of conidia of *U. atrum* was consistently higher in all experiments after 5–6 days than after 18 h. Thus, conidia may survive at least several days under field conditions without losing their germination capacity. During our experiments, conidia of *U. atrum* formed mycelium with a length of up to 239 μm. Length of germ tubes increased with total leaf wetness duration unless individual wetness periods were short as during experiment 7.

These findings indicate that hyphal tips of *U. atrum* may regrow after dry periods with a short lag time. On the other hand, recovery of ascospores of *C. globosum* was mostly low. Even when spores had germinated more than 70% during the first night after spraying in experiments 2 and 8 (Table 1), such spores could not be recovered after 5–6 days. Spores of *C. globosum* might have been washed off leaves during rainfalls. Germination after 5–6 days under field conditions ranged between zero and 10% in the nine experiments. Even in experiments in which leaf wetness periods longer than 15 h had occurred (experiments 6, 8, and 9), no higher germination could be found. When applied to leaf surfaces, ascospores of *C. globosum* may rapidly lose their vigor to germinate under field conditions.

The sporulation potential of *B. cinerea* was consistently lower on dead lily leaves when *U. atrum* had been applied. The antagonistic effect of *U. atrum* occurred in all microclimatic conditions during the period when leaves were exposed to field conditions. *U. atrum* seemed to survive during the long dry periods in experiment 3 as well as during the frequent but short wetness periods during experiment 7 and was able to colonize the substrate during the subsequent incubation period in moist chambers (Fig. 1). Intensive substrate colonization under field conditions was found after the long leaf wetness periods that occurred during experiments 2, 4, 6, 8, and 9; as a result, the antagonist might suppress saprophytic growth of *B. cinerea* during field exposure period as well as during the subsequent incubation period in moist chambers. We conclude from the results obtained in our field experiments that *U. atrum* is well adapted to the rapid changes of the microclimatic conditions in the phyllosphere and is able to colonize necrotic leaf tissue. It can germinate rapidly with high percentages. Germ tubes and hyphal tips seem to withstand dry conditions and regrow rapidly during wet conditions.

Saprophytic antagonists such as *U. atrum* might be plant pathogenic under certain circumstances. Considering that *Ulocladium* spp. are ubiquitous saprophytes colonizing leaf litter and soil (12), reports on plant pathogenicity of *U. atrum* are rare. However, *U. atrum* has been described as the causal agent of cucumber leaf spot in Great Britain (5), of a potato leaf disease in Peru (38) and eastern Turkey (8), and as a possible biocontrol agent of *Orobanche* spp. (28). In our own field experiments with onions, lilies, tulips, and sugar beets, *U. atrum* did not produce any symptoms of disease.

C. globosum is a potential antagonist against a wide range of fungal pathogens. *C. globosum* has high cellulolytic activity (12) and produces potential antifungal antibiotics (11). Applications of conidia of the antagonist to apple leaf litter prevent the ascospore production of *V. inaequalis* and enhance leaf decomposition (24,29). *C. globosum* produces antifungal antibiotics that control apple leaf infection by ascospores of *V. inaequalis*, without spore germination of the antagonist (3).

In our study, *C. globosum* reduced the sporulation potential of *B. cinerea* in three experiments and was competitive with *Cladosporium*-like fungi in two experiments. In all cases where spores of *C. globosum* germinated with high percentages during the night after field application, no antagonism was found later after incubation in moist chambers. Although able to germinate during the first night after application if leaf wetness periods were longer than approximately 15 h, recovery of germinated or ungerminated spores was low after 5–6 days. In contradiction to these low recovery rates, sporulation of *B. cinerea* was signifi-

cantly suppressed by *C. globosum* after incubation of the dead lily leaves in moist chambers subsequent to the field exposure period in experiments 1, 5, and 7. The antagonist might have the potential to germinate under optimum conditions in moist chambers and a low inoculum density may be sufficient for high antagonistic activity. Antagonistic activity with the presence of low densities of *C. globosum* also can be explained by toxins already present in suspensions applied to leaves. Toxins may be stable only under certain environmental conditions (3), resulting in variable antagonistic activity during the nine experiments independent of the presence of an actively growing population of *C. globosum*.

The application of a combination of spores of *C. globosum* and *U. atrum* consistently reduced the sporulation potential of *B. cinerea*, but effects were less marked compared to applications of *U. atrum* alone. In combined applications, both fungi were applied in half the concentration of suspensions used for applications of single antagonists. Thus, the reduced effectiveness of combined applications of the two antagonists may be explained by the lower inoculum rate of *U. atrum* applied. In combined applications, growth of *U. atrum* also may partly be inhibited by toxins produced by *C. globosum*. However, germination of conidia of *U. atrum* was not lower when applied in combination with *C. globosum* compared to applications alone (Table 1).

Gliocladium spp. were antagonistic against many pathogens, including *Botrytis* spp. (14, 31). Intensive research on the antagonistic potential of *Gliocladium* spp. and the closely related *Trichoderma* spp. recently resulted in the development of a biocontrol product based on *T. harzianum* against *B. cinerea* (17). In our study, *G. catenulatum* did not control sporulation of *B. cinerea*. Most of the ungerminated or germinated conidia may not survive on necrotic leaf tissue under field conditions. Even if a limited amount of the inoculum of the antagonist survives during field exposure, it may not suppress sporulation of *B. cinerea* efficiently if the pathogen already has colonized the dead leaf (36). The sensitivity of *G. roseum* to dry periods was found by Park (32). In bioassays on dead onion leaves in moist chambers, *Gliocladium* spp. generally lose their capacity to control sporulation of *Botrytis* spp., when wetness periods in the growth substrate are interrupted for several hours (26).

A. pullulans is a common inhabitant of the phyllosphere of green and senescing leaves with a high tolerance of dry conditions (25,32). The antagonistic potential of *A. pullulans* to prevent leaf infections of onion by *Alternaria porri* was demonstrated by Fokkema and Lorbeer (20). *A. pullulans* was not efficient in suppressing sporulation of *B. cinerea* in our experiments. In bioassays on dead onion leaves, this antagonist generally was less effective under optimum conditions than the other three antagonists used in our study (26).

Daconil M, a combination of the fungicides chlorothalonil and maneb, is a commonly used product that protects crops such as lilies, tulips, and onions effectively from *Botrytis* damage. In our experiments, the fungicides never affected *Botrytis* sporulation on dead lily leaves. However, in three experiments with lily leaves exposed to field conditions for 5–6 days as well as in the experiment with an exposure period of 24 days, a reduction of *Cladosporium*-like fungi was found after fungicide treatment. *Cladosporium* spp. may be more sensitive to one of the fungicidal compounds of the applied fungicide than *Botrytis* spp. High efficacy of chlorothalonil against *Cladosporium* spp. on necrotic leaf tissue of *Lolium perenne* was found by Thomas and Shattock (37). The ineffectiveness of the fungicides against *B. cinerea* may be explained by the high adsorption capacity of chlorothalonil to organic matter. Chlorothalonil might have been adsorbed to cell walls in the necrotic tissue, inactivating the compound. Possibly, heavy rainfalls that occurred during most of the experiments also had washed off the nonadsorbed fraction of fungicides from dead leaves.

During nine experiments in which dead lily leaves had been exposed to field conditions for 5–6 days, *U. atrum* was the only antagonist that consistently suppressed colonization and subsequent sporulation of *B. cinerea* on dead lily leaves. The antagonist showed a great potential to colonize necrotic leaf tissue under field conditions and to survive adverse conditions in the field. *U. atrum* could compete with *B. cinerea* as well as with *Cladosporium*-like fungi as a main group of naturally occurring saprophytes on necrotic leaf tissue. The same results were obtained in an additional experiment in which fungal development was examined after dead lily leaves had been exposed to field conditions for 24 days without subsequent incubation in moist chambers. The high saprophytic competitive ability of *U. atrum* makes this fungus an attractive candidate for the development of a biocontrol product aimed at suppression of sporulation of *Botrytis* spp. on necrotic leaf tissue. To our knowledge, *U. atrum*, has not been described as an antagonist of *Botrytis* spp. or other fungal plant pathogens. Experiments in field grown crops and studies on mass production and formulation are being carried out in our laboratory for further evaluation of the suitability of *U. atrum* as biocontrol agent.

LITERATURE CITED

1. Arauz, L. F., and Sutton, T. B. 1990. Effect of interrupted wetness periods on spore germination and apple infection by *Botryosphaeria obtusa*. Phytopathology 80:1218-1220.
2. Biles, C. L., and Hill, J. P. 1988. Effect of *Trichoderma harzianum* on sporulation of *Cochliobolus sativus* on excised wheat seedling leaves. Phytopathology 78:656-659.
3. Boudreau, M. A., and Andrews, J. H. 1987. Factors influencing antagonism of *Chaetomium globosum* to *Venturia inaequalis*: A case study in failed biocontrol. Phytopathology 77:1470-1475.
4. Braun, P. G., and Sutton, J. C. 1987. Inoculum sources of *Botrytis cinerea* in fruit rot of strawberries in Ontario. Can. J. Plant Pathol. 9:1-5.
5. Butler, D., Griffin, M. J., and Fletcher, J. T. 1979. Leaf spot on cucumber caused by *Ulocladium atrum*. Plant Pathol. 28:96-97.
6. Chou, M. C., and Preece, T. F. 1968. The effect of pollen grains on infections caused by *Botrytis cinerea* Fr. Ann. Appl. Biol. 62:11-22.
7. Clarkson, J. P., and Lucas, J. A. 1993. Screening for potential antagonists of *Pseudocercosporella herpotrichoides*, the causal agent of eyespot disease of cereals. 2. Fungi. Plant Pathol. 42:552-559.
8. Demirci, E., and Doken, M. T. 1989. Studies on the determination and identification of fungal agents of the diseases isolated from the roots, stems and leaves of potatoes in Erzurum region. J. Turk. Phytopathol. 18:51-60.
9. Dickinson, C. H. 1981. Biology of *Alternaria alternata*, *Cladosporium cladosporioides* and *C. herbarum* in respect of their activity on green plants. Pages 169-184 in: Microbial Ecology of the Phylloplane. J. P. Blakeman, ed. Academic Press, London.
10. Diem, H. G. 1971. Effect of low humidity on the survival of germinated spores commonly found in the phyllosphere. Pages 211-219 in: Ecology of Leaf Surface Micro-organisms. T. F. Preece and C. H. Dickinson, eds. Academic Press, London.
11. Di Pietro, A., Gut-Rella, M., Pachlatko, J. P., and Schwinn, F. J. 1992. Role of antibiotics produced by *Chaetomium globosum* in biocontrol of *Pythium ultimum*, a causal agent of damping-off. Phytopathology 82:131-135.
12. Domsch, K. H., Gams, W., and Anderson, T. H. 1980. Compendium of Soil Fungi. Academic Press, London.
13. Doss, R. P., Chastagner, G. A, and Riley, K. L. 1984. Techniques for inoculum production and inoculation of lily leaves with *Botrytis elliptica*. Plant Dis. 68:854-856.
14. Dubos, B. 1992. Biological control of *Botrytis*: State-of-the-art. Pages 169-178 in: Recent Advances in *Botrytis* Research. K. Verhoeff, N. E. Malathrakis, and B. Williamson, eds. Pudoc Scientific Publishers, Wageningen, the Netherlands.
15. Dubos, B., Jailloux, F., and Bulit, J. 1982. L'antagonisme microbien dans la lutte contre la pourriture grise de la vigne. Bull. OEPP 12:171-175.
16. Durell, L. W., and Shields, L. M. 1960. Fungi isolated in culture from soils of the Nevada test site. Mycologia 52:636-641.
17. 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.
18. Fokkema, N. J. 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. Nether. J. Plant Pathol. (Suppl. 1) 77:1-60.
19. Fokkema, N. J. 1993. Opportunities and problems of control of foliar pathogens with micro-organisms. Pestic. Sci. 37:411-416.

20. Fokkema, N. J., and Lorbeer, J. W. 1974. Interactions between *Alternaria porri* and the saprophytic mycoflora of onion leaves. Phytopathology 64:1128-1133.
21. Genstat 5 Committee 1990. Genstat 5 Reference Manual. R. W. Payne, chair, and P. W. Lane, secretary. Clarendon Press, Oxford.
22. Good, H. M., and Zathureczky, P. G. M. 1967. Effects of drying on the viability of germinated spores of *Botrytis cinerea, Cercospora musae,* and *Monilinia fructicola.* Phytopathology 57:719-722.
23. Gullino, M. L., 1992. Chemical control of *Botrytis* spp. Pages 217-222 in: Recent Advances in *Botrytis* Research. K. Verhoeff, N. E. Malathrakis, and B. Williamson, eds. Pudoc Scientific Publishers, Wageningen, the Netherlands.
24. Heye, C. C., and Andrews, J. H. 1983. Antagonism of *Athelia bombacina* and *Chaetomium globosum* to the apple scab pathogen, *Venturia inaequalis.* Phytopathology 73:650-654.
25. Hudson, H. J. 1971. The development of the saprophytic fungal flora as leaves senesce and fall. Pages 447-455 in: Ecology of Leaf Surface Micro-organisms. T. F. Preece and C. H. Dickinson, eds. Academic Press, London.
26. Köhl, J., and Fokkema, N. J. 1994. Fungal interactions on living and necrotic leaves. Pages 321-334 in: Ecology of Plant Pathogens. J. P. Blakeman and B. Williamson, eds. CABI, Oxon, England.
27. Köhl, J., Molhoek, W. M. L., van der Plas, C. H., and Fokkema, N. J. Suppression of sporulation of *Botrytis* spp. as a valid biocontrol strategy. Eur. J. Plant Pathol. In press.
28. Linke, K. H., Scheibel, C., Saxena, M. C., and Sauerborn, J. 1992. Fungi occurring on *Orobanche* spp. and their preliminary evaluation for *Orobanche* control. Trop. Pest Manage. 38:127-130.
29. Miedtke, U., and Kennel, W. 1990. *Athelia bombacina* and *Chaetomium globosum* as antagonists of the perfect stage of the apple scab pathogen (*Venturia inaequalis*) under field conditions. Z. Pflkrankh. Pflschutz 97:24-32.
30. Miller, P. M., and Waggoner, P. E. 1957. Dispersal of spores of *Botrytis cinerea* among strawberries. Phytopathology 47:24-25.
31. Papavizas, G. C. 1985. *Trichoderma* and *Gliocladium*: Biology, ecology, and potential for biocontrol. Annu. Rev. Phytopathol. 23:23-54.
32. Park, D. 1982. Phylloplane fungi: Tolerance of hyphal tips to drying. Trans. Br. Mycol. Soc. 79:174-178.
33. Pfender, W. F., Zhang, W., and Nus, A. 1993. Biological control to reduce inoculum of the tan spot pathogen *Pyrenophora tritici-repentis* in surface-borne residues of wheat fields. Phytopathology 83:371-375.
34. Rotem, J., Wooding, B., and Aylor, D. E. 1985. The role of solar radiation, especially ultraviolet, in the mortality of fungal spores. Phytopathology 75:510-514.
35. Sutton, J. C., James, T. D. W., and Rowell, P. M. 1983. Relation of weather and host factors to an epidemic of Botrytis leaf blight in onions. Can. J. Plant Pathol. 5:256-265.
36. Sutton, J. C., and Peng, G. 1993. Biocontrol of *Botrytis cinerea* in strawberry leaves. Phytopathology 83:615-621.
37. Thomas, M. R., and Shattock, R. C. 1986. Effects of fungicides on *Drechslera* spp. and leaf surface filamentous saprophytic fungi on *Lolium perenne.* Plant Pathol. 35:120-125.
38. Turkensteen, L. J. 1979. *Ulocladium atrum*, el organismo causal de Kasahui, enfermedad foliar de la papa en el altiplano del Peru. Fitopatologia 14:85-86.