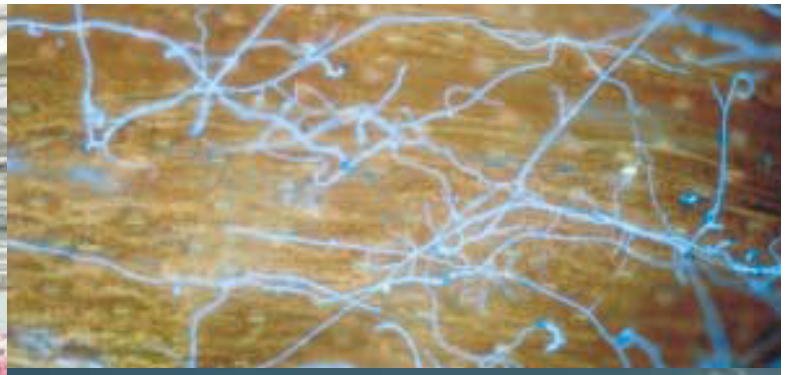
PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Biological control of *Botrytis* spp. by *Ulocladium atrum* through competitive colonisation of necrotic plant tissues

Jürgen Köhl



Biological control of *Botrytis* spp. by *Ulocladium atrum* through competitive colonisation of necrotic plant tissues

Jürgen Köhl

Wageningen, April 2004

Plant Research International B.V.

Address	:	Droevendaalsesteeg 1, Wageningen, The Netherlands P.O. Box 16, 6700 AA Wageningen, The Netherlands
Tel.	:	+31 317 47 70 00
Fax	:	+31 317 41 80 94
E-mail	:	post@plant.wag-ur.nl
Internet	:	http://www.plant.wageningen-ur

Table of contents

Preface

Preface

In this book an attempt is made to discuss concept development for biological control of necrotrophic fungal foliar pathogens and to summarise results obtained in research on the selection, characterisation and use of the antagonist *Ulocladium atrum* in biocontrol of *Botrytis* spp. in necrotic plant tissues. This research has been carried out during the period 1990-2001 at the DLO Research Institute for Plant Protection (IPO-DLO), merged into Plant Research International in 2000, in Wageningen, the Netherlands.
The main results presented in the book have been published earlier in a series of publications in scientific journals or book chapters as listed below. All authors of publications contributed significantly to the progress made in the different research steps. The book has been presented as thesis of postdoctoral lecturing qualification (Habilitationsschrift) at the faculty of agriculture of the Rheinische Friedrich-Wilhelms-Universität in Bonn, Germany, which has been accepted in December 2003.

Substantial funding from different sources allowed the continuation of research on concept development, antagonist screening and antagonist testing in different cropping systems. The main funding sources during the period 1990-2001 were:
Dutch Ministry of Agriculture, Nature Management, and Fisheries (1990 - 2001);
European Commission (FAIR3 PL96-1898 BIOSPORSUPPRESS (1996 - 2001);
Dutch Product Board for Horticulture (Produktschap Tuinbouw) (1996 - 2000);
Technology Foundation (STW) for G.J.T. Kessel (1995 - 1999);
International Agricultural Centre (IAC) scholarships for J. Köhl (1989 - 1990), P.A.G. Elmer (1996), and T. Sesan (2000);
Brazilian Government (CAPES-2959/95-0) for P. Boff (1997 - 2001);
Stichting Nederlandse Uien-Federatie (SNUiF) (1989 - 1991).

Besides the authors of publications listed below, the support of many other colleagues enabled the progress in our work. Our many fruitful discussions on the use of *U. atrum* in disease control with the partners of FAIR3 PL96-1898 BIOSPORSUPPRESS, H.-W. Dehne, P.C. Nicot, B. Dubos, W. van der Werf, M.H. Jijakli, N. Magan, F.M. Dewey, D. Berg and W.J. Ravensberg, contributed significantly to our research. J. de Bree, E. van Remortel, P. Vereijken and S. Burgers analysed data; J. Huizinga, M.R. Holdinga, J. Mekking and M. van den Bogert took care of field experiments; and H. Terburg and H.M. Kleinjan-Meijering helped in lay out of this book. Many experiments in greenhouses or in the field were only possible with the excellent support Applied Plant Research, Research Unit Glasshouse Horticulture, Naaldwijk, and Research Unit Fruit, Zetten, Schoneveld Twello B.V., Twello and Nolina B.V., Woubrugge.

Results of the following publications are presented in the book:

Köhl, J., Krijger, M.C. and Kessel, G.J.T. 1992. Drought tolerance of *Botrytis squamosa*, *B. aclada* and potential antagonist. In: Verhoeff, K., Malathrakis, N.E. and Williamson, W. (eds). Recent Advances in *Botrytis* Research. Pudoc Scientific Publishers, Wageningen. pp 206-210.

Köhl, J., Plas, C.H. van der, Molhoek, W.M.L. and Fokkema, N.J. 1993. Drought tolerance as a major selection criterium for antagonists of *Botrytis* spp. In: Fokkema, N.J., Köhl, J. and Elad, Y. (eds). Biological Control of Foliar and Postharvest Diseases. IOBC/WPRS Bulletin 16(11). pp 169-172.

Köhl, J. and Fokkema, N.J. 1994. Fungal interactions on living and necrotic leaves. In: Blakeman, J.P. and Williamson B. (eds). Ecology of Plant Pathogens. CABI, Oxon. pp 321-334.

Köhl, J., Molhoek, W.M.L., Plas, C.H. van der and Fokkema, N.J. 1995a. Suppression of sporulation of *Botrytis* spp. as a valid biocontrol strategy. Eur. J. Plant Pathol. 101: 251-259.

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

Köhl, J., Plas, C.H. van der, Molhoek, W.M.L. and Fokkema, N.J. 1995c. Effect of interrupted leaf wetness periods on suppression of sporulation of *Botrytis allii* and *B. cinerea* by antagonists on dead onion leaves. Eur. J. Plant Pathol. 101: 627-637.

Köhl, J., Bélanger, R.R. and Fokkema, N.J. 1997. Interaction of four antagonistic fungi with *Botrytis aclada* in dead onion leaves: A comparative microscopic and ultrastructural study. Phytopathology 87: 634-642.

Köhl, J. and Fokkema, N.J. 1998. Strategies for biological control of necrotrophic fungal foliar pathogens. In: Boland, G.J. and Kuykendall, L.D. (eds). Plant-Microbe Interactions and Biological Control. Marcel Dekker, New York. pp 49-88.

Köhl, J., Gerlagh, M., Haas, B.H. de and Krijger, M.C. 1998. Biological control of *Botrytis cinerea* in cyclamen with *Ulocladium atrum* and *Gliocladium roseum* under commercial growing conditions. Phytopathology 88: 568-575.

Kessel, G.J.T. 1999. Biological control of *Botrytis* spp. by *Ulocladium atrum*: an ecological analysis. PhD Thesis Wageningen University, Wageningen, the Netherlands.

Kessel, G.J.T., Haas, B.H. de, Lombaers-van der Plas, C.H., Meijer, E.M.J., Dewey, F.M., Goudriaan, J., Werf, W. van der and Köhl, J. 1999. Quantification of mycelium of *Botrytis* spp. and the antagonist *Ulocladium atrum* in necrotic leaf tissue of cyclamen and lily by fluorescence microscopy and image analysis. Phytopathology 89: 868-876.

Köhl, J. and Gerlagh, M. 1999. Biological control of *Botrytis cinerea* in roses by the antagonist *Ulocladium atrum*. Med. Fac. Landbouww. Univ. Gent 64/3b: 441-445.

Köhl, J., Lombaers-van der Plas, C.H., Molhoek, W.M.L., Kessel G.J. and Goossen-van der Geijn, H.J. 1999. Competitive ability of the antagonist *Ulocladium atrum* and *Gliocladium roseum* at temperatures favourable for *Botrytis* spp. development. BioControl 44: 329-346.

Köhl, J., Gerlagh, M., and Grit, G. 2000. Biocontrol of *Botrytis cinerea* by *Ulocladium atrum* in different production systems of cyclamen. Plant Dis. 84: 569-573.

Boff, P. 2001. Epidemiology and biological control of grey mould in annual strawberry crops. PhD Thesis Wageningen University, Wageningen, the Netherlands.

Boff, P., Kastelein, P., Kraker, J. de, Gerlagh, M. and Köhl, J. 2001. Epidemiology of grey mould in annual waiting-bed production of strawberry. Eur. J. Plant Pathol. 107: 615-624.

Gerlagh, M., Amsing, J.J., Molhoek, W.M.L., Bosker-van Zessen, A.I., Lombaers-van der Plas, C.H. and Köhl, J. 2001. The effect of treatment with *Ulocladium atrum* on *Botrytis cinerea*-attack of geranium (*Pelargonium zonale*) stock plants and cuttings. Eur. J. Plant Pathol. 107: 377-386.

Kessel, G.J.T., Haas, B.H. de, Lombaers-van der Plas, C.H., Ende, J.E. van der, Pennock-Vos, M.G., Werf, W. van der and Köhl, J. 2001. Comparative analysis of the role of substrate specificity in biological control of *Botrytis elliptica* in lily and *B. cinerea* in cyclamen with *Ulocladioum atrum*. Eur. J. Plant Pathol. 107: 273-284.

Köhl, J. and Molhoek, W.M.L. 2001. Effect of water potential on conidial germination and antagonism of *Ulocladium atrum* against *Botrytis cinerea*. Phytopathology 91: 485-491.

Boff, P., Köhl, J., Jansen, M., Horsten, P.J.F.M., Lombaers-van der Plas, C.H. and Gerlagh, M. 2002. Biological control of gray mold with *Ulocladium atrum* in annual strawberry crops. Plant Dis. 86: 220-224.

Köhl, J., Molhoek, W.M.L., Goossen-van de Geijn, H.M. and Lombaers-van der Plas, C.H. 2003. Potential of *Ulocladium atrum* for biocontrol of onion leaf spot through suppression of sporulation of *Botrytis* spp. BioControl 48: 349-359.

Summary

Botrytis spp. are causing economically important losses in the production of vegetables, fruit and ornamentals. The world market for chemical control of *Botrytis* spp. is estimated 200-300 million euro per year. There is an urgent need for other control methods because at future government regulations may restrict fungicide use and organic farming is still lacking efficient tools for *Botrytis* control. Second, resistance of *B. cinerea* populations against fungicides is a common problem so that novel disease control tools are needed for integrated use with fungicides to increase their commercial life-span.

The objective of our research was to develop a concept for biological control of *Botrytis* spp., to select antagonists and to test their use in economically important crops. We expected suppression of pathogen sporulation on necrotic plant tissues to be a valid biocontrol strategy because the periods of interaction during this stage of the life cycle are long and other mechanisms than antibiosis can be exploited. Competitive substrate colonisation under a broad range of microclimatic conditions and persistence during long dry periods were identified as the key success factors for biocontrol agents targeting at saprophytic pathogen development in necrotic tissues. Several research steps were necessary to develop such a biocontrol system:

(1) Epidemiological knowledge on diseases caused by *Botrytis* spp. in different crops was necessary to identify valid target tissues.
(2) Suitable antagonists with a high competitive ability during the colonisation of various necrotic plant tissues had to be selected.
(3) Antagonists with superior ecological competence had to be found.
(4) Knowledge on the mode of action of selected antagonists was needed to optimise targeting and timing of antagonist applications.
(5) The potential of the control strategy and applications of the selected antagonist had to be studied under field and greenhouse conditions.

These subsequent research steps have been carried out successfully. *U. atrum* (isolate 385) has been found to be an antagonist suitable for application on necrotic above-ground plant tissues. *U. atrum* combines a high competitive colonisation ability with a superior ecological competence. Main characteristics are the long persistence of viable conidia on leaf surfaces, the high resistance to interruptions of leaf wetness, and the strong antgonistic activity over a broad temperature range. Application strategies for *U. atrum* have been developed and disease control has been achieved in strawberries, onion, cyclamen, pot roses and *Pelargonium*.

In the meantime, other research groups applied *U. atrum* in grapevine and tomato resulting in significant reductions of grey mould, and investigated methods for mass production of *U. atrum*.

In conclusion, *U. atrum* 385 fulfils all requirements tested so far for a biological control agent so that the development and registration of a biocontrol product based on *U. atrum* 385 is feasible. Further steps are now needed towards commercial exploitation of the results by developing and marketing a biocontrol agent based on *U. atrum*.

1 Introduction

1.1 Microbial suppression of colonisation of necrotic tissues by necrotrophic fungal foliar pathogens

The different stages in the life cycle of pathogens and the vulnerability of each of these stages to biocontrol as well as ecological niche characteristics and antagonists attributes form the basis for the development of biocontrol concepts (Köhl and Fokkema, 1998). With this in mind possibilities and prospects for biocontrol of necrotrophic leaf pathogens through reduction of colonisation of necrotic tissues and subsequent sporulation will be discussed. Profound knowledge of the biology of the pathogen and its epidemiology is a prerequisite for the proper choice of the biocontrol strategies to be followed.

1.1.1 Biology of necrotrophic pathogens

1.1.1.1 Life cycle of necrotrophic plant pathogens and biocontrol strategies

Necrotrophic plant pathogens can only obtain nutrients from dead plant tissue. Consequently, for infection and colonisation of healthy leaves, host tissue has to be killed in advance. The life cycle of necrotrophic pathogens can be separated into three stages: the pathogenic stage during which healthy plant tissues are invaded and killed; the saprophytic and dissemination stage during which mycelium is developing in the necrotic parts of lesions or in other necrotic plant tissues, often resulting in sporulation, and the survival stage during which mycelium, sclerotia or spores are resting in crop debris or soil.

In many cases the infection by spores or mycelium of necrotrophic pathogens is dependent on or stimulated by exogenous nutrients such as pollen grains or honeydew (Chou and Preece, 1968; Fokkema 1973; Fokkema et al., 1983) and mediated by cutinolytic and cellwall-degrading enzymes. Immediately after infection or after a period of latency, the tissue surrounding the infection site is killed by pathogen-produced toxins or enzymes. Subsequently, the pathogen invades the freshly killed tissue utilising its nutrients. Tissue at the margin of the developing lesion is killed by the pathogen so that the lesion grows and the amount of substrate for pathogen colonisation increases. Mycelium within necrotic tissue contributes to the epidemiology of a necrotrophic pathogen in several ways: (1) The mycelium may infect directly adhering non-infected tissues so that the disease spreads within the plant or by direct contact to neighbouring plants. An example is the infection of leaf tissue via pathogen-colonised senesced petals that had fallen onto the leaf (Zhou and Reeleder, 1989). (2) Spores as inoculum for secondary spread of the disease in the

crop may be produced by the mycelium. (3) Sclerotia, melanised survival structures, may be produced that will form initial inoculum in following crops during later seasons. (4) Mycelium may survive during climatic conditions not favourable for fungal growth during summer in arid regions or during winter (Yunis and Elad, 1989; Rotem, 1990; Raposo et al., 2001).

The choice of disease control strategies depends on the sensitivity of the pathogen and the effectivity of the control agent during the different stages of the pathogens life cycle. Considering biological control it makes sense to distinguish between three strategies: (1) microbial suppression of infection directed at non-germinated or germinating spores as the initial stage of the pathogenic phase of the life cycle of the necrotrophic pathogen; (2) microbial suppression of mycelial colonisation and survival of the pathogen during its saprophytic stage in necrotic plant tissues as the source of initial or secondary inoculum initiating new infection cycles; and (3) microbial degradation of sclerotia as the initial inoculum in following growing seasons.

Two main aspects have to be considered that both highly determine the choice of suitable antagonists aimed at the prevention of infection or at the suppression of saprophytic growth: firstly, the time period for possible interactions between antagonist and pathogen. Longer time periods of interaction will favour slower biological mechanisms of interaction not based on immediate killing. Secondly, the ecological characteristics of the niche in which interactions have to take place, affecting the population dynamics of the introduced antagonist.

1.1.1.2 Time period for antagonist-pathogen interactions

Pathogen spores landed on an aerial plant surface may stay ungerminated for periods of days if conditions are not favourable for germination. The longer the exposure to detrimental factors such as UV radiation or high temperatures, the lower the infectivity of the spore was found (Rotem and Aust, 1991; Rotem et al., 1985). Changing nutrient levels on the leaf surface and leaching of nutrients from the spore by bacterial activity may also affect the infectivity of the spore (Brodie and Blakeman, 1976; Blakeman and Brodie, 1976). Probably, the spore becomes more sensitive to antagonistic mechanisms with time. However, the actual time periods for interaction between the ungerminated spore of the pathogen and an antagonist may be short since spore germination as well as antagonist activity is mostly determined by the same favourable microclimatic conditions. Thus, antagonists may interfere with the pathogen spore mainly during germination and during the infection process of the pathogen spore. The time needed for germination and infection depends on the pathogen and on environmental conditions. For *Botrytis cinerea* leaf wetness of 8 h at 15 °C or 4 h at 21 °C is sufficient for the infection of geranium flowers (Sirjusingh and Sutton, 1996). First infections of grape berries by *B. cinerea* can occur after 4 h wetness at temperatures between 12 °C and 30 °C (Broome et al., 1995). For the same pathogen, Nair and Allen (1993) found that at an optimum

temperature of 24 °C 63% of inoculated grape flowers were infected after 1.3 h. To prevent infections, antagonists must interfere with the germinating spores of pathogens during these limited time periods of a few hours before the infection process is completed. After the pathogen has penetrated the host tissue, it has escaped from further antagonistic activity by plant surface inhabitants unless antagonists also act via the plant by inducing resistance.

The period between infection and induction of necrosis can last hours in the case of highly infectious pathogens such as *Sclerotinia sclerotiorum* or weeks if latent periods are part of the disease process as is typical for *B. cinerea* in soft fruit or grapevine before ripening. After induction of necrosis, the pathogen rapidly colonises the killed tissue. The mycelium present in the lesions over a long period may continue to produce conidia as long as it is alive. Antagonism during substrate colonisation and utilisation may not be limited by such short interaction times as found on the leaf surface, since possible time periods are in the order of days or weeks, and if the pathogen mycelium survives in crop debris even in the order of months.

Besides plant tissue killed by the pathogen, also host tissues naturally died during aging or after abiotic or biotic injuries and necrotic tissues of non-hosts, e.g. weeds after herbicide applications, are suitable substrates for saprophytic colonisation by necrotrophic pathogens. Dependent on the crop, such substrates can form an essential source of inoculum of the pathogen. Examples are aged petals of beans, rapeseed, geranium or other crops fallen onto leaves where they act as exogenous nutrient source for mycelium and spores of *B. cinerea* or *S. sclerotiorum* enabling infections of the adjacent green leaf (Sirjusingh et al., 1996; Zhou and Reeleder, 1989). In kiwifruit orchards, shoots injured by wind or frost and subsequently saprophytically colonised by *B. cinerea*, produce conidia which serve as inoculum for fruit contamination later in the growing season (Elmer et al., 1993). As in pathogen-induced lesions, antagonists have long interaction periods of at least days to interfere with pathogen development. Antagonists may also affect the long-term survival of pathogen mycelium in lesions and crop debris during periods unfavourable for pathogen growth but not for the antagonist.

It is interesting that fungicides are mainly designed for interference with the infection process. Their immediate toxic nature makes them suitable for efficient prevention of infection during the limited period of time available. Fungicides are in general not targeted at the saprophytic or the survival stage of the pathogen within necrotic host tissue and crop debris. An active systemic spread of common fungicides in such substrates is impossible and a passive diffusion unlikely as most of the fungicide may be bound to organic compounds of the cell walls and inactivated on the surface. Furthermore, the uptake of chemicals by the pathogen may be much slower during resting stages of the fungus as compared to that of infection structures with high metabolic activity.

1.1.2 Ecological niche characteristics

Both pathogen and antagonist development in the phyllosphere is determined by several abiotic factors such as availability of nutrients, temperature, water availability, UV radiation and the deposition of agrochemicals. In contrast to the much more buffered soil systems, these environmental factors may rapidly change between extremes, e.g. on hot and dry summer days with cool and dewy nights. Detailed information on the microbial ecology of the phyllosphere can be found in the proceedings of several symposia (Preece and Dickinson, 1971; Dickinson and Preece, 1976; Blakeman, 1981; Fokkema and van den Heuvel, 1986; Andrews and Hirano, 1992; Morris et al., 1996).

1.1.2.1 Nutrients

On healthy plant surfaces, important nutrient sources are mainly deposits. Leachates from the host plant may form a nutritional background (Tukey, 1970) but play no important role as long as cell membranes are intact (Schönherr and Baur, 1996). Exogenous nutrient deposits on the leaf may consist of pollen grains, flower remains, insect honeydew and organic and inorganic dust. Pollen grains deposited on the leaf surface leaching high amounts of easily degradable nutrients such as amino acids and sugars, are an important nutrient source in the phyllosphere (Fokkema, 1971). The second important source, mainly containing sugars, is insect honeydew (Fokkema et al., 1983; Dik et al., 1991). The amount of nutrients available from these sources is not stable. Peaks in pollen deposition depend on the developmental stage of the crop and neighbouring vegetation. Similarly, honeydew secretion is not constant but is affected by e.g. aphid population dynamics and developmental stage of the host plant.
With respect to nutrient amounts and quality, necrotic leaf tissues markedly differ from healthy leaf surfaces. In necrotic tissues, amino acid and soluble sugar contents are low since these substances are relocated during senescence to tissues that are still green (Baddeley, 1971) or rapidly leached from the tissue. The remaining cell wall components such as cellulose, hemicellulose and lignin are the principal nutrient sources in necrotic tissues. These sources are more continuously flowing as compared to nutrient sources on green leaves. However, utilisation by saprophytic micro-organisms may be slower and adapted sets of enzymes are needed since highly complex polymers have to be degraded.

1.1.2.2 Microclimate

Water availability and temperature in the boundary layer of green leaves (Burrage, 1971) and in necrotic leaf tissues determine microbial growth. Rapid fluctuations in water availability and temperature are characteristic of these niches and are main factors limiting the development of microbial populations. Microbial colonisers of

leaf surfaces or of necrotic leaf tissue must have the capacity to survive during dry periods (Diem, 1971) and to utilise the limited leaf wetness periods for rapid colonisation of the substrate. Therefore, short lag times for mycelial regrowth (Park, 1982) or cell multiplication of single cell organisms are a prerequisite for successful colonisation.

Temperature and water dynamics can be different on green and necrotic leaf tissues. When exposed to the sun, the temperature on surfaces of healthy leaves will be regulated by water transpiration of the plant, but temperature of dry necrotic leaf tissue can substantially rise above ambient temperatures, e.g. a temperature of 42 °C was measured in necrotic onion leaf tips exposed to the sun while the air temperature was 31 °C (Köhl and Fokkema, 1994). The affinity of dry necrotic leaf tissue to water is low so that wetting of the tissue takes longer compared to green leaf surfaces. However, leaf wetness periods in necrotic tissue may be longer than on leaf surfaces since necrotic tissues have a great capacity to retain water. The ability to be active under conditions of low water potential has been pointed out as a major factor determining the ecological competence of saprophytes colonising necrotic tissues such as straw laying on soil or incorporated into soil (Magan and Lynch, 1986; Pfender et al., 1991). During the initial stage of the drying process of wetted necrotic tissue, its water potential does not substantially change. After most of the water is evaporated, small changes of the water content result in a sharp decrease of the water potential as demonstrated by Köhl et al. (3.2.1.4; 1995c) for dead onion leaves. Different from the more buffered situation in necrotic tissues in or on soil, water potentials between –3 MPa and –7 MPa were found in necrotic onion leaf tips under field conditions only for time periods mostly shorter than 30 min. during the wetting or drying process of above-ground necrotic tissues (Köhl et al., 1993). The ecological competence of saprophytes colonising such above-ground tissues may thus substantially be determined by their ability to withstand such rapid changes between high water potentials suitable for optimum growth and those too low for any microbial growth.

UV radiation is another detrimental factor for microbial colonisation of leaf surfaces. Exposure of spores on leaf surfaces to direct UV radiation reduces their longevity (Rotem et al., 1985). Protection against UV radiation during the precolonisation stage is often achieved by pigmentation or cell clustering of the spores. After having invaded plant tissue, fungal mycelium is protected from the detrimental effect of sunlight (Rotem and Aust, 1991).

1.1.3 Antagonists attributes

1.1.3.1 Mechanisms involved in antagonism

Several mechanisms, operating alone or in concert, are known to be involved in antagonistic interactions in the phyllosphere. Nutrient competition, antibiosis and

mycoparasitism are the major mechanisms, not essentially differing from mechanisms operating in the soil or rhizosphere. Additional mechanisms such as induced resistance, production of biosurfactants, interference with pathogen-related enzymes, and undoubtedly a number of still unknown mechanisms may complete the microbial arsenal, reviewed by Elad (1996). Particularly mechanistic studies have benefited from molecular biology by identifying, deleting and supplementing genes responsible for e.g. antibiotic production.
Knowledge of mechanisms involved in biocontrol is important for estimating and predicting its reliability and selection of better strains. Besides other criteria, the choice of an antagonist with its characteristic mechanisms depends on the stage of the life cycle of the pathogen the antagonist is aimed at. Allowable interaction times and niche characteristics determine the suitability of certain modes of action during different developmental stages of the pathogen.

Nutrient competition. Nutrient competition is involved in all situations in which the antagonist consumes nutrients which otherwise may be utilised by the pathogen. The extent to which this reduces infection may vary with the infection strategy of the pathogen involved. In early studies (Fokkema, 1971) on the role of pollen on infection of rye leaves by *Cochliobolus sativus*, *Septoria nodorum* and *Puccinia recondita* f.sp. *recondita* is was found that spore germination and even more the superficial growth of mycelium of the two necrotrophic pathogens was highly stimulated by the presence of pollen, resulting in more penetration sites and an up to tenfold increase of necrotic leaf area, whereas infection by the biotrophic rust was not enhanced. There was a positive correlation between the superficial mycelium density of *C. sativus* two to three days after inoculation and the necrotic leaf area. Presence of phyllosphere yeasts reduced the enhanced mycelium density and the subsequent necrosis (Fokkema, 1973). Large exogenous nutrient sources such as pollen and aphid honeydew (Fokkema et al., 1983; Dik et al., 1991) can rapidly be removed by phyllosphere yeasts. In the absence of these and similar resources such as flower remains, micro-organisms may compete for leaf exudates and endogenous spore reserves. Blakeman and Brodie (1976) demonstrated that in the phyllosphere bacterial activity similar to soil fungistasis (Lockwood, 1988) inhibits spore germination of *B. cinerea*, not only by competition for exogenous nutrients, particularly amino acids (Brodie and Blakeman, 1975, 1976; Blakeman and Brodie, 1977), but also by utilisation of nutrients leached from ungerminated spores during water uptake. Using ^{14}C-labelled glucose and amino acids and autoradiography Edwards and Blakeman (1984) showed that in the presence of *Sporobolomyces* sp. or *Pseudomonas fluorescens* and *B. cinerea* the nutrients were partitioned in favour of the antagonists. On detached wheat leaves, *Sporobolomyces roseus* separated from conidia of *C. sativus* and the leaf by cellophane inhibited spore germination, but this was restored when the cellophane with the yeasts had been removed, indicating that yeasts may operate by a constant nutrient stress and that no other mechanisms were involved in this interaction (Fokkema, 1984). Subsequent to these results it was questioned

whether microbial nutrient stress could also affect the physiology of the leaf resulting in early senescence. Analysis of field observations and direct studies, however, revealed no detrimental effect of phyllosphere yeasts (Frossard et al., 1983).
Nutrient stress is only a suitable mechanism of antagonism for pathogens which are sensitive to it and of which infection thus can be stimulated by nutrient addition (e.g. 1% sucrose + 0.5% yeast extract). Consequently biotrophic pathogens, e.g. rusts and mildews, are not affected by nutrient competition, but necrotrophic pathogens generally are. *Colletotrichum* spp. may form an interesting group, because *P. fluorescens* increased the number of melanised appressoria of *C. acutatum* (Blakeman and Parbery, 1977) and *C. coccodes*, a potential mycoherbicide of velvetleaf (Fernando et al., 1994, 1996).
On the other hand yeasts reduced infection of maize by *C. graminicola* by 50% by reducing penetration from the appressoria without any effect on germination, superficial mycelium, and appressoria formation (Williamson and Fokkema, 1985).
The outcome of nutrient competition seems very subtle with respect to *Colletotrichum* spp. and may vary with the efficiency to remove the nutrients and prevailing environmental conditions. Competition is mostly studied with respect to C and N sources. The possible involvement of siderophores or artificial iron-chelating agents in suppressing or stimulating leaf pathogens is intriguing (Swinburne, 1981; Fernando et al. 1996) but will not be discussed in detail since the production of bacterial siderophores in the phyllosphere has not been clearly demonstrated.
Unfortunately research on nutrient competition can profit less from molecular techniques than research on other mechanisms in which a single product is involved. Consequently nutrient competition is often the last option, investigated only when other mechanisms seem not to be involved, but this is not indicative of its importance.

Antibiotics. Antibiotic-producing micro-organisms were often considered first in searching for biocontrol agents since the selection of micro-organisms with the potential to produce antibiotics can be realised with little effort in screening on agar or in liquid culture. Inhibition zones can be quantified and antibiotic substances characterised. The screening for antibiotic production by candidate antagonists on artificial media is an adequate tool to detect antibiotics as basic structures for new fungicide groups. In fact the phenylpyrrole group of fungicides is obtained from pyrrolnitrin (De Waard et al., 1993), an antibiotic produced by e.g. *P. cepacia* (Janisiewicz and Roitman, 1988). However, the use of *in vitro* assays for a first selection of antagonists among unknown micro-organisms is questionable. Inhibition zones in *in vitro* interactions seldom predict for field performance of a biocontrol agent. Moreover, these zones may also be caused by stailing products formed by the pathogen (Fokkema, 1973).
Production of antibiotic secondary metabolites is common for many micro-organisms. The effect of antibiotics shows much similarity with the use of fungi-

cides. The presence of antibiotics on the leaf surface produced by antagonists may ensure efficient control of pathogens also during the characteristically short time periods during the infection process, since disorganisation of cytoplasmic structures of host cells can be observed after short interaction times (Bélanger et al., 1995). Biocontrol agents producing antibiotics are often viewed as *in situ* producers of chemical control agents (Upper, 1992). However, the amounts of antibiotics produced by antagonists depend on its population density and on environmental conditions (Leifert et al., 1995).

Several antibiotic-producing bacteria are candidates for use in biological control of necrotrophic leaf pathogens. Examples are *Bacillus brevis* producing gramicidin S with high *in vitro* activity against *B. cinerea* (Edwards and Seddon, 1992) and *B. subtilis* CL27 producing two antibiotics with high activity against the same pathogen (Leifert et al., 1995). Reports on antibiotic production by yeasts to be exploited in biocontrol are rare. The yeast-like fungus *Aureobasidium pullulans* and several yeasts are found to produce a range of antibacterial compounds (McCormack et al., 1994).

Several antibiotic-producing hyphal fungi have been used in biocontrol studies. Examples are *Epicoccum nigrum* producing antibiotic compounds effective against *B. cinerea* (Hannusch and Boland, 1996a), *S. sclerotinia* (Hannusch and Boland 1996b; Zhou and Reeleder, 1989; 1991) and *Monilinia laxa* (Madrigal et al., 1994) and *Chaetomium globosum*, an effective antagonist of *Venturia inaequalis* (Heye and Andrews, 1983; Boudreau and Andrews, 1987), producing two antifungal substances (Di Pietro et al., 1992). The antagonism of a strain of *Trichoderma harzianum* seems also to be based on antibiotics causing disorganisation of the cytoplasm within 12 h and subsequent cell death of *B. cinerea* (Bélanger et al., 1995).

Antagonistic activity may continue for some time also when environmental conditions become unfavourable for antagonist growth. This may explain the stable effect of plant treatments with suspensions of *E. nigrum* on *B. cinerea* and *S. sclerotiorum* on bean leaves under a broad range of temperatures and relative humidities (Hannusch and Boland, 1996a; b). The presence of antibiotics may also explain the antagonistic activity of *C. globosum* against *B. cinerea* under certain field conditions independent of the presence of an actively growing population of the antagonist (3.2.2.4; Köhl et al., 1995b).

Two factors limit the utilisation of antibiotics. Firstly, antibiotics must continuously be present on the surface to protect the leaf from new infections. However, antibiotics are generally not stable under field conditions for long periods or may be bound on plant surfaces. As a consequence, antagonist populations must continuously be present in sufficiently high densities to produce new antibiotics. Bacterial antagonists successfully controlled *Phytophthora infestans* under controlled environmental conditions where high humidities were maintained but not in the field (Jongebloed et al., 1993). This discrepancy between field and controlled environmental conditions in the biocontrol of *P. infestans* suggests that the antibiotics held responsible for the effect, decline under field conditions as rapidly as the antagonistic population. A breakdown by abiotic factors was found for antibiotics pro-

duced by *C. globosum*, an antagonist applied against *V. inaequalis* in apple (Boudreau and Andrews, 1987), explaining why field efficacy of the antagonist was not sufficient. Gramicidin S produced by *B. brevis*, efficiently reducing growth of *B. cinerea in vitro*, was found to be strongly adsorbed to leaf surfaces (Edwards and Seddon, 1992). No disease control activity was found by the inactivated adsorbed antibiotic.
Secondly, the pathogen may build-up resistance against the antibiotic e.g. *Botrytis cinerea*, a pathogen frequently found to become resistant against fungicides, also developed resistance against two antibiotics produced by *Bacillus subtilis* after nine subsequent crops of *Astilbe* had been sprayed with the antagonist (Li and Leifert, 1994).

Mycoparasitism. Mycoparasitism is described for many fungus-fungus relationships. Enzymes degrading fungal cell walls such as chitinases and beta-glucanases are commonly produced by hyperparasites (Elad et al., 1982). Parasitism depends on close contact between antagonist and host, on the secretion of enzymes and on the active growth of the hyperparasite into the host. These processes need time so that it is unlikely that infection structures of pathogens can be parasitised and killed rapidly enough to prevent penetration of the host plant. When studying the chronological events during the interaction between *T. harzianum* and *B. cinerea* at ultra-structural level, Bélanger et al. (1995) found evidence for antibiosis early during interaction within the first 12 h, but clear chitinolytic activity of the antagonist could not be demonstrated before the tenth day of interaction.
Mycoparasitism is often exploited for biological control of biotrophic pathogens (Kranz, 1981), e.g. *Verticillium lecanii* parasitising on rusts (Spencer, 1980) and *Ampelomyces quisqualis* or *Verticillium lecanii* parasitising on powdery mildews of cucumber or roses (Philipp and Crüger, 1979; Verhaar et al., 1993). Parasitism is then aimed at pathogen mycelium already established on the host and reduced sporulation of the pathogen limits its dissemination.
Mycelial development of necrotrophic pathogens can also be controlled by mycoparasites. The mycoparasite *Coniothyrium minitans* penetrates into mycelium of *S. sclerotiorum* that subsequently collapses and becomes necrotic (Whipps and Gerlagh, 1992). The production of beta-1,3 glucanases and chitinases enables the mycoparasite to utilise the host cells; mycelium of the mycoparasite proliferates around dead hyphae of the host fungus. Above all, *C. minitans* is a potent parasite of sclerotia of *S. sclerotiorum* reducing the survival of sclerotia in field trials by c. 90% (Gerlagh et al., 1995). Also the antagonist *Limonomyces roseipellis* has chitinolytic activity and it is suggested that mycoparasitism is one of the mechanisms involved in its antagonism against *Pyrenophora tritici-repentis* in the debris of wheat crops (Pfender, 1988).

Induced resistance. Host resistance induced by phyllosphere micro-organisms as a mechanism of biocontrol has been studied in detail by Schönbeck and Dehne (1986) with respect to powdery and downy mildews and rusts. Cultures filtrates with bacterial metabolites induced in wheat against powdery mildew responses

similar to those associated with partial resistance. Repeated field applications reduced yield losses caused by *Erisyphe graminis* (Dehne et al., 1984). Induced resistance as a mechanism for biocontrol may have the advantage that, once resistance has been induced, high population densities of the antagonists may no longer be required. However, there is only some circumstantial evidence for the involvement of induced resistance in biocontrol of necrotrophic leaf pathogens (Elad et al., 1994 a; b). Induction of resistance is currently particularly important as a mechanism of newly developed environmentally friendly fungicides (Kessmann et al., 1994). Since induction of resistance requires metabolically active plant cells, this mechanism cannot be exploited in necrotic tissues.

Change of leaf surface wettability. The change of the leaf wettability by surface active bacteria is a common natural phenomenon. Such changes can affect the leaf wetness duration, the redistribution of nutrients and micro-organisms and their attachment to leaves (Bunster et al., 1989). Treatments of Chinese cabbage with a suspension containing *B. brevis* significantly reduced infections by *B. cinerea* by 65 to 71% in experiments in polythene tunnels (Edwards and Seddon, 1992). It could be demonstrated that leaf wetness of Chinese cabbage was shorter when sprayed with the bacterial suspension as compared to the water control treatment. Leaf wetness after overhead irrigation was four times shorter for leaves treated with *B. brevis* (Seddon and Edwards, 1993; Seddon et al., 1997). It was postulated that this change of water availability limited the germination of *B. cinerea* conidia. Antagonists producing biosurfactants reducing leaf wetness periods may be utilised against a wide range of fungi since germination and subsequent infection of pathogen spores depends on a minimum time period during which free water is available on the surface.

Interference with enzyme production of pathogen. During the infection process cutinolytic, pectinolytic and cellulolytic enzymes are produced by pathogens to allow penetration through the cuticula and into host tissues. Zimand et al. (1995) found that the enzymatic activity of pectinolytic enzymes, polygalacturonase, pectin-methyl-esterase and pectate-lyase, produced by *B. cinerea* was less in the presence of the antagonist *Trichoderma harzianum* T-39 and that disease of bean leaves was reduced. They stated that the antagonism of that specific isolate is partly based on direct or indirect effects on the enzyme production of the pathogen during the infection process.
The enzyme production of pathogens is increased by leachates from pollen grains as shown for polygalacturonases and cellulases of *Helminthosporium sativum* by Fokkema (1971). Reduced enzyme production may be a result of the absence of such inducing substances on the leaf surface and thus indirectly the effect of nutrient competition between pathogen and antagonist such as yeasts consuming pollen leachates. In experiments with *Colletotrichum graminicola*, the infection frequency on maize leaves was reduced by 50% in the presence of yeasts

(Williamson and Fokkema, 1985) but no reduction of spore germination, superficial growth and appressorium production of the pathogen before host penetration was found as in other yeast-pathogen interactions e.g. with *Cochliobolus sativus* (Fokkema, 1984). Instead, penetration from the appressoria was reduced by 50% and it was suggested that the nutrient sink produced by yeasts led to a reduced production of enzymes involved in cell-wall penetration.

1.1.3.2 Ecological competence as selection criterion for antagonists

The leaf surface environment differs in many aspects from soil as the other habitat in which biological control has been applied. In soil habitats, environmental conditions may vary between sites but are relatively stable in time compared to the rapid fluctuations in the phyllosphere. The introduction of soil inhabiting antagonists into the above-ground environment may fail because they are not adapted to the harsh microclimatic conditions. Since the successful establishment of antagonist populations is a prerequisite for long-lasting control and the control efficacy is generally related to population sizes of the antagonist, the ecological competence of antagonists is a key selection factor for biocontrol agents. Generally, the antagonist should be equally or preferably be better adapted to adverse environmental factors than its target pathogen in order to become a successful biocontrol agent. Valid selection criteria to identify antagonists with high ecological competence can be derived from thorough knowledge of the practical cropping situation. Antagonist inoculum must survive during unfavourable conditions, e.g. high temperatures or dryness. Mechanisms to increase survival are the production of slime, protecting cells from drying, or pigmentation, protecting from detrimental effects of UV radiation (Dickinson, 1976) as also found for colonisers of stone surfaces (Gutiérrez et al., 1995).

Antagonists must reach high growth rates under favourable conditions during leaf wetness at moderate temperatures and with sufficient nutrient supply as well as under marginal conditions e.g. at low temperatures or at low water potentials. Pathogens and their potential antagonists can markedly differ in their activity at low water potentials and low temperatures. Pfender et al. (1991) compared the potential of antagonists to suppress the colonisation of wheat straw by *Pyrenophora tritici-repentis* at low water potentials. They found that relatively few antagonists such as *Acremonium terricola*, *Epicoccum nigrum*, *Myrothecium roridum* and *Stachybotrys* sp. could compete with the pathogen at –7 MPa. In experiments aimed at the suppression of sporulation of *Botrytis* spp. on necrotic leaf tissues, *Gliocladium roseum* and *Trichoderma viride* were highly effective in moist chambers (Köhl et al., 1992). When the water potential of dead leaf tissues was reduced to –6.6 MPa, *G. roseum* was still effective but *T. viride* failed to control *Botrytis* sporulation. Köhl and Schlösser (1988) found that isolates of *T. viride* grew better at low temperatures than those of other *Trichoderma* spp., and destroyed sclerotia of *B. cinerea* even at 5 °C in experiments under controlled conditions (Köhl and Schlösser, 1989).

Particularly for above-ground applications, antagonists must be adapted to rapid changes between unfavourable and favourable conditions with short lag-times for regrowth e.g. to profit from short periods of leaf wetness. Zhang and Pfender (1993) investigated the effect of wetness periods on straw-colonising antagonists suppressing ascocarp formation of *P. tritici-repentis*. The antagonists *Laetisaria arvalis* and *Limonomyces roseipellis* reduced ascocarp formation when wetness periods lasted 24 or 48 h but not when wetness periods were shorter.
Knowledge about the autecology of candidate antagonists helps to restrict the number of isolates that have to be tested under more practical and complex conditions. However, their synecology in the natural systems is the criterion that finally will determine their competitive ability under the practical conditions (Andrews, 1990).

1.1.4 Biological control through microbial suppression of pathogen colonisation of necrotic tissues and subsequent sporulation

The exploitation of antagonistic interactions during the saprophytic stage of a necrotrophic pathogen has the advantage of significantly longer interaction times between antagonist and pathogen as compared to interactions during the infection process of fungal spores (Fokkema, 1993). Theoretically, longer interaction times increase the chance of successful antagonistic interactions and consequently the reliability of biocontrol. Antagonism in necrotic tissue can be based on nutrient competition, mycoparasitism or antibiosis (Heye and Andrews, 1983; Pfender, 1988).
Antagonists in necrotic tissue may control infection of healthy tissue adjacent to necrotic tissue and can suppress sporulation of the pathogen. Although with the latter there is no direct effect on symptom development as with the prevention of infection, the long-term effect on disease epidemics is evident. Antagonists reduce the number of spores produced by the pathogen on the necrotic tissue so that the initial disease pressure or the secondary dissemination of the disease within a crop is limited. This principle of sporulation suppression for biological control has successfully been applied with biotrophic pathogens, where other principles e.g. nutrient competition between antagonist and infecting spore of the pathogen cannot be exploited. Epidemics of *Puccinia recondita* in wheat can be affected by the rust mycoparasite *Eudarluca caricis* so that the final disease severity can be reduced by 60 to 80% as evaluated by Hau and Kranz (1978) using model-computation. *Bacillus subtilis* reduces spore production and spore viability of *Uromyces appendiculatus* on leaves of *Phaseolus vulgaris* and a retardation of rust epidemics by this antagonist is expected (Mizubuti et al., 1995). Powdery mildew of cucumber and roses can be

controlled by *Ampelomyces quisqualis* or *Sporotrix fugilosa*, respectively (Philipp and Crüger, 1979; Bélanger et al., 1994)
The impact of reduced inoculum levels of necrotrophic pathogens on diseases can be demonstrated by results of investigations on the epidemiological impact of debris as primary inoculum source and on the relationships between initial or secondary inoculum quantities and disease progression.

1.1.4.1 Reduction of inoculum load

Debris as carrier of initial inoculum and relationship between initial inoculum and disease progression. Crop debris containing mycelium, sclerotia or fruiting bodies of necrotrophic pathogens are the main sources of initial inoculum for epidemics of diseases such as caused by *Botrytis cinerea* (Braun and Sutton, 1987; Nair and Nadtotchei, 1987; López-Herrera et al., 1994; Yunis and Elad, 1989), *Pyrenophora tritici-repentis* (Adee and Pfender, 1989), *Cercospora zeae-maydis* (De Nazareno et al., 1993), *Venturia inaequalis* (Heye and Andrews, 1983) or *Alternaria macrospora* (Rotem, 1990).
Eradication of the inoculum surviving in debris by means of antagonist application may retard disease progression. In a field experiment, Rotem (1990) investigated the effect of the presence of crop debris as a source of initial inoculum on the epidemic of a disease in cotton caused by *A. macrospora*. The onset of the epidemic was four weeks earlier in the presence of crop debris compared to debris-free plots. After eight weeks all plants showed lesions in the presence of debris compared to only 3% of the plants in debris-free plots. Soil solarisation can be an effective means to eradicate pathogen inoculum, e.g. mycelium and sclerotia of *B. cinerea* in debris within greenhouses as demonstrated by López-Herrera et al. (1994). Tillage systems leaving corn residues on the soil surface favour grey leaf spot development, caused by *C. zeae-maydis*, of the following corn crop (Payne et al., 1987; De Nazareno et al., 1993). In both field studies, the effect of different levels of corn residues on the soil-surface, due to different tillage systems or created by artificial spread in the experimental plots, respectively, on grey leaf spot epidemics were investigated. Significant increases of grey leaf spot development were found with higher residue levels and disease severities correlated with residue amounts as initial inoculum sources.
However, the sole reduction of initial inoculum of polycyclic disease may not be sufficient in all cropping systems. Pathogens such as *U. phaseoli* in bean, *C. arachidicola* in peanut and *B. cinerea* in begonia can compensate initial low inoculum doses by accelerated rates of disease progression (Plaut and Berger, 1981). Furthermore, the progression of epidemics may more depend on environmental conditions than on the level of initial inoculum as shown for leaf spot diseases in alfalfa (*Medicago sativa*) caused by *Leptosphaerulina trifolii*, *Phoma medicaginis*, *Stemphylium botryosum* and *C. medicaginis* (Duthie and Campbell, 1991). Pfender et al. (1993) found that a reduction of the primary inoculum of *P. tritici-repentis* formed on wheat straw by 60 to

80% was not sufficient to control polycyclic epidemics of tan spot in wheat. They postulated that a reduction of more than 90% of initial inoculum is necessary to control tan spot epidemics.
In strawberries, *B. cinerea* produces conidia on overwintering leaves. Such conidia may infect flowers, causing substantial yield losses. In this monocyclic process, control of sporulation of *B. cinerea* by early spring applications of fungicides onto overwintering leaves reduced flower infections later in the season (Jordan and Pappas, 1977; Braun and Sutton, 1986). *Sclerotinia sclerotiorum* produces ascospores arising from sclerotia as primary inoculum and no secondary dissemination via conidia occurs. A reduction of the soil infestation with sclerotia can lead to a reduced disease incidence in following crops (Gerlagh et al., 1995).

Relationship between secondary inoculum and disease progression. Once initiated, disease progression depends on several disease components: infection frequency, latent period, lesion size and sporulation capacity. Applications of antagonists interfering with the pathogen in lesions may substantially reduce spore production per unit area of lesions. Antagonists may additionally slow down lesion expansion.
Leonard and Mundt (1984) applied a model to estimate the epidemiological effects of reproduction per generation and latent period of biotrophic leaf pathogens in relation to breeding of cultivars with increased quantitative polygenic resistance. They found that for pathogens with short latent periods, a reduction of the reproduction per generation is more effective than to increase the latent period.
Sache and de Vallavieille-Pope (1995) analysed the sporulation and infection characteristics of a range of airborne plant pathogens. They stated that for necrotrophic leaf pathogens with short latent periods and large sporulation capacity disease control methods decreasing the sporulation capacity would be of great benefit.
The validity of the reduction of spore production as a strategy for biological control can be illustrated by epidemiological studies of necrotrophic pathogens. Suppression of spore production aimed at a slower build-up of epidemics is utilised in Integrated Pest Management (IPM) programs and breeding programs. When fungicides are applied protectively, infection frequencies may substantially be reduced so that the pathogen cannot develop sporulating lesions. In IPM programs, without continuous presence of fungicides on the leaf, the pathogen may successfully infect the host and complete its cycle resulting in sporulating lesions. In such systems, fungicides should affect the pathogen also during its post-infection development. For peanut late leaf blight (*Cercosporidium personatum*), Labrinos and Nutter (1993) investigated the effect of chlorothalonil and tebuconazole on the disease components. They found that tebuconazole reduced sporulation of *C. personatum* for more than 95% in lesions of field grown peanuts and stated that this will result in a large negative effect on disease progress in the field.
In breeding programs for durable partial resistance, selection of genotypes is aimed at slower disease progression. When analysing the relationships between disease

components and disease progression of early leaf spot (*C. arachidicola*) in several lines of peanuts, Johnson et al. (1986) found that the percentage of lesions with sporulation and the spore production in lesions highly correlate with the rate of disease development. A similar high correlation between spore production in lesions and the rate of disease progression was found in different genotypes of peanuts for peanut late leaf spot (*C. personatum*) (Aquino et al., 1995). Also the slower disease development of grey leaf spot in a moderately resistant genotype of corn as compared to a susceptible genotype was found to be due to a lower number of lesions with sporulation and a lower spore production in lesions of *C. zeae-maydis* (Ringer and Grybauskas, 1995).

1.1.4.2 Naturally occurring suppression of pathogen development in necrotic tissues

Bacteria, yeasts and spores of saprophytic fungi are common in the phyllosphere. Once the leaf is senescing, it becomes available as nutrient source for saprophytes. The different saprophytes present on the substrate, including necrotrophic pathogens during their saprophytic stage, compete during substrate colonisation, where inoculum density and competitive saprophytic ability of the different groups determine the share of the different populations. Hyphal fungi with their ability to spread actively through the substrate by hyphal growth become more dominant compared to single cell organisms. Typical microbial communities of hyphal fungi develop with a succession of different species during the senescence and decomposition of leaves. Primary colonisers are *Cladosporium herbarum*, *C. cladosporioides*, *Alternaria* spp., *Aureobasidium pullulans*, *Epicoccum nigrum* and *Botrytis cinerea* (Hudson and Webster, 1958; Hudson, 1971). Pfender and Wootke (1988) found that *Pyrenophora tritici-repentis*, *Septoria nodorum*, *Alternaria* spp. and *Cladosporium* spp. colonised wheat straw as primary invaders, secondarily followed by *Arcemonium strictum*, *Diplodia* spp. and *Fusarium* spp. Growth and spreading of necrotrophic pathogens, also being primary invaders of necrotic leaf tissues, is limited by this natural phenomenon but its impact on epidemics of diseases is hard to quantify. However, cultural practises making use of naturally occurring saprophytic fungi to reduce necrotrophic pathogens on crop debris demonstrate the importance of naturally occurring disease control in necrotic tissues. The airborne saprophytic fungi *Penicillium*, *Aspergillus*, *Alternaria* and *Stemphylium* can colonise wheat straw before its incorporation into soil. Straw colonised by these saprophytes could not be colonised by the pathogenic *Fusarium roseum* f.sp. *cerealis* 'Culmorum' after burial into soil (Cook, 1970). Pseudothecia produced on leaf litter are a main source of the primary inoculum of *Venturia inaequalis* causing apple scab. The leaf litter can naturally be colonised by common saprophytes. Colonisation by these naturally occurring fungi such as *Cladosporium*, *Alternaria* and *Fusarium* can be enhanced by spraying the litter with urea, leading to an accelerated decomposition with a lower risk of pseudothecia formation (Burchill and Cook, 1971; Thakur and Sharma,

1999; Carisse et al., 2000). Boland and Hunter (1988) found that *Alternaria alternata* and *Cladosporium cladosporioides* were frequently colonising blossoms of field-grown beans. When field-sampled blossoms were inoculated with ascospores of *Sclerotinia sclerotiorum* and incubated at high humidity, the senescent petals were colonised by the saprophytes and about 50% of the blossoms failed to develop white mould. These examples also demonstrate the substantial contribution of naturally occurring saprophytes to disease control.

1.1.4.3 Suppression of pathogen development in necrotic tissues by introduced micro-organisms

Yeasts and bacteria, lacking hyphal growth and being sensitive to dry conditions, are no strong competitors during the colonisation of necrotic tissues and thus are rarely found during the natural succession of such substrates. So far, bacteria and yeasts have not been exploited for biocontrol in necrotic leaf tissues.
On the other hand, representatives of hyphal fungi as the dominating group during the natural microbial succession in senesced leaf tissues have been selected for applications to necrotic plant tissues aimed at the suppression of colonisation of these tissues by necrotrophic pathogens. Different kinds of necrotic plant tissues can serve as substrate and thus as inoculum source of necrotrophic pathogens: the necrotic part of lesions initiated by the pathogen, plant parts naturally senesced such as petals or killed by abiotic injuries such as frost, wind or herbicides, and crop debris. Several antagonistic fungi have been successfully used to control pathogen development on such different substrates.

Necrotic lesions. Although many necrotrophic pathogens sporulate on lesions, microbial interactions within the lesion have hardly been investigated. Biles and Hill (1988) observed a reduction of the sporulation capacity of *Cochliobolus sativus* by 47% when lesions had been sprayed with *T. harzianum* in experiments conducted at 26 °C under continuously moist conditions, the optimum for sporulation of the pathogen. Several saprophytic hyphal fungi, yeasts and a *Pseudomonas* sp. could reduce lesion expansion and sporulation of *B. cinerea* under controlled conditions when applied to established lesions on bean leaves (Elad et al., 1994a; b). Also Szandala and Backhouse (2001) found that antagonists such as *Epicoccum nigrum*, *Gliocladium roseum* and *Trichoderma harzianum* reduce sporulation of *B. cinerea* in bean leaf assays when antagonists were applied up to 120 h after infection by the pathogen. Biocontrol in necrotic lesions requires further research under field conditions and may have been underestimated so far.

Senescing leaves. Peng and Sutton (1991) selected antagonists that reduced sporulation of *B. cinerea* on artificially killed leaf discs of strawberry. Amongst 230 microbial isolates tested, isolates of *A. alternata*, *Gliocladium roseum*, *Penicillium* sp., *Trichoderma viride*, *Colletotrichum gloeosporioides*, *Epicoccum purpurascens* and *Trichothecium*

roseum suppressed *B. cinerea* sporulation by almost 100%. In experiments under field conditions, *G. roseum* consistently reduced sporulation incidence and fruit infection of strawberry. It was found that control of sporulation was best when the antagonist was applied to green leaves before they senesced (Sutton and Peng, 1993). The antagonist is able to penetrate the green leaf and to colonise epidermal cells without harming host plants such as strawberry, red raspberry and roses (Sutton, 1994; Yu and Sutton, 1999; Morandi et al., 2000; Morandi et al., 2001). Once the leaf is dying, the antagonist progressively colonises the dead leaf and excludes *B. cinerea* from the substrate. However, the antagonist is not able to replace *B. cinerea* in leaf tissues that are already colonised by the pathogen.

Senescing petals. Flower remains adhering to developing fruits can saprophytically be colonised by necrotrophic pathogens. They then act as the exogenous nutrient source needed by the pathogen to be able to infect healthy tissue. The application of saprophytes to such flower remains can lead to an exclusion of the pathogen from the necrotic tissue and thus to a protection of the adjacent fruit. This principle was already explored in 1957 by Newhook (1957). He sprayed *C. herbarum* or *Penicillium* sp. on petals adhering to tomato flowers under greenhouse conditions with increased humidity and inoculated the plants seven days later with *B. cinerea*. Fruits of plants not treated with saprophytic antagonists showed 31 to 42% rot, whereas those treated with antagonists rotted for 0 to 3%.
Boland and Inglis (1989) evaluated the saprophytic mycoflora of bean and rapeseed flowers. *Alternaria* spp. and *Cladosporium* spp., isolated from 65 to 100% of surface-sterilised flowers were the dominating fungi. The role of these saprophytes in naturally occurring reduction of white mould development had been demonstrated by Boland and Hunter (1988). The antagonistic properties of saprophytic fungi were evaluated in screening experiments using petals inoculated with *S. sclerotiorum* and the candidate antagonists. The petals were placed on detached bean leaves and lesion development on the leaves was quantified. *Drechslera* sp., *Epicoccum purpurascens*, *Fusarium graminearum* and *F. heterosporum* were found to be the most effective isolates. In similar experiments, Nelson and Powelson (1988) applied *T. hamatum* and *B. cinerea* to detached petals of snap beans that were laid on healthy pods. After incubation under continuously moist conditions, the antagonist treatment reduced pod rot by 94% compared to the untreated control.
Zhou and Reeleder (1989) used *E. purpurascens* in greenhouse and field experiments with snap beans. The antagonist colonised all senescent petals sampled from treated plants, frequently covering the petal surface completely. *E. purpurascens* was able to germinate and to produce mycelium on the surface of both young emerging flowers and senescent flowers (Zhou and Reeleder, 1991). However, no acceleration of senescence of young flowers was observed and penetration of the tissue was only found with senescent petals. Field applications of *E. purpurascens* led to significantly reduced disease incidence and percentage of diseased pots (Zhou and Reeleder, 1989).

Senesced petals of male and female kiwifruit flowers colonised by saprophytically growing *B. cinerea* are an important source of conidia of the pathogen contaminating the surface of the developing fruit (Elmer et al., 1993). Saprophytes such as *A. alternata*, *E. nigrum* and *Ulocladium atrum* successfully reduced sporulation of *B. cinerea* on petals in bioassays under controlled conditions as well as on petals that had been exposed under field conditions (Elmer, Walter and Köhl, unpublished).

Crop residues. Andrews et al. (1983) investigated antagonists interfering with the colonisation of apple leaf by the imperfect stage of *V. inaequalis*. Amongst the selected antagonists, two were also tested against the perfect stage of the fungus. When leaves were inoculated with *Athelia bombacina* or *Chaetomium globosum*, in autumn and thereafter left on the orchard floor, the production of ascospores on such leaves was reduced to 0 or 10% compared to untreated leaves (Heye and Andrews, 1983). Similar results were achieved by Miedtke and Kennel (1990). The antagonist *A. bombacina* also induced softening of leaf tissue, indicating an accelerated decomposition due to a high cellulolytic activity. Carisse et al. (2000) applied *Microsphaeropsis* sp. to overwintering leaves resulting in significant and consistent reduction in ascospore production of *V. inaequalis*. The impact of reduced ascospore productions on apple scab epidemics still has to be demonstrated.

In conservation tillage systems, antagonist applications are a promising means to prevent the build-up of primary inoculum. Reduction of survival of mycelium of pathogens in crop residues may be achieved with the same antagonists as used for the retardation of the dissemination of a disease during the growing season. However, as crop residues often are incorporated into soil or form a layer on the soil, environmental conditions differ from those of above-ground necrotic tissues. Soil fungi such as *Trichoderma* spp., less adapted to the phyllosphere, may also be suitable candidate antagonists. Infested crop residues are the source of initial inoculum for several major diseases of soybean (*Glycine max* (L.) Merr.) in Brazil. When crop residues were treated with *T. harzianum* in an outdoor experiment, the incidence of the pathogens *Macrophomina phaseolina*, *Glomerella glycines* and *Fusarium* spp. such as *F. graminearum* was significantly reduced (Fernandez, 1992). Inoculation of field grown barley stubble, infested with *Drechslera teres*, with *Myrothecium verrucaria*, *T. viride* and an actinomycete reduced sporulation of the pathogen by more than 70%, when incubated under continuous high humidity at 18 °C (Mostafa, 1993). *Pseudocercosporella herpotrichoides*, the causal agent of eyespot in cereals, survives in infested straw and produces conidia infecting subsequent cereal crops. When straw precolonised by the pathogen was inoculated with a *Trichoderma* sp. in pot experiments, the pathogen produced less conidia and less seedlings of wheat were infected (Clarkson and Lucas, 1993).

Pfender and Wootke (1988) analysed the fungal communities of wheat straw, and *Limonomyces roseipellis*, a secondary invader of infected wheat straw, was found to be antagonistic to *Pyrenophora tritici-repentis*, reducing ascocarp and ascospore formation

for 50 to 99% under controlled conditions (Pfender, 1988). The antagonist suppressed ascocarp formation of *P. tritici-repentis* under field conditions by 60 to 80% in three of four field experiments (Pfender et al., 1993). However, these reduction rates obtained for the primary inoculum were not sufficient to achieve disease control since the polycyclic secondary conidial production was more important for the progression of the leaf disease caused by *P. tritici-repentis*.

1.1.5 Conclusions

Until now most research efforts in biological control of necrotrophic foliar pathogens have been focused on interference with infection and have largely tried to imitate the effect of fungicides by sprayings with micro-organisms. This seems to be a useful approach in protecting man-made wounds against pathogen invasion, and it is in this area that biocontrol products are entering the market. Apart from the possibility of proper timing, the desired protection has only to last until the wound has been healed or dried out. The more general protection of healthy leaves in a field crop, however, requires that a biocontrol agent maintains itself in sufficiently high densities on the leaf surface as long as protection is needed. Moreover, the actual time period available for interaction is limited, so that a pathogen may easily escape from antagonism by penetrating the leaf. Therefore, it is understandable that particularly biocontrol aimed at this general leaf protection suffers inconsistency. In principle this is caused by failure of the antagonistic mechanisms involved or by insufficient population densities of the antagonist. Although particularly with respect to soilborne fungi, much research has been devoted to mechanisms of antagonism, it seems that basic knowledge on microbial colonisation is most essential for explaining inconsistency. In spite of the fact that theoretically antagonism based on induced resistance may not require consistently high population densities, there is no example of biocontrol of necrotrophic foliar pathogens not depending on high population densities of the antagonist. Environmental factors largely determine these densities. Computer simulation studies have proven to be a highly valuable tool for explaining and predicting the behaviour of antagonist populations in the phyllosphere. The ability to predict when a biocontrol agent will not be able to control a pathogen will greatly contribute to its reliability.
Biological control aimed at suppression of colonisation and subsequent sporulation seems a strategy superior to microbial reduction of infection because of the longer time periods available for competition in necrotic tissue which will either reduce the sporulation capacity of the pathogen or infection of adjacent healthy tissue. According to Lockwood (1992) microbial competition is based on exploitation, during which resources are depleted by one organism without inhibiting access of another organism, or on interference, during which such access is inhibited by the presence of the first coloniser. Different types of necrotic tissue can be discriminated varying from the necrotic lesion induced by the pathogen to naturally senesced

and moribund plant tissue. In general, the first colonisers, pathogen or antagonist, may have an advantage. Since successful competition within a lesion has been reported, such advantage may not be conclusive. It is surprising how little is know about interactions within lesions that may influence its expansion as well as a part of the pathogens inoculum production.
Necrotrophic pathogens may equally well colonise and sporulate upon naturally died plant tissue but antagonist applications may result in pre-emptive colonisation of this substrate by saprophytes excluding the later arriving pathogen. Several examples have demonstrated the validity of this approach which aims at a retardation of epidemics instead of protection of an individual plant. Biocontrol resulting in reduced sporulation is likely to be consistent, but may not always be sufficient as a stand alone treatment. In integrated control the impact of reduced sporulation on disease might be comparable with the use of tolerant varieties. In further development of sustainable agriculture and conservation tillage the growing amount of crop residue left on the soil surface will considerably increase inoculum production of necrotrophic pathogens with serious epidemiological consequences. Microbial sanitation merely aiming to imitate the mechanical removal of plant debris may reduce this problem.
A great advantage for eventual commercialisation of a biocontrol agent aimed at competition in necrotic tissue may be the until now observed lack of specificity in competition with other saprophytes or saprophytically growing pathogens. If this holds true and also applies to dead plant tissue of different origin, then there is an opportunity for a broad spectrum biocontrol agent.

1.2 Selection, characteristics and use of *Ulocladium atrum* in biocontrol of *Botrytis* spp. in necrotic tissues

Although fungicides are regularly applied to control *Botrytis* spp. in many crops, there is a need for other controls because fungicide use may be limited in the future by government regulations. Second, resistance of *B. cinerea* populations in greenhouse and field crops against several fungicides is a common problem (De Waard et al., 1993; Gullino, 1992). Benomyl-resistant strains of *B. cinerea* were found in cyclamen production in the early seventies (Van Dommelen and Bollen, 1973) and the occurrence of populations resistant to benzimidazoles and dicarboximides (iprodione, procymidone or vinclozolin) has been reported in Greece and France (Lacroix and Gouot, 1981; Pappas, 1982). Third, several fungicides such as iprodione and vinclozolin can have negative side effects especially on ornamental plants such as cyclamen, reducing the yield or the ornamental value of potted plants (Henseler, 1981). Finally, ornamental plants with visible residues of fungicides on the leaves cannot be marketed.

Experimental work was carried out during 1990 and 2001 with the objective to identify a suitable antagonist for the development of a biocontrol agent against *Botrytis* spp. based on the hypothesis that an exploitation of microbial antagonism during the colonisation of necrotic tissues can result in a reliable biocontrol system against necrotrophic *Botrytis* spp. To support this hypothesis, the role of necrotic tissue in *Botrytis* epidemics in three crops, onion, strawberry and cyclamen, was investigated. Candidate antagonists were isolated from necrotic leaf tissues and screened for their antagonistic efficacy. The ecology of the promising antagonistic strains *Ulocladium atrum* 385 and of several *Gliocladium* spp. was studied. Finally, the effect of applications of *U. atrum* 385 on epidemics caused by *Botrytis* spp. was assessed in crops of field grown strawberry and onion as well as greenhouse grown cyclamen, *Pelargonium* and pot roses.

1.2.1 Role of necrotic tissues in *Botrytis* epidemics

Exploiting sporulation suppression of necrotrophic pathogens as a control strategy demands more knowledge of the rate of disease development in the crop compared to a strategy aiming at prevention of infection. In the latter case, a possible control effect can also be demonstrated directly on single plants or even a single leaf. Suppression of sporulation as a strategy for biological control is only feasible (1) if the progression of an epidemic depends on the amount of inoculum in the crop, and (2) if the inoculum is produced mainly inside the antagonist-treated crop and air-borne inoculum from outside the crop does not contribute substantially to disease development.
The role of necrotic leaf tissues as a source of conidial inoculum in *Botrytis* epidemics was studied in manipulative field experiments in onion as models for a polycyclic epidemic and in annual strawberries as model for a monocyclic epidemic. Exploitation of suppression of colonisation of necrotic tissue by necrotrophic pathogens in order to prevent infection of adjacent healthy tissue is only feasible if the pathogen depends on such a bridge to allow establishment of new infections. Manipulative experiments were carried out to study the importance of naturally senesced leaves in *Botrytis* epidemics in cyclamen.

1.2.1.1 Botrytis *leaf spot in onion*

The effectiveness of sporulation suppression as a strategy for biological control of *Botrytis* spp. was studied under field conditions (3.1.1; Köhl et al., 1995a). The polycyclic epidemic of onion leaf spot, caused by *B. cinerea* and *B. squamosa*, was chosen as a model. Suppression of sporulation by antagonists was simulated by the artificial removal of necrotic onion leaf tissue on which *Botrytis* spp. can sporulate. The effect of removing necrotic tissue on the spore load of *Botrytis* spp. in the crop and on the development of leaf lesions was followed. In an additional treatment,

Gliocladium roseum was applied to test the capacity of this antagonist to suppress sporulation of *Botrytis* spp. under field conditions.

1.2.1.2 Grey mould in annual strawberry

Necrotic tissue in the strawberry canopy has been found to be the major inoculum source of *B. cinerea* in perennial strawberry fields (Braun and Sutton, 1987). The relationships between necrotic tissue present in the crop, flower infection and subsequent fruit rot have already been documented in that system (Braun and Sutton, 1987; Jordan, 1978). Under Florida conditions, leaf sanitation of strawberry plants conducted in raised beds reduced *Botrytis* fruit rot but did not increase marketable yield (Mertely et al., 2000). However, the epidemiology in annual strawberry crops using waiting-bed transplants and perennial crops may be different. The effect of removal of necrotic leaves on the load of *Botrytis* conidia in the air, on flower colonisation by *B. cinerea* and fruit rot was quantified in field experiments (3.1.2; Boff et al., 2001).

1.2.1.3 Grey mould in cyclamen

Experiments focused on the role of necrotic tissue in the onset of disease for *B. cinerea* in cyclamen were carried out by Kessel et al. (2001; 3.1.3). The suitability of necrotic tissue as a mutual substrate of *B. cinerea* and *U. atrum* was confirmed in laboratory experiments. The availability of necrotic tissue in cyclamen crops was studied in greenhouse cyclamen crops. Symptomless senescing tissue was manually removed to prevent colonisation of necrotic tissue by the pathogen. The effect of this treatment on leaf rot epidemics caused by *B. cinerea* was compared with untreated controls.

1.2.2 Antagonist screening

1.2.2.1 Screening under controlled conditions

The aim was to select saprophytic antagonists that are able to suppress sporulation of *Botrytis* spp. on necrotic leaf tissue (3.2.1; Köhl et al., 1995c). *B. aclada* and *B. cinerea*, causing onion neck rot and onion leaf spot, respectively, were chosen as model pathogens. A bioassay based on dead segments of onion leaves pre-inoculated with *B. aclada* or *B. cinerea* was developed. During incubation of antagonist-treated leaf segments in moist chambers, leaf wetness periods were interrupted by dry periods to simulate field conditions. The adaptation of antagonists to rapid changes between wet and dry periods in necrotic tissue of above ground plant parts may be a key factor for field performance of antagonists aimed at suppression of sporulation of necrotrophic leaf pathogens.

In the screening under laboratory conditions, 41 fungal isolates belonging to 22 species were tested for suppression of sporulation of *Botrytis* spp. on dead leaf tissue. Under continuous wet conditions, *Gliocladium catenulatum*, *Chaetomium globosum* and *Ulocladium atrum* inhibited sporulation of *Botrytis* spp. completely whereas *Aureobasidium pullulans* was less efficient. In bioassays with interrupted wetness periods, *C. globosum*, *U. atrum* and *A. pullulans* suppressed sporulation of *Botrytis* spp. almost completely, but *G. catenulatum* was distinctly less effective. These four isolates were tested in a subsequent screening step under field conditions (Köhl et al., 1995b).

1.2.2.2 Screening under field conditions

The main objective was to compare the ability of the antagonists *A. pullulans*, *C. globosum*, *G. catenulatum* and *U. atrum* to colonise dead lily leaf tissue and to protect the substrate from colonisation by naturally occurring airborne inoculum of *Botrytis* spp. under field conditions (3.2.2; Köhl et al., 1995b). A sequence of nine experiments was performed under a variety of natural microclimatic conditions. The performance of antagonists was quantified by determining the sporulation potential of *Botrytis* spp. after incubation of the necrotic leaves in moist chambers after five to six days of exposure to field conditions. Determination of germination of spores of *C. globosum* and *U. atrum* and hyphal growth of *U. atrum* on dead lily leaves under field conditions provided additional information on the colonisation potential of the antagonists. In these experiments, *U. atrum* consistently reduced sporulation of *B. cinerea* by more than 90%. This antagonist was chosen for further studies.

1.2.3 Effect of environmental factors on *U. atrum*

The success or failure of antagonists after applications in the open field or in greenhouses mainly depends upon their adaptation to the particular ecological niche. The environment of the phyllosphere is characterised by rapid changes of water availability and temperatures over a broad range (3.3.1.1; Burrage, 1971). To compete successfully with *Botrytis* spp. during the colonisation of necrotic tissues, the ability of antagonists to survive during dry periods or periods with extreme temperatures as well as to grow during leaf wetness periods at moderate temperatures should at least be equal to those of the target organism.
Little information is available on the ecology of *U. atrum* and no information is published about the temperature requirements of the fungus. Thorough knowledge on the ecology of pathogens and their antagonists is a prerequisite for the development of reliable biocontrol systems. Studies were carried out to quantify the effect of temperature and water availability on *U. atrum* as well as on the persistence of *U. atrum* conidia after application on leaves. This information on the effect of temperature and water potential on development of *U. atrum* and its antagonism

against *Botrytis* spp. will help to better understand the dynamics of antagonist populations under field conditions and to develop reliable application strategies. Knowledge on the persistence of conidia on leaf surfaces is needed to optimise timing of the applications of the antagonist and the intervals between applications.

1.2.3.1 Temperature

The objective of our study (3.3.1; Köhl et al., 1999) was to determine the temperature requirements of *U. atrum* in comparison with those of *Botrytis* spp. In part of the studies, the antagonist *G. roseum* was also included for comparison. The conidial germination rates on agar and necrotic lily leaves, the mycelial growth rates on agar and the antagonistic potential to suppress sporulation of *Botrytis* spp. on necrotic leaf tissues of onion, cyclamen and hydrangea as depending on temperature were quantified. Results were related to the microclimatic conditions measured in onion crops in the open field and in cyclamen crops in greenhouses or common during storage of plant stocks.

1.2.3.2 Water availability

Little information is available in the literature about the fate of germlings of antagonistic fungi in the phyllosphere. The objective of the study (3.3.2; Köhl and Molhoek, 2001) was to determine the effect of constant and alternating water potentials on (1) conidia of *U. atrum* during germination and (2) its antagonistic potential against *Botrytis* spp. 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 of the surrounding air, were not considered.

1.2.3.3 Conidia persistence

When *U. atrum* conidia are sprayed onto a crop, a large proportion of the antagonist inoculum is deposited onto healthy green tissues. Since the primary target consists of necrotic leaf parts, inoculum of the antagonist may be considered 'lost' from the system. However, green leaves or parts thereof will potentially become necrotic as a result of natural senescence, pathogen infection, or other biotic or abiotic factors such as freezing injury. If viable antagonist inoculum is present on green tissues in sufficient quantities just prior to the onset of necrosis then rapid colonisation by the antagonist of the necrotic tissue may exclude the pathogen or other competitive saprophytes from that substrate. Therefore in a biological control system aimed at colonisation of necrotic tissues the level of viable antagonist inoculum on green leaves may be of significant importance.

The objective of our study was (1) to investigate the fate of *U. atrum* inoculum sprayed onto green leaves under greenhouse and field conditions and (2) to determine its ability to colonise freshly induced necrotic leaf tissues after exposure on green leaf surfaces. Spore density on the green leaf surface, survival and germination of *U. atrum* conidia and the superficial growth of germ tubes was followed for a period of three weeks in field experiments and ten weeks in greenhouse experiments. Leaf necrosis was artificially induced and colonisation of that substrate by *U. atrum*, *Botrytis* spp. and naturally occurring saprophytes was quantified. Studies were carried out in greenhouse-grown cyclamen crops (3.3.5.1-2; Köhl et al., 1998; Köhl et al., 2000) and field-grown lilies (3.3.5.3; Elmer and Köhl, 1998). In cyclamen, the fate of *U. atrum* conidia on green leaves and their ability to colonise such leaves after senescence was investigated in crops that were irrigated by capillary matting. Experiments on antagonist survival in top-irrigated cyclamen crops complete the studies on conidial persistence. It was hypothesised that frequent top-irrigation may adversely affect antagonist efficacy since conidia may be washed off from leaf surfaces. Furthermore, germinated conidia may become exhausted during frequent wet/dry periods, each possibly not long enough for conidia to germinate and colonise necrotic tissues.

1.2.4 Mode of action of *U. atrum*

1.2.4.1 Fungal interactions: microscopical and ultra-structural studies

Antagonism of *U. atrum* against fungal pathogens was first found during the screening programme described in 3.2.1. Nothing was known about the mechanisms involved in the antagonism by *U. atrum*. A better understanding of the mechanisms is essential for the further exploitation of the antagonist in necrotic tissues of various crops under different environmental conditions. Population densities of the antagonists in the necrotic substrates necessary to achieve sufficient control levels and the timing of antagonist applications may depend on the underlying mechanisms. Furthermore, necrotic tissues from different crops may have a differential effect on the expression of such mechanisms.
The objective of the microscopic and ultra-structural study (3.4.1; Köhl et al., 1997) was to elucidate the mechanisms involved in the antagonistic interactions between a *Botrytis* sp. and *U. atrum* during the saprophytic colonisation of necrotic plant tissues. The antagonistic fungi *Aureobasidium pullulans*, *Chaetomium globosum* and *Gliocladium catenulatum* were included in the study for comparison. Parasitism, antibiosis and/or nutrient competition may be components of the antagonistic activity (Whipps, 1992; Elad, 1996). Whereas *in vitro* tests can be useful to determine enzymatic or antibiotic activity of biocontrol agents, they cannot determine if and how these mechanisms take place when the organisms are interacting on their natural substrate. Microscopic and ultra-structural studies were

applied to analyse and compare the behaviour of the four antagonists as they developed on dead onion leaves.

1.2.4.2 Competitive substrate colonisation: experimental studies and simulation modelling

Studies on competitive interactions between fungi often rely on quantification of sporulation (Biles and Hill, 1988; Elad et al., 1994a, b; Köhl et al., 1995a) or fruiting bodies (Adee et al., 1990; Newton et al., 1998). A disadvantage of such methods is that they quantify the outcome of competition, spore production, rather than the process of resource capture and growth of mycelial biomass. Techniques for visualising and quantifying mycelia of *B. cinerea* and *U. atrum,* co-occurring in the same substrate, have been developed in 1999 (Kessel et al., 1999). This technique was used to quantify time-courses of mycelial biomass of *B. cinerea* and *U. atrum*, growing alone or simultaneously in necrotic cyclamen leaf tissue (3.4.2; Kessel, 1999). 'Head start' experiments were conducted, in which one of the fungi was inoculated after pre-inoculation with the competing fungus. Sporulation of both fungi was quantified to measure the effect of the time of pre-inoculation. Results of these head start experiments are interpreted in the framework of the ecological theory of replacement series (de Wit, 1960; Adee et al., 1990; Klepzig and Wilkens, 1997). Key concepts are the relative yield (RY, yield in the mixture divided by the yield in monoculture) for both competing species, and the relative yield total (RYT, the sum of the relative yield for both species). The behaviour of relative yields in response to treatments provides evidence for the nature and strength of interactions between species (Braakhekke, 1980). In particular, when the RYT equals 1, the two species are purely competing for the same resources. If there is niche differentiation, the RYT will be greater than 1, whereas allelopathic effects result in a RYT smaller than 1.

1.2.5 Use of *U. atrum* for disease control in crops

1.2.5.1 Annual strawberries

Grey mould of strawberry (*Fragaria* x *ananassa* Duch.) incited by *B. cinerea* can cause severe yield losses in any of the strawberry cropping systems used world-wide (Maas, 1984). In the Netherlands, the majority of strawberry field crops are grown using the so-called waiting-bed transplant system (Rosati, 1991). This cropping system is also adopted in other North west European countries. In such a system, the transplants are produced in waiting-beds from August until December and stored at –2 °C until transplanting in the following spring or summer (Rosati, 1991). Advantages of this system are (1) no plant losses during winter, (2) flexible planting schedule resulting in harvest periods with higher expected product prices,

(3) lower risk of build-up of populations of soilborne pathogens because of the reduced cropping period, and (4) better stand quality by selecting transplants that developed a strong multi-crown during the waiting-bed time.
Biological control of strawberry grey mould can be achieved by interfering with the infection process of the pathogen at the blossom stage and during development of fruits (Bhatt and Vaughan, 1963), or after harvest by using yeasts as antagonists (Lima et al., 1997). Another approach consists of suppression of *B. cinerea* inoculum production by *Gliocladium roseum* (Peng and Sutton, 1991). Using a similar approach, Jordan and Pappas (1977) suggested suppression of inoculum production by chemical means. In perennial systems, control of strawberry grey mould by reducing initial inoculum production on necrotic leaves within the crop is possible, since infection of most strawberry flowers takes place in a single infection cycle by conidia produced on necrotic leaves present in the crop during flowering (Sutton, 1990). Because this source of inoculum may play a different role in annual cropping systems, biological control strategies may need to be adjusted accordingly.
The objective of our study (3.5.1; Boff et al., 2002) was to evaluate under field conditions the effectiveness of *U. atrum* in reducing strawberry fruit rot caused by *B. cinerea* in annual strawberry crops with waiting-bed transplants. Attempts were made to optimise timing, initial applications, spraying frequency and suspension concentration.

1.2.5.2 Onions

Onion leaf blight caused by *B. squamosa* can cause significant yield losses in onion production (Sutton et al., 1986). Conidia produced on sclerotia or crop residues can infect leaves resulting in typical small leaf spots. During leaf maturation or after heavy infection, leaf spots can expand and whole leaves can die. During the polycyclic epidemics, secondary inoculum is produced on dead leaf tips or leaves. Regular fungicide applications for leaf blight prevention are common in onion production and several forecasting systems have been developed to optimise fungicide use (Vincelli and Lorbeer, 1989; Sutton et al., 1986; de Visser 1996). *B. cinerea* can also cause leaf spots in onion, usually not resulting in yield losses (Hancock and Lorbeer, 1963). As *B. squamosa*, *B. cinerea* can sporulate on dead leaf tips or leaves.
The objective of our study was to investigate the effect of regular applications of *U. atrum* on epidemics of onion leaf blight and onion leaf spot under field conditions (3.5.2). Sporulation of *Boytytis* spp. on dead onion leaf tissue, the aerial spore load of *Botrytis* spp. and the number of leaf spots was quantified in *U. atrum* treated plots in comparison with water treated control plots and fungicide treated plots.

1.2.5.3 Cyclamen

Botrytis cinerea causes leaf rot as one of the major diseases in cyclamen (*Cyclamen persicum* L.) in the commercial production of potted plants and seeds (Jacob, 1987;

Reimherr, 1985). Conidia of the pathogen are produced in necrotic lesions of infected plants or on crop residues. From such sources, conidia are spread through the air of greenhouses. Growers' activities in greenhouses result in a substantial increase in the density of conidia in the air within a greenhouse (Hausbeck and Pennypacker, 1991a, b).

Since conidia of *B. cinerea* cannot infect healthy cyclamen leaves (Schlösser, 1978), the pathogen initially colonises senescing leaves or necrotic tissues, and produces large numbers of conidia after the plant develops a dense canopy which increases the relative humidity within the canopy. Conidial clusters and mycelia of the pathogen can infect healthy tissues of adjacent petioles. Subsequently, the leaf blade can be colonised through the infected petiole. The disease can spread through the entire plant by attacking petioles. This leads to gaps in the plant canopy reducing their ornamental value or to complete loss of plants.

Control of the greenhouse climate is an important means to reduce the risk of disease epidemics in cyclamen (Jacob, 1987). Potted plants are placed on mats and water is supplied via the mats to avoid wetting above-ground plant parts. However, senescing or dead leaves in contact with the soil surface often are moist enough to allow infection or saprophytic colonisation by *B. cinerea*. In addition, once leaves are wet the relatively low temperature required by cyclamen for growth slows drying. Removal of dead leaves by hand reduces the risk of *B. cinerea* infection; however this procedure is labour intensive. Furthermore, healthy leaves can be injured during handling and the fresh wounds or enhanced formation of senesced leaves can lead to new infections by *B. cinerea*.

A few antagonistic fungi or bacteria have been reported to suppress *B. cinerea* in cyclamen. Bollen (1982) reported that infections by *B. cinerea* are reduced by naturally occurring *Penicillium* spp. When these antagonists were eliminated by fungicides such as benomyl, the damage caused by benomyl-resistant *B. cinerea* populations increased after benomyl applications. Iyozumi et al. (1996) controlled *B. cinerea* on cyclamen petals in the greenhouse by applications of *Serratia marcescens*. The same bacterium suppressed sporulation of *B. cinerea* by more than 85% on leaf blades placed on the soil near the plant base.

The objective of our study (3.5.3.1; Köhl et al., 1998) was to test the fungal antagonists *U. atrum* and *G. roseum* for their ability to suppress *B. cinerea* in commercially-grown cyclamens. In this study, repeated applications of conidial suspensions of *U. atrum* controlled the disease as effectively as the growers' standard fungicide program. These experiments were carried out in a commercial greenhouse where the potted plants on tables were sub-irrigated by capillary matting. No top irrigation had been used. However, cyclamen are produced in a variety of different growing systems, including those with top-irrigation. The purpose of a subsequent study (3.5.3.2; Köhl et al., 2000) was to investigate the potential of *U. atrum* for *B. cinerea* control under 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.

1.2.5.4 Pelargonium

The ornamental crop geranium (*Pelargonium zonale* (L.) Aiton) is susceptible to attack of flowers and senescent leaves by *B. cinerea*. The crop is reproduced by cuttings. The season for reproduction 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 in the Netherlands even choose to interrupt plant reproduction by cuttings during the most unfavourable month of December.
Hausbeck and Pennypacker (1991a, b) have 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*.
In our study (3.5.4; Gerlagh et al., 2001) it was 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.

1.2.5.5 Pot roses

In pot roses, where young cuttings are susceptible to *B. cinerea*, and where two thorough prunings are performed to provoke denser, ramified plants, grey mould by *B. cinerea* can be very troublesome because of the ample availability of wounds and deciduous substratum. Fungicides are routinely applied to control grey mould in pot roses. Experiments were carried out in a Dutch commercial greenhouse to assess the possibilities of *U. atrum* to control colonisation of rose tissue by and sporulation of *B. cinerea* (3.5.5; Köhl and Gerlagh, 1999).

2 Materials and Methods

2.1 Materials

Fungi. *Botrytis aclada* Fres. (syn *B. allii* Munn), *B. cinerea* Pers. ex Pers. and *B. elliptica* Berk. Cooke were isolated from an onion seed, a gerbera flower and a diseased lily plant, respectively. The following fungi (with isolate number) were isolated from necrotic leaf tips of onions grown in a field at our institute: *Alternaria alternata* (Fr.) Keissler (300, 317, 319), *A. infectoria* Simmons (264, 270), *Arthrinium* sp. (anamorph state of *Apiospora montagnei* Sacc.) (242), *A. phaeospermum* (Corda) M. B. Ellis (243, 244), *Aureobasidium pullulans* (de Bary) Arnaud (490), *Chaetomium globosum* Kunze ex Fr. (256), *Cladosporium cladosporioides* (Fres.) de Vries (564), *C. herbarum* (Pers.) Link ex Gray (587, 571, 593), *Penicillium brevicompactum* Dierckx (221), *P. hirsutum* Dierkx (211), *P. spinulosum* Thom (201), *Penicillium* spp. (023, 025), *Sesquicillium candelabrum* (Bonord.) W. Gams (249), *Ulocladium atrum* Preuss (385), *U. chartarum* (Preuss) Simmons (380), *Verticillium nigrescens* Pethybr. (250). *Gliocladium catenulatum* Gil. & Abbott (1814), *G. nigrovirens* Van Beyma (1815) and *G. roseum* Bain. (1813) were isolated from peel of potato tubers. *Trichothecium roseum* (Pers.) Link ex Gray (706) originated from a necrotic leaf of lily, *Cryptococcus luteus* (Saito) Skinner (WCS36) from a rye leaf. *Trichoderma hamatum* (Bon.) Bain aggr. (003, T166), *T. harzianum* Rifai aggr. (T000, 022, T154) and *T. viride* Pers. ex S.F. Gray aggr. (T004, T048, T122, T141, T218, T226) were isolated from soil or bark (Köhl and Schlösser, 1988). *G. catenulatum* (162) and *G. roseum* (160, 161), isolated from roots of red clover, were kindly provided by P. Lüth, Prophyta Biologischer Pflanzenschutz GmbH, Malchow, Germany. *T. harzianum* 39, isolated from a cucumber fruit grown in a greenhouse, was kindly provided by Y. Elad, Volcani Center, Bet Dagan, Israel.

Inoculum produced on agar. Fungi were cultured in petri dishes on oatmeal agar (20 g oatmeal, 15 g agar, 1000 ml tap water) except *A. pullulans* 490 and *C. luteus* WCS36 which were grown on basal yeast agar (10 g bacteriological peptone, 20 g sucrose, 1 g yeast extract, 20 g agar, 1000 ml tap water). Cultures of all fungi were incubated at 18 °C in the dark for 10 to 14 days, except those of *A. pullulans* and *C. luteus,* which were cultured for three days, and of *Alternaria* spp. and *Ulocladium* spp., which were cultured for 21 to 28 days. To obtain spore suspensions, cultures were flooded with sterile tap water containing 0.01% Tween 80. After gently rubbing with a rubber spatula to remove spores from fungal cultures, suspensions were filtered through sterile nylon gauze with a mesh of 200 μm. Concentrations of conidial suspensions were determined with the aid of a haemocytometer and adjusted with sterile tap water containing 0.01% (v/v) Tween 80.

Inoculum produced on oat. Conidia of *U. atrum* isolate 385 were produced on oat grains in autoclavable spawnbags (Type 3LS, Sylvan, Horst, the Netherlands). Oat kernels were moistened in tap water in a vessel overnight (300 g of dry oat

kernels, 300 ml of tap water), excess water was drained through a sieve and the moistened oat kernels were put in a spawnbag. The open end of the bag was inserted into a metal ring, the remaining opening was closed with a cotton plug and the bag was sealed with autoclavable tape. Bags with the oat kernels were autoclaved at 121 °C for 45 min twice at a 24-h interval. The sterilised oats were inoculated with 0.5 ml of a conidial suspension of *U. atrum* (approximately 1 x 10^5 conidia ml^{-1}) and incubated for 28 days at 20 °C in the dark. Bags were shaken every two to three days to mix the kernels 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 x 0.4 mm mesh and agitating these bags for 5 min in a small washing machine (Nova MW 100, Nova, Maastricht, the Netherlands) in 5 l of chilled tap water (5 °C) with 0.01% of Tween 80. The resulting suspension was filtered through nylon gauze with 200 μm mesh to remove mycelial fragments and oat kernel debris. Concentrations of the conidia in the suspensions were determined with the aid of a haemocytometer and adjusted with chilled tap water containing 0.01% of Tween 80 as required. Suspensions were kept at 5 °C until use.

2.2 Methods

2.2.1 Role of necrotic tissues in *Botrytis* epidemics

2.2.1.1 Botrytis *leaf spot in onion*

Field plots. Onion cv Hyton F1 was sown in rows 0.3 m apart on 27 March 1991 in 18 plots of 9 x 12 m, arranged in six blocks of three plots each. Three treatments were allocated at random within each block, so as to give a randomised block design in six replications. Plant density, determined on 13 April 1991, was 93 plants m^{-2}. In order to minimise interplot interference, the plots were separated by 12 m wide strips of sugar beet. Sugar beet was chosen as buffer crop because it does not produce pollen which could stimulate *Botrytis* infection and is not sufficiently tall to cast shadow on the plots which would alter the microclimate in the onions.

Inoculum source. Sclerotia of *B. squamosa*, the primary inoculum in epidemics of onion leaf blight (Ellerbrock and Lorbeer, 1977), were produced on sterile onion leaves placed on water agar. Sclerotia were separated from the leaf tissue using forceps and 2.4 sclerotia m^{-2} were spread on 23 April 1991 by hand over the plots in order to obtain a homogeneous distribution of primary inoculum of *B. squamosa* at the beginning of the season.

Treatments. The effect of the suppression of sporulation of *Botrytis* spp. by antagonists was simulated by artificial removal of necrotic leaf tissue. For this purpose, all onion leaves with important necrotic area (necrotic for more than 50% of their length) were cut off using scissors and removed from the plots at weekly intervals from 5 June until 9 August 1991. Removing necrotic leaf tips also from young leaves (necrotic for less than 50% of their length) might have led to infections and physiological stress of onion plants. Since the amount of necrotic tissue increased drastically at the end of July, the removal of necrotic leaf tissue could only be continued in two arbitrarily chosen blocks. A second treatment consisted of the application of the fungal antagonist *Gliocladium roseum* Bain IPO-1813 that had been isolated from the surface of a potato tuber. In bioassays based on interactions on dead onion leaf segments under humid conditions, *G. roseum* 1813 showed strong antagonism against *B. aclada* and suppressed sporulation of this pathogen completely (3.2.1; Fokkema et al., 1992). The antagonist was sprayed as conidial suspension at weekly intervals from 5 June until 14 August 1991 at a volume of 60 ml m^{-2}. Conidia were produced on autoclaved moist wheat grains in 250-ml conical flasks (30 g dry weight of grains per flask) during 14 days at 18 °C. Suspensions were prepared by adding tap water with 0.01% Tween 80 to the cultures. The suspension was shaken, filtered through a double layer of cheesecloth and the conidial concentration was adjusted to approximately 1×10^6 conidia ml^{-1}. No treatments were carried out in the control plots.

Quantification of necrotic leaf tissue. In all treatments, ten randomly chosen plants (each with 4 to 6 leaves) per plot were sampled at weekly intervals from 11 June until 20 August. The length of green and necrotic leaf parts was measured to estimate the efficacy of the removal of necrotic tissue and to determine the effect of the treatment with *G. roseum* on leaf dieback caused by *B. squamosa*. Since necrosis of leaf tissue is a continuous process during the interval between sampling dates, and since removal of necrotic tissue with more than 50% necrotic leaf length took place at different dates after sampling, the percentage necrotic area which had been removed could only roughly be estimated at about 60%.

Spore load. Spore samplers (Rotorods, Sampling Technologies, Los Altos Hills, CA, USA) were used to trap airborne spores (Edmonds, 1972; Aylor, 1993). These were positioned at a height of 0.3 m in the centre of each plot of the two blocks where necrotic leaf tissue was removed during the whole duration of the experiment. Thus, spores were trapped simultaneously in all treatments in two replications. Additional sampling was carried out to compare density of airborne spores within and outside the plots. For this, Rotorods were placed 0.3 m above the centre of a control plot, within the buffer crop between a control plot and a plot treated with *G. roseum* and within the buffer crop at a distance of 18 m from onion plots.
Between 1 and 21 August 1991, spores were trapped on five days. Three, three, four, two, and three sampling runs, each of 15 min, were carried out on 1, 10, 12,

16, and 21 August 1991, respectively. All runs were done between 11:00 hours and 15:00 hours. Peaks of spore release could be expected at this time of the day coinciding with a decrease in humidity within the crop (Sutton et al., 1978). Conidia were counted on a length of 22 mm of each of the two rods of a Rotorod per sampling run. The numbers of *Botrytis* conidia shorter than 15 µm and those longer than 15 µm were recorded separately in order to distinguish between conidia of *B. cinerea* and *B. squamosa*. According to Ellis (1971) and our own measurements of conidial sizes of several isolates of *B. cinerea* and *B. squamosa* grown on sterile onion leaves, conidia of *B. cinerea* usually are 8 to 14 µm long and those of *B. squamosa* usually are 15 to 21 µm long. The concentration of conidia m^{-3} air was calculated from the conidial counts, the rotation speed and the sampling duration, according to a formula provided by the manufacturer. Under the prevailing sampling conditions, one hundred conidia counted per sampling run on the two rods together represent 147 conidia m^{-3} air.

Disease development. Lesions were counted on the green parts of all leaves of the plants except those of which the necrotic leaf part exceeded 50% of the leaf length and which had been removed as part of the experiment. The surface of the green leaves was measured with a Delta-T Area Measurement System (Delta-T Devices LTD., Burwell, Cambridge, UK) and the number of lesions cm^{-2} was calculated.

Microclimate. Air temperature, relative humidity and leaf wetness duration were measured in the onion crop at 0.35 m height at 60-minute intervals using a Delta-T logger (Delta-T Devices LTD., Burwell, Cambridge, UK) connected with a Valvosensor (Philips, Eindhoven, the Netherlands) and a leaf wetness sensor (LW 100, Bottemanne, Amsterdam, the Netherlands).

Statistics. The statistical package Genstat 5 was used for data analysis (Numerical Algorithm Group, Inc., Oxford, UK). The number of conidia trapped during all sampling runs were pooled per day and analysed for conidia of *B. cinerea* and *B. squamosa* separately. After analysis of variance of log-transformed data, differences between treatments were established with LSD tests.
Progressive increase in number of lesions cm^{-2} leaf was modelled for each plot separately. The exponential growth model $y = \exp(a + bt)$, with y = density of lesions, t = time, a = log (initial density) and b = relative growth rate (RGR), proved to be adequate. Values of a and b for each plot were subjected to analysis of variance.

2.2.1.2 Botrytis *grey mould in annual strawberry*

Field plots. Data were collected in the untreated plots of five field experiments on the control of grey mould of strawberry cv Elsanta (presented as experiments 2, 3,

5, and 6 in 3.5.1; Boff et al., 2002), located on a sandy soil near Wageningen, the Netherlands, with transplanting dates of 10 April 1997 (experiment 2); 27 June 1997 (experiment 3); 06 May 1998 (experiment 5), and 19 June 1998 (experiment 6). There were 5 and 4 untreated replicate plots in 1997 and 1998, respectively, which were randomised with the other treatment plots within blocks. Experiment 8 consisted of a single untreated plot planted at 16 April 1999. All plots consisted of 78 plants in an area of 4.5 m x 4.3 m, surrounded by at least 10 m grass buffer to reduce inter-plot interference. Cold-stored dormant transplants, kept at –2 °C until the day before planting, were transplanted in double rows with three plants per meter row length. Spacing of rows between double rows was 1 m and within double rows 0.5 m. Sprinkler irrigation (1997) and a mist irrigation system (1998 and 1999) were used to ensure water supply for plant development in dry periods from transplanting until beginning of flowering. From beginning of flowering until the first harvest, strawberry plots were irrigated every second night (5 min per 90 min, a total of 40 min per night) to stimulate epidemic development of *B. cinerea*.

Primary inoculum on transplants. Primary inoculum of *B. cinerea* was assessed on the lots of transplants used in experiments 3 and 4. In both experiments, a total of 50 stolons, senescent and dead leaflets were taken from the cold-stored dormant transplants. The sampled plant parts were washed thoroughly with tap water and put separately into plastic trays (50 cm length x 30 cm width x 7 cm height) with wet filter paper at the bottom and closed with a plastic bag. After 14 days incubation at 18 °C in the dark, the incidence of *B. cinerea* was assessed and the area with pathogen sporulation was estimated for each leaflet or stolon unit, using a stereomicroscope with ocular micrometer at 10 x magnification.

Strawberry phenology and necrotic leaf area. The phenology of the strawberry crops was determined by periodical non-destructive measurements from transplanting until harvest, on four (1997) or five (1998) plants per plot, arbitrarily selected and labelled. In the single untreated plot in 1999, ten plants were monitored. The numbers of flowers and fruits per plant were counted on five to eight occasions during each experiment. The area of necrotic leaf tissue was estimated by measuring the length and width of each necrotic leaf part with a millimetre scale ruler and calculating the area as a rectangle. Averages of necrotic leaf area and number of flowers per plant per replicate plot were considered in further data analysis.

Conidial load in the air. The load of *B. cinerea* conidia in the air was monitored using Rotorod samplers. Rotorods were positioned at 0.3 m height in the centre of an untreated plot and 25, 50 and 50 m outside the strawberry plot in the grass buffer in the years 1997, 1998 and 1999, respectively. Spore samples were collected on 2, 5, 3 and 6 different dates in the experiments 3, 5, 6, and 8, respectively. Two to four runs per sampling date with 15 min duration per run were carried out be-

tween 8:00 and 13:30 h, the most likely period for spore release of *B. cinerea* (Jarvis, 1980). In experiments 5 and 6, the spore load was also assessed in a plot in which all senescent and necrotic leaflets had been removed twice per week from transplanting until first harvest (sanitation plot). The spores of one rod per run were stained with cotton blue (2 ml lactic acid + 4 ml glycerol + 1.5 ml cotton blue at 1% + 2 ml demineralised water) and conidia of *B. cinerea* were counted on the 22 mm upper part of a rod using a microscope at 200 x magnification, and the number of conidia of *B. cinerea* per cubic meter of air was calculated (Sampling Technologies, 1989).

Colonisation of necrotic leaves. Samples of 10 to 40 half or whole necrotic leaflets were harvested per plot and incubated under moist conditions to assess the potential sporulation of *B. cinerea* as an estimate of the colonisation of the substrate by the pathogen. In each sample, not more than one leaflet per plant and not more than 5% of the total necrotic leaf area was removed per plot. This limit was considered to be the maximum that could be removed without significantly disturbing the availability of substrate for *B. cinerea* in the field. Six samples of necrotic leaflets were collected per plot in experiment 2 (13 May, 22 May, 30 May, 6 June, 20 June and 9 July, 1997), four samples in experiment 3 (22 July, 29 July, 5 August and 14 August, 1997), three in experiment 5 (7 May, 8 June and 16 June, 1998) and three in experiment 6 (20 June, 21 July and 4 August, 1998). No assessment of sporulation on necrotic leaves was done in experiment 8. The leaflets harvested from each plot were put into a plastic tray (50 cm length x 30 cm width x 7 cm height) with wet filter paper at the bottom and closed with a plastic bag and incubated for 14 days at 18 °C in the dark. The percentage area with sporulation of *B. cinerea* was estimated for each leaflet, using a stereomicroscope at 10 x magnification. The area colonised by *B. cinerea* per plant was estimated from the total necrotic area per plant and the proportion of area sporulating after incubation. In the correlation analyses interpolated values of the leaf area were used when the assessment dates were not exactly the same as the dates on which other variables were assessed.

Flower colonisation. Samples of 20 to 30 flowers per plot were arbitrarily collected, each from different plants, on two (exp. 2, 3 and 8) or three (exp. 5 and 6) sampling dates. Only flowers already opened for three to four days with brown anthers and with petals still attached were included in the sample. The flowers were put into plastic boxes (22 cm length x 14 cm width x 5 cm height) with wet filter paper at the bottom and incubated at 18 °C for 14 days in the dark. All flower parts, except peduncles, were examined for the presence of sporulation of *B. cinerea* with a stereomicroscope, at 10 to 100 x magnification. Flowers were considered colonised by *B. cinerea* when at least one conidiophore with conidia was present on any of the flower parts. The incidence of *B. cinerea* sporulation on flowers was calculated per sampling date per plot.

In addition to the incidence of *B. cinerea* on flowers, the petal area colonised by *B. cinerea* was estimated from separate samples in the experiments 5, 6 and 8. Senescent petals (20 to 25) were arbitrarily collected per sampling date per plot, each petal from a different plant. The number of sampling dates was 3 (11 June, 20 June and 2 July, 1998), 4 (21 July, 2 August, 5 August and 11 August, 1998) and 5 (24 May, 27 May, 28 May, 31 May and 4 June, 1999) for the experiments 5, 6 and 8, respectively. No sampling of petals was done in the experiments 2 and 3. The petals were placed on water agar (15 g agar, 1000 ml tap water) in sterile plastic petri dishes (14 cm diameter) and incubated for 14 days at 18 °C in the dark. The percentage area with *B. cinerea* sporulation was estimated for each petal using a stereomicroscope, at 20 x magnification.

Fruit rot. Grey mould was determined at harvest by counting the number of diseased fruits with *B. cinerea* symptoms (field fruit rot) and the total number of fruits harvested per plot at each harvesting date. All ripe healthy fruit and all fruit with *B. cinerea* symptoms were picked twice per week. Healthy fruits were always picked separately from diseased fruits to avoid contamination during harvesting. For post-harvest evaluations diseased fruits with non-specific symptoms were placed in moist chambers for 72 hours at 18 °C in the dark to allow further development of symptoms. Post-harvest fruit rot was assessed using a sub-sample of ripe symptomless fruit, consisting of twenty arbitrarily selected fruits per plot. Fruits of each replicate were put into a plastic tray (50 cm length x 30 cm width x 7 cm height) with wet filter paper on the bottom. Each tray was closed within a plastic bag and incubated at 4 °C during five days in the dark and subsequently for three days at 18 °C. After incubation, rotten fruits with *B. cinerea* sporulation on any part of the fruit were counted as post-harvest grey mould.

Microclimatic conditions. Relative humidity and temperature were monitored with an electronic sensor (Pow 8-35 VDG, Rotronic AG, Bassersdorf, Switzerland) positioned at 30 cm height within a strawberry plot inside the experimental area. Precipitation was measured by a rain gauge (Casella, London, UK). Wetness of necrotic leaves was measured in the canopy at 5 cm height, with leaf wetness sensors (Köhl et al., 1995b). Data were recorded each half hour by a data-logger (Delta-T Devices Ltd, Cambridge, UK).

Statistics. Correlation analysis among epidemiological and weather variables was conducted by computing Spearman's coefficient of correlation (r_s) (Snedecor and Cochran, 1989). This correlation coefficient was also computed between the observed incidence of *B. cinerea* on flowers and the predicted spore production of *B. cinerea* per strawberry plant in the field over a 5-day period before sampling of flowers. Spore production per leaf area was predicted with a descriptive model as a function of observed wetness duration and average temperature during wetness over the 5-day period. The model equation used was $\log(Y) = -7.03 + 2.22$

[3.02 + (0.0026 log (W) T^3) + (–0.00011 log (W) T^4)], where Y is the number of *B. cinerea* conidia per cm^2 of leaf tissue, W is the duration of leaf wetness in days, and T is the average temperature during the wetness period (equation 7 of Sosa-Alvarez et al, 1995). The spore production of *B. cinerea* per strawberry plant was calculated as the product of predicted spore production per leaf area and the observed necrotic leaf area with potential sporulation per plant. Correlation analysis was also done between observed levels of grey mould in subsequent harvests during a season and predicted values calculated with a descriptive model with observed temperature and wetness duration during the flowering period as inputs. The model equation used was $\ln(Y/(1-Y)) = -3.526 + 0.0228\ WT - 0.1940\ W - 1.7 \times 10^{-5}\ WT^3$, where Y is the proportion of strawberry fruit infected by *B. cinerea*, W is the duration of leaf wetness in hours, and T is the average temperature during the wetness periods (equation 5 of Bulger et al, 1987).
For each experiment separately, mean spore loads of *B. cinerea* in the air monitored at different locations were compared with the paired *t*-test to detect significant differences. In addition, a confidence interval was calculated for the proportion of days with a higher average spore load of *B. cinerea* inside the untreated plots than outside the strawberry plots.

2.2.1.3 Botrytis *leaf rot in cyclamen*

Two greenhouse experiments were carried out to study the effect of removal of necrotic tissue on *Botrytis* epidemics in cyclamen crops. In experiment 1, also the occurrence of tissue types was monitored. In additional treatments, the effect of applications of *U. atrum* was assessed.
Plants of cyclamen cv Super Serie, not previously treated with pesticides, were obtained from a commercial grower 154 (experiment 1) and 70 days (experiment 2) after sowing. Grey mould severity assessed immediately after arrival revealed an average of 0.1 and 0 leaves per plant with sporulating *B. cinerea* in 1 and 2, respectively. Plants were placed on tables with capillary matting at a spacing just wide enough to avoid contact between the leaves. Greenhouse temperature varied within the 15 to 25 °C range set as minimum and maximum temperature. Relative humidity mostly varied between 80 to 90%. No pesticides were used.
Experimental treatments were: (1) spraying the plants with tap water containing 0.01% Tween 80 every four weeks; (2) an untreated control; (3) a treatment in which senescing, symptomless, plant parts were removed at three to four day intervals; and (4) spraying with a conidial suspension of *U. atrum* ($1x10^6$ conidia ml^{-1}) every four weeks. Necrotic plant parts and plant parts with sporulating *B. cinerea* were left on the plant. Experiment 1 included a fifth treatment in which the plants were sprayed with a conidial suspension of *U. atrum* ($1x10^6$ conidia ml^{-1}) once, coinciding with the first application to other spray treatments. Spray treatments were applied 155, 183 and 211 days after sowing in experiment 1 and 140, 168, 196 and 224 days after sowing in experiment 2. All plants were sprayed individually on and

inside the canopy to reach shielded surfaces such as older leaves, petioles and tubers. This technique is generally used to apply fungicides in commercial cyclamen crops.
In experiment 2, *B. cinerea* was introduced by placing six sporulating cultures (three to four weeks old) in petri plates (9 cm in diameter) in each of the greenhouse compartments (± 80 m^3) when the plants were 166 days old. Three cultures were placed underneath each of the two tables at even spaces. The lids of the petri plates were removed for 48 h to allow aerial dispersal of the conidia.
Experiments were designed as randomised block experiments with five and four treatments respectively, each treatment replicated four times. Two greenhouse compartments were used, each containing two blocks. Each replicate (plot) contained 25 plants arranged in five rows of five plants. Plots were placed by 50 to 60 cm apart.
The presence of healthy (green), senescing (yellow but turgid) and necrotic (yellow-brown lacking turgor) leaf blades and petioles was monitored at two-week intervals in the untreated plots in experiment 1. Grey mould severity was assessed at two-week intervals as the number of leaves per plant with sporulation of *B. cinerea*. The last assessment in experiment 1 took place three weeks after the previous assessment.
Disease severity per plot was calculated as the average number of leaves per plant sporulating with *B. cinerea*. Log-linear regression models, with replicate and treatment as classifying variables, were fitted to the data for disease severity. The models assume that the variance of the data is proportional to Poisson variance. Models were fitted to the data using the method of quasi likelihood (MacCullagh and Nelder, 1989). 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 case *F*-tests were significant ($P < 0.05$), treatment means on the log-scale were separated using *t*-tests ($P = 0.05$). The area under the disease severity progress curve (AUDPC) was calculated as the area under the curve of disease severity (Y-axis) against time (X-axis) and analysed using analysis of variance (ANOVA).

2.2.2 Antagonist screening

2.2.2.1 Screening on necrotic onion leaves under controlled conditions

Leaf segments. Symptomless green leaves were removed from 12-week old field-grown plants of onion cv Hyton, dried for several days at 60 °C and stored in paper bags at room temperature in the dark. Dry leaves were cut into segments each 2-cm long, sealed in plastic bags and sterilised by gamma radiation of 4 Mrad.

Conidial suspensions. All fungi were cultured in petri dishes and suspensions were prepared as described above (2.1). Concentrations of conidial suspensions were adjusted with sterile tap water containing 0.01% Tween 80 to 1 x 10^6 spores ml^{-1} for antagonists, 1x10^4 or 1x10^5 conidia ml^{-1} for *B. aclada* and 1 x 10^3, 1 x 10^4 or 1 x 10^5 conidia ml^{-1} for *B. cinerea*.

Bioassay. Onion leaf segments for use in bioassays were washed thoroughly with tap water to remove soluble nutrients. Approximately 100 segments were washed three times in 350-ml aliquots of sterile tap water contained in sterile 500 ml-conical flasks which were shaken at 150 strokes min^{-1} for 30 min. The segments were subsequently blotted dry with sterile filter paper. Four leaf segments were placed in each of a series of moist chambers. Each chamber consisted of a sterile plastic petri dish (90 mm diameter) containing two sterile filter papers (80 mm diameter) and 1 ml sterile tap water. Experiments were carried out in a completely randomised design with five replications (petri dish) for treatments with antagonists and five or ten replications for the control.
Conidial suspensions of *B. aclada* or *B. cinerea* were sprayed on the leaf segments in the moist chambers and the segments were incubated at 18 °C for 24 h (*B. aclada*) or 8 h (*B. cinerea*). Immediately after the incubation periods, spore suspensions of antagonists, or water plus surfactant, were sprayed on the segments. The pathogens and antagonists were applied with sterile atomisers at approximately 5 µl cm^{-2} leaf segment. Thereafter, leaves were further incubated at 18 °C in the dark in continuous wetness periods or in wetness periods that were interrupted 16, 40, or 64 h after the antagonists were applied. To interrupt wetness periods, leaf segments were placed on two layers of sterile dry filter paper in open petri dishes in laminar flow cabinets for 9 h. Temperatures in the cabinets were approximately 20 °C and relative humidity ranged between 30 and 40% RH. After the dry period, the filter paper in each petri dish was wetted with 2 ml sterile tap water, and the dishes were closed and kept at 18 °C in the dark. After a total incubation period of eight days, the leaf area covered with conidiophores of *B. aclada* or *B. cinerea* was estimated using classes from zero to five, that represented, respectively, 0%, 1-5%, >5-25%, >25-50%, >50-75%, and >75-100% of the leaf surface covered with conidiophores of the pathogens. From the number of leaf segments of each class (n_{0-5}) a sporulation index (SI) ranging from zero to hundred was calculated for each replication (petri dish) consisting of four leaf segments ($SI = (0 \times n_0 + 5 \times n_1 + 25 \times n_2 + 50 \times n_3 + 75 \times n_4 + 100 \times n_5) / 4$).
In total, 41 fungal isolates were tested in bioassays with *B. aclada* under continuously wet conditions. Fourteen isolates that had shown strong antagonism in these screening experiments were tested in bioassays (experiments 1 to 5) with an interrupted wetness period. In experiment 5, the effect of antagonists on sporulation of *B. aclada* was also quantified by counting conidia of *B. aclada* produced on dead onion leaves. The antagonists *A. alternata* 317 and 319, *A. pullulans* 490, *C. globosum*

256, *G. catenulatum* 162, *G. roseum* 1813, and *U. atrum* 385 were also tested in bioassays on dead leaf segments of onion that were pre-inoculated with *B. cinerea*.

Number of conidia produced in bioassays. Conidial production of *B. aclada* on the four leaf segments of each of three arbitrarily chosen replications of each treatment was determined in experiment 5. Leaf surface area was measured using an interactive digitiser (Minimop, Kontron, Oberkochen, Germany). Leaves were homogenised in 5 ml of tap water using a planetary micro mill (Pulverisette 7, Fritsch GmbH Laborgerätebau, Idar-Oberstein, Germany) and water was added to obtain 10 ml suspension. The number of conidia of *B. aclada* in four 6.4 µl aliquots of each of the suspensions was counted with the aid of a haemocytometer and the number of conidia of *B. aclada* recovered cm^{-2} leaf surface was estimated. The detection limit for conidia was approximately 5200 conidia cm^{-2} leaf surface as calculated from the average leaf surface of the four leaf segments per replication (petri dish) that had been homogenised, and the volume of the suspension that had been examined.

Drying process of onion leaves during bioassays. The water content of leaf segments was monitored in one of the bioassays with interrupted leaf wetness. Four leaf segments, similarly treated as leaves of the water treatment of bioassays, were placed in ten replications on dry filter paper in the laminar flow cabinet. The segments were weighed at 60-min intervals for 14 h, and after the leaf segments were dried at 105 °C for 6 h, the water content of the leaf segments was estimated.
The relationship between water content and water potential of dead onion leaves was determined in an additional experiment. Necrotic leaves of field-grown onions were cut into 1-cm segments and dried at 105 °C for 24 h. Sterile glass tubes were filled with 500 mg dried leaf segments. Sterile tap water was added to adjust the water content of the tissue in the individual glass tubes to values ranging from 0.0 to 3.5 g water g^{-1} dry segments. Values of water content increased in steps of 0.25 g water g^{-1} dry segments in the range between 0.0 and 2.0 g water g^{-1} dry segments, and in steps of 0.5 g in the range of 2.0 to 3.5 g water g^{-1} dry segments. For each individual value, four replications were prepared. Glass tubes were sealed, shaken thoroughly and stored at 20 °C in the dark for 12 days to allow equilibration of water potential of leaf segments within a tube. Thereafter, water potentials of one subsample per tube were measured with a Decagon thermocouple psychrometer (Decagon Devices Inc., Pullman, WA, USA).

Statistics. Data on sporulation index (SI) and log-transformed data on number of conidia were analysed separately for each experiment by analysis of variance (ANOVA) followed by LSD tests. Treatments causing complete suppression of sporulation (no variance among replications) were excluded from the analysis.

2.2.2.2 Screening on field-exposed necrotic lily leaves

Lily leaves. Asiatic hybrid lilies (*Lilium* L.) cv Mont Blanc were grown in pots during spring in the greenhouse at 20 °C with 16 h light per day. Flowerbuds were removed as usual in bulb production and to prevent pollen deposition on leaves which is known to enhance infection by *B. cinerea* (Chou and Preece, 1968). No infections by *Botrytis* spp. or other diseases occurred on the leaves. Leaves, approximately 8 cm long, from three-month old plants were cut from the stems, dried at 60 °C for 48 h and stored at room temperature in sealed plastic bags. Before leaves were used for the experiments, they were placed in tap water for 30 min to allow water absorption. Subsequently, they were washed thoroughly three times for 30 min in water to remove soluble nutrients using 1000-ml bottles with approximately 100 leaves in 500 ml of tap water on a flask shaker (150 strokes min^{-1}). Washed leaves were placed on dry filter paper to remove most of the water.

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

Experimental field design. Leaf holders were placed in a sugar beet field, each holder in one microplot of 0.3 x 0.3 m from which sugar beets had been removed. The distance between microplots was 5 m in each direction. Each experiment was carried out according to a randomised block design with seven treatments assigned to plots within blocks. The seven treatments were spraying spore suspensions of each of the four antagonists or a mixture of two of the antagonists, the fungicide Daconil M (250 g a.i. chlorothalonil kg^{-1}, 500 g a.i. maneb kg^{-1}; Schering AG, Berlin, Germany) or water as control. All experiments were performed between 26 May and 28 July 1993. The first four experiments had two replications (blocks) and the last five experiments had four replications.
Sugar beet was chosen as buffer crop because of the dense but low growth (approximately 0.5 m height) comparable to that of a lily crop. *Botrytis* spp. cause no leaf diseases in sugar beet, no pollen is produced that could stimulate *Botrytis* spp. as well as naturally occurring and applied antagonists (Fokkema, 1971). Weeds were efficiently controlled by pre- and post-emergence herbicides and mechanically later in the growing season. Sporulation of *B. cinerea* occurred occasionally on dead sugar beet leaves and a few dead weeds during the growing season.

Monitoring of microclimate. Air temperature and relative humidity of the air were measured with an electronic sensor (airprobe YA-100-hygromer, Rontronic AG, Bassersdorf, Switzerland) with an accuracy of < ± 1% in the range between 80 and 100% RH at 25 °C. This sensor was positioned at 35 cm height in the same sugar beet field, approximately 10 m away from the lily leaf holders. Three leaf wetness sensors were used to monitor leaf wetness of dead lily leaves fixed at leaf holders in an additional microplot. Electronic sensors had been developed at our institute for recording leaf wetness of necrotic leaf tissue based on measuring the capacity between two pins inserted into the substrate (Köhl et al., 1995c). All data were recorded at 30-min intervals and stored in a data-logger (Delta-T Logger, Delta-T Devices Ltd, Burwell, Cambridge, United Kingdom). No data on leaf wetness are available for the first experiment. Rainfall per hour and rainfall duration were recorded with a rain gauge at the meteorological station of the Department of Meteorology of Wageningen Agricultural University at a distance of 500 m from the experimental field.

Treatments. *C. globosum* and *G. catenulatum* were grown in petri dishes on oatmeal agar (2.1). *U. atrum* was grown for 28 days on sterilised oat grains in 250-ml Erlenmeyer flasks containing 30 g of oats (dry weight) saturated with water. *A. pullulans* was grown on basal yeast agar. Suspensions applied to the field contained 2 x 10^6 blastospores of *A. pullulans*, ascospores of *C. globosum* or conidia of the other antagonists per millilitre of tap water containing 0.01% of Tween 80. When applied in mixture, spore suspensions of *C. globosum* and *U. atrum* were mixed just before spraying so that the final concentration of each antagonist was 1 x 10^6 spores ml^{-1}. Suspensions were kept in ice water before spraying. Control plots were sprayed with tap water containing 0.01% of Tween 80 or with the fungicide Daconil M applied in a dose of 4 mg ml^{-1} as recommended for *Botrytis* control in lily bulb production. All applications were carried out in the late afternoon with a propane-operated field plot sprayer (AZO, Ede, the Netherlands) with a pressure of 250 kPa. The application rate was 50 ml of suspension m^{-2}, equivalent to 500 l ha^{-1}.

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

Spore germination. Eighteen hours after application of antagonists, the germination rates of spores of *C. globosum* and *U. atrum*, sprayed alone or in combination, were determined on four leaves per treatment. Spores of these antagonists are easy to distinguish from most of the spores of other fungi naturally deposited on the leaf surface. Fungi were stained with cotton blue in lactic acid (2 ml lactic acid, 4 ml glycerine, 3.5 ml distilled water, 15 mg cotton blue) and examined under a microscope with 200 x magnification. Fifty spores were randomly selected per leaf. They were considered as germinated when germ tube lengths were at least half the diameter of a conidium of *U. atrum* or equal to the diameter of an ascospore of *C. globosum*.
The number of spores of *C. globosum* and *U. atrum* cm^{-2} leaf surface was determined in all experiments 18 h and five days (experiments 3, 4, 6, 8, and 9) or six days (experiments 1, 2, 5, and 7) after spraying. The number of spores within a grid of 0.25 mm^2 at 10 randomly chosen parts of each of four leaves was counted using a microscope at 200 x magnification. In all experiments leaves from the control treatment were examined in the same way to monitor a possible background in the field of naturally occurring spores of *C. globosum*, *U. atrum* or other fungi not distinguishable from those of the two antagonists. On average, less than 20 of such spores cm^{-2} were found in the control treatment compared to an average of approximately 1000 spores cm^{-2} leaf surface on treated leaves.

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

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

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

2.2.3 Effect of environmental factors on *U. atrum*

2.2.3.1 Effect of temperature on conidial germination and mycelial growth of U. atrum *in comparison with* Gliocladium *spp.*

Air temperature during leaf wetness periods under field conditions. From 5 July to 29 August 1994, air temperature and leaf wetness of dead leaves of onion and lily were measured in experimental field plots at Wageningen. Air temperature was measured with an electronic sensor (airprobe YA-100-hygrometer, Rontronic AG, Bassersdorf, Switzerland) in the onion crop at a height of 35 cm. Leaf wetness periods for dead leaves positioned within the canopy of the onion or the lily crop were measured with electronic sensors developed at our institute (Köhl et al., 1995b). Data were recorded at 30-min intervals and stored in a data-logger (Delta-T Logger, Delta-T Devices Ltd., Burwell, Cambridge). The same equipment was used in commercial greenhouses in a cyclamen crop in an experiment lasting from August to December 1997.

Conidial germination. *U. atrum* was cultured in petri dishes (90 mm) containing 15 ml oatmeal agar. *G. roseum* 1813 was grown on malt extract agar (1 g malt extract, 15 g agar, 1000 ml tap water). Conidial suspensions were prepared as described above (2.1). Flasks containing the conidial suspensions were kept in ice-water and suspensions were used within 1 h.
Conidial germination over time at different temperatures was determined in petri dishes (50 mm diameter) containing 6 ml agar. For experiments with *U. atrum* water agar (15 g agar, 1000 ml tap water) was used. Since *G. roseum* did not germinate on water agar, 1 g malt extract l^{-1} was added to the agar as an exogenous nutrient source. The petri dishes were placed in incubators at different temperatures 24 h before use. The temperatures were: 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, and 36 °C. Thirty two petri dishes per temperature were sprayed with conidial suspensions using sterile atomisers in a laminar flow cabinet, resulting in a conidial density on the agar surface of approximately 5.6×10^2 conidia cm^{-2}. Petri dishes were immediately returned to the appropriate incubator, so that it can be assumed that the tem-

perature of the agar was at the corresponding temperature from the beginning of the experiment. Two conidial suspensions of each fungus were prepared for each experiment and the second suspension was prepared approximately 12 h after the first one. Petri dishes for incubation at the same temperature were inoculated on the same day in the morning or 12 h later to avoid late night sampling during the course of the experiment.

The total incubation period of the conidia depended on fungus and temperature and was based on results of preliminary experiments. This was 42 h or 4 h 45 min for *U. atrum* and 144 h or 24 h for *G. roseum* for incubation temperatures of 3 °C and 24 °C, respectively. The incubation period was divided into 16 equal time intervals and after each time interval, two arbitrarily chosen petri dishes were sampled. Fungal growth in the sampled dishes was stopped immediately by adding three droplets of household ammonia water (4.8% (w/w) NH_4OH) onto a filter paper (40 mm) placed into the lid of the petri dish and closing it again. Plates were stored at 4 °C until they were assessed.

To study the germination of *U. atrum* conidia on necrotic lily leaves, symptomless green lily leaves were cut from the greenhouse-grown plants and dried at 60 °C for 48 h. The dead leaves were then gamma-irradiated (4 MRad). Before use in experiments, leaves were rehydrated in sterile flasks containing sterile tap water. The surfaces of leaves were blotted dry using sterile filter paper and leaves were placed in sterile petri dishes (55 mm) containing two filter papers (50 mm) moistened with 0.75 ml sterile tap water. Experiments were carried out at 3, 6, 12, 18, 24, 27, 30, and 33 °C and leaf samples were taken during the course of the experiments as described above for experiments with conidia incubated on agar. All experiments were carried out twice.

To assess conidial density and germination on agar or lily leaves, several droplets of cotton blue in lactic acid were added onto the agar or the leaf surface. Conidial germination was quantified using a microscope (200 x magnification) by examining 50 arbitrarily chosen, non-clustered conidia per petri dish or leaf. Conidia were considered germinated when the germ tube was at least as long as the shortest diameter of the conidium. The mean of the germination percentage for two petri dishes or leaves per sampling time was used for data analysis.

Mycelial growth. Mycelial growth rates at 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, and 36 °C were determined for *U. atrum*, *B. aclada* 006, *B. cinerea* 700 and *B. elliptica* 702, and six isolates of *Gliocladium* spp. in petri dishes (90 mm) containing 15 ml malt extract agar (1 g malt extract, 15 g agar, 1000 ml tap water). The agar plates were inoculated in the centre of the dish with an agar disc (5 mm) obtained from the edge of 4- to 10-day old colonies growing on the same medium for each species at 18 °C in the dark. The average of two perpendicular colony diameters was recorded on the day after a new colony had visibly developed on the inoculated agar and again shortly before the growing colony covered the entire plate. The maximum colony diameter measured was 80 mm. For temperatures resulting in slow fungal

development, the last measurement was carried out 26 days after inoculation. The average daily radial growth rate was calculated as half of the diameter differences between the last and the first measurement divided by the number of days between these two measurements. For each fungal isolate, the experiment was carried out twice and data presented are the mean radial growth rates of the two experiments.

Statistics. The cumulative percentage of germinated conidia over time, was described for each temperature separately by the logistic model: $y = C/(1 + \exp(-B(t - M))$ for $t \geq 0$, in which C = maximum germination percentage, B = germination rate parameter, M = time after which 50% of the conidia are germinated, and t = time in hours. From the fitted regression curve for each of the temperatures, the time needed to reach given germination percentages (e.g. 10%, 20%, 50%, 80%, 90%) can be estimated.
Logan curves were fitted to data on mycelial growth at different temperatures. These curves are typically used to describe temperature controlled biological processes. Logan curves have the following general form:

$$Y = a * (\exp(b * X - d)) - \exp(b * (e - d) - (e - X)/c))$$

The response variable Y increases exponentially with temperature X from the value a at the lower threshold temperature d to an optimum temperature with a relative increase of b after which Y declines sharply until the upper lethal temperature e is reached (Logan et al., 1976).

2.2.3.2 Effect of water potential on conidial germination of U. atrum

Conidial suspensions. *U. atrum* was cultured in petri dishes (90 mm) containing 25 ml of oatmeal agar for 28 days at 18°C in the dark. Conidial suspensions were prepared as described above (2.1). 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 agar, 1000 ml tap water) as described by Fokkema (1984) and dried in a sterile laminar flow cabinet. Slides were sprayed with a conidial suspension of *U. atrum* (5×10^5 conidia ml^{-1}; approximately 800 conidia 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 demineralised water or mixtures of demineralised water and glycerol (Dallyn and Fox, 1980) to obtain, at equilibrium, water potentials in the agar layer of –0.28, –1, –3, –5, –7, –10, or –14 MPa, corresponding to a relative humidity 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 centre 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, these were sealed with parafilm and incubated at 18 °C ± 0.5 °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% 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 92.3% 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 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 for each for the treatments was sampled after 0, 2, 4, 6, 8, 10, 12, and 24 h of incuba-

tion 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 using a microscope. 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 fifty arbitrarily chosen conidia was counted and the length of each germ tube was measured using an interactive digitiser (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.

Statistics. The data on conidial germination over time were analysed by fitting the Gompertz curve, an asymmetric logistic curve: $Y = C * \exp[-\exp(-B * (X - M)]$, 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 (–1MPa) for experiments with varying water potentials and *C*, *B* and *M* the parameters to be estimated. In the curve, *C* = upper asymptote (maximum germinated percentage), *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). Since continuous incubation at –10 and –14 MPa did not result in germination, these data were excluded from the 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 analysed using the original structure of the experimental units (Mead, 1988). 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 analysed differently from experiments with multiple interruptions of moist periods. Since the different constant water potentials and the duration of the initial dry period have quantitative treatment levels, the curve parameters *C*, *B* and *M* were analysed 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 LSD tests ($\alpha = 0.05$) for testing pairwise differences between treatment means.

2.2.3.3 *Effect of temperature on antagonism of* U. atrum *against* B. cinerea *and* B. aclada

Effects of temperature on control of sporulation of *B. cinerea* and *B. aclada* by antagonists in dead host tissues were investigated. The effect of *U. atrum* on sporulation of *B. cinerea* and *B. aclada* at different temperatures (6 to 24 °C) was tested on dead onion leaf segments. The effects of *U. atrum* and *G. roseum* on the sporulation of *B. cinerea* were compared at the same range of temperatures on dead leaf segments of cyclamen. Additionally, *U. atrum* was tested at 18, 3 and 1 °C on necrotic leaves of hydrangea inoculated with *B. cinerea*. Each experiment was carried out twice. Symptomless green leaves of field-grown onions cv Hyton and of cyclamen (bulk sample of several cultivars) were dried at 60 °C for several days. Dry leaves were cut into segments 2 cm long for onions and 9 mm discs for cyclamen, sealed in plastic bags and sterilised by gamma-irradiation (4 Mrad). Naturally senesced leaves of hydrangea were dried, cut into segments of 2 x 2 cm and gamma-irradiated. Leaf segments for use in bioassays were washed thoroughly with sterile tap water to remove soluble nutrients. For this purpose, approximately 100 leaf segments were washed three times in 350 ml sterile water in sterile 500-ml conical flasks which were shaken using a flask shaker at 150 strokes min^{-1}. Leaf segments were then blotted dry with sterile filter paper and placed in a sterile petri dish (90 mm). Petri dishes contained four leaf segments on two sterile filter papers (80 mm) moistened with 1 ml sterile tap water. Separate experiments were carried out for leaf segments of onion, cyclamen and hydrangea with five replicate petri dishes per treatment per temperature. Conidial suspensions of *B. aclada* (1×10^5 conidia ml^{-1}), *B. cinerea* (1×10^3, 1×10^4 or 1×10^5 conidia ml^{-1}), *U. atrum* (1×10^6 conidia ml^{-1}) and *G. roseum* 1813 (1×10^6 conidia ml^{-1}), all cultured on oatmeal agar, were prepared as described above. Leaf segments were sprayed with the conidial suspensions using a sterile atomiser in a laminar flow cabinet with a rate of approximately 5 µl cm^{-2} leaf surface. All leaf segments were first sprayed with conidia of *B. aclada* or *B. cinerea* and incubated at 18 °C in the dark for 24 h and 8 h, respectively. After this initial incubation, leaf segments were then sprayed with conidial suspensions of the antagonists or sterile tap water containing 0.01% Tween 80 as the control treatment. For experiments with onion and cyclamen leaves, the petri dishes were subsequently placed at 6, 9, 12, 15, 18, 21, and 24 °C in the dark. For the experiment with hydrangea leaves, the petri dishes were subsequently placed at 1, 3 and 18 °C in the dark. Petri dishes of each temperature were completely randomised within one incubator.

In experiments with onion leaves, the sporulation of *Botrytis* spp. was assessed 8, 12, 21 and 34 days after the antagonist was applied. The coverage of leaf area with conidiophores was estimated using classes from 0 to 5 and a sporulation index (SI)

ranging from zero to hundred was calculated (2.2.2.1; Köhl et al., 1995c). SI's increased in time during the first days of an experiment but then, depending on the incubation temperature, remained constant after 12 to 34 days. For data analysis, SI's were used from the assessment after which no further increase of sporulation was observed. In experiments with cyclamen leaves, *B. cinerea* sporulation was assessed as described above for all temperatures 19 days after antagonists were applied. Since *B. cinerea* produced sclerotia besides conidia on the leaf surfaces, the number of sclerotia was also counted in these experiments after 19 days. In the experiments with hydrangea leaves, sporulation of *B. cinerea* was assessed 21 days after inoculation for leaves incubated at 18 °C and 56 days after inoculation for leaves incubated at 1 or 3 °C.
In one experiment, the number of *B. cinerea* conidia produced on cyclamen leaf segments was determined at day 19. The four leaf discs from each of the five replicates were bulked for each treatment and each temperature. Leaf discs were transferred into 100-ml flasks containing 15 ml tap water with 0.05% Tween 80 and 20% (v/v) ethanol. After shaking with a flask shaker at 200 strokes min^{-1} for 10 min, the concentration of the resulting suspension of *B. cinerea* conidia was counted with the aid of a haemocytometer. From the mean of four subsamples per sample, the number of conidia produced cm^{-1} cyclamen leaf was calculated.

Statistics. The Sporulation Index (SI) for *B. aclada* or *B. cinerea* from bioassays on onion or cyclamen leaves were angular transformed prior to analysis by ANOVA using a split plot model. The experiment, temperature, antagonist treatment and *Botrytis* species or concentration as well as the interactions between temperature, antagonist treatment and *Botrytis* species or concentration were used as explanatory variables. The 2- and 3-factor interaction between experiment and treatment factors were also taken into account in the ANOVA model. The 2-factor interaction between experiment and temperature was used as the main plot residuals for testing temperature effects. The 4-factor interaction between experiment, temperature, antagonist treatment and *Botrytis* species was used as split plot residual for testing the remaining effects. Angular-transformed SI data for the *B. cinerea* bioassay on hydrangea leaves were analysed by ANOVA. Due to inbalance in the design the REML-directive of Genstat 5 was used. In the ANOVA the main effect of experiment and the effect of treatment with *U. atrum*, temperature and concentration, including their interactions, were tested for significance. A conditional LSD test ($\alpha = 0.05$) on the angular scale was used for testing pairwise differences between treatment means. For presentation the means on the angular scale were backtransformed to the original scale.

2.2.3.4 *Effect of water potential on antagonism of* U. atrum *against* B. cinerea

Symptomless green leaves were removed from cyclamen plants (approximately 30 weeks old), placed on dry filter paper and were allowed to senesce for six weeks at

ambient temperature and 50 to 70% RH. Leaves were rehydrated, cut into pieces of approximately 4 x 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, bags were sealed again and stored for one week to allow equilibration of leaf piece water content. To obtain water potentials of approximately –1 MPa, –3 MPa and –7 MPa, 1.5 ml, 0.5 ml or 0.25 ml of sterile tap water per g dry weight of leaf pieces were added, respectively, according to the relationship between water content and water potential available for dead onion leaves (3.2.1.4; Köhl et al., 1995c).
Moist chambers were prepared consisting of sterile plastic petri dishes (140 mm) containing 75 ml water agar with KCl added to obtain a water potential of the agar of –1 MPa, –3 MPa and –7 MPa (Campbell and Gardner, 1971). A sterile glass slide (76 mm x 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 seven 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 seven days at 18 °C to allow equilibration of the water potentials and obtain a precisely controlled water potential in the leaves (Köhl et al., 1992).
On another set of sterile glass slides, conidial suspensions of *B. cinerea* (1 x 10^5 and 1 x 10^6 conidia ml^{-1}) or *U. atrum* (1x 10^6 conidia ml^{-1}) were sprayed at a rate of approximately 1 µl cm^{-2} and were 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 ten 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 cm x 30 cm x 48 cm) with wet filter paper fixed to the walls. Slides with leaf pieces were immediately placed back into the moist chambers and the moist chambers were sealed with parafilm. Leaf pieces were then incubated at 18 °C ± 0.5 °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 (3.2.1; Köhl et al., 1995c). The experiment was repeated.

Statistics. Angular-transformed data of the repeated experiment on antagonism of *U. atrum* towards *B. cinerea* at different water potentials were combined and analysed

by ANOVA followed by LSD tests ($\alpha = 0.05$) for testing pairwise differences between treatment means.

2.2.3.5 *Survival of* U. atrum *conidia on green leaves and competitive colonisation of senesced leaves by* B. cinerea *and* U. atrum

Experiments with greenhouse-grown cyclamen

Treatments. Fifty 20-week old potted cyclamen plants (cv Super Serie) with approximately 15 leaves per plant were grown in a greenhouse at 20 ± 3 °C and 65 ± 20% RH. Petioles of fully developed leaves were labelled with coloured, plastic coated iron rings. Pots were placed on tables on capillary mats. Water was supplied via the mat so that leaves were not wetted. In a second experiment, water was applied by top irrigation using a sprinkler. Plants were irrigated with approximately 1 l m^{-2} at an interval of two to three days. Experimental plots consisted of five plants, each sprayed with a conidial suspension of *U. atrum* with 1 x 10^6 conidia ml^{-1} (2.1) or tap water containing 0.01% Tween 80. The separate experiments with the two different irrigation regimes were arranged in a randomised complete block design, with each treatment replicated five times and each experiment repeated once.

Conidal density and germination. Two (first experiment without top irrigation) or three labelled leaves (other experiments) per sampling date per replicate were arbitrarily selected from the five plants of each replicate. Sampling dates were 0, 2, 4, 7, 10, 14, 21, 28, 42, 56 and 70 days after spraying. Sampled leaves were healthy and without symptoms of *B. cinerea* during the experiment, but *B. cinerea* developed on senesced leaves of the plants. At each sampling date, one of the sampled leaves was cut longitudinally into two pieces. On one half, the density of conidia and the percentage of germinated conidia of *U. atrum* were determined. The other half was incubated at 24 °C for 8 h in the dark in a moist chamber consisting of petri dishes (55 mm) with two moistened filter papers (50 mm) on the bottom and one in the lid to estimate the percentage of conidia of *U. atrum* which lost their ability to germinate.

Conidial density on the upper leaf surface was determined using a microscope at a magnification of 10 x 10. Each half leaf was divided into ten equally sized sections and the conidial density was determined in the centre of each section by counting the number of conidia lying within a grid representing 1 mm^2. The average number of *U. atrum* conidia cm^{-2} of leaf surface was calculated for each leaf. To determine germination of conidia of *U. atrum* on the leaf surface directly after sampling or after additional incubation in a moist chamber, a droplet of 0.01% of Calcofluor White (Fluorescent Brightener 28, F-6259 Sigma; 0.01% w/v in 1 M Tris HCl buffer, pH 8) was added to the leaf surface and the surface was inspected at a magnification of 10 x 10 under a fluorescence microscope (Axioscope with filter 05, Zeiss, Oberkochen, Germany) using a blue/violet (395 to 440 nm) light source. Per

half leaf, 100 arbitrarily chosen, non-clustered conidia were assessed. Conidia were counted as germinated when germ tube length was at least half of the shortest axis of the ellipsoidal conidium.

Competitive substrate colonisation. In experiments without top irrigation, the remaining one (experiment 1) or two (experiment 2) leaves per replicate were placed in an open petri dish (55 mm) on two dry filter papers (50 mm) for 21 days (experiment 1) or 28 to 42 days (experiment 2). Petri dishes were arranged in blocks as described above for plants. After the detached leaves in the petri dishes had senesced (characterised by the loss of turgor and a yellow or brown colour) during a period of three, four or six weeks in the experimental greenhouse, 0.75 ml of tap water were added to each petri dish and the closed dishes were transferred to an incubator. Leaves were incubated at 18 °C in the dark. After 14 days, the coverage of the upper surface of the leaves with conidiophores of *B. cinerea*, *U. atrum* and other fungi was estimated in classes ranging between 0 and 1 (i.e. 0, 0.01, 0.05, 0.1, 0.2, ... 0.8, 0.9, 0.95, 0.99, 1) using a stereomicroscope with 10-40 x magnification (2.2.2.2; Köhl et al., 1995b). In the first experiment, in many cases leaves were not senescent at the assessment date and no fungi developed. Data from such leaves were excluded from the analysis. Data from three sampling dates, based on at least one leaf per replicate, were further analysed. In the second experiment without top irrigation, detached leaves were left in the greenhouse for 28 days. Thereafter, senesced leaves were incubated in a moist chamber. Leaves without symptoms of senescence were left in the greenhouse for another 14 days. At assessment dates, 14 days after incubation in a moist chamber, all leaves were necrotic.
In experiments with top irrigation, two leaves 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 described for plants above 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 above.

Statistics. Analysis of variance (ANOVA) was employed on log-transformed counts of *U. atrum* conidia cm^{-2} and on arcsine transformed data on sporulation of *B. cinerea*. Significant *F*-tests ($P < 0.05$) were followed by two sided LSD tests ($\alpha = 0.05$) for testing pairwise differences between treatment means.

Experiments with field-grown lilies
Field plots and treatments. Bulbs of the Asiatic hybrid lily (*Lilium* cv Mont Blanc) were planted on 18 April 1995 in 5 m x 1 m plots with each plot separated by 8 m buffers of sugar beet. The herbicide chloridazon (Pyramin, 650 g a.i. kg^{-1}, BASF, Germany) was applied on 19 April and thereafter no further pesticide appli-

cations were made. Prior to anthesis, all flower stems in each plot were removed on 5 July by hand as normal commercial practise in bulb production. Ten microplots, each consisting of at least nine individual plants of equal height and growth stage, were selected within each of the two separate 5 m x 1 m plots.
The *U. atrum* suspension was applied on 24 July (experiment 1) to five replicate microplots in the same 5 m x 1 m plot using a propane-powered backpack sprayer (AZO, Ede, the Netherlands) with a single nozzle with a pressure of 250 kPa. Tap water plus 0.01% Tween 80 was applied to five replicate control microplots also located in the same plot. The experiment was set up as a 2 * 3 * 4 factorial split plot design with treatment factors that were: *U. atrum* application (plus and minus), canopy level (top, middle, bottom) and time of inducing necrosis after *U. atrum* application (0, 7, 14 and 21 days) with five replications (microplots). The experiment was repeated in a separate 5 m x 1 m plot on 31 July (experiment 2). All treatments were sprayed in the evening and a metal frame (50 cm x 30 cm x 30 cm) surrounded by polythene plastic was carefully placed around each microplot at the time of application to minimise drift from one microplot to another.

Microclimate measurements. Temperature and relative humidity (RH) of the air were measured with an electronic sensor (airprobe YA-1OO-hygromer, Rontronic AG, Bassersdorf, Switzerland) positioned at a height of 35 cm in an onion field approximately 50 m away from the treated plots. Rainfall was recorded with a rain gauge placed in the same field. Three leaf wetness sensors (Köhl et al., 1995b) were used to monitor leaf wetness of necrotic lily leaves in the lily canopy in an additional microplot. All data were recorded at 30-min intervals and stored in a datalogger (Delta-T Logger, Delta-T Devices Ltd. Burwell, Cambridge, UK).

Survival of *U. atrum* conidia on green lily leaves. One hour, 7, 14 and 21 days after *U. atrum* application, one green leaf was randomly selected per replicate from each of the top, middle and bottom level of a single plant, located approximately 25 cm, 15 cm and 5 cm respectively from the soil. Each leaf was placed into a sterilised plastic vial and samples were immediately returned to the laboratory. Sampled leaves were then cut in two along the axis of the main vein with the aid of a scalpel. In order to determine the density (number of conidia cm^{-2} leaf surface) and percentage of *U. atrum* conidia which had germinated on the leaf surface in the field (field germination), one half of the leaf was placed into an ammonia fumigation chamber to terminate all microbial growth. The fumigation chambers consisted of sterile plastic petri dishes (90 mm diameter) to which five drops of household ammonia (4.8% NH_4OH in water) were added to a single layer of filter paper (Whatman, 50 mm diameter) attached to the lid of the chamber. To determine the percentage of viable *U. atrum* conidia on the leaf surface (germination potential), the other half of the leaf was placed into a high humidity chamber. The chambers consisted of sterile plastic petri dishes (90 mm diameter) containing two sterile filter papers (Whatman, 80 mm diameter), moistened with 1 ml sterile tap water.

Growth was terminated after 18 h incubation at 18 °C in the dark by the addition of ammonia as described above. All leaf samples were stored at –18 °C until measurement.
Half leaf sections were recut to 1 to 2 cm and placed onto glass microscope slides. To each preparate, 100 µl of the fluorochrome Calcofluor White was added to the leaf surface and then examined with a Zeiss Axioskop Fluorescence Microscope (see above). After staining with Calcofluor, little or no background autofluorescence from plant cells was observed and spore morphology was used to distinguish *U. atrum* conidia from other saprophytic species. The number of conidia in an ocular eyepiece grid (representing 0.25 mm^2 of leaf surface) was counted and the average number of conidia per grid calculated from 10 grids. The first observation was always started at an arbitrarily chosen position on the leaf and the other observations were selected at regular intervals along a diagonal transect across each preparate and the results expressed as the number of conidia cm^{-2} of leaf surface.
Field germination (%) and germination potential (%) were determined by counting all conidia that had germinated in several fields of view, representing 0.64 mm^2 of leaf surface. The first observation was always started at an arbitrarily chosen position on the leaf and the other observations were selected at regular intervals along a transect across the surface of each preparate. Clusters of conidia were avoided and a minimum of 50 conidia in total were counted. Germination was assumed to have occurred when the germ tube length was greater than half the diameter of the conidium.

Fungal colonisation of artificially induced necrotic lily leaves. In order to determine the ability of surviving *U. atrum* inoculum on green leaf surfaces to successfully colonise necrotic tissues, a method was developed which induced localised necrosis on tagged green leaves in the field. Zero, 7, 14 and 21 days after *U. atrum* application, necrosis was artificially induced locally on tagged leaves, selected at random approximately 25 cm, 15 cm and 5 cm from the soil surface, representing the top, middle and bottom levels, respectively. There were four lily plants per replicate and five replicates per treatment. Induction of necrosis was achieved using a desiccant herbicide Gramoxone (200 g l^{-1} paraquat dichloride, Zeneca Agrochemicals, UK). Paraquat has been used in several phytopathology studies as a tool to aid the identification of fungi in host tissues (Bannon, 1978; Cerkauskas and Sinclair, 1980). However, paraquat is known to affect some fungal species more than others (Wilkinson and Lucas, 1969) and spore germination may be adversely affected at concentrations as low as 32 µg ml^{-1} paraquat (Michailides and Spotts, 1991). Our preliminary studies indicated that 10 mg l^{-1} paraquat (plus 0.1% Tween 80) was sufficient to induce localised necrosis on healthy green lily leaves but did not affect spore germination (%) or germ tube growth of an isolate of *B. cinerea*, *B. elliptica*, *Alternaria* spp., *Epicoccum purpurascens*, *Ulocladium* spp. or *U. atrum* (Elmer and Köhl, unpublished).

To induce necrosis on healthy green lily leaves in the field, a small piece (1.5 cm^2) of household moisture absorbent cloth was fixed firmly to the underside of tagged leaves and kept in place with the aid of a paper clip. Six hundred µl of paraquat suspension (10 mg l^{-1} plus 0.1% Tween 80) was then carefully pipetted between the cloth and the underside of the leaf. Paraquat treatment was always made after sunset to avoid the absorbent cloth drying too rapidly and the cloth and paper clip were removed 48 h after paraquat application. The first symptoms of tissue necrosis were observed at that stage and complete necrosis of the entire leaf occurred five to seven days after paraquat treatment.
Fourteen days after each application of paraquat (applied on day 0, day 7, day 14 and day 21), one paraquat-treated necrotic leaf was removed from each of the top, middle and bottom zones of four plants per replicate with flame sterilised forceps. The four leaves per canopy level per replicate were placed into the same sterile high humidity chambers. The chambers consisted of sterile plastic petri dishes (110 mm diameter) with two layers of filter paper (Whatman, 100 mm diameter) moistened with 3 ml sterile tap water. The humidity chambers were sealed with parafilm and incubated at 18 °C in the dark for two days. The parafilm was then removed and the necrotic leaves incubated for a further seven days. All samples were then sealed in plastic bags and stored at –18 °C.
The area of necrotic tissue covered with sporulating *U. atrum* and other commonly occurring saprophytes was measured with the aid of a stereo microscope. A 25 mm^2 square was prepared from a clear acetate sheet and placed at random onto the necrotic leaf surface. The area of sporulation in each 25 mm^2 square by *U. atrum*, *Alternaria* spp., *Cladosporium* spp., *Epicoccum* spp., *Stemphylium* spp., and *Botrytis* spp. was then measured for four squares per leaf. Results were expressed as percentage coverage of the surface of necrotic tissue.

Statistics. For the *U. atrum* treated microplots, an analysis of variance (ANOVA) was performed on the observed density counts of conidia after logarithmic transformation using a split-plot model with canopy level, time after *U. atrum* application and their interaction as explanatory variables and random effects for variation between plots, canopy levels within plots and time within canopy levels within plots. The data for percentages of germinated conidia and the percentages of leaf area colonised by *U. atrum* were angular transformed prior to ANOVA using the same split-plot model. Orthogonal polynomials were used to partition the sum of squares for time as well as for the interaction between time and canopy level into the amount that may be explained by a linear relationship of the response with time, the extra amount that may be explained if the relationship was quadratic and the amount represented by deviations from a quadratic polynomial. For the various time intervals separate ANOVA's were performed to percentages of leaf area colonised by *Alternaria* spp. and *Cladosporium* spp., respectively, after angular transformation using a split-plot model with treatment, canopy level and their interaction as explanatory variables and random effects for variation between plots and variation

between canopy levels within plots. *F*-tests for the variance ratios were used to assess treatment effects and pairwise differences between treatment means were assessed using *t*-tests.

2.2.4 Mode of action of *U. atrum*

2.2.4.1 Fungal interactions: microscopical and ultrastructural studies

Onion leaves. Symptomless green leaves were removed from 12-week old field-grown plants of onion cv Hyton, dried for several days at 60 °C and stored in paper bags at room temperature in the dark (2.2.2.1; Köhl et al., 1995c). Dried leaves were cut into 2-cm segments, sealed in plastic bags and sterilised by gamma radiation of 4 Mrad.

Bioassay on dead leaves. Onion leaf segments for use in bioassays were washed thoroughly with tap water to remove soluble nutrients and subsequently blotted dry with sterile filter paper (2.2.2.1; Köhl et al., 1995c). Six leaf segments were placed in each of a series of moist chambers. Each chamber consisted of a sterile plastic petri dish (90-mm diameter) containing two sterile filter papers (80-mm diameter) and 1 ml sterile tap water. The experiment was carried out in a completely randomised design with five replications (petri dish) with ten treatments. The treatments were: each of the four antagonists applied alone or in combination with *B. aclada* and *B. aclada* applied alone. In another control treatment, no fungi were applied to the leaf segments. Conidial suspensions of *B. aclada* or water plus surfactant were sprayed onto the leaf segments in the moist chambers and the segments were incubated at 18 °C for 24 h. Immediately after the incubation periods, spore suspensions of antagonists, or water plus surfactant, were sprayed onto the segments. Thereafter, leaves were further incubated at 18 °C in the dark.
For light and electron microscopy observations, samples were taken six days after application of the antagonists, since preliminary work had established this time period to be optimal. Leaf pieces (2 x 2 mm) were cut from the central part of two randomly selected leaf segments of each replication of each treatment and were fixed and stored in glutaraldehyde (3%, v/v) in 0.1 M sodium cacodylate at pH 7.2. Samples from the same treatments were bulked.
Eight days after antagonists had been applied, the leaf area covered with conidiophores of *B. aclada* was estimated using classes from zero to five and a sporulation index (SI) ranging from zero to 100 was calculated for each replication (petri dish) consisting of four leaf segments (2.2.2.1; Köhl et al., 1995c).

Assay on agar. Petri dishes containing 15 ml of malt extract agar (10 g malt extract, 15 g agar, 1000 ml tap water) were inoculated in four replicates with 5-mm agar discs with mycelium of *B. aclada* or *U. atrum* that had been obtained from the

margins of young colonies of the fungi grown on the same medium. Petri dishes were inoculated with one agar disc, with two discs of the same fungus or with two discs with different fungi. The distance between two discs was 50 mm. Plates were incubated at 18 °C for seven days. After seven days, agar with mycelium was covered with a 1-mm layer of agarose (3%) (van Balen, personal communication). Small pieces of agarose-covered agar with mycelium were cut from the margins of colonies. From plates with two colonies, samples were taken at the margins of both colonies where they had first contact. Samples were fixed and stored in glutaraldehyde (3%, v/v) in 0.1 M sodium cacodylate at pH 7.2.

Light and electron microscopy. Both leaf and agar samples were post-fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer at 4 °C for 1 h, before being dehydrated in an ethanol series and embedded in Epon 812. Sections (0.7 μm) of leaf tissues were cut and mounted on glass slides prior to being stained with toluidine blue (0.96 mg/ml water) or methylene blue (10 mg/ml water) and examined by light microscopy. Ultrathin sections (0.1 μm) of leaf and agar samples were stained for transmission electron microscopy (TEM) with uranyl acetate and lead citrate prior to being examined, using a JEOL 1200 EX electron microscope at an accelerating voltage of 80 kV. For each treatment of bulked samples, a minimum of three samples were cut into serial sections and examined under both light microscopy and TEM. Unless otherwise stated, reported observations were representative of all samples.

2.2.4.2 Competitive substrate colonisation: experimental studies and simulation modelling

Plant material. Thirty-week old cyclamen plants cv Super Serie were obtained from a commercial grower. The plants were placed on tables with capillary matting in a greenhouse at 18 °C. Relative humidity of the air (RH) varied between 80% and 90%. Daylight was supplemented to a total of 10 h of light per day using artificial lighting. Leaves and flowers were removed and the plants were allowed to develop new pesticide- and pollen-free leaves. Pollen is known to enhance infection by *B. cinerea* (Chou and Preece, 1968). No pesticides were used and flower buds were removed. Three batches of fully grown symptomless leaves were picked in December, February and April. Leaves were placed on dry filter paper at ambient temperature and about 60% RH and allowed to senesce. After two weeks, when the leaves were completely dry, they were cut in half, the midvein was removed. The leaf segments were sealed in plastic bags, sterilised by gamma-irradiation (4 Mrad) and stored at room temperature in the dark until use.

Head start experiments. Sterile necrotic cyclamen leaf segments were re-hydrated overnight in sterile tap water at 4 °C. Excess water was removed by blotting the leaves on sterile filter paper. Four leaf segments were placed in each of 150 petri

dishes (90 mm in diameter), adaxial side facing up, on top of a sterile plastic grid above two sterile filter papers (80 mm in diameter) moistened with 1.5 ml of sterile tap water. Leaves in the petri dishes were spray-inoculated with conidial suspensions of *B. cinerea* or *U. atrum* containing 1×10^6 conidia ml^{-1} using atomisers (Desaga, Heidelberg, Germany). Inoculation resulted in average conidial densities of 45 conidia mm^{-2} leaf for *B. cinerea* and *U. atrum* as determined by direct microscopical observation.

Mixed cultures were initiated by spraying the leaves with conidial suspensions of *B. cinerea* and *U. atrum*. *B. cinerea* was applied 24, 12 and 0 h ahead of *U. atrum* and 12, 24, 30, 36, 42, 48 and 72 h after *U. atrum* had been applied. Monocultures served as control treatments. They were initiated using the application intervals used for the mixed cultures but replacing either *B. cinerea* or *U. atrum* by an application of sterile tap water containing 0.01% Tween 80 resulting in *U. atrum* monocultures and *B. cinerea* monocultures, respectively. For each application interval of the fungi in mixed culture, the corresponding single inoculant treatment was used to assess the effect of the challenger fungus. Five replicate petri dishes were included for each application interval and culture type. Petri dishes were incubated at 18 °C in the dark for two weeks after the first spray in a completely randomised design. The experiment was repeated once.

Sporulation of *B. cinerea* and *U. atrum* was quantified two weeks after the first spray treatment. The fraction of the leaf area covered with conidiophores (0, 10, 20,..., 100%) and the relative intensity of the sporulation (0, 10, 20,..., 100%) of *B. cinerea* and *U. atrum* was recorded per leaf using a dissecting microscope at 100 x magnification. Multiplication of the two parameters and scaling of the result yields the Sporulating Leaf Area Corrected for Intensity (SPLACI, 3.5.4; Köhl et al., 1998), an estimate for the number of conidia produced per leaf ranging from 0 to 100%. SPLACI values were averaged for the four leaves per petri dish.

Data analysis was done analogous to de Wit's analysis of replacement series. Sporulation was considered the ecologically relevant yield of the fungi. Based on average SPLACI values per petri dish, the relative yields (RY, yield in mixed culture divided by the yield of the corresponding monoculture) of *B. cinerea* (RY_{bc}) and *U. atrum* (RY_{ua}) as well as their sum, the relative yield total ($RYT = RY_{bc} + RY_{ua}$), were calculated and plotted against the application interval on the x-axis. If inter- and intraspecific competition are equal in intensity, the RY of each species is directly proportional to its biomass proportion in the starting mixture. The sum of the relative yield for both species gives the relative yield total (RYT). The shape of the RYT line is indicative of the type of interaction between both competing species. A horizontal RYT line remaining at 1.0 for all input proportions indicates equal inter- and intraspecific competition. A RYT line that is concave or convex, respectively, indicates stronger and weaker interspecific than intraspecific competition.

Dynamics of mycelial colonisation and sporulation. Sterile necrotic cyclamen leaf segments, prepared as described before, were placed on top of a sterile plastic

grid, adaxial side facing up, above two sterile filter papers (40 mm in diameter) moistened with 0.5 ml of sterile tap water in a sterile plastic petri dish (55 mm in diameter, one leaf per dish). Leaves were spray-inoculated with conidial suspensions (1 x 10^6 conidia ml^{-1}) of *B. cinerea*, *U. atrum* or both fungi, using an atomiser (Desaga, Heidelberg, Germany). Inoculation resulted in average conidial densities of 25 conidia mm^{-2} for *U. atrum* and *B. cinerea* as determined by direct microscopic observation. Following inoculation, petri dishes were incubated in the dark at 18 °C or 24 °C in a completely randomised design.
Leaf samples were processed and sectioned on a cryostat as described by Kessel et al. (1999). Mycelium of *B. cinerea* in the tissue sections was labelled specifically using the IgM monoclonal antibody (MAb) Bc-KH4 (Bossi and Dewey, 1992) and visualised in a secondary step using a μ-specific goat anti-mouse antibody conjugated to FITC (Cat No 1020 - 02, Southern Biotechnology Associates, Birmingham, Al., USA). Mycelium of *U. atrum* in the sections was labelled specifically using the monoclonal antibody (MAb) Ua-PC3 (obtained from F.M. Dewey, Oxford University, Oxford, UK) and visualised in a secondary step using a γ-specific goat anti-rat antibody conjugated to TRITC (Cat No 3030 - 03, Southern Biotechnology Associates, Birmingham, Al., USA). Ua-PC3 is a rat IgG MAb raised against the surface washings of *U. atrum* mycelium. Ua-PC3 recognises an antigen located in the cell wall of *U. atrum* and does not attach to *B. cinerea* mycelium (Karpovich and Dewey, 2001).
Mycelial colonisation was quantified by measuring the relative surface area of the labelled mycelium in the section using image analysis. The measured relative surface area of the mycelium was mathematically converted into the relative volume of the mycelium in the section (Kessel et al., 1999).

Dynamic simulation model. To test whether resource competition can explain the phenomena observed in the experiments, a mathematical model was constructed. In the model, growth of mycelial biomass is determined by the density of 'parent' biomass, and by the density of resources. Two types of resources were distinguished: (1) general resources available to both species during intra- and interspecific competition, and (2) species-specific resources available to one of both species during intraspecific competition (optional). The state variables in the model are U, B, R, R_U, and R_B. These represent, respectively, the biomass density of *U. atrum*, the biomass density of *B. cinerea*, and the density of resources that are accessible to both fungi (R), only accessible to *U. atrum* (R_U), or only accessible to *B. cinerea* (R_B). All these state variables are scaled and have unit dimensions on a scale from 0 to 1, such that U = 1 if the single species carrying capacity for *U. atrum* is reached (assuming R_U to be 0). Likewise, for *B. cinerea*, B = 1 if the single species carrying capacity for *B. cinerea* is reached. The state variables for resource density are scaled such that R = 1 in a non-colonised leaf, whereas R = 0 in a leaf from which all general resources have been depleted. The variables R_U, and R_B are intro-

duced to allow the option of species specific resources. The corresponding equations are (From: Kessel, 1999):

$\frac{dU}{dt} = r_U \bullet U \bullet (R + R_U)$	(equation 1)
$\frac{dB}{dt} = r_B \bullet B \bullet (R + R_B)$	(equation 2)
$\frac{dR}{dt} = -\mu \bullet \frac{dU}{dt} \bullet \frac{R}{R + R_U} - \beta \bullet \frac{dB}{dt} \bullet \frac{R}{R + R_B}$	(equation 3)
$\frac{dR_U}{dt} = -\mu \bullet \frac{dU}{dt} \bullet \frac{R_U}{R + R_U}$	(equation 4)
$\frac{dR_B}{dt} = -\beta \bullet \frac{dB}{dt} \bullet \frac{R_B}{R + R_B}$	(equation 5)

Parameters and initial conditions are defined as follows: r_U = relative growth rate of *U. atrum* [h^{-1}], r_B = relative growth rate of *B. cinerea* [h^{-1}], μ = resource use efficiency of *U. atrum* [–] and ß = resource use efficiency of *B. cinerea* [–]. At t = 0: U = $U_{(0)}$, B = $B_{(0)}$ and R = $R_{(0)}$ = 1. The model was numerically solved (simulated) using the Fortran Simulation Translator, FST (Rappoldt and van Kraalingen, 1996).
Experimental results of the head start experiments were compared with hypothetical scenarios under the assumption that the final fungal biomass level reached in the simulations was proportional to sporulation. Equal interspecific and intraspecific competition was used as a reference scenario. This scenario was implemented using the following settings for initial conditions and parameters: $R_{(0)}$ = 1; $R_{B(0)}$ = $R_{U(0)}$ = 0; r_U = r_B = 0.1; $U_{(0)}$ = $B_{(0)}$ = 0.01 and μ = ß = 1. In a second step the effect of differential relative growth rates (r_u and/or r_b = 0.1, 0.2 or 0.3), differential resources ($R_{(0)}$ and/or $R_{(0)}$ = 0, 0.1 or 0.2) and differences in the initial biomass generated by germinating conidia ($U_{(0)}$ and/or $B_{(0)}$ = 0.0001, 0.001 or 0.01) were simulated.

2.2.5 Use of *U. atrum* for disease control in crops

2.2.5.1 Experiments in annual strawberries

Experimental plot layout. Seven field experiments were conducted with annual strawberry (cv Elsanta) crops, using waiting-bed transplants cold-stored at –2 °C until the day before planting. All experiments were carried out in a randomised complete block design with four to six replications. Each plot in experiments 1 and 4 consisted of four single rows of ten plants per row, with three plants per meter of row length and 0.8 m between rows. Plots were established inside a field of red cabbage serving as buffer crop with a distance of 10 m between plots. Each plot in experiments 2, 3, 5, 6, and 7 consisted of 78 plants in three twin rows of 2 x 13 plants per twin row. The distance between twin rows was 1 m, and 0.5 m within twin rows with three plants per meter of row length. The plots were established within a grass field, separated by 10 m of grass buffer.

Treatments. The control treatment was an unsprayed plot except in experiment 1, where water-containing Tween 80 at 0.01% was applied weekly from transplanting until fruits began to turn red. *U. atrum* was applied at a concentration of 2×10^6 conidia ml^{-1}, except for experiment 7, in which the concentration was 0.5×10^6 conidia ml^{-1}. Application of *U. atrum* began at transplanting (*U. atrum* transplanting) or at opening of the first flower (*U. atrum* flowering) and ended when the first fruits turned red. The frequency in application of *U. atrum* varied from weekly to twice weekly, depending on the experiment. Comparative fungicide programmes started at first open flower and continued until first fruits turned red, with application frequencies varying among experiments. Materials used included iprodione (Rovral WP, Rohm and Haas, France), tolylfluanide (Euparen M, Bayer, the Netherlands), pyrazophos (Curamil, AgrEvo, the Netherlands), thiram (TMTD, Satec, Germany), and pyrimethanil (Scala, AgrEvo, the Netherlands). Crop sanitation treatments were carried out in experiments 5 and 6, and consisted of removal of all senescing leaflets from the strawberry plants twice per week, starting immediately after transplanting until first fruits turned red.
For experiments 1 and 4, conducted at Breda, *U. atrum* and fungicides were applied with a knapsack sprayer using a hollow-cone nozzle and air-pressure at 200 kPa at a rate of 500 l ha^{-1}. For experiments 2, 3, 5, 6, and 7, conducted at Wageningen, *U. atrum* and fungicides were applied with a propane-gas-driven knapsack sprayer at 250 kPa at a rate of 750 l ha^{-1}.

Crop management and cultural practices. In all experiments, interval overhead irrigation was applied during periods of hot, dry weather in order to promote development of *B. cinerea*. Weeding was done mechanically. The soil was covered with straw from beginning of flowering. No insecticides, herbicides, or other fungicides were applied except for experiment 1 in which deltametrin was applied three times

for thrips control and experiment 4 in which transplants were treated with a fungicide (fosetyl-Al plus benomyl) at the time of planting.

Incidence of *B. cinerea* on flowers. In experiments 2, 3, 5, and 6, samples of 20 to 30 flowers, each flower from a different arbitrarily chosen plant, were collected per plot at each of two or three sampling dates. In experiment 7, 50 petals, each petal from a different arbitrarily chosen plant, were collected per plot instead of whole flowers. The flowers were processed and assessed as described in 2.2.1.2.

Harvest and post-harvest disease assessments. Fruits were picked twice per week. Ripe symptomless fruits were harvested first and kept separate from harvested diseased fruits. Fruits with symptoms were also picked when still in the white stage. Healthy and diseased fruits per plot were weighed and counted.

Fruit rot. In experiments 2, 3, 5, 6, and 7, the ripe and white diseased fruits were assessed for characteristic symptoms caused by *B. cinerea, Colletotrichum acutatum* or *Zythia fragariae*. In experiments 1 and 4, symptoms were classified as those of *B. cinerea* and other diseases without further differentiation. Fruits with non-specific symptoms were placed without contacting each other into a plastic tray (50 cm length x 30 cm width x 7 cm height) with wet paper on the bottom. The tray was sealed in a plastic bag, incubated at 20 °C for two days in the dark before symptoms were reassessed. Fruits with characteristic symptoms were then added to the corresponding disease category, and fruits with non-specific symptoms were classified as 'other diseases'.
Post-harvest fruit rot on a sub-sample of symptomless fruits was assessed for experiments 1, 2, 3, 5, and 6. Twenty arbitrarily chosen fruits per plot were placed in a plastic tray (50 cm length x 30 cm width x 7 cm height) with wet paper on the bottom without contact among fruits, sealed in a plastic bag and stored at 4 °C during five days in the dark followed by three days incubation at 20 °C. Fruits were then assessed for grey mould incidence.

Statistics. The statistical analysis was performed by analysis of variance (ANOVA), followed by LSD tests. Values of grey mould incidence on harvested fruit were arcsine transformed prior to analysis of variance.

2.2.5.2 Experiments in onion

Field plots. Two field experiments with onion were conducted. Onion cv Hyton F1 was sown beginning of April 1995 and 1996, respectively, in 15 plots of 9 x 9 m in rows 0.3 m apart. In order to minimise interplot interference, plots were separated by strips of sugar beet of at least 12 m (3.1.1; Köhl et al., 1995a). Plots were arranged in five blocks with plots of three different treatments randomised within blocks.

Treatments. Three treatments were carried out at weekly intervals starting 26 June 1995 and 24 June 1996, respectively, until the end of August (two weeks before harvest); (1) water containing 0.01% Tween 80; (2) conidial suspensions of *U. atrum* (2×10^6 conidia ml^{-1}); and (3) Ronilan (with 2 kg ha^{-1} a.i. vinclozolin). All sprays were applied at 500 l ha^{-1}.

Colonisation of necrotic leaves exposed in onion plots. Leaf supports each with ten 10-cm long leaf segments of artificially killed onion leaves were prepared as described in 2.2.2.2. Leaf supports were positioned in the centre of each onion plot during the field experiment in 1995 with leaves in a vertical position approximately 20 cm above the soil. Per plot, one leaf support was placed before spray applications and one support 60 min after spraying. After seven days, five leaves per holder were sampled and incubated in moist chambers. Sporulation on the leaves of *Alternaria* spp., *Cladosporium* spp., *Botrytis* spp. and *U. atrum* was assessed after ten days incubation at 18 °C (as described in 2.2.2.2 for lily leaves). In total, this operation was repeated weekly ten times during the course of the field experiment.

Colonisation of naturally senesced leaves. During the field experiment in 1995, necrotic parts of onion leaves were sampled randomly on 9 and 18 August (approximately 25 per plot with a total length of 350 cm). Necrotic leaf tissues were placed in moist chambers (45 x 28 x 5 cm polycarbonate chambers with two layers of sterile wet filter paper, enclosed in polyethylene bags). Sporulation of *Botrytis* spp. was assessed as described above directly after sampling and after incubation at 18 °C for 10 days. In 1996, such samples were obtained at 7, 14 and 21 August and processed in the same way.

Aerial spore load. Rotorod spore samplers were used on 19 and 22 August 1996 to quantify the amount of conidia of *Botrytis* spp. in the air above and outside onion plots. One Rotorod device was fixed at a height of 0.3 m in the centre of each plot of the first four blocks of the experiment. Additionally, four Rotorods were placed approximately 12 m away from onion plots in sugar beet to quantify the background load of *Botrytis* spp. conidia. Thus, in total 16 Rotorod devices were used simultaneously. At each day, three 15-min runs were carried out between 10:00 and 13:00 when rapid changes in relative humidity of the air were expected to favour spore release of *Botrytis* spp. (Jarvis, 1980; Lorbeer, 1966). Trapped conidia of *Botrytis* spp. were counted on one rod per run separately for conidia < 15 µm, most probably belonging to *B. cinerea*, and > 15 µm, most probably belonging to *B. squamosa*. The concentration of conidia m^{-3} of air was calculated according to the formula provided by the manufacturer.

Leaf spots. In both experiments, ten randomly chosen plants per plot were sampled weekly from the end of June until harvest. Lesions were counted on all green

leaf parts and the surface of green leaf parts was measured with a Delta-T Area measurement System (Delta-T Devices LTD., Burwell, Cambridge, UK). The number of lesions cm^{-2} of green leaf surface was calculated per plot.

Statistics. Data on spore load m^{-3} of air and log_{10}-transformed numbers of leaf spots cm^{-2} of green leaf surface were analysed separately per assessment date by ANOVA followed by LSD tests ($\alpha = 0.05$).

2.2.5.3 Experiments in cyclamen under commercial growing conditions

Development of a biocontrol system with *U. atrum* and *G. roseum*

Plants. Cyclamen plants (*C. persicum*) cv Super Serie from four lines with different flower colour (wine red, lilac, pink, white with red heart) were used in all experiments. This cultivar has a characteristically compact growth resulting in a very dense canopy. Plants were seeded in trays with soil plugs and transferred into individual 8-cm pots containing a commercial potting soil ('Lentse' special cyclamen mixture, Lent, the Netherlands) about ten weeks after sowing (three-leaf stage). For about six weeks the plants were held on trays. Subsequently, they were placed on tables with mats at wider spacing and spacing was increased further after about six weeks on the tables in order to avoid touching of leaves of different plants. Flowering plants were considered marketable about 30 weeks after sowing when they have 50 to 55 leaves. In the first experiment, treatments were initiated with 19-week old plants three weeks after the plants were placed on the tables. In the second experiment, treatments were initiated with 16-week old plants still on the trays.

Growing conditions. Experiments were conducted in a 3000 m^2 commercial greenhouse at Twello, the Netherlands, in which cyclamen and several other ornamental crops were produced. Minimum temperatures were set at 14 °C during the day and 16 °C during the night. Relative humidity was set at 80%. On warm or rainy days, temperature or relative humidity may exceed the set values due to insufficient capacity of climate control by ventilation and screening.

Treatments. In the first experiment four treatments were arranged in a randomised complete block design with each treatment replicated four times. Two blocks were located on each of two opposite tables. In the second experiment, all blocks were located on the same table. There were no buffers between treatments. In the first experiment, each replicate started with 63 plants placed in nine rows each with seven plants of which the central 25 plants were assessed for disease development. In the second experiment, five treatments were arranged in a randomised complete block design with each treatment replicated four times. Each replication consisted of 24 plants in four rows each with six plants. Plants were numbered to allow disease assessments in time for each individual plant. In both experiments, plants

within each block belonged to the same lines with the same flower colour but flower colours between blocks differed.
Treatments consisted of spraying the cyclamen plants with tap water containing 0.01% of Tween 80; the fungicide programme as applied by the grower in his commercial growing system (described below); conidial suspension of *G. roseum*; and conidial suspension of *U. atrum*. In the second experiment, the fifth treatment consisted of spraying conidial suspensions of *U. atrum* with increased time intervals between applications.
In the first experiment, the water control and the conidial suspensions were applied five times when plants were 133, 153, 174, 189, and 205 days old. The fungicide programme consisted of five applications of three different fungicides sprayed in the alternation: Euparen M (1 g l^{-1}; tolylfluanid at 500 g a.i. kg^{-1}); Sporgon (1 g l^{-1}; prochloraz-manganese at 450 g a.i. l^{-1}); Euparen M; Sporgon; and Rovral Aquaflo (2 ml l^{-1}; iprodione at 500 g a.i. l^{-1}) when plants were 112, 133, 153, 174, and 200 days old. Vertimec (0.5 ml l^{-1}; abamectin at 18 g a.i. l^{-1}) was sprayed three times during the experiment for thrips and mite control when plants were approximately 120, 160, and 200 days old.
In the second experiment, the water control and the conidial suspensions were applied nine times when plants were 109, 130, 151, 166, 179, 193, 207, 221, and 236 days old. In the fifth treatment, *U. atrum* was applied only five times on plants that were 109, 151, 179, 207, 236 days old. During the second experiment, the fungicide programme consisted of five applications of four different fungicides sprayed in the alternation: Daconil (1 ml l^{-1}; chlorothalonil at 500 g a.i. l^{-1}); Euparen M; Sporgon; Rovral Aquaflo; and Rovral Aquaflo when plants were 84, 106, 130, 160, and 225 days old. Control of thrips, mites and *Lepidoptera* larvae was achieved by applying Vertimec (abamectin), Aseptacarex (0.7 ml l^{-1}; pyridaben at 157 g l^{-1}), Mesurol (1 ml l^{-1} methiocarb at 500 g l^{-1}), Vydate (3 ml l^{-1}; oxamyl at 100 g l^{-1}) and Luxan Permethrin (0.4 ml l^{-1}; permethrin at 250 g l^{-1}) in alternation at intervals of approximately 40 days.
Because commercial crops were growing in the greenhouse, heavily infested plants were removed from the experiments to lower the inoculum density when plants were 205 (experiment 1) and 336 days old (experiment 2), respectively.

Application technique. Water and conidial suspensions were applied with a propane-operated sprayer (AZO, Ede, the Netherlands) at 250 kPa. Fungicides were applied with a motor operated sprayer (EP55, Empas, Veenendaal, the Netherlands) at 400 kPa. During spraying, a polystyrene shield was placed around each treatment to prevent drift to neighbouring plants. Each plant was sprayed individually until run-off. At the beginning of the experiments, plant canopies were open so that all leaf surfaces, petioles and the tuber could easily be reached. Later, when plant canopies became dense, plants were sprayed on the canopy but the spray nozzle also was put inside the canopy of each individual plant so as to reach plant surfaces shielded under the dense canopy such as surfaces of older leaves, petioles

and the tuber. Treatment of individual plants is the standard method of fungicide applications.

Assessments. In both experiments, disease was assessed on each day that the antagonists were applied, and a final assessment was made two weeks after the last antagonist application when plants were 219 (experiment 1) and 248 (experiment 2) days old, respectively.
The following parameters were assessed non-destructively in the greenhouse for each plant at each assessment date. The proportion of the area (ranging from > 0 to 1 at intervals of 0.1) covered with conidiophores of the pathogen was estimated separately for each leaf blade and petiole with sporulation of *B. cinerea*. Because areas with conidiophores of *B. cinerea* varied from sparse to complete (maximal density) coverage, the intensity of sporulation of *B. cinerea* also was estimated for each single area with sporulating *B. cinerea*, using a relative scale from > 0 to 1 (sparse sporulation to complete coverage with conidiophores) at intervals of 0.1. The area of leaf blades and the length of petioles were not recorded.
The following parameters were calculated from the data obtained: the percentage of plants with symptoms of *B. cinerea* (disease incidence), the number of petioles per plant with symptoms of *B. cinerea* (disease severity), the estimated equivalent number of leaf blades with *B. cinerea* sporulation (spore producing leaf area, SPLA), the estimated equivalent number of leaf blades with *B. cinerea* sporulation corrected for sporulation intensity (spore producing leaf area corrected for intensity, SPLACI), the estimated equivalent number of petioles with *B. cinerea* sporulation (spore producing petiole area, SPPA), and the estimated equivalent number of petioles with *B. cinerea* sporulation corrected for sporulation intensity (spore producing petiole area corrected for intensity, SPPACI). Because the size of assessed leaves was not measured, areas with sporulation of *B. cinerea* were not expressed in their absolute size but SPLA and SPLACI represent the equivalent number of leaf blade surfaces per plant fully covered with sporulation. The formula used for calculation were:

$$(1)\quad SPLA = \frac{\sum_{i=1}^{n}\sum_{j=1}^{m_i} p_{ij}}{n} \qquad (2)\quad SPLACI = \frac{\sum_{i=1}^{n}\sum_{j=1}^{m_i} w_{ij}\, p_{ij}}{n}$$

where:
i = number of plant, $i = 1 \ldots n$
m_i = number of diseased leaves of plant i
p_{ij} = proportion of j^{th} leaf blade of plant i covered by conidiophores of *B. cinerea*
w_{ij} = weight ($o < w_{ij} \leq 1$) for intensity of sporulation of *B. cinerea* on the ij^{th} leaf blade

Data on *B. cinerea* sporulation on petioles were produced in the same way. For statistical analysis, the mean values of the assessed parameters for the 25 plants per plot (experiment 1) or the 24 plants per plot (experiment 2) were used.

Biocontrol with *U. atrum* in different cropping systems

Plants. Cyclamen were seeded in trays with soil plugs, and transferred at an age of approximately ten weeks into 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.

Growing conditions. Ten experiments were carried out in six different commercial greenhouses located in western and central Netherlands. In all greenhouses the minimum day temperature was set at approximately 14 °C and the minimum night temperature at approximately 16 °C as 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 the type of material used for pots. In all greenhouses plants were irrigated two to three times per week. During the experiments, the spacing of the plants was increased to avoid touching of leaves of different plants except for plants in greenhouse 6 where the spacing was not increased during the last 50 days of the experiments. Under these conditions, leaves of neighbouring plants touched each other and formed a dense canopy layer. Plants with the first flowers open were considered marketable except for plants in greenhouse 3 where 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 randomised 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: (1) tap water containing 0.01% Tween 80, (2) the fungicide programme as applied by the grower in his commercial growing system, (3) inoculation with conidial suspensions of *U. atrum* twice (at the beginning of the experiment and approximately 28 days later), (4) 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 (5) untreated plants which 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 of the antagonist did not differ since the plants were marketable within eight weeks after the beginning of the experiment. In this case, data of the *U. atrum* application at four-week intervals are not presented.
Fungicides applied by different growers were Euparen M (1 g l^{-1}, tolylfluanid at 500 g a.i. kg^{-1}); Daconil M (1 g l^{-1}, chlorothalonil at 250 g a.i. kg^{-1} and maneb at 500 g a.i. kg^{-1}); Rovral (1 ml l^{-1}, iprodione at 500 g a.i. l^{-1}) or Sumico (1 g l^{-1}, carbendazim at 250 g a.i. kg^{-1} and diethofencarb at 250 g a.i. kg^{-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 with 254 days. *U. atrum* was applied at a plant age of 126 and 150 days or of 126, 150, 177, 206 and 233 days. Tween 80-water was applied at a plant age of 126, 150, 177, 206 and 233 days. Chlorothalonil and mancozeb (as Daconil M with 1 g l^{-1}) was applied at a plant age of 143 and 168 days, iprodion (as Rovral with 1 g l^{-1}) at a plant age of 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 with motor-operated sprayers by the different growers. During spraying, frames with polyethylene were placed around each treatment to prevent drift to neighbouring plants. The canopy of the individual plants was sprayed until run-off. 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) was calculated.

Statistics. Linear logistic regression was employed to explain the percentage of diseased plants (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 case *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 LSD tests for normally distributed data. Area under disease progress curves (AUDPC) for DI and DS were analysed by ANOVA.

2.2.5.4 *Experiments in* Pelargonium

Stock plants of *Pelargonium zonale* cv Springtime Irene were grown in a greenhouse at the Research Station for Floriculture and Glasshouse Vegetables (PBG), the Netherlands. In autumn, when they reached the size required for reproduction, a group of 35 to 40 potted stock plants was placed in each of twelve individual greenhouse compartments with temperature set at 18 °C. Light was supplemented whenever natural illumination was too low (< 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) Euparen M (a.i. 50% tolylfluanide), and (3) *U. atrum* (conidial suspension of 1 x 10^6 conidia ml^{-1} in water with 0.01% Tween 80). Each of the two experiments lasted for about 14 weeks. The treatments were randomised within four blocks, each consisting of three individual greenhouse compartments. Stock plants were sprayed until run-off. 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 hrs and subsequently planted in trays with soil. In a first experiment, autumn/winter 1996/1997, 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 experiment in 1997/1998, 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 was made a semi-quantitative assessment (*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; see 2.2.5.3; Köhl et al., 1998). Cuttings were visually assessed for presence of *B. cinerea* and killing by this pathogen at two, four and six weeks after planting.

Aerial spore load. Spore samplers according to Kerssies (1990) were placed in each compartment, four plates in a vertical position at 20 cm above the crop (first experiment) or six in a horizontal position at crop level (second experiment). 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^{-1} 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^{-1}): quintozene, 0.011; maneb, 0.01; chloramphenicol, 0.05; $CuSO_4$, 2.2; fenarimol 0.1 (ml l^{-1}); 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 two weeks *B. cinerea* can be distinguished as dark colonies, whereas only few other fungi succeed to grow. Numbers

of *B. cinerea* colonies per petri dish were counted and means per compartment used for analysis.

Quantification of spore production by *B. cinerea*. Sporulation of *B. cinerea* was quantified by sampling five 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^{-1}). 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 x) after staining with diluted cotton blue. 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 five 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 were assessed by counting with a haemocytometer. In the first experiment, 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 experiment, 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.

Statistics. Data were analysed by 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 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 ($\alpha = 0.05$) 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.

2.2.5.5 Experiments in pot roses

Two experiments were conducted with pot roses cv Moonlight. Pot roses are produced from cuttings at high temperature in well-illuminated greenhouses. Cuttings root in damp greenhouses at 25 °C within ten days. Subsequently they are transferred to less humid conditions. First pruning takes place at the age of three weeks, and is followed by fungicide application to control grey mould. Mildew is controlled by vaporising sulphur during 4 h per day. The second pruning is three weeks after the first, and is followed by a fungicide treatment if conditions are favourable for grey mould, e.g. during short days in winter. Twelve-week old plants are ready for sale, with some flowers open.

Treatments. The first pruning of the first experiment took place on 15 December 1997, the second on 6 January 1998. Treatments were on 16 December and 8 January. Assessment dates were 6 January before pruning and 12 February. Each treatment consisted of 28 pots with four cuttings each and in four replicates. The treatments were: (1) control, non-treated; (2) control, sprayed with water plus 0.01% Tween 80; (3) *U. atrum* sprayed to run-off with 1 x 10^6 conidia ml^{-1} in water plus 0.01% Tween 80; (4) carbendazim sprayed at a concentration of 1 ml (50% a.i.) l^{-1} on day 1 after the first pruning only.
The second experiment was carried out with the same cultivar and the same numbers of pots per treatment as the first. The treatments were: (1) control, water plus 0.01% Tween 80; (2) *U. atrum* sprayed to run-off with 1 x 10^6 conidia ml^{-1} in water plus 0.01% Tween 80; (3) Teldor (fenhexamide) at 1 g (50% a.i.) l^{-1}; (4) Teldor at 10% of recommended dose (= 0.1 g l^{-1}); (5) Teldor at 0.1 g l^{-1} in mixture with *U. atrum* 10^6 conidia ml^{-1}. Treatments were performed on 11 November 1998, one week after the first pruning and 24 November, one day after pruning. Assessments were made on 19 November and 5 January 1999, and they were conducted as indicated above.

Assessments. The first assessment consisted of counting the number of foci of *B. cinerea* per pot. The pathogen was sporulating on leaf residues on the soil of the pot as well as on senescing leaves still attached to the stem of the plant. At the second assessment a layer of dead leaves covered the soil of the pots. The percentage of the leaf residue area covered with sporulating *B. cinerea* and the intensity of sporulation (scale 0 to 10) were assessed. The product of these values was divided by 10 to give a sporulation index (0 to 100). The number of cuttings killed by *B. cinerea* was counted. Data were analysed by analysis of variance followed by an LSD test for comparison of means.

3 Results

3.1 Role of necrotic tissues in *Botrytis* epidemics

3.1.1 *Botrytis* leaf spot in onion

Incidence of *Botrytis* spp. After introducing sclerotia of *B. squamosa* into the field on 23 April 1991, weather was dry for two weeks. No sporulation of *B. squamosa* was found on the introduced sclerotia and many of the sclerotia were attacked by collembola (e.g. *Sminthurus* spp.). After 21 days, no sclerotia could be recovered. Thus, it can be assumed that most infections of *Botrytis* spp. during the field experiment were caused by naturally occurring inoculum. The appearance of lesions caused by *B. cinerea* or *B. squamosa* is rather similar and does not allow a conclusion on the pathogen involved. A majority of conidia of *Botrytis* spp. found on dead leaf tips during the growing season or collected with Rotorods at all trapping periods was smaller than 15 μm, most probably belonging to *B. cinerea*. Overall, only 12.7% of the trapped conidia were larger than 15 μm and conformed to the size of those of *B. squamosa*. *B. cinerea* was isolated from a large number of lesions of onion leaves, sampled regularly during the field experiment, whereas *B. squamosa* was never isolated.
The sugar beet buffer crop was inspected visually at regular intervals for sporulation of *Botrytis* spp. Leaves of sugar beets remained healthy during the experiment, only a small amount of necrotic leaf tissue was found in the sugar beet crop. Sporulation of *Botrytis* spp. was never observed.

Spore load. From the beginning of August 1991 onwards, sporulation of *Botrytis* spp. was abundant on necrotic leaf tips after periods with persistent dew. In experimental plots where approximately 60% of the necrotic tissue had been removed (Fig. 1), the spore load of conidia of *Botrytis* spp. was generally lower than in the control (Fig. 2). Concentrations of airborne spores of *B. cinerea* in the control plots and the plots where necrotic tissue had been removed differed significantly ($P < 0.05$) on 12 and on 16 August 1991. On these dates the average concentration of *B. cinerea* conidia above plots from which necrotic tissue was removed was 50% less than that above the control plots. Treatments with *G. roseum* did not significantly affect the spore load of *B. cinerea*.
The spore load above the buffer crop, between the plots, at a distance of 6 m from the nearest onions, was on average 55% of the spore load above the onion crop. At a distance of 18 m from the onions, the spore load was 40% of the spore load above the onion crop.

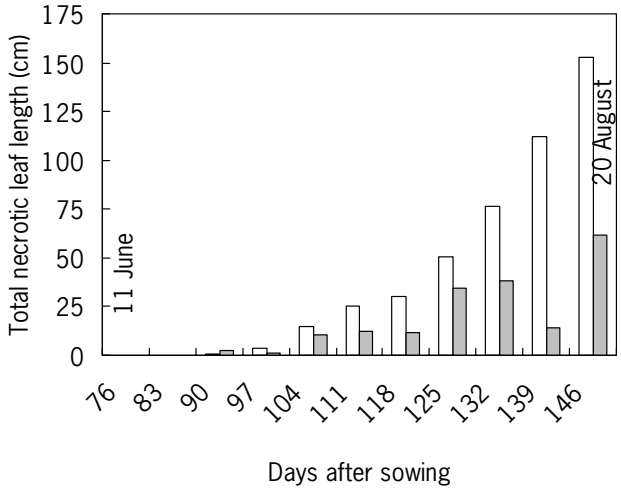


Figure 1. *Total necrotic leaf length per onion plant after removal of leaves with more than 50% necrotic leaf length ( ) in comparison to the control treatment ( ). Data of 2-6 replications with 10 plants each per sampling date. (From: Köhl et al., 1995a.)*

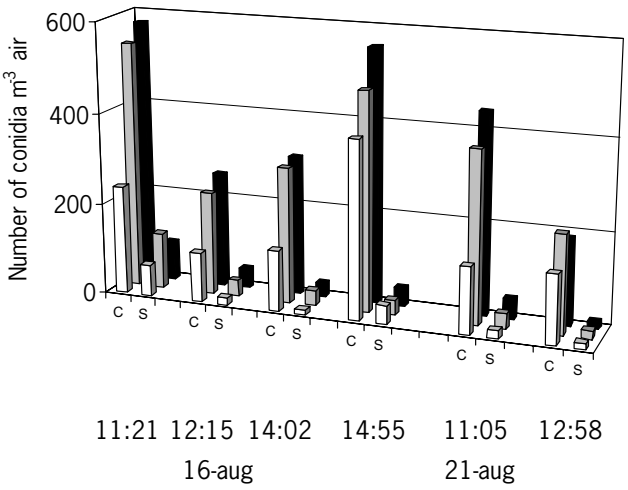


Figure 2. *Concentration of airborne conidia of* Botrytis cinerea *(C) and* B. squamosa *(S) above onion plots from which necrotic leaf tissue was removed at weekly intervals ( ), above plots that were sprayed with conidial suspensions of* Gliocladium roseum *( ), and above control plots ( ). Each bar represents a mean value for two replications per treatment. Local time indicated below the x-axis. Concentrations of airborne spores of B. cinerea in the control plots and the plots where necrotic tissue had been removed differed significantly ($P < 0.05$) on 12 and on 16 August 1991. (From: Köhl et al., 1995a.)*

Leaf lesions. A few *Botrytis* lesions were observed in the first two weeks of June 1991 but no differences were found among the treatments. Until the middle of July 1991, the weather was dry and warm without dew formation during nights and epidemics of *Botrytis* did not progress. Thereafter, rainy weather and dew formation during nights favoured the development of onion leaf spot and the number of lesions increased exponentially (Fig. 3). The initial densities did not differ significantly among treatments, but the relative growth rate (RGR) for the plots where necrotic leaf tissue had been removed was significantly smaller ($P < 0.05$) than for the control and antagonist treatment. Since block differences were not significant, the block effect was pooled with the residual effects. At the last sampling date on 20 August 1991, the number of lesions cm^{-2} of green leaves was 1.1 in the control, 1.1 in the antagonist treatment and 0.6 in the plots where necrotic leaf tissue had been removed. Treatment with *G. roseum* had no effect on the amount of necrotic leaf tissue.

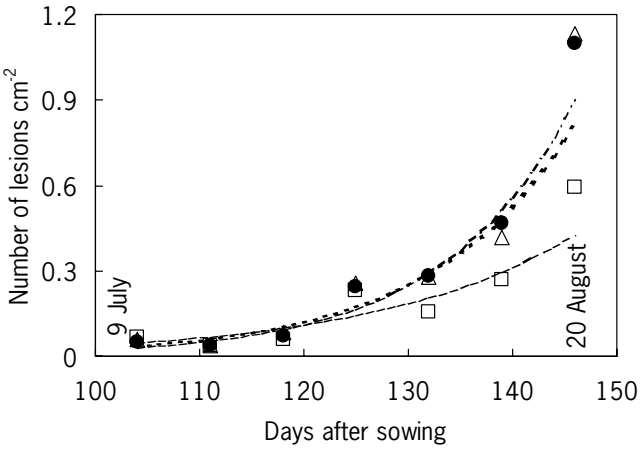


Figure 3. *Number of leaf lesions caused by* Botrytis *spp. on green onion leaves in plots from which necrotic leaf tissue was removed at weekly intervals (—□—), in plots sprayed with* Gliocladium roseum *(---●---), and in control plots (---△---). Means of 2-6 replications with 10 plants each per sampling date; curves were fitted with exponential growth model y = exp (a + b * t). Relative growth rate (parameter b) for plots from which dead leaf tissue was removed was significantly different (P < 0.05) from plots treated with* G. roseum *and control plots. (From: Köhl et al., 1995a.)*

3.1.2 *Botrytis* grey mould in annual strawberries

Conidial load in the air during flowering. The average number of conidia m^{-3} of air was always below 25 (Table 1). In only one of the 39 runs the concentration of conidia was higher than 100 m^{-3} air. A higher spore load of *B. cinerea* inside the untreated strawberry plots as compared to outside the plots was found on 10 of 16 days, a proportion of 0.63. This proportion is not significantly different from the expected value of 0.50, given its large 95% confidence interval (0.35, 0.85). Differences between spore loads inside and outside the strawberry plots were generally less than 10 conidia m^{-3} of air. No significant differences between spore loads inside untreated plots, inside sanitation plots, and outside the strawberry plots were found in any of the experiments. Daily average and daily peak values of the spore load in the air inside untreated plots as estimated from Rotorod samples, were not correlated with the potential spore production of *B. cinerea* on necrotic leaves, calculated with equation 7 of Sosa-Alvarez et al. (1995) as explained in the Materials and Methods section (2.2.1.2).

Substrate availability and inoculum production of *B. cinerea*. The incidence of *B. cinerea* on cold-stored transplants was 26.7% (experiment 5) and 52.6%

Table 1. *Estimated air load of conidia of* Botrytis cinerea *as determined by Rotorod sampling during flowering in plots with annual strawberries at Wageningen, the Netherlands. (From: Boff et al., 2001.)*

Experiment [x]	Number of spores m^{-3} of air per run [y]		
	Control [z]	Outside [z]	Sanitation [z]
3 (1997)	19.5	10.4	-
5 (1998)	8.8	7.0	8.3
6 (1998)	23.8	4.7	7.4
7 (1999)	6.3	10.2	-

[x] *Rotorod sampling was not conducted in experiment 1.*

[y] *Means of 5, 12, 10, and 12 runs, in experiments 3, 5, 6, and 7, respectively; paired t-tests on log-transformed numbers of spores per run did not show significant differences ($P > 0.05$) in mean air load of conidia between the different sampling locations for any experiment.*

[z] *Control = inside untreated strawberry plots; Outside = outside strawberry plots, Rotorods were located 25 m (1997) or 50 m (1998, 1999) from strawberry plots; Sanitation = inside strawberry plots where all dead leaves were removed.*

(experiment 6) on dead leaves, and 29% (experiment 5) and 32.2% (experiment 6) on stolons. The incidence of *B. cinerea* on senescent leaves was 30.3% (experiment 5) and 75% (experiment 6). The area with potential sporulation of *B. cinerea* on dead leaves was 3.5% and 15.6% for experiments 5 and 6, respectively.
The dynamics of necrotic strawberry leaf tissue showed similarity among experiments 2, 3 and 8 and among experiments 5 and 6. In experiments 2, 3 (1997) and 8 (1999) the amount of necrotic leaf tissue increased steadily after transplanting but was below 25 cm² per plant until the beginning of flowering, then increased further during flowering. This trend was not observed in experiments 5 and 6 (1998) in which more necrotic leaf tissue was found during the first three to four weeks after transplanting, whereas the presence of necrotic tissue was lower at flowering. The necrotic leaf area with potential sporulation of *B. cinerea* followed the same dynamics as the total area of necrotic leaf tissue in experiments 3 and 4 but not in experiments 2 and 6. The largest area with potential sporulation of *B. cinerea* per plant (15.5 cm²) was found in experiment 5, 14 days after transplanting. In relation to strawberry phenology, the area with potential sporulation of *B. cinerea* reached its maximum when the first flower appeared in experiments 2 and 5, at the middle of flowering in experiment 3, and at transplanting time in experiment 6. Among the dates of flower sampling, the total necrotic leaf area per plant varied from 16.5 to 137.9 cm², and the leaf area per plant with potential sporulation of *B. cinerea* ranged from 0.7 to 15.4 cm² (interpolated values, Table 2).

Flower colonisation. The incidence of *B. cinerea* on flowers varied in the five experiments from 5.1% (experiment 2, sample 1, 1997) to 96% (experiment 3, sample 2, 1998) (Table 2). The incidence of *B. cinerea* on flowers increased during the season in all experiments except in experiment 6. No significant correlation was found between the incidence of *B. cinerea* on flowers and the total area of necrotic leaf tissue ($r_s = 0.04$, df = 10, $P > 0.05$), the area of necrotic leaf tissue with potential sporulation of *B. cinerea* ($r_s = 0.43$, df = 8, $P > 0.05$), or the percentage area with potential sporulation of *B. cinerea* on necrotic tissue ($r_s = 0.45$, df = 8, $P > 0.05$), at the time of sampling of flowers. The sum of precipitation (rain + irrigation) over a period of five days before sampling of flowers was highly variable among sampling dates, ranging from 4.5 to 127.5 mm (Table 2). However, the incidence of *B. cinerea* on flowers was not positively correlated with this or other climatic variables, such as cumulative precipitation ($r_s = –0.57$ to $–0.08$, df = 10) or daily leaf wetness duration ($r_s = –0.58$ to $–0.85$, df = 10), during 2, 3, 4, 5, 7, 10, or 14 days before sampling of flowers. There was no positive correlation between the predicted levels of spore production of *B. cinerea* on necrotic leaves and the incidence of *B. cinerea* on flowers, calculated either over seven days ($r_s = –0.50$, df = 8) or five days ($r_s = –0.72$, df = 8) before sampling of flowers (Table 2).

Table 2. *Incidence of flower colonisation by* Botrytis cinerea*, cumulative precipitation over the five days preceding sampling of flowers, total area of necrotic leaf tissue, area of necrotic leaf tissue with potential sporulation at the date of flower sampling and predicted number of conidia* [z] *on necrotic tissue per plant calculated over a period of five days before sampling of flowers of untreated strawberry plots, at Wageningen, the Netherlands. (From: Boff et al., 2001.)*

Experiment	Sampling date	Incidence of *Botrytis* on flowers (%)	Cumulative precipitation, –5 to 0 days (mm)	Total necrotic leaf tissue per plant (cm^2)	Necrotic leaf tissue per plant with potential sporulation (cm^2)	Predicted number of conidia of *B. cinerea* per plant [z]
2	11 Jun 1997	5.1	89.5	86.3	1.9	25792
2	17 Jun 1997	14.9	92.0	108.0	1.7	629
3	29 Jul 1997	76.2	11.0	123.6	4.1	162
3	4 Aug 1997	96.0	8.0	137.9	14.5	11
5	8 Jun 1998	63.7	127.5	49.2	16.5	4192
5	15 Jun 1998	70.0	40.5	30.1	8.7	231
5	2 Jul 1998	81.3	92.0	26.1	4.9	23656
6	21 Jul 1998	83.7	4.5	48.0	3.7	2
6	5 Aug 1998	75.0	46.5	16.5	0.8	490
6	11 Aug 1998	60.1	109.0	19.2	0.7	88131
8	6 Jun 1999	60.2	35.5	88.0	-	-
8	9 Jun 1999	65.2	24.5	97.1	-	-

[z] *Calculated with equation 7 of Sosa-Alvarez et al. (1995.)*

Grey mould. Fruit rot at harvest ranged from 1.4% (experiment 3) up to 11.3% (experiment 6), and post-harvest grey mould ranged from 2.1 to 32.6% (Table 3, average values per experiment). Post-harvest fruit rot was typically greater than fruit rot at harvest and no correlation was found between these two variables ($r_s = 0.0$, $P > 0.05$). Fruit rot at harvest was significantly correlated with daily average precipitation during the harvest period ($r_s = 0.9$, $P < 0.05$), but post-harvest fruit rot was not ($r_s = 0.1$, $P > 0.05$). The incidence of *B. cinerea* on flowers was better correlated with post-harvest grey mould ($r_s = 0.6$, $P > 0.05$) than with grey mould at harvest ($r_s = –0.1$, $P > 0.05$), but neither of the correlations were signifi-

cant. The area with sporulation of *B. cinerea* on petals, assessed in three experiments, was significantly correlated with post-harvest fruit rot ($r_s = 1.0$, $P < 0.05$), but not with fruit rot at harvest (Table 3). A significant correlation between the percentage of fruit rot observed in subsequent harvests in the field and the level of fruit rot predicted with the model of Bulger et al. (1987) was found in one experiment (experiment 3, $r_s = 0.71$, $P < 0.05$), but never for post-harvest fruit rot.
In experiments 5 and 6, treatments were carried out in separate plots in which necrotic tissues were removed season-long regularly twice per week (see 3.5.1). In no case, this sanitation resulted in a reduction of the incidence of grey mould at harvest or after subsequent incubation.

Table 3. *Grey mould at harvest (HaRot), post-harvest grey mould (PoRot), % area with potential sporulation of* Botrytis cinerea *on necrotic leaves (LPS),* Botrytis *incidence on flowers (FBI), % area with potential sporulation of* B. cinerea *on petals (Petal), average daily precipitation during flowering and average daily precipitation during harvesting of strawberry untreated plots of five experiments at Wageningen, the Netherlands. (From: Boff et al., 2001.)*

Experiment	HaRot (%) [v]	PoRot (%) [v]	LPS (%) [w]	FBI (%) [x]	Petal (%) [y]	Average daily precipitation (mm) at	
						Flowering [z]	Harvest [z]
2 (1997)	9.5	13.1	9.4	9.9	-	9.6	5.5
3 (1997)	1.4	26.9	5.6	86.1	-	2.0	3.0
5 (1998)	10.1	10.4	22.4	71.7	20.3	14.6	6.2
6 (1998)	11.3	32.6	9.5	72.9	29.4	9.9	11.3
8 (1999)	11.2	2.1	-	62.7	5.8	12.5	5.6

[v] *Average of 10 (exp. 2), 5 (exp. 3), 10 (exp. 5), 8 (exp. 5), and 5 (exp. 8) samples.*
[w] *Average of 6 (exp. 2), 4 (exp. 3), 3 (exp. 5), and 3 (exp. 6) samples.*
[x] *Average of 2 (exp. 2), 2 (exp. 3), 3 (exp. 5), 3 (exp. 6), and 2 (exp. 8) samples.*
[y] *Average of 3 (exp. 5), 4 (exp. 6), and 5 (exp. 8) samples.*
[z] *Period of flowering was 14 May to 23 June, 16 July to 5 August, 31 May to 9 July, 8 July to 13 August, and 16 May to 17 June and period of harvesting was 16 June to 17 July, 11 August to 25 August, 2 July to 3 August, 13 August to 7 September, and 18 June to 12 July in experiments 2, 3, 5, 6, and 8, respectively.*

3.1.3 *Botrytis* grey mould in cyclamen

Numbers of green leaves per plant increased linearly from 31 to 52 during experiment 1. Senescing and necrotic petioles and leaf laminae first appeared about 157 days after sowing (Table 4). Sporulation of *B. cinerea* was typically first found on leaves dead due to senescence.
At the last assessment dates, 1.7 and 2.7 leaves per plant showed symptoms of *B. cinerea* in the untreated control of experiment 1 and 2, respectively. Regular removal of senescing, symptomless leaves significantly reduced disease severity to 0.2 and 0.5 leaves per plant showing symptoms of *B. cinerea*, respectively. *U. atrum* applications carried out in an additional treatment reduced AUDPC values but less effectively than removal of senescing leaves (Table 5). When *B. cinerea* was present in the crop from the start of experiment 1, reduction of the AUDPC values by *U. atrum* was marginal.

Table 4. *Total number of leaves and senescing and necrotic leaf parts in untreated plots of cyclamen greenhouse experiment 1. In brackets the number of plant parts with sporulating* Botrytis cinerea. *Means in the table are based on counts in four replicate plots containing 25 plants each. (From: Kessel et al., 2001.)*

Plant age (days)	Leaves per plant	Senescing plant parts [z]		Necrotic plant parts [z]	
		Laminae	Petioles	Laminae	Petioles
157	30.7	0.6 (0.0)	0.1 (0.0)	0.5 (0.1)	0.4 (0.2)
171	39.3	1.0 (0.0)	0.5 (0.1)	2.2 (1.1)	1.1 (0.6)
183	44.1	1.1 (0.0)	0.6 (0.0)	2.5 (1.1)	1.4 (0.5)
197	45.4	1.0 (0.0)	0.6 (0.1)	3.0 (1.2)	1.9 (0.7)
213	51.6	0.8 (0.0)	0.2 (0.1)	3.4 (1.8)	2.3 (1.7)

[z] *Per plant.*

Table 5. *Effects of* Ulocladium atrum *and the removal of senescing leaves on the Area Under the Disease Progress Curve (AUDPC) for* Botrytis cinerea *severity in cyclamen greenhouse experiments 1 and 2. (From: Kessel et al., 2001.)*

Treatments	AUDPC	
Experiment 1		
Water + 0.01% Tween 80	91.7	a [z]
Not treated	76.4	ab
Removal of senescing leaves	4.0	c
U. atrum at 4-week intervals	56.5	b
U. atrum applied 1x	61.9	b
Experiment 2		
Water + 0.01% Tween 80	66.7	a
Not treated	76.6	a
Removal of senescing leaves	7.4	b
U. atrum at 4-week intervals	34.3	c

[z] *Means from the same experiment followed by a common letter do not differ significantly (P > 0.05) according to ANOVA followed by an LSD test (a = 0.05.)*

3.2 Antagonist screening

3.2.1 Screening on necrotic onion leaves under controlled conditions

3.2.1.1 Bioassays with B. aclada *under continuously wet conditions*

From 18 isolates tested in a first screening, eight isolates suppressed sporulation of *B. aclada* significantly with efficacies of up to 90% (Table 6). All isolates of *Trichoderma harzianum* tested belonged to this group of antagonists. *Gliocladium catenulatum* 1814, *G. roseum* 1813, *T. hamatum* 003 and all isolates of *T. viride* tested consistently reduced sporulation of *B. aclada* by more than 90%. Only the yeast *Cryptococcus luteus* WCS36 caused no significant reduction of the sporulation index (SI) of *B. aclada*.

In a second screening, saprophytes isolated from necrotic leaf tips of field-grown onions and several isolates of *Gliocladium* spp. were tested (Table 6). Conidial suspensions of *B. aclada* contained 1 x 10^5 conidia ml^{-1} instead of 1 x 10^4 conidia ml^{-1} as in the initial screening to allow a better differentiation between strong antagonists. All 23 isolates tested significantly suppressed sporulation of *B. aclada* in each of the two repetitions of the bioassays. *Aureobasidium pullulans* 490, *Cladosporium cladosporioides* 564,

Table 6. *Comparative suppression of sporulation of* Botrytis aclada *by antagonists on dead segments of onion leaves in bioassays under continuously moist conditions. (From: Köhl et al., 1995c.)*

Reduction of sporulation index [w]	First screening [x]	Second screening [y]
Not significant [z]	*Cryptococcus luteus* WCS36	
< 90% [z]	*Gliocladium nigrovirens* 1815 *Trichoderma hamatum* T166 *T. harzianum* 39, T000, 022, T154 *Penicillium* sp. 023, 025	*Aureobasidium pullulans* 490 *Cladosporium cladosporioides* 564 *C. herbarum* 593 *P. hirsutum* 211 *P. spinulosum* 201 *Trichothecium roseum* 706 *Verticillium nigrescens* 250
> 90% [z]	*G. catenulatum* 1814 *G. roseum* 1813 *T. hamatum* 003 *T. viride* T004, T048, T122, T141, T218, T226	*Alternaria infectoria* 264, 270 *A. alternata* 317 *Arthrinium* sp. 242 *A. phaeospermum* 243, 244 *Chaetomium globosum* 256 *C. herbarum* 571, 587 *G. catenulatum* 162 *G. roseum* 1813, 160, 161 *P. brevicompactum* 221 *Sesquicillium candelabrum* 249 *Ulocladium atrum* 385

[w] *Antagonists were sprayed with 10^6 spores ml^{-1} 24 h after application of* B. aclada. *Leaves were incubated for eight days at 18 °C. Sporulation of* B. aclada *was scored using classes 0-5, representing, respectively, 0%, 1-5%, >5-25%, >25-50%, >50-75%, and >75-100% of the leaf area covered with conidiophores of* B. aclada *and a sporulation index (SI) was calculated* ($SI = (0 \times n_0 + 5 \times n_1 + 25 \times n_2 + 50 \times n_3 + 75 \times n_4 + 100 \times n_5) / 4$).

[x] B. aclada *sprayed with 10^4 conidia ml^{-1}.*

[y] B. aclada *sprayed with 10^5 conidia ml^{-1}.*

[z] *Compared to control treatment by LSD test ($a = 0.05$).*

G. roseum 248, *Penicillium hirsutum* 211, *P. spinulosum* 201, *Trichothecium roseum* 706 and *Verticillium nigrescens* 250 showed reduced SI's for up to 90%. Isolates of *Alternaria* spp., *Arthrinium* spp., *Chaetomium globosum*, *C. herbarum*, *P. brevicompactum*, *Sesquicillium candelabrum* and *Ulocladium atrum* consistently suppressed sporulation of *B. aclada* by more than 90%. Strongest antagonism was expressed by four out of five isolates of *Gliocladium* spp., which gave a complete inhibition of *B. aclada*. Additionally, several isolates were tested in single bioassays (data not presented). Four isolates of *Alternaria alternata* and one of each of *G. roseum*, *U. chartarum* and *U. consortiale* (Thüm.) Simmons suppressed sporulation of *B. aclada* by more than 90%, whereas one isolate of *C. herbarum* and two of *U. chartarum* caused reductions of less than 90%.

3.2.1.2 Bioassays with B. aclada *with an interrupted leaf wetness period*

In the five experiments, interruption of the leaf wetness period had no consistent effect on SI of *B. aclada* of water control treatments where only *B. aclada* had been applied (Table 7 and 8). When the leaf wetness period had been interrupted repeatedly, SI was lower compared to incubation under continuously moist conditions in experiment 1 and 2, but not in experiment 3 and 4.

Dry periods interrupting humid conditions had a differential effect on antagonists. *A. alternata* 300, 317 and 319, *C. globosum* 256, *U. atrum* 385 and *U. chartarum* 380 suppressed sporulation of *B. aclada* almost completely under continuously wet conditions and when wetness periods were interrupted 16, 40, or 64 h after application of antagonists. These antagonists were still highly suppressive when wetness periods were interrupted on up to three consecutive days. *A. pullulans* 490 showed high efficacies against *B. aclada* when leaf wetness periods had been interrupted. In contrast, *A. pullulans* 490 did not consistently suppress sporulation of *B. aclada* by more than 90% on leaves that were incubated under continuously wet conditions. These results were consistent with results obtained with this antagonist in preliminary bioassays (Table 6).

On the other hand, *G. roseum* 1813, *G. catenulatum* 162 and *S. candelabrum* 249, all highly efficient under continuously wet conditions, were distinctly less efficient when leaf wetness periods were interrupted 16 h after the antagonists were applied. Under these conditions, *G. roseum* 1813, *G. roseum* 162, *T. harzianum* 39 and *S. candelabrum* 249 reduced the SI of *B. aclada* by 48%, 51%, 72% and 36% (mean values of two repeated experiments), respectively, compared to the SI value of the control treatment that was sprayed with *B. aclada* only and subjected to a dry period after 16 h. Interruption of wetness after 40 h reduced efficacy of the antagonists less than an interruption after 16 h. When wetness was interrupted after 64 h, both isolates of *Gliocladium* spp. completely suppressed sporulation of *B. aclada*, as under continuously wet conditions, but the efficacy of *S. candelabrum* 249 was reduced. *Arthrinium* sp. 242 and *A. phaeospermum* 243 also showed sensitivity to an interrupted leaf wetness period. The effect of an interrupted leaf wetness period on *C.*

Table 7. *Effect of antagonists on sporulation of* Botrytis aclada *on dead segments of onion leaves after interruption of leaf wetness. (From: Köhl et al., 1995c.)*

Treatment [y]	Sporulation index (SI) [z] for *B. aclada* Time of wetness interruption (h after antagonist applied)					
	0	16	40	64	16 and 40	16, 40, and 60
Experiment 1						
Water	91	78	79	78	59	45
A. alternata 319	0	0	0	0	0	0
Arthrinium sp. 242	0	4	0	0	1	7
C. globosum 256	0	0	0	0	0	0
G. roseum 1813	0	42	12	0	45	30
Experiment 2						
Water	79	58	69	89	37	45
A. pullulans 490	8	1	2	1	1	1
G. catenulatum 162	0	30	18	0	31	27
S. candelabrum 249	0	50	34	19	29	43
U. chartarum 380	0	0	1	0	0	0
Experiment 3						
Water	44	59	54	33	48	56
A. phaeospermum 243	5	15	3	1	20	20
G. roseum 1813	0	42	15	2	33	33
T. harzianum 39	0	22	7	1	31	24
U. atrum 385	0	0	0	0	0	0
Experiment 4						
Water	37	47	56	45	43	50
A. alternata 300	0	0	0	0	0	0
A. alternata 317	0	0	0	0	0	0
C. herbarum 571	0	0	0	2	0	0
G. roseum 1813	0	24	11	0	10	21

[y] B. aclada *had been sprayed with* 1×10^5 *conidia* ml^{-1} *24 h before antagonists were sprayed with* 1×10^6 *spores* ml^{-1}. *Leaves were incubated for eight days at 18 °C.*

[z] *See Table 6. LSD (a = 0.05) for experiment 1 to 4 were 16.4, 14.5, 11.5 and 9.8, respectively. Treatments without sporulation of* B. aclada *have been excluded from analysis of variance.*

Table 8. *Effect of antagonists on number of conidia of* Botrytis aclada *produced on dead segments of onion leaves incubated in moist chambers under continuously wet conditions or with an interruption of leaf wetness 16 or 64 h after application of antagonists (Experiment 5). (From: Köhl et al., 1995c.)*

Treatment [v]	Number of conidia of *B. aclada* cm^{-2} leaf area Time of wetness interruption (h after antagonists applied)					
	No		16 h		64 h	
Water	238100	(55) [w]	373400 a [x]	(70)	731100 a	(59)
A. alternata 300	n.d. [y]	(0)	n.d.	(0)	n.d.	(0)
A. alternata 317	n.d.	(0)	n.d.	(0)	n.d.	(0)
A. alternata 319	n.d.	(0)	n.d.	(0)	n.d.	(0)
Arthrinium sp. 242	n.d.	(0)	27400 bc	(13)	n.d.	(0)
A. phaeospermum 243	<8300 [z]	(4)	12500 c	(8)	n.d.	(0)
A. pullulans 490	n.d.	(0)	n.d.	(1)	n.d.	(0)
C. globosum 256	n.d.	(0)	<7800 [z]	(5)	n.d.	(0)
C. herbarum 571	n.d.	(0)	58700 bc	(23)	<9600 [z]	(2)
G. roseum 1813	n.d.	(0)	118000 ab	(24)	n.d.	(0)
G. catenulatum 162	n.d.	(0)	47200 bc	(32)	n.d.	(0)
S. candelabrum 249	<6000 [z]	(0)	27800 bc	(30)	31600 b	(26)
T. harzianum 39	n.d.	(2)	<5200 [z]	(13)	n.d.	(0)
U. atrum 385	n.d.	(0)	<5200 [z]	(0)	n.d.	(0)
U. chartarum 380	n.d.	(0)	n.d.	(0)	n.d.	(0)

[v] B. aclada *had been sprayed with 1 x 10^5 conidia ml^{-1} 24 h before antagonists were sprayed with 1 x 10^6 spores ml^{-1}. Leaves were incubated for eight days at 18 °C.*

[w] *SI (see Table 6); LSD (a = 0.05) for SI was 11.8.*

[x] *Numbers of one column followed by the same letter are not significantly different according LSD test of log-transformed data (a = 0.05).*

[y] *Not detectable at an average detection limit of 5200 conidia cm^{-2} (range from 4000 to 8300 conidia cm^{-2} depending on leaf size).*

[z] *One or two measurements below detection limit.*

herbarum 571 and *T. harzianum* 39 were not consistent in two repetitions of the experiment. More than 2 x 10^5 conidia of *B. aclada cm*$^{-2}$ leaf surface were found on leaves incubated under continuously moist conditions in the control treatment of experiment 5 (Table 8). No conidia of *B. aclada* could be detected on leaves treated with antagonists except a few conidia on leaves that had been treated with *A. phaeospermum* 243 or *S. candelabrum* 249. Considering that the detection limit of the assessment was approximately 5200 conidia cm^{-2} leaf surface, all antagonists reduced conidia production by more than 97%. Conidia of *B. aclada* were not detected when

leaf wetness periods were interrupted 16 h after *A. alternata* 300, 317 and 319, *A. pullulans* 490 and *U. chartarum* 380 were applied. Thus, considering the detection limit, each of these antagonists reduced the number of conidia by more than 98%. After treatments with *C. globosum* 256, *T. harzianum* 39 and *U. atrum* 385 only a few conidia of *B. aclada* were found in one or two replications. On leaves treated with *Arthrinium* sp. 242, *A. phaeospermum* 243, *C. herbarum* 571, *G. catenulatum* 162 or *S. candelabrum* 249 between 1.2×10^4 and 6×10^4 conidia of *B. aclada* were produced cm^{-2} leaf surface. These antagonists significantly reduced conidial production by 84 to 97%. Only *G. roseum* 1813 did not reduce conidial production significantly. When the wetness period was interrupted 64 h after antagonists were applied, conidia production was reduced for more than 99%, so that no conidia could be detected, by all antagonists except *C. herbarum* 571, where a few conidia could be found, and *S. candelabrum* 249, where 3×10^4 conidia cm^{-2} leaf surface were counted compared to 7×10^5 conidia cm^{-2} leaf surface in the control treatment.
A close correlation was found between the log-transformed numbers of conidia of *B. aclada* produced on dead onion leaves (5200 conidia cm^{-2} leaf surface as the average detection limit were considered when no conidia were found) and SI's calculated from the estimated leaf area covered with conidiophores of *B. aclada* (Fig. 4).

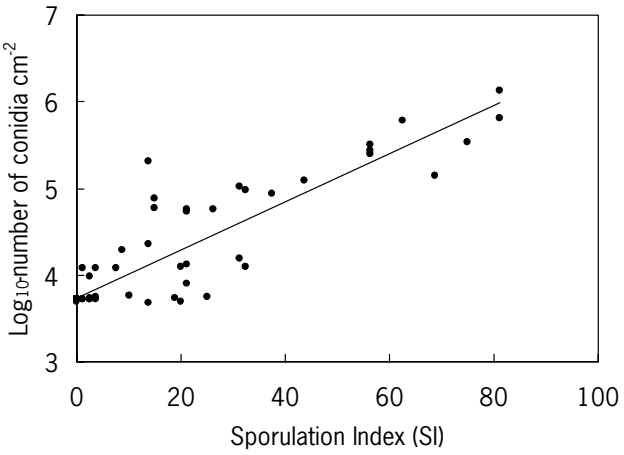


Figure 4. *Relationship between sporulation indexes (SI's) estimated for leaf area of dead onion leaf segments covered with conidiophores of* Botrytis aclada *(y) and numbers of conidia* of B. aclada *produced on dead onion leaf segments (x). Number of conidia cm-2 was log-transformed and a density of 5200 conidia cm-2 was considered as the detection limit when no conidia were recovered (y = 3.730 + 0.028 x; r = 0.915; P < 0.001). (From: Köhl et al., 1995c.)*

3.2.1.3 *Bioassays with* B. cinerea *with interrupted leaf wetness periods*

When conidial suspensions of *B. cinerea* (1 x 10^5 conidia ml^{-1}) were sprayed to the leaf segments 24 h before the antagonists *A. alternata* 317 and 319, *A. pullulans* 490, *C. globosum* 256, *G. catenulatum* 162, *G. roseum* 1813, and *U. atrum* 385 were applied as in bioassays with *B. aclada*, SI values were higher than 90 on leaves not treated with antagonists. Antagonists did not reduce SI values significantly (data not shown). When *B. cinerea* was applied eight hours before the antagonist, antagonists were highly efficient if *B. cinerea* had been sprayed with 1 x 10^3 conidia ml^{-1} (Table 9). The SI value was 63 for leaves not treated with antagonists, but was one or lower for leaves treated with antagonists. When *B. cinerea* was applied at 1 x 10^4 conidia ml^{-1}, the SI value was 64 for leaves not treated with antagonists. The antagonists reduced SI values to 5 or lower. At the highest concentration of *B. cinerea*, 1 x 10^5 conidia ml^{-1}, *C. globosum* 256 suppressed sporulation of *B. cinerea* completely; *A. alternata* 319 and *U. atrum* 385 reduced sporulation from a SI of 85 in the water treatment to 12 and 15, respectively. Treatments with *A. alternata* 317, *G. catenulatum* 162 and *G. roseum* 1813 were only moderately efficient, resulting in SI's between 32 and 53, and *A. pullulans* 490 showed no antagonistic activity. When wetness was interrupted after 16 h, both isolates of *Gliocladium* spp. showed only moderate or no antagonism at all concentration levels of *B. cinerea*. *C. globosum* 256 was also highly

Table 9. Effect of antagonists on sporulation of Botrytis cinerea *on dead segments of onion leaves after interruption of leaf wetness. (From: Köhl et al., 1995c.)*

Treatment	Sporulation index (SI) for *B. cinerea* [x]					
	Leaf wetness period not interrupted			Leaf wetness period interrupted [y]		
	10^3 [z]	10^4	10^5	10^3	10^4	10^5
Water	63	64	85	55	61	53
A. alternata 317	0	2	32	0	1	26
A. alternata 319	0	1	12	1	3	16
A. pullulans 490	0	5	86	0	8	58
C. globosum 256	0	0	0	3	14	48
G. catenulatum 162	0	4	42	18	64	57
G. roseum 016	1	5	53	28	43	42
U. atrum 385	0	1	15	0	0	1

[x] *After incubation of leaves for eight days at 18 °C. SI see Table 6. LSD (a = 0.05) for SI was 16.6.*

[y] *16 h after application of antagonists.*

[z] *Concentration of conidial suspension of* B. cinerea *(conidia ml^{-1}) sprayed 8 h before antagonists were sprayed with 1 x 10^6 spores ml^{-1}.*

effective after a dry period when *B. cinerea* was applied at 1 x 10^3 conidia ml^{-1}, but did not reduce sporulation of *B. cinerea* when *B. cinerea* was applied at the highest concentration with 1 x 10^5 conidia ml^{-1}. *A. alternata* 317 and 319 and *U. atrum* 385 showed no sensitivity to an interruption of leaf wetness and suppressed sporulation of *B. cinerea* as efficiently as under continuously wet conditions.

3.2.1.4 Water availability

It can be concluded from the relationship between water content and water potential in dead onion leaves (Fig. 5) and the gravimetrically measured water content of dead onion leaves during the drying process that under the experimental conditions the water potential of dead onion leaf segments was lower than –5 MPa after approximately 5 h and approximately –12 MPa after 7 h of drying (Fig. 6).

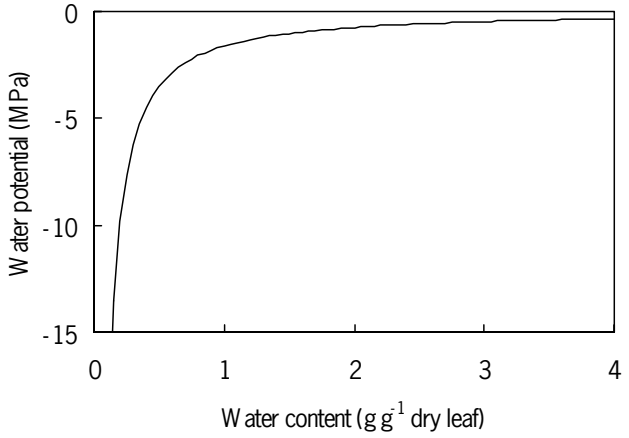


Figure 5. Relationship between water content (y) and water potential (x) in dead onion leaves. ($y = -1.610\ x^{-1.121}$; $r = 0.952$, $P < 0.001$). (From: Köhl et al., 1995c.)

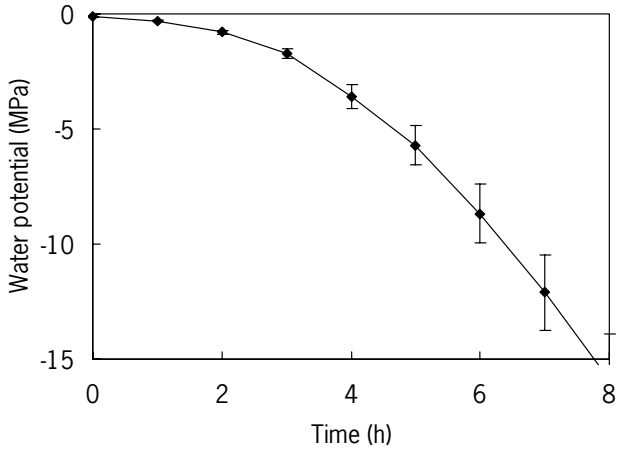


Figure 6. *Water potential of dead onion leaf segments during the drying process in bioassays with an interrupted leaf wetness period. Water content of dead onion leaves was measured gravimetrically and water potential was calculated using the formula given in Fig. 5. Bars indicate standard error of the mean. (From: Köhl et al., 1995c.)*

3.2.2 Screening on field-exposed necrotic lily leaves

3.2.2.1 Microclimatic conditions

The microclimatic conditions varied considerably during the nine experiments (Köhl et al., 1995b). Frequency and duration of leaf wetness periods of dead lily leaves, which seem to have a major effect on spore germination and colonisation, varied considerably between the experiments. In experiment 3, leaf wetness periods longer than 1 h occurred only during two nights. In the experiments 8 and 9, long leaf wetness periods after frequent and heavy rainfalls occurred on each day, with wetness periods lasting sometimes longer than 24 h. In experiment 7, the dew periods were short, but leaf wetness was frequently caused by slight rainfalls. A total of 11 leaf wetness periods were recorded, most of them lasting not longer than 2 to 3 h. Temperatures ranged between 2 °C and 31 °C during the nine experiments. Temperatures below 10 °C during leaf wetness periods were recorded during experiments 2, 3, and 7. Temperatures exceeded 30 °C during experiment 6.

3.2.2.2 Germination of spores of U. atrum *and* C. globosum *18 h after application*

Spores of *U. atrum* and *C. globosum* reached a germination of almost 100% during warm, wet nights, with wetness periods of dead leaves of 18 h and temperatures

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

3.2.2.3 Substrate colonisation by U. atrum *and* C. globosum

After five days (experiments 3, 4, 6, 8, and 9) or six days (experiments 1, 2, 5, and 7) exposure to field conditions, on the average approximately 800 conidia of *U. atrum* cm^{-2} leaf surface could be recovered. More than 50% of the conidia of *U. atrum* recovered on dead lily leaves germinated in all experiments, except in experiment 3 (Table 11). With increasing germination, the number of germ tubes produced per germinated conidium increased from 1.4 (experiment 3) to 2.6 (experiment 9). In all experiments, except in experiments 1, 3 and 7, germ tubes had branched so that up to 4.6 hyphal tips (experiment 8) were produced per germinated conidium. The overall average of mycelial length produced per germinated conidium was 108 µm. In general, mycelial growth appeared to depend on the total duration of leaf wetness, e.g. mycelium length per germinated conidium was 19 µm in experiment 3 with 24.5 h of total leaf wetness duration, and 239 µm in experiment 9 with 73.0 h of total leaf wetness duration. However, only 28 µm per germinated conidium was produced during 51.5 h of total leaf wetness duration in experiment 7, when frequent but short leaf wetness periods had occurred.
Recovery of ascospores of *C. globosum* after five to six days was distinctly lower than for *U. atrum*. On average, less than 200 ascospores cm^{-2} leaf surface were found except in experiment 7, in which approximately 1000 ascospores cm^{-2} leaf surface were recovered. Spores had not germinated in experiments 1, 3, and 7 which was similar to the observations after 18 h. Germination less than 10% was found in experiment 5, 6, and 9 (as after 18 h) but also in experiments 2, 4, and 8 where germination rates of 20 to 87% were found after 18 h. On average, 1.2 germ tubes per germinated ascospore with a length of approximately 60 µm had been formed. Branching of germ tubes did not occur.

Table 10. *Percentage germination of spores of* Ulocladium atrum *and* Chaetomium globosum *on dead lily leaves exposed in the field for 18 h in relation to microclimatic conditions. (From: Köhl et al., 1995b.)*

Experiment	Spray date and time	Germination (%) after 18 h [t]				Duration of leaf wetness period (h) [u]		Temperature range [v]
		C. globosum		*U. atrum*				
		Alone [w]	Mixture [x]	Alone [w]	Mixture [x]	Total	>10 °C [y]	
1	26 May; 18:00	2 ± 1	<1	36 ± 14	12 ± 12	- [z]	-	-
2	02 June; 19:00	70 ± 7	50 ± 9	95 ± 5	95 ± 1	18.0	18.0	12.0 - 17.1
3	09 June; 20:00	0	0	0	0	0.0	0.0	-
4	16 June; 19:00	27 ± 23	18 ± 18	71 ± 9	77 ± 13	15.0	11.5	9.4 - 15.2
5	23 June; 19:00	0	0	11 ± 2	16 ± 15	18.0	8.5	5.4 - 16.5
6	01 July; 20:00	0	0	<1	<1	8.5	8.5	10.5 - 23.9
7	07 July; 17:00	0	0	2 ± 2	0	9.0	3.5	4.4 - 19.7
8	15 July; 16:00	87 ± 13	67 ± 27	99 ± 1	90 ± 7	18.0	18.0	15.5 - 16.7
9	22 July; 18:00	2 ± 2	0	16 ± 11	24 ± 22	4.0	4.0	11.6 - 11.9

[t] *Mean ± standard deviation of two replications with two leaves each (experiments 1 to 4) or four replications with one leaf each (experiments 5 to 9).*

[u] *During 18 h after spraying.*

[v] *During leaf wetness period.*

[w] *2×10^6 spores ml^{-1} were sprayed.*

[x] U. atrum *and* C. globosum *were sprayed at 1×10^6 spores ml^{-1} each.*

[y] *With temperatures >10 °C.*

[z] *Not recorded.*

Table 11. *Germination and germ tube growth of conidia of* Ulocladium atrum *after five days (experiments 3, 4, 6, 8, and 9) or six days (experiments 1, 2, 5, and 7) on dead lily leaves exposed to field conditions* [w]. *(From: Köhl et al., 1995b.)*

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

[w] *Conidia of* U. atrum *were sprayed at 2 × 10^6 conidia ml^{-1}.*

[x] *Mean ± standard deviation of two replications with two leaves each (experiments 1 to 4) or four replications with one leaf each (experiments 5 to 9). Fifty spores were examined on each leaf.*

[y] *Per germinated conidium.*

[z] *Mean ± standard deviation of two replications with two leaves each (experiments 1 to 4) or four replications with one leaf each (experiments 5 to 9). Twenty spores were examined on each leaf.*

3.2.2.4 Sporulation potential of Botrytis *spp.*

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

Sporulating conidiophores of *B. cinerea* (sporulation potential) in the water control treatment covered between 5 and 30% of the leaf area in the nine experiments (Table 12). This may be explained by the differential effect of varying microclimatic conditions on colonisation and the naturally occurring inoculum load of *B. cinerea.*

U. atrum consistently reduced the sporulation potential of *B. cinerea*. The reduction of sporulation of *B. cinerea* by *U. atrum* was statistically significant compared to the control treatment in eight out of nine experiments ($P < 0.05$), which were carried out under markedly different microclimatic conditions and with probably varying inoculum pressure of the pathogen. Even at the highest level of sporulation of *B. cinerea* in experiment 4, *U. atrum* caused a 90% reduction in sporulation potential of the pathogen. *C. globosum* significantly reduced sporulation potential of *B. cinerea* in three experiments. The mixture of *C. globosum* and *U. atrum*, both antagonists applied in half

the concentration of that when sprayed alone, reduced sporulation potential of *B. cinerea* (statistically significant in five out of nine experiments), except in experiment 3. However, effects were mostly less marked compared to those of *U. atrum* when sprayed alone. *G. catenulatum* reduced *Botrytis* sporulation only in experiment 4. *A. pullulans* never reduced sporulation of *Botrytis*. Daconil M showed no fungicidal activity against *B. cinerea* on necrotic tissue of lily leaves.

3.2.2.5 *Sporulation potential of* Cladosporium-*like and* Alternaria-*like fungi*

Cladosporium-like and *Alternaria*-like fungi were the dominating saprophytic fungi sporulating on the leaves (Table 12). *U. atrum* caused a statistically significant reduction of the leaf area covered with *Cladosporium*-like fungi ($P < 0.05$) compared to the control treatment in five experiments. No effect of *U. atrum* was found in the last two experiments when *Cladosporium*-like fungi covered less than 10% of the leaf surface. *C. globosum* gave varying results. In two experiments *Cladosporium*-like fungi were significantly ($P < 0.05$) reduced; in six experiments no effect on *Cladosporium*-like fungi was found, and in one experiment *Cladosporium*-like fungi were even stimulated significantly ($P < 0.05$) by *C. globosum*. *C. globosum* and *U. atrum* sprayed in mixture affected sporulation of *Cladosporium*-like fungi in five experiments. *G. catenulatum* and *A. pullulans* had no effect on the occurrence of *Cladosporium*-like fungi, except in experiment 5 in which *A. pullulans* stimulated *Cladosporium*-like fungi. Daconil M significantly ($P < 0.05$) reduced the occurrence of *Cladosporium*-like fungi in three experiments but not the sporulation of *B. cinerea*.

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

Table 12. *Effect of antagonists and Daconil M (chlorothalonil and maneb) on sporulation of* Botrytis cinerea, Cladosporium*-like fungi and* Alternaria*-like fungi (including* Ulocladium *and* Stemphylium*) on dead lily leaves. Dead lily leaves were exposed to field conditions for five (experiments 3, 4, 6, 8, and 9) or six days (experiments 1, 2, 5, and 7) and subsequently incubated in a moist chamber at 18 °C for eight days. (From: Köhl et al., 1995b.)*

Treatment [x]	Percentage leaf area covered with conidiophores [y]		
	B. cinerea	*Cladosporium*-like	*Alternaria*-like
Experiment 1			
Water	15 a [z]	49 ab	23 b
Daconil M	16 a	45 ab	32 b
A. pullulans	25 a	62 a	26 b
C. globosum	5 bc	42 ab	32 b
G. catenulatum	14 ab	39 ab	22 b
U. atrum	2 c	31 b	91 a
C. globosum / *U. atrum* in mixture	6 bc	34 b	75 a
Experiment 2			
Water	24 b	19 bc	12 b
Daconil M	31 ab	29 ab	22 b
A. pullulans	32 ab	22 bc	18 b
C. globosum	31 ab	48 a	25 b
G. catenulatum	45 a	10 c	9 b
U. atrum	1 c	0 d	82 a
C. globosum / *U. atrum* in mixture	2 c	0 d	80 a
Experiment 3			
Water	19 a	52 a	20 c
Daconil M	13 a	53 a	36 bc
A. pullulans	14 a	50 a	32 bc
C. globosum	15 a	5 b	19 c
G. catenulatum	17 a	49 a	29 bc
U. atrum	1 b	7 b	49 ab
C. globosum / *U. atrum* in mixture	17 a	33 a	59 a

Table 12. Continued.

Treatment [x]	Percentage leaf area covered with conidiophores [y]		
	B. cinerea	*Cladosporium*-like	*Alternaria*-like
Experiment 4			
Water	30 a	61 a	30 c
Daconil M	26 ab	36 bc	28 c
A. pullulans	26 ab	38 abc	30 c
C. globosum	34 a	43 ab	26 c
G. catenulatum	15 bc	61 a	40 bc
U. atrum	3 d	26 bc	97 a
C. globosum / *U. atrum* in mixture	6 cd	21 c	60 b
Experiment 5			
Water	23 a	32 bc	53 bc
Daconil M	16 ab	25 bcd	66 b
A. pullulans	17 ab	51 a	47 c
C. globosum	10 b	23 cd	49 c
G. catenulatum	24 a	38 ab	63 bc
U. atrum	2 c	19 cd	97 a
C. globosum / *U. atrum* in mixture	2 c	16 d	92 a
Experiment 6			
Water	5 a	29 a	57 b
Daconil M	2 ab	9 c	49 bc
A. pullulans	2 ab	12 bc	38 c
C. globosum	6 a	37 a	65 b
G. catenulatum	4 ab	23 ab	65 b
U. atrum	1 b	11 bc	87 a
C. globosum / *U. atrum* in mixture	2 ab	12 bc	85 a
Experiment 7			
Water	14 a	48 ab	67 b
Daconil M	11 ab	28 cd	55 bc
A. pullulans	11 ab	33 bc	46 c
C. globosum	5 bc	24 cd	53 bc
G. catenulatum	14 a	50 a	59 bc
U. atrum	2 c	21 cd	96 a
C. globosum / *U. atrum* in mixture	2 c	16 d	96 a

Table 12. Continued.

Treatment [x]	Percentage leaf area covered with conidiophores [y]		
	B. cinerea	*Cladosporium*-like	*Alternaria*-like
Experiment 8			
Water	6 ab	6 a	31 b
Daconil M	5 ab	11 a	29 b
A. pullulans	1 bc	9 a	19 b
C. globosum	7 a	6 a	24 b
G. catenulatum	6 a	7 a	30 b
U. atrum	1 c	5 a	51 a
C. globosum / *U. atrum* in mixture	3 abc	11 a	50 a
Experiment 9			
Water	5 ab	7 a	24 bc
Daconil M	3 ab	3 a	23 bc
A. pullulans	3 ab	7 a	13 c
C. globosum	4 ab	5 a	25 bc
G. catenulatum	5 a	5 a	29 b
U. atrum	1 b	5 a	49 a
C. globosum / *U. atrum* in mixture	3 ab	5 a	47 a

[x] *2×10^6 spores ml^{-1} were sprayed. When sprayed in mixture,* C. globosum *and* U. atrum *were sprayed at 1×10^6 spores ml^{-1}.*

[y] *Mean of two replications (experiments 1 to 4) or four replications (experiments 5 to 9) with five leaves each. Total percentage of leaf area can be more than 100 when fungal colonies overlapped.*

[z] *For each experiment, numbers within a column with a common letter did not differ significantly (LSD test, a = 0.05).*

3.2.2.6 Fungal sporulation on dead lily leaves under field conditions

Additional leaf holders were placed in microplots on 22 June and were sprayed weekly from 23 June onwards with the antagonists or the fungicide. On 16 July, after 24 days exposure to field conditions, almost no conidiophores of *B. cinerea* were found. Two groups of saprophytes dominated on such leaves (Table 13). *Cladosporium*-like fungi covered approximately 31 to 46% of the surfaces of leaves of the control or treated with *A. pullulans*, *C. globosum*, or *G. catenulatum*. On leaves treated with Daconil M or *U. atrum*, significantly less sporulation of *Cladosporium* spp. was found with 14 and 13%, respectively. On the other hand, the leaf area covered with conidiophores of *Alternaria*-like fungi, including *Ulocladium* and *Stemphylium*, increased from 10% in leaves not treated with *U. atrum* to above 40% when

U. atrum had been sprayed on leaves. *Alternaria*-like fungi on leaves sprayed with *U. atrum* had the same colour and the same conidial characteristics as the applied isolate of *U. atrum*, unlike leaves not treated with this antagonist.

Table 13. Effect of antagonists and Daconil M (chlorothalonil and maneb) on the percentage area of dead lily leaves covered with conidiophores of Botrytis cinerea, Alternaria- *and* Cladosporium-*like saprophytes after 24 days field exposure* [x]*. (From: Köhl et al., 1995b.)*

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

[x] *Leaves were treated in four replications with five leaves each with antagonists or fungicide at day 1, 9, 15, and 23 of field exposure.*

[y] *Including* Ulocladium *and* Stemphylium.

[z] *Numbers within a column with a common letter do not differ significantly (LSD test, a = 0.05).*

3.3 Effect of environmental factors on *U. atrum*

3.3.1 Effect of temperature on conidial germination and mycelial growth of *U. atrum* in comparison with *Gliocladium* spp.

3.3.1.1 Air temperature in onion, lily, and cyclamen crops during leaf wetness periods

From 5 July to 29 August 1994, 50 wetness events in dead leaves were recorded in both the onion and the lily crops. The mean duration of leaf wetness periods was 4.9 h in onion and 9.7 h in lily. The total sum of hours with leaf wetness during the experiment was 246 in onion and 484 in lily. Dead leaves were wet for 18% and 36% of the total duration of the experiment in the onion and lily canopies,

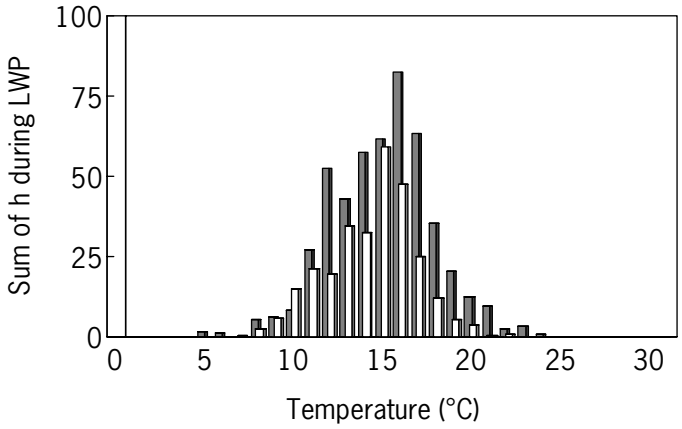


Figure 7. Air temperatures during leaf wetness periods (LWP) of dead leaves in an onion canopy (□) and lily canopy (▭) recorded from 5 July to 29 August 1994 in an experimental field at Wageningen, NL. (From: Köhl et al., 1999.)

respectively. Air temperatures ranged between 7.7 and 23.0 °C with a mean of 14.8 °C during leaf wetness periods in the onion canopy and between 4.3 and 23.8 °C with a mean of 14.5 °C during leaf wetness periods in the lily canopy (Fig. 7). In cyclamen, no leaf wetness periods were recorded but the relative humidity (RH) was > 90% for 19% of the total duration of the experiment. During such periods with > 90% RH, the air temperature within the canopy ranged between 15.8 and 18.5 °C with a mean of 16.8 °C.

3.3.1.2 Conidial germination

The percentage of conidial germination was greater than 95% for *U. atrum* during experiments on both agar and lily leaves and for *G. roseum* during the first experiment on agar; in the second experiment, only 60% of *G. roseum* conidia germinated. For the various temperatures, the increase of conidial germination over time was adequately described by the logistic model. The model accounted for 99.0 and 99.3% for the experiments with *U. atrum* on agar and 98.2% for the experiments on lily leaves. For *G. roseum* the values were 99.3 and 95.0% for the two experiments on agar. The optimum temperature was between 27 and 33 °C for both fungi in all experiments (Fig. 8).
The speed of germination of *U. atrum* conidia was the same on water agar and on lily leaves for all temperatures except for one experiment at 3 °C, in which germination on agar was slower. This was most likely due to insufficient temperature control in the incubator during the experiment.

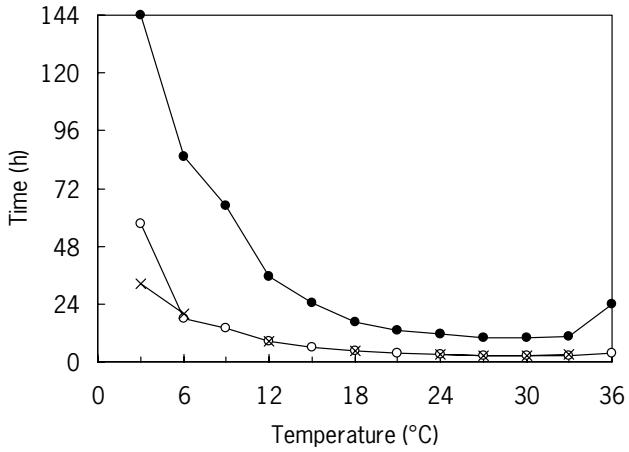


Figure 8. *Effect of temperature on the time needed for conidia of* Ulocladium atrum *on water agar (—○—) and on dead lily leaves (— × —) or of* Gliocladium roseum *on malt agar (—●—) to reach a germination percentage of 50%. (From: Köhl et al., 1999.)*

On agar and lily leaves, conidia of *U. atrum* needed 2.6 h at optimum temperature to reach a germination percentage of 50%. Conidia of *G. roseum* on agar (first experiment) needed 10.0 h to reach this germination percentage. The differences in time needed for conidial germination for the two fungi were larger at low temperatures. At 6 °C, 50% of the *U. atrum* conidia germinated on agar within 18.1 h and on lily leaves within 19.8 h (means of two experiments). The estimated time for *G. roseum* was 85 h (first experiment).

3.3.1.3 Mycelial growth

The parameters of the fitted Logan-curves show that *B. aclada*, *B. cinerea* and *B. elliptica* had their optimum for mycelial growth at 23.4, 24.6 and 23.8 °C, respectively (Fig. 9). The estimated maximum temperature was 30.0 °C for *B. cinerea* and *B. aclada* and 32.0 °C for *B. elliptica*. Mycelial growth of *U. atrum* was at its optimum at 27.9 °C. The estimated maximum temperature was 35.7 °C. *G. roseum* 1813 had its optimum mycelial growth at 28.3 °C and an estimated maximum at 36.0 °C. Results obtained with four other *Gliocladium* spp. were similar.

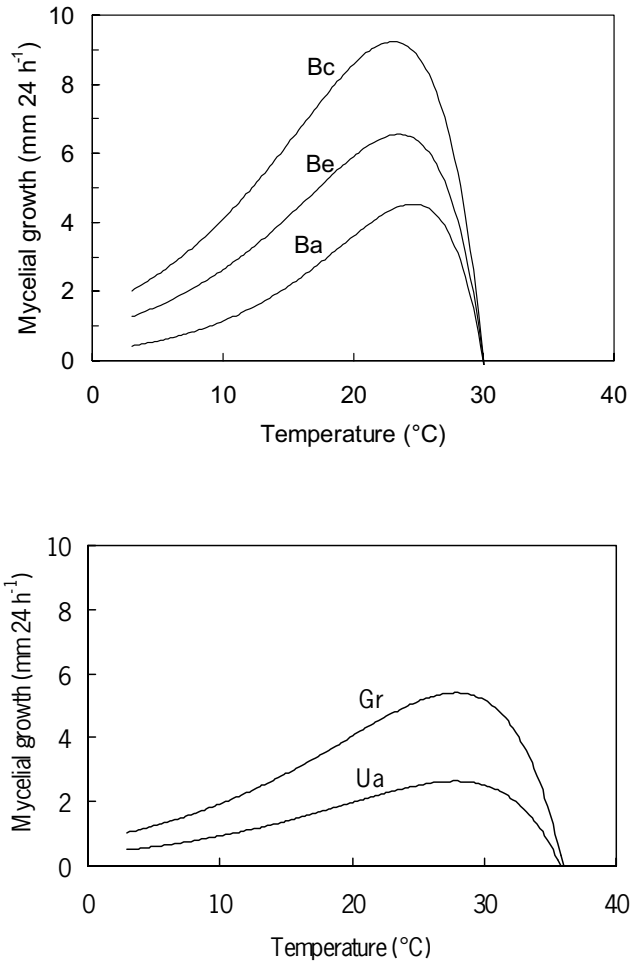


Figure 9. *Radial growth rate of mycelium of* Botrytis aclada *(Ba),* B. cinerea *(Bc),* B. elliptica *(Be) and* Ulocladium atrum *(Ua) and* Gliocladium roseum *1813 (Gr) at different temperatures on malt extract agar plates. Logan curves were fitted to data obtained from measured growth rates at temperatures of 3 to 36 °C at intervals of 3 °C in five replicates. R^2 for the different fungi were 87.3, 97.9, 96.3, 93.2, and 87.5, respectively. Repeated experiments gave similar curves. (From: Köhl et al., 1999.)*

3.3.2 Effect of water potential on conidial germination of *U. atrum*

3.3.2.1 Conidial germination at constant water potentials

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

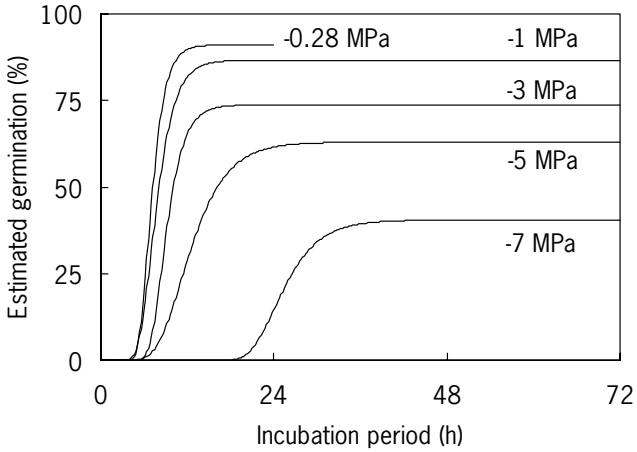


Figure 10. Estimated germination percentage of conidia of Ulocladium atrum *on water agar slides at different water potentials (MPa). Gompertz curves were fitted to data obtained from repeated experiments. For parameter estimates see Table 14. Adjusted* R^2*'s for the different treatments ranged between 0.76 and 0.98. (From: Köhl and Molhoek, 2001.)*

Table 14. Parameter estimates of Gompertz curves fitted to the germination percentage of conidia of Ulocladium atrum *incubated on agar slides at different water potentials (MPa). (From: Köhl and Molhoek, 2001.)*

Water potential (MPa)	Gompertz curve 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 (± standard error).*

3.3.2.2 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's 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 (Fig. 11 and 12). An exception was the treatment in which the moist incubation of conidia was interrupted after 8 h by a dry period at –42 MPa (Fig. 12), for which no 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 15). In the experiments with a dry period at –42 MPa, similar results

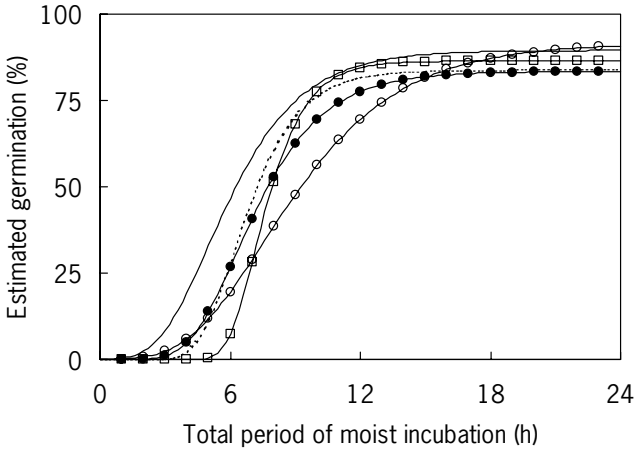


Figure 11. *Estimated germination percentage of conidia of* Ulocladium atrum *incubated on agar slides under moist conditions (–1 MPa) interrupted by a dry period at –10 MPa during the initial stage of germination. Initial period of moist incubation was 0 (—), 2 (---), 4 (□), 6 (○), or 8 h (●). Sum of hours of initial moist period and dry period was 24 h. Gompertz curves were fitted to data obtained from repeated experiments. For parameters see Table 15. Adjusted R^2's for the different treatments ranged between 0.91 and 0.99. (From: Köhl and Molhoek, 2001.)*

were obtained for parameter *C*, which ranged between 88 and 91% (Table 16). 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.

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 h or 32 h under moist conditions, the majority of the

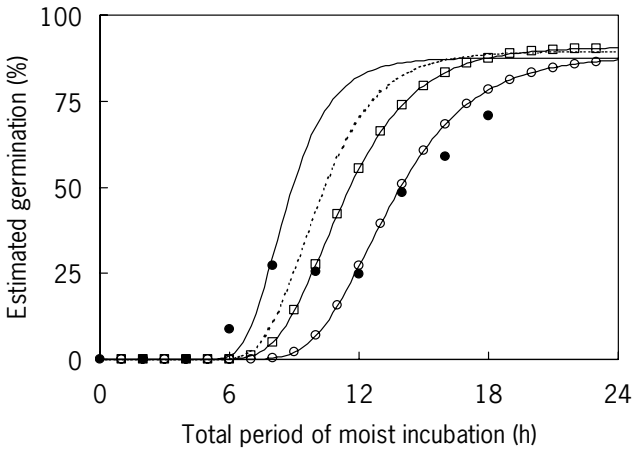


Figure 12. *Estimated germination percentage of conidia of* Ulocladium atrum *incubated on agar slides under moist conditions (–1 MPa) interrupted by a dry period at –42 MPa during the initial stage of germination. Initial period of moist incubation was 0 (—), 2 (---), 4 (□) or 6 (○). Sum of hours of 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 (●). For parameters see Table 16. Adjusted R^2's for the different treatments ranged between 0.90 and 0.99. (From: Köhl and Molhoek, 2001.)*

first germ tubes were 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. 13). Values for germination percentages determined at the beginning of each dry period (–42 MPa) and at the end of the dry period (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 to the control treatment with continuously moist incubation with 87% (Table 17). 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 significant from treatments with two or three dry periods, showing that conidial germination was slower after multiple interruptions of the germination

Table 15. 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. (From: Köhl and Molhoek, 2001.)*

Duration (h) [x]	Gompertz curves parameters [y]		
	M	*B*	*C*
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)
t probability	0.111	0.834	0.816

[x] *Duration of initial moist period; sum of initial moist period plus dry period was 24 h.*

[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 duration of initial moist incubation; slope parameter (± standard error).*

process. It can be calculated from the fitted curves that a germination percentage of 37% 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 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 h 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 μm for the majority of the conidia.

Table 16. *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. (From: Köhl and Molhoek, 2001.)*

Duration (h) [x]	Gompertz curve parameters [y]		
	M	*B*	*C*
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)
t probability	< 0.001	0.021	0.785

[x] *Duration of initial moist period; sum of initial moist period plus dry period was 24 h.*

[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 duration of initial moist incubation; slope parameter (± standard error).*

Table 17. *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. (From: Köhl and Molhoek, 2001.)*

Interruption [y]	Gompertz curve parameters [z]		
	M	*B*	*C*
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
$F_{prob.}$	< 0.001	0.004	0.103

[y] *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.*

[z] *Means of two experiments: M = point of inflection, B = slope parameter, and C = upper asymptote.*

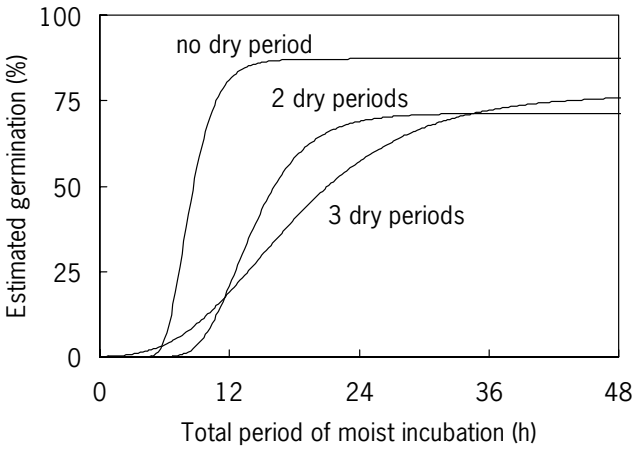


Figure 13. *Estimated germination percentage of conidia of* Ulocladium atrum *continuously incubated on agar slides under moist conditions (–1 MPa) or incubated with two or three moist-dry cycles (6 h at –1 MPa and 18 h at –42 MPa) at the beginning of the experiment followed by continuously moist incubation. Gompertz curves were fitted to data obtained from repeated experiments. For parameter estimates see Table 17. Adjusted R^2's for the different treatments ranged between 0.82 and 1.00 for the different treatments. (From: Köhl and Molhoek, 2001.)*

3.3.3 Effect of temperature on antagonism of *U. atrum* against *B. cinerea* and *B. aclada* on necrotic leaf tissues

3.3.3.1 Antagonism of U. atrum *on dead onion leaves*

The effect of *U. atrum* on the sporulation of *B. aclada* and *B. cinerea* on dead onion leaf segments was quantified after incubation at temperatures between 6 and 24 °C. There was a highly significant ($P < 0.001$) interaction between experiment, treatment and *Botrytis* species. No evidence ($P > 0.05$) was found for effects for temperature or interaction with temperature. The sporulation indices (SI) for *Botrytis* spp. sporulation were high, e.g. 74 for *B. aclada* in the water control treatment (mean for all temperatures) in the first experiment (Table 18). SI's were low in the second experiment, e.g. 37 for *B. aclada* in the water control treatment (mean for all temperatures). *U. atrum* reduced the SI for *B. aclada*, compared to the water control by 86% and 99% in experiments 1 and 2, respectively. Sporulation of *B. cinerea* was reduced by *U. atrum* in both experiments by 99% when *B. cinerea* was applied at 1×10^3 conidia ml^{-1} and by 96% and 99% when *B. cinerea* was applied at 1×10^4 conidia ml^{-1} (averages for all temperatures). T-tests for pairwise differences be-

tween treatment means in the 3-way table of means for experiment, treatment and *Botrytis* spp. showed that in both experiments a treatment with *U. atrum* in all cases resulted in significantly ($P < 0.05$) lower SI's compared to the water control treatment.

Table 18. *Effect of* Ulocladium atrum *on the sporulation index (SI) for* Botrytis aclada *and* B. cinerea *on dead onion leaves incubated at different temperatures. (From: Köhl et al., 1999.)*

Treatment [y]	SI [z] for *Botrytis* spp. after incubation at						
	6 °C	9 °C	12 °C	15 °C	18 °C	21 °C	24 °C
Experiment 1							
B. cinerea (10^3)	58	86	83	85	89	88	81
B. cinerea (10^3) + *U. atrum*	1	1	0	0	2	2	0
B. cinerea (10^4)	84	86	89	89	94	94	86
B. cinerea (10^4) + *U. atrum*	5	2	2	6	5	5	3
B. aclada	79	74	70	73	83	80	74
B. aclada + *U. atrum*	20	24	9	6	8	6	5
Experiment 2							
B. cinerea (10^3)	26	30	39	25	30	6	17
B. cinerea (10^3) + *U. atrum*	0	0	0	0	0	1	0
B. cinerea (10^4)	19	21	24	44	46	5	8
B. cinerea (10^4) + *U. atrum*	0	0	1	0	0	1	0
B. aclada	24	49	44	43	31	37	37
B. aclada + *U. atrum*	1	1	1	0	0	1	0

[y] *Suspensions containing 1×10^3 or 1×10^4 conidia ml^{-1} of* B. cinerea, *1×10^4 conidia ml^{-1} of* B. aclada *and 1×10^6 conidia ml^{-1} of* U. atrum *were applied.*

[z] *SI was measured 12 to 34 days after* B. cinerea *inoculation when there was no further increase in sporulation at each incubation temperature. In both experiments, SI for* U. atrum *treated leaves was significantly less than for leaves not treated with* U. atrum *for both* Botrytis *spp. at all temperatures (LSD test; a = 0.05).*

3.3.3.2 *Antagonism of* U. atrum *and* G. roseum *on dead cyclamen leaves*

Significant ($P < 0.05$) interactions between experiment and antagonist treatment were found. *U. atrum* significantly ($P < 0.05$) reduced the SI of *B. cinerea* at all temperatures except at 18 °C in both experiments and at 21 °C in experiment 2 (Table 19). However, control efficacy was on average below 40% at temperatures above 15 °C but increased to between 50 and 90% as temperatures decreased. *G.*

roseum reduced the SI for *B. cinerea* significantly ($P < 0.05$) in both experiments at 24 and 21 °C. At temperatures below 21 °C, no significant control of *B. cinerea* sporulation was achieved with *G. roseum*.
A highly significant ($P < 0.001$) relationship was found between the log-transformed number of conidia produced cm^{-2} (x) and the average SI for the corresponding leaf discs (y) ($y = 4.228 + 0.019\ x$; $R^2 = 0.909$).
In the second experiment, *B. cinerea* formed up to two sclerotia per leaf segment when incubated at temperatures above 9 °C (Table 19). The number of sclerotia formed on leaves incubated at temperatures above 9 °C treated with one of the

Table 19. *Effect of* Ulocladium atrum *and* Gliocladium roseum *on the sporulation index (SI) and sclerotia production of* Botrytis cinerea *on dead cyclamen leaves incubated at different temperatures. (From: Köhl et al., 1999.)*

Treatment [x]	SI [y] for *B. cinerea* after incubation at						
	6 °C	9 °C	12 °C	15 °C	18 °C	21 °C	24 °C
Experiment 1							
B. cinerea	64 a [z]	73 a	66 a	62 a	69 a	54 a	76 a
B. cinerea + *U. atrum*	12 b	26 b	37 b	43 b	59 a	49 a	57 b
B. cinerea + *G. roseum*	57 a	71 a	74 a	71 a	62 a	31 b	16 c
Experiment 2							
B. cinerea	25 a	63 a	64 a	62 a	49 a	68 a	79 a
B. cinerea + *U. atrum*	1 b	12 b	18 b	28 b	39 a	37 b	41 b
B. cinerea + *G. roseum*	22 a	66 a	71 a	65 a	41 a	18 c	3 c
	Number of sclerotia of *B. cinerea* per leaf segment after incubation at						
	6 °C	9 °C	12 °C	15 °C	18 °C	21 °C	24 °C
B. cinerea	0.0	0.0 a	0.8 a	1.9 a	1.9 a	1.9 a	1.7 a
B. cinerea + *U. atrum*	0.0	0.0 a	0.0 c	0.2 c	0.6 b	0.4 b	0.3 b
B. cinerea + *G. roseum*	0.0	0.1 a	0.3 b	0.9 b	0.3 b	0.0 c	0.0 c

[x] *Suspensions containing 1×10^3 conidia ml^{-1} of* B. cinerea *and 1×10^6 conidia ml^{-1} of* U. atrum *or* G. roseum *were applied.*

[y] *SI was assessed after 19 days for all treatments.*

[z] *Values within a column of the same experiment with the same letter do not differ significantly (LSD test; $a = 0.05$).*

antagonists was always lower compared to leaves from the control treatment. In most cases the number of sclerotia was reduced by more than 70% by either antagonist.

3.3.3.3 *Antagonism of* U. atrum *on dead hydrangea leaves*

B. cinerea sporulated on dead hydrangea leaves when incubated at 18 °C in the moist chambers within three weeks (Table 20). When incubated at 1 or 3 °C, *B. cinerea* sporulation started after several weeks. A significant ($P < 0.05$) interaction between temperature, concentration and treatments with *U. atrum* was observed. For all three temperatures and concentrations of *B. cinerea*, the application of *U. atrum* resulted in a significant decrease in the SI of *B. cinerea*. This effect was less pronounced at the highest concentration of *B. cinerea* applied (1 x 10^5 conidia ml^{-1}).

Table 20. *Effect of* Ulocladium atrum *on the sporulation index (SI) for* Botrytis cinerea *on dead hydrangea leaves incubated at different temperatures. (From: Köhl et al., 1999.)*

Treatment [w]	SI [x] for *Botrytis* spp. after incubation at		
	1 °C	3 °C	18 °C
B. cinerea (10^3)	38.0 a [y]	58.7 a	57.0 a
B. cinerea (10^3) + *U. atrum*	2.0 b	4.6 b	2.9 b
B. cinerea (10^4)	40.4 a	51.9 a	74.4 a
B. cinerea (10^4) + *U. atrum*	4.9 b	5.9 b	4.9 b
B. cinerea (10^5)	n.d. [z]	58.7 a	71.4 a
B. cinerea (10^5) + *U. atrum*	n.d.	25.2 b	43.7 b

[w] *Suspensions containing 1 x 10^3, 1 x 10^4 or 1 x 10^5 conidia ml^{-1} of* B. cinerea *and 1 x 10^6 conidia ml^{-1} of* U. atrum *were applied.*

[x] *Means of two experiments. SI of* B. cinerea *was assessed 21 days after* B. cinerea *inoculation for leaves incubated at 18 °C and 56 days after inoculation for leaves incubated at 1 or 3 °C.*

[y] *Values of each concentration of* B. cinerea *within a column with the same letter do not differ significantly (LSD test; a = 0.05).*

[z] *Not determined.*

3.3.4 Effect of water potential on antagonism of *U. atrum* against *B. cinerea* on cyclamen leaves

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 incu-

bated at –7 MPa (Table 21). 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.

Table 21. *Effect of* Ulocladium atrum *on the sporulation index (SI) of* Botrytis cinerea *on cyclamen leaves at different water potentials (MPa). (From: Köhl and Molhoek, 2001.)*

Treatment [y]	SI for *B. cinerea* after incubation at		
	–1 MPa	–3 MPa	–7 MPa
B. cinerea 10^5	17.2 a [z]	4.6 a	0.05 b
B. cinerea 10^5 + *U. atrum*	0.4 c	0.1 c	0.00 b
B. cinerea 10^6	15.3 a	6.1 a	0.63 a
B. cinerea 10^6 + *U. atrum*	3.0 b	1.0 b	0.01 b

[y] B. cinerea *was applied at 10^5 or 10^6 conidia ml^{-1}*. U. atrum *was applied at 10^6 conidia ml^{-1}*.

[z] *Backtransformed means of two experiments. Values within a column followed by the same letter do not differ significantly (LSD test after angular transformation; a = 0.05).*

3.3.5 Persistence of *U. atrum* conidia on green leaves and subsequent competitive colonisation of senesced leaves by *B. cinerea* and *U. atrum*

3.3.5.1 Greenhouse-grown cyclamen without top irrigation

Persistence on leaves. On green cyclamen leaves treated with conidial suspensions of *U. atrum* (1 x 10^6 conidia ml^{-1}), 3880 and 4070 conidia cm^{-2} of leaf were found directly after spraying for each experiment (Fig. 14). In the first experiment, the number of conidia cm^{-2} of leaf remained stable for eight weeks, but at day 70 significantly ($P < 0.05$) fewer conidia were found (1120 conidia cm^{-2} of leaf). In the second experiment, the conidial density was stable until the end of the experiment and was 3165 at day 70. On leaves of the water control, conidia of *U. atrum* were found only on rare occasions in both experiments. The mean conidial density for all assessment dates was below 0.03 conidia cm^{-2} leaf.

The percentage of germinated conidia of *U. atrum* on the healthy cyclamen leaves in the greenhouse remained below 12% during the first experiment (Fig. 15). During the second experiment, the percentage of germinated conidia increased after day 7

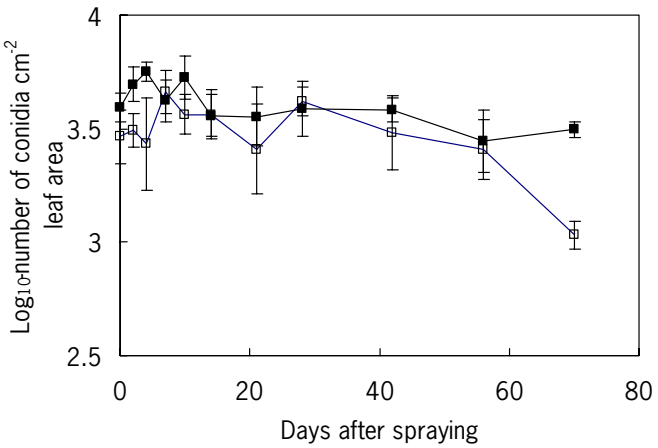


Figure 14. *Density of conidia of* Ulocladium atrum *on green cyclamen leaves under greenhouse conditions (— □ — experiment 1, — ■ — experiment 2). Leaves were sprayed with a conidial suspension (1 × 10⁶ conidia ml⁻¹) at day 0. Means ± standard errors of the mean of five leaves (replicates) per assessment date. (From: Köhl et al., 1998.)*

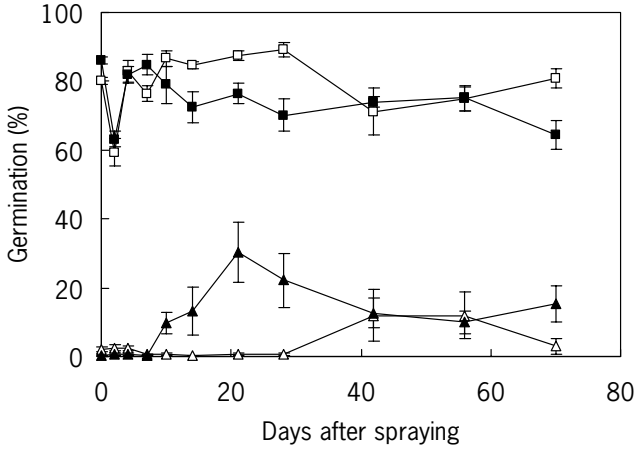


Figure 15. *Germination of conidia of* Ulocladium atrum *on green cyclamen leaves under greenhouse conditions directly after sampling (—△— experiment 1, —▲— experiment 2) or after incubation in a moist chamber at 24 °C for 8 h (— □— experiment 1, — ■— experiment 2). Leaves were sprayed with a conidial suspension (1 × 106 conidia ml-1) at day 0. Means ± standard errors of the mean of 100 conidia on each of five leaves (replicates) per assessment date. (From: Köhl et al., 1998.)*

from 0.4% to 10%. Thereafter, percentages of germinated conidia ranged between 10% and 30%, but there was no trend of a further increase of the germination over time. After incubation of leaf samples in moist chambers, the percentage of germinated conidia of *U. atrum* was consistently high during both experiments: 80% and 86% at the beginning and 81% and 64% at the end of experiment 1 and 2, respectively.

Competitive colonisation of dead cyclamen leaves by *B. cinerea* and *U. atrum.* In experiment 1, *B. cinerea* sporulation was significantly lower at the three analysed sampling dates on *U. atrum*-treated leaves than on leaves of the water control. At sampling day 42, 56, and 70, the coverage of the leaf area with sporulating *B. cinerea* was 42, 58, and 33% on the water-treated leaves but 12, 0, and 10% on *U. atrum*-treated leaves, respectively. In the second experiment, no difference in fungal colonisation was found for leaves that had been left senescing for 28 or 42 days in the greenhouse, and the data of such leaves were pooled for data analysis. Leaves were highly contaminated by *B. cinerea* inoculum produced on infected cyclamen plants present in the greenhouse, and sporulation of the pathogen was found on 84% of the area of leaves from the water control (mean of all sampling dates; Fig. 16). *B. cinerea* sporulation was significantly reduced by the treatment with

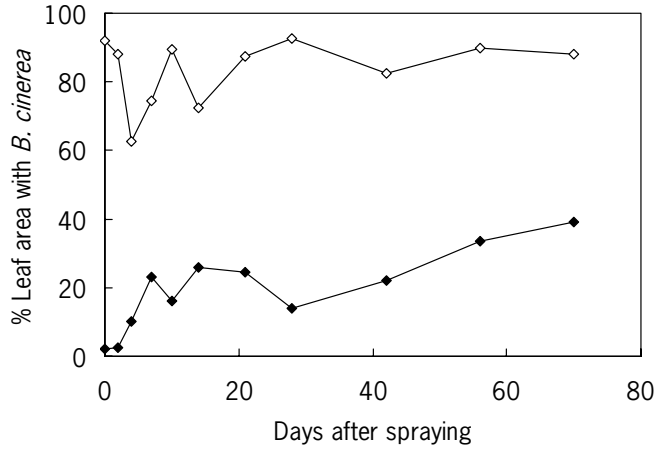


Figure 16. *Percentage of leaf area with* Botrytis cinerea *sporulation on necrotic cyclamen leaves (experiment 2). Leaves were sprayed with a conidial suspension of* Ulocladium atrum *(1 x 106 conidia ml¹) (—◆—) or with water (—◇—). Healthy green leaves of plants in an experimental greenhouse were treated at day 0, detached from plants at the indicated sampling date, allowed to senesce for 28 to 42 days in the greenhouse, and subsequently incubated in a moist chamber (14 days, 18 °C). The two treatments differed significantly ($P < 0.05$) at all assessment dates according to LSD tests. (From: Köhl et al., 1998.)*

U. atrum compared to the water control for all sampling dates. On average, 19% of the leaf area was covered with *B. cinerea* sporulation. The reduction in sporulation of *B. cinerea* by *U. atrum* ranged from 95% on leaves removed at day 0 to 56% on leaves sampled at day 70 after application of *U. atrum* conidia.

3.3.5.2 Greenhouse-grown cyclamen with top irrigation

Persistence on leaves. Densities of *U. atrum* conidia on green cyclamen leaves were 6900 and 6700 conidia cm^{-2} at the beginning of experiments 1 and 2, respectively. Densities decreased to 1700 and 3500 conidia cm^{-2} during the first 10 days of the two experiments and thereafter remained relatively stable (Fig. 17; data presented as Log_{10} numbers). At the end of both experiments, approximately 3000 conidia cm^{-2} were found. On untreated leaves, densities were below 1 conidium 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. 18). 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 colonisation 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 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. 19a). 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. 19b). Thereafter, efficacies ranged between 51 and 96%, except for the last sampling date of experiment 1 where the efficacy was only 29%.

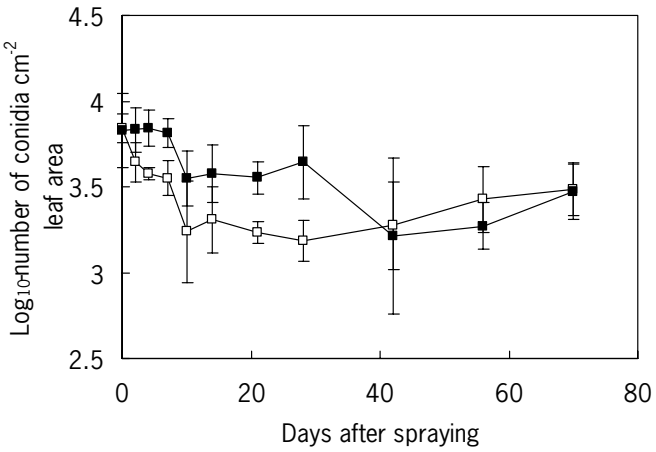


Figure 17. *Density of conidia of* Ulocladium atrum *on green leaves of cyclamen plants (cv Super Serie) under greenhouse conditions with top irrigation three times per week. (— □ — experiment 1; — ■ — experiment 2). Leaves were sprayed with a conidial suspension (1 × 10⁶ conidia ml⁻¹) at day 0. Means ± standard errors of the mean of five leaves (replicates) per assessment date. (From: Köhl et al., 2000.)*

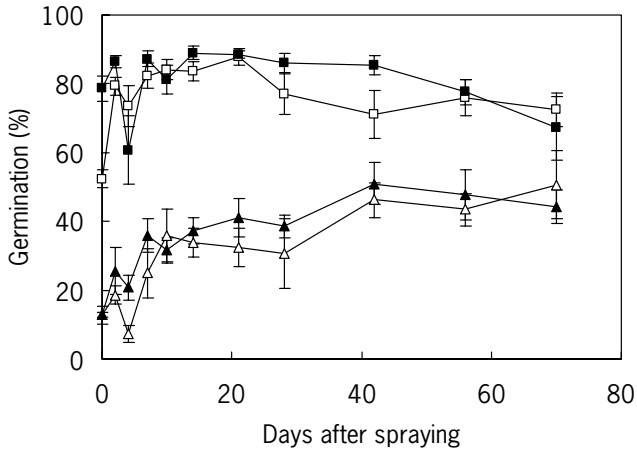


Figure 18. *Germination of conidia of* Ulocladium atrum *on green leaves of cyclamen plants (cv Super Serie) under greenhouse conditions with top irrigation three times per week. Leaves were sprayed with a conidial suspension (1 × 10⁶ conidia ml⁻¹) at day 0. Germination was assessed directly after sampling (—△— experiment 1; —▲— experiment 2) or after incubation in moist chamber at 24 °C for 8 h (— □ — experiment 1; — ■ — experiment 2). Means ± standard errors of the mean for 100 conidia on each of five leaves (replicates) per assessment date. (From: Köhl et al., 2000.)*

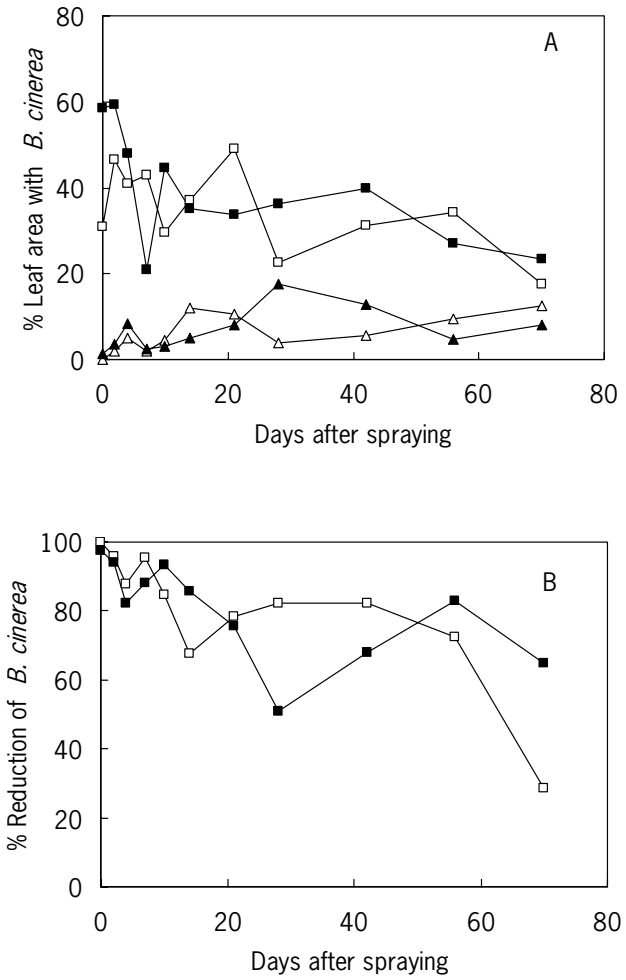


Figure 19. *Effect of an* Ulocladium atrum *treatment on sporulation of* Botrytis cinerea *on dead cyclamen leaves. Green leaves of cyclamen plants were sprayed with a conidial suspension of U. atrum (1 x 10^6 conidia ml^{-1}) at day 0 and kept under greenhouse conditions with top irrigation three times per week. Leaves were detached from plants at the indicated sampling date, allowed to senesce for 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) Percentage of leaf area with B. cinerea sporulation on dead cyclamen leaves treated with U. atrum (—△— experiment 1; —▲— experiment 2) or with Tween-water (— □— experiment 1; — ■— experiment 2). The two treatments differed significantly at all assessment dates (LSD test; a = 0.05), except for the last sampling date for experiment 1. (B) Efficacy of U. atrum treatment in B. cinerea control (— □— experiment 1; — ■— experiment 2). (From: Köhl et al., 2000.)*

3.3.5.3 Field-grown lilies

Microclimatic conditions. In the first week of experiment 1 five wetness periods of necrotic lily leaves were recorded and four of these were longer than 10 h (Table 22). In contrast, leaf wetness periods were relatively short and only one rain event was recorded in the first week of experiment 2 (week 2, Table 22). The following weeks were characterised by relatively short leaf wetness periods. The only significant rain event (rainfall > 5 mm) was recorded on 27 July 1995. Several minor rain events (≤ 0.5 mm) were also recorded but these did not result in leaf wetness inside the dense lily canopy. During leaf wetness periods, temperatures ranged between 7.2 °C and 24.8 °C with an average of 14.3 °C. Temperatures above 25 °C consistently occurred during the five weeks of experimentation.

Conidia density. The background number of *U. atrum* conidia on green leaves at each level in the lily canopy in the water-treated control plots was very low (< 60 cm^{-2}) at all sampling times in experiment 1 and less than 160 cm^{-2} at all sampling times in experiment 2. The number of conidia on lily leaves sampled directly after *U. atrum* application was 4184, 2664 and 816 cm^{-2} for leaves at the top, middle and bottom canopy levels, respectively, in experiment 1 (Fig. 20a). Significantly ($P < 0.01$) less conidia were found on leaves from the bottom of the canopy compared to the middle or top levels of the canopy. In experiment 2, the number of conidia on lily leaves sampled directly after spraying was 14040, 4720 and 2464 cm^{-2} for leaves at the top, middle and bottom levels of the canopy, respectively (Fig. 20b). In this experiment, the number of *U. atrum* conidia cm^{-2} at each leaf level was significantly different ($P < 0.05$). The number of *U. atrum* conidia cm^{-2} on lily leaves declined over time in both experiments and after 21 days were 672, 1264 and 336 conidia cm^{-2} on leaves at the top, middle and bottom level of the lily canopy, respectively (experiment 1). In experiment 2, the number of *U. atrum* conidia cm^{-2} declined to 5112, 1920 and 512 on leaves at the top, middle and bottom levels of the lily canopy, respectively. There were significant ($P < 0.01$) effects of canopy level and linear time ($P < 0.001$) based on the calculated P values of the F-tests in both experiments. The decline in number of conidia cm^{-2} over time was best described by a linear polynomial with different intercepts for canopy levels. Since there were no significant ($P < 0.05$) differences in the rate of decline of conidia density on leaves at each canopy level, a single slope value (b) is presented (Fig. 20). Despite large differences in the initial density of conidia on green leaf surfaces in experiment 1 and 2, the rate of decline in both experiments was similar and was not significantly ($P > 0.05$) different.

Field germination and germination potential. Seven days after *U. atrum* application, the germination of conidia on green leaf surfaces in the field (mean of all leaf levels) was 81% and 60% in experiment 1 and experiment 2, respectively (Table 23). Field germination was significantly ($P < 0.05$) lower on leaves sampled from the top level of the canopy than from the middle or bottom levels in

Table 22. Microclimate measurements during field experimentation (Wageningen, NL, 24 July - 4 September 1995). (From: Elmer and Köhl, 1998.)

Week no. [y]	Wetness periods of necrotic lily leaves			Rain events		Average temperature (°C) recorded during:	
	Total number	Average duration (h)	Total duration (h)	Total number	Total amount (mm)	the whole period [z]	leaf wetness periods [z]
1	5	17.1	85.5	5	11.0	20.5 (13.0-29.8)	17.9 (13.0-24.8)
2	5	6.7	33.5	1	0.2	21.4 (12.0-30.3)	14.8 (12.0-19.3)
3	5	5.0	25.0	1	0.2	19.3 (8.5-30.5)	12.7 (8.5-18.8)
4	7	6.9	48.0	4	0.8	19.3 (7.2-30.1)	13.0 (7.2-19.8)
5	9	7.1	64.0	12	6.0	18.3 (9.0-29.7)	14.4 (9.0-19.0)
6	7	3.1	22.0	12	7.4	13.5 (5.4-20.2)	12.9 (11.3-16.7)

[y] *Experiment 1 and 2 were started on 24 and 31 July, respectively.*

[z] *Values are the average temperature and figures in parenthesis are the range.*

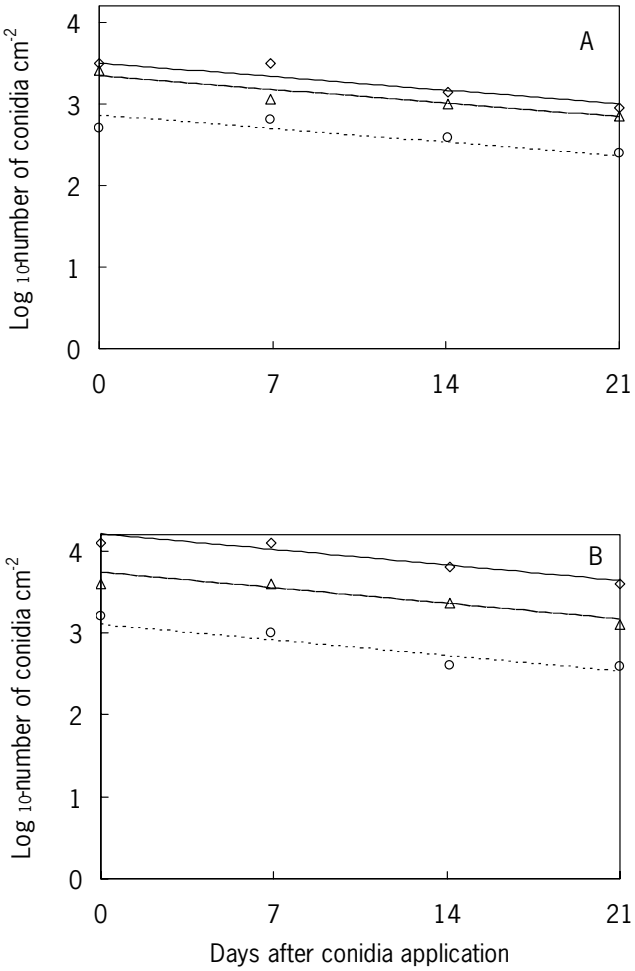


Figure 20. *Number of conidia of* Ulocladium atrum *on leaves at the top (— ◇—), middle (---△---) and bottom (··· ○···) level of the lily canopy in experiment 1 (A) and experiment 2 (B). The regression equation was* $\log_{10}$ *(number of conidia* cm^{-2}*) = a + b(time). Intercept values (a) were 3.50, 3.35, and 2.87 (LSD = 0.2945; a = 0.05) for leaves located at the top, middle and bottom canopy levels (experiment 1) and were 4.21, 3.75, and 3.11 (LSD = 0.2250; a = 0.05) for leaves located at the top, middle and bottom canopy levels in experiment 2. Slope (b) values were –0.0238 (Standard error = 0.005) and –0.0271 (Standard error = 0.005) for experiment 1 and experiment 2, respectively. (From: Elmer and Köhl, 1998.)*

Table 23. *Germination of* Ulocladium atrum *conidia on green lily leaves (cv Mont Blanc) at different canopy levels in the field (field germination) and after subsequent incubation at high humidity (germination potential). (From: Elmer and Köhl, 1998.)*

Sampling time [x]	Canopy level	Field germination (%)	Germination potential (%) [y]
Experiment 1			
Day 7	Top	75 b [z]	83 b
	Middle	83 ab	89 a
	Bottom	85 a	91 ab
Day 14	Top	72 b	80 b
	Middle	85 a	85 ab
	Bottom	76 b	91 a
Day 21	Top	62 b	75 b
	Middle	78 a	76 b
	Bottom	71 ab	90 a
Experiment 2			
Day 7	Top	56	86
	Middle	62	79
	Bottom	62	93
Day 14	Top	58	67
	Middle	58	74
	Bottom	47	72
Day 21	Top	43	64
	Middle	54	56
	Bottom	56	62

[x] *Time after application of* U. atrum.

[y] *Percentage germination after sampling and additional incubation in high humidity chambers at 18 °C in the dark for 18 h.*

[z] *Values within the same column and each sampling time with common letters do not differ significantly (LSD test of angular-transformed values; a = 0.05). There were no significant (P > 0.05) differences for percentage germination for sample time and canopy level variables.*

experiment 1. In contrast, canopy level had no significant ($P > 0.05$) effect on field germination in experiment 2. In both experiments there were no significant ($P > 0.05$) time and canopy level interactions.

At the time of *U. atrum* application, germination potential was 100% in both experiments. Germination potential was significantly ($P < 0.05$) greater than field germination at all sampling times in experiment 1 (Table 23). Seven days after application, germination potential declined to 88% (mean of three canopy levels) but thereafter there were no significant ($P > 0.05$) changes over time. Germination

potential of conidia on leaves at the bottom level of the canopy was significantly ($P < 0.05$) higher than conidia deposited on the top level of the canopy at all sampling times. In experiment 2, germination potential (mean of all canopy levels) was 26% higher than the percentage field germination at the 7-day assessment. In experiment 2, germination potential declined significantly ($P < 0.01$) from 86% (mean of all canopy levels) at day 7 to 61% at day 21, and in contrast to experiment 1, there were no significant ($P > 0.05$) canopy level effects.

Colonisation of artificially induced necrotic lily leaves by *U. atrum*. The ability of surviving *U. atrum* inoculum on green leaf surfaces to successfully colonise necrotic tissues was measured after paraquat induction of necrosis on tagged green leaves. Necrosis induction was not successful immediately after *U. atrum* application (day 0) in experiment 1 and *U. atrum* colonisation of necrotic tissue was not recorded. On leaves in the control treatment without *U. atrum* application, the leaf area colonised by *Ulocladium* spp. was 1.6% and 4.4% in experiment 1 and experiment 2, respectively. Up to 74% of the necrotic leaf area was colonised by *U. atrum* after the leaves were treated with this antagonist (Fig. 21). Leaf area colonisation by *U. atrum* was always highest on the leaves from the top level of the canopy and lowest for leaves sampled from the bottom canopy level in both experiments. This pattern was consistent for all times of necrosis induction and there was a significant ($P < 0.05$) effect of canopy level on *U. atrum* colonisation.

The colonisation of necrotic leaves by *U. atrum* decreased over time. When necrosis was induced immediately after antagonist application (day 0), colonisation of the leaf area by *U. atrum* was 74%, 54% and 26% for leaves sampled from the top, middle and bottom canopy levels, respectively (Fig. 21b, experiment 2). In contrast, when necrosis was induced 21 days after application of the antagonist, colonisation was 40%, 16% and 7% for the three canopy levels, respectively. Seven days after antagonist application (day 7, Fig. 21a), leaf area colonisation by *U. atrum* was 43%, 31% and 14% for leaves sampled from the top, middle and bottom canopy levels, respectively, in experiment 1. When necrosis was induced 21 days after application of the antagonist (day 21, Fig. 21a), colonisation was 29%, 10% and 6% for the top middle and bottom canopy levels, respectively. Despite a reduction of *U. atrum* colonisation 21 days after antagonist application colonisation was still greater than in untreated control treatments (17%, 1%, and 1%), experiment 1, and 2%, 2%, and 1%, experiment 2, for top middle and bottom levels, respectively.

For both experiments, ANOVA indicated that there were significant ($P < 0.05$) linear and quadratic effects over time. A quadratic polynomial in time with different intercepts and slopes for the linear effect was the best model which fitted the data for both experiments. However, pairwise comparisons within canopy levels indicated that the decrease of colonisation by *U. atrum* in time was not significant ($P > 0.05$) in all cases (Fig. 21).

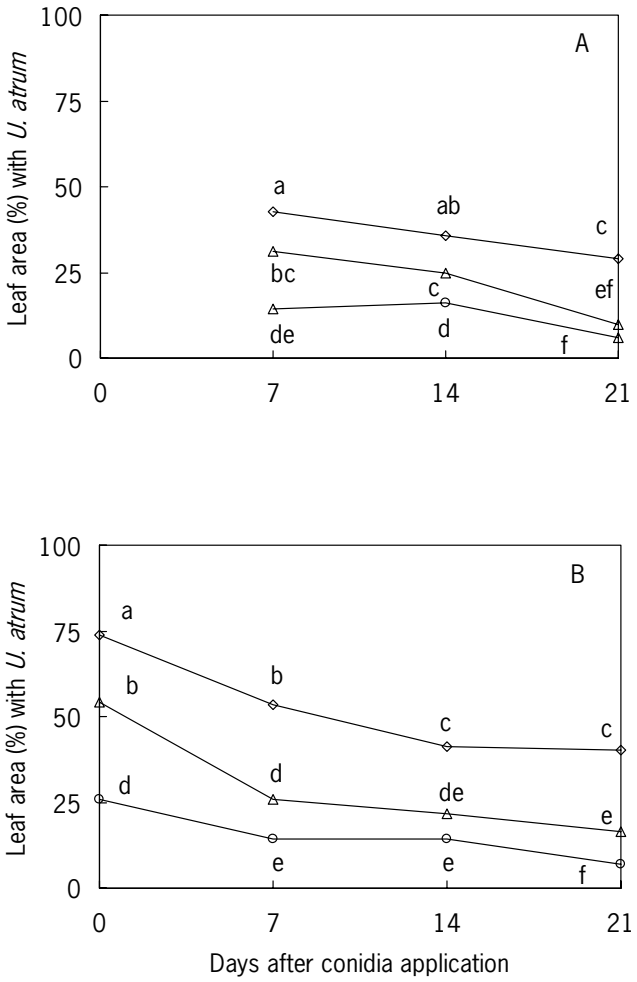


Figure 21. *Colonisation of necrotic lily leaves by* Ulocladium atrum *sampled from the top (— ◇—), middle (—△—) and bottom (— ○—) levels of the canopy in experiment 1 (A) and experiment 2 (B). Leaves were sprayed with U. atrum at day 0. Necrosis was induced at day 0, 7, 14 and 21. Leaves were sampled 14 days after induction of necrosis, incubated for nine days at 18 °C in moist chambers and the leaf area covered with conidiophores of U. atrum was estimated. Values with common letters do not differ significantly at P > 0.05 (t-test of angular-transformed values). (From: Elmer and Köhl, 1998.)*

***U. atrum* conidial density and colonisation relationship.** For *U. atrum* treated leaves, there was a significant ($P < 0.001$) relationship between the density of *U. atrum* conidia (germinated and ungerminated) assessed on the leaves prior to induc tion of necrosis and the leaf area (%) covered with *U. atrum* found after induction of necrosis, field exposure for a further 15 days, and subsequent incubation in moist chambers in the laboratory (Fig. 22).

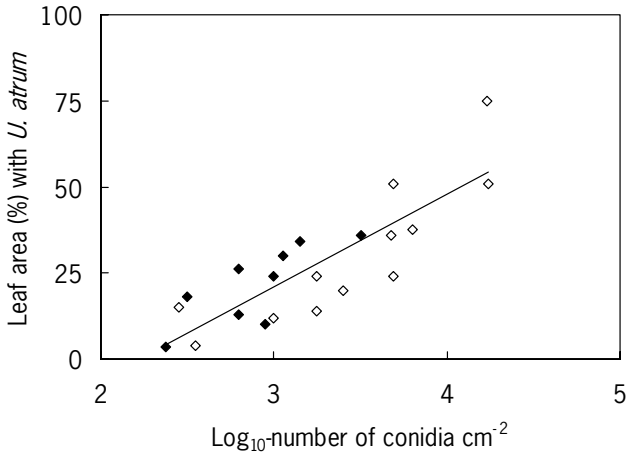


Figure 22. *Relationship between density of* Ulocladium atrum *conidia on the green leaf surface of lilies prior to induction of necrosis and leaf area (%) covered with conidiophores of U. atrum. Number of conidia cm^{-2} of leaf tissue was log-transformed. Regression equation is of the form % leaf area with U. atrum = –66.23 + 29.61 (log_{10}-number of conidia cm^{-2}); $R^2 = 0.713$; $P < 0.001$). Data from experiment 1 (◆) and experiment 2 (◇) were pooled for analysis. (From: Elmer and Köhl, 1998.)*

Colonisation of artificially induced necrotic lily leaves by commonly occurring saprophytic fungi. The dominant saprophytic fungi detected on lily leaves after artificial induction of necrosis were *Alternaria* spp. and *Cladosporium* spp. Several other saprophytic fungi such as *Epicoccum* spp., *Stemphylium* spp. and *Gonatobotrys* spp. covered only 1% or less of the area of necrotic leaves (mean of all leaves) in the untreated water control plots. *Botrytis* spp. were found sporadically and only on three and five leaves throughout the entire periods of experiment 1 and 2, respectively. The leaf area covered with conidiophores of *Botrytis* spp. for all leaves in the untreated plots was less than 0.05% in both experiments. The effect of *U. atrum* application to green lily leaves on subsequent colonisation of leaf tissue by common saprophytic fungi after induction of necrosis was investigated for *Alternaria* spp. and *Cladosporium* spp. The occurrence of other saprophytic fungi was sporadic and was therefore not analysed further.

In the absence of *U. atrum*, and after incubation in high humidity chambers, *Alternaria* spp. sporulation on necrotic leaves was 16.3% (experiment 1) and 18.4% (experiment 2) of the leaf area when averaged over all times of necrosis induction and canopy level. Leaves sampled from the bottom canopy level were generally less colonised by *Alternaria* spp. and the occurrence of *Alternaria* spp. tended to increase with time (Table 24).

Significant ($P < 0.01$) treatment and leaf level interactions were detected (at each necrosis induction time) and the statistical comparisons are summarised for each canopy level at each necrosis induction time. The application of *U. atrum* resulted in significantly ($P < 0.05$) reduced colonisation of lily leaves by *Alternaria* spp. at all times of necrosis induction on leaves from the top and the middle canopy levels and in two out of the seven observations on leaves from the bottom level. *Alternaria* spp. sporulation was reduced by between 63 and 78% in experiment 1 and by 51 and 90% in experiment 2 on leaves from the top and middle canopy levels. *U. atrum* suppression of *Alternaria* spp. declined over time in experiment 2 (90% at day 0 and 66% at day 21). This decline was statistically not further analysed since there was evidence that *Alternaria* spp. colonisation in the control treatment increased over time (16% at day 0 to 31% at day 21, Table 24).

In the absence of *U. atrum*, and after incubation in high humidity chambers, *Cladosporium* spp. sporulation on necrotic leaves was 7.4% (experiment 1) and 12.0% (experiment 2) of the leaf area when averaged over all times of necrosis induction and canopy level. In contrast to *Alternaria* spp., there did not appear to be any trend of *Cladosporium* spp. colonisation over time. The application of *U. atrum* resulted in significantly ($P < 0.05$) reduced colonisation of *Cladosporium* spp. on lily leaves by up to 97% when necrosis was induced on the same day as the *U. atrum* application (Table 25). The suppression of *Cladosporium* spp. by *U. atrum* tended to decrease over time and was generally lower on leaves at the bottom canopy level. When necrosis was induced 21 days after *U. atrum* application, there were no significant ($P > 0.05$) reductions of *Cladosporium* spp. sporulation in both experiments

Table 24. *Effect of* Ulocladium atrum *application on colonisation of artificially induced necrotic lily leaf tissue by* Alternaria *spp. (From: Elmer and Köhl, 1998.)*

Treatment	Canopy level	Percentage leaf area covered with conidiophores of *Alternaria* spp. [u]							
		0 days [v]		7 days		14 days		21 days	
Experiment 1									
Water	Top	-	[w]	10.5	* [x]	18.0	*	27.7	*
U. atrum		-		3.8	(64) [y]	5.3	(71)	6.0	(78)
Water	Middle	-		12.8	*	20.1	*	24.8	*
U. atrum		-		4.8	(63)	6.5	(68)	8.4	(66)
Water	Bottom	-		6.1	n.s. [z]	10.3	n.s.	16.4	*
U. atrum		-		5.2	(15)	6.7	(35)	8.2	(50)
Experiment 2									
Water	Top	16.1	*	17.8	*	29.4	*	30.5	*
U. atrum		1.6	(90)	2.9	(84)	7.0	(76)	10.5	(66)
Water	Middle	20.8	*	19.4	*	19.4	*	20.2	*
U. atrum		3.2	(85)	5.7	(71)	9.4	(52)	10.0	(51)
Water	Bottom	10.4	n.s.	11.5	n.s.	15.8	*	10.0	n.s.
U. atrum		6.3	(39)	5.9	(49)	6.6	(58)	7.3	(27)

[u] *Assessed after leaf samples were incubated in high humidity chambers at 18 °C in the dark for nine days.*

[v] *Necrosis was induced 0, 7, 14 and 21 days after application of* U. atrum *onto green leaves.*

[w] *Not assessed.*

[x] *Significantly different at* $P < 0.05$ *from* U. atrum *treatment (t-test of angular-transformed values).*

[y] *Figures in parentheses are percentage reduction of* Alternaria *spp. sporulation in comparison to the water treatment.*

[z] *n.s. = no significant difference.*

Table 25. *Effect of* Ulocladium atrum *application on colonisation of artificially induced necrotic lily leaf tissue by* Cladosporium *spp. (From: Elmer and Köhl, 1998.)*

Treatment	Canopy level	Percentage leaf area covered with conidiophores of *Alternaria* spp. [u]							
		0 days [v]		7 days		14 days		21 days	
Experiment 1									
Water	Top	-	[w]	5.2	* [x]	5.4	*	10.4	n.s.
U. atrum		-		0.8	(85) [y]	2.0	(63)	4.6	(56)
Water	Middle	-		6.1	n.s. [z]	5.5	*	9.3	n.s.
U. atrum		-		3.8	(38)	2.7	(51)	11.5	(–24)
Water	Bottom	-		7.3	n.s.	6.7	*	10.8	n.s.
U. atrum		-		4.9	(33)	3.3	(51)	11.9	(–10)
Experiment 2									
Water	Top	9.8	*	8.7	*	12.7	*	13.7	n.s.
U. atrum		0.3	(97)	1.0	(89)	2.8	(78)	10.2	(26)
Water	Middle	8.1	*	7.9	*	10.1	*	21.9	n.s.
U. atrum		3.6	(56)	2.7	(66)	5.5	(46)	21.2	(3)
Water	Bottom	11.5	*	7.9	n.s.	10.1	n.s.	21.7	n.s.
U. atrum		4.1	(64)	6.6	(16)	11.2	(–11)	19.2	(12)

[u] *Assessed after leaf samples were incubated in high humidity chambers at 18 °C in the dark for nine days.*
[v] *Necrosis was induced 0, 7, 14 and 21 days after application of* U. atrum *onto green leaves.*
[w] *Not assessed.*
[x] *Significantly different at $P < 0.05$ from* U. atrum *treatment (t-test of angular-transformed values).*
[y] *Figures in parentheses are percentage reduction of* Cladosporium *spp. sporulation in comparison to the water treatment.*
[z] *n.s. = no significant difference.*

3.4 Mode of action of *U. atrum*

3.4.1 Fungal interactions: microscopical and ultra-structural studies

Bioassay. Under constant humid conditions, all four antagonists efficiently suppressed the sporulation of *B. aclada* as in earlier experiments (Köhl et al., 1995c). The sporulation index for *B. aclada* was 41.8 (with a standard deviation of 5.8) when the pathogen had been sprayed alone. On leaf segments treated with *B. aclada*, no sporulation of *B. aclada* was observed when *G. catenulatum* or *C. globosum* had been applied and the sporulation index was 0.8 and 0.3 for leaf segments treated with *U. atrum* or *A. pullulans*, respectively. *B. aclada* was not observed on leaf segments that had been treated with antagonists without *B. aclada*. Growth of all antagonists, resulting in sporulation of *U. atrum* and *G. catenulatum* and blastospore formation of *A. pullulans*, could be observed on the leaf segments whether or not treated with the pathogen. No fungal development was observed on leaves that had not been inoculated with any of the fungal suspensions.

Agar plate assay. Colonies of *B. aclada* or *U. atrum* grown alone showed a regular radial growth. When two colonies of the same fungus had been inoculated on a petri dish, colonies were slightly flattened where growing towards each other. Similar colony shapes were observed when *U. atrum* and *B. aclada* were grown in combination on the same petri dishes. There were no distinct inhibition zones between colonies of the same or the two different fungi. However, the colonies did not seem to grow into each other during the seven days of the experiment.

3.4.1.1 Light microscopy

Colonisation of dead onion leaves. Despite the fact that tissues and cells of irradiated dead onion leaves were quite disorganised, it was possible to observe a differential pattern of colonisation among the five fungi six days after their inoculation. For instance, in all samples examined, *B. aclada* was found throughout the leaf section including the vascular tissue. In the same manner, *U. atrum* was found to be a very aggressive coloniser of onion leaves and was consistently observed in dense concentrations throughout the leaf tissues.
On the other hand, both *C. Globosum*, and to an extent, *G. catenulatum* appeared to be confined primarily to the outer portion of the leaf surface. In all examined samples, *C. globosum* appeared less dense than *B. aclada* or *U. atrum*. *G. catenulatum* was found occasionally, although in low concentrations, in the parenchymal tissues and around the veins. *A. pullulans* was characteristically concentrated in the leaf stomata

(or between epidermal cells) with a few fungal propagules being randomly dispersed underneath the epidermal cells.

Interactions between *Botrytis aclada* and the fungal antagonists. Of the four interactions, it was nearly impossible to generate salient information under light microscopy because of the difficulty of precisely differentiating the fungi since differential staining techniques (2.2.4.2) were not yet available for these studies. In the case of *Botrytis-Gliocladium*, differences in mycelium diameter between the two species made it somewhat easier to separate them. From all samples studied, it was obvious that *B. aclada* was unable to colonise onion leaves to the extent observed in control treatments when competing against *G. catenulatum*. In some instances, cells of *B. aclada* appeared dead, as evident by their lack of reaction with methylene blue.
For the *Botrytis-Ulocladium* interaction, abundant colonisation and sporulation by the antagonist suggested that *U. atrum* had completely saturated the substrate. The antagonist was as abundant as in the controls with the distinction that more spores were consistently observed on the leaf surface. In this situation, typical dictyospores of *U. atrum* covering the leaf surface made it easy to identify the antagonist. In the numerous samples studied, it was impossible to confirm conclusively the presence of *B. aclada* under light microscopy.

3.4.1.2 Transmission electron microscopy

Botrytis-Ulocladium. In samples of dead onion leaves inoculated with *U. atrum* alone, the fungus was observed easily within tissues. Its cells were characterised by a granular cytoplasm containing several large lipid bodies of variable electron density; its cell wall was covered by a regular hairy mucilage making it easy to discriminate from the cells of *B. aclada* (Fig. 23a). The fungus seemed to colonise all dead leaf tissues and it was capable of degrading the plant cell walls (Fig. 23a, arrowhead). In the numerous samples observed, it was impossible to obtain conclusive evidence of the presence of *B. aclada* when both fungi were inoculated onto onion leaves (Fig. 23b). On the other hand, *U. atrum* was always conspicuously present and degraded the cell walls of onion leaves.
Because of the inability to observe interactions *in planta* between *B. aclada* and *U. atrum*, *in vitro* confrontations were studied in an attempt to understand the basis of the antagonistic activity of *U. atrum*. In control samples, as observed in the bioassay, neither *B. aclada* nor *U. atrum* were markedly altered by a self-confrontation. Confrontations between the pathogen and the antagonist failed to show typical reactions of parasitism or antibiosis in the bioassay. On the other hand, TEM observations at the zone of contact revealed that fungal cells from both fungi showed varying degrees of alteration at the immediate zone of contact. This ranged from detached plasmalemma to slight disorganisation of the cytoplasm (Fig. 23c, d). These alterations subsided gradually away from the contact zone and neither fungus appeared to overcome the other.

Botrytis-Gliocladium. Ultrastructural observations of dead onion leaves inoculated with *B. aclada* showed that fungal cells were of regular shape and contained a dense cytoplasm (Fig. 24a). Typical organelles such as nuclei and mitochondria were clearly visible and the cells were surrounded by a thick fibrillar mucilage. By comparison, *G. catenulatum* cells were characteristically smaller than those of the pathogen (thus confirming light microscope observations) and were covered by a thin and irregular mucilage (Fig. 24b). Their cytoplasm was regular and contained a few large electron-dense lipid bodies.
In the instances where it was possible to observe both fungi in close vicinity, cells of *B. aclada* had lost all turgor pressure and were typically devoid of cytoplasmic content (Fig. 24c). They were reduced to empty shells being shaped according to the presence of *G. catenulatum* hyphae. The cell wall of the pathogen were thicker compared to controls (Fig. 24a), but were otherwise unaltered and no samples revealed the presence of the antagonist within *B. aclada* cells (Fig. 24c).

Botrytis-Chaetomium. In dead onion leaves inoculated with *C. globosum* alone, cross-sections of fungal cells revealed a dense and uniform cytoplasm with a dark lateral lipid body that sometimes covered most of the section (Fig. 25a). Leaf cell walls adjacent to fungal propagules were either degraded or characterised by a loose fibrillar appearance, suggestive of cellulolytic activity by the antagonist.
In the samples inoculated with both the pathogen and the antagonist, simultaneous presence of both fungi was rarely observed under TEM. In longitudinal sections, lipid bodies of *C. globosum* were quite numerous and distinctive, making it easy to identify the antagonist (Fig. 25b). Based on our observations, *C. globosum* was predominant over *B. aclada* within all treated leaf tissues. In a few instances, dead fungal cells completely devoid of their cytoplasm, appearing as empty hyphal shells, were observed in the proximity to *C. globosum* (Figs. 25b, c). However, because of the advanced stage of degradation of these former cells, it was impossible to confirm whether they belonged to *B. aclada*.

Botrytis-Aureobasidium. In dead onion leaves inoculated with *A. pullulans*, cells of the antagonist were typically found in clusters (Fig. 26a) as observed under light microscopy. They were characterised by a very dense cytoplasm and the presence of dark lipid bodies.
Close contact between *B. aclada* and *A. pullulans* cells revealed that the pathogen was affected by the presence of the antagonist (Fig. 26b). In these observations, cells of *B. aclada* had lost their integrity and the cytoplasmic content of damaged cells had retracted away radially from the cells of *A. pullulans*. At the point of direct contact between the pathogen and the antagonist cells, the cell wall of *B. aclada* had lost some of its definition and appeared distended and fibrillar (Fig. 26b, open arrow).

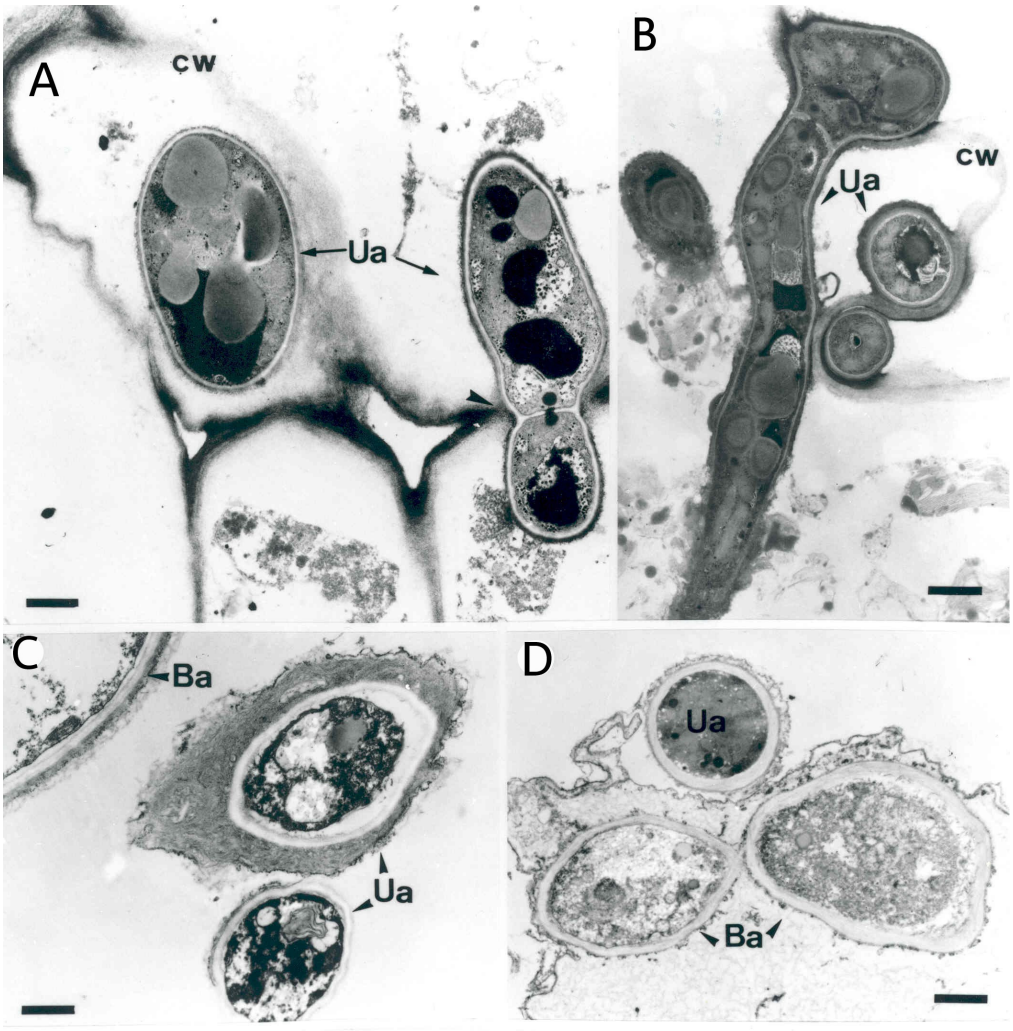


Figure 23. *Transmission electron micrographs of (A)* Ulocladium atrum *(Ua) cells in dead onion (Allium cepa cv Hyton) leaf tissues, (B)* U. atrum *cells in dead onion leaf tissues inoculated with both* Botrytis aclada *(Ba) and* U. atrum *and (C) and (D)* B. aclada *and* U. atrum *cells taken at the meeting point of two colonies growing on agar plates. Leaf samples were taken six days after inoculation* with U. atrum. *Bar = 1 µm. (A) Plant cell walls (cw) of onion leaves are highly degraded in the presence of* U. atrum. *Note the passage of an Ua cell through the plant cell wall (arrowhead). (B) In the interaction* B. aclada-U. atrum *(Ua), only cells of the antagonist are observed within the plant tissues. Note the degradation of the plant cell walls (cw). (C) and (D) Cells of both fungi show varying degrees of alteration when grown in confrontation on agar medium. (From: Köhl et al., 1997.)*

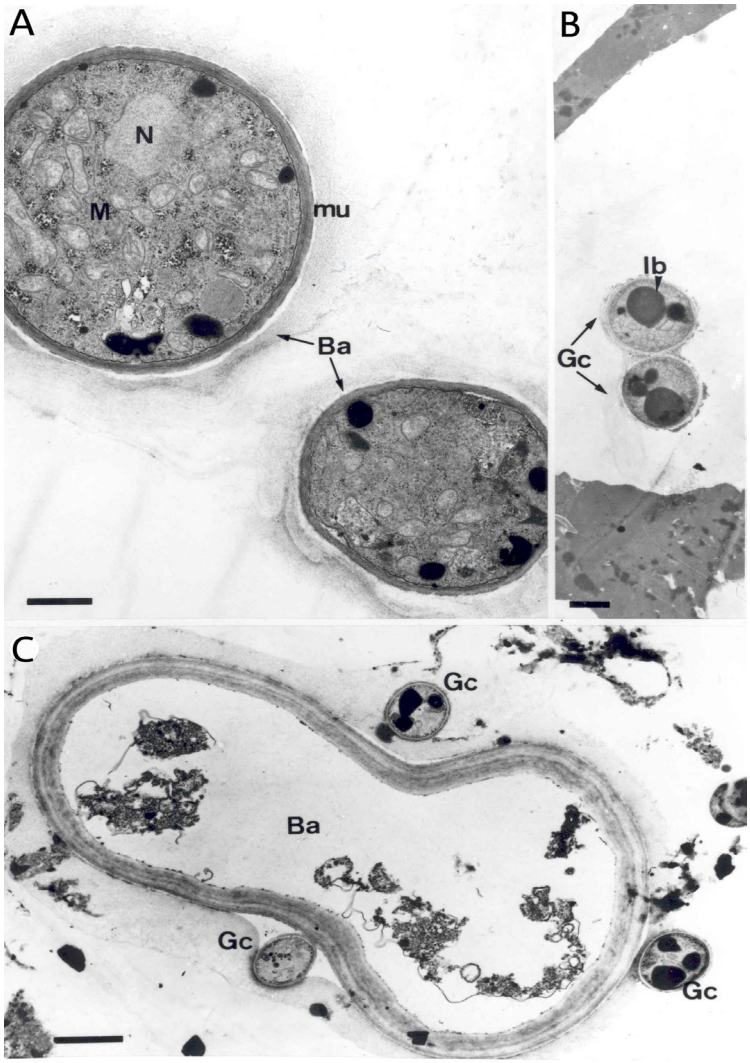


Figure 24. *Transmission electron micrographs of (A)* Botrytis aclada *cells, (B)* Gliocladium catenulatum *cells and (C) the interaction between* B. aclada *and* G. catenulatum *cells in dead onion (*Allium cepa *cv Hyton) leaf tissues. (A)* Botrytis aclada *cells (Ba) surrounded by a thick fibrillar mucilage (mu) and characterised by a dense cytoplasm filled with distinct organelles such as a nucleus (N) and mitochondria (M). Bar = 1 µm. (B) Cells of* Gliocladium catenulatum *(Gc) showing typical large lipid bodies (lb) and surrounded by a thin and irregular mucilage. Bar = 1 µm. (C) A dead cell of* B. aclada *(Ba) surrounded by three cells of* G. catenulatum *(Gc). External pressure by Gc induces wall deformation. Note thickness of the cell wall of Ba. Bar = 2 µm. (From: Köhl et al., 1997.)*

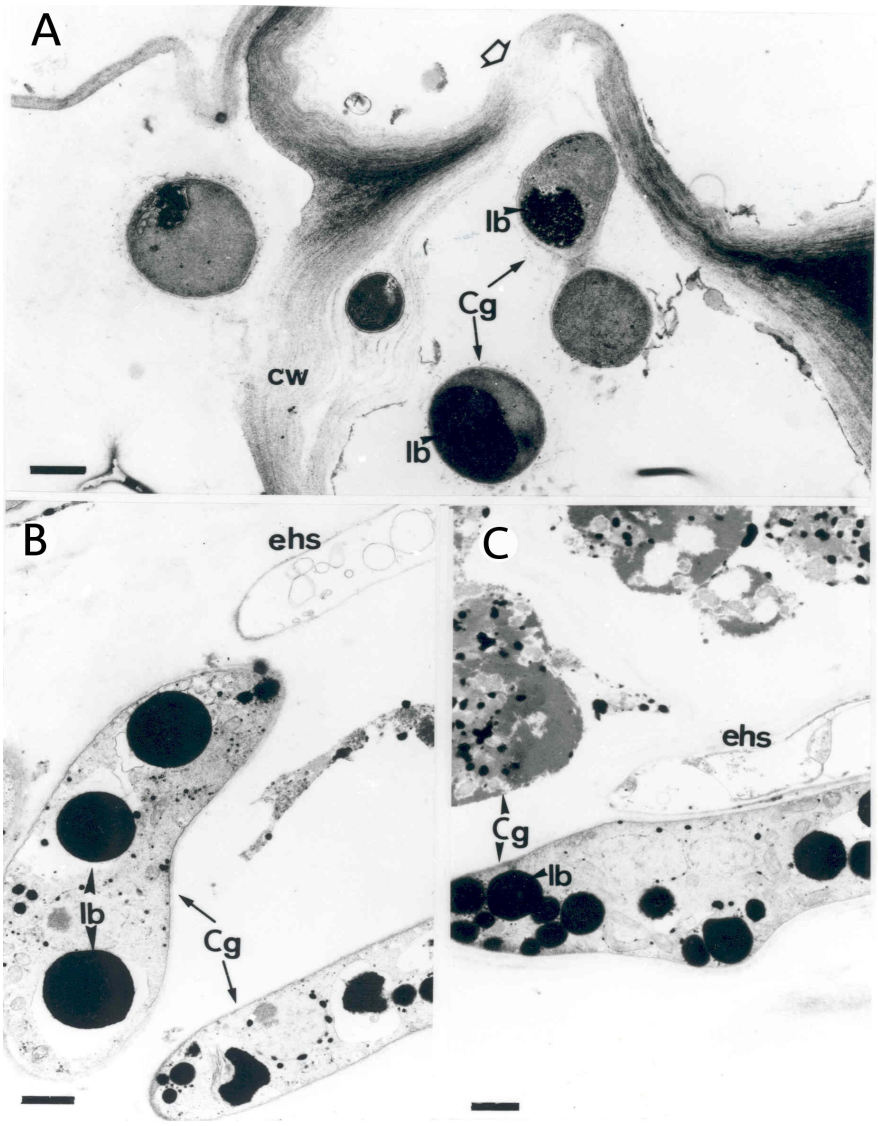


Figure 25. *Transmission electron micrographs of (A)* Chaetomium globosum *cells in dead onion (*Allium cepa *cv Hyton) leaf tissues and (B) and (C)* C. globosum *cells and dead fungal cells, six days after inoculation of the antagonist on dead onion leaf tissues that had been inoculated with* Botrytis aclada. *Bar = 1 µm. (A)* C. globosum *(Cg) is characterized by a dense cytoplasm and the presence of large and electron dense lipid bodies (lb). The plant cell wall (cw) is highly degraded and near complete disruption in certain areas (open arrow). (B) and (C) Presence of empty hyphal shells (ehs) in proximity of* C. globosum *cells. Note typical lipid bodies (lb) of the antagonist. (From: Köhl et al., 1997.)*

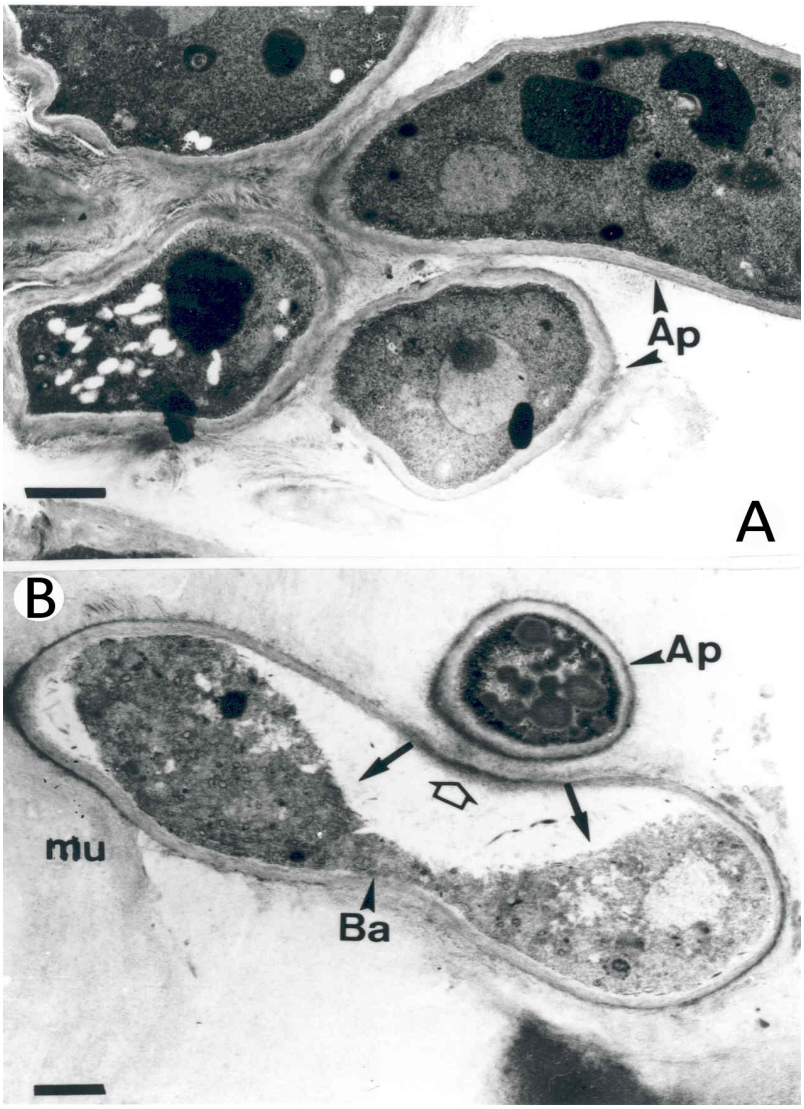


Figure 26. *Transmission electron micrographs of (A).* Aureobasidium pullulans *cells in dead onion (*Allium cepa *cv Hyton) leaf tissues and (B)* A. pullulans *and* B. aclada *cells in dead onion leaf tissues inoculated with both the pathogen and the antagonist. Samples were taken six days after inoculation with* A. pullulans. *Bar = 1 µm. (A) Cells of* A. pullulans *(Ap) are frequently found in clusters and characterized by a very dense cytoplasm. (B) Interaction between* B. aclada *(Ba) and* A. pullulans *(Ap). Note Ba cytoplasm retracted radially from Ap (arrows). Note change in Ba cell wall definition and density at point of direct contact with Ap (open arrow). (From: Köhl et al., 1997.)*

3.4.2 Competitive substrate colonisation: experimental studies and simulation modelling

3.4.2.1 Head start experiments

The yields (sporulation) of monocultures of *B. cinerea* and *U. atrum* on necrotic cyclamen leaf tissue were not influenced by differences in total incubation time caused by the different application intervals (dotted lines in Fig. 27a). Therefore, it can be concluded that two weeks after the first spray application, sporulation was fully developed and stable. Sporulation of *B. cinerea* was not significantly reduced by the presence of the antagonist when *B. cinerea* and *U. atrum* were applied simultaneously or when *B. cinerea* was inoculated before *U. atrum*. With increasing application advantages for *U. atrum*, *B. cinerea* sporulation was reduced until it was completely suppressed at advantages of 48 h or more for *U. atrum* (Fig. 27a).
Sporulation of *U. atrum* was not significantly reduced by *B. cinerea* when *U. atrum* had an application advantage of 12 h or more. With simultaneous inoculation or earlier application of *B. cinerea*, sporulation of *U. atrum* was suppressed although *B. cinerea* was not capable of suppressing sporulation of *U. atrum* completely at the application intervals tested. *U. atrum* sporulation seems to stabilise at a low level in the interval of 12 to 24 h advantage for *B. cinerea*, despite the dominating presence of *B. cinerea* (Fig. 27a).
The relative yields (RY) of *U. atrum* and *B. cinerea* increased with growing application advantages. The intersection between both RY lines was displaced upward and slightly to the right when compared to the reference position of the intersection for equal competitive abilities at 0 hours and 50% relative yield (Fig. 27b). As indicated by the standard errors of the average relative yield totals (RYT), the RYT was significantly higher than 1 for application intervals of 0 and 12 h advantages for *U. atrum*. For longer or shorter advantages for *U. atrum* the RYT did not differ significantly from 1. The convex shape of the RYT line indicates stronger intraspecific competition than interspecific competition, i.e. some degree of niche differentiation between both fungi.

3.4.2.2 Dynamics of internal mycelial colonisation

Internal mycelial biomass followed a more or less sigmoidal time course but maximum colonisation levels were not stable between experiments and in some cases colonisation levels were observed to decrease during the latest phases of colonisation. Variation within experiments might be related to differences in leaf quality between leaves within a batch as reported by Kessel et al. (1999). Variation between experiments might be related to differences in leaf quality between batches. Leaves for the different experiments were harvested in December, February and April, respectively.

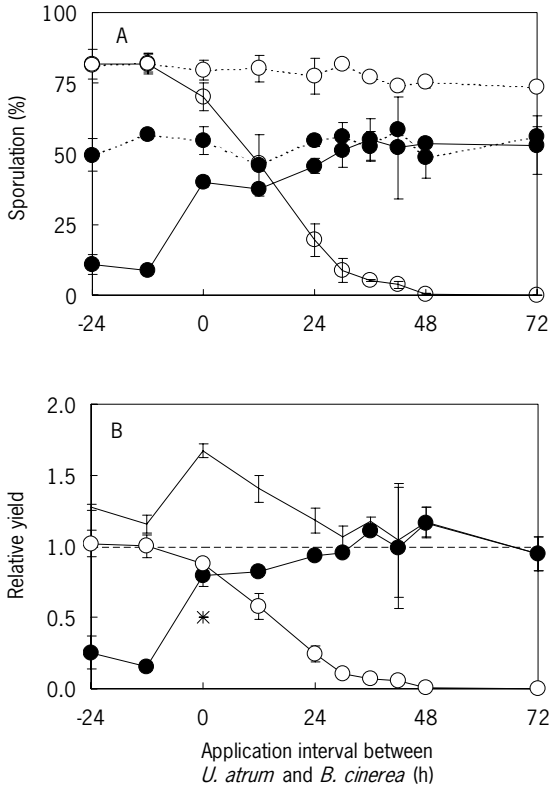


Figure 27. *(A) Sporulation measured as SPLACI (0 to 100%) of* Botrytis cinerea *and* Ulocladium atrum *on sterilised necrotic cyclamen leaf tissue after two weeks incubation at 18 °C. Leaves were sprayed with conidial suspensions (1 x 10^6 conidia ml^{-1}) of B. cinerea and* U. atrum, B. cinerea *and sterile tap water containing 0.01% Tween 80 or* U. atrum *and sterile tap water containing 0.01% Tween 80 at different application intervals ranging from 24 h application advantage for* B. cinerea *(–24 h) to 72 h application advantage for* U. atrum *(72 h). (* ----●---- *)* = U. atrum *sporulation in monoculture. (* ----○---- *)* = *B. cinerea sporulation in monoculture. (* —●— *)* = U. atrum *sporulation in mixed culture with* B. cinerea. *(* —○— *)* = B. cinerea *sporulation in mixed culture with* U. atrum. *(B) Relative yields (*RY*, average sporulation in mixed culture divided by the average sporulation in the corresponding monoculture) and relative yield total (*RYT*) for sporulation of* U. atrum *and* B. cinerea *in mixed cultures. (* —●— *)* = *Experimental* RY *of* U. atrum. *(* —○— *)* = *Experimental* RY *of* B. cinerea. *(* —— *)* = *Experimental* RYT. *(- - -)* = *theoretical* RYT *in case of equal intra- and interspecific competition. (*)* = *theoretical intersection of* RY *lines for B. cinerea and* U. atrum *in case of equal intra- and interspecific competition. Error bars represent standard error of the mean. (From: Kessel, 1999.)*

For *B. cinerea* and *U. atrum* it was observed that in the latest phases of colonisation the clearly visible fluorescing hyphae were replaced by a halo of fluorescence without any apparent structure as if the hyphae had dissolved and the antigen had dispersed in the tissue. The decrease of colonisation levels during later phases of colonisation could thus be caused by lysis of the hyphal elements. Observations on even later phases of colonisation were not possible due to high levels of maceration of the cyclamen tissue. During the first 60 h of colonisation, colonisation levels displayed a consistent exponential increase. No significant effects of fungal species on the colonisation rate were detected. During competitive substrate colonisation, fungal colonisation dynamics followed the same patterns as in monoculture with a trend towards lower maximum colonisation levels in mixed cultures than in monocultures.

3.4.2.3 Simulation

Under the assumption that the final fungal biomass level reached in the simulations is proportional to sporulation, the general behaviour of the simulation model was in agreement with experimental results from the head start experiments. Simulated and measured RY lines displayed a sigmoid shape over the range of application intervals (Fig. 28a). The simulated reference scenario of equal inter- and intraspecific competition (Fig. 28a) resulted in a close resemblance of the simulated and measured RY's for *U. atrum*. The simulated RY's for *B. cinerea* however were underestimated in this scenario. A major difference between the experimental results and the simulated results was found in the shape of the RYT line. The experimental RYT line was convex whereas the simulated RYT lines were straight horizontal lines.

The effect of small changes of the model parameters, giving one of the fungi a competitive advantage, on the simulation results is summarised in Fig. 28. The response of the model system is described using five characteristics of the RY curves and RYT: (1) the steepness of both RY curves, (2) the position of the intersection of both RY curves, (3) the value of the RYT, (4) the lowest RY achieved and (5) the head start needed by each of the fungi to completely suppress the competitor. The relative growth rates (r_U and r_B) are the only parameters controlling the steepness of the RY curves. Higher relative growth rates resulted in shorter head starts needed for complete exclusion of the competitor (Fig. 28b). Differences in the relative growth rate of *U. atrum* and *B. cinerea* result in a lateral displacement of the intersection between both RY curves (Fig. 28c, d). Changes in the relative growth rates did not influence the value of the RYT or the minimum colonisation level achieved.

The availability of species specific resources ($R_B \neq 0$ and / or $R_U \neq 0$) raised both the minimum colonisation level of the species to whom the specific resources were accessible and the RYT. Availability of species-specific resources also caused the

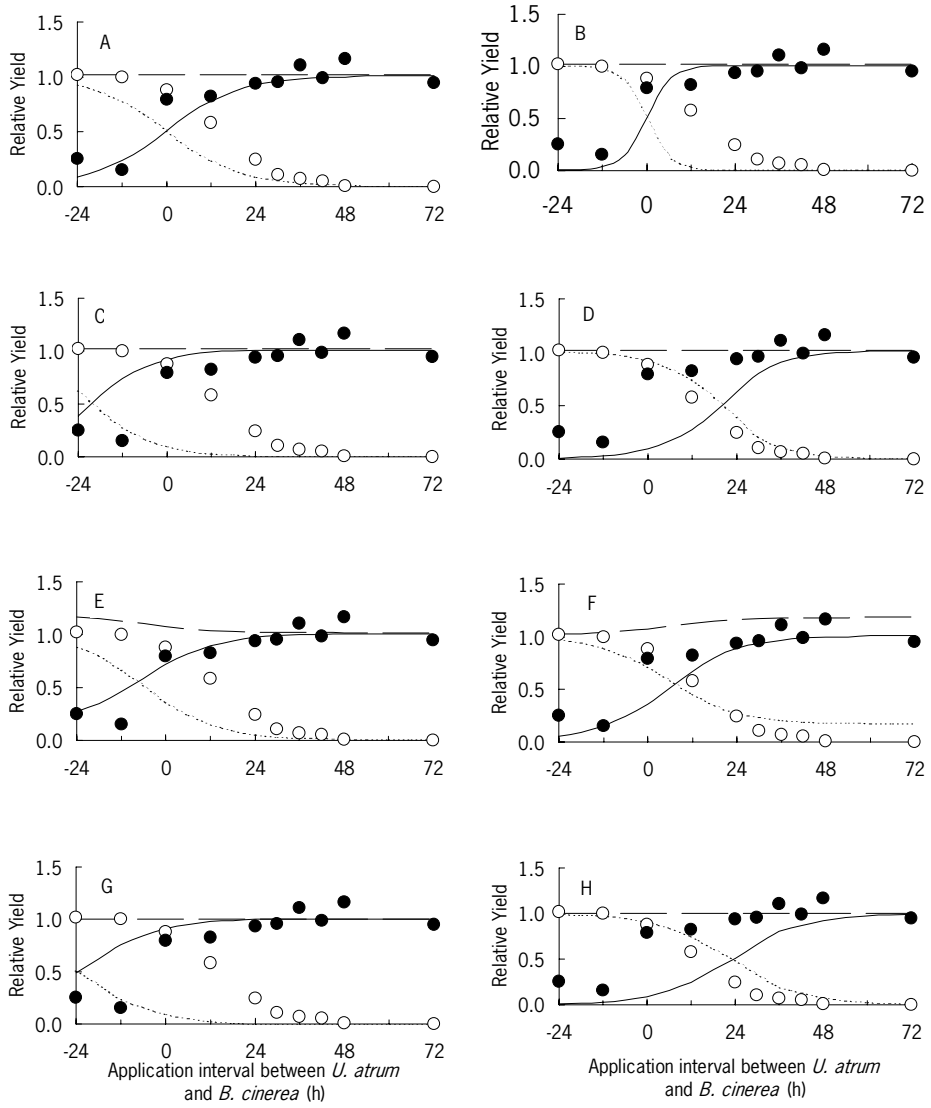


Figure 28. *Relative yield (RY) of* Botrytis cinerea *and* Ulocladium atrum *colonising necrotic cyclamen leaves in simulated and experimental head start experiments. (●) =* U. atrum *experimental* RY. *(○) =* B. cinerea *experimental* RY. *(------) =* B. cinerea *simulated* RY. *(———) =* U. atrum *simulated* RY. *(——— ———) = simulated* RYT. *(A) Reference scenario with equal inter- and intraspecific competition. Model parameters:* $R = 1$; $R_B = R_U = 0$, $r_U = r_B = 0.1$; $U_0 = B_0 = 0.01$ *and* $\mu = \beta = 1$. *All other figures were generated with identical model parameters except for the following: (B)* $r_U = r_B = 0.3$. *(C)* $r_U = 0.2$, $r_B = 0.1$. *(D)* $r_U = 0.1$, $r_B = 0.2$. *(E)* $R_B = 0$, $R_U = 0.2$. *(F)* $R_B = 0.2$, $R_U = 0$. *(G)* $U_0 = 0.001$, $B_0 = 0.0001$. *(H)* $U_0 = 0.0001$, $B_0 = 0.001$. *(From: Kessel, 1999.)*

intersection between both RY curves to shift laterally and upward (Fig. 28e, f). Extra species-specific resources did only marginally affect the steepness of both RY curves.
Differences in initial biomass for both fungi caused the intersection between both RY lines to shift laterally (Fig. 28g, h). The head start needed for complete suppression of the competitor shifted laterally along with the intersection of the RY lines. Minimum colonisation levels and the RYT value were not influenced by differences in initial biomass.

3.5 Use of *U. atrum* for disease control in crops

3.5.1 Experiments in strawberries

3.5.1.1 Grey mould at harvest

In total, seven field experiments were carried out (Table 26). The incidence of grey mould in untreated plots was generally low, from 1.4 to 12.3% of the total fruits (Tables 27 and 28). The application of *U. atrum* weekly at 2 x 10^6 conidia ml^{-1} from transplanting until first fruits turned red significantly reduced the percentage of grey mould in comparison to the control in experiments 1, 2 and 4 (Table 27). *U. atrum* weekly sprayed from transplanting and twice weekly from beginning of flowering significantly reduced grey mould in comparison to the control in experiment 5, but not in experiment 6 (Table 28). *U. atrum* applied weekly from the beginning of flowering at 2 x 10^6 conidia ml^{-1} gave significant reduction of grey mould in comparison to the control in experiment 2 but not in experiment 4 (Table 28). *U. atrum* at 2 x 10^6 conidia ml^{-1}, applied twice per week from the beginning of flowering, reduced grey mould in comparison to the control in experiment 5 but not in experiment 6 (Table 28). In experiment 7 (data not presented), *U. atrum* applied at 0.5 x 10^6 conidia ml^{-1} from the beginning of flowering until first fruits turned red, did not reduce grey mould in comparison to the control when applied at two- or four-day intervals. However, the incidence of grey mould in the control treatment (2.2%) was low. The effects of applications of *U. atrum* at 2 x 10^6 conidia ml^{-1} starting at transplanting were superior to those starting at flowering in only one experiment (experiment 4, Table 27). Fungicide programmes reduced grey mould significantly more than *U. atrum* applied at 2 x 10^6 conidia ml^{-1} from the beginning of flowering in four experiments (experiment 2 and 4, Table 27; experiment 5 and 6, Table 28). Crop sanitation by removing senescent and necrotic leaflets did not reduce grey mould in comparison to the control (experiment 5 and 6, Table 28). *U. atrum* spray programmes effectively reduced grey mould in four of seven experiments. In two of seven experiments at least one *U. atrum* spray programme gave

Table 26. Location, crop rotation and date of transplanting and harvesting of strawberry experiments carried out from 1996 to 1999 in the Netherlands. (From: Boff et al., 2002.)

Experiment	Location	Previous crop	Transplanting date	No. of replicates	Harvesting period
1	Breda	Strawberry	2 July 1996	6	29 Aug to 4 Sep 1996
2	Wageningen	Grass	10 April 1997	5	16 Jun to 7 Jul 1997
3	Wageningen	Grass	27 June 1997	5	11 Aug to 25 Aug 1997
4	Breda	Strawberry	25 June 1997	6	14 Aug to 26 Aug 1997
5	Wageningen	Grass	6 May 1998	4	02 Jul to 03 Aug 1998
6	Wageningen	Grass	19 June 1998	4	13 Aug to 07 Sep 1998
7	Wageningen	Grass	21 May 1999	4	15 Jul to 09 Aug 1999

the same or better control than the fungicide programme (experiment 1 and 4, Table 27). In experiment 3, the lack of treatment effects can be explained by the extremely low incidence of fruit rot (1.4% in the control) due to hot, dry conditions.

3.5.1.2 Post-harvest grey mould

Incidence of grey mould in the control treatment after post-harvest incubation (8.8 to 38%) was higher than at harvest (1.4 to 12%), and in two of five experiments treatment effects were found (experiments 1 to 3 and 5 to 6; Tables 27 and 28). *U. atrum* spray programmes either from transplanting or from the beginning of flowering reduced post-harvest grey mould in comparison to the control only in one (experiment 2) of five experiments. The fungicide programme reduced post-harvest grey mould more effectively than the *U. atrum* spraying programme only in one experiment (experiment 6, Table 28). Removal of senescent and necrotic leaflets from strawberry plants (crop sanitation) did not reduce post-harvest grey mould in comparison to the control (experiments 5 and 6, Table 28).

3.5.1.3 Incidence of B. cinerea on flowers and petals

The incidence of *B. cinerea* on flowers from plants treated with *U. atrum* starting at transplanting time was lower than the control in one of two samplings in experiment 2, in two of the three samplings in experiment 5, and in no sampling from experiment 3 or 6 (Table 29). There was no significant ($P > 0.05$) difference between the *U. atrum* and the fungicide treatment, except for sample 2, experiment 5, when the fungicide programme resulted in a lower incidence of *B. cinerea* (Table 29).

Table 27. *Effect of applications of conidial suspensions of* Ulocladium atrum *or fungicide programme on the incidence of grey mould on strawberry fruit in 1996 and 1997. (From: Boff et al., 2002.)*

Treatments	Disease incidence at harvest (%) [w]				Disease incidence post-harvest (%) [w]				Number of applications			
	Experiment				Experiment				Experiment			
	1	2	3	4	1	2	3	4	1	2	3	4
Untreated control [x]	12.3 a	5.4 a	1.4 a	8.1 a	38 a	16.2 a	21.2 a	-	8	0	0	0
U. atrum weekly (starting at transplanting) [y]	4.8 b	2.6 b	1.1 a	4.7 b	30 a	10.3 b	17.6 a	-	8	11	6	6
U. atrum weekly (starting at beginning flowering) [y]	-	3.4 b	2.0 a	7.1 a	-	7.8 b	21.2 a	-	-	5	3	3
Fungicides weekly (starting at beginning flowering) [z]	11.5 a	1.3 c	1.5 a	3.9 b	37 a	5.7 b	15.8 a	-	4	5	3	4

[w] *Values in the same column followed by the same letter are not significantly different based on LSD tests (a = 0.05).*

[x] *The control for experiment 1 was sprayed with water plus Tween 80 at 0.01%.*

[y] U. atrum *was applied at weekly intervals at 2 × 10⁶ conidia ml⁻¹.*

[z] *Experiment 1: iprodione (0.75 kg ha⁻¹), 2 applications of tolylfluanide (0.5 kg ha⁻¹) and pyrazophos (0.15 l ha⁻¹) in alternation; experiment 2: thiram (2 kg ha⁻¹), 3 applications of tolylfluanide (0.75 kg ha⁻¹) and pyrimethanil (0.8 l ha⁻¹) in alternation; experiment 3: thiram (2 kg ha⁻¹), tolylfluanide (0.75 kg ha⁻¹) and pyrimethanil (0.8 l ha⁻¹) in alternation; experiment 4: 2 applications of tolylfluanide (0.75 kg ha⁻¹) and 2 applications of iprodione (0.75 kg ha⁻¹) in alternation.*

Table 28. Effect of applications of conidial suspensions of U. atrum *or fungicide programme on the incidence of grey mould on strawberry fruits in 1998. (From: Boff et al., 2002.)*

Treatments	Disease incidence at harvest (%) [w]		Disease incidence post-harvest (%) [w]		Number of sprays	
	Experiment		Experiment		Experiment	
	5	6	5	6	5	6
Untreated control	8.3 a	11.3 ab	8.8 a	33.2 a	-	-
U. atrum (starting at transplanting) [x]	5.9 b	8.8 b	14.9 a	29.3 a	12	8
U. atrum (starting at beginning flowering) [y]	5.6 b	10.6 b	6.9 a	26.8 a	6	5
Fungicide (starting at beginning flowering) [z]	3.6 c	3.0 c	7.5 a	19.3 b	6	5
Crop sanitation (starting at transplanting)	8.3 a	13.9 a	8.8 a	31.4 a	-	-

[w] *Values in the same column followed by the same letter are not significantly different based on LSD tests (a = 0.05).*

[x] U. atrum *was applied weekly from transplanting and twice weekly from first green bud appearance at 2 × 10⁶ conidia ml⁻¹.*

[y] U. atrum *at 2 × 10⁶ conidia ml⁻¹ or fungicide was applied twice weekly from first open flowers.*

[z] *Experiment 5: 3 applications of tolylfluanide (0.75 kg ha⁻¹) and 3 applications of iprodione (0.75 kg ha⁻¹) in alternation; experiment 6: 3 applications of tolylfluanide (0.75 kg ha⁻¹) and 2 applications of iprodione (0.75 kg ha⁻¹) in alternation.*

Table 29. Effect of applications of conidial suspensions of U. atrum*, fungicides or crop sanitation on the incidence of B. cinerea on strawberry flowers. (From: Boff et al., 2002.)*

Experiment	Interval between the previous spray and sampling day	Incidence of *B. cinerea* on flowers / petals [w]							
		Control untreated		*U. atrum* (from transplanting)		*U. atrum* (from beginning of flowering)		Fungicide (from beginning of flowering) [x]		Crop sanitation [y]	
Experiment 2 (1997)											
sample 1 (10 Jun)	5	5.1	a	3.0	a	3.0	a	10.0	a	-	
sample 2 (16 Jun)	3	14.9	a	1.3	a	1.3	a	6.0	ab	-	
Experiment 3 (1997)											
sample 1 (29 Jul)	7	76.2	a	87.0	a	93.8	a	83.0	a	-	
sample 2 (4 Aug)	6	96.0	a	92.0	a	90.0	a	97.0	a	-	
Experiment 5 (1998)											
sample 1 (08 Jun)	3 (only treat 2) [z]	63.7	a	33.7	b	83.7	a	62.5	a	65.0	a
sample 2 (15 Jun)	4	70.0	a	42.5	bc	51.2	b	21.3	a	62.5	ab
sample 3 (03 Jul)	3	81.3	a	66.2	ab	64.0	ab	51.8	a	72.5	a
Experiment 6 (1998)											
sample 1 (21 Jul)	5 (only treat 2) [z]	83.7	a	72.5	a	83.7	a	81.2	a	80.0	a
sample 2 (5 Aug)	1	75.0	a	53.7	a	63.7	a	53.7	a	67.5	a
sample 3 (11 Aug)	4	60.1	a	51.2	a	53.7	a	55.0	a	66.2	a

[w] *Values in the same row followed by the same letter are not significantly different based on LSD test (a = 0.05).*
[x] *Experiments 1-4: see Table 27; experiments 5 and 6: see Table 28.*
[y] *Removal of senescent and necrotic leaflets twice per week from transplanting until first fruits turned red.*
[z] *In sample 1 (experiments 5 and 6), only treatment 2 had been sprayed before this sampling date.*

In experiment 7, the incidence of *B. cinerea* on petals from plots treated with *U. atrum* or fungicides was lower as compared to untreated plots. However, these differences were statistically significant only for sample 1. The incidence of *B. cinerea* on petals did not differ between the untreated plots and plots with crop sanitation (Table 29).

3.5.1.4 Fruit quality

Healthy fruit weight, as measure of quality, did not differ among the treatments, except in experiment 5 in which plants treated twice weekly with *U. atrum* at 2 x 10^6 conidia ml^{-1} from first flowering presented significantly smaller fruits (8.8 g per fruit) than the control (10.3 g per fruit) and in experiment 7, in which plants sprayed with fungicides presented significantly larger fruits (13.2 g) than the untreated control plants (11.6 g) (other data not presented).

3.5.2 Experiments in onion

3.5.2.1 Fungal sporulation on necrotic onion leaf segments exposed on onion field plots

On necrotic onion leaf segments exposed in 1995 on onion plots treated with water, conidial suspensions of *U. atrum* or Ronilan, sporulation of *Botrytis* spp. was found only sporadically even after moist incubation for ten days at 18 °C for all ten periods in which such experiments had been carried out. Sporulation after moist incubation of *Alternaria* spp., *Cladosporium* spp. and *U. atrum* varied for the different periods (Fig. 29). In most cases, high values for sporulation of *Alternaria* spp. coincided with low values for *Cladosporium* and vice versa. On leaf segments treated with conidial suspensions of *U. atrum*, the sporulation of *Alternaria* spp. and *Cladosporium* spp. was lower than on leaf segments from control plots treated with water. Such effects were not found for Ronilan treated leaf segments. Colonisation of *U. atrum* (and naturally occurring fungi which cannot be distinguished from *U. atrum* microscopically) was high with approximately 50% coverage on average of all experiments for *U. atrum* treated leaf segments, but below 7% for leaf segments from control or Ronilan treated plots. Colonisation of leaf segments not sprayed with conidial suspensions of *U. atrum* but exposed on *U. atrum* treated plots tended to have only a slightly higher colonisation by *U. atrum* than leaf segments sampled from plots not treated with *U. atrum*. Such leaf segments exposed on *U. atrum* treated plots but not sprayed with *U. atrum* were covered by *Alternaria* spp. and *Cladosporium* spp. to the same extend as leaves from water treated control plots (data not shown).

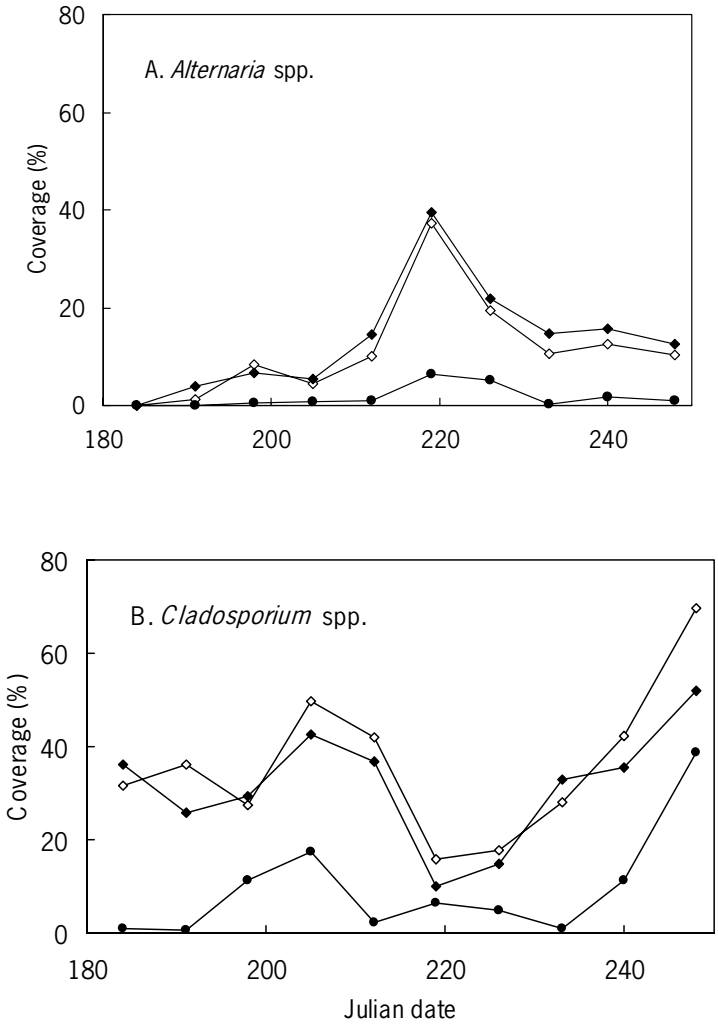


Figure 29. *Colonisation of necrotic onion leaf segments exposed to field plots before treatment with water (—◊—), Ronilan (—◆—) or* Ulocladium atrum *(2×10^6 conidia ml^{-1}) (—●—) or after treated with* U. atrum *(—○—). Leaf segments fixed to leaf supports were left on the field plots for seven days, incubated in moist chamber for ten days at 18 °C and leaf surface covered by fungal sporulation was assessed for (A)* Alternaria *spp., (B)* Cladosporium *spp., and (C)* U. atrum. *Field experiment 1995. (From: Köhl et al., 2003.)*

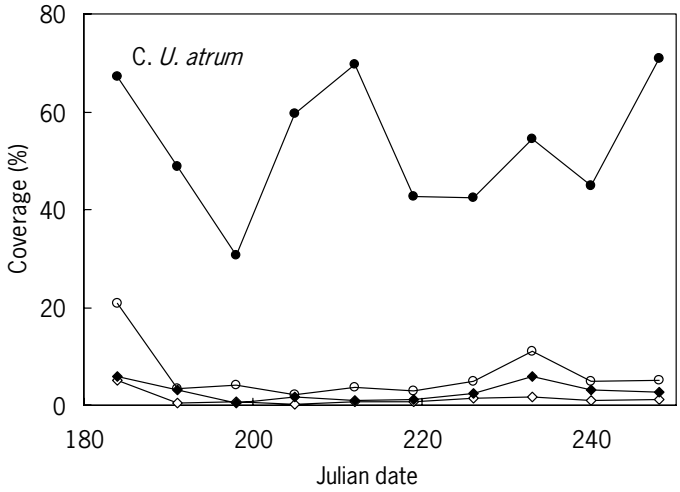


Figure 29. Continued.

3.5.2.2 Colonisation of necrotic leaves

Necrotic leaf parts sampled during both field experiments were incubated in a moist chamber to stimulate *Botrytis* spp. sporulation. On all samples, *Botrytis* spp. sporulated only incidentally after incubation in a moist chamber except for leaves sampled on 21 August 1996. On necrotic leaf tips sampled at this date from water treated plots, 3.0% of the area was covered with sporulating *Botrytis* spp. after moist incubation, whereas on necrotic leaf parts from *U. atrum* or Ronilan treated plots statistically significantly less sporulation was found with 0.2 and 0.3% coverage with sporulating *Botrytis* spp. (LSD test; $\alpha = 0.05$).

3.5.2.3 Aerial spore load

During the field experiment in 1996, spore load of *Botrytis* spp. above onion plots and outside plots in the buffer crop was quantified (Table 30). Spore counts in general were low with a maximum of 150 spores (sized <15 μm) m^{-3} of air as compared to counts made during a leaf spot epidemic in 1991 with a maximum value of nearly 600 spores m^{-3} of air (3.1.1; Fig. 2). For all runs, the spore load outside the experimental field was the same as for plots treated with *U. atrum* or Ronilan. Spore load in water treated control plots was statistically significantly higher in four assessments and tended to be higher in another two assessments. In the other runs, no differences in spore load of *Botrytis* spp. were found inside and outside experimental plots.

Table 30. *Aerial load with conidia of* Botrytis *spp. above and outside onion plots treated weekly with* Ulocladium atrum [w] *or Ronilan in comparison to a water treated control. (From: Köhl et al., 2003)*

Treatment	Number of conidia of *Botrytis* spp. m^{-3} of air					
	<15 µm [x]	>15 µm	<15 µm	>15 µm	<15 µm	>15 µm
19 Aug	Run 1 (11:44) [y]		Run 2 (12:19)		Run 3 (12:56)	
Water	150 a [z]	31 n.s.	149 a	14 n.s.	141 n.s.	19 n.s.
U. atrum	76 b	14	99 b	16	126	15
Ronilan	92 b	16	86 b	9	101	13
outside	85 b	15	89 b	8	121	13
22 Aug	Run 1 (9:56)		Run 2 (10:40)		Run 3 (11:18)	
Water	102 a	39 a	73 n.s.	9 n.s.	137 n.s.	9 n.s.
U. atrum	49 b	11 b	75	7	58	4
Ronilan	52 b	16 b	61	9	44	4
outside	73 ab	8 b	87	5	87	5

[w] *Conidial suspensions with 2 × 10^6 conidia ml^{-1} were sprayed at 500 l ha^{-1}.*
[x] *Spore length.*
[y] *Starting time of 15 min-run.*
[z] *Values of the same column and sampling day with a common letter do not differ statistically (LSD test; a = 0.05).*

3.5.2.4 Leaf spots

In both years, the number of leaf spots cm^{-2} of green leaf tissue was low. Lesions did not expand and no symptoms of leaf die back were observed. In 1995, maximum values at the end of the growing season were approximately 0.1 leaf spot cm^{-2} of green leaf surface (data not presented). A statistically significant reduction of the number of leaf spots by approximately 40% was found for both *U. atrum* and Ronilan treated plots as compared to the water control only one week before 50% of the leaves had lodged (data not presented). In 1996, 0.2 spots cm^{-2} of green leaf surface were counted on plants from the water treated control when 50% of the leaves had lodged (Fig. 30). The number of leaf spots cm^{-2} of green leaf surface was statistically significantly reduced by 60% in both *U. atrum* treated and Ronilan treated plots.

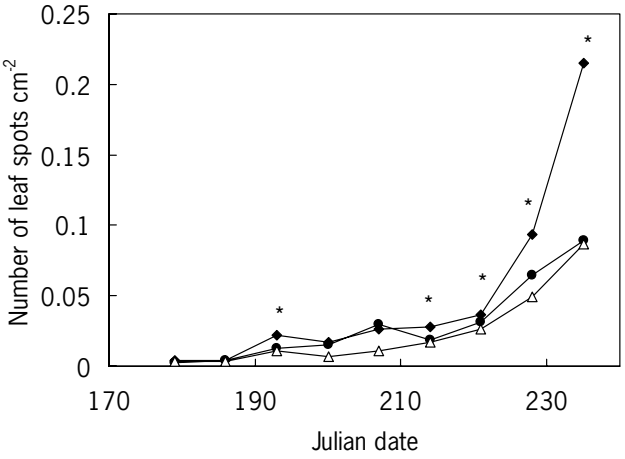


Figure 30. *Number of leaf spots caused by* Botrytis squamosa *or* B. cinerea *in onion treated at weekly intervals with conidial suspensions of* Ulocladium atrum *(2 x 10⁶ conidia ml⁻¹) (—●—) or Ronilan (—△—) in comparison with a water treated control (—◆—). Statistically significant differences between control and treatments with* U. atrum *or Ronilan are indicated with asterisks (LSD test; a = 0.05). Field experiment 1996. (From: Köhl et al., 2003.)*

3.5.3 Experiments in cyclamen under commercial growing conditions

3.5.3.1 Development of a biocontrol system with U. atrum *and* G. roseum

In the first experiment, no sporulation of *B. cinerea* was found at the beginning of the experiment. Sporulation initially was observed on dead leaf tissue of 153-day old plants. The estimated leaf area with *B. cinerea* sporulation corrected for sporulation intensity (SPLACI; 2.2.5.4) increased with time in all treatments (Fig. 31a). When plants were near marketable age at 205 days, more than 0.8 leaves per plant of the control treatment were completely covered with sporulating *B. cinerea*. SPLACI was reduced significantly to 0.15 by the application of *U. atrum* or the fungicides. Disease incidence increased with time, and more than 80% of the water-treated plants showed symptoms of *B. cinerea* at a plant age of 205 days (Fig. 31b). Treatments with *U. atrum* or fungicides resulted in significant disease suppression with disease incidences of 40% and 46%, respectively. The number of diseased petioles per plant increased during the experiment in all treatments, but was significantly reduced by treatment with *U. atrum* (0.9) or fungicides (0.7) as compared to the control (3.5) at a plant age of 205 days (Fig. 31c). On average, approximately 1.8 petioles per plant treated with *G. roseum* were infected.

Several heavily infected plants of the different treatments were removed from the greenhouse at a plant age of 205 days to reduce the inoculum density for neighbouring commercially grown crops. SPLACI, disease incidence, and disease severity of the remaining plants of the control treatment increased during the subsequent 14 days but SPLACI and disease severity increased slower for plants treated with *U. atrum* (Fig. 31a-c).

In the second experiment, treatments were started with younger plants (plant age of 109 days) that did not show symptoms caused by *B. cinerea*. *B. cinerea* sporulation first occurred at a plant age of 151 days. During the last month of experiment 2, disease development was more rapid than in the first experiment (Fig. 32). When plants were close to marketability (plant age of 236 days), approximately 1.4 leaves per plant of the water control were completely covered with *B. cinerea* (Fig. 32a). SPLACI was significantly reduced to below 0.7 leaves by the application of *U. atrum* (applied at intervals as in experiment 1), *G. roseum* (applied at a five times higher dose than in experiment 1) and the fungicides. *U. atrum* applied at increased intervals significantly reduced SPLACI assessed at a plant age of 179, 193, 207, and 221 days, but not of 236 days. Disease incidence in the treatments with antagonists or fungicides was significantly reduced to 50 to 60% as compared to 77% in the control treatment at a plant age of 236 days (Fig. 32b). Disease severity based on diseased petioles per plant showed the same pattern (Fig. 32c). Antagonist and fungicide treatments significantly reduced the number of diseased petioles from 3.9 in the water control to 1.5 to 2.3 with no significant differences between these treatments. After heavily diseased plants were removed when plants were 236 days old, the disease severity of the remaining control plants increased from 1.5 to 3.0 during the next 14 days. For *U. atrum*-treated plants, the disease severity increased from 1.1 to 1.6 (regular application intervals) and from 0.9 to 1.5 (increased application intervals), respectively.

For both experiments, the different treatments had similar effects on sporulation of *B. cinerea* on both leaf blades (SPLA, SPLACI) and petioles (SPPA, SPPACI) as on disease severity (Fig. 31 and 32, Table 31). Significant block effects, at least partly to be attributed to possible differences in resistance against *B. cinerea* of lines with different flower colour, were found for several assessment dates during experiment 2 but not during experiment 1; e.g., disease severity was twice as high in one block consisting of plants with wine red coloured flowers, than in the three other blocks, consisting of plants with pink, white with red heart, or lilac coloured flowers.

Occasionally, epiphytic colonies of *U. atrum* were found on the lower side of leaves where humidity in the canopy allowed epiphytic growth. Such colonies could be wiped off without any sign of leaf damage underneath the colony.

Table 31. *Effect of* Ulocladium atrum, Gliocladium roseum, *and fungicides on the spore-producing leaf area (SPLA), spore-producing petiole area (SPPA), and spore-producing petiole area corrected for intensity (SPPACI)* [x] *of cyclamen plants cv Super Serie grown in a commercial greenhouse. (From: Köhl et al., 1998.)*

Treatment [y]	SPLA		SPPA		SPPACI	
Experiment 1						
Control	1.31	a [z]	2.82	a	1.73	a
Fungicides	0.25	b	0.46	c	0.23	b
G. roseum	0.76	a	1.27	b	0.78	b
U. atrum	0.25	b	1.03	bc	0.63	b
Experiment 2						
Control	1.86	a	3.01	a	2.08	a
Fungicides	0.83	b	1.33	bc	0.77	bc
G. roseum	0.97	b	0.90	c	0.59	c
U. atrum (2-3 weekly)	1.05	b	1.75	bc	0.88	bc
U. atrum (4-6 weekly)	1.46	ab	2.02	ab	1.38	ab

[x] *SPLA is defined as the estimated equivalent number of leaf blades per plant with conidiophores of* Botrytis cinerea*; SPPA is defined as the estimated equivalent number of petioles per plant with conidiophores of* B. cinerea*; SPPACI is defined as the estimated equivalent number of petioles per plant completely covered with conidiophores of* B. cinerea. *Plant age at assessment date was 205 days (experiment 1) and 236 days (experiment 2).*

[y] *In experiment 1, conidial suspensions of* U. atrum *(1×10^6 conidia ml^{-1}) and* G. roseum *(2×10^6 conidia ml^{-1}) were applied at a plant age of 133, 153, 174, 189, and 205 days. The fungicides tolylfluanid, prochloraz-manganese, tolylfluanid, prochloraz-manganese, and iprodione were applied in alternation when plants were 112, 133, 153, 174, and 200 days old. In experiment 2, conidial suspensions of* U. atrum *(1×10^6 conidia ml^{-1}) and* G. roseum *(1×10^7 conidia ml^{-1}) were applied at intervals of two to three weeks at a plant age of 109, 130, 151, 166, 179, 193, 207, 221, and 236 days. In an additional treatment* U. atrum *was applied at intervals of four to six weeks at a plant age of 109, 151, 179, 207, and 236 days. Chlorothalonil, tolylfluanid, prochloraz-manganese, iprodione, and iprodione were applied in alternation when plants were 84, 106, 130, 160, and 225 days old.*

[z] *In the two experiments, 25 and 24 plants of each of four replicates were assessed. Means in the same column and the same experiment with a common letter do not differ significantly ($P < 0.05$) according to log-linear regression followed by t-tests.*

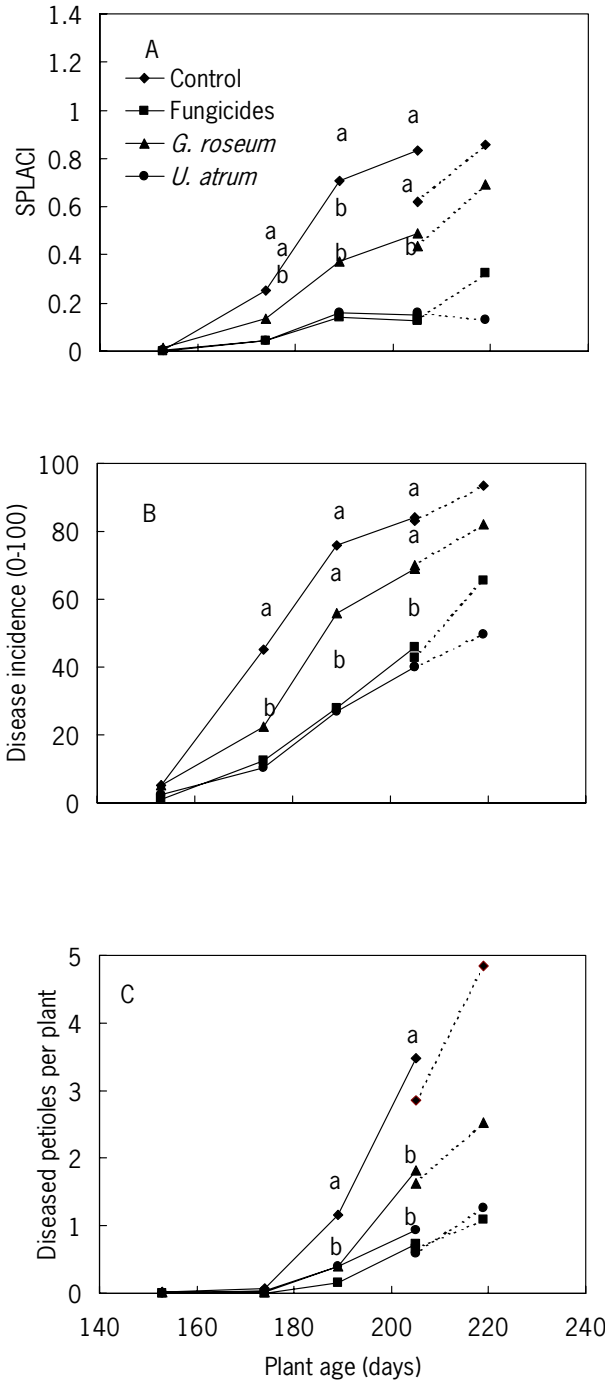


Figure 31.
Effect of treatments with Ulocladium atrum, Gliocladium roseum, *and a fungicide programme on (A) spore-producing leaf area corrected for intensity (SPLACI); (B) disease incidence; and (C) disease severity of* Botrytis cinerea *on cyclamen plants cv Super Serie under commercial growing conditions. Conidial suspensions of* U. atrum *(1×10^6 conidia ml^{-1}) and* G. roseum *(2×10^6 conidia ml^{-1}) were applied at a plant age of 133, 153, 174, 189, and 205 days. Tolylfluanid, prochloraz-manganese, tolylfluanid, prochloraz-manganese, and iprodione were applied in alternation when plants were 112, 133, 153, 174, and 200 days old. SPLACI is defined as the estimated equivalent number of leaf blades per plant completely covered with conidiophores of* B. cinerea, *and disease incidence is defined as the percentage of plants with symptoms of* B. cinerea*; disease severity is defined as the average number of petioles with symptoms of* B. cinerea *per plant. Dotted lines represent remaining plants after heavily diseased plants had been removed at a plant age of 205 days. Twenty-five plants of each of four replicates were assessed. Values of the same assessment date with a common letter do not differ significantly ($P < 0.05$) according to logistic or log-linear regression followed by t-tests. (From: Köhl et al., 1998.)*

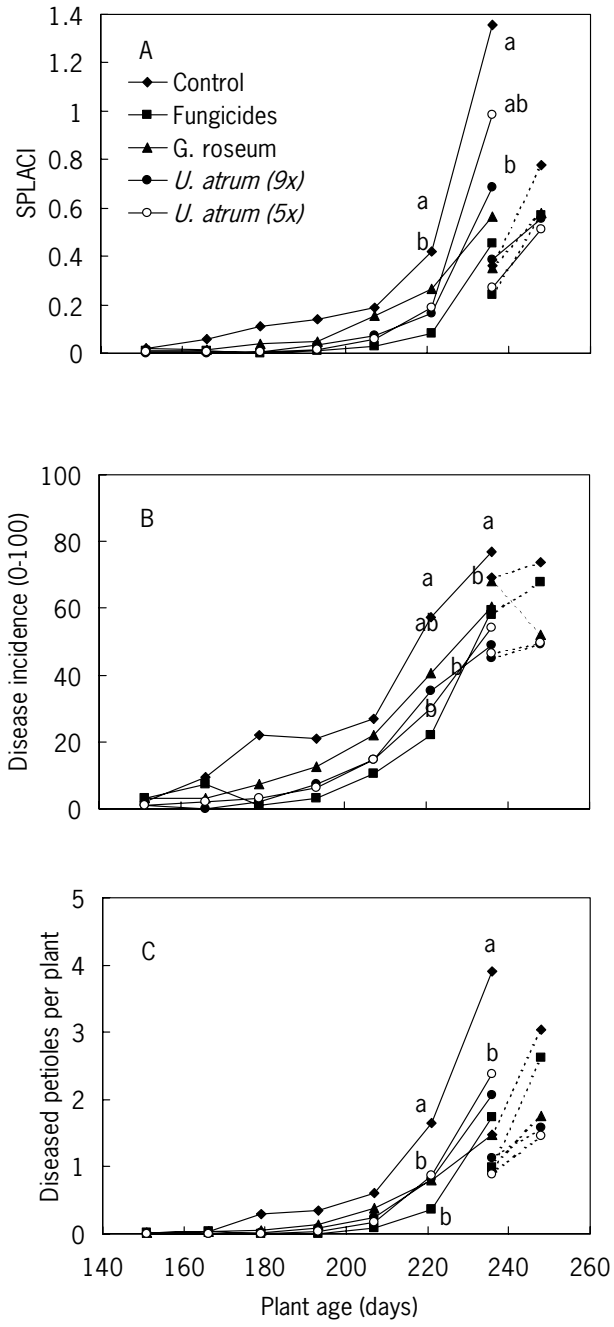


Figure 32.
Effect of treatments with Ulocladium atrum, Gliocladium roseum *and a fungicide programme on (A) spore-producing leaf area corrected for intensity (SPLACI); (B) disease incidence; and (C) disease severity of* Botrytis cinerea *on cyclamen plants cv Super Serie under commercial growing conditions. Conidial suspensions of* U. atrum *(1×10^6 conidia ml^{-1}) and* G. roseum *(1×10^7 conidia ml^{-1}) were applied at intervals of two to three weeks at a plant age of 109, 130, 151, 166, 179, 193, 207, 221, and 236 days. In an additional treatment* U. atrum *was applied at intervals of four to six weeks at a plant age of 109, 151, 179, 207, and 236 days. Chlorothalonil, tolylfluanid, prochloraz-manganese, iprodione, and iprodione were applied in alternation when plants were 84, 106, 130, 160, and 225 days old. SPLACI is defined as the estimated equivalent number of leaf blades per plant completely covered with conidiophores of* B. cinerea, *disease incidence is defined as the percentage of plants with symptoms of* B. cinerea*; disease severity is defined as the average number of petioles with symptoms of* B. cinerea *per plant. Dotted lines represent remaining plants after heavily diseased plants had been removed at a plant age of 236 days. Twenty-four plants of each of four replicates were assessed. Values of the same assessment date with a common letter do not differ significantly ($P < 0.05$) according to logistic or log-linear regression followed by t-tests. (From: Köhl et al., 1998.)*

3.5.3.2 *Biocontrol with* U. atrum *in different cropping systems*

Grey mould occurred in all ten experiments with different cropping systems (Table 32). Disease incidence (DI) at marketing ranged between 41% and 87% in the untreated controls for experiments carried out in greenhouses 1-5 (Table 33), 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.

Table 32. Growing systems of cyclamen crops in greenhouses 1 to 6. (From: Köhl et al., 2000.)

Green-house number	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 of 150 days on gutters	Top irrigation; from plant age of 150 days intermittent flow irrigation	
4	Plastic	Ground on polyethylene foil; from plant age of 190 days on gutters	Top irrigation; from plant age of 190 days intermittent flow irrigation	Additional tubes 30 cm above plants
5	Plastic	Tables	Capillary matting plus top irrigation	
6 [z]	Clay	Tables	Capillary matting plus top irrigation	

[z] *Data of experiments not presented in Table 33.*

Table 33. *Effect of treatments of cyclamen with* Ulocladium atrum *or fungicides on* Botrytis cinerea *disease development (AUDPC) and disease manifestation at marketable age[o]. (From: Köhl et al., 2000.)*

Treatment	Disease development (AUDPC)		Disease manifestation 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
U. atrum 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/diethocarb, iprodione, iprodione	381 bc	7 bc	19 bc	0.4 bc
U. atrum 2x	643 ab	18 ab	34 ab	1.1 ab
U. atrum 4x	175 c	6 c	11 c	0.3 c
Greenhouse 3; cv Pastel [v]				
Untreated	9424 a	279 a	87 a	5.5 ab
Tween-water	9290 a	295 a	93 a	6.7 a
Iprodione	10001 a	330 a	96 a	7.4 a
U. atrum 2x	8032 b	209 b	89 a	4.4 bc
U. atrum 5x	7365 b	164 b	69 b	3.2 c
Greenhouse 4; cv Anglia [w]				
Untreated	3424 a	64 a	52 a	2.0 a
Tween-water	2974 a	64 a	43 ab	1.5 ab
U. atrum 2x	1767 b	32 b	29 bc	0.7 b
U. atrum 4x	1518 b	27 b	17 c	0.4 b
Greenhouse 4; cv Miracle [x]				
Untreated	1954 a	42 a	70 a	2.3 a
Tween-water	1837 a	41 a	71 a	2.1 a
U. atrum 2x	409 b	7 b	21 b	0.4 b
U. atrum 3x	882 b	18 b	26 b	0.6 b

Table 33. Continued.

Treatment	Disease development (AUDPC)		Disease manifestation at marketing	
	DI [p]	DS [q]	DI	DS
Greenhouse 5; cv Marvel [y]				
Untreated	3011 a	92 a	76 ab	3.6 a
Tween-water	2841 a	89 a	78 a	3.2 a
Tolylfluanid	2555 a	69 ab	67 ab	2.6 a
U. atrum 2x	1885 b	49 b	65 ab	2.4 a
U. atrum 3x	1802 b	44 b	63 b	2.2 a
Greenhouse 5; cv Sierra [z]				
Untreated	2409 b	54 b	78 a	2.7 a
Tween-water	3244 a	73 a	85 a	3.3 a
U. atrum 2x	1577 c	27 c	51 b	1.2 b
U. atrum 3x	1816 c	28 c	43 b	0.9 b

[o] U. atrum *was applied as conidial suspension containing 0.01% Tween 80 at 1 × 10⁶ conidia ml⁻¹. 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 was applied at plant ages of 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 (LSD test; a = 0.05).*

[u] *Experiment started with 77-day old plants and plants were marketable at 189 days.* U. atrum *was applied at plant ages of 77 and 105 days (U. atrum 2x) or of 77, 105, 136, and 163 days (*U. atrum *4x). Tween-water was applied at plant age of 77, 105, 136, and 163 days. Carbendazim plus dithiofencarb (as Sumico with 1 g l⁻¹; at 250 g a.i. kg⁻¹ and 250 g a.i. kg⁻¹, respectively), iprodione (as Rovral with 1 ml l⁻¹; at 500 g a.i. l⁻¹) and iprodione were applied in alternation at a plant age of 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 of 133 and 157 days (*U. atrum *2x) or of 133, 157, 184, 211, and 240 days (*U. atrum *5x). Tween-water was applied at plant ages of 133, 157, 184, 211, and 240 days. Iprodione (as Rovral with 1 ml l⁻¹; at 500 g a.i. l⁻¹) was applied at a plant age of 149 days.*

[w] *Experiment started with 126-day old plants and plants were marketable with 229 days.* U. atrum *was applied at plant ages of 126 and 154 days (*U. atrum *2x) or of 126, 154, 182, and 208 days (*U. atrum *4x). Tween-water was applied at plant ages of 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 of 119 and 147 days (*U. atrum *2x) or of 119, 147, and 175 days (*U. atrum *3x). Tween-water was applied at plant ages of 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 of 140 and 167 days (*U. atrum *2x) or of 140, 167, and 195 days (*U. atrum *3x). Tween-water was applied at plant ages of 140, 167, and 195 days. Tolylfluanid (as Euparen M with 1 g l^{-1}; at 500 g a.i. kg^{-1}) was applied at a plant age of 147 days.*

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

When *U. atrum* was applied at four-week intervals, DI at marketing was significantly lower than in the water controls in seven of the ten experiments. In the three experiments carried out in greenhouse 6, five *U. atrum* applications at four-week intervals showed no effect. Disease severity (DS) at marketing ranged between 0.8 and 11.0 in the untreated controls in the ten 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 to applications at four-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 four-week intervals were found on the disease progression as expressed by AUDPC for both DI and DS in five out 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 AUDPC's significantly in greenhouse 2, but not in greenhouses 3, 5, and 6. In no case did fungicide applications give a better control than *U. atrum* applied at four-week interval.

3.5.4 Experiments in *Pelargonium*

3.5.4.1 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 rose to about ten per plant at the end of the first experiment, and 16 for the second experiment. In the first experiment, 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) (Fig. 33). However, only a fraction of colonised leaves was really covered

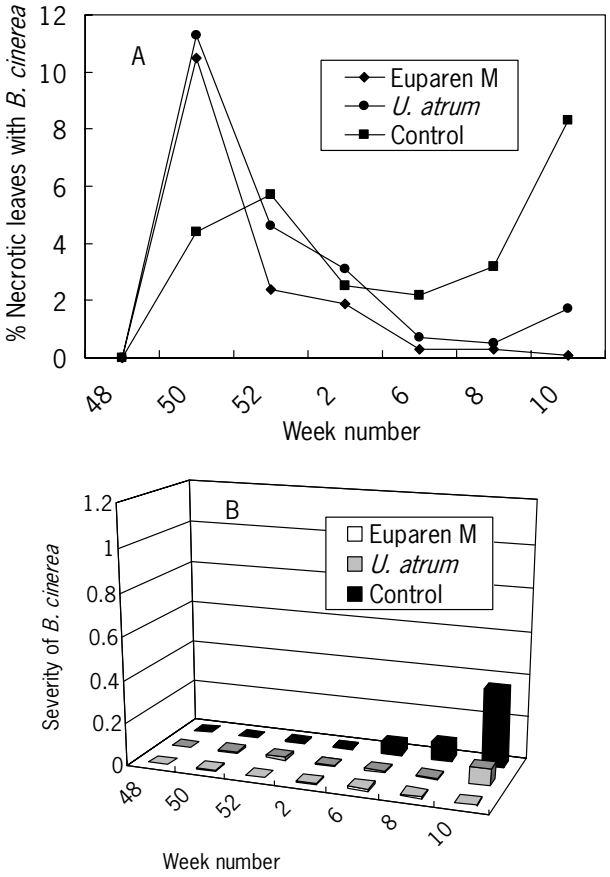


Figure 33. *Effect of treatments (Control, Euparen M and* Ulocladium atrum*) on the development of* Botrytis cinerea *incidence (A) and severity (B) on necrotic leaves for the first experiment (1996/97). Incidence is expressed as percentage of the necrotic leaves on which* B. cinerea *occurs, severity as the equivalent number of leaves with maximum colonisation by* B. cinerea. *(See 2.2.5.3). (From Gerlagh et al., 2001.)*

by *B. cinerea* (¼ and ¾ 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 experiment, with the exception of the final weeks (Fig. 33). 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 34).

In the second experiment 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

Table 34. *Summary of statistical data. The statistical significance is indicated by F values. Probabilities between 0.05 and 0.10 are between brackets. P >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. (From Gerlagh et al., 2001.)*

Category	Severity [w]	Conidia [x]		Spore load [y]	% Dead cuttings		
		Necrotic	Green		4 weeks	6 weeks	4 weeks [z]
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	

[w] *Severity of* Botrytis cinerea *on necrotic leaves.*

[x] *Counts of conidia on necrotic and green leaves respectively.*

[y] *Spore load (CFU of* B. cinerea*) in the air as captured on agar plates.*

[z] *Cuttings were collected in one separate greenhouse, and were scored four and six 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).*

43), twice as much as in the fungicide treatment, but the colonisation steadily decreased to less than 10% in all treatments at week 51 (Fig. 34). Correction of these figures by weighting for percentage area colonised by *B. cinerea* and intensity of *B.*

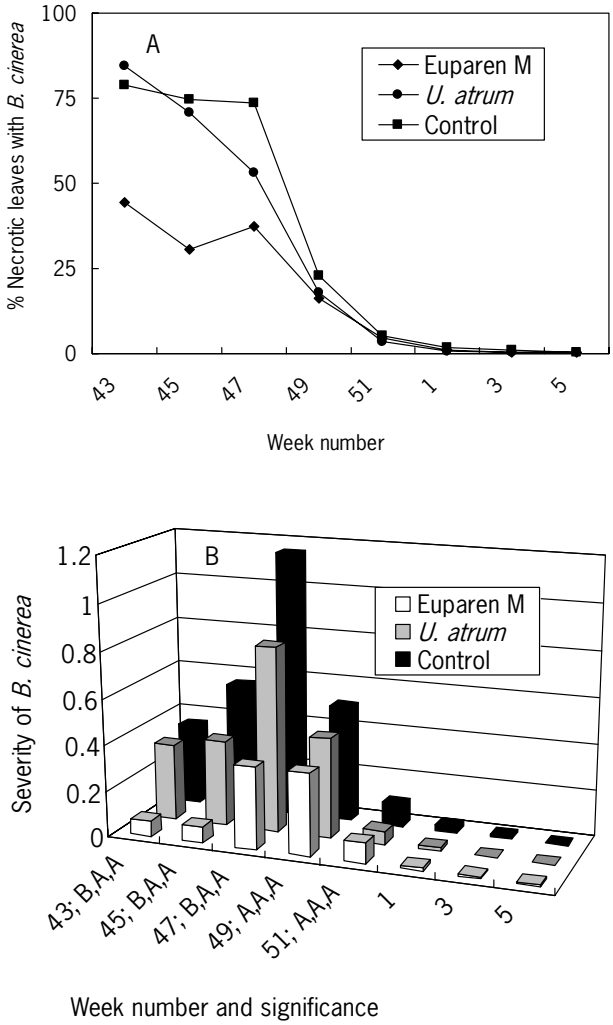


Figure 34. *Effect of treatments (Control, Euparen M and* Ulocladium atrum*) on the development of* Botrytis cinerea *incidence (A) and severity (B) on necrotic leaves for the second experiment (1997/98). Incidence is expressed as percentage of the necrotic leaves on which* B. cinerea *occurs, severity as the equivalent number of leaves with maximum colonisation by* B. cinerea *(See 2.2.5.4). 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. (From Gerlagh et al., 2001.)*

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; Fig. 34). Effects of treatment, week and their interaction were all highly significant (Table 34) for weeks 43 to 51. In weeks 43 to 47, Euparen M treatment was more effective than *U. atrum*, which was indistinguishable from the control.

3.5.4.2 Conidia trapped in the air

In the first experiment, significant numbers of *B. cinerea* conidia were trapped from the air only at two occasions. 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 (Fig. 35). On average for six samplings, the treatment effect was just significant, however, only the fungicide reduced the number of conidia compared to the control. In the second experiment, 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 (Fig. 35).

3.5.4.3 Conidia counts on necrotic and green leaves

Both experiments 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 conidia numbers (Fig. 36 and 37). Data based on numbers of *B. cinerea* conidia per sample (the sampled leaves of all five plants per greenhouse compartment put together), per gram of tissue (first experiment) or per surface area (second experiment) yielded similar results (data not shown).
In the first experiment, 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) (Fig. 36). *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 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 conidia per sample, respectively, and both *U. atrum* and fungicide treatments significantly reduced the conidial count compared to the control.
In the second experiment, high numbers of conidia of *B. cinerea* on necrotic leaves were counted at several sampling dates from the start of the experiment, but they decreased with time (Fig. 37). In week 45, the numbers were 86×10^6, 29×10^6 and12×10^6, respectively, for control, *U. atrum* and fungicide treatment. In week 5, only 4.5, 1.3 and 1.6 times 10^6 were found. On green leaves, the highest number of conidia of *B. cinerea* for the control was 79 cm^{-2} in week 45. *U. atrum* and fungicide treatments gave 30 and 14 conidia cm^{-2} at that date. On average over four sam-

plings, the reduction of number of conidia by *U. atrum* and fungicide treatments compared to control was significant, with values of 5.3, 5.5 and 13.2 respectively.

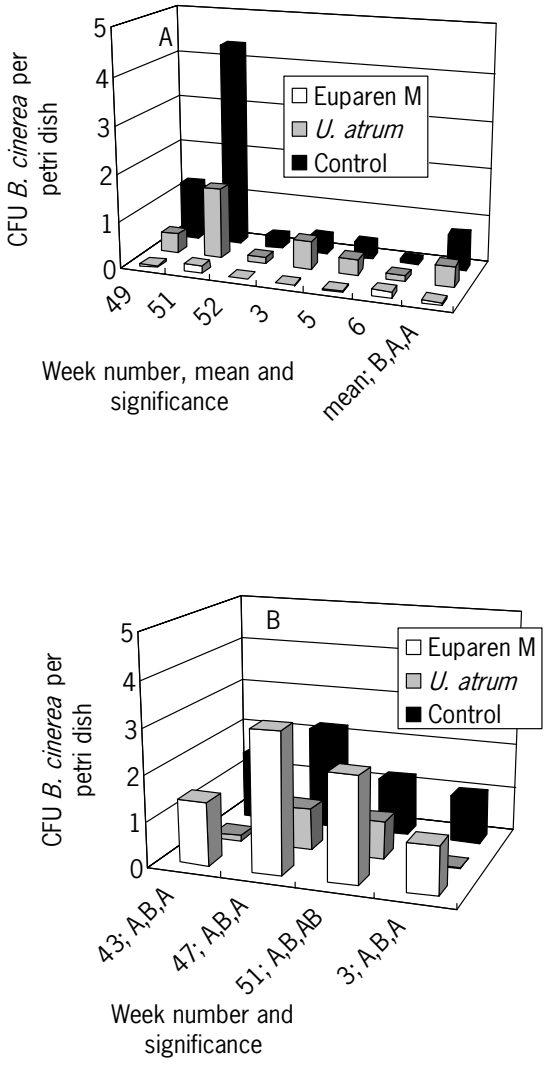


Figure 35. *Effect of treatments (Control, Euparen M and* Ulocladium atrum*) on numbers of conidia of* Botrytis cinerea *in the air of greenhouse compartments. The spore traps, according to Kerssies (1990), were incubated for 24 h. (A) Experiment 1996/'97. (B) Experiment 1997/'98. Statistical significance 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. (From Gerlagh et al., 2001.)*

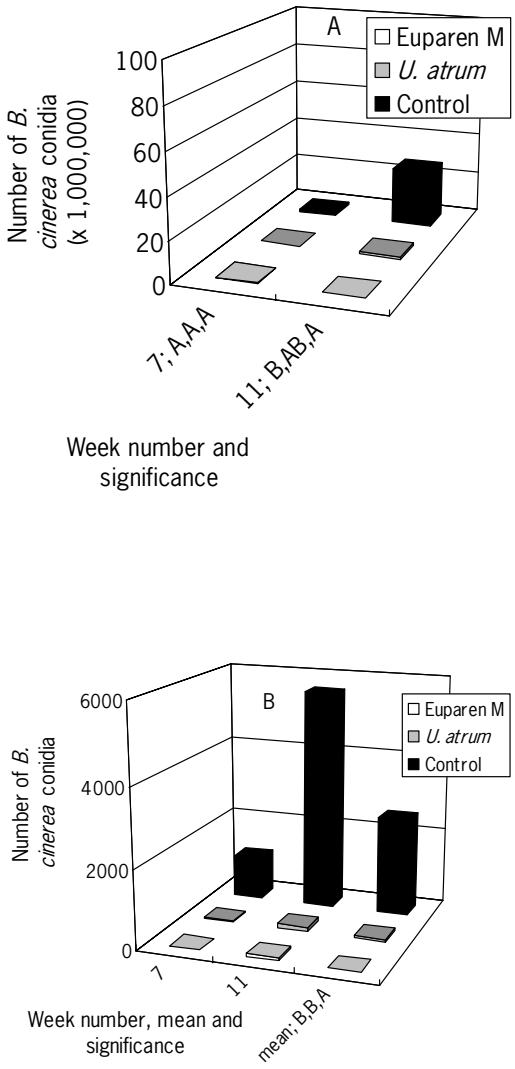


Figure 36. *Effect of treatments (Control, Euparen M and* Ulocladium atrum*) on numbers of conidia of* Botrytis cinerea *on (A) necrotic leaves (figures for a sample consisting of all necrotic leaves of five plants) and (B) young green leaves (figures for a sample of 25 leaves, five from each of five 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. (From Gerlagh et al., 2001.)*

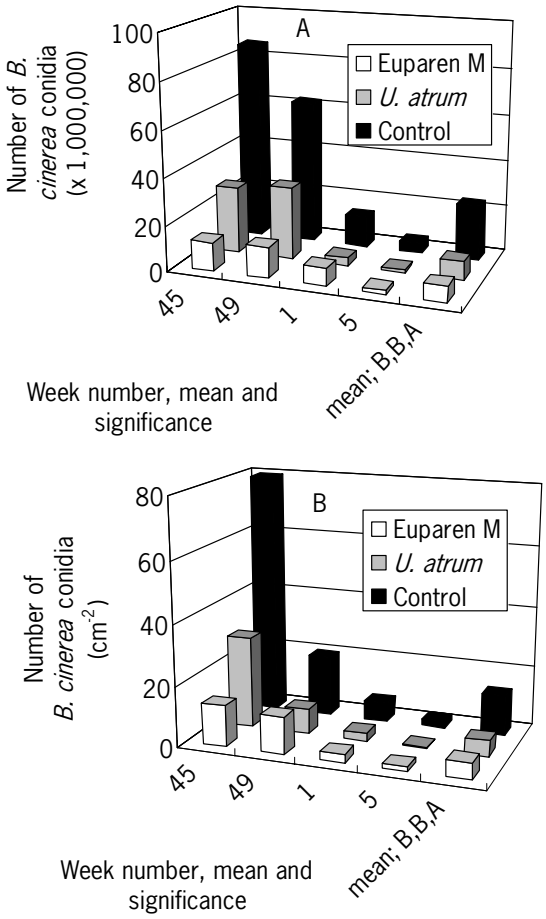
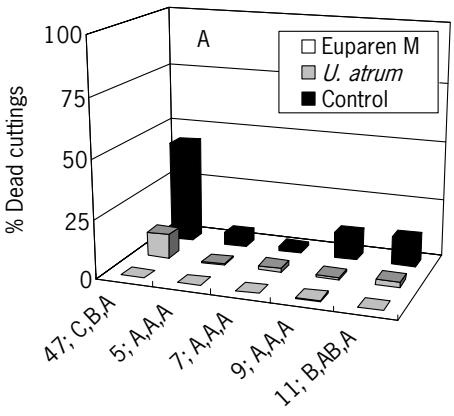
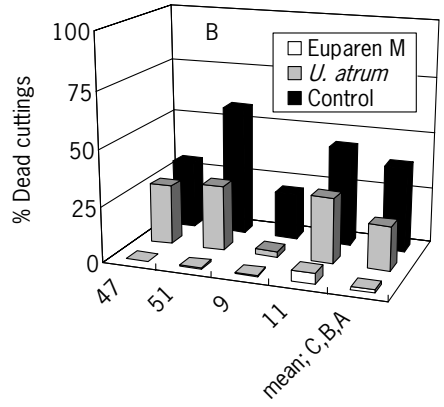


Figure 37. Effect of treatments (Control, Euparen M and Ulocladium atrum*) on numbers of conidia of* Botrytis cinerea *on (A) necrotic leaves (figures for a sample consisting of all necrotic leaves of five plants) and (B) young green leaves (figures expressed cm² leaf surface area for a sample of 25 leaves, five from each of five 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 ≤ 0.05). Treatments with the same letter are not significantly different. (From Gerlagh et al., 2001.)*

3.5.4.4 Cuttings

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





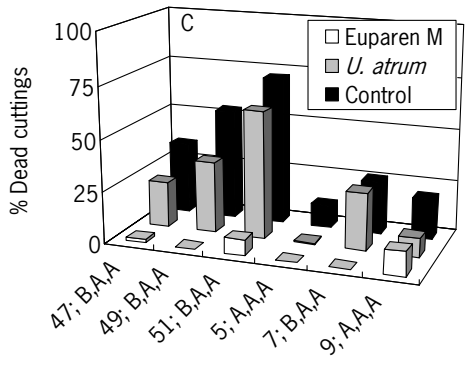


Figure 38.
Effect of treatments (Control, Euparen M and Ulocladium atrum*) on attack of cuttings by* Botrytis cinerea *leading to death. Data for four- and six-week old cuttings. Four-week old cuttings were incubated under different conditions. (Week number = week in which the cuttings were harvested from the stock plants and planted.) First experiment (1996/97). (A) Four-week old cuttings in individual greenhouse compartments; (B) four-week old cuttings all collected in a separate greenhouse; (C) six-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. (From Gerlagh et al., 2001.)*

In the first experiment, 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 (Fig. 38). This was most pronounced with four-week old cuttings in

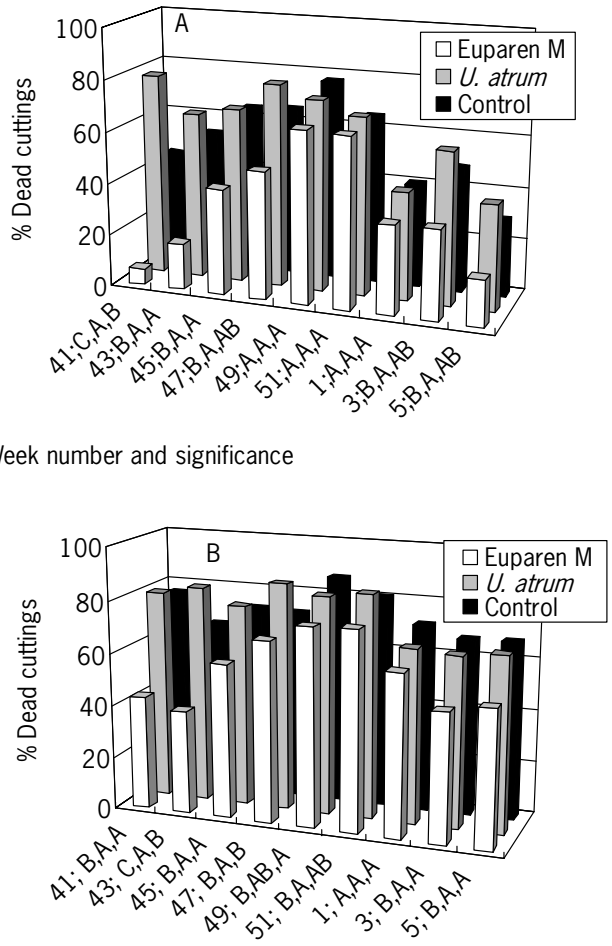


Figure 39. *Effect of treatments (Control, Euparen M and* Ulocladium atrum*) on attack of cuttings by* Botrytis cinerea *leading to death. Data for four- and six-week old cuttings. (Week number = week in which the cuttings were harvested from the stock plants and planted.) Second experiment (1997/98). (A) Four-week old cuttings all collected in a separate greenhouse; (B) six-week old cuttings all 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. (From Gerlagh et al., 2001.)*

the separate greenhouse. For six-week old cuttings taken at week 51, maximum percentage death was 72, 61 and 7%, respectively, for control, *U. atrum* and Euparen M treatment. With the exception of week 47, four-week old cuttings which remained in the small individual greenhouse compartments tended to show less attack by *B. cinerea* than those in the common (separate) greenhouse.
In the second experiment, depending on the harvest date, already about 50% of the cuttings was dead within four weeks, and 65 to 87% of the cuttings of all harvest dates with both control and *U. atrum* treatment were killed at six weeks (Fig. 39). With 39 to 76% of the cuttings of any harvest date killed, the fungicide treatment was significantly better, although very high loss rates still occurred.

3.5.5 Experiments in pot roses

Two experiments were carried out with pot roses in a commercial greenhouse. In both experiments *B. cinerea* sporulated abundantly on senescent and dead leaves, but seldom killed cuttings. For all treatments except *U. atrum* the number of foci of *B. cinerea* per pot in the first experiment varied between 1.33 and 1.43 in the first experiment. The *U. atrum* treatment significantly ($P < 0.05$) reduced this number to 0.54 (Fig. 40). The sporulation index (SI) at the second assessment varied from 16.5 for the *U. atrum* treatment to 34.1 for the non-treated control. Both controls had significantly more sporulation than the treatment with the antagonist. With SI = 26.1 the fungicide treatment did not differ from any of the other treatments (Fig. 41). Three *B. cinerea* isolates from the greenhouse showed equal linear growth on carbendazim amended agar as a resistant isolate, whereas a wild-type isolate of *B. cinerea* (culture collection Plant Research International) did not grow at the relevant concentration of the fungicide (data not shown).
Results of the second experiment showed a strong reduction of *B. cinerea* foci and SI by the use of fenhexamide as a fungicide, whatever its concentration. The effect did not differ significantly from the effect of *U. atrum* (values for foci were 0.22, 0.20 and 0.32, respectively). The mixture of the low concentration of fenhexamide with *U. atrum* did not affect *B. cinerea* compared to the control (0.42 and 0.53, respectively; Fig. 42); neither did *U. atrum* alone. At the second assessment date the SI was extremely low for fenhexamide (0.7; Fig. 43). Fenhexamide at low dose, whether alone or as a mixture with *U. atrum* led to a significantly higher sporulation index (5.0 and 6.3, respectively). *U. atrum* alone was somewhat less effective (SI = 10.0), but significantly better than the control (SI = 24.5).

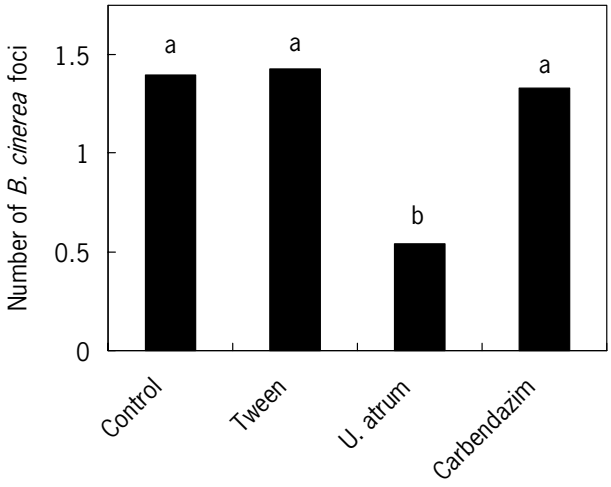


Figure 40. *Effect of treatment of pot roses on number of* Botrytis cinerea *foci per pot (first experiment) (Tween = water plus 0.01% Tween 80). Columns headed by the same letter are not statistically different (LSD test; a = 0.05). (From: Köhl and Gerlagh, 1999.)*

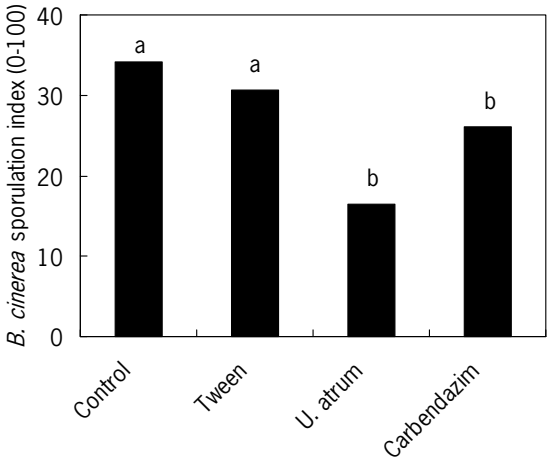


Figure 41. *Effect of treatment of pot roses on the sporulation of* Botrytis cinerea *(first experiment). Tween = water plus 0.01% Tween 80. Sporulation index = percentage area of leaf residue with sporulating* B. cinerea *multiplied by the intensity of sporulation (scale 0 - 10) and divided by 10). Columns headed by the same letter are not statistically different (LSD test; a = 0.05). (From: Köhl and Gerlagh, 1999.)*

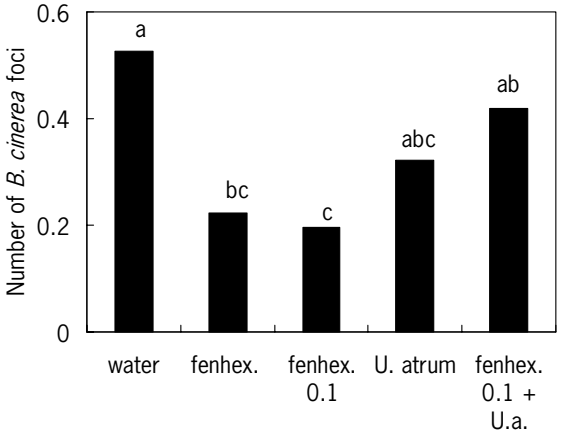


Figure 42. *Effect of treatment of pot roses on number of* Botrytis cinerea *foci per pot (second experiment). Fenhex. = fenhexamide; fenhex. 0.1 = fenhexamide at 10% of recommended dose; water = water plus 0.01% Tween 80). Columns headed by the same letter are not statistically different (LSD test; a = 0.05). (From: Köhl and Gerlagh, 1999.)*

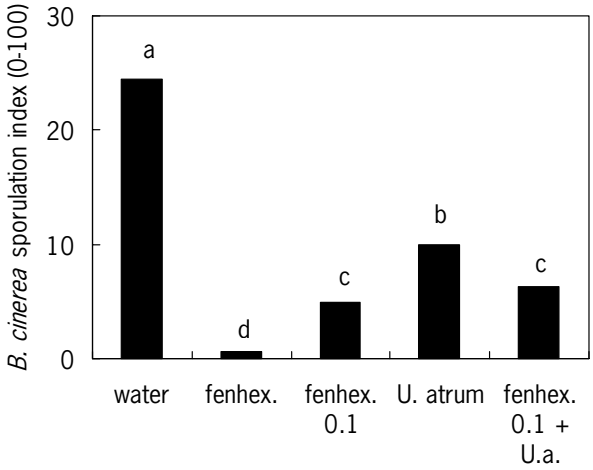


Figure 43. *Effect of treatment of pot roses on the sporulation of* Botrytis cinerea *(second experiment). Fenhex. = fenhexamide; fenhex. 0.1 = fenhexamide at 10% of recommended dose; water = water plus 0.01% Tween 80. Sporulation index = percentage area of leaf residue with sporulating* B. cinerea *multiplied by the intensity of sporulation (scale 0-10) and divided by 10). Columns headed by the same letter are not statistically different (LSD test; a = 0.05). (From: Köhl and Gerlagh, 1999.)*

4 Discussion

4.1 Introduction

When our research on biological control of *Botrytis* spp. started in the early 1990's, a control strategy was developed which differed from strategies commonly applied using chemical control or biological control methods interfering with diseases during the infection process. In this strategy only a short part of the pathogen life cycle is considered as target of control efforts. The control agent needs to persist on the plant tissue to prevent infection whenever pathogen inoculum arrives and conditions are suitable for infection. To achieve reliable control of infection, mechanisms of control agents must work quickly within the few hours needed by the pathogen for infection and must be persistently present on the plant tissue as long as new pathogen inoculum may arrive and tissue is susceptible to infection.
Antagonists usually act much slower than fungicides. Furthermore, introduced populations of antagonists such as yeasts or bacteria on leaves often decrease rapidly within a few days (Fokkema, 1971; Dik et al., 1991; Jongebloed et al., 1993). Only antagonists excreting strong antibiotics killing the target pathogen can achieve the rapid control needed to prevent infection.
When studying the whole life cycle of pathogens other stages can be identified during which antagonist applications may be more powerful if time periods of interactions are longer and other mechanisms than antibiosis can be exploited. In the case of necrotrophic pathogens we expected suppression of pathogen sporulation on necrotic plant tissues to be a valid biocontrol strategy (Köhl and Fokkema, 1998). Targeting biocontrol agents against specific stages of the life cycle of the pathogen needs antagonists with specific characteristics. Competitive substrate colonisation under the broad range of microclimatic conditions and persistence during long dry periods were identified as the key success factors for biocontrol agents targeting saprophytic pathogen development in necrotic tissues. Research was necessary in several steps to develop such a biocontrol system:

(1) Epidemiological knowledge on diseases caused by *Botrytis* spp. in different crops was necessary to identify valid target tissues (3.1; 4.2). Only if necrotic tissues that are significant inoculum sources during *Botryis* epidemics can be targeted with a biocontrol agent such a strategy may result in disease control.
(2) Suitable antagonists with a high competitive ability during the colonisation of various necrotic plant tissues had to be selected (3.2; 4.3).
(3) Antagonists with superior ecological competence had to be found (3.3; 4.4).
(4) Knowledge on the mode of action of selected antagonists was needed to optimise targeting and timing of antagonist applications to different target substrates of different crops (3.4; 4.5).
(5) The potential of the control strategy and applications of the selected antagonist had to be studied under field and greenhouse conditions (3.5; 4.6).

These subsequent research steps have been carried out successfully. *U. atrum* has been found to be an antagonist suitable for application on necrotic above-ground plant tissues and application strategies for *U. atrum* in several crops have been developed to achieve significant disease control. Further steps are needed towards commercial exploitation of the results by developing and marketing a biocontrol agent based on *U. atrum* (4.7).

4.2 Role of necrotic tissues in *Botrytis* epidemics

Conidia of *B. cinerea* are common atmospheric microbes (Gregory and Hirst, 1957). Main sources of conidia of *B. cinerea* are diseased plants of field crops or the natural vegetation, plant debris and sclerotia. A certain background level of airborne conidia of *B. cinerea* may always be present in the field, even when no disease is present in the crop. Inoculum produced outside a crop may initiate an epidemic in a field crop, but once an epidemic has started and sporulation occurs inside the field, airborne inoculum from outside the field becomes relatively less important as shown by our experiment in onion (3.1).
For other crops such as perennial strawberries and raspberries it has been demonstrated that necrotic plant tissue inside the crop serves as the main inoculum source during an epidemic of *B. cinerea* (Braun and Sutton, 1987, 1988; Jarvis 1962a, 1962b, 1962c; Jordan and Pappas, 1977; Miller and Waggoner, 1957). In kiwifruit orchards, senescing male flowers and prunings on the ground were found to be the main sources of inoculum of *B. cinerea* (Elmer et al., 1993). A significant correlation was found between inoculum potential inside a plot and external kiwifruit contamination.
Control of polycyclic diseases can be aimed at reduction of either initial or subsequent inoculum. Disease control may only be achieved when the progression of an epidemic mainly depends on inoculum density and when control agents can reach main sources of inoculum. Reduction of inoculum of *Botrytis* spp., which is produced outside the crop is not feasible because inoculum sources are spread over large areas, but inoculum produced inside the crop, such as on crop debris or on sclerotia, could be accessible for biological control. Antagonists such as cold-tolerant *Trichoderma* spp. may destroy overwintering sclerotia (Köhl and Schlösser, 1989). A reduction of initial inoculum may delay disease development. Adee and Pfender (1989) demonstrated a positive relationship between the amount of local initial inoculum of *Pyrenophora tritici-repentis* and the disease progress curve of tan spot in wheat in field experiments with different levels of primary inoculum. However, a 60 to 80% reduction of ascospore formation as reached with applications of the antagonist *Limonomyces roseipellis* was not sufficient to control tan spot because of secondary conidiation (Pfender et al., 1993). For three other polycyclic pathosystems, including *B. cinerea* on *Begonia semperflorens*, Plaut and Berger (1981) found that initially low inoculum densities were compensated for by higher rates of disease increase compared to initially high inoculum den-

sities. Thus, reduction of initial inoculum alone by biological control or other means may not always result in efficient disease control.
Control strategies aimed at reduction of the initial inoculum and subsequent conidial inoculum of a polycyclic pathogen may be more successful because they interfere with the multiplication of the pathogen during the whole epidemic. Control of *B. cinerea* by suppressing sporulation was suggested by Jarvis (1962a, 1962c) by using a chemical antisporulant or by means of plantation hygiene, which may limit potential sporulation sites for *B. cinerea*. Jordan and Pappas (1977) suppressed sporulation of *B. cinerea* in the field by spraying fungicides on strawberry debris before flowering. Consequently, the number of conidia trapped with Rotorods inside treated plots during flowering was drastically reduced and strawberry fruit rot was reduced by 66%. Braun and Sutton (1986) also controlled strawberry fruit rot by pre-blossom fungicide applications on dead host leaves.
We studied the role of necrotic tissues in manipulative experiments for epidemics of leaf spot in onion (3.1.1), grey mould in strawberry (3.1.2) and leaf rot in cyclamen (3.1.3). Necrotic leaves were identified as the important inoculum source of *Botrytis* spp. in onion and cyclamen but not in annual strawberries.

Onions. The artificial removal of necrotic leaves in an onion crop reduced the spore load of *B. cinerea* above the experimental plots and retarded the epidemic of onion leaf spot compared to the control treatment (Fig. 2). The relationship between the amount of necrotic tissue as substrate for *Botrytis* to sporulate on, the spore load above a plot, and number of leaf lesions, indicated that the progression of the onion leaf spot epidemic largely depended on the rate of inoculum production inside the crop (Fig. 3). From these relationships it can be concluded that application of antagonists to suppress sporulation of *Botrytis* spp. on necrotic leaf tissue could be an effective strategy for biocontrol.
The counts of spores recovered in traps positioned between the experimental plots and at a distance of 18 m from onion plots indicated that there was a gradient in the concentration of airborne spores for several meters away from diseased onions. Consequently, even though plots were separated by a 12-m buffer strip of sugar beet, interplot interference may have occurred during the experiments. A treatment at field level without interplot interference from nearby untreated control plots could have resulted in even greater disease suppression in response to reduced spore production in the plots.

Strawberries. Necrotic leaf tissue in the crop is the major source of conidial inoculum for flower infection by *B. cinerea* in perennial strawberry crops (Jordan, 1978; Braun and Sutton, 1987). The amount of necrotic leaf residue produced in annual strawberry crops in our experiments was much less than in perennial strawberry production systems (3.1.2). The maximum we found was a necrotic leaf area of about 150 cm^2 per plant, which is equivalent to a dry weight of about 2 g per

plant (Table 2). In perennial crops, dry weights were found to vary between 10 and 25 g (Sutton et al., 1988).
Despite the considerable incidence of *B. cinerea* on senescent and necrotic leaves of transplants (25 to 75%), the relatively low amount of necrotic leaf substrate for spore production and the low levels of actual and potential spore production observed, suggest that necrotic leaves were not a major inoculum source of *B. cinerea* in the annual strawberry crops we studied. This conclusion is supported by the absence of significant differences in the air load of conidia inside and outside the crops, and between crops with and without necrotic leaves (Table 1). In this respect the epidemiology of *B. cinerea* in the annual waiting-bed strawberry production system differs from the epidemiology as described for perennial and overwintering strawberry crops.
The incidence of *B. cinerea* on sampled flowers was high in almost all experiments. According to Braun and Sutton (1987, 1988) the inoculum for flower colonisation by *B. cinerea* in spring originates mainly from necrotic leaves inside the crop. We did not find significant correlations between the incidence of *B. cinerea* on flowers and total necrotic leaf area present during flowering, the leaf area with potential sporulation of *B. cinerea*, or predicted amount of sporulation based on colonised leaf area and weather conditions (Sosa-Alvarez et al., 1995) (Table 2). An explanation for the absence of positive correlations is that the amount of conidia produced locally on necrotic leaves in the untreated plots was too low for a marked increase of the inoculum pressure as compared to its background level. The finding that removal of all necrotic leaf tissue from strawberry plots did not affect the incidence of *B. cinerea* on flowers (3.5.1.1; Table 29; Boff et al., 2002) confirms this explanation. However, when conditions for symptom expression are suitable, the level of flower colonisation determines the level of grey mould by *B. cinerea*, as demonstrated by the correlation between post-harvest grey mould and petal colonisation (Table 3). Control strategies for grey mould in annual strawberry cropping systems using waiting-bed transplants should therefore aim to protect flowers from colonisation by inoculum of *B. cinerea*.

Cyclamen. Necrotic tissue, dead due to senescence, is present in cyclamen crops during the second half of the growing period (Table 4). *B. cinerea* sporulation was typically first observed on this type of leaf. Regular removal of the precursor of this type of tissue, senescing, symptomless cyclamen leaves, resulted in a significant reduction of the AUDPC (Table 5). Apparently, cyclamen leaves, dead from senescence, play an important role in the onset of disease. Saprophytic colonisation of these leaves by an antagonist could exclude later saprophytic colonisation by *B. cinerea* and result in a biocontrol effect. Mycelial contact infections from *B. cinerea*-colonised necrotic tissue to the petioles that are the least resistant (Schlösser, 1978) and from petiole to petiole could then generate the typical symptom of infected clusters of leaves.

Colonisation and subsequent sporulation of *B. cinerea* on necrotic leaf tissue of cyclamen results not only in a high inoculum potential within the canopy of individual plants but also increases the spore load within the greenhouse. It is likely that microbial suppression of sporulation can be exploited for disease control within a greenhouse when all potential sources of spore production within a greenhouse compartment are treated. The application of antagonists may then affect the spreading of the disease within the canopy of the individual plant as well as between plants.

4.3 Antagonist screening

The bioassay method developed for antagonist screening, based on interactions between *Botrytis* spp. and antagonistic fungi on dead leaf tissue, was an effective tool to select such antagonists (3.2.1). Onion leaves that senesced and dried naturally in the field were not suitable for standardised experiments because they frequently were already colonised by naturally occurring saprophytes. Symptomless green leaves of field-grown onions that were killed by drying and subsequently sterilised gave reproducible results in the bioassays. Senescing tissue of leaves attached to the plant contains lower amounts of nutrients than tissue of healthy leaves because during senescence nutrients are translocated to younger tissue (Baddeley, 1971) or because soluble nutrients are leached. Therefore, leaf segments used in bioassays were thoroughly washed to remove soluble nutrients creating a substrate comparable to naturally senesced tissue. Under field conditions, necrotic leaf tissue may already partly be colonised by *Botrytis* spp. before antagonists arrive. Therefore, leaves used in the bioassays were inoculated with conidia of *Botrytis* spp. 8 to 24 h before antagonists were applied to select antagonists with strong competitive abilities.

The method used to estimate sporulation intensity of *Botrytis* spp. gave reproducible results and was less time-consuming than counting conidia produced on the substrate per square centimetre. Because the estimated leaf area covered with conidiophores correlated closely with the log-transformed numbers of conidia produced on the substrate (Fig. 4), this method was reliable to detect distinct reductions of sporulation as were found in the bioassays. However, the method may not be effective when antagonists affect the sporulation index (SI) only slightly in the upper range of the scale. It is possible that moderately competitive antagonists suppress the number of conidia produced per conidiophore of *Botrytis* spp. whereas highly competitive antagonists suppress chiefly the number of conidiophores produced by the pathogen.

Under constantly humid experimental conditions most of the antagonists suppressed sporulation of *B. aclada* efficiently in the initial screening experiments (Table 6). Complete inhibition of sporulation of *B. aclada* was common with a range of antagonists even when dead leaves had been incubated with the pathogen for 24 h

before antagonists had been applied and the inoculum density of antagonists was only ten-fold of that of the pathogen. Only saprophytic fungi were included in the screening that had been isolated from necrotic leaf parts or that belong to a group of well-known antagonists, *Trichoderma* spp. and *Gliocladium* spp. *Trichoderma* spp. and *Gliocladium* spp. have been shown to be antagonistic against a wide range of fungal pathogens (Papavizas, 1985) including *Botrytis* spp. (Dubos, 1992; Sutton et al., 1997). Saprophytes isolated from necrotic leaf tips of field-grown plants had to compete with other naturally occurring saprophytes in the field during the fungal succession in the substrate. Thus, it could be assumed that such fungi show a high competitive saprophytic ability also in bioassays.

Antagonists with high competitive saprophytic ability in necrotic leaf tissue may be suppressive to a wide range of *Botrytis* spp. and necrotrophic pathogens. While *B. aclada* was used in most of the experiments to select antagonists, antagonists showed the same differentiation of antagonism and sensitivity to interrupted wetness periods when tested with *B. cinerea* (Table 9). However, reduction of sporulation of *B. cinerea* was less compared to *B. aclada* when applied 24 h before the antagonists.

The performance of antagonists under field conditions is determined mainly by their ecological competence. The environmental conditions of above-ground necrotic leaves are harsh for the development of both pathogen and saprophytic antagonists. Water availability, extreme temperatures, UV radiation and nutrient depletion are among the factors limiting fungal development (Burrage, 1971; Diem, 1971; Park, 1982; Rotem et al., 1985). Frequent and rapid fluctuations of the water potential may be highly restrictive for fungal development in the field, where periods of leaf wetness or high humidity are usually interrupted daily by dry periods (3.3.1.1). In general, fungal hyphae, especially fungal germ tubes, are sensitive to dry periods, but fungi can essentially differ in their potential to regrow and in the lag time needed for regrowth (Park, 1982). Antagonists aimed at suppression of sporulation of *Botrytis* spp. and other necrotrophic leaf pathogens on necrotic leaf tissue may only be reliable under field conditions if they are able to survive during dry periods and to start regrowth rapidly with only short lag times after conditions become favourable for fungal growth again. Bioassays with interrupted leaf wetness periods were suitable to differentiate antagonists according to their sensitivity to dry conditions during the colonisation process.

Two main factors determine the efficacy of antagonists after an interruption of leaf wetness: (1) their mycelial growth rates and competitive ability at low water potentials during the drying process, and (2) their survival during, and rapid regrowth after, dry periods when water potentials of the substrate are too low to allow fungal growth. Under our experimental conditions, the water potential of the dead leaves decreased continuously after wet leaves had been placed on dry filter paper in open petri dishes placed in a laminar flow cabinet. The drying process lasted approximately 7 h until the water potential was lower than –12 MPa, at which fungal growth is slow or stops (Alderman and Lacy, 1984; Magan and Lacey, 1984; Magan

and Lynch, 1986; Hocking et al., 1994). As a consequence, antagonists sensitive to low water potentials may colonise the substrate to a lower extent and may not be as competitive as antagonists growing also at lower water potentials. Under the experimental conditions, antagonists and *Botrytis* spp. also had to survive several hours in the substrate at water potentials too low for fungal growth, so that aspects of drought tolerance, growth at low water potential and survival during dry periods were tested in combination in these bioassays.

Antagonists originating from necrotic leaf tissue such as *A. alternata* and *U. atrum* were highly antagonistic even after leaf wetness periods had been interrupted repeatedly on three consecutive days (Table 7). On the other hand, soil-borne fungi such as *Gliocladium* spp. and *T. harzianum* showed sensitivity to interrupted leaf wetness periods especially during the early stage of colonisation on the first day after application. Germinating spores and germ tubes of these fungi may be more sensitive to dry periods than well-developed mycelium formed later during the colonisation process.

Antagonist screening was completed in subsequent experiments in which the performance of four selected antagonists, *A. pullulans*, *C. globosum*, *G. catenulatum* and *U. atrum*, under field conditions was studied using artificially killed lily leaves as substrate. *U. atrum* germinated rapidly on dead lily leaves during the night after spraying, provided temperatures during wetness periods were not below 10 °C (3.2.2; Table 10). Germination rates of almost 20% were reached within 4 h and germination rates above 90% were found after wetness periods of 18 h. Germination of ascospores of *C. globosum* was also high 18 h after spraying when leaf wetness periods lasted 15 h or longer. Almost no germination occurred when wetness periods were 9 h or shorter. Germination rates of spores of both antagonists were also recorded during an experiment with onions at the same location (data not presented). On naturally senescent leaf tips of field-grown onions, germination of spores of *U. atrum* and *C. globosum* were comparable to those found on the dead lily leaves fixed onto holders; they evidently were also highly dependent on favourable microclimatic conditions. Recovery of conidia of *U. atrum* was high after field exposure of five to six days. Apparently conidia of this antagonist were not washed off the leaves during rainfall that occurred during all experiments except experiment 3. Germination of conidia of *U. atrum* was consistently higher in all experiments after five to six days than after 18 h. Thus, conidia may survive at least several days under field conditions without losing their germination capacity. During our experiments, conidia of *U. atrum* formed mycelium with a length of up to 239 µm (Table 11). Length of germ tubes increased with total leaf wetness duration unless individual wetness periods were short, as during experiment 7.

These findings indicate that hyphal tips of *U. atrum* may regrow after dry periods with a short lag time. On the other hand, recovery of ascospores of *C. globosum* was mostly low. Even when spores had germinated for more than 70% during the first night after spraying in experiments 2 and 8 (Table 10), such spores could not be recovered after five to six days. Spores of *C. globosum* may have been washed from

leaves during rainfall. Germination after five to six days under field conditions ranged between 0 and 10% in the nine experiments. Even in experiments where leaf wetness periods longer than 15 h had occurred (experiments 6, 8, and 9), no higher germination could be found. When applied to leaf surfaces, ascospores of *C. globosum* may rapidly lose their vigour to germinate under field conditions.

The sporulation potential of *B. cinerea* was consistently lower on dead lily leaves when *U. atrum* had been applied (Table 12). The antagonistic effect of *U. atrum* occurred in all microclimatic conditions during the period when leaves were exposed to field conditions. *U. atrum* seemed to survive during the long dry periods in experiment 3 as well as during frequent but short wetness periods during experiment 7 and was able to colonise the substrate during the subsequent incubation period in moist chambers. Intensive substrate colonisation under field conditions was found after long leaf wetness periods that occurred during experiments 2, 4, 6, 8, and 9; as a result, the antagonist might suppress saprophytic growth of *B. cinerea* during the field exposure period as well as during the subsequent incubation period in moist chambers. Results of the field experiments show that *U. atrum* is well adapted to the rapid changes in the microclimatic conditions in the phyllosphere and is able to colonise necrotic leaf tissue. It can germinate rapidly with high germination percentages. Germ tubes and hyphal tips seem to withstand dry conditions and regrow rapidly during wet conditions.

C. globosum is a potential antagonist against a wide range of fungal pathogens. *C. globosum* has a high cellulolytic activity (Domsch et al., 1980) and produces potential antifungal antibiotics (Di Pietro et al., 1992). Applications of conidia of the antagonist to apple leaf litter prevented ascospore production of *Venturia inaequalis* and enhanced leaf decomposition (Heye and Andrews, 1983; Miedtke and Kennel, 1990). *C. globosum* produces antifungal antibiotics that control apple leaf infection by ascospores of *V. inaequalis*, without spore germination of the antagonist (Boudreau and Andrews, 1987).

In our study, *C. globosum* reduced the sporulation potential of *B. cinerea* in three experiments and was competitive with *Cladosporium*-like fungi in two experiments. In all cases where spores of *C. globosum* germinated with high percentages during the night after field application, no antagonism was found later after incubation in moist chambers. Although able to germinate during the first night after application if leaf wetness periods were longer than approximately 15 h, recovery of germinated or ungerminated spores was low after five to six days. In contradiction to these low recovery rates, sporulation of *B. cinerea* was significantly suppressed by *C. globosum* after incubation of the dead lily leaves in moist chambers subsequent to the field exposure period in the experiments 1, 5, and 7. The antagonist may have the potential to germinate under the optimum conditions in moist chambers and a low inoculum density may be sufficient for a high antagonistic activity. Toxins already present in suspensions applied to leaves can also explain antagonistic activity with the presence of low densities of *C. globosum*. Toxins may be stable only under certain environmental conditions (Boudreau and Andrews, 1987), resulting in vari-

able antagonistic activities during the nine experiments, independent of the presence of an actively growing population of *C. globosum*.
The application of a combination of spores of *C. globosum* and *U. atrum* consistently reduced the sporulation potential of *B. cinerea*, but effects were less marked compared to applications of *U. atrum* alone. In combined applications, both fungi were applied in half the concentration of the suspensions used for applications of single antagonists. Thus, the reduced effectiveness of combined applications of the two antagonists may be explained by the lower inoculum rate of *U. atrum* applied. In combined applications, toxins produced by *C. globosum* may also partly inhibit growth of *U. atrum*. However, germination of conidia of *U. atrum* was not lower when applied in combination with *C. globosum* compared to applications alone (Table 12).
Gliocladium spp. are antagonistic against many pathogens, including *Botrytis* spp. (Dubos, 1992; Papavizas, 1985; Sutton et al., 1997). Intensive research on the antagonistic potential of *Gliocladium* spp. and the closely related *Trichoderma* spp. resulted in the development of the biocontrol product Trichodex based on *T. harzianum* against *B. cinerea* (Elad et al., 1993). In our study, *G. catenulatum* did not control sporulation of *B. cinerea*. Most of the ungerminated or germinated conidia may not survive on necrotic leaf tissue under field conditions. Even if a limited amount of the inoculum of the antagonist survives during field exposure, it may not suppress sporulation of *B. cinerea* effectively if the pathogen already had colonised the dead leaf (Sutton and Peng, 1993). A high sensitivity of *G. roseum* to dry periods was found by Park (1982). This was also found in bioassays on dead onion leaves in moist chambers (3.2.1.2; Table 7). *Gliocladium* spp. generally lost their capacity to control sporulation of *Botrytis* spp. when wetness periods in the growth substrate were interrupted for several hours.
A. pullulans is a common inhabitant of the phyllosphere of green and senescing leaves with a high tolerance to dry conditions (Hudson, 1971; Park, 1982). The antagonistic potential of *A. pullulans* to prevent leaf infections of onion by *Alternaria porri* was demonstrated by Fokkema and Lorbeer (1974). *A. pullulans* was not efficient in suppressing sporulation of *B. cinerea* in our experiments. In bioassays on dead onion leaves, this antagonist generally was less effective under optimum conditions than the other three antagonists used in our study (Table 12).
Daconil M, a combination of the fungicides chlorothalonil and maneb, is a commonly used product that protects crops such as lilies, tulips and onions effectively from *Botrytis* damage. In our experiments, the fungicides never affected *Botrytis* sporulation on dead lily leaves. However, in three experiments with lily leaves exposed to field conditions for five to six days as well as in the experiment with an exposure period of 24 days, a reduction of *Cladosporium*-like fungi was found after fungicide treatment. *Cladosporium* spp. may be more sensitive to one of the fungicidal compounds of the applied fungicide than *Botrytis* spp. A high efficacy of chlorothalonil against *Cladosporium* spp. on necrotic leaf tissue of *Lolium perenne* L. was found by Thomas and Shattock (1986). The ineffectiveness of the fungicides

against *B. cinerea* may be explained by the high adsorption capacity of chlorothalonil to organic matter. Chlorothalonil may have been adsorbed to cell walls in the necrotic tissue, inactivating the compound. Possibly, heavy rainfalls that occurred during most of the experiments have also washed off the nonadsorbed fraction of fungicides from dead leaves.
During nine experiments in which dead lily leaves were exposed to field conditions for five to six days, *U. atrum* was the only antagonist that consistently suppressed colonisation and subsequent sporulation of *B. cinerea* on dead lily leaves. The antagonist showed a great potential to colonise necrotic leaf tissue under field conditions and to survive adverse conditions in the field. *U. atrum* could compete with *B. cinerea* as well as with *Cladosporium*-like fungi as a main group of naturally occurring saprophytes on necrotic leaf tissue. The same results were obtained in an additional experiment in which fungal development was examined after dead lily leaves had been exposed to field conditions for 24 days without subsequent incubation in moist chambers. The high saprophytic competitive ability of *U. atrum* makes this fungus the most attractive candidate amongst fungi tested in the screening programme for the development of a biological control product against *Botrytis* spp. exploiting antagonistic interactions in necrotic plant tissues. To our knowledge, *U. atrum* had not been described as antagonist of *Botrytis* spp. or other fungal plant pathogens before.

4.4 Effect of environmental factors on *U. atrum*

Strong saprophytic competitive ability during colonisation of above-ground necrotic tissues depends on two parameters: (1) a high enzymatic activity for substrate utilisation (Berto et al., 2001), and (2) the adaptation to the harsh microclimatic conditions in the phyllosphere. Before applying antagonists in large-scale field experiments, their ecological attributes should be known in order to prevent failure under the prevailing environmental conditions. In our studies, we investigated the effect of different constant temperatures as well as constant and fluctuating water potentials. The persistence of conidia of *U. atrum* was followed under three very different environmental conditions in field-grown lily and greenhouse-grown cyclamen without and with top irrigation.

4.4.1 Temperature

No information was available in the literature on temperature requirements of *U. atrum*. For the closely related *U. cucurbitae*, an optimum temperature of 30 °C for conidial germination as well as for mycelial growth was reported (Zitter and Hsu, 1992). Conidia of *U. cucurbitae* germinate quickly reaching a germination percentage of 90% within 2 h on water agar. In our experiments, we also found that conidia of

U. atrum germinate rapidly (Fig. 8). Within 5 h a germination percentage of at least 50% is reached over a broad temperature range (18 to 36 °C).
The optimum temperature for germination was between 27 and 30 °C. Even at temperatures as low as 3 °C, conidial germination occurred within 24 h. The speed of germination was similar on both water agar and necrotic lily leaves. The rapid germination of conidia of *U. atrum* over a broad temperature range may enable this antagonist to produce sufficient hyphal biomass during the limited hours of leaf wetness in the crop to establish itself in the substrate. In contrast, antagonists needing time periods for germination much longer than the average duration of leaf wetness periods coinciding with low temperatures may suffer from interruptions of wetness periods before becoming established in the substrate and as a consequence may fail to colonise the necrotic tissue under such conditions.
G. roseum germinated only in the presence of exogenous nutrients and not on water agar. This may be due to the small size of the conidia, possibly containing only little nutrient resources. The germination process of conidia of *G. roseum* was much slower than that of *U. atrum*. At 15 °C, the mean temperature recorded during leaf wetness periods under Dutch growing season conditions (3.3.1), *G. roseum* conidia needed 24 h to reach a germination percentage of 50%. Conidia of *U. atrum* reached that level already after 6 h. Sutton and Peng (1993) also reported slow germination of *G. roseum* conidia which required 48 h to reach 80% germination at 10 °C. However, this fungus reduced sporulation of *B. cinerea* on necrotic strawberry leaf discs in bioassays over a broad temperature range between 10 and 25 °C in their study. Jackson et al. (1991) found a temperature optimum for mycelial growth between 25 and 30 °C for *G. virens* (currently reclassified as *Trichoderma virens*). A large variation in growth rates and antagonistic activity at low temperatures was found for closely related *Trichoderma* spp. (Köhl and Schlösser, 1988; 1989). It may be possible also to select more cold-tolerant strains of *G. roseum*.
Our results for *Botrytis* spp. mycelial growth are in accordance with other published data showing that *Botrytis* spp. have a relatively low optimum temperature and are able to grow and sporulate in the range of temperatures that typically occur during leaf wetness periods under Dutch growing conditions (Fig. 9). The optimum temperature for flower infection of strawberry and berry infection of grapes by *B. cinerea* and for leaf infection of onion by *B. squamosa* is 20 °C (Bulger et al., 1987; Nair and Allen, 1993; Broome et al., 1995; Alderman and Lacy, 1983). The optimum for conidia production of *B. cinerea* on strawberry leaf discs and tomato stems was 17 °C (Sosa-Alvarez et al., 1995; O´Neill et al, 1997). At higher temperatures, conidia production decreased and was almost zero at 25 °C in their studies. Since spore production of *Botrytis* spp. was not determined over time in our study, the data obtained from our bioassays give no information on the optimum temperature for speed of sporulation.
The bioassays conducted on dead onion leaves showed that *U. atrum* suppressed sporulation of *B. aclada* and *B. cinerea* over the entire temperature range studied (6 to

24 °C; Table 18). High antagonistic potential against *B. cinerea,* even at temperatures as low as 1 °C, was found in the experiments with *U. atrum* on hydrangea leaves (Table 20). On cyclamen, control efficacy of *U. atrum* was also high at temperatures lower than 18 °C. Different from the results obtained on onion leaves, control efficacy of *U. atrum* on cyclamen leaves was much lower at 18 °C or at higher temperatures in the repeat experiments (Table 19). In addition to conidia, *B. cinerea* also produced sclerotia in both experiments. Sclerotia production increased with temperature and was significantly higher on control leaves. The formation of sclerotia by *B. cinerea* may have resulted in reduced sporulation of the pathogen at higher temperatures especially on leaves of the control treatments. On the other hand, dead leaf tissues obtained from different hosts may have a differential effect on the outcome of the competitive substrate colonisation by *U. atrum* and *B. cinerea.*

The comparison of *U. atrum* and *G. roseum* in the bioassays on cyclamen leaves clearly showed that *U. atrum* was the more efficient antagonist at 18 °C or lower temperatures whereas *G. roseum* was a better antagonist at temperatures higher than 18 °C. Contradictory to the results obtained in bioassays, the shapes of curves fitted to data on mycelial growth rates on agar for the two antagonists were very similar (Fig. 9). *U. atrum* had the same high optimum temperature as *G. roseum* and the decline of the *U. atrum* growth rate with declining temperature was similar to that for *G. roseum*. *Botrytis* spp. had lower optimum temperatures for growth than both antagonists. The production and activity of enzymes involved in the colonisation of necrotic leaf tissues by fungi or their growth on an artificial medium such as malt extract agar may differ in their temperature requirements.

Our experiments were carried out at constant temperatures but temperatures of above-ground necrotic tissues are characterised by rapid diurnal changes. Under Dutch growing conditions, temperatures recorded during leaf wetness periods often fluctuate from below 5 °C at night to above 20 °C during daylight before the canopy dries (Fig. 7). It has not been investigated whether such changes have any adverse effect on growth rates of the pathogen or antagonists during the competitive substrate colonisation. Morton and Eggins (1977) found for several wood-inhabiting fungi that diurnal temperature cycles result in growth rates similar to growth rates estimated from those determined at constant temperatures and no adverse effects of temperature fluctuations were found.

The two antagonists compared in our study showed clearly different temperature requirements. *U. atrum*, although having the same high optimum temperature as *G. roseum*, was less affected by lower temperatures than *G. roseum*. *U. atrum* suppressed sporulation of *B. cinerea* in bioassays conducted at temperatures down to 1 °C. Considering the temperatures recorded for leaf wetness periods under Dutch growing conditions and the temperature requirements of *Botrytis* spp. for substrate colonisation and sporulation, *U. atrum* would therefore be a better antagonist than *G. roseum* for applications in the open field or in greenhouse crops such as cyclamen grown at moderate temperatures. Only under conditions with long leaf wetness periods with temperatures above 18 °C in heated greenhouses, the application of the *G. roseum*

isolate may result in a better control of *B. cinerea* sporulation than that obtained with *U. atrum*. The differential temperature requirements of both antagonists may partly explain the higher antagonistic efficacy of *U. atrum* compared to a *Gliocladium* sp. applied to necrotic lily leaves in the open field (3.2.2) and applied to cyclamen crops in cold greenhouses (3.5.3).

4.4.2 Water potential

Little information is available in the literature about the fate of germlings of saprophytic fungi in the phyllosphere. More information is published on leaf pathogens that 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 (1974) 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 germlings 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 to ungerminated conidia (Becker and Burr, 1994). Germlings of *Cercospora kikuchii*, causing leaf blight in soybean (Schuh, 1993), *Botrytis cinerea*, *Cercospora musae* and *Monilinia fructicola* survived dry periods (Good and Zathureczky, 1967). In contrast, germlings of *Botryosphaeria obtusa*, causing leaf spot in apple, were extremely sensitive to interruptions of leaf wetness and did not resume growth (Auruz and Sutton, 1990). Also, germinated pycnidiospores of *Mycosphaerella pinodes* were found to be sensitive to interruption of leaf wetness period before appressorium formation on pea leaves (Roger et al., 1999).
Survival of germlings during dry periods can differ for dematiaceous and hyaline fungi (Diem, 1971). A high percentage of germlings of dematiaceous fungi such as *Alternaria tenuis*, *Stemphylium botryosum*, *Helminthosporium sativum* and *Cladosporium herbarum* survived dry periods (Diem, 1971), but germlings of hyaline species such as *Aspergillus* sp., *Penicillium* sp. and *Colletotrichum graminicola* died during dry periods. Park (1982) 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 (1982) found that phylloplane fungi could not grow at a water potential below –15 MPa but survived under such conditions. Nonphylloplane fungi can seriously be affected by such low water potentials. Hjeljord and Tronsmo (pers. comm.) found that conidia of *T. harzianum* were killed when exposed to desiccation during germi-

nation and poorly germinated under greenhouse conditions on leaves (Hjeljord et al., 2000).

It was 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 (3.2.1) and in the field (3.2.2). 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 colonisation 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 (PEG). 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 (Magan, 1988; Magan and Lynch, 1986). To avoid such interactions, methods were applied in our study to control the water potential of a thin water agar layer via equilibrium with the relative air humidity, which was controlled by media with adjusted water potential, but separated from the fungal growth medium (Pfender et al., 1991).

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 (Table 14; Fig. 10). Endogenous nutrients play a crucial role during germination under water stress and the establishment of the antagonist *Epicoccum nigrum* after application in the field (Pascual et al., 1996). 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 (Table 15; Fig. 11). 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*) showed no statistically significant difference between treatments. Conidia incubated first at –42 MPa needed longer to start regrowth (Table 16; Fig. 12). The delay for conidia that had an initial moist incubation, was 1.3 to 4.3 h longer than for 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 pregerminated 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 the 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 to conidia incubated with or without a single dry interruption of moisture (Table 17; Fig. 13). 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 to 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 (1981) for *Alternaria alternata*, 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 since 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, video-microscopy 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 was 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. The 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 (Table 21).

This study into 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 (3.2.1, 3.2.2), demonstrates that *U. atrum* tolerates water stress and thus is adapted to the ecological niche of leaf surfaces and above-ground necrotic tissues. The results help to understand the fate of conidial inoculum of the antagonist after field applications. Based on this knowledge on the ecological competence of the antagonist, application frequencies and densities can be optimised to achieve sufficiently high population

densities of the antagonist on the target substrates with economically sound dosages.

4.4.3 Persistence of conidia

U. atrum survived for several days on necrotic tissues in the field without any loss of germination capacity and results indicated that *U. atrum* conidia were not affected by microclimate conditions during that time (3.2.2). In order to gain a better understanding of the behaviour of *U. atrum* in the phyllosphere, we obtained information in subsequent studies on the density, survival and colonisation ability of *U. atrum* conidia on the green leaf surfaces of cyclamen grown in greenhouses under different irrigation regimes (3.3.5.1 and 3.3.5.2) and of Asian lilies in the field as influenced by (1) the vertical nature of the lily canopy, and (2) the duration of exposure to field conditions (3.3.5.3).
The number of *U. atrum* conidia cm^{-2} on green lily leaves at the top and middle canopy levels was not significantly different (experiment 1) immediately after *U. atrum* application to lily microplots with a propane powered backpack (Fig. 20a). However, conidia density was significantly less on green leaves at the lower canopy level compared to the middle and top levels. In the second experiment, the number of conidia was significantly different at each level of the canopy (Fig. 20b). In both experiments the number of conidia detected on leaves at the bottom level of the canopy was 20% (experiment 1) and 18% (experiment 2) of the number deposited onto leaves at the top of the lily canopy. An uneven vertical distribution of antagonist inoculum may have significant epidemiological consequences since primary infections in host canopies may commence on the older leaves at the bottom of the canopy where host and microclimate conditions are likely to be more conducive to infection and sporulation. These results indicate that the spraying system used in these studies resulted in uneven vertical distribution of antagonist inoculum in the lily canopy and that further evaluation of application methods may be required to improve penetration of the antagonist suspension.
After antagonist application, the number of *U. atrum* conidia on green leaf surfaces declined at a similar rate at each canopy level in both experiments (Fig. 20). This result is in contrast to earlier findings in 1994 which indicated that a loss of *U. atrum* conidia from the top of the lily canopy coincided with an accumulation of conidia on the bottom leaves of the canopy (Köhl et al., unpublished). The 1994 growing season was characterised by more frequent rain events than in 1995 and indicates that rain splash may be an important mechanism for redistribution of antagonist inoculum. Rain splash has been shown to be an efficient mechanism for spreading large numbers of pathogen propagules from a source of inoculum but the relative role of rain and wind dispersal has only been described for some pathogens (Fitt et al., 1989; Vloutoglou et al., 1995). The relative importance of rain versus wind as a dispersal mechanism for redistributing antagonists such as *U.*

atrum is poorly understood. The use of molecular markers may enable better investigation of the spatial and temporal dynamics of *U. atrum* as was recently reported for the antagonist *Trichoderma harzianum* (Bowen et al., 1996).

The number of *U. atrum* conidia on green lily leaf surfaces after 21 days exposure to field conditions declined substantially by 70% and 73% in experiment 1 and 2, respectively (Fig. 20). Only one significant rain event was recorded over the duration of these experiments and suggests that other factors may also be responsible for the movement of conidia. The use of stickers in fungicide formulations is common and has improved chemical persistence on plant surfaces. The formulation of *U. atrum* used in these studies was a spore suspension in water with Tween 80 and it is likely that improved adhesion of antagonist spores could be achieved by improving the formulation of the antagonist suspension.

Germination of *U. atrum* conidia on green leaves in the field reached a maximum of 81% and 60% seven days after antagonist application in experiment 1 and 2 (Table 23). Field germination was lower in experiment 2 than in experiment 1. Results obtained in earlier studies (3.2.2) suggested that germination on necrotic leaf surfaces was dependent upon the duration of leaf wetness and temperatures above 10 °C. Low temperatures during the wetness periods were reported to hamper germination. In the first seven days of experiment 1, five leaf wetness periods with an average duration of 17.1 h were recorded (Table 22). The average temperature during the leaf wetness periods was 17.9 °C. In contrast, the average duration of leaf wetness in the first seven days of experiment 2 was 6.7 h with an average temperature of 14.8 °C. Shorter leaf wetness duration may have been the factor responsible for a reduction of conidia germination on green lily leaf surfaces.

Long-term survival on green leaf surfaces is a necessary attribute for many successful saprophytic species since spores that land on the leaf surface must wait for the onset of senescence in order to penetrate the host tissue. Conidial viability (germination potential) declined slightly (100 to 88%) after seven days exposure to field conditions (mean of three canopy levels, experiment 1). Thereafter, there were no significant changes in germination potential even after 21 days (Table 23). Germination potential was significantly higher on leaves from the bottom level of the lily canopy (experiment 1), but germination potential was not affected by canopy level in experiment 2. It is possible that direct exposure to UV radiation may increase conidial mortality and host tissue shading may provide some protection. Spore survival in the field was also reported to be greater on the underside of leaves than on the upper leaf surfaces (Ceasar and Pearson, 1983; Rotem et al., 1985). This suggests that there is a need to study further *U. atrum* survival on the underside of leaves at different canopy levels.

The success of a potential antagonist is not only dependent upon antagonist inoculum remaining viable for prolonged periods of time but also on retention of cellular functions required for rapid colonisation of the host substrate. In our study, we found a significant decline in the capacity of germ tubes to produce new extension growth, indicating a loss of germling vigour, when *U. atrum* conidia were

incubated in a high humidity chamber after an exposure to field conditions for 21 days on green leaves (data not presented). In addition, the number of new germ tubes arising from conidia exposed in the field for 21 days was also significantly reduced. A reduction of germ tube development may affect the ability of *U. atrum* to remain competitive in the phyllosphere and subsequently colonise host tissue at the onset of senescence.

In our study the ability of surviving *U. atrum* inoculum on green leaf surfaces to successfully colonise necrotic tissues was measured after paraquat induction of necrosis on tagged green leaves. Colonisation by *U. atrum* was consistently highest on necrotic leaves at the top level of the canopy and consistently lower on the necrotic leaves sampled from the bottom canopy level in both experiments (Fig. 21). This pattern was also consistent for all times of necrosis induction and indicates that the density of *U. atrum* conidia on the green leaf surface prior to artificial induction of necrosis had a profound effect on the level of necrotic lily leaf colonisation. Necrotic leaf colonisation by *U. atrum* decreased over time (Fig. 21) from 51% (averaged over the three canopy levels) when necrosis was induced immediately after antagonist application (day 0) to 21% (averaged over the three canopy levels) when necrosis was induced 21 days after antagonist application (experiment 2). A significant decline in colonisation may have been due to a combination of factors. Conidial density declined significantly over time and regression analysis indicated a significant relationship between *U. atrum* density on green leaves, prior to artificial induction of necrosis, and subsequent *U. atrum* colonisation of necrotic lily tissue (Fig. 22). A reduction in germination potential, germ tube elongation and number of germ tubes per conidium may have also contributed to a decline in colonisation after 21 days in the field.

The ability of *U. atrum* inoculum to compete with common saprophytic species on artificially induced necrotic tissues was measured. When necrosis was induced immediately after antagonist application (Table 24 and 25, day 0, experiment 2), *U. atrum* completely colonised necrotic lily leaves and outcompeted commonly occurring saprophytic *Alternaria* spp. and *Cladosporium* spp. The ability of *U. atrum* to significantly reduce colonisation by *Alternaria* spp. and *Cladosporium* spp. was maintained throughout the 14 days (*Cladosporium* spp.) and 21 days (*Alternaria* spp.) of each experiment. The reduction of *Alternaria* spp. colonisation on necrotic tissues was generally less on leaves at the bottom level of the canopy. Lower numbers of *U. atrum* conidia reaching the bottom leaves of the canopy may account for a reduction of competitive ability since basic ecological principles state that the effects of competition are density-dependent (Begon et al., 1986; Kessel, 1999). Our results suggested that when the density of *U. atrum* was relatively low (approximately 1000 conidia cm^{-2}), the reduction of *Alternaria* spp. colonisation compared to the water treatment was not significant. This is potentially important information and indicates that high densities of *U. atrum* conidia (approximately greater than 4000 cm^{-2}) on green leaves should be maintained to ensure that colonisation by naturally occurring saprophytes is effectively suppressed. Such high numbers are practical

and were achieved immediately after field application to the top leaves of the canopy (experiment 1) and the top and middle leaves in experiment 2. This information is required in order to optimise *U. atrum* frequency of application and application methodology.

In our study we found that *U. atrum* had the ability to survive and persist in the lily phyllosphere for up to 21 days in the field. In addition, *U. atrum* colonised artificially induced necrotic tissues and competed successfully against naturally occurring saprophytes on Asian lily leaves in the Netherlands. Unfortunately, *Botrytis* spp. were not present in our experimental plots in 1995, so that the effect of persisting *U. atrum* inoculum on colonisation of necrotic lily tissue by *Botrytis* spp. could not be quantified.

Our studies into the persistence of *U. atrum* conidia on cyclamen leaves under greenhouse conditions demonstrated that propagules of this antagonist have the potential to survive on the surface of green leaves for at least seventy days under dry conditions (3.3.5.1). When such green leaves senesced seventy days after the antagonist application and thus became available as substrate for saprophytic colonisation by both *B. cinerea* and *U. atrum,* propagules of the antagonist still had the potential to compete successfully with the pathogen (Fig. 16). Because the amount of *B. cinerea* inoculum on the leaves was not standardised during the experiments, the amount of suppression obtained with antagonist propagules exposed to greenhouse conditions for different time periods cannot directly be compared.

At the beginning of the study on conidial persistence in cyclamen canopies it was hypothesised 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 favour *B. cinerea* (Grantzau and ter Hell, 1993). 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.

No indications were found that top irrigation affected the persistence of *U. atrum* inoculum (3.3.5.2). Under these conditions, a significant part of the conidia had the ability to attach to green leaves of cyclamen and to survive (Fig 17 to 19). An antagonistic activity of the *U. atrum* propagules against *B. cinerea* in necrotic tissue could even be found when conidia had been sprayed onto green leaves ten weeks before necrosis was induced by removing the leaves from the plants. Given that leaves were allowed to senesce slowly under dry conditions during the six weeks after removal from the plant, *U. atrum* retained its antagonistic potential for a total of sixteen weeks after application. These findings on the persistence of *U. atrum* propagules confirm the results obtained in lilies in the open field (3.3.5.3).

4.5 Mode of action of *U. atrum*

Two different studies were carried out to elucidate the mode of action of *U. atrum* antagonism against *Botrytis* spp. Firstly, ultrastructural observations on the interference between saprophytically growing *B. aclada* and *U. atrum* were made (Köhl et al., 1997). Observations on three other antagonists were included in this study for comparison. Secondly, the mechanistic principles behind the interaction between *B. cinerea* and *U. atrum* were elucidated by studies on internal substrate colonisation using a recently developed technique for immunolocalisation of fungal mycelium in necrotic tissues (Kessel et al., 1999) and external sporulation in time series experiments. Results were integrated in a dynamic simulation to check the validity of the hypothesis of nutrient competition as the prevailing antagonistic mechanism between *B. cinerea* and *U. atrum* (Kessel, 1999).

4.5.1 Fungal interactions: microscopical and ultra-structural studies

All four antagonists tested in this study suppressed sporulation of *B. aclada* almost completely in the bioassay on necrotic onion leaves. This agrees with results obtained earlier (3.2.1) that had shown the relative biocontrol potential of *Aureobasidium pullulans, Chaetomium globosum, Gliocladium catenulatum* and *Ulocladium atrum*. However, microscopic observations of the interactions have highlighted some important differences among the four fungi in terms of their antagonistic activity against *B. aclada*. This information is of key importance in establishing the respective behaviour of the antagonists and how this behaviour can influence the possible success of biocontrol of *Botrytis* spp.

Based on our light and electron microscope studies, *A. pullulans* grew in a yeastlike manner. Clusters of cells, most probably blastospores, were formed on the leaf surface or inside stomata whereas hyphae were rarely found inside the tissue. This can be explained by the limited formation of hyphae in solid media by this dimorphic fungus (Andrews et al., 1994). Considering this colonisation pattern, it is unlikely that nutrient competition within the substrate would play a major role in antagonism since the substrate would only be depleted locally at the site where colonies are formed. On the other hand, *A. pullulans* could be an efficient antagonist for the protection of leaf surfaces and man-made wounds, as previously reported (Falconi and Mendgen, 1994; Fokkema and Lorbeer, 1974). The *in vitro* production of antibiotic compounds by *A. pullulans* has been described (Bhatt and Vaugham, 1963; McCormack et al., 1994). In the present work, in the rare instances where close contact between *A. pullulans* and *B. aclada* was found, our observations appeared to support a chemical antibiosis by *A. pullulans* (Fig. 26).

The colonisation of dead onion tissues by *C. globosum* was poor and its hyphae were mostly restricted to the leaf surface. However, the ultrastructural studies indicated

that this antagonist, which is known for its strong cellulolytic activity (Domsch et al., 1980), has the potential to degrade plant cell walls (Fig. 25). *C. globosum* was previously found to be antagonistic against several plant pathogens such as *Pythium ultimum* (Di Pietro et al., 1992) and *Venturia inaequalis* (Heye and Andrews, 1983) and is known to produce two antifungal substances (Di Pietro et al., 1992). The production of antibiotics by *C. globosum* may also explain its antagonistic activity against *B. cinerea* under certain field conditions regardless of the presence of an actively growing population of the antagonist on the substrate (3.2.2). In our ultrastructural studies, highly disorganised fungal cells were associated with cells of *C. globosum*, whereas such dead cells were not found when the antagonist was grown alone. This would support the manifestation of an *in situ* antibiotic activity by *C. globosum*. However, it was not possible to confirm whether these dead cells belonged to *B. aclada*, but it can be assumed, supported by the information given in the literature and our observations, that the antagonist did induce cell death of *B. aclada*. Because of its limited colonisation potential, it would appear that antibiosis, if not exclusively, is at least an important component of the biocontrol ability of *C. globosum*.

In previous experiments, *G. catenulatum* displayed strong antagonism against *Botrytis* spp. under continuously moist conditions favourable for fungal growth (3.2.1). Our studies revealed that *G. catenulatum* could colonise all parts of necrotic tissues, but that this colonisation was not very extensive. Nevertheless, colonisation of *B. aclada* was considerably reduced in the presence of the antagonist. Antibiosis was clearly one of the modes of action of *G. catenulatum* based on cell reactions of *B. aclada* which were typical of toxic activity such as cytoplasm degradation and loss of turgor pressure (Fig. 24) (Hjlaoui et al., 1994; Yoder, 1980). No clear signs of hyperparasitism such as cell wall degradation at sites of contact between hyphae of the two organisms were found. While no information is given in the literature about the mode of action of *G. catenulatum*, such information abounds about other species of *Gliocladium* and the closely related *Trichoderma* spp. (Chet, 1987; Papavizas, 1985). For instance, *G. virens* has been described as an antibiotic producer and its activity against *P. ultimum* and *R. solani* is strongly related to the antibiotics it produces (Howell and Stipanovic, 1995). Di Pietro et al. (1993) suggested that enzymes such as beta-glucanases provided a synergistic effect with the toxins but evidence of hydrolytic activity of *G. virens in situ* has never been reported. In addition, while some *Trichoderma* spp. have been shown to degrade their host cells enzymatically, it is well established that they produce a number of antibiotic substances (Ghisalberti and Sivasithamparam, 1991). In the chronological events associated with the antagonism of *T. harzianum* against *B. cinerea,* Bélanger et al. (1995) showed that antibiosis preceded penetration and suggested that the enzymatic cell wall degradation was more a saprophytic action than the cause of death in *B. cinerea*.

In the case of *U. atrum,* there were no previous reports on microscopic studies of the colonisation of necrotic plant tissue or possible mechanisms of antagonism by this fungus. *U. atrum* mycelium was found to be present and distributed throughout

necrotic leaf tissues and *B. aclada* seemed to be completely excluded from that niche. Ultrastructural studies showed that *U. atrum* degraded plant cell walls actively so that it could grow through the dead plant cells walls (Fig. 23a, b). Because *B. aclada* could not be detected in necrotic leaf tissues in the presence of *U. atrum*, it was not possible to determine if antibiosis and/or parasitism complemented its competition for substrate in onion leaves. However, when the interaction between both fungi was studied on agar, there were no obvious signs of hyperparasitism or antibiosis as the fungi seemed unaltered (Fig. 23c, d).

4.5.2 Competitive substrate colonisation: experimental studies and simulation modelling

The dynamics of internal mycelial colonisation were found to follow logistic growth patterns. The logistic equation is the most widely used equation to describe the evolution of biomass in fungal solid fermentation (Smits, 1998). However, large standard errors and observed decline of colonisation levels in the latest phases of the process urge for some reservation regarding this conclusion. The large standard errors may be due to the variable nutritional status of the cyclamen leaves used for the study. *B. cinerea* and *U. atrum* displayed similar colonisation patterns and maximum colonisation levels within experiments. The nutritional status of the necrotic cyclamen leaves was thus equally stimulating or hampering both fungi and did not result in a competitive advantage for *B. cinerea* or *U. atrum*. Necrotic leaves in cyclamen and especially in other crops are likely to differ significantly in their nutritional composition. Consequently this must be taken into account when assessing the perspectives for biocontrol of *B. cinerea* using *U. atrum*. The decline in colonisation levels observed after reaching carrying capacity was supported by visual observations. Autolysis of cell walls, initiated in the ageing mycelium by nutrient deprivation as described for *Schizophyllum commune* (Wessels and Sietsema, 1984) is a likely cause of this phenomenon.

The mechanistic principles behind the interaction of *U. atrum* and *B. cinerea* were analysed using head start experiments. Factors potentially influencing the outcome of a replacement experiment such as active host resistance, host susceptibility or pathogenicity factors (Newton et al., 1998) are unlikely to interfere in necrotic tissue. Potential effects of inoculum density on the competitive interaction (Firbank and Watkinson, 1985; Snaydon, 1991) were not taken into account. The chosen inoculum densities represented a worst case scenario for the antagonist with realistic conidial densities for *U. atrum* and unrealistically high conidial densities for *B. cinerea*.

The position of the two experimental RY lines and the RYT line in Fig. 27, which is located well above the reference line for equal inter- and intraspecific competition, implies that the available resources for *U. atrum* in a necrotic cyclamen leaf include all the essential resources available to *B. cinerea* plus a small amount of re-

sources specifically available to *U. atrum*. Alternatively, *U. atrum* could benefit from by-products of enzymatic degradation of the tissue by *B. cinerea*, which would otherwise not have been available to the antagonist.
The hypothesis on nutrient competition as the dominant antagonistic mechanism between *B. cinerea* and *U. atrum* and on the nutrient utilisation spectra of both fungi were checked using simulation. In general, comparison of experimental and model results supported the hypothesis of nutrient competition as the dominant competitive mechanism between *U. atrum* and *B. cinerea*. A major difference between model results and experimental results was found in the shape of the RYT line. The experimental RYT line (Fig. 27), based on sporulation, was convex whereas the model RYT lines (Fig. 28), based on mycelial biomass, were horizontal straight lines. The convex shape of the experimental RYT line indicates a less than proportional reduction of sporulation of *U. atrum* and *B. cinerea* in competitive situations (Fig. 28) and thus a stimulating effect of competition on sporulation. Alternatively, the convex shape of the RYT line could be caused by (1) by-products of enzymatic degradation of the tissue produced by one of the species and beneficial to the other (Rayner and Webber, 1984), or (2) differences in metabolic capabilities of the fungi during their development. The shape of the RYT line also indicates that toxins are unlikely to be involved in the competitive relationship between *U. atrum* and *B. cinerea*. In case toxins would have been involved the RYT line would be concave and fall below 1 (Fig. 27; Braakhekke, 1980).
Competitive interactions comprise interactions based on primary resource capture and combative interactions. For the present system we find that (1) *B. cinerea* and *U. atrum* both belong to the primary colonisers of necrotic plant tissue; (2) mycelial contact between both species does not negatively affect the mycelia (3.4.1; Köhl et al., 1997); (3) *U. atrum* and *B. cinerea* grow and sporulate in mixed cultures; (4) *U. atrum* pre-emptively colonises necrotic cyclamen leaf tissue and excludes *B. cinerea*; and (5) the outcome of the race for resources between *B. cinerea* and *U. atrum* in necrotic cyclamen tissue can be decided within the very first phase of the colonisation process. All these facts point towards a competitive interaction based on primary resource capture.
Both studies (Köhl et al., 1997; Kessel, 1999) indicate that *U. atrum* relies exclusively on competition for gaining access to its ecological niche over *Botrytis* spp. The ability to colonise and to utilise necrotic tissues rapidly may enable *U. atrum* to exclude competitors from the substrate without any other antagonistic interaction. These findings may have practical implications for the proper timing of application of the antagonist and for predicting its limitations. Most efficient control of substrate colonisation and subsequent sporulation of the pathogen may be achieved on freshly senesced tissues or in freshly pathogen-induced lesions where mycelium of the pathogen is not established in high density to allow profuse sporulation. Since the antagonist seems to lack the ability to kill fungal cells by toxins or by hyperparasitism, a curative application of *U. atrum* would be unlikely to reduce the pathogen population already established in high densities. However, nutrient com-

petition resulting in reduced spore yields of the pathogen may also play a role when *U. atrum* colonises substrates where *Botrytis* mycelium is already established.
The exploitation of biological control agents with mechanisms that are mainly based on competition for nutrients and space can provide distinct advantages against necrotrophic plant pathogens that rely on their saprophytic phase for establishing a critical base population. Firstly, the antagonistic interaction is not specific for certain pathogen-antagonist relationships. For example, *U. atrum* is known to compete with and suppress sporulation of a range of *Botrytis* species and other saprophytic fungi such as *Cladosporium* spp. and *Alternaria* spp. (3.2.2; Köhl et al., 1995b). Secondly, there is no strict necessity for the simultaneous presence of antagonist and pathogen. The antagonist can utilise the limited amounts of nutrients in necrotic tissues long before the pathogen arrives and thus restricts access to the substrate. Organisms acting strictly by hyperparasitism depend on the presence of the pathogen for activity. In addition, the process can be relatively slow and often only activated when the pathogen itself is under stress. In the case of antibiosis, while efficient against biotrophs such as powdery mildews (Paulitz and Bélanger, 2001), it is also subject to a precise timing of release against necrotrophs because antibiotics produced early before arrival of the pathogen may be degraded or may be adsorbed to cell surfaces (Boudreau and Andrews, 1987). Thirdly, it is unlikely that resistance against competition will be developed by *Botrytis* populations. This point is of particular significance considering the ability of *B. cinerea* to develop resistance against fungicides (Gullino, 1992; Rosslenbroich and Stuebler, 2000). This phenomenon was also reported against two antibiotics produced by *Bacillus subtilis* after nine subsequent crops of *Astilbe* had been sprayed with the antagonist (Li and Leifert, 1994).

4.6 Use of *U. atrum* for disease control in crops

4.6.1 Strawberries

U. atrum was effective in reducing grey mould at harvest in at least one spray programme per experiment in four out of seven experiments (3.5.1). In experiments 3 and 7, *U. atrum* failed to show an effect on grey mould, probably due to the low incidence of grey mould (1.4 and 2.2%, respectively).
Compared to applications during flowering only, season-long applications of *U. atrum* resulted in an improved control of grey mould at harvest only in one out of five experiments (Table 27 and 28). Bhatt and Vaughan (1963) studied the biocontrol of grey mould in strawberry by *Cladosporium* spp. The effect of the fungus on reduction of fruit rot at harvest was attributed to prevention of establishment of *B. cinerea* on the senescent and dead flower organs of strawberry. The same mechanism may be involved in the interaction between *U. atrum* and *B. cinerea*. In this

case, targeting applications to flower parts would be effective to reduce *B. cinerea* infection. Moreover, the small amount of necrotic leaves produced inside annual strawberry crops may mean that the inoculum produced on these tissues does not play an important role in the epidemiology of grey mould in such cropping systems (3.1.2; Boff et al., 2001). This could explain the limited additional effect of *U. atrum* treatments before flowering, although the antagonist is able to colonise and suppress the sporulation of *B. cinerea* on dead strawberry leaves (Berto et al., 2001; Köhl et al., unpublished). Crop sanitation by removing senescent and necrotic leaf tissue in our system did not reduce *Botrytis* infection in comparison to the untreated control as it did in similar experiments in onion crops (3.1.1) and in another perennial strawberry system using raised beds (Mertely et al., 2000).
Shorter application intervals of *U. atrum* may result in better control. In the strawberry crops new flowers are produced each day and flower parts start to senesce after three to five days. Since the mode of action of *U. atrum* is by substrate competition (3.4; Kessel, 1999), the antagonist should reach the site of colonisation on the flower parts before or at the same time as the pathogen. This explains the need of frequent applications to reach all flowers at an early stage.
U. atrum tested under low disease pressure in the field showed its potential to control grey mould by regularly spraying conidia from transplanting or flowering through to the beginning of fruit ripening. Further studies should evaluate the performance of the antagonist on strawberry crops under conditions more favourable to grey mould development. In such experiments, treatments with other antagonists described for grey mould control in strawberry, such as *Gliocladium roseum* (syn. *Clonostachys rosea*) (Sutton, 1994) and *Paenibacillus polymyxa* (Helbig, 2001) should be included for comparison. The integrated use of *U. atrum* together with *Serratia plymuthica* antagonistic to several soilborne diseases of strawberry (Kurze et al., 2001) should also be investigated. Testing bees or bumble bees as vectors for *U. atrum* application to strawberry flowers is another attractive option in future exeriments (Sutton, 1994; Kovach et al., 2000).

4.6.2 Onion

Spray applications of conidia of *U. atrum* resulted in colonisation of necrotic onion leaves under field conditions (3.5.2). This was demonstrated in ten subsequent experiments with artificially killed leaves exposed on onion plots. Colonisation of necrotic leaf tissue by *U. atrum* resulted in significant reduction of colonisation of other naturally occurring saprophytic fungi such as *Alternaria* spp. and *Cladosporium* spp. It can be assumed that under conditions more conducive for *Botrytis* spp. also colonisation of onion leaf tissue by *Botrytis* spp. may be lower after *U. atrum* treatments as observed in similar experiments on lily leaves exposed to field conditions (3.2.2).

On necrotic leaf tissues of onion crops, at one sampling date substantial sporulation of *Botrytis* spp. was observed after additional moist incubation of leaf tissues during both experiments. For this single observation a clear reduction in the potential to sporulate was found for leaves from *U. atrum* or Ronilan-treated plots. This indicates that crop treatments with *U. atrum* may lower the risk of rapid build-up of epidemics. Results on aerial load of *Botrytis* spp. conidia confirm this conclusion (Table 30). Although not visually observed on sampled leaf tissues, *Botrytis* spp. produced and released conidia as proven by the significantly higher spore load of *Botrytis* spp. above control plots as compared to the spore load outside experimental plots above the buffer crop. Treatments with *U. atrum* significantly reduced the aerial spore load of *Botrytis* spp. down to levels as found above the buffer crops. This proves a substantial reduction of sporulation by *U. atrum* within the onion crop.
Competitive colonisation of necrotic onion leaf tissue by *U. atrum* suppressed colonisation and sporulation of *Botrytis* spp. in treated plots, which may result in a slower epidemic of onion leaf spot. Although leaf spot epidemics were mild in both years, *U. atrum* and Ronilan-treated plots tended to have lower amounts of leaf spots as compared to water-treated plots (Fig. 30). In summary, results show that *U. atrum* has the potential to slow down epidemics of leaf spot under field conditions. However, further research is needed under more severe disease pressure to investigate the relevance of this finding for commercial onion production.
Sporulation of *U. atrum* was not observed on leaf tissues if not further incubated in a moist chamber. Conidia of *U. atrum* were not trapped with Rotorods. The level of tissue colonisation by *U. atrum* of artificially killed leaf segments exposed after spray applications on *U. atrum* treated onion plots did not differ from *U. atrum* colonisation of leaf tissues exposed on plots not treated with *U. atrum* (Fig. 29). These results indicate that the antagonist may not spread secondarily in the crop after sporulation. Repeated applications of the antagonist may thus be necessary.
Under controlled conditions, *U. atrum* suppressed sporulation of different *Botrytis* spp. including *B. cinerea*, *B. aclada*, (3.2.1; Nielsen, 2000) and *B. squamosa* (Köhl et al., unpublished). Further studies in field-grown onions should include assessments of the possible effect of *U. atrum* on *B. aclada* to obtain information on the potential of the antagonist to also control neck rot caused by *B. aclada* (Maude, 1984).

4.6.3 Cyclamen

Regular application of conidial suspensions of *U. atrum* (1 x 10^6 conidia ml^{-1}) suppressed the incidence and severity of *B. cinerea* rot in cyclamen under commercial growing conditions (3.5.3.1; Fig. 31). Increasing the intervals between applications of the antagonist from two to four weeks in the second experiment resulted in similar control levels of disease incidence and disease severity (Fig. 32). Regular applications of conidial suspensions of *G. roseum* (2 x 10^6 conidia ml^{-1}) suppressed

the disease half as effectively as *U. atrum* (experiment 1) and were similar to *U. atrum* treatments when applied at 1 x 10^7 conidia ml^{-1} (experiment 2). Such antagonist applications reached control levels similar to those of the standard fungicide programme used by the grower.
The results of the experiments in the different commercial greenhouses (3.5.3.2) show that *U. atrum* controls *B. cinerea* when sprayed at four-week intervals (Table 33), 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 (3.3.5.2; Fig. 17 to 19).
Further experiments under commercial growing conditions with antagonist applications only to green leaves in an early growth stage of the cyclamen crop are needed and may provide information as to whether propagule survival of *U. atrum* is sufficient for a persistent disease control during cyclamen production. Such early applications will reduce labour costs, especially because an overhead treatment of the plants will be sufficient to reach all surfaces. An individual treatment of the heart of the plant as necessary for fungicide or antagonist application during the later developmental stage of the plants will then not be necessary. An early application of *U. atrum* on plants before the dense canopy is formed also reduces the risk of black spots from dried droplets of the conidial suspension on marketable plants as found when plants are sprayed a couple of weeks before marketing.

4.6.4 *Pelargonium*

***B. cinerea* severity on stock plants.** The experiments have been performed during two seasons at periods considered risky for development of *B. cinerea* epidemics. Due to high RH in greenhouses in autumn, senescent and necrotic leaves of geranium stock plants are easily colonised by *B. cinerea*. The start of the first experiment was rather late, which caused the experiment to partly escape from the high humidity which usually prevails during October to 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 (Fig. 33). A cold and dry spell in the middle of the experimental 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 to occur at the end of the experiment. The second experiment started earlier and developed a higher inci-

dence 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 (Fig. 34).

***B. cinerea* spores in the air.** The results of the spore traps to monitor the inoculum pressure in the greenhouse 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 (Fig. 35). 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. 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 experiment and by *U. atrum*, but not the fungicide treatment in the second experiment cannot be explained.

***B. cinerea* conidia counts on necrotic and green leaves.** The counts of conidia of *B. cinerea* on necrotic and green leaves, both in the first and second experiment are in full agreement with each other (Fig. 36 and 37). They consistently show suppression of the pathogen by the antagonist as well as by the fungicide. In both experiments these estimates of *B. cinerea* severity show highest values in the weeks when visual estimates of the stock plants gave highest values too. In the first experiment the number of conidia of *B. cinerea* on green leaves is low; in the second it is several times higher. The data of the two years as presented cannot be compared directly, since only in the second year the number of spores was 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 as in 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, 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 et al., 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 experiment low *B. cinerea* inoculum pressure in the middle of the experiment, combined with increasing daylength may be held responsible for relatively low loss of cuttings due to *B. cinerea*. The second experiment started earlier, and *B. cinerea* on stock plants was present at a high level for several weeks at the beginning of the experiment. Contamination of cuttings with

conidia of *B. cinerea,* as represented by the counts of spores on green leaves, was high in 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 experiment 2 (Fig. 39). The reduction of the number of spores of the pathogen on green leaves by these treatments compared to the control seems effective in the first, but not in the second experiment. Two factors may account for this. First, a minimum number of conidia is required for infection, even of highly susceptible young leaves (Sirjusingh et al., 1996). Second, when inoculum pressure is high the significant reduction brought about by *U. atrum* or fungicide may not yet be enough for a substantial reduction of infection. It is generally observed with biological control that the effect is better at low than at high disease pressure (cf. McQuilken et al., 1990). In the two experiments described it seems that the spore load in the first experiment was often below the critical level, even in the control. In the second experiment it was above the threshold, even in the *U. atrum* and fungicide treatments.

Integrated control. The results of the present experiments show a potential for *U. atrum* in an integrated programme. Several management practices can lead to lessen the pressure of *B. cinerea*. A dry atmosphere is primordial. This can be obtained by changes in greenhouse climate by plastic on the soil or heating under the plant tables (Hausbeck and Moorman, 1996; Hausbeck et al., 1996), or by lower 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 intervention on its own resulted already 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 well cause a strong effect by bringing the pressure of *B. cinerea* under the critical threshold, and thus positively affect the success rate of cuttings.

4.6.5 Pot roses

In both experiments treatment of pot roses with *U. atrum* resulted in effective control of *B. cinerea* (3.5.5; Fig. 40 and 42). In the first experiment the fungicide carbendazim was not effective at all. This has to be attributed to the resistance of the greenhouse population of *B. cinerea* to this specific fungicide. In the second trial the fungicide Teldor (fenhexamide) proved highly effective, even at a dose of 10% of the recommended concentration. It even surpassed the effect of *U. atrum*.
The positive results of the present experiments hold promise for a potential practical application of *U. atrum* as a biocontrol agent replacing fungicides in the production of pot roses. This is in agreement with other observations (Yohalem, 1997; 2000). There may be a potential risk, however, of wound infection by a saprophyte

on the fresh pruning wounds of pot roses. In the present production system we have delayed the treatment with *U. atrum* to one day after pruning, and we have never observed any damage by the biological control agent.
In a related crop, cut roses, *B. cinerea* is a great problem with certain cultivars in some periods of the year. The cropping system in greenhouses is such that cleaning and pruning the plants leaves a mass of vegetative tissue such as stem pieces and leaves on the soil. This results in colonisation by saprophytes, but also by *B. cinerea*, and consequently a heavy spore load of the pathogen in the air. The risk in this case lies in contamination of the flowers. Post-harvest handling of the flowers creates conditions which often induce colonisation of rose petals by *B. cinerea*, leading to brown specks, which may coalesce and finally cause rotting of the complete flower. The commercial circuit is very sensitive to this type of unacceptable damage. Growers may be able to reduce the risk of flower contamination by treatment of vegetative residues with *U. atrum*, thus limiting sporulation of *B. cinerea* and reducing the spore load in the air. Trials on this aspect are expensive, because they need to be done at the scale of whole greenhouse compartments. But the problem to be solved is sufficiently important to plead for further research in this direction.

4.6.6 Other crops or diseases

The potential of *U. atrum* to control *Botrytis* spp. has been tested in several other crops such as tomato, grapevine and lily, and against several other pathogens in various crops.
In tomato, *B. cinerea* is causing severe damage after stem infection leading to girdling. Yield losses can be substantial since entire plants can be killed. This disease is a major limiting factor in tomato production in greenhouses and plastic tunnels with limited climate control as common in Mediterranean countries. In this production system, regular leaf removal creates pruning wounds on stems which are potential entrance sites for *B. cinerea*. In a series of experiments carried out by L. Fruit and P.C. Nicot in Southern France (Fruit and Nicot, 1999; Fruit, 2001) in greenhouses and plastic tunnels, *U. atrum* was applied to stem wounds artificially inoculated also with conidia of *B. cinerea*. Applications of *U. atrum* protected stems from infection as effective as a fungicide applied in separate treatments whereas stem lesions occurred on 30-40% of water-treated stems serving as control in the different experiments. Such a consistently high level in control efficacy was also found in experiments carried out under controlled conditions in a broad range of combinations of temperature and relative humidity (Fruit, 2001; Nicot et al., in press). The high potential of *U. atrum* in protecting wounds from invasion by *B. cinerea* had not been expected at the beginning of the experiments because the antagonists had been selected for competitive colonisation of necrotic plant tissues but not of wounds. The mode of action of *U. atrum* in wound tissue is not yet

known but investigations on antagonistic interactions in tomato stem wounds are ongoing (Nicot, pers. comm.).
Studies on biocontrol of grey mould in grapevine were carried out by Schoene et al. (2000) in 1997 and 1998 with two German white grape varieties at two different locations. In all three field experiments development of grey mould epidemics was slower in plots treated three to four times with conidial suspensions of *U. atrum* than in water-treated control plots. The percentage of infected berries at harvest was significantly reduced by *U. atrum* applications. Antagonist applications reached the same efficacy as conventional fungicide programmes.
U. atrum did not control *B. elliptica* on lily cv. Mont Blanc, which is highly susceptible to the pathogen causing lily fire on leaves (Kessel et al., 2001). In several field experiments it was found that *B. elliptica* was stronger in competitive substrate colonisation of necrotic tissue of pathogen-incited lesions than *U. atrum*. Within such lesions, applications of *U. atrum* did not reduce sporulation of the pathogen. On naturally senesced tissue, *U. atrum* was the superior competitor and sporulation of *B. elliptica* was significantly reduced. However, it was demonstrated that such leaf tissues play no important role as inoculum source during lily fire epidemics because the majority of conidia is produced within pathogen-incited lesions. Consequently, epidemics of lily fire could not be retarded by *U. atrum* applications.
Alternaria radicina can cause black spots on stored carrots. The pathogen can be transmitted by seed. Experiments are initiated to study the use of *U. atrum* in carrot seed production to control *A. radicina*. In bioassays under controlled conditions, *U. atrum* suppressed seed colonisation by *A. radicina* (Meekes et al., 2002). Preliminary results of field experiments show that applications of *U. atrum* to carrot flowers resulted in colonisation of seeds by *U. atrum* and a moderate reduction of *A. radicina* infection of seeds (Meekes, pers. comm.).
In cabbage, ring spot (*Mycosphaerella brassicicola*) can lead to severe losses of both yield and quality. The pathogen can produce ascospores in lesions on leaves. This inoculum is important for secondary spreading of the disease in crops. Under controlled conditions, *U. atrum* could colonise lesions caused by *M. brassicicola* and such lesions expanded slower than untreated control lesions (Meekes et al., 2002). Field experiments so far did not lead to conclusive results.
In several field experiments, Lennartz et al. (1998) applied *U. atrum* to wheat. Moderate control effects were found on epidemics caused by *Leptosphaeria nodorum* or *Mycosphaerella graminicola*.

4.7 Perspectives for development of a biocontrol agent based on *U. atrum*

Applications of *U. atrum* in various crops showed the high potential of the antagonist in controlling diseases caused by *B. cinerea*. This makes the antagonist an attractive candidate for the development of a biological control agent. For the

development of a biocontrol agent by commercial companies for use by growers several prerequisites must be fulfilled. The market size must justify the investments, production of spores must be economically sound and criteria considered during registration procedures such as toxicological and ecotoxicological aspects must be fulfilled.

For a biocontrol agent against *B. cinerea* market sizes are large because major high value crops suffer from grey mould and regular fungicide applications are common in grey mould control. Alternative disease control methods are needed to protect the environment but also because of the occurrence of fungicide resistance in *B. cinerea* populations (Gullino, 1992; Rosslenbroich and Stuebler, 2000). For use in integrated crop protection systems, a biocontrol agent must be compatible with chemical control agents, e.g. in grapevine production under German conditions grey mould control with *U. atrum* should be compatible with chemical control of downy mildew. Schoene et al. (1999) tested the effect of a broad range of fungicides on *U. atrum in vitro* and in the field. It could be demonstrated that *U. atrum* is less sensitive than target pathogens to many fungicides so that the integrated use of antagonist and fungicides is feasible. This opens a substantial market for a biocontrol agent in integrated production systems of grapes and vegetables.

Economically sound inoculum production is one of the key factors in commercialisation of biocontrol agents. *U. atrum* conidia can easily be produced in solid state fermentation on oat or other cereal substrates. In our studies, we used autoclavable plastic bags with microfilters for gas exchange. Autoclaved and inoculated cereal substrates were incubated for four weeks at 18 °C to obtain mature conidia. Our procedure yielded sufficient conidia to carry out field experiments. However, this protocol will not be suitable for commercial inoculum production because fermentation units were small and incubation periods were long. Designing procedures for up-scaling conidia production in solid state fermentors with growing conditions allowing fast production of high quality conidia will be necessary for the development of a biocontrol agent based on *U. atrum*. Frey and Magan (2001) developed a method to produce conidia and mycelial fragments in oat meal broth. The quality of inocula, quantified by germination tests on water stressed media, could be manipulated by alternating the water potential of the growth media. Inocula were viable for a period of at least six months. However, conidia produced on solid substrate in the same study had a shelf life of at least 12 months. Further investigations are needed to develop production methods for solid state fermentation or liquid fermentation which allow high spore yields in short fermentation runs.

Possible negative side effects of biocontrol agents have to be investigated during the registration procedures according to governmental regulations. Toxicological data on *U. atrum* have not been published in medical literature. Results of toxicological and ecotoxicological studies will be needed for registration. Saprophytic antagonists such as *U. atrum* might be plant pathogenic under certain circumstances. Considering that *Ulocladium* spp. are ubiquitous saprophytes colonising leaf litter and soil (Domsch et al., 1980), reports on plant-pathogenicity of *U. atrum* are rare. How-

ever, *U. atrum* has been described as causal agent of cucumber leaf spot in Great Britain (Butler et al., 1979), of a potato leaf disease in Peru (Turkensteen, 1979) and eastern Turkey (Demirci and Doken, 1989), and as a possible biological control agent of *Orobanche* spp. (Linke et al., 1992). In our own experiments in crops such as strawberries, onions, lilies, tulips, cyclamen, pelargonium and pot roses, *U. atrum* did not produce any symptoms of disease. Disease symptoms caused by *U. atrum* were neither described by Schoene et al. (2000) in grapevine or Fruit and Nicot (1999) in tomato.

In conclusion, *U. atrum* isolate 385 fulfils all requirements tested so far for a candidate biological control agent so that the development and registration of a biocontrol product based on *U. atrum* 385 is feasible.

5 Literature cited

Adee, E.A. and Pfender, W.F. 1989. The effect of primary inoculum level of *Pyrenophora tritici-repentis* on tan spot epidemic development in wheat. Phytopathology 79: 873-877.

Adee, S.R., Pfender, W.F. and Hartnett, D.C. 1990. Competition between *Pyrenophora tritici-repentis* and *Septoria nodorum* in the wheat leaf as measured with the de Wit replacement series. Phytopathology 80: 1177-1182.

Alderman, S.C. and Lacy, M.L. 1983. Influence of dew period and temperature on infection of onion leaves by dry conidia of *Botrytis squamosa*. Phytopathology 73: 1020-1023.

Alderman, S.C. and Lacy, M.L. 1984. Influence of temperature and water potential on growth of *Botrytis allii*. Can. J. Bot. 62: 1567-1570.

Andrews, J.H. 1990. Biological control in the phyllosphere: Realistic goal or false hope? Can. J. Plant Pathol. 12: 300-307.

Andrews, J.H., Berbee, F.M. and Nordheim, E.V. 1983. Microbial antagonism to the imperfect stage of the apple scab pathogen, *Venturia inaequalis*. Phytopathology 73: 228-234.

Andrews, J.H., Harris, R.F., Spear, R.N. and Nordheim, E.V. 1994. Morphogenesis and adhesion of *Aureobasidium pullulans*. Can. J. Microbiol. 40: 6-17.

Andrews, J.H. and Hirano, S.S. 1992. Microbial Ecology of Leaves. Springer, New York.

Aquino, V.M., Shokes, F.M., Gorbet, D.W. and Nutter, F.W. Jr. 1995. Late leaf spot progression on peanut as affected by components of partial resistance. Plant Dis. 79: 74-78.

Auruz, L.F. and Sutton, T.B. 1990. Effect of interrupted wetness periods on spore germination and apple infection by *Botryosphaeria obtusa*. Phytopathology 80: 1218-1220.

Aylor, D.E. 1993. Relative collection efficiency of Rotorod and Burkhard spore samplers for airborne *Venturia inaequalis* ascospores. Phytopathology 83: 1116-1119.

Baddeley, M.S. 1971. Biochemical aspects of senescence. In: Preece, T.F. and Dickinson, C.H. (eds), Ecology of Leaf Surface Micro-organisms. Academic Press, London, pp 415-429.

Bannon, E. 1978. A method of detecting *Septoria nodorum* on symptomless leaves of wheat. Irish J. Agric. Res. 17: 323-324.

Bashi, E. and Rotem, J. 1974. Adaptation of four pathogens to semi-arid habitats as conditioned by penetration rate and germinating spore survival. Phytopathology 64: 1035-1039.

Becker, C.M. and Burr, T.J. 1994. Discontinuous wetting and survival of conidia of *Venturia inaequalis* on apple leaves. Phytopathology 84: 372-378.

Begon, M., Harper J.L. and Townsend, C.R. 1986. Ecology: Individuals, Populations and Communities. Blackwell Scientific Publishers, Oxford.

Bélanger, R.R., Dufour, N., Caron, J. and Benhamou, N. 1995. Chronological events associated with the antagonistic properties of *Trichoderma harzianum* against *Botrytis cinerea*: Indirect evidence for sequential role of antibiosis and parasitism. Biocontrol Sci. Technol. 5: 41-53.

Bélanger, R.R., Labbé, C. and Jarvis, W.R. 1994. Commercial scale control of powdery mildew with a fungal antagonist. Plant Dis. 78: 420-424.

Berto, P., Jijakli, H.M. and Lepoivre, P. 2001. Possible role of colonization and cell wall-degrading enzymes in the differential ability of three *Ulocladium atrum* strains to control *Botrytis cinerea* on necrotic strawberry leaves. Phytopathology 91: 1030-1036.

Bhatt, D.D. and Vaughan, E.K. 1963. Inter-relationships among fungi associated with strawberries in Oregon. Phytopathology 53: 217-220.

Biles, C.L. and Hill, J.P. 1988. Effect of *Trichoderma harzianum* on sporulation of *Cochliobolus sativus* on excised wheat seedling leaves. Phytopathology 78: 656-659.

Blakeman, J.P. 1981. Microbial Ecology of the Phylloplane. Academic Press, London.

Blakeman, J.P. and Brodie, I.D.S. 1976. Inhibition of pathogens by epiphytic bacteria on aerial plant surfaces. In: Dickinson, C.H. and Preece, T.F. (eds) Microbiology of Aerial Plant Surfaces. Academic Press, London, pp 529-557.

Blakeman, J.P. and Brodie, I.D.S. 1977. Competition for nutrients between epiphytic micro-organisms and germination of spores of plant pathogens on beetroot leaves. Physiol. Plant Pathol. 10: 29-42.

Blakeman, J.P. and Parbery, D.G. 1977. Stimulation of appressorium formation in *Colletotrichum acutatum* by phyllosphere bacteria. Physiol. Plant Pathol. 11: 313-325.

Bodreau, D.D. and Andrews, J.H. 1987. Factors influencing antagonism of *Chaetomium globosum* to *Venturia inaequalis*: A case study in failed biocontrol. Phytopathology 77: 1470-1475.

Boff, P., Kastelein, P., de Kraker, J., Gerlagh, M. and Köhl, J. 2001. Epidemiology of grey mould in annual waiting-bed production of strawberry. Eur. J. Plant Pathol. 107: 615-624.

Boff, P., Köhl, J., Jansen, M., Horsten, P.J.F.M., Lombaers-van der Plas, C.H. and Gerlagh, M. 2002. Biological control of gray mold with *Ulocladium atrum* in annual strawberry crops. Plant Dis. 86: 220-224.

Boland, G.J. and Hunter, J.E. 1988. Influence of *Alternaria alternata* and *Cladosporium cladosporioides* on white mold of bean caused by *Sclerotinia sclerotiorum*. Can. J. Plant Pathol. 10: 172-177.

Boland, G.J. and Inglis, G.D. 1989. Antagonism of white mold (*Sclerotinia sclerotiorum*) of bean by fungi from bean and rapeseed flowers. Can. J. Bot. 67: 1775-1781.

Bollen, G.J. 1982. Fungicide resistance and microbial balance. In: Dekker, J. and Georgopoulos, S.G. (eds), Fungicide Resistance in Crop Protection. Pudoc Agricultural Publishing and Documentation, Wageningen, pp 161-176.

Bossi, R. and Dewey, F.M. 1992. Development of a monoclonal antibody-based immuno-detection assay for *Botrytis cinerea*. Plant Pathol. 41: 472-482.

Boudreau, M.A. and Andrews, J.H. 1987. Factors influencing antagonism of *Chaetomium globosum* to *Venturia inaequalis*: A case study in failed biocontrol. Phytopathology 77: 1470-1475.

Bowen, J.K., Crowhurst, R.N., Templeton, M.D. and Stewart, A. 1996. Molecular markers for a *Trichoderma harzianum* biological control agent - introduction of the hygromycin B resistance gene and the beta-glucuronidase gene by transformation. New Zeal. J. Crop Hort. 24: 219-228.

Braakhekke, W.G. 1980. On coexistence: a causal approach to diversity and stability in grassland vegetation. PhD thesis, Agricultural University Wageningen.

Braun, P.G. and Sutton, J.C. 1986. Management of strawberry grey mould with fungicides targeted against inoculum in crop residues. Proceedings British Crop Protection Conference - Pests and Diseases, pp. 915-921.

Braun, P.G. and Sutton, J.C. 1987. Inoculum sources of *Botrytis cinerea* in fruit rot of strawberries in Ontario. Can. J. Plant Pathol. 9: 1-5.

Braun, P.G. and Sutton, J.C. 1988. Infection cycles and population dynamics of *Botrytis cinerea* in strawberry leaves. Can. J. Plant Pathol. 10: 133-141.

Brodie, I.D.S. and Blakeman, J.P. 1975. Competition for carbon compounds by a leaf surface bacterium and conidia of *Botrytis cinerea*. Physiol. Plant Pathol. 6: 125-135.

Brodie, I.D.S. and Blakeman, J.P. 1976. Competition for exogenous substrates *in vitro* by leaf surface micro-organisms and germination of conidia of *Botrytis cinerea*. Physiol. Plant Pathol. 9: 227-239.

Broome, J.C., English, J.T., Marois, J.J., Latorre, B.A. and Aviles, J.C. 1995. Development of an infection model for *Botrytis* bunch rot of grapes based on wetness duration and temperature. Phytopathology 85: 97-102.

Bujold, I., Paulitz, T.C. and Carisse, O. 2001. Effect of *Microsphaeropsis* sp. on the production of perithecia and ascospores of *Gibberella zeae*. Plant Dis. 85: 977-984.

Bulger, M.A., Ellis, M.A. and Madden, L.V. 1987. Influence of temperature and wetness duration on infection of strawberry flowers by *Botrytis cinerea* and disease incidence on fruit originating from infected flowers. Phytopathology 77: 1225-1230.

Bunster, L., Fokkema, N.J. and Schippers, B. 1989. Effect of surface-active *Pseudomonas* spp. on leaf wettability. Appl. Environ. Microbiol. 55: 1340-1345.

Burchill, R.T. and Cook, R.T.A. 1971. The interaction of urea and mirco-organisms in suppressing the development of perithecia of *Venturia inaequalis* (Cke.) Wint. In: Preece, T.F. and Dickinson, C.H. (eds), Ecology of Leaf Surface Micro-organisms. Academic Press, London, pp 471-483.

Burrage, S.W. 1971. The micro-climate at the leaf surface. In: Preece, T.F. and Dickinson C.H. (eds), Ecology of Leaf Surface Micro-organisms. Academic Press, London, pp 91-101.

Butler, D., Griffin, M.J. and Fletcher, J.T. 1979. Leaf spot on cucumber caused by *Ulocladium atrum*. Plant Pathol. 28: 96-97.

Caesar, A.J. and Pearson, R.C. 1983. Environmental factors affecting survival of ascospores of *Sclerotinia sclerotiorum*. Phytopathology 73: 1024-1030.

Campbell, G.S. and Gardner, G.A. 1971. Psychrometric measurement of soil water potential: Temperature and bulk density effects. Proceedings Soil Science Society of America 35: 8-12.

Carisse, O., Philion, V., Rolland, D. and Bernier, J. 2000. Effect of fall application of fungal antagonists on spring ascospore production of the apple scab pathogen, *Venturia inaequalis*. Phytopathology 90: 31-37.

Cerkauskas, R.F. and Sinclair, J.B. 1980. Use of Paraquat to aid detection of fungi in soybean tissues. Phytopathology 70: 1036-1038.

Chet, I. 1987. *Trichoderma*-application, mode of action and potential as a biocontrol agent of soilborne plant pathogenic fungi. In: Chet, I. (ed), Innovative Approaches to Plant Disease Control. John Wiley and Sons, New York, pp 137-160.

Chou, M.C. and Preece, T.F. 1968. The effect of pollen grains on infections caused by *Botrytis cinerea* Fr. Ann. appl. Biol. 62: 11-22.

Clarkson, J.P. and Lucas, J.A. 1993. Screening for potential antagonists of *Pseudocercosporella herpotrichoides,* the causal agent of eyespot disease of cereals. 2. Fungi. Plant Pathol. 42: 552-559.

Cook, R.J. 1970. Factors affecting saprophytic colonization of wheat straw by *Fusarium roseum* f.sp. *cerealis* 'Culmorum'. Phytopathology 60: 1672-1676.

Dallyn, H. and Fox, A. 1980. Spoilage of material of reduced water activity by xerophylic fungi. In: Gould, G.H. and Corry, J.E.L. (eds) Society of Applied Bacterial Technology. Series no. 15, pp 129-139.

De Nazareno, N.R.X., Lipps, P.E. and Madden, L.V. 1993. Effects of levels of corn residues on the epidemiology of gray leaf spot of corn in Ohio. Plant Dis. 77: 67-70.

De Waard, M.A., Georgopoulos, S.G., Hollomon, D.W., Ihsii, H., Leroux, P., Ragsdale, N.N. and Schwinn, F.J. 1993. Chemical control of plant diseases: problems and prospects. Annu. Rev. Phytopathol. 31: 403-421.

De Wit, C.T. 1960. On competition. Verslagen Landbouwkundige Onderzoekingen Wageningen 66: 1-82.

Dehne, H.-W., Stenzel, K. and Schönbeck, F. 1984. Zur Wirksamheit induzierter Resistenz unter praktischen Anbaubedingungen. III. Reduktion Echter Mehltaupilze auf induziert resistente Pflanzen. Z. Pflanzenkr. Pflanzensch. 91: 258-265.

Demirci, E. and Doken, M.T. 1989. Studies on the determination and identification of fungal agents of the diseases isolated from the roots, stems and leaves of potatoes in Erzurum region. Journal of Turkish Phytopathology 18: 51-60.

Di Pietro, A., Gut-Rella, M., Pachlatko, J.P. and Schwinn, F.J. 1992. Role of antibiotics produced by *Chaetomium globosum* in biocontrol of *Pythium ultimum*, a causal agent of damping-off. Phytopathology 82: 131-135.

Di Pietro, A., Lorito, M., Hayes, C.K., Broadway, R.M. and Harman, G.E. 1993. Endochitinase from *Gliocladium virens*: isolation, characterization and synergistic antifungal activity in combination with gliotoxin. Phytopathology 83: 308-313.

Dickinson, C.H. 1976. Fungi on the aerial surfaces of higher plants. In: Dickinson, C.H. and Preece, T.F. (eds), Microbiology of Aerial Plant Surfaces. Academic Press, London, pp 293-324.

Dickinson, C.H. 1981. Biology of *Alternaria alternata*, *Cladosporium cladosporioides* and *C. herbarum* in respect of their activity on green plants. In: Blakeman, J.P. (ed), Microbial Ecology of the Phylloplane. Academic Press, London, pp 169-184.

Dickinson, C.H. 1986. Adaptations of micro-organisms to climatic conditions affecting aerial plant surfaces. In: Fokkema, N.J. and Heuvel, J. van den (eds), Microbiology of the Phyllosphere. Cambridge University Press, Cambridge, pp 77-100.

Dickinson, C.H. and Preece, T.F. 1976. Microbiology of Aerial Plant Surfaces. Academic Press, London.

Diem, H.G. 1971. Effect of low humidity on the survival of germinated spores commonly found in the phyllosphere. In: Preece, T.F. and Dickinson, C.H. (eds), Ecology of Leaf Surface Micro-organisms. Academic Press, London, pp 211-219.

Dik, A.J., Fokkema, N.J. and Pelt, J.A. van 1991. Consumption of aphid honeydew, a wheat yield reducing factor, by phyllosphere yeasts under field conditions. Neth. J. Pl. Pathol. 97: 209-232.

Dommelen, L. van and Bollen, G.J. 1973. Antagonisms between benomyl-resistant fungi on cyclamen sprayed with benomyl. Acta Bot. Neerl. 22: 169-170.

Domsch, K.H., Gams, W. and Anderson, T.H. 1980. Compendium of Soil Fungi. Academic Press, London.

Dubos, B. 1992. Biological control of *Botrytis*: state-of-the-art. In: Verhoeff, K., Malathrakis, N.E. and Williamson, B. (eds), Recent Advances in *Botrytis* Research. Pudoc Scientific Publishers, Wageningen, pp 169-178.

Duthie, J.A. and Campbell, C.L. 1991. Effects of plant debris on intensity of leaf spot diseases, incidence of pathogens and growth of alfalfa. Phytopathology 81: 511-517.

Edmonds, R.L. 1972. Collection efficiency of Rotorod samplers for sampling fungus spores in the atmosphere. Plant Dis. Rep. 56: 704-708.

Edwards, M.C. and Blakeman, J.P. 1984. An autoradiographic method for determing nutrient competition between leaf epiphytes and plant pathogens. J. Microscopy 133: 205-212.

Edwards, S.G. and Seddon, E. 1992. *Bacillus brevis* as a biocontrol agent against *Botrytis cinerea* on protected Chinese cabbage. In: Verhoeff, K., Malathrakis, N.E. and Williamson, B. (eds), Recent Advances in *Botrytis* Research. Pudoc Scientific Publishers, Wageningen, pp 267-271.

Elad, Y. 1996. Mechanisms involved in the biological control of *Botrytis cinerea* incited diseases. Eur. J. Plant Pathol. 102: 719-732.

Elad, Y., Chet, I. and Henis, Y. 1982. Degradation of plant pathogenic fungi by *Trichoderma harzianum*. Can. J. Microbiol. 28: 719-725.

Elad, Y., Zimand, G., Zaqs, Y., Zuriel, S. and Chet, I. 1993. Use of *Trichoderma harzianum* in combination or alternation with fungicides to control cucumber grey mould (*Botrytis cinerea*) under commercial greenhouse conditions. Plant Pathol. 42: 324-332.

Elad, Y., Köhl, J. and Fokkema, N.J. 1994a. Control of infection and sporulation of *Botrytis cinerea* on bean and tomato by saprophytic yeasts. Phytopathology 84: 1193-1200.

Elad, Y., Köhl, J. and Fokkema, N.J. 1994b. Control of infection and sporulation of *Botrytis cinerea* on bean and tomato by saprophytic bacteria and fungi. Eur. J. Plant Pathol. 100: 315-336.

Ellerbrock, L.A. and Lorbeer, J.W. 1977. Sources of primary inoculum of *Botrytis squamosa*. Phytopathology 67: 363-372.

Ellis, M.B. 1971. Dematiaceous Hyphomycetes. Commonw. Mycol. Inst., Kew.

Elmer, P.A.G. and Köhl, J. 1998. The survival and saprophytic competitive ability of the *Botrytis* spp. antagonist *Ulocladium atrum* in lily canopies. Eur. J. Plant Pathol. 104: 435-447.

Elmer, P.A.G., Boyd-Wilson, K., Cook, D., Gaunt, R.E., Frampton, C. and Pyke, N. 1993. Sources of *Botrytis cinerea* inoculum in kiwifruit orchards and the relationship between total inoculum potential, external fruit contamination and stem end rot of kiwifruit. Abstracts of the 6th International Congress of Plant Pathology, Montreal, Canada, p 110.

Falconi, C. J. and Mendgen, K. 1994. Epiphytic fungi on apple leaves and their value for control of the postharvest pathogens *Botrytis cinerea*, *Monilinia fructigena* and *Penicillium expansum*. Z. Pflanzenkr. Pflanzensch. 101: 38-47.

Fernandez, M.R. 1992. The effect of *Trichoderma harzianum* on fungal pathogens infesting soybean residues. Soil Biol. Biochem. 24: 1027-1029.

Fernando, W.G.D., Watson, A.K. and Paulitz, T.C. 1994. The role of phylloplane *Pseudomonas* spp. in the enhancement of disease control caused by *Colletotrichum coccodes* on velvetleaf. Biol. Control 4: 125-131.

Fernando, W.G.D., Watson, A.K. and Paulitz, T.C. 1996. The role of *Pseudomonas* spp. and competition for carbon, nitrogen and iron in the enhancement of appressorium formation by *Colletotrichum coccodes* on velvetleaf. Eur. J. Plant Pathol. 102: 1-7.

Firbank, L.G. and Watkinson, A.R. 1985. On the analysis of competition within two-species mixtures of plants. J. Appl. Ecol. 22: 503-517.

Fitt, B.D.L., McCartney, H.A., Walklate, P.J. 1989. Role of rain in dispersal of pathogen inoculum. Annu. Rev. Phytopathol. 27: 241-270.

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

Fokkema, N.J. 1973. The role of saprophytic fungi in antagonism against *Drechslera sorokiniana* (*Helminthosporium sativum*) on agar plates and on rye leaves with pollen. Physiol. Plant Pathol. 3: 195-205.

Fokkema, N.J. 1984. Competition for endogenuous and exogenuous nutrients between *Sporobolomyces roseus* and *Cochliobolus sativus*. Can. J. Bot. 62: 2463-2468.

Fokkema, N.J. 1993. Opportunities and problems of control of foliar pathogens with micro-organisms. Pestic. Sci. 37: 411-416.

Fokkema, N.J. and Heuvel, J. van den 1986. Microbiology of the Phyllosphere. Cambridge University Press, Cambridge.

Fokkema, N.J. and Lorbeer, J.W. 1974. Interactions between *Alternaria porri* and the saprophytic mycoflora of onion leaves. Phytopathology 64: 1128-1133.

Fokkema, N.J., Riphagen, I., Poot, R.J. and Jong, C. de 1983. Aphid honeydew, a potential stimulant of *Cochliobolus sativus* and *Septoria nodorum* and the competitive role of saprophytic microflora. Trans. Br. mycol. Soc. 81: 355-363.

Fokkema, N.J., Gerlagh, M. and Köhl, J. 1992. Biological control of *Sclerotinia sclerotiorum* and *Botrytis* spp. In: Tjamos, E.C., Papavizas, G. and Cook, R.J. (eds), Biological Control of Plant Diseases - Progress and Challenge for the Future (Proc. Nat. Advanced Research Workshop). Plenum Press, New York, pp 267-271.

Frossard, R., Fokkema, N.J. and Tietema, T. 1983. Influence of *Sporobolomyces roseus* and *Cladosporium cladosporioides* on leaching of ^{14}C-labelled assimilates from wheat leaves. Trans. Br. mycol. Soc. 80: 289-296.

Frey, S. and Magan, N. 2001. Production of the fungal biocontrol agent *Ulocladium atrum* by submerged fermentation: accumulation of endogenous reserves and shelf-life studies. Appl. Microbiol. Biotechnol. 56: 372-377.

Fruit, L. 2001. Etude des facteurs d'efficacité d'un agent de lutte biologique (*Ulocladium atrum*) pour la protection des plaies d'effeuillage sur tomate contre *Botrytis cinerea*. PhD ThesisUniversite Claude Bernard, Lyon, France.

Fruit, L. and Nicot, P. 1999. Biological control of *Botrytis cinerea* on tomato stem wounds with *Ulocladium atrum*. Integrated Control in Glasshouses. IOBC Bulletin 22, pp. 81-84.

Gerlagh, M., Goossen-van der Geijn, H.M., Verdam, B. and Fokkema, N.J. 1995. Biological control of white mould (*Sclerotinia sclerotiorum*) in various crops by application of *Coniothyrium minitans*. In: Whipps, J. M. and Gerlagh, T. (eds), Biological Control of Sclerotium-forming Fungi, IOBC/WPRS Bulletin 18(3), pp 13-17.

Gerlagh, M., Amsing, J.J., Molhoek, W.M.L., Bosker-van Zessen, A.I., Lombaers-van der Plas, C.H. and Köhl, J. 2001. The effect of treatment with *Ulocladium atrum* on *Botrytis cinerea*-attack of geranium (*Pelargonium zonale*) stock plants and cuttings. Eur. J. Plant Pathol. 107: 377-386.

Ghisalberti, E.L. and Sivasithamparam, K. 1991. Antifungal antibiotics produced by *Trichoderma* spp. Soil Biol. Biochem. 23: 1011-1020.

Good, H.M. and Zathureczky, P.G.M. 1967. Effects of drying on the viablility of germinated spores of *Botrytis cinerea*, *Cercospora musae*, and *Monilinia fructicola*. Phytopathology 57: 719-722.

Grantzau, E. and Hell, B. ter 1993. *Botrytis* bei Cyclamen. Einfluss von Bewässerungssystem, Düngung oder Substrat. Gartenbau und Gartenwirtschaft 93: 542-543.

Gregory, P.H. and Hirst, J.M. 1957. The summer air-spora at Rothamsted in 1952. J. Gen. Microbiol. 17: 135-152.

Gullino, M.L. 1992. Chemical control of *Botrytis* spp. In: Verhoeff, K., Malathrakis, N.E. and Williamson, B. (eds), Recent Advances in *Botrytis* Research. Pudoc Scientific Publishers, Wageningen, pp 217-222.

Hajlaoui, M.R., Traquair, J.A., Jarvis, W.R. and Bélanger, R.R. 1994. Antifungal activity of extracellular metabolites produced by *Sporothrix flocculosa*. Biocontrol Sci. Technol. 4: 229-237.

Hancock, J.G. and Lorbeer, J.W. 1963. Pathogenesis of *Botrytis cinerea*, *B. squamosa*, and *B. allii* on onion leaves. Phytoapthology 53: 669-673.

Hannusch, D.J. and Boland, G.J. 1996a. Interactions of air temperature, relative humidity and biological control agents on grey mold of bean. Eur. J. Plant Pathol. 102: 133-142.

Hannusch, D.J. and Boland, G.J. 1996b. Influence of air temperature and relative humidity on biological control of white mold of bean (*Sclerotinia sclerotiorum*). Phytopathology 86: 156-162.

Hau, B. and Kranz, J. 1978. Modellrechnungen zur Wirkung des Hyperparasiten *Eudarluca caricis* auf Rostepidemien. Z. Pflanzenkr. Pflanzensch. 85: 131-141.

Hausbeck M.K. and Moorman, G.W. 1996. Managing *Botrytis* in greenhouse-grown flower crops. Plant Dis. 80: 1212-1219.

Hausbeck, M.K. and Pennypacker, S.P. 1991a. Influence of grower activity and disease incidence on concentrations of airborne conidia of *Botrytis cinerea* among geranium stock plants. Plant Dis. 75: 798-803.

Hausbeck, M.K. and Pennypacker, S.P. 1991b. Influence of grower activity on concentrations of airborne conidia of *Botrytis cinerea* among geranium cuttings. Plant Dis. 75: 1236-1243.

Hausbeck, M.K., Pennypacker, S.P. and Stevenson, R.E. 1996. The effect of plastic mulch and forced heated air on *Botrytis cinerea* on geranium stock plants in a research greenhouse. Plant Dis. 80: 170-173.

Helbig, J. 2001. Biological control of *Botrytis cinerea* Pers. ex. Fr. in strawberry by *Paenibacillus polymyxa* (isolate 18191). J. Phytopathol. 149: 265-273.

Henseler, K. 1981. Fungizide beeinträchtigen den Ertrag. Möglichkeiten der *Botrytis*-Bekämpfung bei Schnittcyclamen. Gartenbau und Gartenwirtschaft 81: 382.

Heye, C.C. and Andrews, J.H. 1983. Antagonism of *Athelia bombacina* and *Chaetomium globosum* to the apple scab pathogen, *Venturia inaequalis*. Phytopathology 73: 650-654.

Hjeljord, L.G., Stensvand, A. and Tronsmo, A. 2000. Effect of temperature and nutrient stress on the capacity of commercial *Trichoderma* products to control *Botrytis cinerea* and *Mucor piriformis* in greenhouse strawberries. Biol. Control 19: 149-160.

Hocking, A.D., Miscamble, B.F. and Pitt, J.I. 1994. Water relations of *Alternaria alternata*, *Cladosporium cladosporioides*, *Cladosporium sphaerospermum*, *Curvularia lunata* and *Curvularia pallescens*. Mycol. Res. 98: 91-94.

Howell, C.R. and Stipanovic, R.D. 1995. Mechanisms in the biocontrol of *Rhizoctonia solani*-induced cotton seedling disease by *Gliocladium virens*-antibiosis. Phytopathology 85: 469-472.

Hudson, H.J. 1971. The development of the saprophytic fungal flora as leaves senesce and fall. In: Preece, T.F. and Dickinson, C.H. (eds), Ecology of Leaf Surface Micro-organisms. Academic Press, London, pp 447-455.

Hudson, H.J. and Webster, J. 1958. Succession of fungi on decaying stems of *Agropyron repens*. Trans. Br. mycol. Soc. 41: 165-177.

Iyozumi, H., Komagata, T., Hirayae, K., Tsuchiya, K., Hibi, T. and Akutsu, K. 1996. Biological control of cyclamen gray mould (*Botrytis cinerea*) by *Serratia marcescens* B2. Ann. Phytopathol. Soc. Jpn. 62: 559-565.

Jackson, A.M., Whipps, J.M. and Lynch, J.M. 1991. Effects of temperature, pH and water potential on growth of four fungi with disease biocontrol potential. World J. Microbiol. Biotechnol. 7: 494-501.

Jacob, M. 1987. Bekämpfung bedeutender Schaderreger bei der Produktion von Cyclamen. Gartenbau 34: 281-283.

Janisiewicz, W.J. and Roitman, J. 1988. Biological control of blue mold and gray mold on apple and pear with *Pseudomonas cepacia*. Phytopathology 78: 1697-1700.

Jarvis, W.R. 1962a. The epidemiology of *Botrytis cinerea* Pers. in strawberries. Proceedings 16th International Horticultural Congress Vol.III, pp 258-262.

Jarvis, W.R. 1962b. The infection of strawberry and raspberry fruits by *Botrytis cinerea* Fr.. Ann. Appl. Biol. 50: 569-575.

Jarvis, W.R. 1962c. The dispersal of spores of *Botrytis cinerea* Fr. in a rasberry plantation. Trans. Br. mycol. Soc. 45: 549-559.

Jarvis, W.R. 1980. Epidemiology. In: Coley-Smith, J.R., Verhoeff, K. and Jarvis, W.R. (eds), The Biology of *Botrytis*. Acad. Press, London, UK, pp 219-250.

Johnson, C.S., Beute, M.K. and Ricker, M.D. 1986. Relationship between components of resistance and disease progress of early leaf spot on Virginia-type peanut. Phytopathology 76: 495-499.

Jongebloed, P.H. J., Kessel, G.J.T., Molhoek, W.M.L., Plas, C.H. van der and Fokkema, N.J. 1993. Biological control of *Phytophthora infestans* with compost extracts and selected bacterial antagonists. In: Fokkema, N.J., Köhl, J. and Elad, Y. (eds), Biological Control of Foliar and Postharvest Diseases IOBC/WPRS Bulletin 16(11), pp 16-20.

Jordan, V.W.L. 1978. Epidemiologie und Bekämpfung des Grauschimmels (*Botrytis cinerea*) an Erdbeeren. Pflanzenschutz-Nachrichten 31: 1-10.

Jordan, V.W.L. and Pappas, A.C. 1977. Inoculum suppression and control of strawberry *Botrytis*. Proceedings British Crop Protection Conference - Pests and Diseases: 341-348.

Karpovich-Tate, N. and Dewey, F.M. 2001. Quantification of *Ulocladium atrum* in necrotic plant tissues by monoclonal antibody-based enzyme-linked immunosorbent assay. Mycol. Res. 105: 567-574.

Kerssies, A. 1990. A selective medium for *Botrytis cinerea* to be used in a spore trap. Neth. J. Plant Pathol. 96: 247-250.

Kessel, G.J.T. 1999. Biological control of *Botrytis* spp. by *Ulocladium atrum*: an ecological analysis. PhD ThesisWageningen University, Wageningen.

Kessel, G.J.T., Haas, B.H. de, Lombaers-van der Plas, C.H., Meijer, E.M.J., Dewey, F.M., Goudriaan, J., Werf, W. van der and Köhl, J. 1999. Quantification of mycelium of *Botrytis* spp. and the antagonist *Ulocladium atrum* in necrotic leaf tissue of cyclamen and lily by fluorescence microscopy and image analysis. Phytopathology 89: 868-876.

Kessel, G.J.T., Haas, B.H. de, Lombaers-van der Plas, C.H., Ende, J.E. van der, Pennock-Vos, M.G., Werf, W. van der and Köhl, J. 2001. Comparative analysis of the role of substrate specificity in biological control of *Botrytis elliptica* in lily and *B. cinerea* in cyclamen with *Ulocladium atrum*. Eur. J. Plant Pathol. 107: 273-284.

Kessmann, H., Staub, T., Hofmann, C., Maetzke, T., Herzog, J., Ward, E., Uknes, S. and Ryals, J. 1994. Induction of systemic acquired disease resistance in plants by chemicals. Annu. Rev. Phytopathol. 32: 439-459.

Klepzig, K.D. and Wilkens, R.T. 1997. Competitive interactions among symbiotic fungi of the southern pine beetle. Appl. Environ. Microbiol. 63: 621-627.

Köhl, J. and Fokkema, N.J. 1994. Fungal interactions on living and necrotic leaves. In: Blakeman, J.P. and Williamson, B. (eds), Ecology of Plant Pathogens. CABI, Oxon, pp 321-334.

Köhl, J. and Fokkema, N.J. 1998. Strategies for biological control of necrotrophic fungal foliar pathogens. In: Boland, G.J. and Kuykendall, L.D. (eds), Plant-Microbe Interactions and Biological Control. Marcel Dekker, Inc, New York, pp. 49-88.

Köhl, J. and Gerlagh, M. 1999. Biological control of *Botrytis cinerea* in roses by the antagonist *Ulocladium atrum*. Med. Fac. Landbouww. Univ. Gent 64/3b: 441-445.

Köhl, J. and Molhoek, W.M.L. 2001. Effect of water potential on conidial germination and antagonism of *Ulocladium atrum* against *Botrytis cinerea*. Phytopathology 91: 485-491.

Köhl, J. and Schlösser, E. 1988. Occurrence and temperature requirements of four *Trichoderma* species from different regions and substrates. Angew. Bot. 62: 301-309.

Köhl, J. and Schlösser, E. 1989. Decay of sclerotia of *Botrytis cinerea* by *Trichoderma* spp. at low temperatures. J. Phytopathol. 125: 320-326.

Köhl, J., Krijger, M.C. and Kessel, G.J.T. 1992. Drought tolerance of *Botrytis squamosa*, *B. aclada* and potential antagonist. In: Verhoeff, K., Malathrakis, N.E. and Williamson, W. (eds), Recent Advances in *Botrytis* Research. Pudoc Scientific Publishers, Wageningen, pp 206-210.

Köhl, J., Plas, C.H. van der, Molhoek, W.M.L. and Fokkema, N.J. 1993. Drought tolerance as a major selection criterium for antagonists of *Botrytis* spp. In: Fokkema, N.J., Köhl, J. and Elad, Y. (eds), Biological Control of Foliar and Postharvest Diseases IOBC/WPRS Bulletin 16(11), pp 169-172.

Köhl, J., Molhoek, W.M.L., Goossen-van de Geijn, H.M. and Lombaers-van der Plas, C.H. 2003. Potential of *Ulocladium atrum* for biocontrol of onion leaf spot through suppression of sporulation of *Botrytis* spp. BioControl 48: 349-359.

Köhl, J., Molhoek, W.M.L., Plas, C.H. van der and Fokkema, N.J. 1995a. Suppression of sporulation of *Botrytis* spp. as a valid biocontrol strategy. Eur. J. Plant Pathol. 101: 251-259.

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

Köhl, J., Plas, C.H. van der, Molhoek, W.M.L. and Fokkema, N.J. 1995c. Effect of interrupted leaf wetness periods on suppression of sporulation of *Botrytis allii* and *B. cinerea* by antagonists on dead onion leaves. Eur. J. Plant Pathol. 101: 627-637.

Köhl, J., Bélanger, R.R. and Fokkema, N.J. 1997. Interaction of four antagonistic fungi with *Botrytis aclada* in dead onion leaves: A comparative microscopic and ultrastructural study. Phytopathology 87: 634-642.

Köhl, J., Gerlagh, M., Haas, B.H. de and Krijger, M.C. 1998. Biological control of *Botrytis cinerea* in cyclamen with *Ulocladium atrum* and *Gliocladium roseum* under commercial growing conditions. Phytopathology 88: 568-575.

Köhl, J., Lombaers-van der Plas, C.H., Molhoek, W.M.L., Kessel G.J. and Goossen-van der Geijn, H.J. 1999. Competitive ability of the antagonist *Ulocladium atrum* and *Gliocladium roseum* at temperatures favourable for *Botrytis* spp. development. BioControl 44: 329-346.

Köhl, J., Gerlagh, M. and Grit, G. 2000. Biocontrol of *Botrytis cinerea* by *Ulocladium atrum* in different production systems of cyclamen. Plant Dis. 84: 569-573.

Kovach, J., Petzoldt, R. and Harman, G.E. 2000. Use of honey bees and bumble bees to disseminate *Trichoderma harzianum* 1295-22 to strawberries for *Botrytis* control. Biol. Control 18: 235-242.

Kranz, J. 1981. Hyperparasitism of biotrophic fungi. In: Blakeman, J.P. (ed), Microbial Ecology of the Phylloplane. Academic Press, London, pp 327-352.

Kurze, S., Bahl, H., Dahl, R. and Berg, G. 2001. Biological control of fungal strawberry diseases by *Serratia plymuthica* HRO-C48. Plant Dis. 85: 529-534.

Labrinos, J.L. and Nutter, F.W. Jr. 1993. Effects of a protectant versus a systemic fungicide on disease components of peanut late leaf spot. Plant Dis. 77: 837-845.

Lacroix, L. and Gouot, J.M. 1981. Etude en serre de la resistance de *Botrytis cinerea* a l'iprodione. Med. Fac. Landbouww. Rijksuniv. Gent 46: 979-989.

Leifert, C., Li, H., Chidburee, S., Hampson, S., Workman, S., Sigee, D., Epton, H.A.S. and Harbour, A. 1995. Antibiotic production and biocontrol activity by *Bacillus subtilis* CL27 and *Bacillus pumilus* CL45. J. Appl. Bacteriol. 78: 97-108.

Lennartz, B., Schoene, P. and Oerke, E.C. 1998. Biocontrol of *Botrytis cinerea* on grapevine and *Septoria* spp. on wheat. Med. Fac. Landbouww. Univ. Gent 63/3b: 963-970.

Leonard, K.J. and Mundt, C.C. 1984. Methods for estimating epidemiological effects of quantitative resistance to plant diseases. Theor. Appl. Genet. 67: 219-230.

Li, H. and Leifert, C. 1994. Development of resistance in *Botryotinia fuckeliana* (de Bary) Whetzel against the biological control agent *Bacillus subtilis* CL27. Z. Pflanzenkr. Pflanzensch. 101: 414-418.

Lima, G., Ippolito, A., Nigro, F. and Salerno, M. 1997. Effectiveness of *Aureobasidium pullulans* and *Candida oleophila* against postharvest strawberry rots. Postharv. Biol. Tech. 10: 169-178.

Linke, K.H., Scheibel, C., Saxena, M.C. and Sauerborn, J. 1992. Fungi occurring on *Orobanche* spp. and their preliminary evaluation for *Orobanche* control. Tropical Pest Manage. 38: 127-130.

Lockwood, J.L. 1988. Evolution of concepts associated with soilborne plant pathogens. Annu. Rev. Phytopathol. 26: 93-121.

Lockwood, J.L. 1992. Exploitation competition. In: Carroll, G.C. and Wicklow, D. T. (eds), The Fungal Community. Marcel Dekker, New York, pp 243-261.

Logan, J.A., Wollkind, D.J., Hoyt, S.C. and Tanigoshi, L.K. 1976. An analytical model for description of temperature dependant rate phenomena in arthropods. Environ. Entomol. 5: 1133-1140.

López-Herrera, C.J., Verdú-Valiente, B. and Melero-Vara, J.M. 1994. Eradication of primary inoculum of *Botrytis cinerea* by soil solarization. Plant Dis. 78: 594-597.

Lorbeer, J.W. 1966. Diurnal periodicity of *Botrytis squamosa* conidia in the air. Phytopathology 56: 887.

Maas, J.L. 1984. Compendium of strawberry diseases. American Phytopathological Society, St. Paul, USA.

MacCullagh, P. and Nelder, J.A. 1989. Generalized Linear Models. 2nd ed. Chapman and Hall, London.

Madrigal, C., Pascual, S. and Melgarejo, P. 1994. Biological control of peach twig blight (*Monilinia laxa*) with *Epicoccum nigrum*. Plant Pathol. 43: 554-561.

Magan, N. 1988. Effects of water potential and temperature on spore germination and germ-tube growth in vitro and in straw leaf sheaths. Trans. Br. mycol. Soc. 90: 97-107.

Magan, N. and Lacey, J. 1984. Effect of temperature and pH on water relations of field and storage fungi. Trans. Br. mycol. Soc. 82: 71-81.

Magan, N. and Lynch, J.M. 1986. Water potential, growth and cellulolysis of fungi involved in decomposition of cereal residues. J. Gen. Microbiol. 132: 1181-1187.

Maude, R.B., Shipway, M.R., Presley, A.H. and O'Connor, D. 1984. The effects of direct harvesting and drying systems on the incidence and control of neck rot (*Botrytis allii*) in onions. Plant Pathol. 33: 263-268.

McCormack, P.J., Wildman, H.G. and Jeffries, P. 1994. Production of antibacterial compounds by phylloplane-inhabiting yeasts and yeastlike fungi. Appl. Environ. Microb. 60: 927-931.

McQuilken, M.P., Whipps, J.M. and Cooke, R.C. 1990. Control of damping-off in cress and sugar-beet by commercial seedcoating with *Pythium oligandrum*. Plant Pathol. 39: 452-462.

Mead, R. 1988. The Design of Experiments. Cambridge University Press, Cambridge, UK.

Meekes, E.T.M., Köhl, J., Molhoek, W.M.L., Goossen-van der Geijn, H.M.G. and Gerlagh, M. 2002. Biologische bestrijding van bovengrondse plantenziekten met *Ulocladium atrum*. In: Wijnands, F.G., Schröder, J.J., Sukkel, W. and Booij, R. (eds), Biologisch Bedrijf Onder de Loep – Biologische Akkerbouw en Vollegrondsgroenteteelt in Perspectief, Praktijkonderzoek Plant en Omgeving, Lelystad, pp 103-112.

Mertely, J.C., Chandler, C.K., Xiao, C.L. and Legard, D.E. 2000. Comparison of sanitation and fungicides for management of *Botrytis* fruit rot of strawberry. Plant Dis. 84: 1197-1202.

Metz, C., Oerke, E.-C. and Dehne, H.-W. (in press). Biological control of grey mould (*Botrytis cinerea*) with the antagonist *Ulocladium atrum*. Med. Fac. Landbouww. Univ. Gent.

Michailides, T.J. and Spotts, R.A. 1991. Effects of certain herbicides on the fate of sporangiospores of *Mucor piriformis* and conidia of *Botrytis cinerea* and *Penicillium expansum*. Pestic. Sci. 33: 11-22.

Miedtke, U. and Kennel, W. 1990. *Athelia bombacina* and *Chaetomium globosum* as antagonists of the perfect stage of the apple scab pathogen (*Venturia inaequalis*) under field conditions. Z. Pflanzenkr. Pflanzensch. 97: 24-32.

Miller, P.M. and Waggoner, P.E. 1957. Dispersal of spores of *Botrytis cinerea* among strawberries. Phytopathology 47: 24-25.

Mizubuti, E.S.G., Maffia, L.A., Muchovej, J.J., Romeiro, R.S. and Batista, U.G. 1995. Epidemiological aspects of *Uromyces appendiculatus* on dry bean (*Phaseolus vulgaris*) after treatment with *Bacillus subtilis*. J. Phytopathol. 143: 689-691.

Morandi, M.A.B., Sutton, J.C. and Maffia, L.A. 2000. Effects of host and microbial factors on development of *Clonostachys rosea* and control of *Botrytis cinerea* in rose. Eur. J. Plant Pathol. 106: 439-448.

Morandi, M.A.B., Maffia, L.A. and Sutton, J.C. 2001. Development of *Clonostachys rosea* and interactions with *Botrytis cinerea* in rose leaves and residues. Phytoparasitica 29: 103-113.

Morris, C., Nicot, P. and Nguyen-The, C. 1996. Aerial Plant Surface Microbiology. Plenum Press, New York.

Morton, L.H.G. and Eggins, H.O.W. 1977. The effect of constant, alternating and fluctuating temperatures on the growth of some wood inhabiting fungi. Int. Biodeterior. 13: 116-122.

Mostafa, M.M. 1993. Biological control of *Drechslera teres*: ability of antagonists to reduce conidia formation, coleoptile infection and leaf infection in barley (*Hordeum vulgare*). Cryptogamie Mycol. 14: 287-295.

Nair, N.G. and Allen, R.N. 1993. Infection of grape flowers and berries by *Botrytis cinerea* as a function of time and temperature. Mycol. Res. 97: 1012-1014

Nair, N.G. and Nadtotchei, A. 1987. Sclerotia of *Botrytis* as a source of primary inoculum for bunch rot of grapes in New South Wales, Australia. J. Phytopathol. 119: 42-51.

Nelson, M.E. and Powelson, M.L. 1988. Biological control of grey mold of snap beans by *Trichoderma hamatum*. Plant Dis. 72: 727-729.

Newhook, F.J. 1957. The relationship of saprophytic antagonism to control of *Botrytis cinerea* Pers. on tomatoes. New Zeal. J. Sci. Technol. 38: 473-481.

Newton, M.R., Kinkel, L.L. and Leonard, K.J. 1998. Constraints on the use of de Wit models to analyze competitive interactions. Phytopathology 88: 873-878.

Nicot, P.C., Decognet, V., Fruit, L., Bardin, M. and Trottin, Y. (in press). Combined effect of microclimate and dose of application on the efficacy of biocontrol agents for protection of pruning wounds on tomatoes against *Botrytis cinerea*. In: Elad, Y., Köhl, J. and Shtienberg, D. (eds), Influence of A-biotic and Biotic Factors on Bio-control Agents, IOBC/WPRS Bulletin.

Nielsen, K. 2000. Molecular characterisation and biological control of grey mould (*Botrytis* spp.) in onion. PhD thesis Danish Institute of Agricultural Sciences, Slagelse, Denmark.

O´Neill, T.M., Shtienberg, D. and Elad, Y. 1997. Effect of some host and microclimate factors on infection of tomato stems by *Botrytis cinerea*. Plant Dis. 81: 36-40.

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: Biology, ecology, and potential for bio-control. Annu. Rev. Phytopathol. 23: 23-54.

Pappas, A.C. 1982. Inadequate control of grey mould on cyclamen by dicarboximide fungicides in Greece. Z. Pflanzenkr. Pflanzensch. 89: 52-58.

Park, D. 1982. Phylloplane fungi: Tolerance of hyphal tips to drying. Trans. Br. mycol. Soc. 79: 174-178.

Pascual, S., Magan, N., and Melgarejo, P. 1996. Improved biological control of peach twig blight by physiological manipulation of *Epicoccum nigrum*. Proceedings of the Brighton Crop Protection Conference - Pests and Diseases: 411-412.

Paulitz, T.C. and Bélanger, R.R. 2001. Biological control in greenhouse systems. Annu. Rev. Phytopathol. 39: 103-133.

Payne, G.A., Duncan, H.E. and Adkins, C.R. 1987. Influence of tillage on development of gray leaf spot and number of airborne conidia of *Cercospora zeae-maydis*. Plant Dis. 71: 329-332.

Peng, G. and Sutton, J.C. 1991. Evaluation of microorganisms for biocontrol of *Botrytis cinerea* in strawberry. Can. J. Plant Pathol. 13: 247-257.

Pfender, W.F. 1988. Suppression of ascocarp formation in *Pyrenophora tritici-repentis* by *Limonomyces roseipellis*, a basidiomycete from reduced-tillage wheat straw. Phyto-pathology 78: 1254-1258.

Pfender, W.F., Sharma, U. and Zhang, W. 1991. Effect of water potential on microbial antagonism to *Pyrenophora tritici-repentis* in wheat residue. Mycol. Res. 95: 308-314.

Pfender, W.F. and Wootke, S.L. 1988. Microbial communities of *Pyrenophora*-infested wheat straw as examined by multivariate analysis. Microbial Ecol. 15: 95-113.

Pfender, W.F., Zhang, W. and Nus, A. 1993. Biological control to reduce inoculum of the tan spot pathogen *Pyrenophora tritici-repentis* in surface-borne residues of wheat fields. Phytopathology 83: 371-375.

Philipp, W.D. and Crüger, G. 1979. Parasitismus von *Ampelomyces quisqualis* auf Echten Mehltaupilzen an Gurken und anderen Gemüsearten. Z. Pflanzenkr. Pflanzensch. 86: 129-142.

Plaut, J.L. and Berger, R.D. 1981. Infection rates in three pathosystem epidemics initiated with reduced disease severities. Phytopathology 71: 917-921.

Preece, T.F. and Dickinson, C.H. 1971. Ecology of Leaf Surface Micro-organisms. Academic Press, London.

Raposo, R., Gomez, V., Urrutia, T. and Melgarejo, P. 2001. Survival of *Botrytis cinerea* in southeastern Spanish greenhouses. Eur. J. Plant Pathol. 107: 229-236.

Rappoldt C. and Kraalingen, D.W.G. van 1996. The Fortran Simulation Translator, FST version 2.0. Introduction and reference manual. Quantitative Approaches in Systems Analysis No 5. DLO-Research Institute for Agrobiology and Soil Fertility, The C.T. de Wit Graduate School for Production Ecology, Wageningen.

Rayner, A.D.M. and Webber, J.F. 1984. Interspecific mycelial interactions - an overview. In: Jennings, D.H. and Rayner, A.D.M. (eds), The Ecology and Physiology of Fungal Mycelium. Cambridge University Press, Cambridge, pp 383-417.

Reimherr, P. 1985. Atlas der Krankheiten und Schädlinge bei Cyclamen. Gartenbau und Gartenwirtschaft 85: 1780-1790.

Ringer, C.E. and Grybauskas, A.P. 1995. Infection cycle components and disease progress of gray leaf spot on field corn. Plant Dis. 79: 24-28.

Roger, C., Tivoli, B. and Huber, L. 1999. Effects of interrupted wet periods and different temperatures on the development of ascochyta blight caused by *Mycosphaerella pinodes* on pea (*Pisum sativum*) seedlings. Plant Pathol. 48: 10-18.

Rosati, P. 1991. The strawberry in Europe. In: Dale, A. and Luby, J.J. (eds), The Strawberry into the 21st Century. Proceedings of the third North American Strawberry Conference, Houston, USA, 14-16 February 1990, Timber Press, Oregon, USA, pp 27-35.

Rosslenbroich, H.-J. and Stuebler, D. 2000. *Botrytis cinerea* – history of chemical control and novel fungicides for its management. Crop Prot. 19: 557-561.

Rotem, J. 1990. Overwintering of *Alternaria macrospora* in cotton debris. Phytoparasitica 18: 143-152.

Rotem, J. and Aust, H.J. 1991. The effect of ultraviolet and solar radiation and temperature on survival of fungal propagules. J. Phytopathol. 133: 76-84.

Rotem, J., Wooding, B. and Aylor, D.E. 1985. The role of solar radiation, especially ultraviolet, in the mortality of fungal spores. Phytopathology 75: 510-514.

Sache, I. and Vallavieille-Pope, C. de 1995. Classification of airborne plant pathogens based on sporulation and infection characteristics. Can. J. Bot. 73: 1186-1195.

Schoene, P., Lennartz, B. and Oerke, E.-C. 1999. Fungicide sensitivity of fungi used for biocontrol of perthotrophic pathogens. In: Lyr, H., Russell, P.E. and Dehne, H.-W. (eds), Modern Fungicides and Antifungal Compounds. Proceedings of the 12th International Reinhardsbrunn Symposium, Friedrichsroda, Germany, pp 477-482.

Schoene, P., Oerke, E.-C. and Dehne, H.-W. 2000. A new concept for integrated control of grey mould (*Botrytis cinerea*) in grapevine. Proceedings British Crop Protection Conference - Pests and Diseases: 1031-1036.

Schlösser, E. 1978. Entwicklungsstadien von Alpenveilchenblättern (*Cyclamen persicum*) - Besiedlung durch *Botrytis cinerea*. Z. Pflanzenkr. Pflanzensch. 85: 179-185.

Schönbeck, F. and Dehne, H.-W. 1986. Use of microbial metabolites inducing resistance against plant pathogens. In: Fokkema, N.J. and Heuvel, J. van den (eds), Microbiology of the Phyllosphere. Cambridge University Press, Cambridge, pp 363-375.

Schönherr, J. and Baur, P. 1996. Cuticle permeability studies: A model for estimating leaching of plant metabolites to leaf surfaces. In: Morris, C., Nicot, P. and Nguyen-The, C. (eds), Aerial Plant Surface Microbiology. Plenum Press, New York, pp 1-23.

Schuh, W. 1993. Influence of interrupted dew periods, relative humidity, and light on disease severity and latent infections caused by *Cercospora kikuchii* on soybean. Phytopathology 83: 109-113.

Seddon, B. and Edwards, S.G. 1993. Analysis of and strategies for the biocontrol of *Botrytis cinerea* by *Bacillus brevis* on protected Chinese cabbage. In: Fokkema, N.J., Köhl, J. and Elad, Y. (eds), Biological Control of Foliar and Postharvest Diseases IOBC/WPRS Bulletin 16(11), pp 38-41.

Seddon, B., Edwards, S.G., Markellou, E. and Malathrakis, N.E. 1997. Bacterial antagonist - fungal pathogen interactions on the plant aerial surface. In: Gange, A.C. and Brown V.K. (eds), Multitrophic Interactions in Terrestrial Systems. Blackwell Scientific Publishers, pp 5-25.

Shtienberg, D., Elad, Y., Niv, A., Nitzani, Y. and Kirshner, B. 1998. Significance of leaf infection by *Botrytis cinerea* in stem rotting of tomatoes grown in non-heated greenhouses. Eur. J. Plant Pathol. 104: 753-763.

Sirjusingh, C. and Sutton, J.C. 1996. Effects of wetness duration and temperature on infection of geranium by *Botrytis cinerea*. Plant Dis. 80: 160-165.

Sirjusingh, C., Sutton, J.C. and Tsujita, M.J. 1996. Effects of inoculum concentration and host age on infection of geranium by *Botrytis cinerea*. Plant Dis. 80: 154-159.

Smits, J.P. 1998. Solid-state fermentation, modelling fungal growth and activity. PhD thesis, Wageningen Agricultural University, Wageningen.

Snaydon, R.W. 1991. Replacement or additive designs for competition studies? J. Appl. Ecol. 28: 930-946.

Snedecor, G.W. and Cochran, W.G. 1989. Statistical Methods. Iowa State Univ. Press, 8th ed., Iowa, USA.

Sosa-Alvarez, M., Madden, L.V. and Ellis, M.A. 1995. Effects of temperature and wetness duration on sporulation of *Botrytis cinerea* on strawberry leaf residues. Plant Dis. 79: 609-615.

Spencer, D.M. 1980. Parasitism of carnation rust (*Uromyces dianthi*) by *Verticillium lecanii*. Trans. Br. mycol. Soc. 74: 191-194.

Sutton, J.C. 1990. Epidemiology and management of *Botrytis* leaf blight of onion and gray mold of strawberry: a comparative analysis. Can. J. Plant Pathol. 12: 100-110.

Sutton, J.C. 1994. Biological control of strawberry diseases. Advances in strawberry research 13: 1-12.

Sutton, J. C. and Peng, G. 1993. Biocontrol of *Botrytis cinerea* in strawberry leaves. Phytopathology 83: 615-621.

Sutton, J.C., Swanton, C.J. and Gillespie, T.J. 1978. Relation of weather variables and host factors to incidence of airborne spores of *Botrytis squamosa*. Can. J. Bot. 56: 2460-2469.

Sutton, J.C., James, T.D.W. and Rowell, P.M. 1986. BOTCAST: A forcasting system to time the initial fungicide spray for managing *Botrytis* leaf blight of onions. Agr. Ecosyst. Environ. 18: 123-143.

Sutton, J. C., Li, D.-W., Peng, G., Yu, H., Zhang, P. and Valdebenito-Sanhueza, R.M. 1997. *Gliocladium roseum*, a versatile adversary of *Botrytis cinerea* in crops. Plant Dis. 81: 316-328.

Swinburne, T.R. 1981. Iron and iron chelating agents as factors in germination, infection and aggression of fungal pathogens. In: Blakeman, J. P. (ed), Microbial Ecology of the Phylloplane. Academic Press, London, pp 227-243.

Szandala, E.S. and Backhouse, D. 2001. Suppression of sporulation of *Botrytis cinerea* by antagonists applied after infection. Austr. Plant Pathol. 30: 165-170.

Thakur, V.S. and Sharma, R.D. 1999. Effect of urea on microbial degradation of apple leaf litter and its relationship to the inhibition of pseudothecial development of *Venturia inaequalis*. Indian J. Agr. Sci. 69: 147-151.

Thomas, M.R. and Shattock, R.C. 1986. Effects of fungicides on *Drechslera* spp. and leaf surface filamentous saprophytic fungi on *Lolium perenne*. Plant Pathol. 35: 120-125.

Tukey, H.B. Jr. 1970. The leaching of substances from plants. Annu. Rev. Plant Phys. 21: 305-324.

Turkensteen, L.J. 1979. *Ulocladium atrum*, el organismo causal de Kasahui, enfermedad foliar de la papa en el altiplano del Peru. Fitopatologia 14: 85-86.

Upper, C.D. 1992. Manipulation of microbial communities in the phyllosphere. In: Andrews, J.H. and Hirano, S.S. (eds), Microbial Ecology of Leaves. Springer, New York, pp 451-463.

Verhaar, M.A., Strien, P.A.C. van and Hijwegen, T. 1993. Biological control of cucumber powdery mildew (*Sphaerotheca fuliginea*) by *Verticillium lecanii* and *Sporothrix* cf. *flocculosa*. In: Fokkema, N.J., Köhl, J. and Elad, Y. (eds), Biological Control of Foliar and Postharvest Diseases, IOBC/WPRS Bulletin 16(11), pp 79-81.

Vincelli, P.C. and Lorbeer, J.W. 1989. BLIGHT-ALERT: A weather-based predictive system for timing fungicide applications on onion before infection periods of *Botrytis squamosa*. Phytopathology 79: 493-498.

Visser, C.L.M. de 1996. Field evaluation of a supervised control system for *Botrytis* leaf blight in spring sown onions in the Netherlands. Eur. J. Plant Pathol. 102: 795-805.

Vloutoglou, I., Fitt, B.D.L. and Lucas, J.A. 1995. Periodicity and gradients in dispersal of *Alternaria linicola* in linseed crops. Eur. J. Plant Pathol. 101: 639-653

Wessels, J.G.H. and Sietsema, J.H. 1984. Wall structure and growth in *Schizophyllum commune*. In: Jennings, D.H. and Rayner, A.D.M (eds), The Ecology and Physiology of the Fungal Mycelium. Cambridge University Press, Cambridge, pp 27-48.

Whipps, J.M. 1992. Status of biological disease control in horticulture. Biocontrol Sci. Techn. 2: 3-24.

Whipps, J.M. and Gerlagh, M. 1992. Biology of *Coniothyrium minitans* and its potential for use in disease biocontrol. Mycol. Res. 96: 897-907.

Wilkinson, V. and Lucas, R.L. 1969. Influence of herbicides on the competitive ability of fungi to colonise plant tissues. New Phytopathol. 68: 701-708.

Williamson, M.A. and Fokkema, N.J. 1985. Phyllosphere yeasts antagonize penetration from appressoria and subsequent infection of maize leaves by *Colletotrichum graminicola*. Neth. J. Pl. Pathol. 91: 265-276.

Yoder, O.C. 1980. Toxins in pathogenesis. Annu. Rev. Phytopathol. 18: 103-129.

Yohalem, D. 1997. Prospects for the biological control of foliar plant diseases in Danish greenhouses. 14th Danish Plant Protection Conference. Pests and Diseases. Special Report Statens Planteavlsforsog. no. 8, pp 199-206.

Yohalem, D.S. 2000. Microbial management of early establishment of grey mould in pot roses. DJF-rapport 12: 97-102.

Yu, H. and Sutton, J.C. 1999. Density dynamics of *Gliocladium roseum* in relation to biological control of *Botrytis cinerea* in red raspberry. Can. J. Plant Pathol. 21: 23-32.

Yunis, H. and Elad, Y. 1989. Survival of dicarboximide-resistant strains of *Botrytis cinerea* in plant debris during summer in Israel. Phytoparasitica 17: 13-21.

Zhang, W. and Pfender, W.F. 1993. Effect of wetting-period duration on ascocarp suppression by selected antagonistic fungi in wheat straw infested with *Pyrenophora tritici-repentis*. Phytopathology 83: 1288-1293.

Zhou, T. and Reeleder, R.D. 1989. Application of *Epicoccum purpurascens* spores to control white mold of snap bean. Plant Dis. 73: 639-642.

Zhou, T. and Reeleder, R.D. 1991. Colonization of bean flowers by *Epicoccum purpurascens*. Phytopathology 81: 774-778.

Zimand, G., Elad, Y., Gagulashvilly, N. and Chet, I. 1995. Effect of the biocontrol agent *Trichoderma harzianum* T-39 on the pathogenicity of *Botrytis cinerea*. Phytoparasitica 23: 241.

Zitter, T.A. and Hsu, L.W. 1992. Influence of temperature and fungicide on germination, growth, and virulence of *Ulocladium cucurbitae* on cucumber. Phytopathology 82: 358-362.