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The effect of treatment with *Ulocladium atrum* on *Botrytis cinerea*-attack of geranium (*Pelargonium zonale*) stock plants and cuttings

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Abstract

In two successive seasons, the effect of treatment of geranium stock plants with the competitive saprophytic fungus *Ulocladium atrum* as a biocontrol agent against *Botrytis cinerea* was compared to a fungicide treatment with Euparene M. *B. cinerea* incidence and severity on the stock plants, *B. cinerea* spore load in the air around stock plants and death of cuttings due to *B. cinerea* were scored. *B. cinerea* incidence and severity were much stronger in the second than the first experiment. This was quantitatively expressed by higher numbers of conidia of *B. cinerea* monitored in the second than the first year, both on necrotic (a maximum for the control of 27.5×10^6 spores per sample – all necrotic leaves of five plants – in experiment 1 against 86×10^6 in experiment 2) and green leaves, but numbers of conidia of *B. cinerea* recovered from the air were only slightly different. The death rate of cuttings was moderate in the first and extremely high in the second experiment. For the fungicide treatment, maximum sample values of 7% and 76% of 6-week old cuttings were killed in the first and the second experiment respectively. Treatment with *U. atrum* was effective in reducing all parameters studied. With the exception of the spore load of *B. cinerea* in the air and the success of cuttings, the effect of *U. atrum* varied from as good as the fungicide to half as effective. In the first trial, only Euparene M reduced spore load in the air, in the second trial only *U. atrum* consistently did so. In the first trial *U. atrum* reduced death of 4-week old cuttings, though less than fungicide (1.2, 20 and 38% killed with fungicide treatment, *U. atrum* treatment and control respectively). In the second trial only the fungicide reduced loss of cuttings. The impact of the data on the integration of *U. atrum* in a control system of *B. cinerea* in geranium is discussed.

Introduction

The necrotrophic fungus *Botrytis cinerea* Pers. ex Pers. is a damaging pathogen of a wide variety of hosts. It thrives at moderate temperatures and high humidity. The ornamental crop of geranium (*Pelargonium zonale* (L.) Aiton) is susceptible to attack of flowers and senescent leaves. The crop is reproduced by cuttings. The season for reproduction of geranium in Dutch horticultural practice is in autumn and winter when daylight is minimal and humidity often high. This combination favours sporulation of *B. cinerea* on stock plants, leading to contamination of cuttings and attack and killing of cuttings by grey mould. Some growers even prefer to interrupt plant reproduction by cuttings during the most unfavourable month of December.

Hausbeck and Pennypacker (1991a,b) studied the spore load of the air in greenhouses with geranium in relation to growers' activities and disease incidence. A combination of heating and covering the pots of stock plants with plastic strongly reduced sporulation of *B. cinerea* on necrotic leaves (Hausbeck et al., 1996). Sirjusingh and Tsujita (1996) provided data of specific age-related susceptibility of leaves which point to the ease of attack of cuttings by *B. cinerea*.

The traditional prevention of fungal diseases by spraying fungicides is under attack from public opinion and policy makers. Fungicides against *B. cinerea* have a supplementary drawback, because the pathogen easily develops resistance towards chemicals. During the last decade, competitive colonisation of substrate has been developed as a strategy to suppress epidemics of grey mould through reduction of sporulation by *B. cinerea* (Fokkema, 1993; Köhl et al., 1995a). *Ulocladium atrum* Preuss proved to be an excellent candidate antagonist to compete with *B. cinerea* (Köhl et al., 1995b; 1998; 1999). In the present study, we tried to reduce the inoculum pressure of *B. cinerea* by spraying geranium stock plants with *U. atrum*. It was supposed that this may lead to reduced colonisation of necrotic leaves, reduced spore load of the air, reduced deposition of *B. cinerea* conidia on healthy, young leaves of geranium, and therefore, reduced attack of cuttings by grey mould.

Materials and methods

Material and experimental set-up

Stock plants of the *Pelargonium zonale* cv Springtime Irene were grown in a glasshouse at the Research Station for Floriculture and Glasshouse Vegetables (PBG). In autumn, when they had reached the size required for reproduction, a group of 35–40 stock plants was placed in each of 12 individual glasshouse compartments with the temperature set at 18 °C. Light was supplemented whenever natural illumination was too little (<150 W m^{-2}). Relative humidity varied between 55 and 95% . Treatments varied between compartments and consisted of fortnightly spraying with (1) water with 0.01% Tween 80 (control), (2) Euparene M (a.i. 50% tolylfluanide) and (3) *U. atrum* (conidial suspension of 1×10^6 conidia per ml in water with 0.01% Tween 80). The treatments were randomised within 4 blocks, each consisting of three individual glasshouse compartments. Stock plants were sprayed until run-off. Conidia of *U. atrum*, isolate 385, originating from a necrotic leaf tip of onion, were produced on oat grains in 3 l autoclavable spawnbags (Van Leer, Sacherie de Pont-Audemer, France). Oat kernels were moistened in tap water in a vessel overnight (300 g of dry oat kernels and 300 ml water), excess water was drained through a sieve, and the moistened oat kernels were put in a spawnbag. The bags were autoclaved twice at 121 °C for 45 min at 24 h interval. The sterilised oats were inoculated with 0.5 ml of a conidial suspension of *U. atrum* (approximately 10^5 conidia per ml) and incubated for 28 days at 20 °C in the dark. Bags were shaken every 2–3 days to mix the contents and to avoid formation of aerial mycelium. Suspensions of conidia of *U. atrum* were prepared by transferring the incubated oat kernels into nylon gauze bags with 1 by 0.4 mm mesh and agitating these bags for 5 min in a small washing machine (Nova Miniwash Super 2000 SR; Nova, Maastricht, the Netherlands) in 5 l of chilled tap water (5 °C) with 0.01% Tween 80. The resulting suspension was filtered through a nylon gauze with 200 µm mesh to remove mycelial fragments and oat debris (Köhl et al., 1998). The conidial concentration was adjusted to 1×10^6 conidia per ml.

Every second week, the day after spray treatments, about two cuttings per stock plant were harvested. According to horticultural practice they were left on the greenhouse bench for 24 h and subsequently planted in trays with soil. In a first trial, autumn/winter 1996/97, half of the cuttings from plants in each compartment were left in a tray in that compartment. A tray with the other half was put in one big compartment at 18/16 °C (day/night) together with cuttings originating from the other compartments. In the second trial in 1997/98 all cuttings were collected together in one greenhouse compartment.

Assessments

The stock plants were visually assessed every second week for numbers of necrotic leaves and presence of sporulation of *B. cinerea* on leaves. This assessment was made semi-quantitative (*B. cinerea* severity on necrotic leaves) by estimating the equivalent number of leaves (blades and petioles) with *B. cinerea* sporulation corrected for sporulation intensity (spore producing leaf area corrected for intensity; 'SPLACI' in Köhl et al., 1998). In formula:

$$\text{Severity} = \frac{\sum_{i=1}^{n} \sum_{j=1}^{m_i} w_{ij} p_{ij}}{n} \qquad \text{(Formula 1)}$$

in which i = number of plant, $i = 1, \ldots, n$; m_i = number of *B. cinerea*-colonised leaves of plant i; p_{ij} = proportion of jth necrotic leaf of plant i covered by conidiophores of *B. cinerea*; and w_{ij} = weight ($0 \leq w_{ij} \leq 1$) for intensity of sporulation on the ijth necrotic leaf.

Cuttings were visually assessed for the presence of *B. cinerea* and killing by this pathogen at 2, 4 and 6 weeks after planting.

Aerial spore load

Spore samplers according to Kerssies (1990) were placed in each compartment, 4 plates in a vertical position at 20 cm above the crop (first trial) or, 6 in a horizontal position at crop level (second trial). This method of spore sampling is based on capturing *B. cinerea* on Petri dishes with a selective medium. The medium, as defined by Kerssies (1990) was composed of (in g/l distilled water): $NaNO_3$, 1.0; K_2HPO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 0.2; KCl, 0.15; glucose, 20.0 and agar 25.0. After sterilisation the selective chemical substances were added (g/l): quintozene, 0.011; maneb, 0.01; chloramphenicol, 0.05; $CuSO_4$, 2.2; fenarimol 0.1 (ml/l); tannic acid, 5.0. The pH was adjusted to pH 4.5 with 5.0 M NaOH. Fortnightly, sets of plates were left open for periods of 24 h. After incubation of the dishes in the dark at 20 °C for 2 weeks *B. cinerea* can be distinguished as dark colonies, whereas only a few other fungi grew. The numbers of *B. cinerea* colonies per Petri dish were counted and means per compartment were used for analysis.

Quantification of spore production by B. cinerea

Sporulation of *B. cinerea* was quantified by sampling 5 random stock plants per compartment every month just before spraying. Five young green leaves were collected per plant, which could be expected not to have been in contact with the last *U. atrum* or fungicide spray. The five sampled leaves were put in 250 ml erlenmeyer flasks and washed in 150 ml 0.05% Tween 80 by shaking for 10 min. on a flask shaker (Stuart Scientific SF1, Britain) at high speed (150 strokes/min). The liquid contents of five flasks from one compartment were combined and passed through a cellulose nitrate filter with 5 µm pore size. The conidia of *B. cinerea* were counted on the filter under the microscope (200×) after staining with diluted cotton blue (1.5 ml 1% cotton blue in water, 2 ml lactic acid, 4 ml glycerol, 20 ml distilled water). All the necrotic leaves of the same five plants were carefully collected in a bottle with 20% alcohol and 0.01% Tween 80 in water, about 15 ml per 5 leaves, trying to prevent detachment of fungal spores during collection as much as possible. The bottles with liquid and necrotic leaves were shaken for 10 min. The number of conidia in the liquid was assessed by counting with a haemacytometer. In the first trial, the liquid was filtered through a paper filter, and the weight of dead tissue determined after drying of the filter plus debris to allow calculation of spore number per gram dry weight of necrotic leaves. In the second trial, the surface area of necrotic and green leaves was estimated by transforming scored leaf diameter to surface by means of a grading line based on the measurement of diameter and surface of 90 leaves as measured by means of an electronic surface measuring device (Delta-T device, Cambridge, Britain). The numbers of conidia were calculated per surface area by means of the surface measured as described. The sampled plants were put back between the others to minimise the effect of sampling on the microclimate, but they were excluded from further use for cuttings or assessments. Each experiment lasted for about $3\frac{1}{2}$ months.

Statistics

Data were analysed using Genstat 5 (Numerical Algorithmus Group Inc., Oxford) ANOVA. Time series of observations, mostly fortnightly, were considered as sub-plots. However, when too many zero values were scored the week was excluded from the analysis. To stabilise variance, figures were log-transformed, except for the data on spore load in the air which were analysed after square root transformation, and the percentage data which were analysed after angular transformation. Back-transformed data were represented in the figures. Whenever treatment $*$ week interactions were significant, significant differences between treatments at a given date are based on LSD-values ($P < 0.05$) and indicated by different letters following the week number in the figures. When treatment effect was significant, but the interaction treatment $*$ week was not, the average for the weeks considered was added to the graphs.

Results

Visual assessment

At the start of the experiments, the stock plants had no necrotic leaves. However, when the plants grew older they gradually developed necrotic leaves. Their number

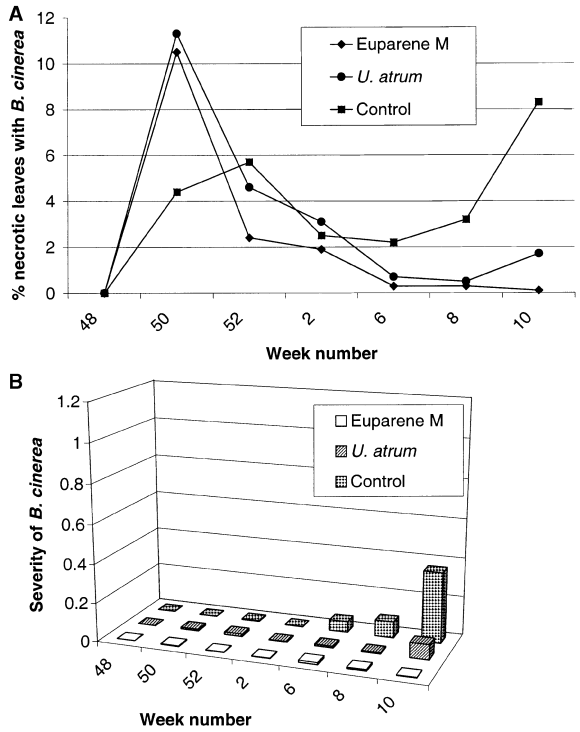

Figure 1. Effect of treatments (control, Euparene M and *U. atrum*) on the development of *B. cinerea* incidence (A) and severity (B) on necrotic leaves for the first experiment (1996/97). Incidence is expressed as percentage of the necrotic leaves on which *B. cinerea* occurs, severity as the equivalent number of leaves with maximum colonization by *Botrytis cinerea*. (see text, Formula 1)

rose to about 10 per plant at the end of the first trial, and 16 at the end of the second trial.

In the first trial, the incidence of *B. cinerea* sporulation on the necrotic leaves was only 4.4% for the control in week 50 (1996) and 8.3% in week 10 (1997) (Figure 1). However, only a fraction of colonised leaves was really covered by *B. cinerea* ($\frac{1}{4}$ and $\frac{3}{4}$ respectively), and the intensity of colonisation was far less than maximum (weight <1 in formula 1). This resulted in insignificant values for *B. cinerea* severity on the stock plants in the first trial, with the exception of the final weeks (Figure 1). For the control, it reached a maximum of 0.36 in week 10 against only 0.08 for the *U. atrum* treatment and less than 0.01 for the fungicide treatment. However, due to high variation within treatments, differences were not statistically significant (Table 1).

In the second trial, about 80% of the necrotic leaves in both the control and the *U. atrum* treatment were colonised by *B. cinerea* at the first sampling date (week 43), twice as much as in the fungicide treatment, but the colonisation steadily decreased to less than 10% in all treatments at week 51 (Figure 2). Correction of these figures by weighting for percentage area colonised by *B. cinerea* and intensity of *B. cinerea* sporulation led to a peak severity of *B. cinerea* for the control at week 47. At the same time, the other treatments peaked at a lower level (1.16, 0.80 and 0.35 respectively for control, *U. atrum* and fungicide) (Figure 2). Effects of treatment, week and their interaction were all highly significant (Table 1) for weeks 43–51. In weeks 43–47, Euparene M treatment was more effective than *U. atrum*, which was indistinguishable from control.

Spores trapped in the air

In the first trial, only on two occasions were significant numbers of *B. cinerea* spores trapped from the air. At week 51, the number was 4.3 per Petri dish for the control, 1.5 for *U. atrum* treatment and lowest (0.2) for the fungicide treatment, (Figure 3). On average for 6 samplings, the treatment effect was just significant, however, only the fungicide reduced the number of conidia compared to control. In the second trial, a more regular pattern of numbers of conidia of *B. cinerea* was obtained. *U. atrum* treatment resulted in lower numbers of conidia of *B. cinerea* in the air than with the control in three out of four samples collected under similar conditions. However, fungicide treatment did not differ from control (Figure 3).

Spore counts on necrotic and green leaves

Both trials showed a reduction of *B. cinerea* conidia on necrotic tissue and on young green leaves of stock plants by *U. atrum* and fungicide treatments compared to control when assessed by counting spore numbers (Figures 4 and 5). Data based on numbers of *B. cinerea* spores per sample (the sampled leaves of all five plants per glasshouse compartment put together), per gram of tissue (first trial) or per surface area (second trial) yielded similar results (data not shown).

In the first trial, only one sampling date (week 11) yielded significant numbers of conidia of *B. cinerea* per sample on necrotic leaves of control plants (27.5×10^6) (Figure 4). *U. atrum* (1.3×10^6) and fungicide (0.07×10^6) suppressed *B. cinerea*. Only two samples of green leaves yielded enough spores for significant

Table 1. Summary of statistical data. The statistical significance is indicated by F values. Probabilities between 0.05 and 0.10 are between brackets. >0.10 is indicated as n.s. (non significant). Since statistical analysis is complicated by zero values some weeks had to be excluded from analysis; the weeks considered are indicated

Category	Severity[1]	Conidia[2]		Spore load[3]	% Dead cuttings		
		Necrotic	Green		4 weeks	6 weeks	4 weeks[4]
1996/97							
Treatment	n.s.	(0.062)	0.002	0.05	< 0.001	0.029	(0.082)
Week	n.s.	0.018	0.022	<0.001	0.003	<0.001	<0.001
Treat∗week	n.s.	0.047	n.s.	n.s.	n.s.	0.006	<0.001
Weeks considered	6, 8, 10	7, 11	7, 11	49, 51, 52, 3, 5, 6	47, 51, 9, 11	47, 49, 51, 5, 7, 9	47, 5, 7, 9, 11
1997/98							
Treatment	0.006	0.034	0.026	0.001	<0.001	<0.001	
Week	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Treat∗week	<0.001	n.s.	n.s.	<0.001	<0.001	<0.001	
Weeks considered	43, 45, 47, 49, 51	45, 49, 1, 5	45, 49, 1, 5	43, 47, 51, 3	41, 43, 45, 47, 49, 51, 1, 3, 5	41, 43, 45, 47, 49, 51, 1, 3, 5	

[1]Severity of *B. cinerea* on necrotic leaves.
[2]Counts of conidia on necrotic and green leaves respectively.
[3]Spore load (CFU of *B. cinerea*) in the air as captured on agar plates.
[4]Cuttings were collected in one separate greenhouse, and were scored 4 and 6 weeks after their collection, except for part of the cuttings in 1996/97 which were placed in the same compartments as the stock plants (last column).

data. The control had its peak value at 5622 conidia per sample in week 11 against 119 and 71 for *U. atrum* and fungicide treatments. The mean of weeks 7 and 11 was 2510, 67 and 8 respectively, and both *U. atrum* and fungicide treatments significantly reduced the conidial count compared to control.

In the second trial (Figure 5) high numbers of *B. cinerea* on necrotic leaves were counted at several sampling dates from the start of the experiment, but they decreased with time. In week 45, the numbers were 86×10^6, 29×10^6 and 12×10^6 respectively for control, *U. atrum* and fungicide treatment. In week 5, only 4.5, 1.3 and 1.6×10^6 conidia were found. On green leaves, the highest number of conidia of *B. cinerea* for the control was 79 per cm^2 in week 45. *U. atrum* and fungicide treatments gave 30 and 14 at that date. On average over 4 samplings, the reduction of number of spores by *U. atrum* and fungicide treatments compared to control was significant, with values of 5.3, 5.5 and 13.2 respectively.

Cuttings

Cuttings were progressively killed by *B. cinerea*. At 2 weeks, few cuttings were killed, at 4 weeks a considerable number were dead, and at 6 weeks this number was still higher.

In the first trial, the loss of cuttings due to *B. cinerea* attack was moderate, and fungicide treatment was more effective than *U. atrum*, whereas control had the highest loss (Figure 6). This was most pronounced with 4-week-old cuttings in the separate greenhouse. For 6-week-old cuttings taken at week 51, the maximum percentage death was 72, 61 and 7% respectively for control, *U. atrum* and Euparene M treatment. With the exception of week 47, 4-week-old cuttings which remained in the small individual greenhouse compartments tended to have less attack by *B. cinerea* than those in the common (separate) greenhouse.

In the second trial, depending on the harvest date, about 50% of the cuttings was dead within four weeks, and 65–87% of the cuttings of all harvest dates with both control and *U. atrum* treatment were killed at 6 weeks (Figure 7). With 39–76% of the cuttings of any harvest date killed, the fungicide treatment was significantly better, though, very high losses still occurred.

Discussion

B. cinerea *severity on stock plants*

The trials were performed during two seasons at periods considered risky for development of *B. cinerea*

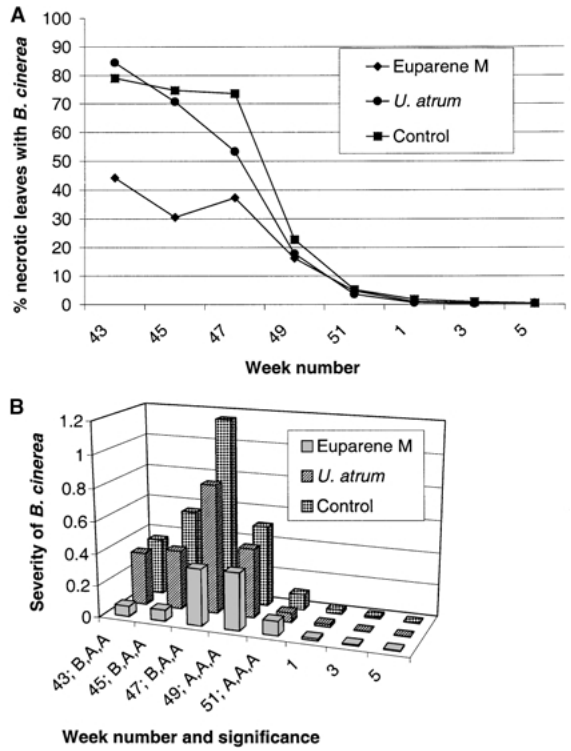

Figure 2. Effect of treatments (control, Euparene M and *U. atrum*) on the development of *B. cinerea* incidence (A) and severity (B) on necrotic leaves for the second experiment (1997/98). Incidence is expressed as percentage of the necrotic leaves on which *B. cinerea* occurs, severity as the equivalent number of leaves with maximum colonisation by *Botrytis cinerea* (see text, Formula 1). Statistical significance of treatments expressed by letters following the week number because of significant treatment∗week interactions ($P \leq 0.05$). Treatments with the same letter are not significantly different.

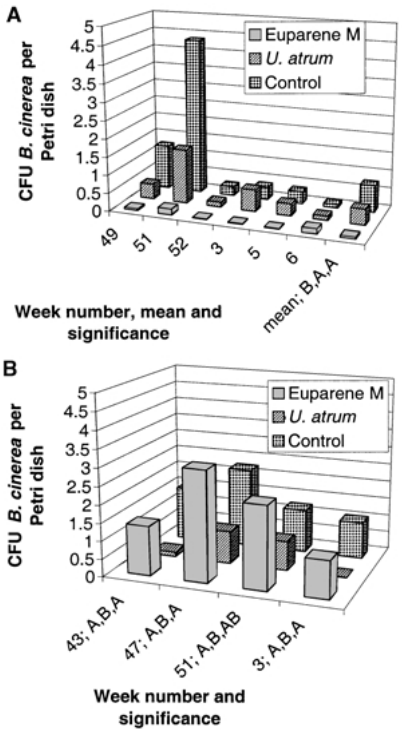

Figure 3. Effect of treatments (control, Euparene M and *U. atrum*) on numbers of conidia of *B. cinerea* in the air of greenhouse compartments. The spore traps, according to Kerssies (1990), were incubated for 24 h. (A) Experiment 1996/97. (B) Experiment 1997/98. Statistical significance of treatment effects is expressed by letters following the week number in case of significant treatment∗week interactions ($P \leq 0.05$) or following the label 'mean' when the treatment effect but not the treatment∗week interaction was significant. Treatments with the same letter are not significantly different.

epidemics. Due to high RH in greenhouses in autumn, senescent and necrotic leaves of geranium stock plants are easily colonised by *B. cinerea*. In the first trial, the start of the experiment was rather late, which caused the experiment to partly escape from the high humidity which usually prevails during October–December. The few necrotic leaves during the first weeks of the experiment had a low percentage *B. cinerea* incidence, and the intensity of the pathogen was low. A cold and dry spell in the middle of the trial period reduced *B. cinerea* still further, but towards the end of the experiment the percentage of necrotic leaves colonised by *B. cinerea* and the intensity of colonisation increased again. In combination with a higher number of necrotic leaves, this led to the highest *B. cinerea* severity occurring at the end of the experiment.

The second trial started earlier and developed a higher incidence of *B. cinerea* on necrotic leaves of the stock plants, leading to a high *B. cinerea* pressure in the first half of the experimental period.

B. cinerea *spores in the air*

The results of the spore traps to monitor the inoculum pressure in the glasshouse air did not correspond to the severity of *B. cinerea* as visually assessed, nor to the more precise counts of *B. cinerea* spores on necrotic and green leaves. The average numbers of colonies formed per Petri dish after 24 h exposure are very low indeed. This is the more striking since the plates were also exposed on days of manipulation of the stock plants for spraying or collection of cuttings.

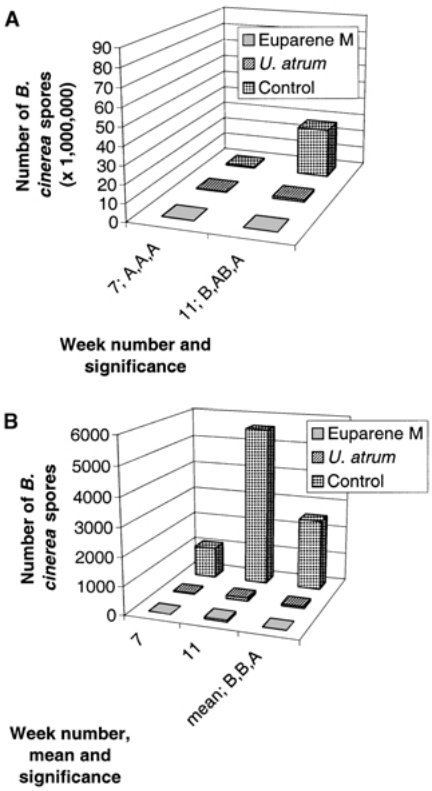

Figure 4. Effect of treatments (control, Euparene M and *U. atrum*) on numbers of conidia of *B. cinerea* on necrotic leaves ((A) figures for a sample consisting of all necrotic leaves of 5 plants) and young green leaves ((B) figures for a sample of 25 leaves, 5 from each of 5 plants) of stock plants. First experiment (1996/97). Statistical significance of treatment effects is expressed by letters following the week number in case of significant treatment∗week interactions ($P \leq 0.05$) or following the label 'mean' when the treatment effect but not the treatment∗week interaction was significant. Treatments with the same letter are not significantly different.

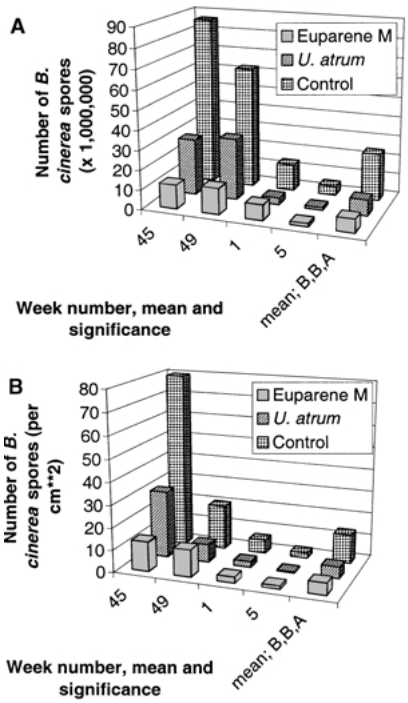

Figure 5. Effect of treatments (control, Euparene M and *U. atrum*) on numbers of conidia of *B. cinerea* on necrotic leaves ((A) figures for a sample consisting of all necrotic leaves of 5 plants) and young green leaves ((B) figures expressed per cm^2 leaf surface area for a sample of 25 leaves, 5 from each of 5 plants) of stock plants. Second experiment (1997/98). Statistical significance of treatment effects is expressed by letters following the label 'mean' since the treatment effect but not the treatment*week interaction was significant ($P \leq 0.05$). Treatments with the same letter are not significantly different.

At such occasions, clouds of spores are to be expected (Hausbeck and Pennypacker, 1991a,b). Also, the significant suppression of the *B. cinerea* spore load by the fungicide in the first trial and by *U. atrum*, but not the fungicide treatment in the second trial cannot be explained.

B. cinerea *spore counts on necrotic and green leaves*

The counts of spores of *B. cinerea* on necrotic and green leaves, both in the first and second trial are in full agreement with each other. They consistently show suppression of the pathogen by the antagonist as well as by the fungicide. In both trials, estimates of *B. cinerea* severity showed highest values in the weeks when visual estimates of the stock plants gave highest values. In the first trial, the number of conidia of *B. cinerea* on green leaves was low, in the second it was several times higher. The data of the two years cannot be compared directly, since only in the second year was the number of spores more accurately scored per cm^2. However, a comparison is possible by multiplication of the 1997/98 data by 14.8, the average surface area of green leaves measured in the samples, and 25, the number of leaves per sample. This calculation shows that control leaves had about twice the spore load in 1997/98 compared to 1996/97, but in the *U. atrum* and fungicide treatments the difference is a factor 100 (means of two samples in 1996/97: control 2510, *U. atrum* 67,

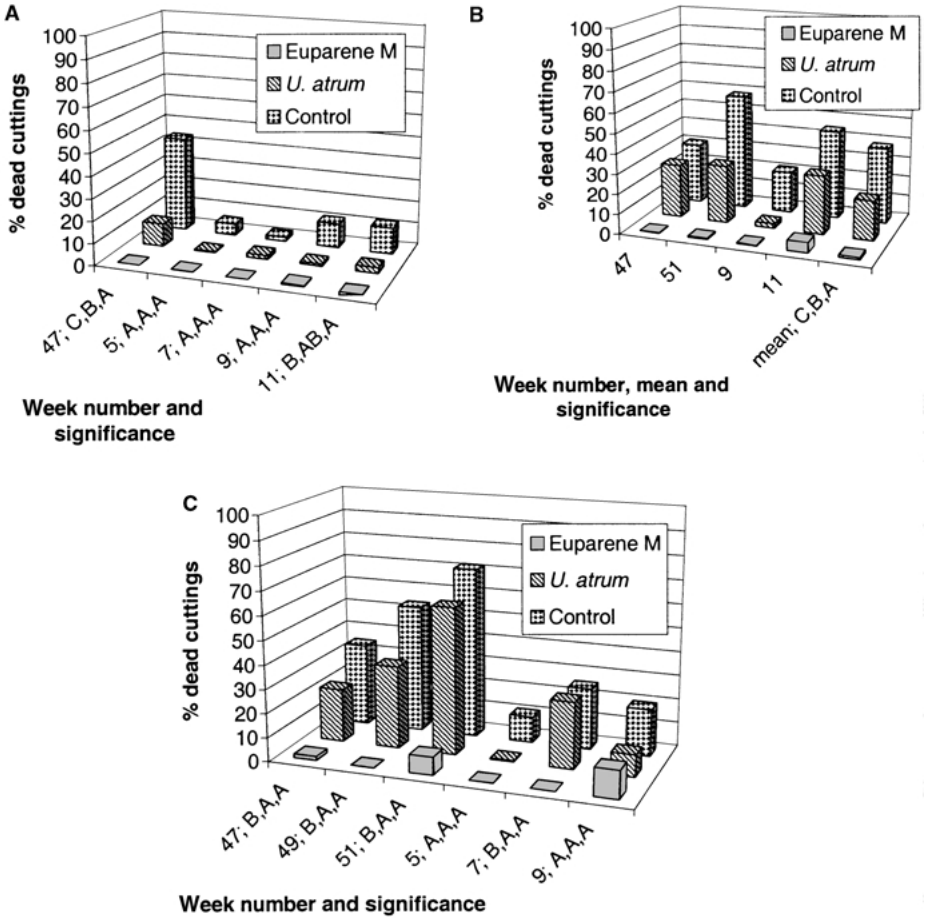

Figure 6. Effect of treatments (control, Euparene M and *U. atrum*) on attack of cuttings by *B. cinerea* leading to death. Data for 4- and 6-week-old cuttings. 4-week-old cuttings were incubated in different conditions. (Week number = week in which the cuttings were harvested from the stock plants and planted.) First experiment (1996/97). (A) 4-week-old cuttings in individual greenhouse compartments; (B) 4-week-old cuttings all collected in a separate greenhouse; (C) 6-week-old cuttings all collected in a separate greenhouse. Statistical significance of treatment effects is expressed by letters following the week number in case of significant treatment∗week interactions ($P \leq 0.05$) or following the label 'mean' when the treatment effect but not the treatment∗week interaction was significant. Treatments with the same letter are not significantly different.

fungicide 8; means of four samples in 1997/98 after correction: control 4884, *U. atrum* 2035, fungicide 1961).

Death of cuttings

Cuttings of geranium may get infected by *B. cinerea* on the wound surface or through contamination of the young top leaves with conidia. Normally, green leaves are not readily infected by conidia without exogenous nutrient supply, but very young geranium leaves show increased susceptibility compared to older ones (Sirjusingh and Tsujita, 1996). December is also the month with light at a minimum, which causes poor conditions for cutting vigour, leading to increased susceptibility to *B. cinerea* as demonstrated by Shtienberg et al. (1998) in shading experiments with tomato. In the first trial low *B. cinerea* inoculum pressure in the middle of the experiment, combined with increasing daylength may have been responsible for relatively low loss of cuttings due to *B. cinerea*. The second trial started earlier, and *B. cinerea* on stock plants was present at a high level for several weeks right from the start of the trial. Contamination of cuttings with conidia of *B. cinerea,* as represented by the counts of spores on green leaves, was high in

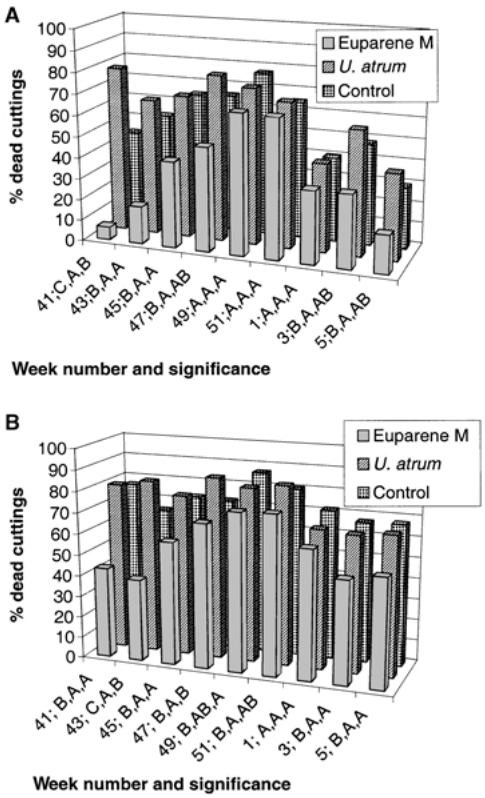

Figure 7. Effect of treatments (control, Euparene M and *U. atrum*) on attack of cuttings by *B. cinerea* leading to death. Data for 4- and 6-week-old cuttings. (Week number = week in which the cuttings were harvested from the stock plants and planted.) Second experiment (1997/98). (A) 4-week-old cuttings collected in a separate greenhouse; (B) 6-week-old cuttings collected in a separate greenhouse. Statistical significance of treatment effects is expressed by letters following the week number because of significant treatment∗week interactions ($P \leq 0.05$). Treatments with the same letter are not significantly different.

November/December 1997, which, in combination with poor light conditions may explain the extremely high loss of cuttings. The higher contamination in the second year might explain the high percentages cuttings killed even for *U. atrum* and fungicide treatments in trial 2. The reduction in the number of spores of the pathogen on green leaves by these treatments seems effective in the first, but not in the second trial. Two factors may account for this result. First, a minimum number of conidia is required for infection (Sirjusingh and Tsujita, 1996). Second, when inoculum pressure is high the significant reduction brought about by *U. atrum* or fungicide may not be enough for a substantial reduction of infection. It is generally observed with biological control that the effect is better at low disease pressure (McQuilken et al., 1990). In the two trials described, it seems that the spore load in the first experiment was often below the critical level, even in the control. In the second trial it was above the threshold, even in the *U. atrum* and fungicide treatments.

Integrated control

The results of the present trials show a potential for *U. atrum* in an integrated programme. Several management practices can reduce the pressure of *B. cinerea*. A dry atmosphere is most important. This can be obtained by changes in glasshouse climate by placing plastic on the soil, installing heating under the plant tables (Hausbeck and Moorman, 1996; Hausbeck et al., 1996), or by reducing the plant density. Hausbeck et al. (1996) have shown that the combination of two practices, plastic cover and heating, was much more effective than a single treatment, though each practice resulted in a significant effect. An additional decrease in spore production by *B. cinerea* by application of the competitor *U. atrum* may be another contribution to reduce the spore contamination of the cuttings. It could cause a strong effect by bringing the pressure of *B. cinerea* under the critical threshold, and thus positively affect the success rate of cuttings.

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