Effect of Water Potential on Conidial Germination and Antagonism of *Ulocladium atrum* Against *Botrytis cinerea*

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ABSTRACT

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The saprophytic fungus *Ulocladium atrum* was selected for its ability to competitively exclude *Botrytis* spp. from aboveground necrotic plant tissues which can play a crucial role in the epidemiology of diseases caused by necrotrophic *Botrytis* spp. Fungal growth in necrotic aboveground tissues can be hampered by fluctuating water availability. Adaptation to these adverse conditions is a key factor for the successful establishment of an antagonist population in this niche. Conidia of *U. atrum* germinated at water potentials between -1 and -7 MPa on water

agar. Germinated conidia survived one to three interruptions of moist incubation by periods at -10 or -42 MPa. The speed of germination was significantly slower with interruption of the moist period as compared with the control, which had continuously moist incubation. However, the maximum germination percentage was the same for conidia incubated with or without interruption of the moist incubation. In bioassays with necrotic cyclamen leaves at -1 , -3 , and -7 MPa, *U. atrum* significantly reduced the sporulation of *B. cinerea* by more than 80%. The results demonstrate that *U. atrum* tolerates water stress during competitive substrate colonization with *B. cinerea*. The antagonist is, therefore, an attractive candidate for field applications on aboveground tissues.

The saprophytic fungus *Ulocladium atrum* Preuss was selected as an antagonist against *Botrytis* spp. (17,18). It competes with the pathogen in its saprophytic stage in necrotic aboveground plant tissues. Such necrotic tissues can play a crucial role in *Botrytis* epidemics, where they serve as substrate for conidia production (e.g., dead strawberry leaves [4]), or as a bridge allowing the pathogen to attack neighboring healthy tissue (e.g., dead leaves in cyclamen plants [14] and necrotic flower parts in strawberry and grapes [12,28]). *U. atrum* has been used successfully for biological control of *B. cinerea* under field conditions in grapes (26) and strawberry (13) as well as in greenhouse crops such as cyclamen (14) and tomato (10).

As the niche for the antagonistic interaction, aboveground plant tissues are characterized by variable microclimatic conditions, often adverse for fungal growth. Water availability and temperature can change very rapidly. Data obtained during field experiments during several summers in the Netherlands showed that wetness periods of necrotic leaf tissues frequently occur, but that the duration of wetness periods can vary between a few minutes and more than 24 h. Wetness periods had an average duration of 4.9 h measured in onion as an open crop and 9.7 h in lilies as a dense crop (16). To obtain reliable biological control, antagonists must be able to survive and to compete with the pathogen under these conditions.

The antagonist *U. atrum* is effective over a broad range of temperatures (16) and antagonism was found in bioassays conducted with interrupted leaf wetness periods (18). Little information is available in the literature about the fate of germlings of antagonistic fungi in the phyllosphere. The objective of the study was to determine the effect of constant and alternating water potentials on conidia of *U. atrum* during germination. Experiments were

carried out under conditions leading to equilibration of the water potential in the growth substrate and the surrounding atmosphere. More complex situations, in which the water potentials of the substrate and the surrounding atmosphere differ (e.g., when a dry necrotic leaf is exposed to a sudden increase of the relative humidity (RH) of the surrounding air), were not considered.

This information will help to better understand the dynamics of antagonist populations under field conditions, which is a prerequisite for the development of reliable application strategies.

MATERIALS AND METHODS

Conidial suspensions. *U. atrum* (isolate number 385), isolated from necrotic leaf tips of onions (18), and *Botrytis cinerea* Pers.:Fr. (isolate number 700), isolated from a gerbera flower, were cultured in petri dishes (90 mm) containing 25 ml of oatmeal agar (20 g of oatmeal, 15 g of agar, 1,000 ml of tap water) for 28 and 14 days, respectively, at 18°C in the dark. To obtain conidial suspensions, cultures were flooded with sterile tap water containing 0.01% (vol/vol) Tween 80. After gently rubbing with a rubber spatula, the resulting suspension was filtered through sterile nylon gauze with a mesh of 200 μm . Concentrations of the conidial suspensions were determined with the aid of a hemacytometer and adjusted with sterile tap water containing 0.01% Tween 80 to 5×10^5 conidia per ml. Flasks containing the conidial suspensions were kept in ice water and suspensions were used within 1 h.

Conidial germination at different constant and alternating water potentials. Glass slides were covered with a thin layer of water agar (15 g of agar, 1 liter of tap water) as described by Fokkema (9) and dried in a sterile laminar flow cabinet. Slides were sprayed with a conidial suspension of *U. atrum* (5×10^5 conidia per ml; approximately 800 conidia per cm^2) and left to dry in the sterile flow cabinet for 30 min. Subsequently, each agar slide was transferred to a moist chamber, consisting of a petri dish (90 mm) containing 15 ml of demineralized water or mixtures of demineralized water and glycerol (6) to obtain, at equilibrium,

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water potentials in the agar layer of -0.28, -1, -3, -5, -7, -10, or -14 MPa, corresponding to RHs of 99.8, 99.3, 97.9, 96.4, 95.0, 92.9, and 90.0%. Glycerol-water solutions were freshly prepared for each experiment. A lid of a smaller petri dish (60 mm) had been fixed in the center of the petri dish to support the agar slide so that there was no contact between the water-glycerol mixture and the agar layer. Immediately after transfer of the agar slides to the moist chambers, the latter were sealed with Parafilm and incubated at $18 \pm 0.5^\circ\text{C}$ in the dark. After the incubation period, fungal growth in the sampled dishes was stopped immediately by adding 1 ml of household ammonia (4.8% [wt/wt] NH_4OH) onto a filter paper (40 mm) placed into the small petri dish and closing the petri dish again. Agar slides were stored at 5°C until microscopic examination.

Experiments were carried out with constant or alternating water potentials. In the experiment with incubation at constant water potentials, agar slides were incubated in petri dishes containing water-glycerol mixtures as described above. Incubation periods were, based on results of preliminary experiments, 4, 6, 8, 12, 16, 24, 36, 48, and 72 h for each water potential, except at -0.28 MPa, where the longest incubation period was 24 h, and at -10 and -14 MPa, where incubation periods were 24, 48, and 72 h. For each incubation period at each water potential, three arbitrarily chosen agar slides were sampled. The experiment was repeated.

For experiments with alternating water potentials, agar slides were inoculated and incubated in petri dishes containing water-glycerol mixtures, as described above. Water potentials of the agar layer were changed by rapidly transferring agar slides to petri dishes containing the appropriate glycerol-water mixtures. Conidia on agar slides were incubated under conditions of high water availability at -1 MPa (equivalent to 99.3% RH) which were interrupted by one period of low water availability at -10 MPa (equivalent to 92.9% RH). Agar slides were incubated first in petri dishes at -1 MPa for 0, 2, 4, 6, or 8 h and subsequently at -10 MPa for 24, 22, 20, 18, or 16 h, respectively. Thereafter, agar slides were transferred again to petri dishes containing glycerol-water mixtures at -1 MPa. In a similar experiment, agar slides were incubated at -42 MPa (equivalent to 70% RH) during periods of low water availability. Three arbitrarily chosen agar slides were sampled for each treatment (duration of incubation before period of low water availability started) after a total incubation time at high water availability (-1 MPa) of 0, 2, 4, 6, 8, 10, 12, 24, or 32 h. Additionally, three agar slides of each treatment were always sampled immediately after incubation periods at low water availability. The experiment was repeated for both water potential levels during periods at low water availability.

In another experiment, conidia on agar slides were incubated at high water availability (-1 MPa) interrupted by two or three periods of low water availability (-42 MPa). The incubation period at -1 MPa lasted 6 h, followed by a period of 18 h at -42 MPa. These cycles were repeated two or three times and were then followed by continuous incubation at -1 MPa. Conidia incubated continuously under moist conditions served as the control. For each of the three treatments, three sets of agar slides were incubated, each prepared with a suspension of *U. atrum* conidia obtained from a separate petri dish. Such sets of petri dishes were allocated to three blocks within the incubator. Agar slides prepared for the different sampling times were arranged randomly within each block. One agar slide per block of each for the treatments was sampled after 0, 2, 4, 6, 8, 10, 12, and 24 h of incubation at -1 MPa. The next samples were taken after 72 h for the control treatment; after 36 and 84 h for the treatment with two dry periods; and after 30, 42, and 90 h for the treatment with three dry periods. Additionally, three agar slides of each treatment were always sampled immediately after incubation periods at low water availability. The experiment was repeated.

Germination was assessed from 100 arbitrarily chosen conidia per slide stained with cotton blue in lactic acid (2 ml of lactic

acid, 4 ml of glycerol, 2 ml of distilled water, 15 mg of cotton blue) using a microscope ($\times 200$; Axioscope, Zeiss, Oberkochen, Germany). Conidia with germ tubes longer than half of the minimum diameter of a conidium were considered to have germinated. For several treatments, the number of germ tubes of 50 arbitrarily chosen conidia was counted and the length of each germ tube was measured using an interactive digitizer (Minimop, Kontron, Oberkochen, Germany). When more than one germ tube was present the longest was defined as the first and the next longest as the second germ tube.

Antagonism on cyclamen leaves at different water-potentials. Symptomless green leaves were removed from cyclamen plants (approximately 30 weeks old), placed on dry filter paper, and allowed to senesce for 6 weeks at ambient temperature and 50 to 70% RH. Leaves were rehydrated and cut into pieces of approximately 4 by 4 mm which were dried between dry filter paper in a plant press to obtain flattened leaf pieces. One hundred dry leaf pieces were weighed, sealed in plastic bags and gamma irradiated (4 Mrad). Fourteen days before the beginning of experiments, sterile water was added to the plastic bag with the leaf pieces and the bags were sealed again and stored for 1 week to allow equilibration of leaf piece water content. To obtain water potentials of approximately -1, -3, and -7 MPa, 1.5, 0.5, or 0.25 ml of sterile tap water per gram of leaf pieces (dry weight) was added, respectively, according to the relationship between water content and water potential available for dead onion leaves (18).

Moist chambers were prepared consisting of sterile plastic petri dishes (140 mm) containing 75 ml of water agar with KCl added to obtain a water potential of the agar of -1, -3, and -7 MPa (5). A sterile glass slide (76 by 26 mm) was placed on the agar and separated from the agar by a sterile plastic grid to avoid diffusion of KCl onto the slide surface. After incubation for 7 days at 20°C in wetted plastic bags, leaf pieces were transferred into moist chambers with the corresponding water potential. Ten leaf pieces were placed on the glass slide in each moist chamber, sealed with parafilm, and stored for 7 days at 18°C to allow equilibration of the water potentials and obtain a precisely controlled water potential in the leaves (15).

On another set of sterile glass slides, conidial suspensions of *B. cinerea* (1×10^5 and 1×10^6 conidia per ml) or *U. atrum* (1×10^6 conidia per ml) were sprayed at a rate of approximately $1 \mu\text{l}/\text{cm}^2$ and subsequently allowed to dry in a sterile laminar flow cabinet. The five treatments consisted of spraying each of the three conidial suspensions alone and spraying the conidial suspension of the antagonist followed by spraying conidial suspensions of *B. cinerea* at the two different concentrations. In this case, the second spray was carried out after the first suspensions had been allowed to dry on the slide. The 10 leaf pieces per moist chamber at the different water potentials were transferred rapidly, using sterile forceps, onto glass slides containing dried conidia of the different fungi. To ensure a relatively stable water potential in the leaf pieces during this operation, this was carried out in a glass box (30 by 30 by 48 cm) with wet filter paper fixed to the walls. Slides with leaf pieces were immediately placed back into the moist chambers and moist chambers were sealed with parafilm. Leaf pieces were then incubated at $18 \pm 0.5^\circ\text{C}$ in the dark. Each of the five fungal treatments was carried out for each of the three water potentials on three glass slides in separate moist chambers (replicates). After incubation at 18°C in the dark for 14 and 28 days, fungal sporulation on the leaf pieces was assessed using sporulation classes, and a sporulation index (0 to 100) was calculated (14). The experiment was repeated.

Statistics. The data on conidial germination over time were analyzed by fitting the Gompertz curve, an asymmetric logistic curve: $Y = C \times \exp\{-\exp(-B \times [X - M])\}$, where Y = germination percentage; X = total incubation period in hours at constant water potentials for experiments with continuous incubation at different

water potentials or moist condition (–1 MPa) for experiments with varying water potentials; and C , B , and M = the parameters to be estimated. In the curve, C = upper asymptote (maximum germinated conidia), B = rate of increase in percentage of germinated conidia (slope parameter), and M = point of inflection (time needed to achieve 37% of the maximum germination percentage). Continuous incubation at –10 and –14 MPa did not result in germination; therefore, these data were excluded from analyses. During the fitting process, the lower asymptotes were forced to zero as the minimum level of germination percentage. Using the estimates of the parameters in the curve, the expected germination percentage can be calculated.

Curves were fitted to the data for the repeated experiments with constant water potentials, with one interruption of the incubation under moist conditions (–1 MPa) by a dry period (–10 or –42 MPa) and with multiple interruptions of the moist period. A separate curve was fitted for each treatment of each block (if relevant) in each experiment. The parameters of the fitted Gompertz curves were then analyzed using the original structure of the experimental units (21). Due to the different experimental designs, experiments with continuous incubation under moist conditions or with one interruption of the incubation under moist conditions (–1 MPa) were analyzed differently from experiments with multiple interruptions of moist periods. The different constant water potentials and the duration of the initial dry period have quantitative treatment levels; therefore, the curve parameters C , B , and M were analyzed using linear regression. The experiments with multiple interruptions of the moist period resulted in a total of 18 curves. The treatment effect on the curve parameters C , B , and M , was tested in an analysis of variance, taking the blocks and different experiments into account. Significant F tests ($P < 0.05$) were followed by least significant difference (LSD) tests ($\alpha = 0.05$) for testing pairwise differences between treatment means.

Angular transformed data of the repeated experiment on antagonism of *U. atrum* toward *B. cinerea* at different water potentials were combined and analyzed by analysis of variance (ANOVA) followed by LSD tests ($\alpha = 0.05$) for testing pairwise differences between treatment means.

RESULTS

Conidial germination at constant water potentials. Conidial germination of *U. atrum* was observed between –0.28 and –7 MPa (Fig. 1), but no germination was observed within 72 h at –10 and –14 MPa. Gompertz curves fitted to the data had values for an

Fig. 1. Estimated germination percentage of conidia of *Ulocladium atrum* on water agar slides at different water potentials (MPa). Gompertz curves were fitted to data obtained from repeated experiments. Adjusted R^2 for the different treatments ranged between 0.76 and 0.98.

adjusted R^2 (given as proportion) ranging between 0.76 and 0.98. The initial germination process was slower with decreasing water potential. The point of inflection M , estimating the time needed to reach 37% of the maximum germination percentage, significantly ($P < 0.001$) increased with decreasing water potential (Table 1). Conidia needed 6.6 h at –0.28 MPa to reach this point but 24 h at –7 MPa. The speed of germination, as represented by the slope parameter B , was affected and significantly ($P = 0.003$) decreased with decreasing water potential. The estimated maximum germination percentage (upper asymptote C) was high for conidia incubated at –0.28 with 91% but significantly ($P < 0.001$) decreased with decreasing water potential. At –7 MPa, less than half of the maximum germination percentages were found as compared to –0.28.

Conidial germination at alternating water potentials. In the first set of experiments, conidia on agar slides were incubated in a moist environment (–1 MPa) and this moist incubation was interrupted once by a period of dry conditions (–10 or –42 MPa) at different times during the initial stage of germination between 0 and 8 h. Gompertz curves fitted to the data had adjusted R^2 ranging between 0.91 and 0.99 for experiments with dry periods at –10 MPa and between 0.90 and 0.99 for experiments with dry periods at –42 MPa (Figs. 2 and 3). An exception was the treatment in which the moist incubation of conidia was interrupted after 8 h with a dry period at –42 MPa (Fig. 3), for which no

TABLE 1. Parameter estimates of Gompertz curves fitted to the germination percentage of conidia of *Ulocladium atrum* incubated on agar slides at different water potentials (MPa)

Water potential	Gompertz curves parameters ^y		
	M	B	C
–0.28	6.6	0.78	91
–1	7.0	0.57	87
–3	8.5	0.60	74
–5	11.2	0.29	63
–7	24.0	0.30	41
Analysis ^z			
Slope	–2.318 (± 0.430)	0.069 (± 0.016)	7.200 (± 1.210)
t probability	<0.001	0.003	<0.001

^y Means of two experiments; M = point of inflection, B = slope parameter, and C = upper asymptote.

^z Linear regression analysis between Gompertz curve parameters and water potential; slope parameter ($\pm$ standard error).

Fig. 2. Estimated germination percentage of conidia of *Ulocladium atrum* incubated on agar slides under moist conditions (–1 MPa) interrupted by a dry period at –10 MPa during the initial stage of germination. Initial period of moist incubation was 0 (solid line), 2 (dashed line), 4 ($\square$), 6 ($\circ$), or 8 h ($\bullet$). Sum of hours of initial moist period and dry period was 24 h. Gompertz curves were fitted to data obtained from repeated experiments. Adjusted R^2 for the different treatments ranged between 0.91 and 0.99.

Gompertz curve was fitted. For this treatment, a lag time of approximately 4 h was observed for the continuation of the germination process after the dry period.

Values for germination percentages determined at the beginning of a dry period (–10 or –42 MPa) and at the end of a dry period (data not shown) did not differ significantly, indicating that the germination process did not continue during dry periods. In the experiment with dry periods at –10 MPa, no statistically significant effects were found attributable to the increase of the duration of the initial moist incubation on the estimated maximum level for the germination percentage (*C*), ranging between 85 and 91%; the slope parameter (*B*); and the point of inflection (*M*) (Table 2). In the experiments with a dry period at –42 MPa, similar results were obtained for parameter *C*, which ranged between 88 and 91% (Table 3). Values of the point of inflection (*M*) significantly ($P < 0.001$) increased and the slope parameter (*B*) significantly ($P = 0.021$) decreased with increasing duration of initial moist incubation. In the treatment with continuously moist conditions, conidia needed 8.1 h to reach 37% of the estimated maximum germination percentage. With a dry period, these periods were 1.3 to 4.3 h longer.

Fig. 3. Estimated germination percentage of conidia of *Ulocladium atrum* incubated on agar slides under moist conditions (–1 MPa) interrupted by a dry period at –42 MPa during the initial stage of germination. Initial period of moist incubation was 0 (solid line), 2 (dashed line), 4 (□) or 6 h (○). Sum of hours of initial moist period and dry period was 24 h. Gompertz curves were fitted to data obtained from repeated experiments, except for treatment with initial period of moist incubation of 8 h, for which means of observed germination percentage are presented (●). Adjusted R^2 for the different treatments ranged between 0.90 and 0.99.

TABLE 2. Parameter estimates of Gompertz curves fitted to the germination percentage of conidia of *Ulocladium atrum* incubated on agar slides under moist conditions (–1 MPa) interrupted by a dry period at –10 MPa during the initial stage of germination

Duration (h) ^y	Gompertz curves parameters ^x		
	<i>M</i>	<i>B</i>	<i>C</i>
0	5.3	0.56	91
2	6.9	1.06	85
4	7.6	0.85	87
6	7.2	0.33	90
8	6.5	0.79	87
Analysis ^z			
Slope	0.225 (±0.125)	–0.013 (±0.058)	–0.114 (±0.474)
<i>t</i> probability	0.111	0.834	0.816

^x Means of two experiments; *M* = point of inflection, *B* = slope parameter, and *C* = upper asymptote.

^y Duration of initial moist period; sum of moist plus dry period was 24 h.

^z Linear regression analysis between Gompertz curve parameters and duration of initial moist incubation; slope parameter (±standard error).

For the control treatment without interruption of moist incubation and the treatment with a dry period (–42 MPa) after 8 h of incubation under moist conditions, the number of germ tubes and their length was determined after 8 h and after the maximum germination percentage had been reached after 24 h for conidia incubated without interruption of the moist period and after 32 h for conidia incubated with one interruption of the moist period. The average number of germ tubes per germinated conidium was 1.1 after 8 h and increased to 1.5 after 24 h when conidia were incubated under continuously moist conditions. When a dry period started after 8 h of incubation under moist conditions, the number of germ tubes was 1.3. After a total of 32 h of moist incubation, 1.3 germ tubes were observed per germinated conidium. The majority of germ tubes were shorter than 50 µm after an incubation period of 8 h for both treatments. When conidia of both treatments had been incubated for 24 or 32 h, respectively, under moist conditions, the majority of the first germ tubes was longer than 250 µm and the distribution of germ tube lengths had the same pattern for both treatments (data not shown). Also, the distribution of the length of second germ tubes had the same pattern for both treatments.

In another repeated experiment, conidia on agar slides were incubated in a moist environment (–1 MPa) and this moist incubation was interrupted two or three times by a period of dry conditions (–42 MPa). The Gompertz curves fitted well to the data with an adjusted R^2 ranging between 0.82 and 1.00 (Fig. 4). Values for germination percentages determined at the beginning of each dry period (–42 MPa) and at the end of the dry period (data not shown) did not differ significantly, indicating that the germination process did not continue during dry periods. The estimated maximum levels for percentage germination (*C*) tended to be lower, with 71 and 77% for conidia which were incubated with two or three dry periods (–42 MPa) as compared with the control treatment with continuously moist incubation (87%) (Table 4). However, these differences were not significant ($P = 0.103$). The slope parameters (*B*) and the point of inflection (*M*) for the control treatment differed statistically significantly from treatments with two or three dry periods, showing that conidial germination was slower after multiple interruptions of the germination process. It can be calculated from the fitted curves that a germination percentage of 50% was reached under continuously moist incubation after 8.7 h. When moist conditions were interrupted two or three times, 16.2 and 21.1 h of moist incubation were needed, respectively. The germination process was thus retarded by approximately 4 h per interruption of the moist period.

In this experiment, germinated conidia had 1.6 germ tubes when maximum germination was reached after 24 h for conidia incubated under continuously moist conditions. For conidia incubated

TABLE 3. Parameter estimates of Gompertz curves fitted to the germination percentage of conidia of *Ulocladium atrum* incubated on agar slides under moist conditions (–1 MPa) interrupted by a dry period at –42 MPa during the initial stage of germination

Duration (h) ^y	Gompertz curves parameters ^x		
	<i>M</i>	<i>B</i>	<i>C</i>
0	8.1	0.71	88
2	9.4	0.54	90
4	10.4	0.44	91
6	12.4	0.38	88
Analysis ^z			
Slope	0.702 (±0.105)	–0.054 (±0.017)	0.129 (±0.451)
<i>t</i> probability	<0.001	0.021	0.785

^x Means of two experiments; *M* = point of inflection, *B* = slope parameter, and *C* = upper asymptote.

^y Duration of initial moist period; sum of moist plus dry period was 24 h.

^z Linear regression analysis between Gompertz curve parameters and duration of initial moist incubation; Slope parameter (±standard error).

with three moist–dry cycles, the number of germ tubes and germ tube length was assessed immediately after the last cycle (after a total of 18 h of moist incubation) and after a total of 42 h of moist incubation. Germinated conidia had 1.2 and 1.4 germ tubes after 18 and 42 h of total moist incubation. The germ tube length for germinated conidia after a total of 18 h of moist incubation was below 50 μm for the majority of the conidia. After a total of 42 h of moist incubation, germ tubes length was $>250 \mu\text{m}$ for the majority of the conidia.

Antagonism on cyclamen leaves at different water-potentials. The sporulation intensity of *B. cinerea* decreased with water potential but sporulation was also found on leaves treated with the higher dose of *B. cinerea* and incubated at -7 MPa (Table 5). Significant interactions between treatment and water potential occurred. The sporulation of *B. cinerea* was significantly reduced by *U. atrum* at all water potentials, except for leaves inoculated with the low dose of *B. cinerea* and incubated at -7 MPa , which showed only sparse sporulation of the pathogen. Similar results were obtained in both replicate experiments.

DISCUSSION

Little information is available in the literature about the fate of germings of saprophytic fungi in the phyllosphere. More information is published on leaf pathogens which are exposed to periods of low water availability. However, in contrast to saprophytes, once the moist period is long enough for infection, pathogens may escape such adverse microclimatic conditions by penetrating host tissue. Bashi and Rotem (2) showed that pathogens developed different survival strategies under semi-arid conditions. *Phytophthora infestans* rapidly penetrates host leaves to escape from dry conditions; *Stemphylium botryosum* f. sp. *lycopersici* produces drought-resistant germ tubes. High drought resistance of germings is also reported for other fungal leaf pathogens. The majority of germinated conidia of *Venturia inaequalis* survived on apple leaves during dry periods, but survival was reduced compared with ungerminated conidia (3). Germings of *Cercospora kikuchii*, causing leaf blight in soybean (27), *Botrytis cinerea*, *Cercospora musae*, and *Monilinia fructicola* survived dry periods (11). In contrast, germings of *Botryosphaeria obtusa*, causing leaf spot in apple, were extremely sensitive to interruptions of leaf wetness and did not resume growth (1). Also, germinated pycnidiospores of *Mycosphaerella pinodes* were found to be sensitive to inter-

ruption of leaf wetness period before appressorium formation on pea leaves (25).

Survival of germings during dry periods can differ for dematiaceous and hyaline fungi (8). A high percentage of germings of dematiaceous fungi such as *Alternaria tenuis*, *S. botryosum*, *Helminthosporium sativum*, and *Cladosporium herbarum* survived dry periods (8), but germings of hyaline species such as *Aspergillus*, *Penicillium*, and *Colletotrichum graminicola* died during dry periods. Park (22) compared the time fungal hyphal tips needed for regrowth after dry periods. Phyllosphere inhabitants such as *A. alternata*, *Aureobasidium pullulans*, *B. cinerea*, *Cladosporium cladosporioides*, and *Epicoccum purpurascens* initiated hyphal tip growth within 60 min of moist incubation. Hyphal tips of soil fungi such as *Fusarium solani*, *Gliocladium roseum*, *Humicola grisea*, and *Trichoderma harzianum* and storage fungi such as *Penicillium* spp. were all nonviable after a dry period. Park (22) found that phylloplane fungi cannot grow at water potential below -15 MPa but survive under such conditions. Non-phylloplane fungi can be seriously affected by such low water potentials. L. Hjeljord and A. Tronsmo (personal communication) found that conidia of *T. harzianum* were killed when exposed to desiccation during germination.

It is known from earlier studies that *U. atrum* survives and successfully competes with *Botrytis* spp. on necrotic leaves after interruptions of leaf wetness periods under controlled conditions (18) and in the field (17). However, the effect of interruptions of wetness periods on the development of germ tubes and the effect of low water potentials on conidial germination and competitive colonization of necrotic leaves with *Botrytis* spp. has not been studied before. Water potential relationships in fungi can be studied on artificial media in which the osmotic or matric conditions are altered by adding salts, glycerol, or polyethylene glycol. Direct effects of these additions on fungal metabolism cannot be excluded and may explain the interactions often found between water potential and additives when fungal development is quantified (19,20). To avoid such interactions, methods were applied in

Fig. 4. Estimated germination percentage of conidia of *Ulocladium atrum* continuously incubated on agar slides under moist conditions (-1 MPa) or incubated with two or three moist–dry cycles (6 h at -1 MPa and 18 h at -42 h) at the beginning of the experiment followed by continuously moist incubation. Gompertz curves were fitted to data obtained from repeated experiments. Adjusted R^2 for the different treatments ranged between 0.82 and 1.00 for the different treatments.

TABLE 4. Parameter estimates of Gompertz curves fitted to the germination percentage of conidia of *Ulocladium atrum* incubated on agar slides under moist conditions (-1 MPa) interrupted by two or three dry periods (-42 MPa) during the initial stage of germination

Interruption ^z	Gompertz curves parameters ^y		
	<i>M</i>	<i>B</i>	<i>C</i>
0 dry periods	7.7	0.61	87
2 dry periods	12.7	0.30	71
3 dry periods	14.6	0.13	77
LSD ($\alpha = 0.05$)	2.6	0.24	15
<i>F</i> _{prob.}	<0.001	0.004	0.103

^y Means of two experiments; *M* = point of inflection, *B* = slope parameter, and *C* = upper asymptote.

^z Interruption of initial moist incubation. During each moist–dry cycle, conidia were incubated for 8 h at -1 MPa followed by 18 h at -42 MPa . LSD = least significant difference.

TABLE 5. Effect of *Ulocladium atrum* on the sporulation of *Botrytis cinerea* on cyclamen leaves at different water potentials (MPa)

Treatment ^z	Sporulation index (0–100) ^y		
	-1 MPa	-3 MPa	-7 MPa
<i>B. cinerea</i> 10^5	17.2 a	4.6 a	0.05 b
<i>B. cinerea</i> 10^5 + <i>U. atrum</i>	0.4 c	0.1 c	0.00 b
<i>B. cinerea</i> 10^6	15.3 a	6.1 a	0.63 a
<i>B. cinerea</i> 10^6 + <i>U. atrum</i>	3.0 b	1.0 b	0.01 b

^y Backtransformed means of two experiments. Values within a column followed by the same letter do not differ significantly (least significant difference-test after angular transformation; $\alpha = 0.05$).

^z *B. cinerea* was applied at 10^5 or 10^6 conidia per ml and *U. atrum* was applied at 10^6 conidia per ml.

the present study to control the water potential of a thin water agar layer via equilibrium with the RH of the air, which was controlled by media with adjusted water potential, but separated from the fungal growth medium (24).

When conidia of *U. atrum* were incubated on water agar slides, the speed of germination and the estimated maximum germination (upper asymptote *C*) decreased with decreasing water potential. Endogenous nutrients play a crucial role during germination under water stress and the establishment of the antagonist *Epicoccum nigrum* after application in the field (23). Conidia of the *U. atrum* inoculum used in the present study may contain different amounts of endogenous nutrients, possibly accumulated during maturation. As a consequence, the fraction of conidia with low nutrient level may remain ungerminated under stress conditions.

Germinating conidia were not killed when humid incubation was interrupted by one dry period and the maximum germination percentage (*C*) was the same for conidia incubated with or without an interruption of moist conditions. Significant differences were found for the time needed to reach 37% of the level of the maximum germination percentage (point of inflection *M*). After incubation at -10 MPa, conidia and germ tubes needed 1.6 to 2.3 h longer to take up water again from the surrounding humid air and to start regrowth when they were first incubated under moist conditions for 2 to 8 h, as compared to the control conidia incubated without interruption of the moist period. Thereafter, the speed of the germination process (slope parameter *B*) did not differ statistically significantly between treatments. Conidia incubated first at -42 MPa needed longer to start regrowth. The delay for conidia which had an initial moist incubation was 1.3 to 4.3 h longer as compared to conidia incubated without interruption of the moist period. The speed of germination tended to be slower with longer initial incubation period under moist conditions. Conidia initially incubated for 4 to 6 h under moist conditions needed longer periods to continue with germination than conidia incubated initially for 2 or 8 h under moist conditions. It can be concluded that pre-germinated conidia of *U. atrum* survive a dry period but continue the germination process at lower speed after such a dry period. The most vulnerable stage is reached after an initial moist incubation period at 18°C of 4 to 6 h, when germination percentage was 0 to 10% and germ tubes were shorter than 20 µm. Conidia were more stressed when incubated with two or three moist-dry cycles, resulting in a slower germination process as compared with conidia incubated with or without a single dry interruption of moisture. However, germinated conidia survived the repeated moist-dry cycles and reached the same high maximum germination percentage as conidia incubated without a moist-dry cycle.

Conidia incubated without or with one or three moist-dry cycles had the same number of germ tubes. After moist incubation following dry periods, germ tube length significantly increased as compared with their length before dry periods, which indicates that conidia of *U. atrum* do not produce new germ tubes after dry periods but that the existing germ tubes already formed during the initial humid period continued growth. In contrast to our results, renewed germination was found by Dickinson (7) for *Alternaria alternata*, a fungus closely related to *U. atrum*. Our attempts to follow viability and growth of individual germ tubes more directly failed. Techniques using vital stains such as fluoresceine diacetate (FDA) were not reliable because germ tubes showed no reaction directly after a dry period but the same germ tubes were stained after several hours of moist incubation. A reliable differentiation between living and dead germ tubes during dry periods was not possible. Attempts were also made to measure the speed of hyphal tip growth by following individual germ tubes in time during and after dry periods. Therefore, videomicroscopy combined with automated image analysis was used. However, germ tubes rapidly grew out of focus and germ-tube length could not be measured.

To study the antagonism of *U. atrum* against *B. cinerea* at different water potentials, a method was developed which guaranteed

a constantly controlled water potential of the substrate. Conidia were applied to glass slides in water suspensions which were allowed to evaporate before the conidia came into contact with the necrotic leaves with adjusted water potentials; hence, the water potential of the leaves was not changed. These results show that *U. atrum* can successfully compete with *B. cinerea* not only under conditions of optimum water availability but also when water availability is suboptimal.

This study on the effect of moist-dry cycles on germinating conidia of *U. atrum* and the effect of different constant water potentials on conidial germination and antagonism against *B. cinerea*, together with earlier studies (17,18), demonstrates that *U. atrum* tolerates water stress and thus, with respect to this attribute, is adapted to the ecological niche of leaf surfaces and above-ground necrotic tissues. These results help us to understand the fate of conidial inoculum of the antagonist after field applications. Based on this knowledge of the ecological competence of the antagonist, application frequencies and densities can be optimized to achieve sufficiently high population densities of the antagonist on the target substrates with economically sound dosages.

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