Biocontrol of *Botrytis cinerea* by *Ulocladium atrum* in Different Production Systems of Cyclamen

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ABSTRACT

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Ten experiments in six different commercial greenhouses were conducted to study the effect of spraying conidial suspensions of the saprophytic fungus *Ulocladium atrum* (1×10^6 conidia per ml) on infection of leaves and flower petioles of cyclamen by *Botrytis cinerea* (gray mold). The greenhouses represented the range of Dutch growing systems of cyclamen, differing considerably in the arrangement of plants, irrigation system, heating system, and material of pots. Applications of *U. atrum* suspensions were carried out at 4-week intervals only twice on young plants or were continued during the whole growing season until 4 weeks before plants were marketable. The antagonistic treatments were compared with untreated or water-treated controls and with fungicide applications as applied by growers. After applications of *U. atrum* at 4-week intervals, disease development was significantly reduced in experiments carried out in five different greenhouses. In no case did treatments with fungicides give better control than *U. atrum* treatments. Two applications of *U. atrum* resulted in sufficient control when plants were marketed within 60 days after the last application. In one greenhouse with an extremely high disease pressure, neither *U. atrum* nor fungicide applications controlled leaf rot. In additional experiments, the fate of *U. atrum* conidia on leaves of cyclamen grown in a system with top-irrigation three times per week was studied during a period of 70 days. The number of conidia per square centimeter of green leaves declined by 50% during the first 10 days of the experiment but remained stable during the following 60 days. The percentage of germinated conidia on green leaves increased during the experiment to approximately 50%. After additional incubation of leaf samples in moist chambers, more than 75% of the conidia had germinated, indicating that viable inoculum was present on leaves during the whole experiment. Artificial necrosis of leaves was induced by removing leaves from the plants. *U. atrum* colonized these leaves and competed successfully with *B. cinerea* on such leaves, even when they had been removed 70 days after the *U. atrum* application. Our results show that *U. atrum* has the potential to control leaf rot of cyclamen under a broad range of commercial growing conditions.

Botrytis cinerea causes leaf and fruit infections in numerous crops, including ornamentals. Its control in many ornamental crops depends on fungicides, but is often restricted because of the threat of development of fungicide resistance by the pathogen (1,5,11), negative side effects of fungicides on plant growth, or visible residuals on plant surfaces (6). Alternatively to fungicides, antagonists can be utilized to achieve disease control. Biocontrol of *B. cinerea* by yeasts, bacteria, and filamentous fungi has been found in many crops (2).

The saprophytic fungus *Ulocladium atrum* has the potential to compete sapro-

phytically with *Botrytis* spp. during the colonization of necrotic plant tissues (9). The inoculum potential of *B. cinerea* can be reduced by antagonistic interaction, leading to slower disease epidemics (8). Its high ecological competence makes *U. atrum* an attractive candidate for applications in fields and greenhouses (3,10).

In cyclamen (*Cyclamen persicum*), naturally senesced leaves within the dense canopy play a crucial role in *Botrytis* epidemics. Since healthy leaves are normally resistant to conidial infections (12), *B. cinerea* depends on dead tissues for initial entry into the plant. Stimulated by this food base, the inoculum potential of the pathogen increases within the canopy of the single plant to such a level that healthy petioles and leaf blades can then be infected. The resulting gaps in the canopy reduce the ornamental value of the plant or lead to complete plant loss. Such losses are common, especially during the autumn production when environmental factors, such as high humidity, favor the disease.

In the cyclamen system, biocontrol of *B. cinerea* by *U. atrum* can be achieved by competitively excluding the pathogen from

colonizing necrotic leaves present within the cyclamen canopy. This was recently shown by Köhl et al. (7). In their study, repeated applications of conidial suspensions of *U. atrum* controlled the disease as effectively as the grower's standard fungicide program. These experiments were carried out in a commercial greenhouse, where potted plants on tables were subirrigated by capillary matting. No top-irrigation was used.

However, cyclamen are produced in a variety of different growing systems, including those with top-irrigation. The purpose of our study was to investigate the potential of *U. atrum* for *B. cinerea* control in the broad range of commercial growing conditions used in cyclamen production in the Netherlands. It was not intended to compare the effect of different growing systems or cultivars on *B. cinerea* development. Experiments on antagonist survival in top-irrigated crops completed the studies on biocontrol in the various production systems. It was hypothesized that frequent top-irrigation may adversely affect antagonist efficacy since conidia may be washed off leaf surfaces. Furthermore, germinated conidia may become exhausted during frequent wet-dry periods, each possibly not long enough for conidia to germinate and colonize necrotic tissues.

MATERIALS AND METHODS

Inoculum production. Conidia of *U. atrum* isolate 385, originating from a necrotic onion leaf tip (10), were produced on oat grains in autoclavable mushroom spawnbags (Type 3LS; Van Leer Ltd., Poole, Dorset, UK) and conidial suspensions containing 1×10^6 conidia per ml were prepared as described by Köhl et al. (7).

Plants. Cyclamen were seeded in trays with soil plugs and transferred at approximately 10 weeks to individual pots containing commercial potting soil.

Plants were 77 to 168 days old at the beginning of the experiments. At that stage, the oldest leaves were beginning to senesce, and plants were almost free from symptoms of *B. cinerea*. Only plants in greenhouse 3 had exceptionally high numbers of necrotic leaves (approximately 0.5 per plant), and symptoms of *B. cinerea* were found on approximately 25% of the plants. Varieties used in the individual experiments are shown in Table 1.

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Table 1. Effect of treatments of cyclamen with *Ulocladium atrum* or fungicides on *Botrytis cinerea* disease development (AUDPC) and disease manifestation at marketable age^o

Treatment	Disease devel. (AUDPC)		Disease at marketing	
	DI ^p	DS ^q	DI	DS
Greenhouse 1; cv. Laser ^r				
Untreated	nd ^s	nd	41 a ^t	0.8 a
Tween-water	nd	nd	45 a	0.9 a
<i>U. atrum</i> 2x	nd	nd	17 b	0.3 b
Greenhouse 2; cv. Super Serie ^u				
Untreated	830 a	24 a	46 a	1.4 a
Tween-water	851 a	29 a	51 a	1.9 a
Carbendazim/ dithiofencarb, iprodione, iprodione	381 bc	7 bc	19 bc	0.4 bc
<i>U. atrum</i> 2x	643 ab	18 ab	34 ab	1.1 ab
<i>U. atrum</i> 4x	175 c	6 c	11 c	0.3 c
Greenhouse 3; cv. Pastel ^v				
Untreated	9,424 a	279 a	87 a	5.5 ab
Tween-water	9,290 a	295 a	93 a	6.7 a
Iprodione	10,001 a	330 a	96 a	7.4 a
<i>U. atrum</i> 2x	8,032 b	209 b	89 a	4.4 bc
<i>U. atrum</i> 5x	7,365 b	164 b	69 b	3.2 c
Greenhouse 4; cv. Anglia ^w				
Untreated	3,424 a	64 a	52 a	2.0 a
Tween-water	2,974 a	64 a	43 ab	1.5 ab
<i>U. atrum</i> 2x	1,767 b	32 b	29 bc	0.7 b
<i>U. atrum</i> 4x	1,518 b	27 b	17 c	0.4 b
Greenhouse 4; cv. Miracle ^x				
Untreated	1,954 a	42 a	70 a	2.3 a
Tween-water	1,837 a	41 a	71 a	2.1 a
<i>U. atrum</i> 2x	409 b	7 b	21 b	0.4 b
<i>U. atrum</i> 3x	882 b	18 b	26 b	0.6 b
Greenhouse 5; cv. Marvel ^y				
Untreated	3,011 a	92 a	76 ab	3.6 a
Tween-water	2,841 a	89 a	78 a	3.2 a
Tolyfluanid	2,555 a	69 ab	67 ab	2.6 a
<i>U. atrum</i> 2x	1,885 b	49 b	65 ab	2.4 a
<i>U. atrum</i> 3x	1,802 b	44 b	63 b	2.2 a
Greenhouse 5; cv. Sierra ^z				
Untreated	2,409 b	54 b	78 a	2.7 a
Tween-water	3,244 a	73 a	85 a	3.3 a
<i>U. atrum</i> 2x	1,577 c	27 c	51 b	1.2 b
<i>U. atrum</i> 3x	1,816 c	28 c	43 b	0.9 b

^o *U. atrum* was applied as conidial suspension containing 0.01% Tween 80 with 1×10^6 conidia per ml. The water control contained 0.01% Tween 80. No data provided for three experiments in greenhouse 6, where *U. atrum* or fungicides were not effective.

^p Disease incidence (DI) measured as the percentage of plants with *B. cinerea* symptoms.

^q Disease severity (DS) measured as the number of leaves or flower petioles per plant with *B. cinerea* symptoms.

^r Experiment started with 168-day-old plants, and plants were marketable at 224 days. *U. atrum* and Tween-water were applied at plant ages 168 and 196 days (*U. atrum* 2x).

^s Not determined because DI and DS were assessed only three times.

^t Values of the same experiment within one column with a common letter do not differ statistically ($P > 0.05$).

^u Experiment started with 77-day-old plants, and plants were marketable at 189 days. *U. atrum* was applied at plant ages 77 and 105 days (*U. atrum* 2x) or 77, 105, 136, and 163 days (*U. atrum* 4x). Tween-water was applied at ages 77, 105, 136, and 163 days. Carbendazim plus dithiofencarb (as Sumico at 1 g/liter; at 250 g a.i./kg and 250 g a.i./kg, respectively), iprodione (as Rovral at 1 ml/liter; at 500 g a.i./liter), and iprodione were applied in rotation at ages 111, 125, and 153 days, respectively.

^v Experiment started with 133-day-old plants, and plants were marketable at 263 days. *U. atrum* was applied at plant ages 133 and 157 days (*U. atrum* 2x) or 133, 157, 184, 211, and 240 days (*U. atrum* 5x). Tween-water was applied at ages 133, 157, 184, 211, and 240 days. Iprodione (as Rovral with 1 ml/liter; at 500 g a.i./liter) was applied at 149 days.

^w Experiment started with 126-day-old plants, and plants were marketable at 229 days. *U. atrum* was applied at plant ages 126 and 154 days (*U. atrum* 2x) or 126, 154, 182, and 208 days (*U. atrum* 4x). Tween-water was applied at ages 126, 154, 182, and 208 days.

^x Experiment started with 119-day-old plants, and plants were marketable at 196 days. *U. atrum* was applied at plant ages 119 and 147 days (*U. atrum* 2x) or 119, 147, and 175 days (*U. atrum* 3x). Tween-water was applied at ages 119, 147, and 175 days.

^y Experiment started with 140-day-old plants, and plants were marketable at 214 days. *U. atrum* was applied at plant ages 140 and 167 days (*U. atrum* 2x) or 140, 167, and 195 days (*U. atrum* 3x). Tween-water was applied at ages 140, 167, and 195 days. Tolyfluanid (as Euparene M at 1 g/liter; at 500 g a.i./kg) was applied at 147 days.

^z Experiment started with 140-day-old plants, and plants were marketable at 222 days. *U. atrum* was applied at plant ages 140 and 167 days (*U. atrum* 2x) or 140, 167, and 195 days (*U. atrum* 3x). Tween-water was applied at ages 140, 167, and 195 days.

Growing conditions. Ten experiments were carried out in six different commercial greenhouses located in western and central Netherlands. In all greenhouses, the minimum temperature during daytime was set at approximately 14°C and during nighttime at approximately 16°C, as is usual in the Dutch cyclamen production system.

The growing systems in the different greenhouses differed considerably in the arrangement of plants, irrigation system, heating system, and type of material used for pots (Table 2). In all greenhouses, plants were irrigated two to three times per week. During the experiments, plant spacing was increased to avoid touching of leaves of different plants, except in greenhouse 6, where spacing was not increased during the last 50 days of the experiments. Under these conditions, leaves of neighboring plants touched each other and formed a dense canopy layer. Plants with the first flowers open were considered marketable, except for those in greenhouse 3, where the first flowers were removed and plants were left in the greenhouse for an additional 30 days.

Treatments. In most experiments, five treatments were arranged in a randomized complete block design, with each treatment replicated four times. Each replication of a treatment consisted of 24 plants arranged in three or four rows. Between treatments, one untreated row of plants served as a buffer. The treatments consisted of spraying the plants with: (i) tap water containing 0.01% (vol/vol) Tween 80; (ii) the fungicide program as applied by the grower in his commercial growing system (as described in Table 1); (iii) inoculation with conidial suspensions of *U. atrum* twice (at the beginning of the experiment and approximately 28 days later); (iv) inoculation with conidial suspensions of *U. atrum* at intervals of approximately 28 days from the beginning of the experiment until approximately 28 days before plants were marketable; and (v) untreated plants that served as controls. No fungicide treatments were made in greenhouses 1 and 4 and in one of the two experiments in greenhouse 5. In these cases, data of untreated plants that had been planned for fungicide treatments are not presented. In the experiment in greenhouse 1, the two different spraying strategies for the antagonist did not differ since the plants were marketable within 8 weeks after the beginning of the experiment. In this case, data of the *U. atrum* application at 4-week intervals are not presented.

Fungicides applied by different growers were Euparene M (1 g/liter, tolyfluanid at 500 g a.i./kg; Bayer AG, Leverkusen, Germany); Daconil M (1 g/liter, chlorothalonil at 250 g a.i./kg and maneb at 500 g a.i./kg; AgrEvo, Frankfurt, Germany); Rovral (1 ml/liter, iprodione at 500 g a.i./liter; Rhone-Poulenc Agrochimie,

Lyon, France); or Sumico (1 g/liter, carbendazim at 250 g a.i./kg and diethofencarb at 250 g a.i./kg; AgrEvo).

Application dates for experiments in greenhouses 1 to 5 are given in Table 1. In greenhouse 6, three experiments were carried out, each with a different cultivar. Plants were 126 days old at the beginning of the experiment and were marketable within 254 days. *U. atrum* was applied at plant ages of 126 and 150 days or at 126, 150, 177, 206, and 233 days. Tween-water was applied at plant ages of 126, 150, 177, 206, and 233 days. Chlorothalonil and mancozeb (as Daconil M at 1 g/liter) were applied at plant ages of 143 and 168 days, and iprodione (as Rovral at 1 g/liter) was applied at 190 and 240 days.

Application technique. Water and antagonist suspensions were applied with a propane-operated sprayer (AZO, Ede, the Netherlands) at 250 kPa. Fungicides were applied by growers using motor-operated sprayers. During spraying, frames with polyethylene were placed around each treatment to prevent drift to neighboring plants. The canopies of individual plants were sprayed until runoff. Later, when the canopy of plants became very dense, the spray nozzle was also placed inside the canopy to reach all surfaces of leaves, petioles, and the tuber with the suspension. Growers also use this technique for fungicide applications.

Assessments. In each experiment, disease was assessed on days when *U. atrum* was applied, and a final assessment was made approximately 28 days after the last treatment, when plants were marketable. The numbers of leaves or flower petioles with sporulation of *B. cinerea* were recorded for each plant. From these data, the percentage of plants with disease symptoms of *B. cinerea* (disease incidence, DI) and the number of diseased leaves and flower petioles per plant (disease severity, DS) were calculated.

Survival of *U. atrum* conidia on top-irrigated green leaves of cyclamen plants and competitive colonization of senesced leaves by *B. cinerea* and *U. atrum*. Fifty 20-week-old potted cyclamen plants (cv. Super Serie) were grown in a greenhouse at $20 \pm 3^\circ\text{C}$ and $65 \pm 20\%$ relative humidity (RH). Pots were placed on a table on capillary mats. Petioles of fully developed leaves were labeled with colored, plastic-

coated iron rings. Water was applied by top-irrigation using a sprinkler. Plants were irrigated at approximately 1 liter/m² at intervals of 2 to 3 days. Experimental plots consisted of five plants, each sprayed with a conidial suspension of *U. atrum* (prepared as described above) or tap water containing 0.01% Tween 80. The experiment was arranged in a randomized complete block design, with each treatment replicated five times and the experiment repeated once.

Three labeled leaves per replicate were sampled at day 0, 2, 4, 7, 10, 14, 21, 28, 42, 56, and 70 after spraying. Conidial density and conidial germination of *U. atrum* directly after sampling and after additional incubation in a moist chamber at 24°C for 8 h were determined on one leaf per replicate at each of the sampling dates as described by Köhl et al. (7). The other two leaves sampled per replicate at each of the sampling dates were placed in open petri dishes on two dry filter papers. Petri dishes were arranged in blocks in the greenhouse as previously described for plants but were not irrigated. Forty-two days after sampling, when all these leaves had senesced, 0.75 ml of sterile tap water was added to the filter paper in each petri dish, and the closed petri dishes were transferred to an incubator. Leaves were incubated for 14 days in the dark at 18°C . Thereafter, the coverage of the leaf area with conidiophores of *B. cinerea* was estimated as described by Köhl et al. (7).

Statistics. Linear logistic regression was employed to explain the percentage of DI in response to the classifying variable: replicate and treatment. Log-linear regression models with the same classifying variables were fitted to the data on the number of diseased leaves and flower petioles per plant (DS). Overall effects of replicate and treatment were assessed using *F* tests for the ratio of the mean deviance for the particular effect and the mean residual deviance. In cases where *F* tests were significant ($P < 0.05$), treatment means on the logit or log-scale were separated by two-sided *t* tests ($P < 0.05$), corresponding to least significant difference (LSD) tests for normally distributed data.

Area under disease progress curves (AUDPC) for DI and DS were analyzed by ANOVA. ANOVA was also employed on angular-transformed data on sporulation of

B. cinerea on dead leaves of the experiment on the survival of *U. atrum* inoculum. Significant *F* tests ($P < 0.05$) were followed by two-sided LSD tests ($P < 0.05$) for testing pairwise differences between treatment means.

All analyses were performed with the Genstat 5 program (Numerical Algorithms Group Inc., Oxford, UK).

RESULTS

Disease control. Gray mold occurred in all 10 experiments. DI at marketing ranged between 41 and 87% in the untreated controls for experiments carried out in greenhouses 1 to 5 (Table 1), and was above 90% in the three experiments carried out in greenhouse 6 (data not shown). The experiments were not designed to compare the effect of different growing systems or cultivars on *B. cinerea* development; therefore, no conclusions can be drawn about the reasons for such a variable level of disease between experiments.

When *U. atrum* was applied at 4-week intervals, DI at marketing was significantly lower than in the water controls in seven of the 10 experiments. In the three experiments carried out in greenhouse 6, five *U. atrum* applications at 4-week intervals showed no effect. DS at marketing ranged between 0.8 and 11.0 in the untreated controls in the 10 experiments, with exceptionally high values above 7.0 in greenhouse 6. A significant reduction in DS after four weekly applications of *U. atrum* was found for five experiments. Again, there was no effect by *U. atrum* in greenhouse 6. The application of *U. atrum* twice at the beginning of the experiment was less effective on DI and DS at marketing when compared with applications at 4-week intervals when crops were marketed more than 60 days after the second treatment (greenhouses 2, 3, 4, and 6). Significant effects of *U. atrum* applications at 4-week intervals were found on the disease progression as expressed by AUDPC for both DI and DS in five of the nine experiments where AUDPC could be determined. The significant effects of only two applications of *U. atrum* at the beginning of the experiments were found more often on disease development (AUDPC) than on DI and DS at marketing.

Fungicide applications reduced DI and DS at marketing and AUDPCs signifi-

Table 2. Growing systems in greenhouses 1 to 6

Greenhouse no.	Pot	Plant arrangement	Irrigation	Heating
1	Clay	Concrete floor	Ebb and flow	Additional tubes 30 cm above plants
2	Plastic	Tables	Capillary matting	
3	Plastic	Tables; from plant age 150 days on gutters	Top-irrigation; from plant age 150 days intermittent flow irrigation	
4	Plastic	Ground on polyethylene foil; from plant age 190 days on gutters	Top-irrigation; from plant age 190 days intermittent flow irrigation	Additional tubes 30 cm above plants
5	Plastic	Tables	Capillary matting plus top-irrigation	
6 ^a	Clay	Tables	Capillary matting plus top-irrigation	

^a Data of experiments not presented in Table 1.

Fig. 1. Density of conidia of *Ulocladium atrum* on green leaves of cyclamen plants (cv. Super Serie) under greenhouse conditions with top-irrigation three times per week (□ = experiment 1; ■ = experiment 2). Leaves were sprayed with a conidial suspension (1×10^6 conidia per ml) at day 0. Means $\pm$ standard errors of the mean of five leaves (replicates) per assessment date.

Fig. 2. Germination of conidia of *Ulocladium atrum* on green leaves of cyclamen plants (cv. Super Serie) under greenhouse conditions with top-irrigation three times per week. Leaves were sprayed with a conidial suspension (1×10^6 conidia per ml) at day 0. Germination was assessed directly after sampling (Δ = experiment 1; $\blacktriangle$ = experiment 2) or after incubation in moist chamber at 24°C for 8 h (□ = experiment 1; ■ = experiment 2). Means $\pm$ standard errors of the mean for 100 conidia on each of five leaves (replicates) per assessment date.

cantly in greenhouse 2, but not in greenhouses 3, 5, and 6. In no case did fungicide applications give better control than *U. atrum* applied at 4-week intervals.

Survival of *U. atrum* conidia on cyclamen leaves. Densities of *U. atrum* conidia on green cyclamen leaves were 6,900 and 6,700 conidia per cm^2 at the beginning of experiments 1 and 2, respectively. Densities decreased to 1,700 and 3,500 conidia per cm^2 during the first 10 days of the two experiments and thereafter remained relatively stable (Fig. 1; data presented as Log10 numbers). At the end of both experiments, approximately 3,000

conidia per cm^2 were found. On untreated leaves, densities were below 1 conidium per cm^2 for all sampling dates.

The percentage of conidia germinated on the green leaves under greenhouse conditions increased during both experiments to a level of approximately 50% (Fig. 2). After an additional incubation of the sampled leaves in a moist chamber, the percentage of germinated conidia increased to approximately 75% for most sampling dates with no reduction in percentages at the end of the experiment. Formation of new conidia of *U. atrum* on the leaf surfaces was not observed.

Competitive colonization of dead leaves by *B. cinerea* and *U. atrum*. In both experiments, for all sampling dates, dead leaves were naturally contaminated in the greenhouse with *B. cinerea*, resulting in a leaf coverage with sporulating *B. cinerea* after incubation in a moist chamber ranging from 17.5 to 59.5% on leaves not treated with *U. atrum* (Fig. 3A). Significantly less ($P < 0.05$) *B. cinerea* sporulation was found on *U. atrum*-treated leaves for all sampling dates, except for leaves sampled 70 days after *U. atrum* application in experiment 1. The efficacy of the *U. atrum* treatment was highest on leaves sampled at the beginning of the experiment, with 100 and 97% for experiments 1 and 2, respectively (Fig. 3B). Thereafter, efficacy ranged between 51 and 96%, except for the last sampling date of experiment 1, where efficacy was only 29%.

DISCUSSION

In an earlier study, *U. atrum* showed significant potential to control *B. cinerea* in commercially grown cyclamen (7). These results were all obtained in the same greenhouse, where no top-irrigation was applied. Results presented in our study confirmed these findings but add essential novel information on the potential of the antagonist in a variety of cyclamen growing systems.

At the beginning of the study, it was hypothesized that *U. atrum* may be less effective in situations where top-irrigation is applied, since conidia may be washed off. Furthermore, top-irrigation, especially in combination with capillary matting, results in a higher relative humidity within the canopy and thus may favor *B. cinerea* (4). On the other hand, during periods with low relative humidity in the greenhouse, top-irrigation will result in leaf wetness periods of only a few hours, allowing conidia of *U. atrum* to germinate. Under these alternating conditions, such germinated conidia may become exhausted after several wet-dry cycles because of insufficient nutrient supply and thus be lost.

The results of the experiments in the different commercial greenhouses show that *U. atrum* controls *B. cinerea* when sprayed at 4-week intervals, except when the disease pressure of *B. cinerea* was extremely high, as in greenhouse 6. Under such conditions, even fungicide applications did not control the disease.

No indications were found that top-irrigation, as applied in greenhouses 3, 4, 5, and 6, affected the efficacy of *U. atrum* applications. This finding is in line with the results obtained in experiments where the survival of *U. atrum* conidia in the cyclamen canopy was monitored in a growing system with top-irrigation. Under these conditions, a significant part of the conidia had the ability to attach to green leaves of cyclamen and to survive. An-

Fig. 3. Effect of an *Ulocladium atrum* treatment on sporulation of *Botrytis cinerea* on dead cyclamen leaves. Green leaves of cyclamen plants were sprayed with conidial suspension of *U. atrum* (1×10^6 conidia per ml) at day 0 and kept under greenhouse conditions with top-irrigation three times per week. Leaves were detached from plants at the indicated sampling date, allowed to senesce during 42 days in the greenhouse (not irrigated) and exposed to natural deposition of *B. cinerea* conidia, and subsequently incubated in a moist chamber (14 days, 18°C). (A) Percent leaf area with *B. cinerea* sporulation on dead cyclamen leaves treated with *U. atrum* (Δ = experiment 1; $\blacktriangle$ = experiment 2) or with Tween-water ($\square$ = experiment 1; $\blacksquare$ = experiment 2). The two treatments differed significantly ($P < 0.05$) at all assessment dates according to LSD, except for the last sampling date for experiment 1. (B) Efficacy of *U. atrum* treatment in *B. cinerea* control ($\square$ = experiment 1; $\blacksquare$ = experiment 2).

tagonistic activity of *U. atrum* propagules against *B. cinerea* was found in necrotic tissue when conidia had been sprayed onto green leaves 10 weeks before necrosis was induced by removing leaves from plants. Given that leaves were allowed to senesce slowly under dry conditions during the 6 weeks after removal from the plant, *U. atrum* retained its antagonistic potential for a total of 16 weeks after application. These findings on the persistence of *U. atrum* propagules confirm results obtained in

lilies in the open field. In those experiments, antagonistic activity of *U. atrum* was also found at the end of the experiment, 3 weeks after application of the antagonist (3).

Further research on *U. atrum* applications in cyclamen will concentrate on the optimization of spraying intervals and concentrations. This information may help to develop an economically feasible biological control based on *U. atrum*. Furthermore, information on the compatibility

of *U. atrum* with fungicides is needed. The combined application of the antagonist with compatible fungicides may further improve disease control. With such an integrated approach, disease control may also be possible in situations with a high disease pressure (as in greenhouse 6) or when *B. cinerea* already is established in the canopy before the first applications are carried out (as in greenhouse 3).

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