Epidemiology of *Botrytis* spotting on gerbera and rose flowers grown under glass

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Epidemiology of *Botrytis* spotting on gerbera and rose flowers grown under glass

Proefschrift

ter verkrijging van de graad van
doctor in de landbouw- en milieuwetenschappen
op gezag van de rector magnificus,
dr. C.M. Karssen,
in het openbaar te verdedigen
op woensdag 22 juni 1994
des namiddags te één uur dertig in de Aula
van de Landbouwuniversiteit te Wageningen

ISN 167359

This thesis is available as publication of the Research Station for Floriculture ('Mededelingen no. 102'), Linnaeuslaan 2a, 1431 JV Aalsmeer, the Netherlands

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Kerssies, Albert

Epidemiology of *Botrytis* spotting on gerbera and rose flowers grown under glass / Albert Kerssies. - [S.l. : s.n.]. - Ill.
Thesis Wageningen. - With ref. - With summary in Dutch.
ISBN 90-9006952-6
NUGI 821
Subject headings: Botrytis cinerea / gerberas / roses.

Cover design: Ineke Nijburg

Stellingen

1. Klimaatfactoren hebben nauwelijks invloed op de gevoeligheid van in kassen geteelde bloemen voor *Botrytis cinerea*.
   Dit proefschrift

2. De invloed van instraling op schimmelstructuren wordt onvoldoende als onderzoekfactor meegenomen in de fytopathologie.
   Dit proefschrift

3. De rol van de cuticula van bladeren en bloemen als barrière voor schimmels wordt door veel onderzoekers sterk overschat.
   P.E. Kolattukudy (1985), Annual Review of Phytopathology 23: 223-250;
   Dit proefschrift.

4. In onderzoek waar gebruik wordt gemaakt van sporevang-methoden die geen onderscheid kunnen maken tussen levende en dode sporen wordt de infectiedruk overschat.
   Dit proefschrift.

5. De waarde van het holistisch epidemiologisch onderzoek wordt in de fytopathologie onderschat.
   J.C. Zadoks (1990), Plant Disease 74: 82

6. Het feit dat de term 'biologische bestrijding' in de sierteelt voornamelijk met betrekking tot insekten wordt gebruikt is typerend voor de manier waarop schimmels worden bestreden in de sierteelt.

7. Lange termijn praktijk onderzoek is in de ogen van tuinders een contradictio in terminis.

8. Het telen van potplanten op eb-vloed systemen is een uitstekende manier om verspreiding van *Phytophthora* soorten tegen te gaan.

9. Manipulatie van fysiologische factoren voor en tijdens vermeerdering van planten heeft pas zin als ook de effecten in teelt- en naoogstfase zijn vastgesteld.

10. De toenemende strijd om onderzoekgelden tussen instituten en proefstations heeft een negatieve invloed op de diepgang van het onderzoek van instituten.

11. De wijze waarop de meeste onderzoekers hun resultaten overdragen op congressen lijkt meer op een dia-diarree en een sheet-show dan op een presentatie.
D. Bloch (1994), Intermediar 10: 49

12. Het dragen van een colbert en een stropdas lijkt de geloofwaardigheid van een voordracht van een onderzoeker te verhogen.

Stellingen behorende bij het proefschrift 'Epidemiology of *Botrytis* spotting on gerbera and rose flowers grown under glass', Albert Kerssies.

Wageningen, 22 juni 1994

Hebreeën 11: 3

Door het geloof verstaan wij, dat de wereld door het woord Gods tot stand gebracht is, zodat het zichtbare niet onstaan is uit het waarneembare.

abstract

Experiments described in this thesis were performed to improve the knowledge on the epidemiology of *Botrytis cinerea* in glasshouses, with gerbera and rose as model systems. *B. cinerea* is an airborne fungus, with conidia as the most important propagules in glasshouses. Conidia of *B. cinerea* are always present in glasshouse air, day and night, troughout the year. The numbers of conidia present in the glasshouse air depend mainly on the production system, wet or dry production system, amount of senescing plant material on the ground, periods with windows open for ventilation. The horizontal and vertical distribution of conidia in glasshouses is fairly uniform, irrespective of the crop. Deposition of conidia on cut flowers takes place in the glasshouse during the production stage. Sedimentation by gravity is a major mechanism. The shape of the flower is one of the factors that affect the number of conidia deposited on the flower surface. Temperature has an effect both on the susceptibility of flowers to *B. cinerea* and the infectivity of conidia of *B. cinerea*, whereas relative humidity and radiation only have an effect on the infectivity of conidia. No clear relation between season and susceptibility of flowers to *B. cinerea* was found. Cuticle and wax on flowers do not seem to be important factors for the susceptibility of flowers to *B. cinerea*. These epidemiological studies lead to a warning system against *B. cinerea* damage in the post-harvest stage. If the daily mean relative humidity in the glasshouse for days 6, 7 and 8 before the day of the flower harvest exceeds 70% and the daily mean global radiation outside the glasshouse for days 1, 2 and 3 before the harvest day is below 1500 $Jcm^{-2}day^{-1}$, there is great risk of *B. cinerea* damage on cut flowers in the post-harvest stage.

Key words: *Botrytis cinerea*, Burkard volumetric spore trap, conidia, cuticula, deposition, dispersal, distribution, epidemiology, flowers, gerbera, glasshouse, global radiation, ornamentals, production system, regression analysis, relative humidity, rose, spore trap, temperature, turgor, vapour pressure deficit, warning system, wax.

Voorwoord

Het proefschrift is klaar!
Bij het promotie-onderzoek en het schrijven van dit proefschrift heb ik steun en medewerking gehad van vele mensen. Ik wil al deze mensen hartelijk bedanken. Een aantal wil ik met name noemen.
Professor Zadoks wil ik bedanken voor zijn vele goede en vernieuwende ideeën en de nauwgezette wijze waarop hij het onderzoek en de concept-artikelen van kritische en opbouwende opmerkingen heeft voorzien.
Herman Frinking wil ik bedanken voor de prettige eerste-lijn begeleiding en voor het kritisch beschouwen van het onderzoek en de concept-artikelen. Mijn belangstelling voor de epidemiologie van ziekten veroorzaakt door schimmels, is mede door Herman sterk toegenomen. De artikelen die hij heeft geschreven over het gedrag van schimmelsporen in kassen zijn voor mijn onderzoek zeer bruikbaar geweest.
Het bestuur en de directie van het Proefstation voor de Bloemisterij wil ik bedanken voor de mogelijkheid die zij mij hebben gegeven om een gedeelte van het onderzoek dat ik gedaan heb met een proefschrift af te ronden.

Veel collega's hebben mijn concept-artikelen kritisch doorgeworsteld. Hartelijk dank daarvoor. In het bijzonder wil ik Joke Fransen bedanken voor de kritische en nauwgezette wijze waarop zij de artikelen heeft gelezen. Verder wil ik hiervoor Nollie Marissen, Hendrik-Jan van Telgen, Cor Vonk Noordegraaf en Rick van Gorsel bedanken.

Zonder de steun van de assistenten Monique Dil en Ineke Bosker waren veel experimenten niet geslaagd. Honderden liters bruine drab hebben ze gemaakt en in petrischalen gegoten, in de kas opgehangen, uit de kas gehaald, in de klimaatcel geplaatst en vervolgens de kolonies geteld. Kolonies en lesies, honderden, duizenden, gek werden ze ervan. Toch werd elk vlekje geteld en genoteerd. Marco ten Hoope wil ik bedanken voor het meehelpen bij het vangen van *Botrytis*-sporen tijdens het 'assistentie-loze' tijdperk.

'Rozenteler' Kees Boer en zijn 'medewerker' Frits Akse wil ik bedanken voor het liefdevol verzorgen van het rozengewas, waarin veel experimenten zijn uitgevoerd. Tot groot verdriet van 'professor' Kees werden veel van zijn troetelkindjes mishandeld in het kader van het onderzoek. Klaas van Dam wil ik bedanken voor het telen van de vele mooie gerbera's, die na de oogst meestal direct ziek werden gemaakt.

Mijn collega-onderzoekers van de kerngroep Gewasbescherming wil ik bedanken voor hun meeleven.

De onderzoekers van de kerngroep Naoogst, Caspar Slootweg, Nollie Marissen en Rick

van Gorsel wil ik bedanken voor het onderwijs in de eerste beginselen van de waterhuishouding van snijbloemen en in het meten van de waterpotentiaal en de osmotische potentiaal. Soms dachten ze dat ik een nieuwe naoogst-onderzoeker was.

Jesus Salinas wil ik bedanken voor het bijbrengen van de eerste beginselen van de relatie *Botrytis cinerea* - Gerbera en voor het belangeloos afstaan van de meest agressieve isolaten van *B. cinerea*.

De mannen van de technische dienst wil ik bedanken voor de technische ondersteuning tijdens het onderzoek, in de vorm van meetboxen, lichtmeters, dataloggers, en hulp bij vaak haperende klimaatkasten.

Adri Verlind en Margriet Stapel hebben mij ondersteuning gegeven bij het statistisch verwerken van de gegevens en daardoor een essentiële bijdrage geleverd voor het slagen van dit proefschrift.

Mijn ouders wil ik bedanken dat zij mij de gelegenheid hebben gegeven om te studeren en de voor enthousiaste belangstelling die zij altijd hebben getoond.

Tenslotte wil ik Ineke bedanken voor de ruimte en de ondersteuning die zij mij heeft gegeven om dit proefschrift te schrijven. Het was altijd weer goed om thuis te komen.

Bovenal dank ik God voor de capaciteiten die Hij mij heeft gegeven om dit werk te kunnen doen.

Contents

GENERAL INTRODUCTION

Gerbera and rose

In the Netherlands most cutflower crops are grown in heated glasshouses, in which the temperature and the relative humidity are computer controlled. In 1992, 4000 ha of cutflowers were grown under glass, of which approximately 900 ha were roses and 180 ha were gerberas (Anonymus, 1993). In 1992, 340 ha of roses and 82 ha of gerberas were grown on artificial substrate (e.g. rockwool). In the Netherlands approximately 250 different rose cultivars and 350 gerbera cultivars are grown commercially. The rose cultivars differ in susceptibility to *Botrytis cinerea*. Except for the cultivar Delphi, all the gerbera cultivars are susceptible to *B. cinerea*.

Roses and gerberas in glasshouses are preferably grown at temperatures of 18-20°C. In fall and winter the daily mean temperature is below 20°C, in spring it fluctuates between 18 and 22°C and in summer the daily mean temperature can increase well over 30°C due to solar radiation. The daily mean relative humidity fluctuates between 50% in winter and 90% in fall. In spring and summer the temperature and the relative humidity show a daily pattern, low temperature (<20°C) and high relative humidity (>80%) at night and high temperature (>20°C) and low relative humidity (<70%) at day.

In glasshouses many fungi can attack rose and gerbera plants. In roses important root rot and wilting diseases are caused by *Phytophthora* sp., *Gnomonia radicicola* and *Verticillium dahliae* and important foliar diseases are caused by *Sphaerotheca pannosa* (powdery mildew) and *Botrytis cinerea* (grey mold). In gerbera important root rot and wilting diseases are caused by *Phytophthora cryptogea, Fusarium* sp., and *Myrothecium roridum*, and important foliar diseases are caused by *Oidium* spp. (powdery mildew) and *Botrytis cinerea* (grey mold).

Botrytis cinerea

Botrytis cinerea Pers. ex Pers. is an air-borne fungus and belongs to the *Deuteromycota* (Fungi Imperfecti). *B. cinerea* is the asexual (anamorphic) stage and *Sclerotinia fuckeliana* (de Bary) Whetz. is the sexual (teleomorphic) stage in the life cycle of the fungus. The asexual stage, often referred to as the grey mold fungus, consists of vegetative hyphae, sclerotia, macroconidia and microconidia. The sexual stage consists of a reproductive body, the apothecium, containing ascospores in linear asci (Faretra and Grindle, 1993).

B. cinerea is a pathogen to a wide variety of economically important plants, such as vegetables, ornamentals, bulbs and fruits and a saprophyte on senescing and dead plant material. Infection occurs through wounds, via decaying or dead plant tissue, or by direct penetration of the undamaged host (Verhoeff, 1980).

B. cinerea is a major air-borne pathogen in ornamentals grown under glass. *B. cinerea* causes damage to other ornamentals than gerbera and rose, such as Chrysanthemum and to potted plants such as Saintpaulia, Cyclamen and Poinsettia (De Jong, 1985, 1986). Problems with *B. cinerea* in glasshouses in the Netherlands mainly occur during fall and winter, when the temperature is low (<20°C) and the relative humidity is high (>80%; Bakker, 1986; Damen, 1986).

Infection by *B. cinerea* can take place in the glasshouse during the production stage, particularly on dying and dead plant material. In the post-harvest stage *B. cinerea* is a pathogen on flowers. Deposition of conidia of *B. cinerea* on flowers mainly occurs in the glasshouse, during production. Flowers can function as spore traps, horizontal (gerbera) or vertical (rose) (Fig. 1). After deposition on the flowers, conidia remain dormant until a thin water film is available for germination. Necrotic lesions (spotting), caused by young colonies, occur on flower buds and petals during the post-harvest period. These symptoms are encouraged by a relative humidity above 93% (Salinas *et al.*, 1989), for instance during the transport stage when flowers are packed into boxes and rapid changes in temperature occur owing to transfer from cold storage into non-cooled trucks and then into cold store again after transport. Within 24 h of harvest many lesions occur at 18 to 25°C (Salinas *et al.*, 1989). *B. cinerea* in rose flowers can infect whole petals, but not in gerbera flowers (hypersensitive reaction; Pie and De Leeuw, 1991). On susceptible roses 1-3 lesions/flower are enough to colonise and destroy a flower, whereas with gerbera's 50-100 lesions are necessary for declassification of the flower.

Use of fungicides (benzimidazoles, dicarboximides) in the glasshouse may increase the risk of fungicide resistance developing (Gullino & Garibaldi, 1987; Witte & De Jong, 1985), of flower quality loss and of chemical residues on the flowers. Quality loss caused by *B. cinerea* during the post-harvest period is hard to avoid. Chemical control of *B. cinerea* during post-harvest is difficult, especially when the numbers of conidia on the flower surface are high (Dirkse, 1980).

Distribution and dispersal of air-borne fungi in glasshouses

Conidia, ascospores, mycelial fragments and sclerotia are all important for dispersal of *B. cinerea* (Jarvis, 1980), although mainly conidia are important for dispersal in glasshouses. Conidia are dispersed by air currents, water droplets and insects. Conidium release occurs mainly during rapid changes in relative humidity (Jarvis, 1980; van Holsteijn, 1985; Winspear *et al.*, 1970). According to Jarvis (1980), the density of *B. cinerea* conidia in the air during rapid changes in relative humidity can increase to $2*10^4$ conidia/m^3 air.

Studies on dispersal of plant pathogens (e.g. *B. cinerea*) in glasshouses are few (Frinking and Scholte, 1983). Hirst (1959) was one of the first to monitor densities of air-borne spores, and *B. cinerea* conidia were amongst those trapped. Frinking and

Scholte (1983) showed that the complex dispersal process involves aspects of pathogen, host, environment and activity of man. These authors suggest that crop density, crop structure and crop height are important in the dispersal of spores. Frinking *et al.* (1987) studied the dissemination of *Lycopodium* spores in a glasshouse divided into three multiple bays. They found that more air movements owing to open ventilation windows caused a rapid cleaning of air while in a glasshouse with less air movements owing to closed ventilation windows spores remained suspended for a long time, and stated that the air movement in a closed glasshouse is sufficient to counteract gravity but insufficient to impact the spores onto plants or trap surfaces. Frinking *et al.* (1987) suggest that the patterns of air movement in glasshouses differ according to the spatial arrangement of the crop canopy. Hausbeck and Pennypacker (1991) showed that grower activity in a greenhouse with geraniums resulted in peak conidial concentrations in the greenhouse air. Air-borne epidemics often start with the entry of one or more fungal spores from the outside environment (Frinking, 1991). Zandvoort (1968) showed that inoculum of *Puccinia horiana* can as readily enter the glasshouse as it escapes the glasshouse, apparently by way of ventilation windows and other openings. The same is suggested by Schepers (1984) for conidia of *Sphaerotheca fuliginea.* Frinking (1991) claimed a continuous exchange of air between the glasshouse and its outside environment, because of wind speeds outside the glasshouse, which normally exceed those within the glasshouse, and because of differences in temperature. De Jong (1990) showed a linear relationship between the air flow within and the wind-speed outside a glasshouse. According to Hirst (1959) glasshouses may act as important spore emitters by means of convection through open ventilators. Zadoks (1967) stated that no greenhouse is airtight, a fact widely known and rarely published. Low windspeeds inside the glasshouse together with high windspeeds outside cause pressure differences between inside and outside, and in that way a suction force is able to 'clean' the glasshouse from air-borne micro-organisms, but this system can also introduce new micro-organisms from the outside air (Frinking *et al.*, 1987).

These studies suggest that conidia of *B. cinerea* in cut flowers grown in computer controlled ventilated glasshouses can enter and leave the glasshouses easily. In seasons with high ventilation rates (spring and summer) the number of *B. cinerea* conidia in glasshouses is probably lower than in the other seasons with low ventilation rates. The dispersal of conidia of *B. cinerea* in a gerbera crop probably differs from the dispersal in a rose crop. The number of conidia in a recently planted crop (free from conidia of *B. cinerea*) is probably lower than in an old crop.

Deposition on plant surfaces results from the interaction of several mechanisms, sedimentation, impaction and turbulence (Gregory, 1973; Zadoks and Schein, 1979). In still air, all spores in the air sink with a constant and characteristic 'terminal velocity' (Gregory, 1973). *B. cinerea* has a 'terminal fall velocity' of 0.22-0.45 cm/s. Gregory (1973) concluded that the effect of sedimentation is strong at wind-speeds lower than 2

m/s and slight at wind-speeds of 2 m/s and upwards. Outdoors the effect of 'terminal fall velocity' is usually masked by the speed and turbulence of the wind, but wind-speed and turbulence in glasshouses hardly ever exceed 1.0 m/s (Frinking, 1991). In glasshouse air the deposition of conidia on plant surfaces is probably mainly due to sedimentation.

Role of cuticle in the infection process

Environmental parameters can affect plant wax and cuticle development of leaves and flowers (Baker, 1974; Kolattukudy, 1985; Skoss, 1955). Marois *et al.* (1988) showed that in California the susceptibility of rose flowers to *B. cinerea* was significantly higher in December, January, and February than in October and November, when temperature and radiation level were higher.

According to Kolattukudy (1985) and Köller (1991) the enzymatic hydrolysis of the polyester cutin by cutinase, an enzyme secreted during the initial step of host invasion, is a requirement for penetration of plant cuticles and, therefore, an essential factor of fungal pathogenicity. Salinas (1990) treated gerbera flowers, inoculated with conidia of *B. cinerea,* with antibodies raised against purified cutinase of *B. cinerea.* These gerbera flowers were completely protected against the infection of *B. cinerea.* Treatments with these antibodies can be a possibility in controlling *B. cinerea* during post-harvest, but treatments with these antibodies are too expensive.

According to Nicholson & Epstein (1991) cutinases are not important in the penetration process, but are involved in the adhesion process. If there is no adhesion the spore pushes itselves up when it tries to penetrate the plant surface. These authors stated that adhesion of fungal spores to the host cuticle is an essential prepenetration process that determines the success of infection and disease development. They stated that cutinases are involved in this adhesion process. This is a fast process, as within 2 minutes an ungerminated spore can adhere to the plant surface. When the cutinases were inhibited no infection took place. After adhesion of a conidium to the flower surface other processes than enzymatic hydrolysis of the cuticle are probably involved in the infection process. Edlich *et al.* (1989) found a positive correlation between pathogenicity of *B. cinerea* and the intensity of active types of oxygen released by the fungus.

Aim and outline of this thesis

In 1994, *B. cinerea* in ornamentals is still controlled mainly by fungicides. The pesticide use in ornamentals in the Netherlands has to be reduced for 65% in 2000, according to the Multi-Year Crop Protection Plan published by the Dutch government (Anonymus, 1991). More knowledge on the epidemiology of *B. cinerea* in glasshouses is needed in order to take specific measures (e.g. fungicide treatment) at the right time and to reduce fungicide use (Fransen, 1992). For these epidemiological studies, sampling systems for *B. cinerea* in glasshouses had to be developed (Chapter 1). Little is known about factors

influencing dispersal and distribution of *B. cinerea* in ornamentals in glasshouses. Relations between environmental factors (temperature, relative humidity, radiation and windspeed) and dispersal of *B. cinerea* in ornamentals are unknown yet. All experiments described in this thesis were performed to increase the knowledge on the epidemiology of *B. cinerea* in glasshouses, using gerbera and rose as model systems. The epidemiology of *B. cinerea* in gerbera (cultivars Terrafame and Delphi) and rose crops (cultivar Sonia) was studied and compared, because the physical structures of these canopies differs. The glasshouses used for the experiments and described in Chapters 2, 3 and 4 are shown in Fig. 2, 3 and 4.

With the aim to improve the efficiency of aerobiological studies in glasshouses the horizontal and vertical distributions of air-borne conidia of *B. cinerea* in gerbera and rose crops grown under glass were studied (Chapters 3 and 4). The differences in impaction of *B. cinerea* conidia on rose and gerbera flowers and on spore traps placed at different angles were studied. The numbers of *B. cinerea* conidia in glasshouse air during a 24 h period in rose and gerbera crops were determined (Chapter 6).

To develop a warning system for the numbers of conidia of *B. cinerea* on the surface of cutflowers the dispersal of conidia in gerbera and rose crops growing under glass and the effects of environmental factors on dispersal of conidia and on infection of gerbera and rose flowers during storage were studied (Chapters 2 and 4). For the same purpose, the effect of vapour pressure deficit (VPD), temperature and radiation on the susceptibility of gerbera flowers to *B. cinerea* during the post-harvest period, on the water relations of gerbera flowers during the post-harvest period and on the infectivity of conidia of *B. cinerea* were studied (Chapter 5).

The possible effect of season (related to certain levels of relative humidity, temperature and total global radiation) on area dry weight, wax and cuticle of gerbera and rose petals and on pre-harvest flowering period of gerbera and rose flowers was studied. The effect of season on number of lesions on harvested gerbera and rose flowers, grown under glass, was considered. The area dry weight, wax and cuticle of petals of susceptible and less susceptible gerbera and rose cultivars was investigated (Chapter 7).

The conclusions based on the results of these experiments can probably be used by gerbera and rose growers to reduce the problems caused by *B. cinerea*. With more knowledge of the horizontal and vertical distributions of *B. cinerea* in glasshouses more efficient sampling strategies can be developed for scouts and for research purposes. With a reliable (computer controlled) warning system for the infection pressure of *B. cinerea* in glasshouses appropriate measures can be taken at the right time to control *B. cinerea* in the glasshouse or during the post-harvest period. *B. cinerea* can be controlled by chemicals or by climatic measures. Possible differences in wax and cuticle of flower petals between the seasons and between the cultivars may be used in breeding resistance programs for *B. cinerea*. When *B. cinerea* can be better controlled without the use of huge amounts of chemicals the fungicide resistance development of *B. cinerea* can be

decreased.

With the knowledge collected in this thesis, problems in cut flowers grown in glasshouses caused by *B. cinerea* should not be a problem anymore, because a reliable warning system for *B. cinerea* can be developed.

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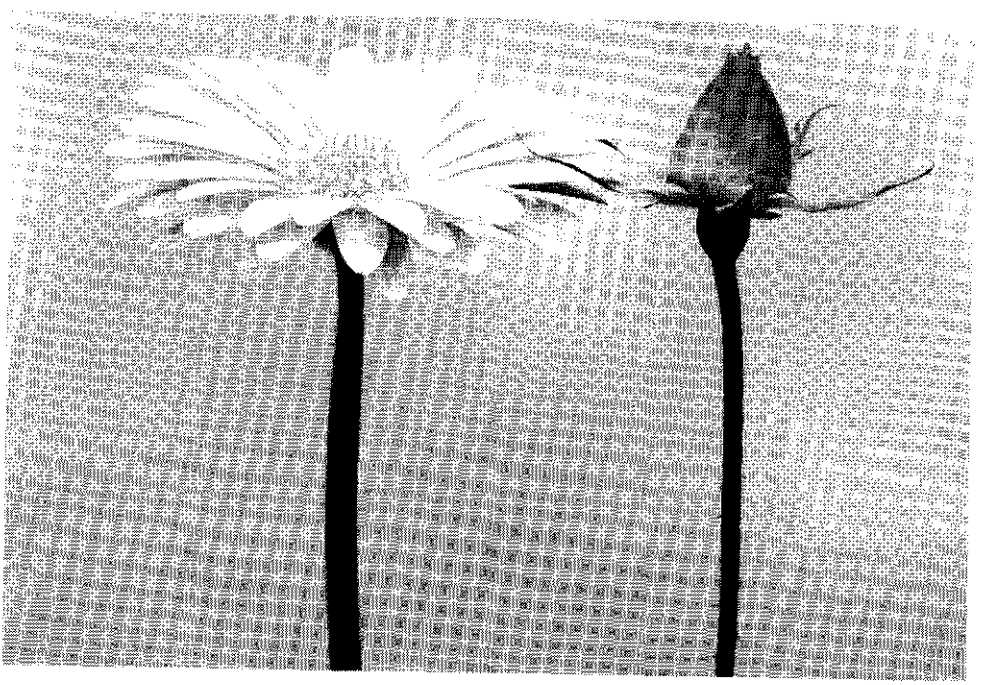

Fig. 1. Gerbera and rose flowers.

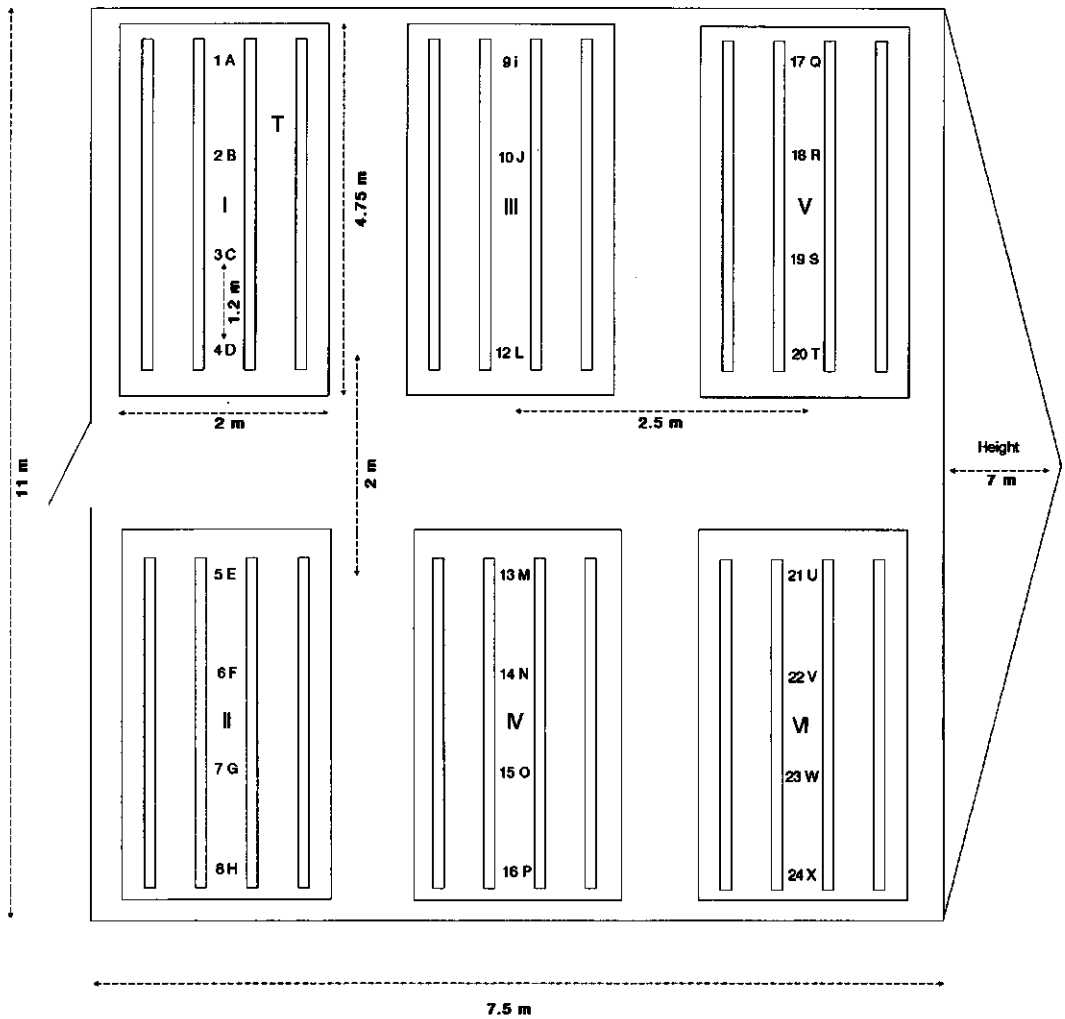

Fig. 2. Sketch of the gerbera glasshouse of 100 m² in Aalsmeer. A-X: spore traps within the crop; 1-24: spore traps 0.5 m above the crop; I-VI: spore traps 1.5 m above the crop.

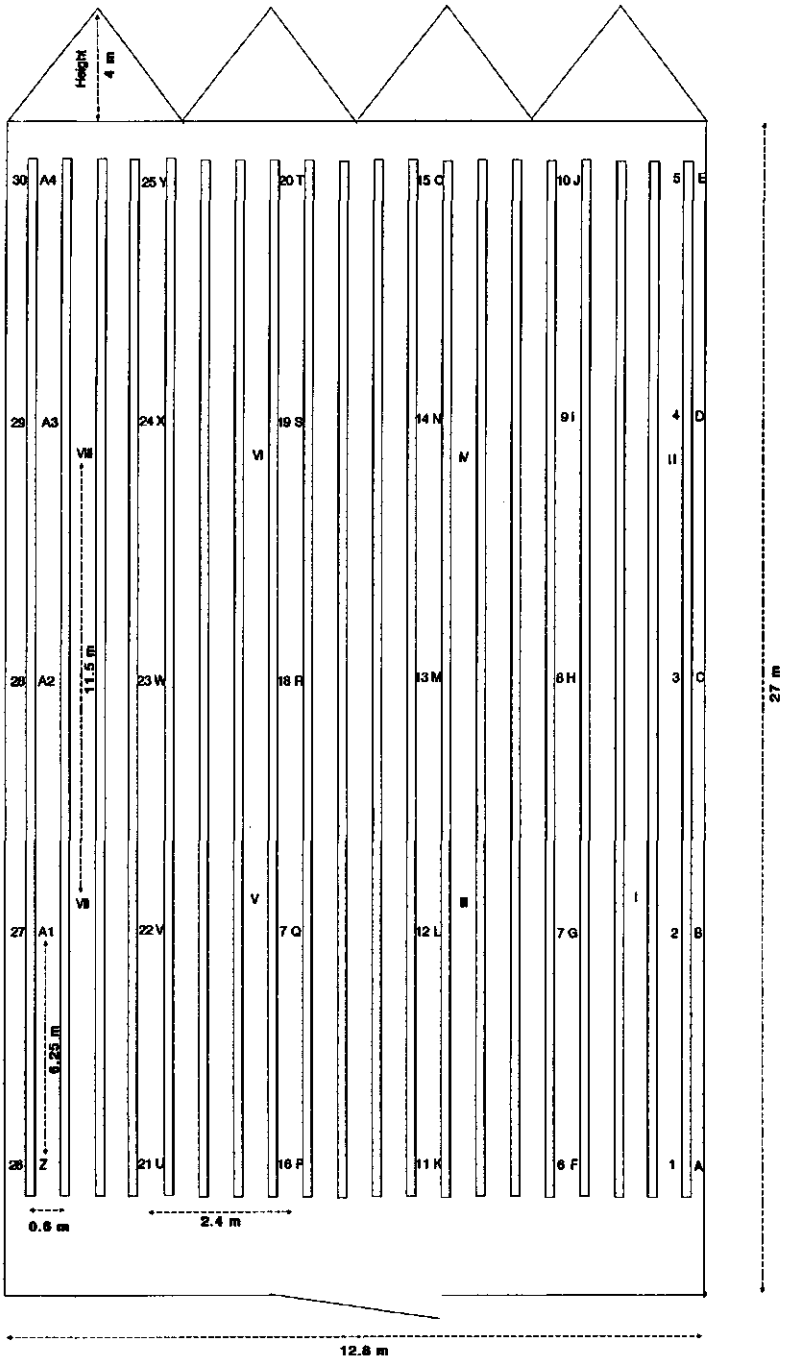

Fig 3. Sketch of the gerbera glasshouse of 350 m² in Vleuten. A-A4: spore traps within the crop; 1-30: spore traps 0.5 m above the crop; I-VIII: spore traps 1.5 m above the crop.

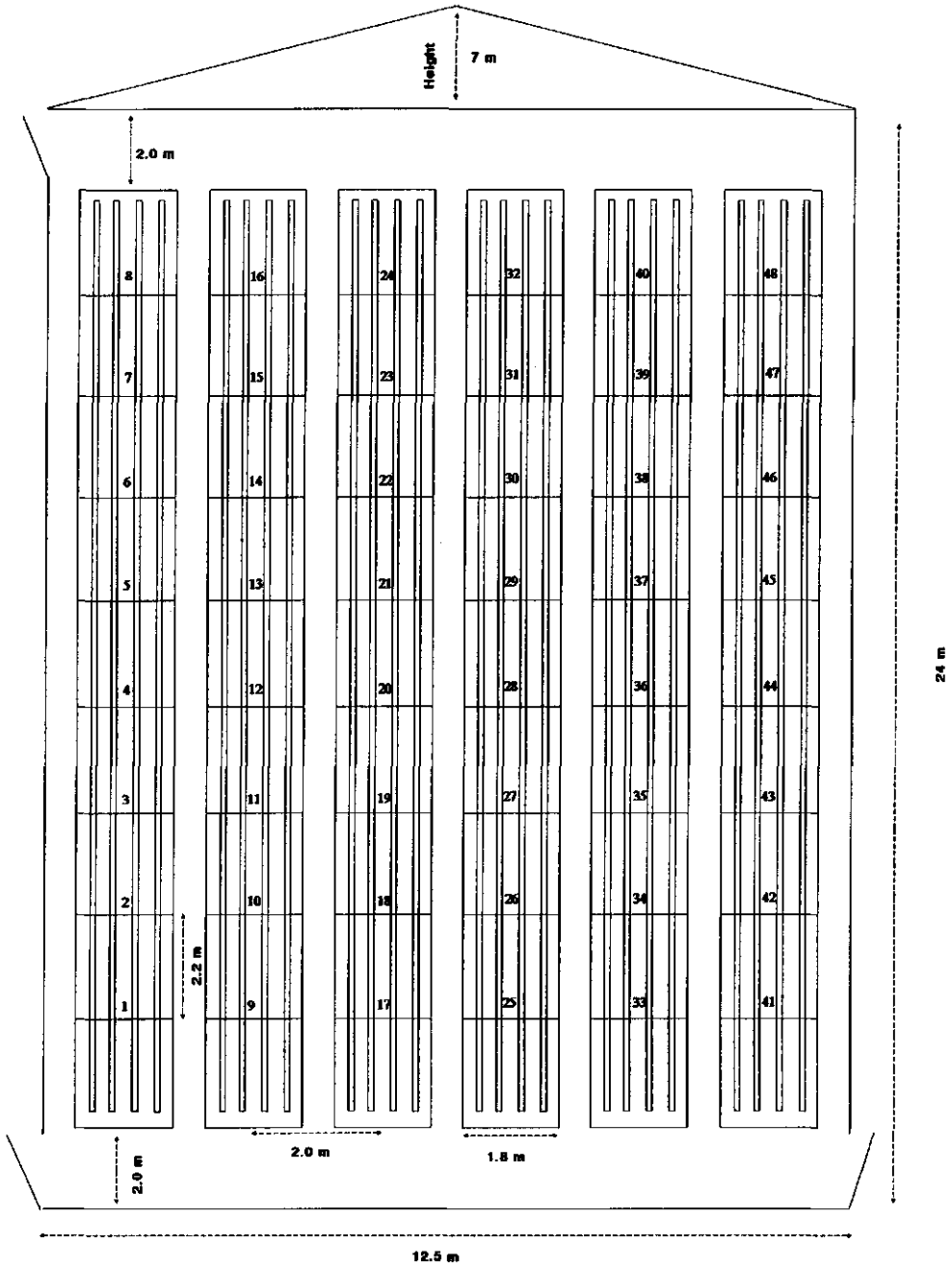

Fig 4. Sketch of the rose glasshouse of 300 m² in Aalsmeer. 1-48: spore traps at flower level, 1 m above soil level.

Chapter 1

A selective medium for *Botrytis cinerea* to be used in a spore trap

Short Communication

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Kerssies, A., 1990. A selective medium for *Botrytis cinerea* to be used in a spore trap. Netherlands Journal of Plant Pathology 96: 247-250

Abstract

A selective medium has been developed for the use in spore-traps to study the dispersion of *B. cinerea* on gerbera grown in glasshouses.

Additional keywords: dispersal, gerbera

To study the dispersion of air-borne fungi in glasshouses different types of spore-traps were used (Frinking et al, 1987; Hirst, 1959; Jarvis, 1962). These traps were based on air-suction or on impaction on sticky surfaces by natural airflows. In the case presented here fungal spores in glasshouse air were trapped using Petri dishes containing an agar-based culture medium to study the dispersion of the air-borne spores of *B. cinerea* on gerbera grown in glasshouses.

In glasshouse air, spores of many different fungi are present. When an universal medium was used, for example Potato Dextrose Agar (PDA), it was very difficult to recognize colonies of specific fungi such as *Botrytis cinerea.* Within 24 hours of incubation fast growing fungi such as *Mucor* spp. covered the whole surface of the universal medium, suppresssing other fungi. For this reason it is necessary to use a selective medium in the spore-trapping device.

A selective medium for *B. cinerea* was described by Kritzman and Netzer (1978). They developed their medium for the isolation and identification of *Botrytis* spp. from soil and onion seed. The medium contains tannic acid as a substrate for polyphenol-oxidase (PPO) activity. PPO activity of growing mycelium of a culture of *Botrytis allii* was found to convert the tannin in the medium into a dark-brown pigment. Other fungi were found to have PPO too, but were inhibited by PCNB (pentachloronitrobenzene), Maneb and $CuSO_4$. Germination of conidia, hyphal elongation and mycelium growth of *B. allii* and *B. cinerea* were not prevented on the selective medium. Brown pigmented colonies could be identified 48 hours after incubation at 24°C (Kritzman and Netzer, 1978). This selective medium is likely to be very useful for the study of the development of *B. cinerea.*

The following selective medium for *B. cinerea* is an adjustment of the selective medium used by Kritzman and Netzer. The basal medium, which was almost the same as that used by Kritzman and Netzer, consists of the following components (g/l distilled water): $NaNO_3$, 1.0; K_2HPO_4, 1.2; $MgSO_4.7H_2O$, 0.2; KCl, 0.15; glucose, 20.0 and agar, 25.0. The medium was sterilized for 20 minutes at 121 °C. After cooling till 65 °C the following ingredients were added (g/l distilled water): Terrachlor (PCNB, pentachloro-benzene 75% WP), $15*10^{-3}$; Maneb (manganese ethylene bisdithiocarbamate), $1*10^{-2}$; chloramphenicol (antibiotic), $5*10^{-2}$; $CuSO_4$, 2.2; Rubigan (fenarimol), 0.1 ml/l and tannic acid, 5.0. The pH of the supplemented basal medium (SBM) was adjusted to 4.5 with 5.0 N NaOH. Rubigan was added into the selective medium to suppress air-borne

spores of *Penicillium* spp. At pH 4.5 colonies of *B. cinerea* formed mycelium, conidiophores and conidia. At pH 6.5, used by Kritzman and Netzer (1978) *B. cinerea* was not able to form conidia.

Germination and mycelial growth of *B. cinerea* was not prevented on this selective medium. It is therefore reliable and useful for monitoring *B. cinerea*-spores in a glasshouse environment.

The SBM was put into plastic Petri dishes (O 9cm), 20-25 ml per Petri dish. These Petri dishes were mounted in spore-traps. The traps were custom-made. Each trap was built around a wooden block. To each of the four sides of the block the bottomhalf of a Petri dish containing the selective medium was attached (Fig. 1).

The traps were placed in the greenhouse for eight hours. After incubation for seven days at 20°C and under a lightperiod of 24 hours per day dark brown spots were counted as colony forming units of *B. cinerea* (Fig. 2). Other fungi were inhibited by PCNB, Maneb, $CuSO_4$ and Rubigan. *Penicillium* spp. and *Trichoderma* spp. were still able to grow on the selective medium, but very slowly. They did not convert the medium to a dark-brown colour. With this selective medium it is possible to count more than 200 plates, with 10 to 15 colony forming units of *B. cinerea*, per hour.

After fourteen days the dark brown *Botrytis*-colonies started to produce conidia. Conidia from these colonies tested on gerbera-petals all formed small lesions, typical for infection of *B. cinerea* (Salinas et al, 1989).

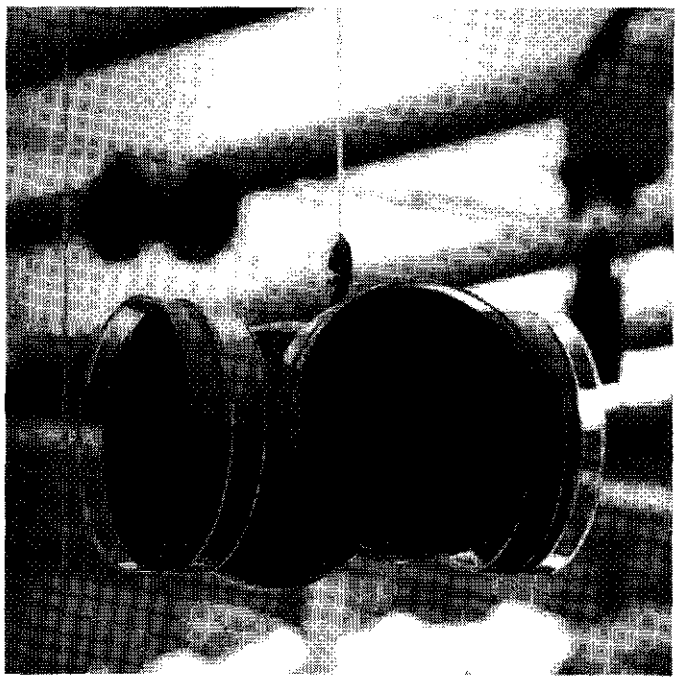

Fig. 1. Spore-trap device.

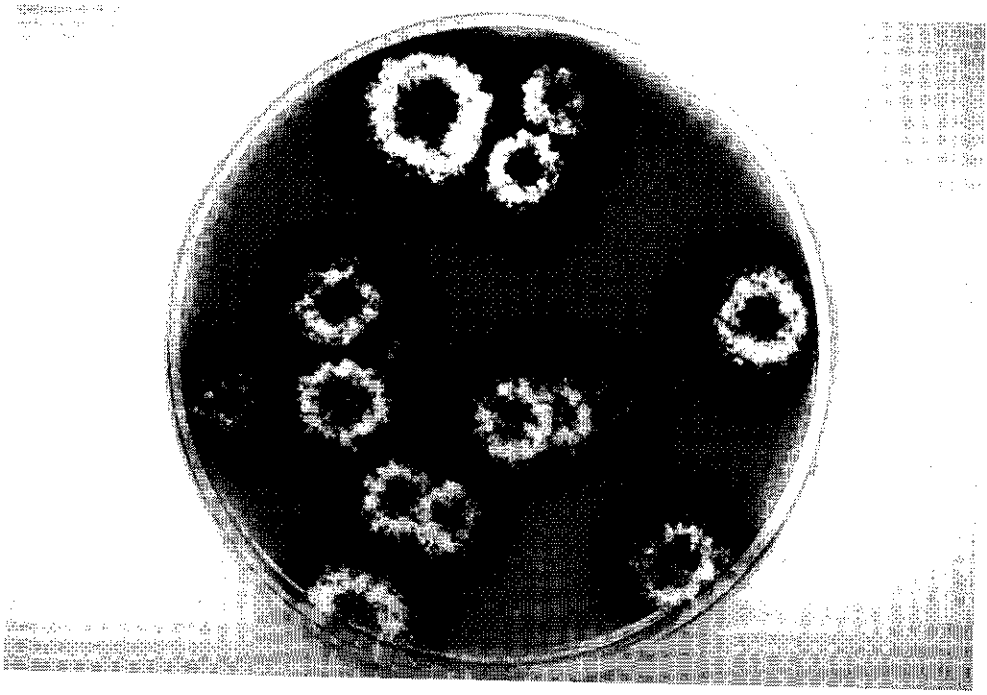

Fig. 2. Colonies of Botrytis cinerea, turning the colour of the selective medium into dark-brown.

Acknowledgements

The author is indebted to Ms Monique Dil and Mr Jan Tolsma for making the selective medium, to Ms Ineke Bosker for collecting useful data and to Dr J.J. Fransen, Ir H.D. Frinking and Dr W.E. Fry for critical reading of this short communication.

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Chapter 2

Influence of environmental conditions on dispersal of *Botrytis cinerea* conidia and on post-harvest infection of gerbera flowers grown under glass

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Kerssies, A., 1993. Influence of environmental conditions on dispersal of *Botrytis cinerea* conidia and on post-harvest infection of gerbera flowers grown under glass. Plant Pathology 42: 754-762

Abstract

Dispersal of *Botrytis cinerea* in a gerbera crop grown in two glasshouses 30 km apart was studied over a period of 18 months, in 1988 and 1989. Conidia were caught in spore traps consisting of agar in petri dishes exposed at different heights in the crop in each glasshouse. No seasonal patterns could be identified in the spore catches, assessed as colonies on the agar traps after incubation. The number of lesions caused by conidial infection of gerbera flowers following incubation, however, showed a distinct pattern. In spring and early summer few lesions were recorded whereas at other times of the year many lesions appeared. In linear regression analysis, variation in numbers of colonies (spore catches) could not be explained by environmental factors recorded during the experiments. Linear regression accounted for 77 and 81% of the variation in the number of lesions on flowers in the two glasshouses, in terms of relative humidity (positively correlated), global radiation outside the glasshouse (negatively correlated) and age of the crop (positively correlated). Despite differences in the systems by witch the gerbera crop was produced and in the spore catches, the numbers of lesions on gerbera flowers in the two glasshouses were significantly correlated though not significantly different from each other.

Additional keywords: colonies, lesions, relative humidity, global radiation, linear regression.

Introduction

The fungus *Botrytis cinerea* Pers. ex Pers., the imperfect stage of Sclerotinia fuckeliana (Ellis & Waller, 1974), infects a wide variety of plants. Infection takes place through wounds, through decaying or dead plant tissue, and by direct penetration of the undamaged host (Verhoeff, 1980). Conidia, ascospores, mycelial fragments and sclerotia are important for dispersal (Jarvis, 1980), although only conidia are probably important in the spread of diseases in glasshouses. Conidia are dispersed by air currents, water droplets and insects. Conidium release occurs mainly by rapid changes in relative humidity (Winspear *et al.*, 1970; Jarvis, 1980; van Holsteijn, 1985).

Botrytis cinerea causes damage to ornamentals such as gerbera, rose, chrysanthemum and pot plants such as SaintPaulia (De Jong, 1985, 1986). Necrotic lesions (spotting) occur on flower buds and petals, and are caused by early infections. These symptoms are encouraged by a relative humidity above 93% (Salinas *et al.*, 1989), as when flowers are packed into boxes and rapid changes in temperature occur owing to transfer from cold storage into trucks and then back into store after transport. Many lesions occur within 24 h of harvest between 18 to 25°C (Salinas *et al.*, 1989). Damage caused by *B.*

cinerea in the post-harvest phase is difficult to avoid. Use of fungicides in the glasshouse may increase the risks of fungicide resistance developing (Gullino & Garibaldi, 1987; De Witte & De Jong, 1985), of damaging the flowers and of chemical residues on the flowers.

Little is known about the factors influencing spore dispersal in *B. cinerea* on ornamentals under glass. Frinking and Scholte (1983) showed that the dispersal process is complex because many factors relating to pathogen, host, environment and man are involved. The aims of this study were to investigate the patterns of conidia dispersal in a gerbera crop growing under glass and the effects of environmental factors on dispersal and infection of gerbera flowers during storage and transport.

Materials and methods

Measurement of environmental conditions. The relative humidity and the temperature in and above the crop were measured over 1 h periods using psychrometers coupled to a data logger and thermohygrographs. Global radiation outside the glasshouse ($Jcm^{-2}.day^{-1}$) was measured by a Kipp solarimeter 8 m above the ground at the Research Station for Floriculture in Aalsmeer. The windspeed outside the glasshouse in Aalsmeer was measured over 1-h periods using a tachometer 8.5 m above the ground (De Jong, 1990). In the study of De Jong a linear relationship was demonstrated between the air flux within and the average wind-speed outside a glasshouse.

Flowering period of gerbera. In January, March and June to October, 1989, the period over which gerbera blooms (GB) in a glasshouse was measured by observing five gerbera flowers from the first day they opened until the day they were cut (harvest).

Dispersal of Botrytis cinerea. Experiments were done to examine dispersal of *B. cinerea* conidia in a gerbera crop, from planting (April 1988) to removal (October 1989). Gerbera crops were grown in two glasshouses, one (glashouse A) of 100 m^2 in Aalsmeer with 480 plants of the cv. Terrafame and the other (glasshouse V) of 350 m^2 in Vleuten with 2000 plants of the cvs. Rosamunde and Maria. The two glasshouses were 30 km apart. In glasshouse A, six tables each supported four rows of 20 gerbera plants growing on rockwool on an irrigation mat. In glasshouse V, 20 rows of gutters (Van Weel *et al.*, 1990) with rockwool were installed, each with 100 plants. At planting (t=0, see Figs. 1-4) *B. cinerea* was introduced into the glasshouses. Petri dishes containing heavily sporulating colonies of *B. cinerea* on potato dextrose agar were exposed in glasshouses A (two dishes) and V (four dishes) for 7 days and were removed before spore trapping started. The density of *B. cinerea* conidia in the air of the glasshouses was studied between April 1988 and October 1989 using the following two methods.

1. Spore traps were constructed by attaching the bases of Petri dishes containing a selective medium for *B. cinerea* (Kritzman and Netzer, 1978; Kerssies, 1990) to the four sides of a wooden cube (9*9*9cm). They were distributed within and above the crops in a regular spatial pattern. Each week (twice-weekly during winter) fresh traps were placed in the glasshouses for 8 h during the day (08.30 - 16.30 hours) and for 16 h during the night (16.30 - 08.30 hours, glasshouse A only). The dishes were incubated for 7 days at 20°C under fluorescent light (Pope, FTD 36W/30, 8 $\mu mol/m^2.s$) after which the number of dark brown colonies was recorded (Kerssies, 1990).
2. The number of lesions on petals of gerbera flowers was counted after cutting. In addition, each week (twice-weekly during winter) 24 flowers near the spore traps were harvested in glasshouse A and 30 flowers in glasshouse V. The surfaces of the upper 10 petals of each flower were exposed to the glasshouse air for 6 to 13 days, dependent of the season. They were then placed on wet paper in plastic boxes and incubated at 20°C under fluorescent light. After 3 days, *B. cinerea* lesions were counted under a microscope (10x magnification, Salinas *et al.*, 1989).

Regression analysis. The mean numbers of colonies on 53 trapping occasions over 545 days and the mean numbers of lesions on gerbera petals in 36 samples over 371 days (glasshouse A) were the dependent variables ($\ln(N+1)$ transformed) in regressions against environmental variables (Madden & Ellis, 1988). These data constituted of a time series. Successive observations were treated as largely independent, because (i) the length of the infection cycle of *B. cinerea* was shorter than the time between two successive observations which were clearly separated in time (1 or 2 weeks), (ii) the crop was fully grown at the start of observations, and (iii) there were large differences between subsequent measurements.

The following independent variables were used in the regressions: RH, daily mean relative humidity within the crop (%); T, daily mean temperature within the crop (°C); S, total daily global radiation outside the glasshouse ($J/cm^2.day$); W, daily mean wind-speed outside the glasshouse (m/s); GB, the bloom period of gerbera (days); t, age of the crop in number of days from the start of the experiment. Daily mean values of RH, T, S and W were calculated for each of the 14 days before a trap exposure day (MRH, MT, MS and MW). By means of linear regression analysis (procedure RSELECT in the statistical program GENSTAT) the subsets of independent variables which gave the best fit for the mean numbers of colonies and for the mean number of lesions were selected. The procedure RSELECT selects the best subset of variables in regression according to Mallow's C_p as the criterion for goodness of fit (Montgomery & Peck, 1982). The variables used in the best equation determined with the data from glasshouse A were also used for the data of glasshouse V (only for numbers of lesions), where lesions on petals were counted 37 times over a period of 390 days.

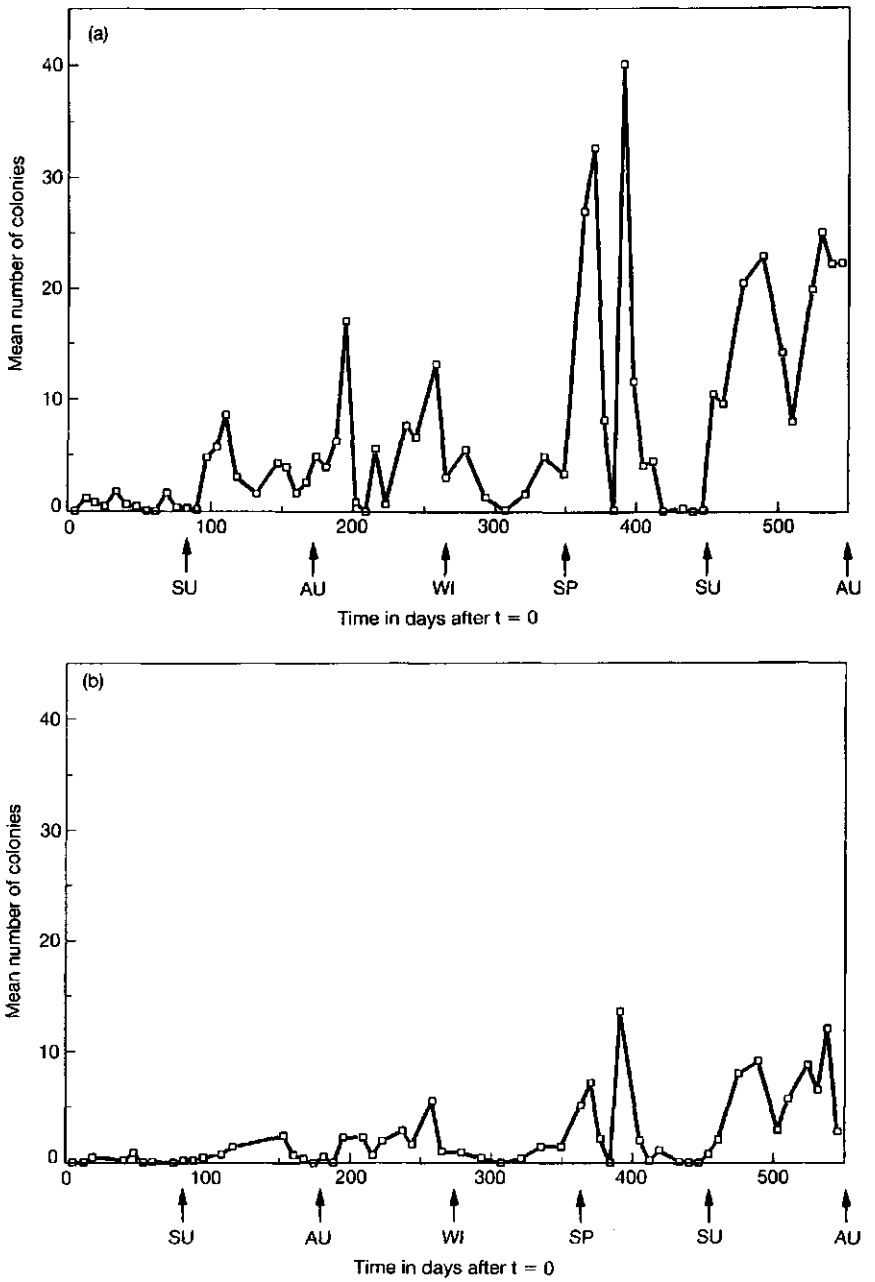

Fig.1. Mean number of colonies per spore trap (n=24), 0.5 m above the crop, (a) from 08.30 to 16.30 hours and (b) from 16.30 to 08.30 hours in glasshouse A, in 1988-1989. SP, SU, AU, WI: beginning of spring (21/3), summer (21/6), autumn (21/9) and winter (21/12), respectively.

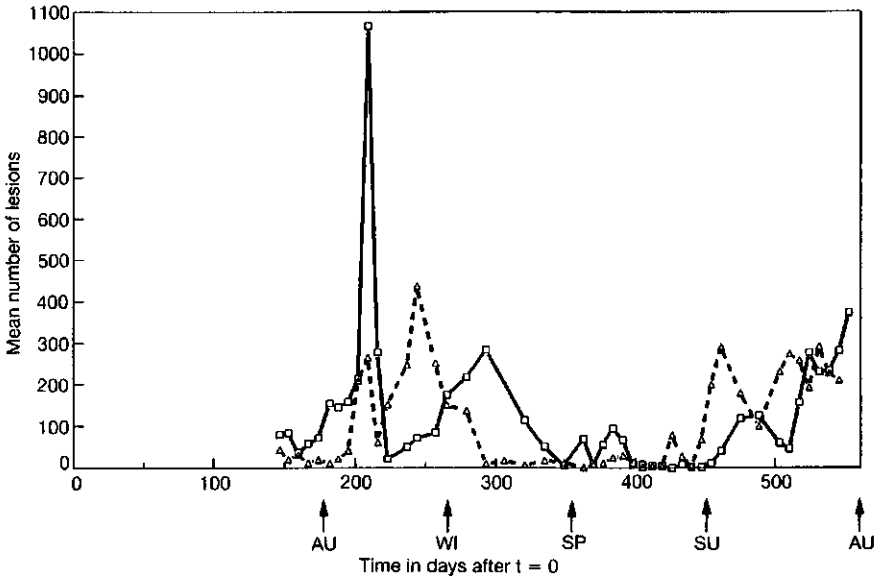

Fig. 2. Mean number of lesions formed on 10 petals per flower in glasshouse A (□ , n = 24 flowers) and V (△ , n = 30 flowers, t = t-110 days), in 1988-1989. SP, SU, AU, WI: beginning of spring (21/3), summer (21/6), autumn (21/9) and winter (21/12), respectively.

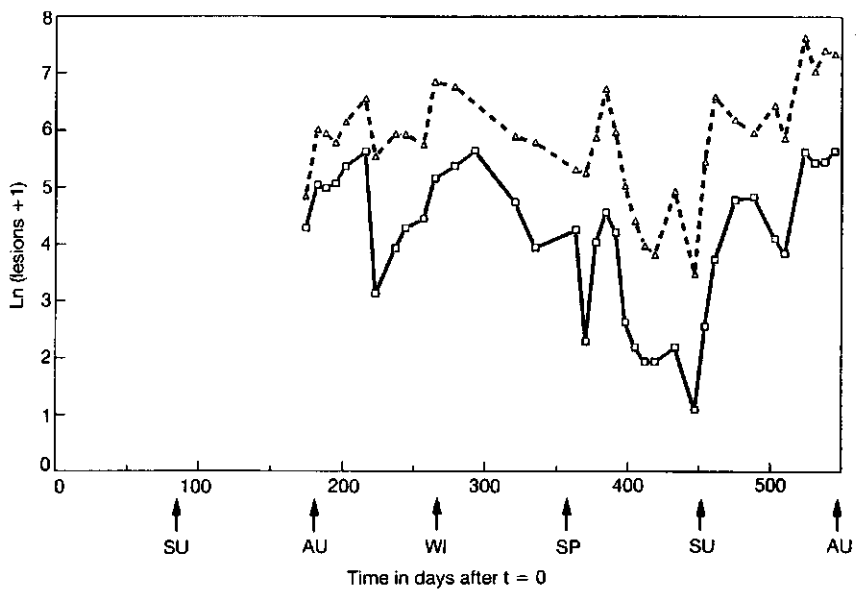

Fig. 3. Observed (□) and fitted (△) (equation (2) used) numbers of lesions (ln(N+1) transformed) on 10 petals per gerbera flower over time, in glasshouse A. SP, SU, AU, WI: beginning of spring (21/3), summer (21/6), autumn (21/9) and winter (21/12), respectively.

Results

Environmental conditions. In glasshouse A the daily mean relative humidity was < 80% in spring, > 85% in autumn, and 80% - 85% in winter and summer. The daily mean temperature was < 16°C in winter, > 21°C in summer and 16°C - 21°C in spring and autumn. In glasshouse V the daily mean relative humidity showed the same seasonal pattern, but was 10% - 20% lower while the daily mean temperature was similar to that in glasshouse A. The total daily global radiation was low (< 600 J/cm^2.day) during autumn and winter. During spring it increased rapidly up to 3000 J/cm^2.day early in summer. The wind-speed outside the glasshouses varied during the experiment without a clear pattern.

Flowering period of gerbera. The flowering period (GB) in glasshouse A was 5.6 days (minimum) in July and 13.2 days (maximum) in January.

Colonies. Figures 1a and 1b show the mean number of colonies per spore trap, 0.5 m above the crop, between 08.30 and 16.30 hours (day) and between 16.30 and 08.30 hours (night), respectively, in glasshouse A for weekly or biweekly exposures over 545 days. Data within and 1.5 m above the crop are not shown since the number of colonies showed the same pattern as 0.5 m above the crop. The number fluctuated from 0 - 40 (four Petri dishes) in the daytime and from 0 - 15 at night. During the first 90 days of the experiment (13 trapping occasions) there were less than five colonies per spore trap. At the end of the trapping period (after t=350) there were up to 40 colonies. High and low numbers of colonies occurred frequently between successive weeks during this latter period. The number of colonies in the daytime was about 3 - 4 times greater than at night. The mean number of colonies over time trapped in glasshouse V between 8.30 and 16.30 hours (day) was low compared to those in glasshouse A. Peaks could hardly be distinguished. Mean numbers fluctuated over time from 0 - 7 colonies per spore trap.

Significant linear correlations were found between the mean number of colonies, in day and night, of the three different spore trap heights in glasshouse A (Table 1), and between the mean number of colonies, in the daytime, of the three different spore trap heights in glasshouse V (Table 2).

Lesions. The mean number of lesions formed on 10 petals of a single gerbera flower varied from 0 - 1100 in glasshouse A (n=24) and from 0 - 450 in glasshouse V (n=30) (Fig. 2). Data over the first 146 days in glasshouse A and the first 43 days in glasshouse V are omitted because of poor incubation. During spring and early summer (t=350 to t=460 in glasshouse A and t=200 to t=330 in glasshouse V) very few lesions occurred, though many appeared at other times. Towards the end of the recording period the number of lesions increased. Apart from the exceptionally large peak at t=209 in

Table 1. Linear correlations between the mean number of colonies per spore trap (ln(N+1) transformed), in day (D) and night (N) periods, from three different spore trap heights.

		1	2	3	4	5	6
DO	1	1·00					
D50	2	0·85	1·00				
D150	3	0·79	0·94	1·00			
NO	4	0·85	0·71	0·61	1·00		
N50	5	0·77	0·81	0·76	0·85	1·00	
N150	6	0·71	0·76	0·75	0·76	0·94	1·00

(0: within; 50: 0·5 m above and 150: 1·5 m above the crop) in glasshouse A (For $P \leqslant 0{\cdot}05$, $r \geqslant 0{\cdot}27$ at $n = 53$).

Table 2. Linear correlations between the mean number of colonies per spore trap (ln(N+1) transformed), in the day, from three different spore trap heights.

		1	2	3
0	1	1·00		
50	2	0·88	1·00	
150	3	0·84	0·87	1·00

(0: within; 50: 0·5 m above and 150: 1·5 m above the crop) in glasshouse V (For $P \leqslant 0{\cdot}05$, $r \geqslant 0{\cdot}28$ at $n = 47$).

glasshouse A, the numbers of lesions were similar for the two glasshouses. The mean number of lesions over time fluctuated less than the mean number of colonies between successive exposure periods.

Regression analysis. Fluctuations in the number of colonies could not be satisfactorily accounted for by regressions incorporating any of the independent variables. The adjusted R^2 values were all below 0.4. Of both linear and non-linear regression models examined for the number of lesions, the best for data in glasshouse A utilized the three

variables, MRH, MS and t, and gave an adjusted R^2 of 0.81 ($P \leq 0.05$):

$$Y = -7.3(\pm 1.4) + 0.14(\pm 0.03)MRH - 0.00077(\pm 0.00015)MS + 0.0029(\pm 0.0006)t \quad (1),$$

where Y is ln(N+1) of the total number of lesions on 10 gerbera petals from one gerbera flower, MRH is the mean RH for days 6, 7 and 8 before the day of harvesting gerbera flowers and MS is the mean global radiation for days 1, 2 and 3 before the harvest day. For the data of glasshouse V the best regression equation utilized the same variables and explained 77% of variation in number of lesions (adjusted R^2 of 0.77; $P \leq 0.05$):

$$Y = -5.2(\pm 1.2) + 0.12(\pm 0.03)MRH - 0.00063(\pm 0.00015)MS + 0.0051(\pm 0.0012)t \quad (2).$$

The values of the variables of the two linear regression models are significantly different at $P \leq 0.05$, indicating that the glasshouse had a significant effect on the model. No significant effects were found for the interaction terms glasshouse * MH, glasshouse * MS and glasshouse * t. The best linear regression model for the number of lesions on gerbera petals for the data of both glasshouses combined was (adjusted $R^2=0.77$; $P \leq 0.05$):

$$Y = -6.6(\pm 1.5) + 0.12(\pm 0.03)MRH - 0.00074(\pm 0.00017)MS + 0.0042(\pm 0.001)t + 0.98(\pm 0.23)G \quad (3),$$

where G is a weighing factor (0-1) of the glasshouse effect. This is the effect of differences between glasshouses A and V in size, height, production system, location etc. The effects of varying values of MRH, MS and t in equation (3) on the numbers of lesions are shown in Table 3. Variable G was kept at 0.5. When MRH was high (90%) and MS was low (250 J/cm^2.day) or when MRH was high (90%) and t was high (500 days), the number of lesions exceeded 150. Equation (2) and (1) were used to estimate values from glasshouse A and V respectively (Figs. 3 and 4). The observed and estimated numbers of lesions on gerbera petals over time were significantly correlated ($P \leq 0.05$ at n=34) in either glasshouse. Figures 3 and 4 show that the levels of the observed and fitted curves were significantly different at $P \leq 0.05$, as stated earlier.

Table 3. Number of lesions at different values of MRH (%), MS (J/cm².day) and t (days) used in the linear regression equation (3).

MRH	*MS*	*t*	Number of lesions
60	250	200	5
60	250	500	21
60	2500	200	0
60	2500	500	3
90	250	200	232
90	250	500	811
90	2500	200	43
90	2500	500	154

The variable G was kept at a value 0·5.

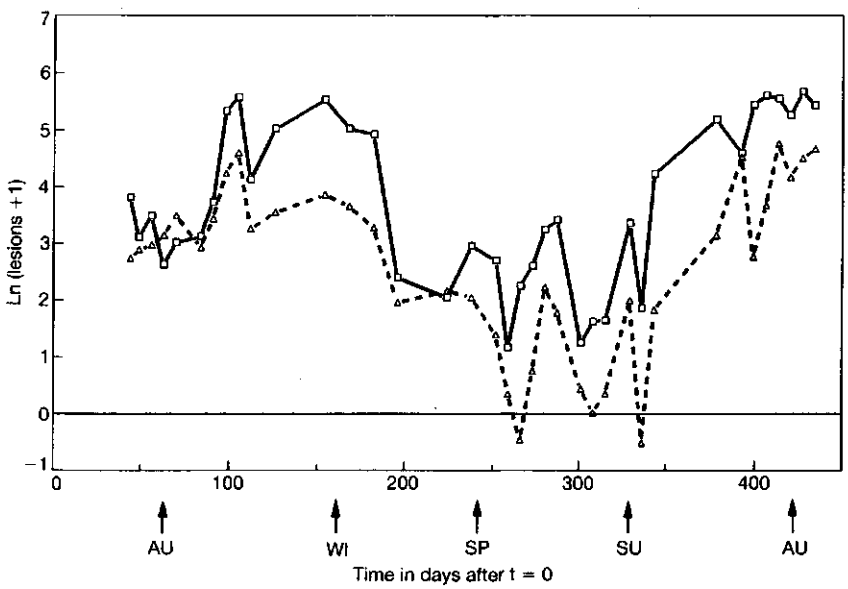

Fig. 4. Observed (□) and fitted (△) (equation (1) used) numbers of lesions (ln(N+1) transformed) on 10 petals per gerbera flower over time, in glasshouse V. SP, SU, AU, WI: beginning of spring (21/3), summer (21/6), autumn (21/9) and winter (21/12), respectively.

Discussion

In glasshouse A, the increasing number of trapped colonies over time can be attributed to the increasing amount of dead gerbera tissue as the gerbera crop ages (> 6 months). *B. cinerea* can enter senescing leaves as a saprophyte (Blakeman, 1980) and can produce an enormous number of conidia on colonized leaves (Jarvis, 1980). Conidial production is also enhanced by increases in relative humidity in a crop to 85 - 100% when the canopy closes. *B. cinerea* needs high relative humidity for germination, colonization of dead leaf tissue and sporulation (Blakeman, 1980).

The experienced changes in the numbers of trapped colonies over time are probably caused by changes in environmental conditions in the glasshouse. However, fluctuations in the number of colonies could not be explained by regressions incorporating any of the independent variables. The spore traps used were based on impaction. This means that fewer spores would be caught when there is less turbulence, given the same number of spores in the glasshouse air. Differences in the numbers of colonies between day and night in glasshouse A can be attributed to increased air movements in the glasshouse during the day owing to people working, open doors and windows and higher temperatures (A. Kerssies, personal observation).

Positive correlations between the three trapping heights suggest that trapping spores at a single height, e.g. at flower level, is sufficient to reflect the overall patterns of airborne spores.

The large differences in the numbers of colonies between glasshouse A and V were due to differences in systems of plant production. In glasshouse A, the gerbera plants were placed on rockwool on an irrigation mat to which superfluous water was drained. Dead leaves fell on to the irrigation mat within the crop. In glasshouse V, the plants were placed on rockwool on gutters 0.5 m above the ground from which superfluous water drained and the dead leaves fell to the ground, 0.5 m under the crop. The relative humidity within the crop in glasshouse A was 10% higher than that in glasshouse V so that relatively wet, dead leaves in the crop of glasshouse A provided a better substrate for germination, colonization and sporulation of *B. cinerea*. In glasshouse V, fewer wet dead leaves were found within the crop and fewer spores of *B. cinerea* were able to infect and form new sources of inoculum.

The number of lesions over time fluctuated less than the number of colonies. The exposure time of gerbera flowers (7 - 14 days) was longer than the exposure time of the spore traps (< 16 h), which were more sensitive to rapid changes in glasshouse conditions. Although Salinas *et al.* (1989) showed that one conidium could induce a lesion on a gerbera petal, significant correlations between numbers of colonies and lesions were not found in this study. This may have been because of differences in exposure time between the traps and flowers and also differences in opportunities for spore germination.

The linear regression models for the numbers of lesions on gerbera petals in both glasshouses suggests that relative humidity (positively correlated), radiation intensity (negatively correlated) and age of the crop (positively correlated) had an effect on the numbers of lesions. It is not clear whether these effects were based on causality. Vincelli and Lorbeer (1988a,b; 1989) found an effect of relative humidity, temperature and calendar date on disease severity of *Botrytis squamosa* in onion. They applied these variables in their weather-based predictive system. The significantly correlated observed and estimated values in Figs 3 and 4 show that equations (1) and (2) have predictive value. However, the type of glasshouse also had a significant effect on the number of lesions. Therefore, equation (3) in which the glasshouse effect is incorporated should be used.

The variables of temperature, flowering period and wind speed are not included in the final linear regression models. The factors temperature (R^2=0.78; $P \leq 0.05$; n=32) and flowering period (R^2=0.78; $P \leq 0.05$; n=32) were significantly correlated with total daily global radiation.

In both glasshouses, the numbers of lesions was more seasonally dependent than the number of colonies (Figs 1 and 2). The numbers of lesions on the same days of the year in glasshouses A and V, 30 km apart, were significantly correlated and not significantly different, except for the exceptional peak of 1100 lesions in glasshouse A. Reasons for this high peak are not understood. These results suggest that rather than the density of spores (as indicated by the number of colonies) and the level of relative humidity being the most important variables regulating number of lesions, other factors are responsible, such as a combination of radiation and relative humidity, the structure and composition of the flower cuticle, or the water relations (water and osmotic potential) in the flower.

In spring and early summer, flowers may possess a thicker cuticle and more wax owing to high radiation and low relative humidity (Skoss, 1995; Baker, 1974; Kolattukudy, 1985); germinated spores of *B. cinerea* may have greater difficulty penetrating the cuticle in spring and summer than in autumn and winter. Marois *et al.* (1988) showed that in California (USA) the susceptibility of rose flowers to *B. cinerea* was significantly higher in December, January, and February than in October and November, when temperature and radiation were higher. Also, conidial germination in *B. cinerea* on the gerbera flower surface could be affected by radiation and relative humidity. According to Hennebert and Giles (1958), exposure to direct sunlight can accelerate the decline in viability of conidia of *B. cinerea*.

Further research is needed on the causal relations of relative humidity, temperature, global radiation and age of the crop on germination and penetration of *B. cinerea* conidia, on the structure and composition of the cuticle of gerbera flowers and on the water relations in gerbera flowers.

Acknowledgements

The author is indebted to Prof. Dr. J.C. Zadoks, Ir. H.D. Frinking, Dr. J.J. Fransen and Dr. C. Vonk Noordegraaf for critically reading the manuscript, to Ir. M. Stapel and Ms. A. Verlind for helping with the statistical analyses and to Ms. M.C. Dil and Mr. J. Tolsma for assistance in the experimental part of the work.

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Chapter 3

Horizontal and vertical distribution of airborne conidia of *Botrytis cinerea* in a gerbera crop grown under glass

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Kerssies, A., 1993. Horizontal and vertical distribution of airborne conidia of *Botrytis cinerea* in a gerbera crop grown under glass. Netherlands Journal of Plant Pathology 99: 303-311.

Abstract

The horizontal and vertical distribution of airborne conidia of *Botrytis cinerea* in a gerbera crop in two glasshouses (100 m^2 and 350 m^2) was studied during 18 months in 1988 and 1989. Conidia of *B. cinerea* were caught in simple spore traps consisting of agar in Petri dishes placed in a regular pattern at three different heights in the glasshouse and counted as colonies, after incubation. Lesions due to conidial infection were counted on gerbera petals. The horizontal and vertical distribution of conidia of *B. cinerea* in a gerbera crop grown under glass was fairly uniform in both distinct glasshouses. Conidia of *B. cinerea* trapped in a glasshouse can originate from sources inside and outside the glasshouse. No significant interaction was found between location and time for the colony counts and for the log transformed (ln(N+1)) lesion counts. The results of this study suggest that spore trapping at one height and at a limited number of locations and dates is sufficient for efficient monitoring of *B. cinerea* in a glasshouse.

Additional keywords: lesions, trapping.

Introduction

Botrytis cinerea Pers. ex Pers. causes damage to a wide variety of plants including ornamentals like gerbera, rose, chrysanthemum and several pot plant species such as Saintpaulia (Kerssies, 1993). Conidia, ascospores, mycelial fragments and sclerotia are important for dispersal (Jarvis, 1980), although only conidia seem to play the main role in disease dispersal in glasshouses. Conidia are dispersed by air currents, water droplets and insects. Necrotic lesions ('spotting') on flower buds and petals are caused by early infections of plant tissue. Studies on dispersal of plant pathogens (e.g. *B. cinerea*) in glasshouses are scarce (Frinking and Scholte, 1983). Hirst (1959) was one of the first to monitor densities of air-borne spores, and *B. cinerea* conidia were amongst those trapped. Frinking et al. (1987) studied the dissemination of particles in a glasshouse divided in three multiple bays using *Lycopodium* sp. Little is known about factors influencing dispersal of *B. cinerea* in ornamentals in glasshouses. Frinking and Scholte (1983) showed that the complex dispersal process involves aspects of the pathogen, host, environment and the activity of man. Hausbeck and Pennypacker (1991) showed that grower activity in a greenhouse with geraniums resulted in peak conidial concentrations in the greenhouse atmosphere.

Dispersal of conidia in a gerbera crop growing under glass and the effects of environmental factors on dispersal of conidia and on infection of gerbera flowers during storage have been published (Kerssies, 1993). In this former study no seasonal pattern was found in the numbers of colonies developed from trapped conidia, but the numbers of

lesions on the flowers were season-dependent. In spring and early summer few lesions were produced, whereas many lesions appeared in other seasons. Linear regression accounted for 77 and 81% of the variation in the number of lesions on gerbera flowers in the two glasshouses in terms of relative humidity (positively correlated), total incident solar radiation outside the glasshouse (negatively correlated) and age of the crop (positively correlated). The aim of the present study was to investigate the horizontal and vertical distribution of airborne conidia of *B. cinerea* in a gerbera crop growing under glass in order to improve the efficiency of aerobiological studies in glasshouses.

Materials and methods

Distribution of Botrytis cinerea. The distribution of *B. cinerea* in a gerbera crop grown under glass on rockwool was studied in two glasshouses, one of 100 m^2 in Aalsmeer (glasshouse A; 480 plants, cv. Terrafame) and one of 350 m^2 in Vleuten (glasshouse V; 2000 plants, cvs. Rosamunde and Maria). In glasshouse A, six tables each supported four rows of 20 gerbera plants growing on rockwool on an irrigation mat. In glasshouse V, 20 rows of gutters with rockwool were installed, each with 100 plants. *B. cinerea* was introduced into the glasshouses. Petri dishes containing heavily sporulating colonies of *B. cinerea* on potato dextrose agar were exposed in glasshouse A (two dishes, between location 10 and 11 and between location 14 and 15) and V (four dishes, at location 7, 9, 22 and 24) for seven days and were removed before spore trapping started. The densities of *B. cinerea* conidia in the air of the glasshouses were studied between April 1988 and October 1989 using two methods (Kerssies, 1993). In method 1, spore traps were distributed within and above the crops in a regular pattern (Kerssies, 1990). In glasshouse A 24 trap locations within the crop, 24 trap locations 0.5 m above the crop and six trap locations 1.5 m above the crop were used. In glasshouse V 30 trap locations within the crop, 30 trap locations 0.5 m above the crop and eight trap locations 1.5 m above the crop were used. Each week (2-weekly during winter) fresh traps were placed in the glasshouses for 8 h during the day (08.30 - 16.30) and for 16 h during the night (16.30 - 08.30, glasshouse A only). The dishes were incubated for seven days at 20°C under fluorescent light (Pope, Ftd 36W/30, 8 $\mu molm^{-2}s^{-1}$) after which the number of colonies (dark brown) were recorded (Kerssies, 1990).
In method 2, each week (2-weekly during winter) 24 flowers near the spore traps were harvested in glasshouse A and 30 flowers in glasshouse V. The surfaces of the upper ten petals of each flower were exposed to the glasshouse air. The duration of exposure of the flower petals to the glasshouse air varied from 6 days in July to 13 days in January. Thereafter the petals were placed on wet paper in plastic boxes and incubated at 20°C under fluorescent light. After three days, *B. cinerea* lesions were counted under a microscope with a magnification of 10.

In glasshouse A spore traps were exposed 63 times over a period of 545 days at daytime and 55 times over a period of 545 days at night. Flowers were harvested and lesions were counted 46 times over a period of 405 days. In glasshouse V spore traps were exposed 49 times over a period of 439 days, at daytime only. Flowers were harvested and lesions were counted 45 times over a period of 371 days. After removal the crop (end of experiment) from glasshouse A spores were trapped inside (six spore traps) and outside (five spore traps) the glasshouse during 6 consecutive weeks.

Statistical analysis. In search of systematic spatial differences in numbers of colonies through time, Poisson regression models were fitted to the number of colonies per trap location per counting date (for each trapping height) or per trap height per counting date. Correlations were calculated between the numbers of colonies at different trap locations through time.

Spatial differences in numbers of lesions on gerbera petals were tested by analysis of variance. The log transformed (ln(N+1)) numbers of lesions on gerbera flowers per harvest location per counting date was taken as the explanatory variable. Again, correlations were calculated between the lesion counts at different harvest locations through time.

Results

Colonies on spore traps, horizontal distribution. The numbers of colonies on spore traps per counting date, at daytime, showed few significant differences ($P \leq 0.05$) between locations of one trapping height, in either glasshouse (Fig. 1a,b,c, glasshouse A and Fig. 2a,b,c, glasshouse V). The numbers of colonies per counting date, at night, in glasshouse A (not shown) again showed few significant differences between locations of one trapping height. In glasshouse A, at daytime, the numbers of colonies on spore traps at 0.5 m above the crop showed that the locations 17-24 were significantly higher ($P \leq 0.05$; Fig. 3). The locations 17-24 were at the rear end of the glasshouse. In glasshouse V, the numbers of colonies per counting date of a few trapping locations were significantly different within the crop only. No clear pattern could be observed.

Most of the spore trap locations, per trap height, were significantly correlated at $P \leq 0.05$ with regard to the numbers of colonies per counting date (Table 1).

Colonies on spore traps, vertical distribution. In neither glasshouse significant differences ($P \leq 0.05$) were found in the numbers of colonies per counting date between spore traps, at any location, within the crop and 0.5 m above the crop. The numbers of colonies did not show significant differences ($P \leq 0.05$) between the spore trapping heights, by day or by night in either glasshouse (Table 2). In glasshouse A the spore

trap heights were significantly different between day and night at P≤0.05 for the numbers of colonies per counting date (Table 2).

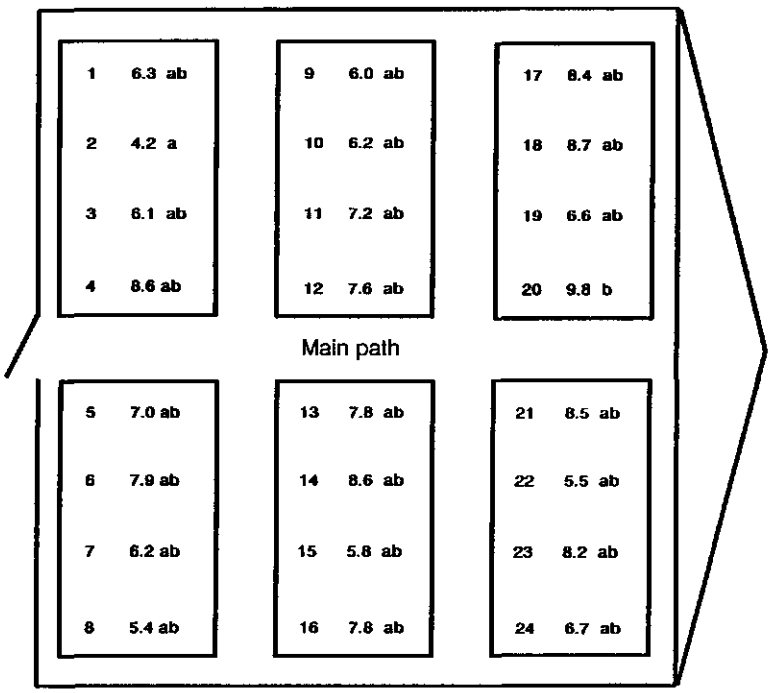

Fig. 1A. Mean number of colonies per spore trap (n=63) for the 24 trap locations, within the crop, trapped from 08.30 to 16.30 in glasshouse A. Location numbers refer to traps 1-24. Different letters indicate significant differences (P≤0.05).

Lesions. Analysis of variance of the log transformed (ln(N+1)) numbers of lesions per harvest location per counting date showed few significant differences (P≤0.05) between the harvest locations in either glasshouse (Fig. 4a,b). In glasshouse A the lesion density (log transformed (ln(N+1)) of the twelve harvest locations at the left side of the main path (3.81) and of the twelve harvest locations at the right side of the main path (3.65) were significantly different (P≤0.05). In glasshouse V no clear pattern could be observed.

In glasshouse A and V 91% and 85%, respectively, of the harvest locations were significantly correlated at P≤0.05 with regard to the log transformed numbers of lesions per counting date.

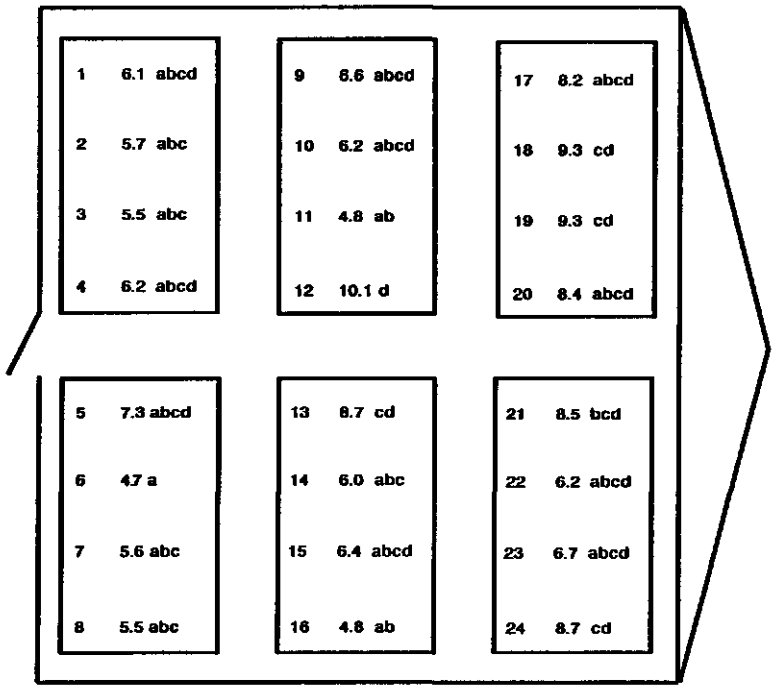

Fig. 1B. Mean number of colonies per spore trap (n=63) for the 24 trap locations, 0.5 m above the crop, trapped from 08.30 to 16.30 in glasshouse A. Location numbers refer to traps 1-24. Different letters indicate significant differences ($P \leq 0.05$).

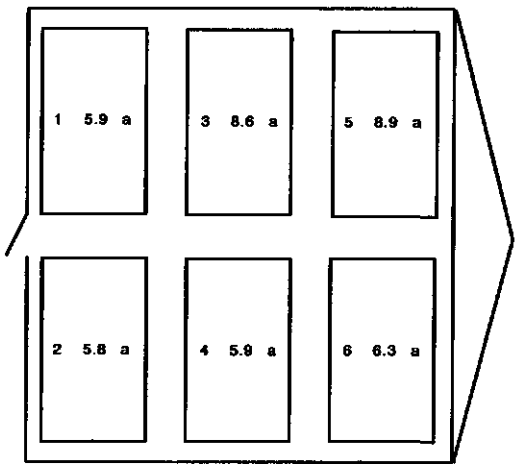

Fig. 1C. Mean number of colonies per spore trap (n=63) for the 24 trap locations, 1.5 m above the crop, trapped from 08.30 to 16.30 in glasshouse A. Location numbers refer to traps 1-6. Different letters indicate significant differences ($P \leq 0.05$).

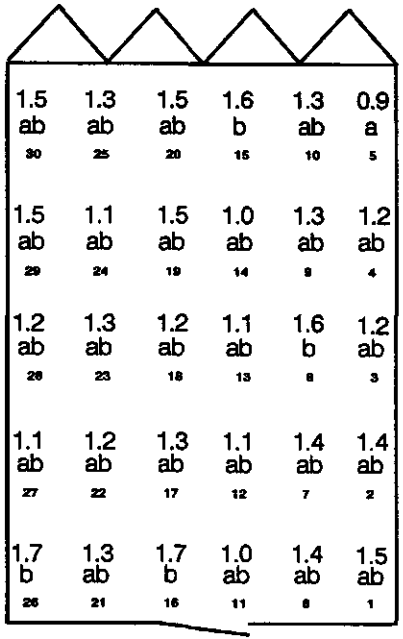

Fig 2A. Mean number of colonies per spore trap ($n = 49$) for the 30 trap locations, within the crop, trapped from 08.30 to 16.30 in glasshouse V. Location numbers refer to traps 1–30. Different letters indicate significant differences ($P \leq 0.05$).

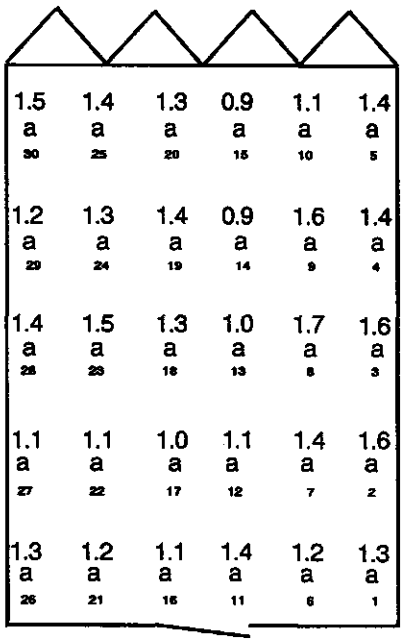

Fig 2B. Mean number of colonies per spore trap ($n = 49$) for the 30 trap locations, 0.5 m above the crop, trapped from 08.30 to 16.30 in glasshouse V. Location numbers refer to traps 1–30. Different letters indicate significant differences ($P \leq 0.05$).

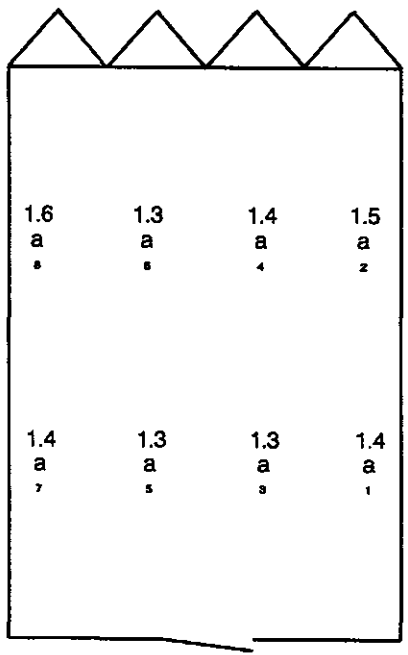

Fig 2C. Mean number of colonies per spore trap ($n = 49$) for the eight trap locations, 1.5 m above the crop, trapped from 08.30 to 16.30 in glasshouse V. Location numbers refer to traps 1–8. Different letters indicate significant differences ($P \leq 0.05$).

No significant interaction was found between location and time for the numbers of colonies and for the log transformed numbers of lesions. No relation was found between the distribution of sources of conidia (wet, dead leaves; rarely found) and the distribution of trapped conidia in the glasshouses.

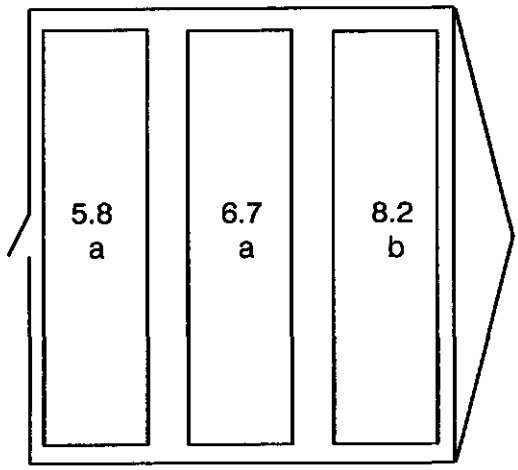

Fig. 3. Mean number of colonies per eight spore traps (n=63) for the trap locations 1-8, 9-16 and 17-24, 0.5 m above the crop, trapped from 08.30 to 16.30 in glasshouse A. Different letters indicate significant differences ($P \leq 0.05$).

Table 1. Percentage significant linear correlations between spore trap locations for trap height through time, in glasshouse A ($P \leq 0.05$; n=53) and glasshouse V ($P \leq 0.05$; n=47) for the numbers of colonies. D: day; N: night; 0: spore traps within the crop; 50: spore traps 0.5 m above the crop; 150: spore traps 1.5 m above the crop.

Glasshouse	Significant linear correlations (%)
Glasshouse A	
D0	95
D50	99
D150	100
N0	90
N50	99
N150	100
Glasshouse V	
D0	81
D50	94
D150	86

Table 2. Mean number of colonies per spore trap height in the day and night periods in glasshouse A and in the day in glasshouse V. D: daytime; N: night; 0: spore traps within the crop; 50: spore traps 0.5 m above the crop; 150: spore traps 1.5 m above the crop. Different letters indicate significant differences ($P \leq 0.05$; Anova).

Glasshouse	Spore trap height	Mean number of colonies
Glasshouse A ($n = 55$)	D0	7.2 a
	D50	6.9 a
	D150	6.9 a
	N0	2.5 b
	N50	2.2 b
	N150	2.1 b
Glasshouse V ($n = 49$)	D0	1.3 a
	D50	1.3 a
	D150	1.4 a

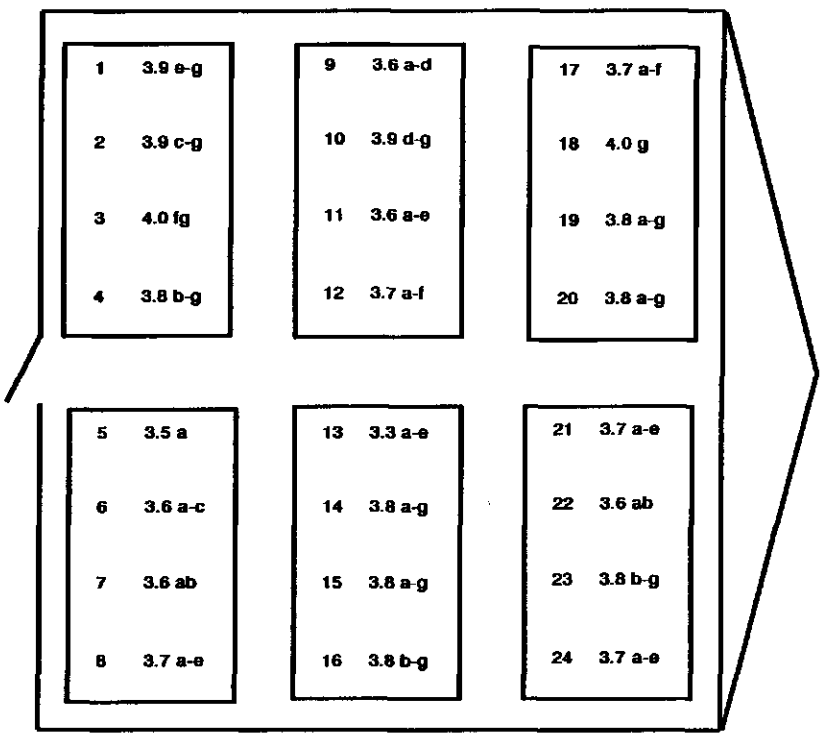

Fig. 4A. Log transformed (ln(N+1) mean number of lesions on gerbera petals at each harvest location (n=46) in glasshouse A. 1-24: harvest location numbers. Different letters indicate significant differences ($P \leq 0.05$).

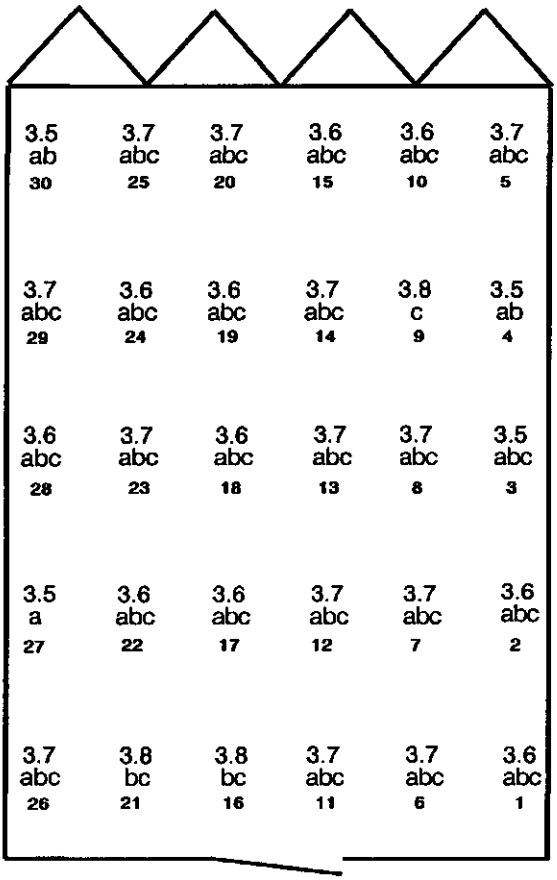

Fig. 4B. Log transformed (ln(N+1) mean number of lesions on gerbera petals at each harvest location (n=45) in glasshouse V. 1-30: harvest location numbers. Different letters indicate significant differences ($P \leq 0.05$).

Discussion

The horizontal and vertical distributions of conidia of *B. cinerea* in a gerbera crop grown under glass, counted as colonies or as lesions on petals, were fairly uniform in both glasshouses A (100 m^2) and V (350 m^2), with high and low levels of trapped conidia. The significantly higher numbers of colonies per counting date, 0.5 m above the crop, at the rear of glasshouse A, could have been caused by air movements due to draught from the open door at the front in combination with ventilation through the windows. The significantly higher numbers of lesions on petals in glasshouse A at the left side of the glasshouse (as viewed from the main path) might have been caused by

increased turbulence, since at the left side of the glasshouse the windows were more frequently open than at the right side. Although glasshouse V is more than three times larger than glasshouse A the numbers of colonies and lesions counts on petals in glasshouse V showed fewer significant differences. Probably, in glasshouse V more turbulence occurred due to the presence of more opening windows per m^2, resulting in a more regular distribution of colonies of *B. cinerea* within the glasshouse.

The lack of significant differences between trapping locations and between trapping heights in the numbers of colonies and between harvest locations in the numbers of lesions on petals suggests that conidia of *B. cinerea* are transported rapidly within the glasshouse (Frinking *et al.*, 1987).

Presumably, inoculum of *B. cinerea* can also be transported from inside the glasshouse to the outside and vice versa. After removal the crop from glasshouse A conidia were trapped in- and outside the glasshouse during six consecutive weeks. During the first four weeks, the numbers of colonies inside and outside decreased, respectively from 11 to 1 and from 18 to 2 colonies per trap, but after four weeks they increased to 10 and to 5 colonies per trap, respectively. Air from outside, containing conidia of *B. cinerea*, can enter the glasshouse through open windows, doors and small holes. On spore traps at a distance of 50 m from glasshouse A, high numbers of spores of *B. cinerea* were trapped (8 spores per spore trap, n=14), though less than in glasshouse A (14 spores per spore trap, n=14, $P \leq 0.05$). Pady and Kelly (1954) and Richards (1956) reported that *B. cinerea* is one of the fungi most frequently trapped in air. Conidia of *B. cinerea* trapped outside the experimental glasshouse can originate from other glasshouses or from the field. This statement is in agreement with results of other authors. Zadoks (1967) stated that fungi can enter and leave glasshouses easily. Frinking (1991) stated that there is a continuous exchange of air between the glasshouse and the outside environment. Schepers (1984) suggested that conidia of *Sphaerotheca fuliginea* will also be transported easily into and out of glasshouses.

The results of this study suggest that measuring *B. cinerea* in a glasshouse with spore traps at a single height and at a limited number of locations is sufficient. Kerssies (1993) showed that in spring and early summer very few lesions were counted, whereas in the other seasons many lesions were present. Therefore, trapping *B. cinerea* spores to monitor spore behaviour is mainly necessary in fall and winter.

Acknowledgements

The author is indebted to Professor Dr. J.C. Zadoks, Ir. H.D. Frinking, Dr. J.J. Fransen and Dr. C. Vonk Noordegraaf for critically reading the manuscript, to Ir. M. Stapel and Ms. A. Verlind for helping with the statistical analyses and to Ms. M.C. Dil and Mr. J. Tolsma for assistance in the experimental part of the work.

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Chapter 4

Influence of environmental conditions on dispersal of *Botrytis cinerea* conidia and on post-harvest infection of rose flowers grown under glass

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Kerssies, A., Bosker-van Zessen, A.I. and Frinking, H.D., 1994. Influence of environmental conditions on dispersal of *Botrytis cinerea* conidia and on post-harvest infection of rose flowers grown under glass. Submitted to European Journal of Plant Pathology.

Abstract

Dispersal and horizontal distribution of *Botrytis cinerea* in a rose crop in a glasshouse of 300 m^2 was studied in 1991 and 1992. Conidia of *B. cinerea* were caught in spore traps consisting of an agar medium selective for *B. cinerea* in Petri dishes placed within the crop, at flower height 1 m above the ground. Spore catches were counted as colonies, after incubation. Lesions due to conidial infection were counted on petals of rose flowers, also after incubation. Relative humidity and temperature within the glasshouse and global radiation and windspeed outside were recorded during the experiments. The horizontal distribution of *B. cinerea* in a rose crop grown under glass was fairly uniform in both years. In 1991 a clear seasonal pattern in the number of colonies could not be found. In 1992 the number of colonies were high in August, September and October. The number of lesions on rose flowers showed a distinct pattern in both years. In August, September and October many lesions were counted whereas in the other months few lesions appeared.

In linear regression analysis, variation in numbers of colonies (spore catches) could not be explained by environmental factors recorded during the experiments. Linear regression accounted for 76 and 63% of the variation in the number of lesions on rose flowers in 1991 and 1992, in terms of relative humidity (positively correlated), global radiation outside the glasshouse (negatively correlated) and numbers of colonies on spore traps (positively correlated).

The results in the rose crop suggest that RH, global radiation and spore density in glasshouses are important variables in regulating the numbers of lesions during storage and transport. The numbers of spores in glasshouses are dependent on the production system. A glasshouse with a system resulting in wet dead tissue on the ground give higher amounts of spores in the glasshouse air and through that high numbers of lesions on flowers. On roses outside the glasshouses very high numbers of lesions were counted sometimes, mostly during and after rain showers, as a result of rain-deposition of spores onto the flowers.

Additional keywords: colonies, lesions, relative humidity, global radiation, linear regression.

Introduction

The fungus *Botrytis cinerea*, the imperfect stage of *Sclerotinia fuckeliana* (Ellis & Waller, 1974), infects a wide variety of plants. Infection takes place through wounds, via decaying or dead plant tissue, and by direct penetration of the undamaged host (Verhoeff, 1980). Conidia are dispersed by air currents, water droplets and insects. *B.*

cinerea causes damage to ornamentals such as gerbera, rose, chrysanthemum and pot plants such as saintpaulia (De Jong, 1985, 1986). Necrotic lesions ('spotting') occur on flower buds and petals are caused by early infections. These symptoms are encouraged by a relative humidity above 93% (Salinas *et al.*, 1989), as when flowers are packed into boxes and rapid changes in temperature occur owing to transfer from cold storage into trucks. Many lesions occur within 24 h after harvest at 18 to 25°C (Salinas et al., 1989). Damage caused by *B. cinerea* in the post-harvest phase is difficult to avoid. Use of fungicides in the glasshouse may increase the risk of damaging the flowers, of chemical residues on the flowers and of selection for fungicide resistance in the fungus (Gullino & Garibaldi, 1987; Witte & De Jong, 1985).

Studies on dispersal of plant pathogens in glasshouses are few (Frinking & Scholte, 1983). Hirst (1959) was among the first to monitor densities of air-borne spores, and *B. cinerea* conidia were amongst those trapped. Hausbeck and Pennypacker (1991) showed that grower activity in a greenhouse with geraniums resulted in peak conidial concentrations in the greenhouse air. Frinking *et al.* (1987) studied the dissemination of particles in a glasshouse divided into three bays using *Lycopodium* sp. Frinking *et al.* (1987) studied the dissemination of *Lycopodium* spores in a glasshouse divided into three multiple bays. They found that more air movements owing to open ventilation windows caused a rapid cleaning of air while in a glasshouse with less air movements owing to closed ventilation windows spores remained suspended for a long time. Frinking *et al.* (1987) suggest that the patterns of air movement in glasshouses differ according to the spatial arrangement of the crop canopy. Little is known about the factors influencing dispersal of spores of *B. cinerea* in ornamentals grown in glasshouses. Frinking and Scholte (1983) showed that the complex dispersal process involves aspects of pathogen, host, environment and human activity. Studies on the dispersal and the horizontal and vertical distributions of conidia of *B. cinerea* in a gerbera crop growing under glass and the effects of environmental factors on dispersal and infection of gerbera flowers during postharvest were published (Kerssies, 1993 a,b). The structure of a rose crop is totally different from a gerbera crop. The aims of the present study were to investigate the patterns of *B. cinerea* conidia dispersal and distribution in a rose crop growing under glass and the effects of environmental factors on dispersal and infection of rose flowers during storage and transport. Some observations were made on the dispersal of *B. cinerea* conidia outside glasshouses.

Materials and methods

Measurement of environmental conditions. Dry en wet bulb temperatures were measured continuously using 9 psychrometers, distributed within the crop, 0.5 m above the ground, coupled to a data logger. The temperature in the crop was measured and

averaged over 1 h periods and the relative humidity was calculated and averaged over 1 h periods. The total incoming global radiation outside the glasshouse ($Jcm^{-2}day^{-1}$) was measured by a Kipp solarimeter 8 m above the ground at the Research Station for Floriculture in Aalsmeer. The windspeed outside the glasshouse in Aalsmeer was measured continuously and averaged over 1 h periods using a tachometer 8.5 m above the ground (De Jong, 1990).

Dispersal of Botrytis cinerea. Experiments were done to examine dispersal and horizontal distribution of *B. cinerea* conidia in a rose crop, during 1991 and 1992. The rose crop was grown in a 300 m^2 glasshouse (E4) in Aalsmeer with 2424 plants of the cv. 'Sonia'. Six tables each supported four rows of gutters (Van Weel *et al.*, 1990) with rockwool, with 101 rose plants per gutter. The density of *B. cinerea* conidia in the air of the glasshouse was studied in 1991 and 1992 using two methods.

1. Spore traps were constructed by attaching the bases of Petri dishes containing a selective medium for *B. cinerea* (Kritzman and Netzer, 1978; Kerssies, 1990) to the four sides and the bottom of a wooden cube (9*9*9cm). Fourty-eight spore traps were distributed within the crop, at flower height, 1 m above the ground, in a regular spatial pattern. Each week fresh Petri dishes were placed in the glasshouses for 24 h. The dishes were incubated for 7 days at 20°C under fluorescent light (Pope, FTD 36W/30, 8 $\mu molm^{-2}s^{-1}$) and the numbers of dark brown colonies were recorded (Kerssies, 1990).

2. The number of lesions on rose flowers was counted after harvest. In addition, each week 3 flowers (if available) near each spore trap were harvested. Before harvesting, the flowers had been exposed to the glasshouse air until ripening for 4 to 13 days, according to the season. They were then placed in plastic boxes with wet paper and incubated at 20°C under fluorescent light. After 3 days, *B. cinerea* lesions were counted.

Three additional spore traps were used each week in 1992 and five flowers (cv. Sonia) were harvested from week 23 to week 52 in 1992 in a neighbouring glasshouse (E3; 300 m^2) planted with a range of rose cultivars. Outside glasshouse E4 four spore traps were exposed (three at a distance of 1 meter and one 40 meter south-west from the glasshouse) each week in 1991 and 1992. In the vicinity of two spore traps (one at a distance of 1 meter and one 40 meter south-west from the glasshouse) 5 flowers were placed in plastic tubes containing 32 ml of water for 4 days from week 23 to week 52 in 1992.

Statistical analysis. The mean numbers of colonies (Poisson distributed) on 50 trapping occasions and the mean numbers of lesions on rose flowers (Poisson distributed) in 41 samples (the first 9 weeks the rose-plants did not produce flowers) in glasshouse E4 over a period of 365 days (1991) were the dependent variables in regressions against environmental variables (Madden & Ellis, 1988). These data constituted a time series. Successive observations can be treated as largely independent (Kerssies, 1993). The

following independent variables were used in the regressions: RH, daily mean relative humidity within the crop, at flower height, 1 m above the ground (%); V, daily mean vapour pressure deficit within the crop, at flower height, 1 m above the ground (VPD); T, daily mean temperature within the crop, at flower height, 1 m above the ground (°C); S, total daily global radiation outside the glasshouse ($Jcm^{-2}day^{-1}$); W, daily mean windspeed outside the glasshouse (ms^{-1}); t, age of the crop in number of days from the start of the experiment (= planting date); CFU, mean numbers of colonies on the spore traps (Ln (N+1)) adjusted to the mean numbers of lesions on the flowers counted 1 week later (only for the regression analysis of the mean numbers of lesions). Daily mean values of RH, V, T, S and W were calculated for each of the 14 days before a trap exposure day (MRH, MV, MT, MS and MW). By means of linear Poisson regression analysis (procedure RSELECT in the statistical program GENSTAT, Payne *et al.*, 1987) the subsets of independent variables which gave the best fit for the mean numbers of colonies and for the mean numbers of lesions were selected. The procedure RSELECT selects the best subset of variables in regression according to Mallow's Cp as the criterion for goodness of fit (Montgomery & Peck, 1982). The variables used in the best equation determined with the data from 1991 were also used for the data of 1992, where the mean numbers of colonies on spore traps (CFU) and the mean numbers of lesions on petals were counted 42 and 48 times, respectiveley, over a period of 366 days.

In search of significant spatial differences in colony and lesion densities through time, Poisson regression models were fitted to the numbers of colonies per trap location and to the number of lesions per harvest location per counting date.

Linear correlations over time were calculated between locations inside and outside the glasshouses, for the mean numbers of colonies on spore traps and for the mean numbers of lesions on rose flowers.

Results

Environmental conditions. The daily mean temperature, the daily mean VPD and the daily mean relative humidity in glasshouse E4 and the total daily global radiation outside the glasshouse, in 1991 and 1992, are shown in Table 1. The averaged windspeed outside the glasshouses varied during the experiment without a clear pattern.

Table 1. Daily mean temperature, daily mean VPD and daily mean relative humidity within the rose crop, at flower height 1 m above the ground, in glasshouse E4 and total daily global radiation outside the glasshouse, in 1991 and 1992, grouped per season.

Season	Temperature (°C)	VPD (kPa)	Relative humidity (%)	Total global radiation ($Jcm^{-2}day^{-1}$)
Spring	19-22	5-12	65-75	>700 and <2700
Summer	20-25	5-8	70-80	>700 and <2700
Autumn	17-20	4-6	70-85	<700
Winter	15-18	5-10	55-70	<700

Table 2a. Linear correlations between the mean numbers of colonies per spore trap (Ln(N+1) transformed) inside and outside E4, in 1991 (for $P \leq 0.05$, $r \geq 0.34$ at $n=35$).

Inside E4	1	1.00	
Outside E4	2	0.49	1.00
		1	2

Table 2b. Linear correlations between the mean numbers of colonies per spore trap (Ln(N+1) transformed) in E3, E4 and outside E4, in 1992 (for $P \leq 0.05$, $r \geq 0.30$ at $n=42$).

Inside E3	1	1.00		
Inside E4	2	0.59	1.00	
Outside E4	3	0.47	0.85	1.00
		1	2	3

Table 2c. Linear correlations between the mean numbers of lesions per rose flower (Ln(N+1) transformed) in E3, E4 and outside E4, in 1992 (for $P \leq 0.05$, $r \geq 0.38$ at n=27).

Inside E3	1	1.00		
Inside E4	2	0.28	1.00	
Outside E4	3	0.07	0.52	1.00
		1	2	3

Colonies. The colony counts for locations averaged over a year (n=50 in 1991 and n=42 in 1992) in glasshouse E4 showed few significant differences ($P \leq 0.05$) between trapping locations, in either year (Fig. 1; data of 1991 not shown). No clear pattern could be observed.

Figure 2 shows the colony counts for trapping dates averaged over locations (n=48) in 1991 and 1992 in glasshouse E4 for weekly exposures over 365 and 366 days. The numbers fluctuated from 0 - 18 (summation over the five Petri dishes per trap) in 1991 and 1992. In 1991 no clear pattern could be observed. In 1992 the numbers were high (>5 colonies/spore trap) in August, September and October. High numbers of colonies (>5 colonies/spore trap) were counted at day 79, 184, 205, 219, 261, 289 and 338 in 1991 and at day 183, 232, 239, 246, 274 and 288. On these days and 1-4 days before the counting date, the relative humidity was high (≥75%), the temperature and the total daily global radiation were variable. The high relative humidity could have been caused by fungicide treatments against powdery mildew or by a high relative humidity outside the glasshouse.

The colony counts for trapping dates averaged over locations (n=3) in glasshouse E3 fluctuated from 0 - 36 in 1992 (Fig. 3). No clear pattern could be observed, except for the low number of colonies in spring. The colony counts for trapping dates averaged over locations (n=4) outside glasshouse E4 fluctuated from 0 - 20 in 1991 and from 0 - 37 in 1992 (Fig. 4). In 1991 no clear pattern could be observed. In 1992 the numbers were high (>20 colonies/spore trap) in August, September and October. On these days and 1-4 days before the counting date, the relative humidity was high (≥75%), the temperature and the total daily global radiation were variable.

The mean numbers of colonies on spore traps in- and outside E4 were significantly correlated in 1991 and 1992 (Table 2a,b). The mean numbers of colonies in E3, E4 and outside the glasshouses were significantly correlated in 1992 (Table 2b).

Most of the peaks in the mean numbers of colonies outside E4 are similar to the peaks inside E4 at the same counting dates (at t= 184, 219, 289, 310 and 338 in 1991 and at t= 41, 156, 183, 232, 246, 274 and 228 in 1992; Fig. 2 and 4). In 1992 some peaks in the mean numbers of colonies are counted at the sime time in E4 and E3, but

other peaks are counted in E3 (at t= 15, 93, 120, 188 and 316) and not in E4 (Fig. 2 and 3). Most of the peaks in the mean numbers of colonies outside E4 are also similar to peaks inside E3 (at t= 41, 93, 120, 156, 183, 232, 246, 288 and 316 in 1992, Fig. 3 and 4). Peaks in E3 and outside E4 were higher (maximum of 36 cfu/trap) than peaks in E4 (maximum of 18 cfu/trap).

Lesions. The lesion counts for locations averaged over counting dates in glasshouse E4 showed few significant differences ($P \leq 0.05$) between the harvest locations in either year (Fig. 5; data of 1991 not shown). No clear pattern could be observed.

The lesion counts per counting date averaged over locations in glasshouse E4 varied from 0 - 42 in 1991 (n=48) and from 0 - 9 in 1992 (n=48) (Fig. 6). In 1991 the numbers were high (>7 lesions/flower) in September and October. In the other months the numbers were <3 lesions/flower. In 1992 the numbers were high (>4 lesions/flower) in August, September and October. In the other months the numbers were <3 lesions/flower, except for one peak in February when the glasshouse was cleaned inside with water. The lesion counts per counting date averaged over locations fluctuated less between successive exposure periods than the mean number of colonies (Fig. 2 and 6). The mean numbers of lesions formed on a single rose flower in glasshouse E3 varied from 2 - 60 in 1992 (Fig. 7, n=5). Outside the glasshouses the numbers varied from 0 - 310 in 1992 (Fig. 8, n=5). Very high numbers of lesions on roses (>100 lesions/flower) outside were counted on days with rain showers.

Table 3. Calculated mean numbers of lesions per rose flower at different values of MRH, MS and CFU using equation (3). The variable Year was kept at a value of 0.5.

MRH (%)	MS ($Jcm^{-2}day^{-1}$)	CFU/ spore trap	# Lesions/ flower
60	250	1	0.2
60	2500	10	0.1
60	250	10	1.0
60	2500	1	0.0
90	250	1	21.9
90	2500	10	11.1
90	250	10	89.5
90	2500	1	2.7

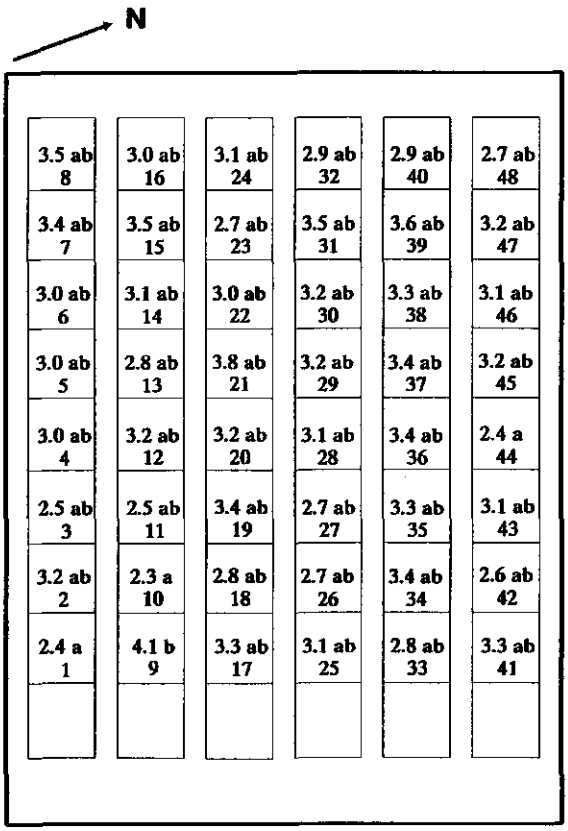

Fig. 1. Spore trap positions in a rose glasshouse. Colony counts for locations averaged over 1992 (n=42). 1-48: location numbers. Colony counts with the same letters are not signficantly different ($P \leq 0.05$).

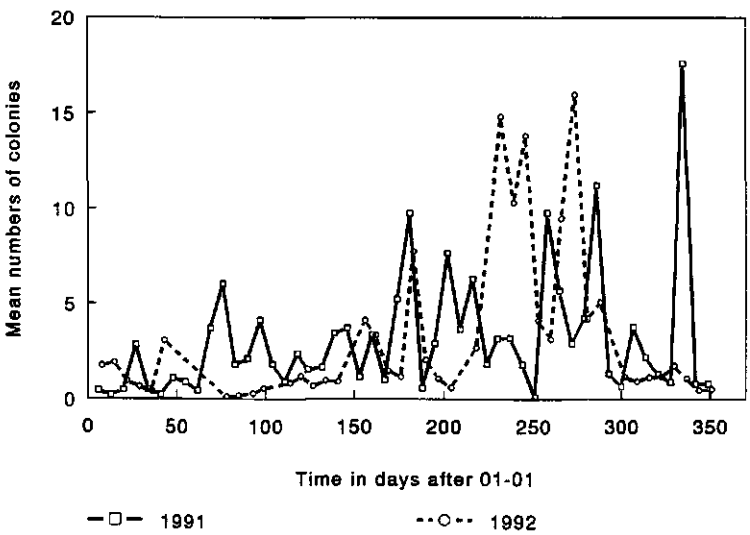

Fig. 2. Colony counts for trapping dates averaged over locations (n=48) in glasshouse E4, in 1991 and 1992.

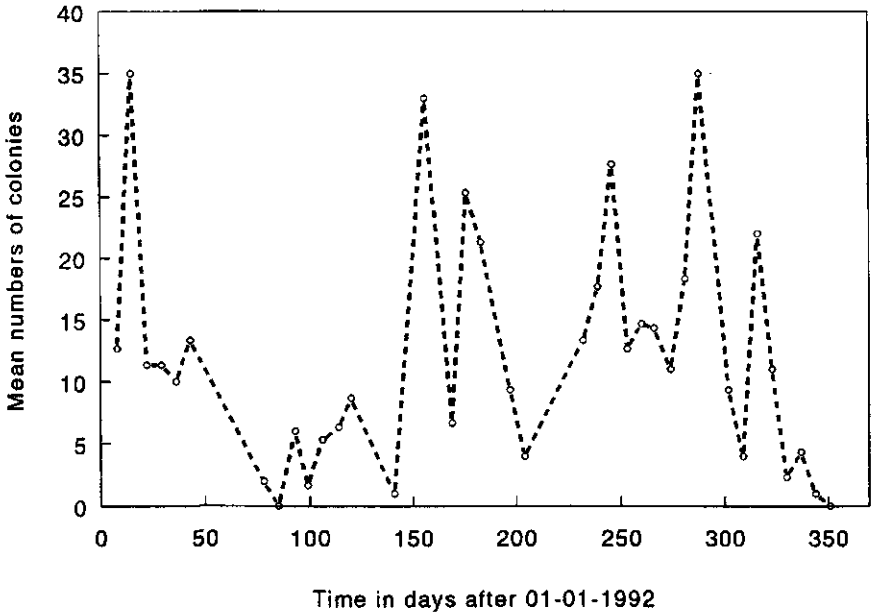

Fig. 3. Colony counts for trapping dates averaged over locations (n=3) in glasshouse E3, in 1992.

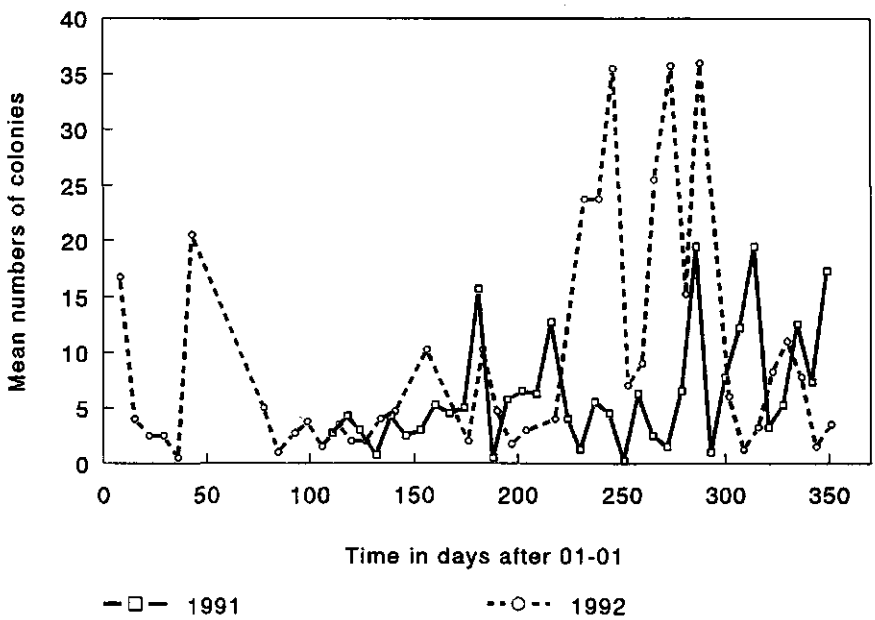

Fig. 4. Colony counts for trapping dates averaged over locations (n=4) outside glasshouses, in 1991 and 1992.

In 1992 the mean numbers of lesions on roses in- and outside E4 were significantly correlated (Table 2c). The mean numbers of lesions on roses in E3 were not significantly correlated with those in and outside E4 (Table 2c).

Most of the peaks in the mean numbers of lesions on roses outside E4 are similar to peaks inside E4 (at t= 231, 238, 245, 266 and 280 in 1992; Fig. 6 and 8). Most of the peaks in E3 are not similar to peaks in- and outside E4 (Fig. 6, 7 and 8). Peaks in E3 and outside E4 were higher (maximum of 60 lesions/flower in E3 and 310 outside E4) than peaks in E4 (maximum of 42 lesions/flower). The high peak (60 colonies/spore trap) in E3 at t=308 was probably caused by a combination of wet tissue on the ground resulting in high numbers of spores in the glasshouse air and a high relative humidity.

No significant interaction was found between location and time for the numbers of colonies and for the log transformed numbers of lesions.

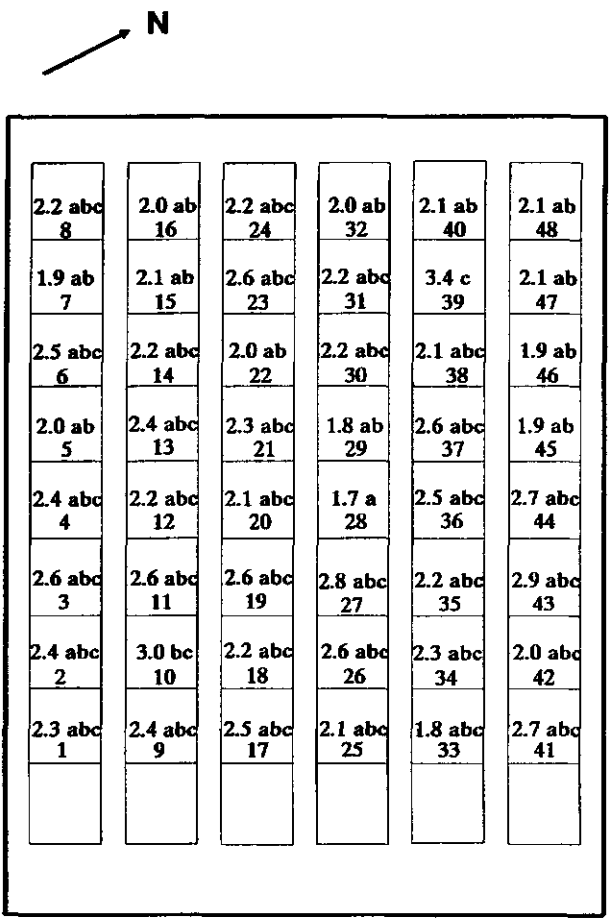

Fig. 5. Spore trap positions in a rose glasshouse. Lesion counts on rose flowers for locations averaged over counting dates in 1992 (n=48). 1-48: harvest location numbers. Lesion counts with the same letters are not significantly different ($P \leq 0.05$).

Regression analysis. Fluctuations in the number of colonies in glasshouse E4 could not be explained by regressions on any of the independent variables separately. The adjusted R^2 values were all below 0.4. Of all linear and non-linear regression models examined for the number of lesions in glasshouse E4 the best model for the 1991 data utilized the three variables, MRH, MS and CFU, and gave an adjusted R^2 of 0.76 ($P \leq 0.05$):

$$Y = -12.3(\pm 1.8) + 0.17(\pm 0.02)*MRH - 0.00098(\pm 0.00026)*MS + 1.01(\pm 0.22)*CFU \quad (1),$$

where Y is ln(N+1) of the mean number of lesions per rose flower, MRH is the mean RH for days 4, 5, 6 and 7 before the day of harvesting rose flowers and MS is the mean global radiation for days 1, 2 and 3 before the harvest day. For the data in 1992 the best regression equation utilized the same variables and gave an adjusted R^2 of 0.63 ($P \leq 0.05$):

$$Y = -6.1(\pm 1.9) + 0.09(\pm 0.03)*MRH - 0.00056(\pm 0.00017)*MS + 0.46(\pm 0.14)*CFU \quad (2).$$

The regression equations for the data in 1991 and 1992 are rather similar, but the partial regression coefficients tested pairwise of the two linear regression models are significantly different at $P \leq 0.05$ indicating that the year had a significant effect on the model. Both equations predict higher numbers of lesions in September, October and November and lower numbers in the other months. Therefore, it is better to use the best linear regression model for the number of lesions on rose flowers for the data of both years combined (adjusted $R^2=0.70$; $P \leq 0.05$):

$$Y = -10.3(\pm 1.4) + 0.15(\pm 0.02)*MRH - 0.00093(\pm 0.00017)*MS + 0.61(\pm 0.13)*CFU + 0.27(\pm 0.18)*Z \quad (3),$$

in which Z is the year effect, with Z being 0 or 1. The effects of varying values of MRH, MS and CFU in equation (3) on the numbers of lesions are shown in Table 3. When MRH was high (90%) and MS was low (250 $Jcm^{-2}day^{-1}$) or when MRH was high (90%) and CFU was high (≥10 colonies per trap), the number of lesions per flower exceeded 11 lesions. The observed and estimated numbers of lesions on rose petals over time were significantly correlated ($P \leq 0.005$ at n=41 or n=48) in either year.

Equation (1, 1991) and (2, 1992) were used to estimate values for 1992 and 1991, respectively (Figs. 9 and 10). In Fig. 9, at t=294 the fitted number of lesions shows no peak (4.8 lesions/flower), as the observed number of lesions does (20.4 lesions/flower). On this counting date the relatively low MRH (73%) resulted in the relatively low number of fitted lesions. In Fig. 10, at t=41 the fitted number of lesions shows no peak (1.2 lesions/flower), as the observed number of lesions does (7.1 lesions/flower). On this

counting date a combination of relatively low MRH (73%) and low numbers of colonies (0.5 colonies/trap) resulted in the low number of fitted lesions. At t=253 the fitted number of lesions shows a peak (8.5 lesions/flower), as the observed number of lesions does not (3.6 lesions/flower). On this counting date a combination of relatively high MRH (75%) and high numbers of colonies (14) resulted in the high number of fitted lesions. On these counting dates the number of lesions were not well explained by the regression models. They were probably explained by the other 30% which was not included in the regression models. However, the observed and estimated numbers of lesions on rose petals over a period of 1 year (Fig. 9 and 10; 1991-1992 and 1992-1991, respectively) were significantly correlated ($P \leq 0.05$ at n=38). Figures 9 and 10 illustrate that the partial regression coefficients of the observed and fitted curves were significantly different at $P \leq 0.05$, as stated before. In September and October, 1991, the numbers of lesions were higher than in the same period in 1992, but in both years the numbers were higher than in the other months. The pattern of peaks and valleys rather than their absolute values is considered important here. In both years the numbers were high (>4 lesions per flower), when the RH was high and the global incoming radiation was low.

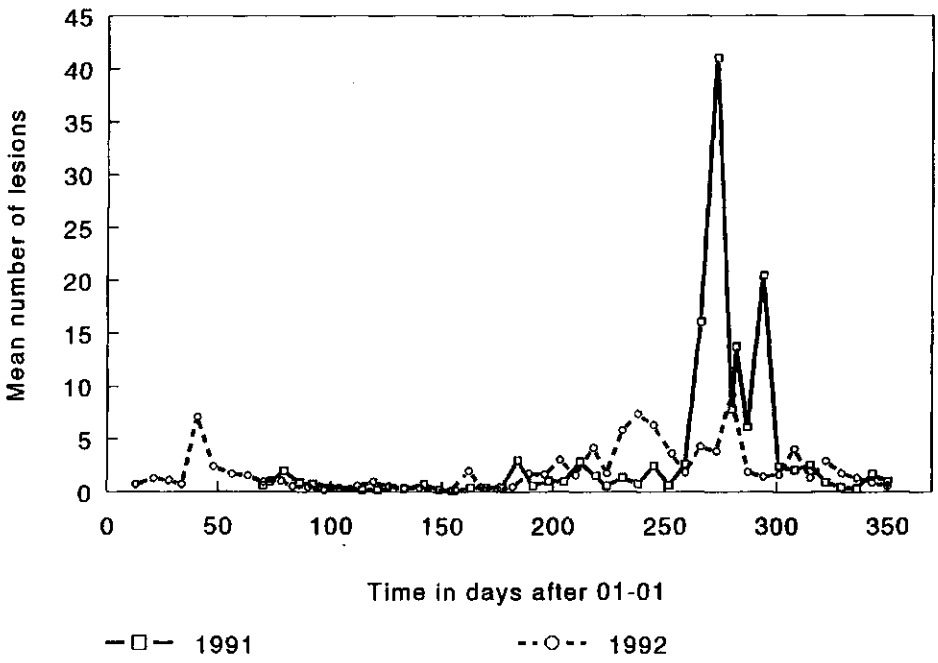

Fig. 6. Lesion counts per rose flower per counting date averaged over locations in glasshouse E4 (n=144 flowers), in 1991 and 1992.

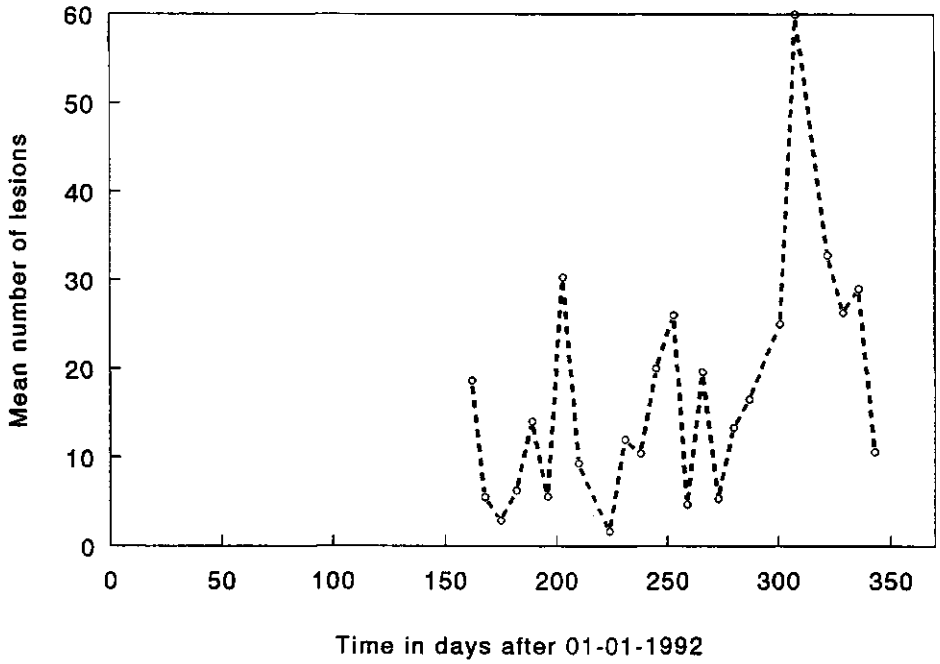

Fig. 7. Lesion counts per rose flower per counting date averaged over locations in glasshouse E3 (n=5 flowers), in 1992.

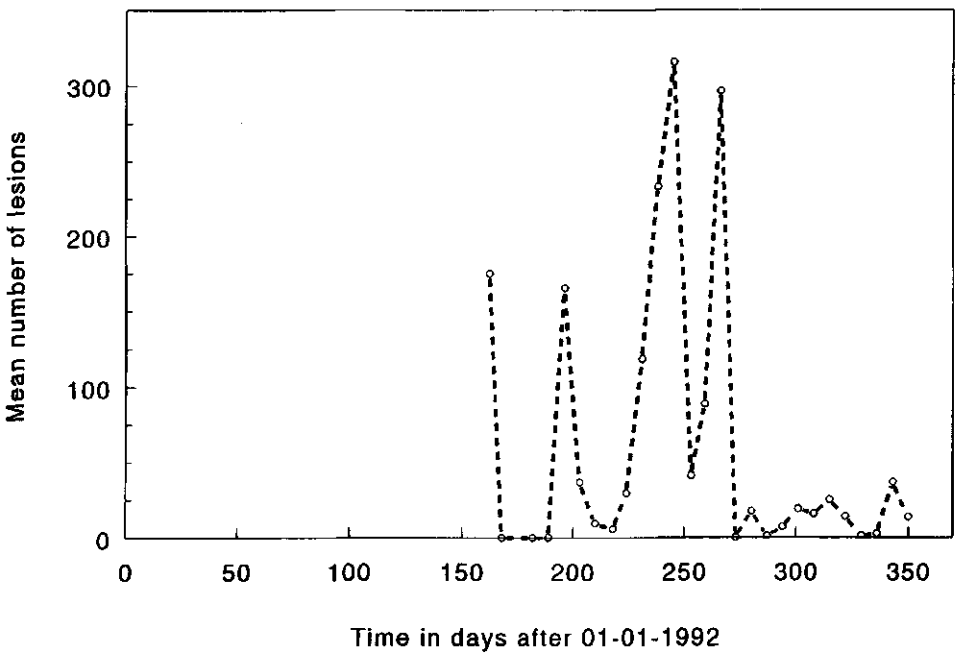

Fig. 8. Lesion counts per rose flower per counting date averaged over locations outside glasshouses (n=10 flowers), in 1992.

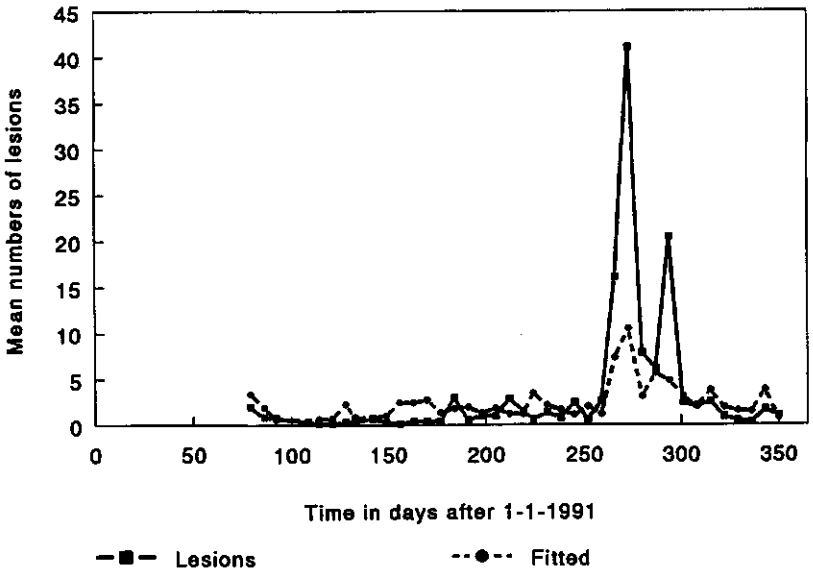

Fig. 9. Observed (1991) and fitted (equation (2) of 1992 used) numbers of lesions per rose flower.

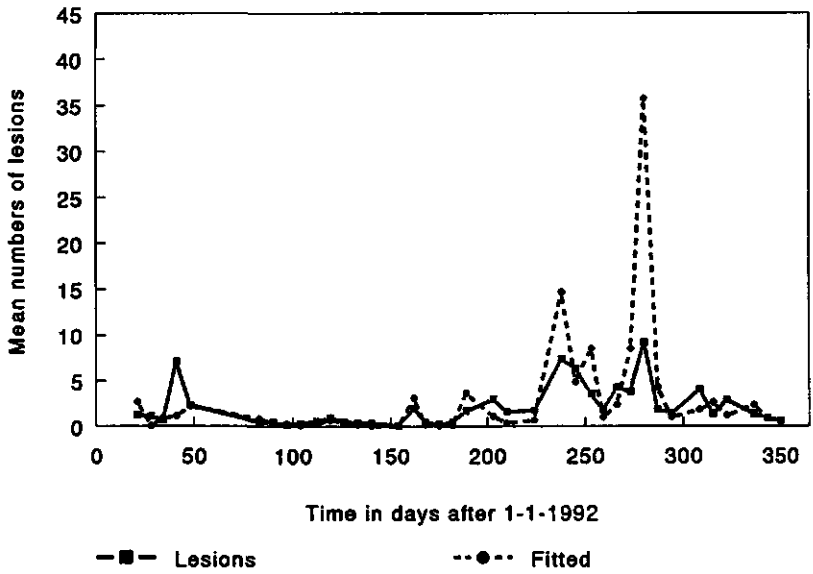

Fig. 10. Observed (1992) and fitted (equation (1) of 1991 used) numbers of lesions per rose flower.

Discussion

Horizontal distribution of colonies and lesions on flowers

The horizontal distribution of *B. cinerea* spores in a rose crop grown under glass, counted as colonies or as lesions on petals, was fairly uniform in both years. This is in agreement with results from gerbera (Kerssies 1993b). The lack of significant differences in the colony numbers between trapping locations and in lesion numbers on rose flowers from different harvest locations suggests that spores of *B. cinerea* could be dispersed rapidly through the glasshouse (Frinking *et al.*, 1987). This lack of differences does not depend on the crop grown in the glasshouse, because in two totally different crops, a dense and low (0.6 m) gerbera crop and an open and high (1.5 m) rose crop, location effects were absent. These results suggest that observing *B. cinerea* in cut flowers grown in a glasshouse with spore traps at one height (only tested at different heights in a gerbera crop) and at a limited number of locations is sufficient, irrespective of the crop. No significant interaction was found between location and time for the log transformed numbers of colonies and for the log transformed numbers of lesions.

Colonies, changes over time

The changes in the number of colonies on spore traps over time in the rose crop are probably caused by changes in environmental conditions, such as radiation induced variables as RH and air-movements, in and outside the glasshouse (Frinking et al., 1987; Frinking, 1991). In 1991 and 1992, high numbers of colonies were counted in autumn when the RH was high (≥75%) and the total daily global radiation was low (>1000 $Jcm^{-2}day^{-1}$). Fluctuations in the number of colonies could not be explained by regressions incorporating any of the independent variables separately. In the glasshouse with roses the numbers of colonies did not depend on the age of the crop, as with gerbera's, where the amount of dead gerbera tissue increases as the gerbera crop ages (Kerssies, 1993a).

Peaks in the number of colonies in glasshouse E3 and outside were higher than in glasshouse E4. In glasshouse E3, dead tissue on the ground was wetted frequently, due to the production system. Conidia on this wet dead tissue were able to germinate and grow and the resulting mycelium can produce many spores (A. Kerssies, personal observation). This causes a higher amount of conidia in the glasshouse air and higher peaks in the numbers of colonies. In glasshouse E4 conidia were also found on the dead tissue, but this tissue remained dry most of the time. These conidia were not formed on the dead tissue, but they were deposited from the glasshouse air. Outside the glasshouse more sporulation of *B. cinerea* on dead tissue than in E4 could be observed and higher air-movements were recorded than inside (A. Kerssies, personal observation). Frinking (1991) had found that air movements in a glasshouse hardly ever exceed 0.5 to 0.6 m/s, while outside glasshouses air movements can be >10 m/s (Kerssies, personal observation). Higher windspeeds can cause a higher release of spores in the air and these spores

can give more colonies on the spore traps. Gregory and Lacey (1963) found that the total number of spores blown away in a given time is roughly proportional to the wind-speed.

The higher similarity in the pattern of peaks (numbers of colonies on spore traps) between inside E4 and outside than between E3 and outside is probably due to the very low amount of spore production inside E4. The spore production in E3, with the wet dead tissue on the ground, probably exceeded the numbers of spores coming from outside the glasshouse. These observations suggest that the pattern of peaks in E3 was more dependent on the amount of spore production in the glasshouse than on the spores from outside. In E4 hardly no spore production was observed, and the pattern of peaks depended more on the spore production outside. Leakage of air in a modern glasshouse through openings other than ventilation windows is strongly influenced by wind-speed outside (Fernández and Bailey, 1992) and is for example 0.028 (change of total amount of glasshouse air per hour) at a wind-speed outside of 1 m/s (Groen, 1988). Zandvoort (1968) showed that inoculum of *Puccinia horiana* can as readily enter the glasshouse as it escapes the glasshouse, apparently by way of ventilation windows and other openings. Frinking (1991) claimed a continuous exchange of air between the glasshouse and its outside environment, because of wind speeds outside the glasshouse, which normally exceed those within the glasshouse, and because of differences in temperature. De Jong (1990) showed a linear relationship between the air flux within and the wind-speed outside a glasshouse. Hirst (1959) stated that glasshouses may act as important spore emitters by means of convection through open ventilators.

Lesions, changes over time

With roses, the number of lesions over time fluctuated less than the number of colonies, as with gerbera (Kerssies, 1993). The exposure time of rose flowers (4 - 12 days) was longer than the exposure time of the spore traps (24 hours) and the latter were thus more sensitive to rapid changes in glasshouse conditions. The number of lesions on roses were higher in glasshouse E3 and outside the glasshouses than in glasshouse E4. The higher numbers of lesions in E3 were probably due to the wet tissue on the ground resulting in more spore production and more spores in the glasshouse air and on the flowers. On roses outside the glasshouses very high numbers of lesions were counted sometimes, mostly during and after rain showers, as a result of rain-deposition of spores onto the flowers.

Linear regression models

The linear regression models for the number of lesions on rose petals in both years suggest that relative humidity (positively correlated), radiation intensity (negatively correlated) and numbers of colonies (positively correlated) in the glasshouse had an effect on the numbers of lesions during post-harvest. The number of lesions on gerbera

petals were explained by relative humidity, radiation intensity and age of the crop. The age of the crop was tested but not included in the model for the numbers of lesions on rose petals, as it was not significant. The absence of age in the model can be explained by the fairly stable amount of dead rose tissue in the glasshouse, which remained dry during most of the time. Conidial germination in *B. cinerea* on the rose flower surface might have been affected by radiation and relative humidity. According to Hennebert and Giles (1958), under field conditions exposure to direct radiation can accelerate the decline in viability of conidia of *B. cinerea*.

The significant correlations between observed and estimated values in figures 9 and 10 show that the equations (1) and (2) have a predictive value, even though the year had a significant effect on the level of the number of lesions. Therefore it is better to use equation (3) in which this year effect is incorporated, than (1) or (2). The equations (1) and (2) produced an increase of lesions on rose flowers at the same time of the year. Only the observed numbers were different, in 1991 a maximum of 43 and in 1992 a maximum of 10. In both years the numbers were high (>4 lesions per flower), when the RH was high and the global incoming radiation was low.

The linear regression models for gerbera and rose can be used in an integrated pest management system for *B. cinerea* as a warning system to reduce the use of fungicides in ornamentals grown in glasshouses (Fransen, 1993), see e.g. Table 3. Further research is needed on the causal relations of relative humidity, temperature and global radiation on germination and penetration of *B. cinerea* conidia and on the structure and composition of the cuticle of rose flowers.

The numbers of spores in glasshouse-air do not depend so much on the crop, but rather depend on the production system. A system resulting in moist dead tissue on the ground can give high amounts of spores in the glasshouse air. The numbers of lesions on flowers depends probably on the position of the receptive area of the flowers (Kerssies, personal observation). Further research is needed on the relation between the position of the receptive area of flowers and the amount of spores trapped on the flowers.

Acknowledgements

The authors are indebted to Professor J.C. Zadoks, Ir. H.D. Frinking and Dr. J.J. Fransen for critically reading the manuscript, to Ir. M. Stapel and Ms. A. Verlind for helping with the statistical analyses and to Mr. M. ten Hope for assistance in the experimental part of the work.

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Chapter 5

Effects of temperature, vapour pressure deficit and radiation on infectivity of conidia of *Botrytis cinerea* and on susceptibility of gerbera petals

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Kerssies, A., 1994. Effects of temperature, vapour pressure deficit and radiation on infectivity of conidia of *Botrytis cinerea* and on susceptibility of gerbera petals. European Journal of Plant Pathology. Accepted.

Abstract

The effect of vapour pressure deficit, temperature and radiation on the postharvest susceptibility of gerbera flowers to *B. cinerea*, on the water relations of gerbera flowers and on the lesion formation after conidial infection of *B. cinerea* was studied. The temperature range in which *B. cinerea* could germinate and grow *in vitro* is 5-30°C. In climate chamber experiments flowers had more lesions of *B. cinerea* at temperatures of 20 and 25°C than at 10 and 15°C. At 15, 20 and 25°C the infectivity of *B. cinerea* conidia was negatively affected during a storage-period of 7 days. At a vapour pressure deficit (VPD) of 200 Pa significantly more conidia of *B. cinerea* were infective than at 800 Pa. At a VPD of 800 Pa the susceptibility of gerbera flowers for *B. cinerea* was not significantly different than at 200 Pa. High radiation levels in glasshouses in spring and summer negatively influenced the infectivity of conidia of *B. cinerea* on the flower surface, but did not affect the susceptibility of gerbera flowers for *B. cinerea*. In spring and early summer conidia lost their infectivity at high radiation levels, high temperatures and high levels of VPD. In summer gerbera flowers could be more susceptible to *B. cinerea* because of high temperatures in glasshouses, but the negative effect of radiation on the conidia of *B. cinerea* seemed to overrule the temperature effect. Thus, the numbers of lesions in spring and summer can be low compared with the numbers in other seasons, although the numbers of *B. cinerea* colonies on spore traps can be high. The effect of temperature on the susceptibility of gerbera flowers can probably be explained by changes of water status in the petals. At higher temperatures the number of lesions and the turgor (= water potential - osmotic potential) in the petals increased. Temperatures <10°C during lesion formation (RH>95% and VPD<50 Pa) had a temporary negative effect on the number of lesions. After 3 days of incubation the numbers of lesions were about equal (≥30 lesions/cm^2) from 5 to 20°C. At 30°C no lesion formation was observed even after 3 days.

Additional keywords: Water potential, osmotic potential, turgor, climate chamber.

Introduction

The fungus *Botrytis cinerea* Pers. ex Pers., the imperfect stage of *Sclerotinia fuckeliana* (Ellis & Waller, 1974), causes damage to a wide variety of plants. Infection takes place through wounds, via decaying or dead plant tissue, and by direct penetration of the undamaged host (Verhoeff, 1980). *B. cinerea* causes damage to ornamentals such as gerbera, rose, chrysanthemum and potted plants among which saintpaulia (De Jong, 1985, 1986). Conidia play an important role in dispersal of *B. cinerea* in glasshouses. Necrotic lesions ('spotting') on flower buds and petals are caused by early infections. Salinas *et al.* (1989) showed that these symptoms are encouraged by a relative humidity (RH) above 93% which only occurs during postharvest conditions. Below 93% RH no

lesion formation was observed. They also found that germination of conidia and lesion formation occurred between 4 and 25°C; at 30°C, germination and lesion formation did not occur. Between 18 and 25°C many lesions became visible within 1 day after inoculation; at 4°C it took 2 to 3 days before lesions could be seen. They did not test the effect of low RH's (≤90%), which are more common in glasshouses than high RH's, and the effect of temperatures at low RH's on infection of *B. cinerea* conidia on gerbera flowers.

The dispersal of conidia of *B. cinerea* in a gerbera crop growing under glass and the effects of environmental factors in the glasshouse on dispersal and infection of gerbera flowers during the postharvest period were studied (Kerssies, 1993). The best linear regression model showed that susceptibility of gerbera flowers to lesion formation during the postharvest period was affected by three variables during development of the gerbera flowers (R^2=0.81; P≤0.05): RH in the glasshouse (positively correlated), global radiation outside the glasshouse (negatively correlated) and age of the crop (positively correlated). Marois *et al.* (1988) showed that in California (USA) the susceptibility of rose flowers to *Botrytis* blight was affected by RH and temperature during development of the flowers. In the fall, when RH was lower and temperature was higher, the rose flowers were less susceptible than in winter.

The aim of the present study was to investigate the effect of glasshouse levels of vapour pressure deficit (VPD), temperature and radiation on the susceptibility of gerbera flowers to *B. cinerea* during the post-harvest period, on the water relations of gerbera flowers during the post-harvest period and on the infectivity of conidia of *B. cinerea*.

Materials and methods

Germination and growth rate

The effect of temperature on germination of conidia and on growth of mycelium of *B. cinerea in vitro* was determined at six different temperatures: 5, 10, 15, 20, 25 and 30°C. All plates were grown under fluorescent light (Pope, FTD 36W/30, 8 $\mu molm^{-2}s^{-1}$).

For the effect of temperature on germination of conidia (**Experiment 1**) 10 potato dextrose agar (PDA) plates per temperature were inoculated with 100-125 freshly harvested conidia per plate, obtained from a 7-day old *B. cinerea* culture. Two days after inoculation the germination percentage was determined as number of colonies on PDA. For the effect of temperature on mycelial growth (**Experiment 2**), 5 potato dextrose agar plates per temperature were infested at the centre by means of a mycelial plug, obtained from a 3-day old *B. cinerea* culture. Three days after inoculation the radial growth of *B. cinerea* mycelium was measured in cm^2. The experiments were repeated twice.

Non-linear regression analysis was applied for the percentage germinated conidia and for

the mycelial growth, with temperature as the independent variable.

Flowers, inoculation and incubation

Gerbera flowers (cv. 'Terrafame') were grown on rockwool in a glasshouse of 100 m^2. For all experiments isolate Bc-16 of *B. cinerea* was used, obtained from an infected gerbera flower. Cultures were grown on potato dextrose agar under fluorescent light (Pope, FTD 36W/30, 8 $\mu molm^{-2}s^{-1}$) for 7 to 9 days at 20°C (Salinas *et al.*, 1989). Conidia were freshly harvested in sterilized, distilled water and adjusted to a density of $1*10^4$ conidia per ml. Flowers were inoculated with 1 ml conidial suspension in a Potter (1952) spray tower, resulting in approximately 90 conidia/cm^2 petal and 30 lesions/cm^2 petal, and air dried for 10 minutes before placing them in a climate chamber or in a plastic box (RH>95%, VPD<50 Pa). For each combination of temperature, VPD and day four flowers were used. After one or more days (maximum of 7 days) in the climate chamber (= storage period; during this period no lesions occurred) the upper 10 petals of each flower were placed on wet paper in plastic boxes (RH>95%, VPD<50 Pa) and incubated at 20°C under fluorescent light (Pope, FTD 36W/30, 8 $\mu molm^{-2}s^{-1}$). After 1 day incubation at RH>95% (VPD<50 Pa), *B. cinerea* lesions were counted under a microscope (10x magnification, Salinas *et al.*, 1989). Resulting numbers of lesions per cm^2 on gerbera petals were subjected to analysis of variance (ANOVA).

Temperature, Vapour Pressure Deficit and lesion formation

The effects of temperature and VPD on flowers and on conidia of *B. cinerea* was observed, both expressed as numbers of lesions after incubation at RH>95% (VPD<50 Pa). Young (just open) gerbera flowers were inoculated before or seven days after placing them in a climate chamber (= storage period). The stems of the flowers were placed in plastic tubes each containing 32 ml distilled water.

In **experiment 3** the effect of temperature on lesion formation was studied. The flowers were kept in a climate chamber for seven days (= storage period) at four different temperatures: 10, 15, 20 or 25°C and a VPD of 400 Pa. Experiment 3 was repeated at least three times.

In **experiment 4** the effect of VPD at different temperatures on lesion formation was studied. The flowers were kept in a climate chamber for seven days (= storage period) at two different VPD's: 200 or 800 Pa and at three different temperatures: 15, 20 or 25°C. Experiment 4 was repeated at least twice.

Seven days after placing the flowers in a climate chamber the upper ten petals of each flower were incubated at RH>95% (VPD<50 Pa) and 20°C, for experiments 3 and 4. From each flower the water potential (in kPa) of three other petals was measured by using a pressure chamber (Meeteren, 1980a). Fifteen other petals were placed at -20°C. After thawing, the petals were squeezed for sap extraction. The osmotic potential (in kPa) of the sap was measured by cryoscopy using a Gonotec Osmomat 030 osmometer

(Meeteren, 1980a). The turgor (in kPa) was calculated with the formula: turgor = water potential - osmotic potential (neglecting the matrix potential). Values of water potential, osmotic potential and turgor in the petals were subjected to analysis of variance (ANOVA).

In **experiment 5** the effect of temperature and time on lesion formation was studied, from day 0 to day 7, at 15 or 25°C and a VPD of 400 Pa. Every day the numbers of lesions on the flowers after incubation at RH>95% (VPD<50 Pa) and 20°C, the water potential and the osmotic potential were determined. The experiments were repeated at least twice. Non-linear regression analysis was applied for the mean numbers of lesions and for the turgor in petals with the storage period as the independent variable, except for the turgor in petals at 15°C to which no regression could be applied.

In **experiment 6** the effect of temperature on lesion formation during incubation at RH>95% (VPD< 50 Pa) was studied. After inoculation, the upper 10 petals of each flower were placed on wet paper in plastic boxes and incubated at 5, 10, 15, 20, 25 or 30°C in the dark. After 1 and 3 days, *B. cinerea* lesions were counted under a microscope (10x magnification). The experiments were repeated once.

Radiation in the glasshouse and lesion formation

For radiation experiments a part of the crop in a glasshouse of 100 m^2 was grown under a double screen of white cheesecloth. The total global incoming radiation ($Watt/m^2$) inside and outside the screen was recorded with a tube solarimeter (type TSL, spectrum: 300-2500 nm). The screen reduced the total global incoming radiation by approximately 35%.

In **experiment 7** the influence of radiation on the infectivity of *B. cinerea* conidia was studied. Young (just open) gerbera flowers outside the screen were harvested and inoculated. After inoculation the stems of the flowers were put in tubes containing 32 ml distilled water and placed in the glasshouse, inside or outside the screen (5 flowers per treatment). After four days the upper 10 petals of each flower were incubated at RH>95% (VPD<50 Pa) and 20°C. After 1 day, lesions were counted. The experiment was repeated 14 times under different radiation intensities, from March until December 1992.

In **experiment 8** the influence of radiation on the susceptibility of gerbera flowers to infection by *B. cinerea* was studied. Flowers were harvested inside and outside the screen (6 flowers per treatment), inoculated and incubated at RH>95% (VPD<50 Pa) and 20°C. After 1 day, lesions were counted. The experiment was repeated 19 times under different radiation intensities, from March until December 1992.

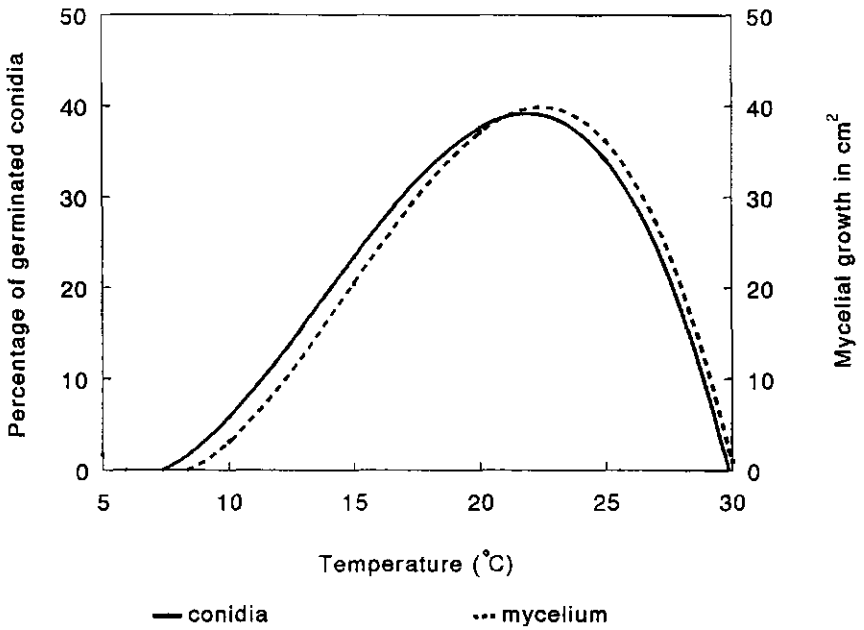

Fig. 1. Experiment 1, Fitted values of percentage germinated conidia (=Y_1) of B. cinerea, in vitro, at different temperatures (= X); Experiment 2, Fitted values of radial mycelial growth in cm^2 (=Y_2) of B. cinerea, in vitro, at different temperatures (= X);
*Y_1=18.3(±11.3)-7.3(±2.6)*X+0.80(±0.17)*X^2-0.019(±0.003)*X^3; R^2=0.84, P≤0.05.*
*Y_2=33.1(±8.1)-10.7(±1.8)*X+0.99(±0.12)*X^2-0.022(±0.002)*X^3; R^2=0.95, P≤0.05.*

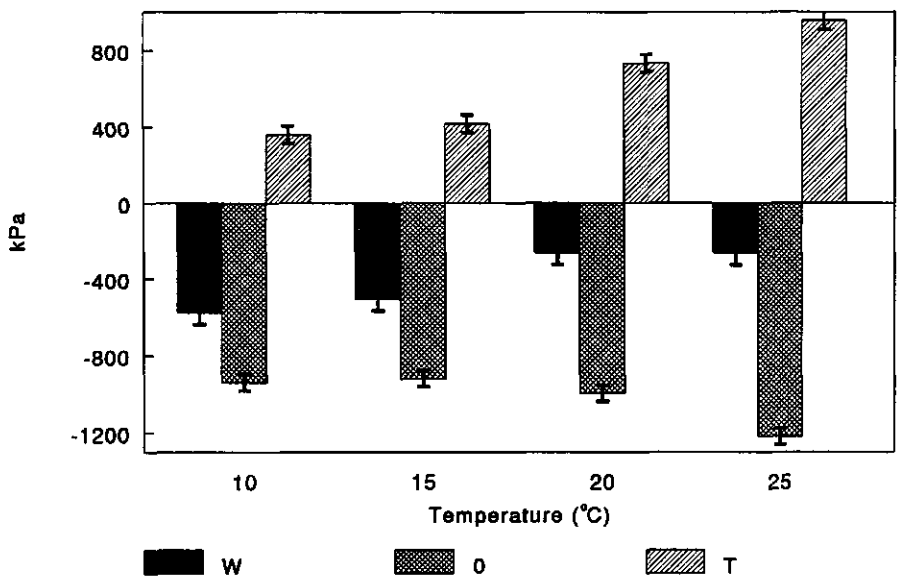

Fig. 2. Experiment 3. Water potential (W), Osmotic potential (O) and Turgor (T) in gerbera petals (VPD=400 Pa) at different temperatures, after the 7-day storage period in a climate chamber.

Results

Germination and growth rate

The fitted percentage of germinated conidia (**Experiment 1**) and the fitted mycelial growth (**Experiment 2**) of *B. cinerea* showed an optimum between 20-25°C (Fig. 1). The partial regression coefficients of the two non-linear regression equations were tested pairwise and were not significantly different at $P \leq 0.05$.

Temperature and lesion formation

Experiment 3. When flowers grown at 20°C were placed in a climate chamber for 7 days, higher temperatures were significantly more favourable for lesion formation (Table 1). This was observed for flowers inoculated before and after the 7-days storage period. At 20 and 25°C the numbers of lesions were high, >13 lesions/cm^2 compared to those at 10 and 15°C (≤8.4 lesions/cm^2). At temperatures of 15, 20 and 25°C the numbers of lesions increased significantly when flowers were inoculated after the 7-days storage period (Table 1). No significant interaction was found between temperature and storage period. From 10 to 25°C the water potential increased, the osmotic potential decreased and the turgor increased in the petals, after the 7 day storage period (Fig. 2).

VPD and lesion formation

Experiment 4. When flowers were inoculated and stored in a climate chamber for 7 days at different temperatures, a VPD of 200 Pa was significantly more favourable to lesion formation than a VPD of 800 Pa at 15 or 20°C, but not at 25°C (Table 2). When flowers were inoculated after the 7-days storage period no significant differences were observed in the numbers of lesions between the two VPD-levels, at any temperature (Table 2). Significantly more lesions were formed on flowers inoculated after the 7-days storage period than before in two cases: at both VPD levels at 25°C and at a VPD of 800 Pa at 15 and 20°C. After the 7-days storage period the water potential, the osmotic potential and the turgor in the petals were not significantly different at any VPD-level or temperature (Table 3). In experiment 4 no significant interactions were found between VPD and temperature.

Temperature, storage time and lesion formation

Experiment 5. Storage from one to seven days at different temperatures and a VPD of 400 Pa influenced subsequent lesion formation (Fig. 3). The first 4 days the numbers of lesions decreased at 15 and 25°C, after 4 days the numbers increased at 25°C but remained stable at 15°C. The partial regression coefficients of the two non-linear regression models at 15°C and 25°C for the mean numbers of lesions on the petals are significantly different at $P \leq 0.05$.

The water potential of the petals decreased at 15°C from -317 kPa at day 1 to -515

kPa at day 7. At 25°C the water potential decreased slightly during the first 3 days, but after 3 days it increased from -428 kPa to -265 kPa. The osmotic potential was fairly stable at 15°C, but at 25°C it decreased from -421 kPa at day 1 to -610 at day 7. The turgor was fairly stable during the first 3 days, but at 25°C it increased after 3 days. At 15°C the turgor decreased slightly (Fig. 4).

Table 1. Experiment 3. Temperature and lesion formation on gerbera petals.
With storage of conidia = flowers inoculated with conidia of B. cinerea before the 7-days storage period.
Without storage of conidia = flowers inoculated with conidia of B. cinerea after the 7-days storage period.

Temperature	Mean numbers of lesions/cm^2	
	With storage	Without storage
10°C	5.2 a[1] A[2]	5.6 a A
15°C	6.2 a A	8.4 a B
20°C	13.1 b A	19.0 b B
25°C	16.0 b A	21.6 b B

[1] ***Significant differences within columns.***
[2] ***Significant differences within rows.***
Means followed by a common letter are not significantly different ($P \leq 0.05$).

Temperature and lesion formation during incubation at RH>95%

Experiment 6. Temperatures ≤10°C during lesion formation (RH>95%) had a negative effect on the numbers of lesions after 1 day but not after 3 days (Fig. 5). After 1 day of incubation the numbers of lesions were high (>23 lesions/cm^2) between 10 to 20°C. At 5°C and ≥25°C the numbers of lesions were low (<13 lesions/cm^2). After 3 days of incubation the numbers of lesions were about equally high (≥30 lesions/cm^2) from 5 to 20°C. At 30°C no lesion formation was observed even after 3 days.

Table 2. Experiment 4. VPD and lesion formation on gerbera petals, at different temperatures.
With storage of conidia = flowers inoculated with conidia of B. cinerea before the 7-days storage period.
Without storage of conidia = flowers inoculated with conidia of B. cinerea after the 7-days storage period.

Temperature	VPD (Pa)	RH (%)	Mean numbers of lesions/cm2	
			With storage	Without storage
15°C	800	53	5.5 a[1] A[2]	8.1 a B
	200	88	7.5 b A	8.4 a A
20°C	800	65	10.7 a A	18.6 a B
	200	91	14.3 b A	19.2 a A
25°C	800	75	17.0 a A	22.3 a B
	200	94	16.0 a A	20.5 a B

[1] Significant differences between the two VPD-levels at the same temperature.
[2] Significant differences between inoculation times at the same temperature and VPD. Means followed by a common letter are not significantly different ($P \leq 0.05$).

Radiation in the glasshouse and lesion formation

Experiment 7 In spring and summer, when radiation levels in the glasshouse were high, the numbers of lesions after incubation at RH>95% and 20°C were significantly lower on plants tested outside the screen (>900 Watt/m^2) than inside the screen (<670 Watt/m^2). In autumn and winter when the radiation levels inside and outside the screen were low (<300 Watt/m^2) no significant differences were observed between the numbers of lesions on inoculated flowers tested inside and outside the screen (Table 4).

Experiment 8. In spring and summer and in autumn and winter no significant differences were observed between numbers of lesions on flowers, after inoculation and incubation, grown inside or outside the screen (Table 5).

Table 3. Experiment 4. Waterpotential (W), Osmotic potential (O) and Turgor (T) in gerbera petals, at different VPD's and temperatures, after the 7-days storage period.

Temperature	VPD (Pa)	RH (%)	W (kPa)	O (kPa)	T (kPa)
15°C	800	53	-625 a	-994 a	370 a
	200	88	-518 a	-955 ab	438 a
20°C	800	65	-314 b	-1197 abc	878 b
	200	91	-245 b	-1111 abc	867 b
25°C	800	75	-425 ab	-1312 bc	887 b
	200	94	-260 b	-1161 c	902 b

Means in each column followed by a common letter are not significantly different ($P \leq 0.05$).

Table 4. Experiment 7. Mean numbers of lesions per cm^2 on gerbera petals, after inoculation with conidia of B. cinerea, exposure to global radiation for 4 days and subsequent incubation at 20°C (RH>95%). Means in each column followed by a common letter are not significantly different ($P \leq 0.05$).

Season	Global radiation ($Watt/m^2$)	Mean numbers of lesions/cm^2
Spring and Summer	948 (outside screen)	9 a
	666 (inside screen)	18 b
Autumn and Winter	216 (outside screen)	17 b
	147 (inside screen)	18 b

Table 5. Experiment 8. Mean numbers of lesions per cm² on gerbera petals, after exposure to global radiation inside or outside the screen for 5 to 11 days, inoculation with conidia of B. cinerea (approximately 90 conidia/cm²) and incubation at 20°C(RH>95%). Means followed by a common letter are not significantly different (P≤0.05).

Season	Global radiation (Watt/m²)	Mean numbers of lesions/cm²
Spring and Summer	854 (outside screen)	22 a
	603 (inside screen)	23 a
Autumn and Winter	305 (outside screen)	23 a
	208 (inside screen)	20 a

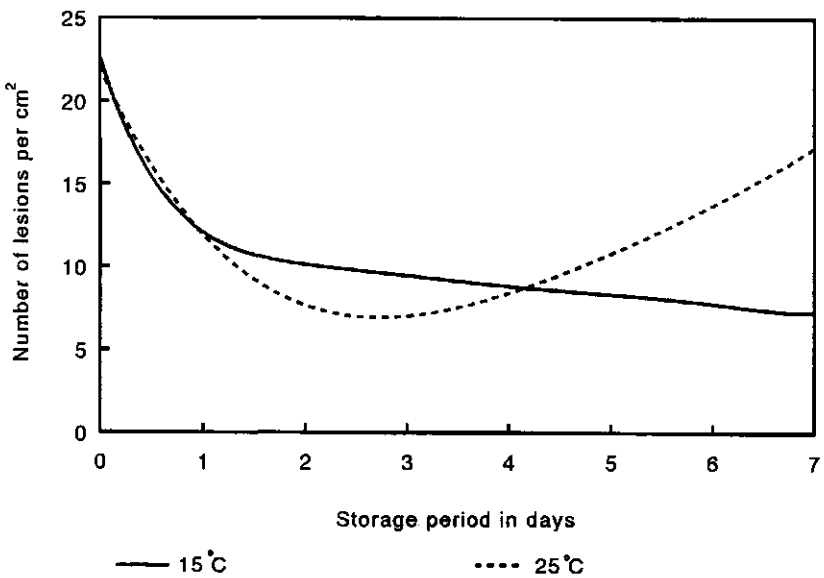

Fig. 3. Experiment 5. Fitted values of mean numbers of lesions (=Y) on gerbera petals after different storage periods (days=X) at
*15°C (Y= 11.0(±2.0) +11.7(±2.4)*0.14(±0.18)expX-0.5(±0.4)*X; R^2=0.80, P≤0.05),*
*25°C (Y=-10.4(±12.4)+32.5(±12.1)*0.57(±0.15)expX+3.9(±1.7)*X; R^2=0.71, P≤0.05).*

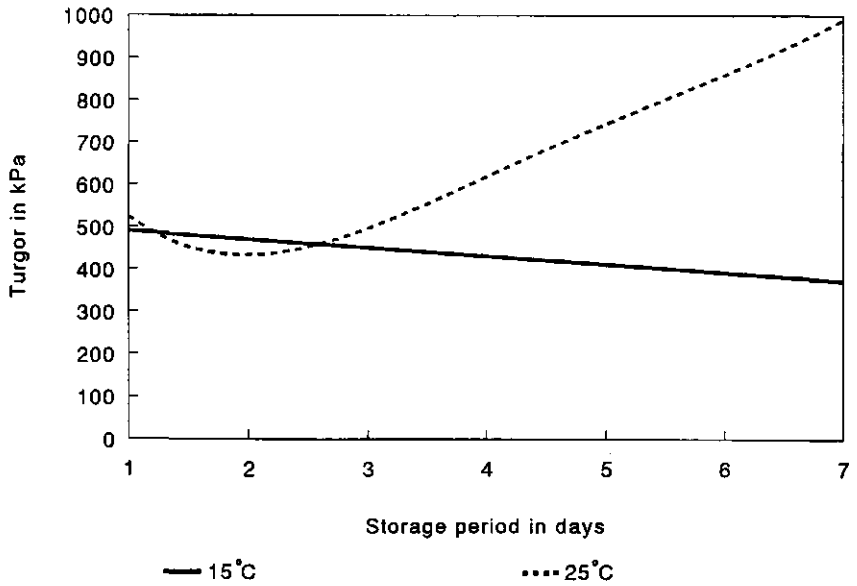

Fig. 4. Experiment 5. Mean values of the turgor in gerbera petals after different days of storage at 15°C and fitted values of the turgor (=T) in gerbera petals after different storage periods (days=X) at 25°C
($T=114(\pm 93)+1264(\pm 1018)*0.23(\pm 0.23)expX-125(\pm 16)*X$; $R^2=0.88$, $P \leq 0.05$).

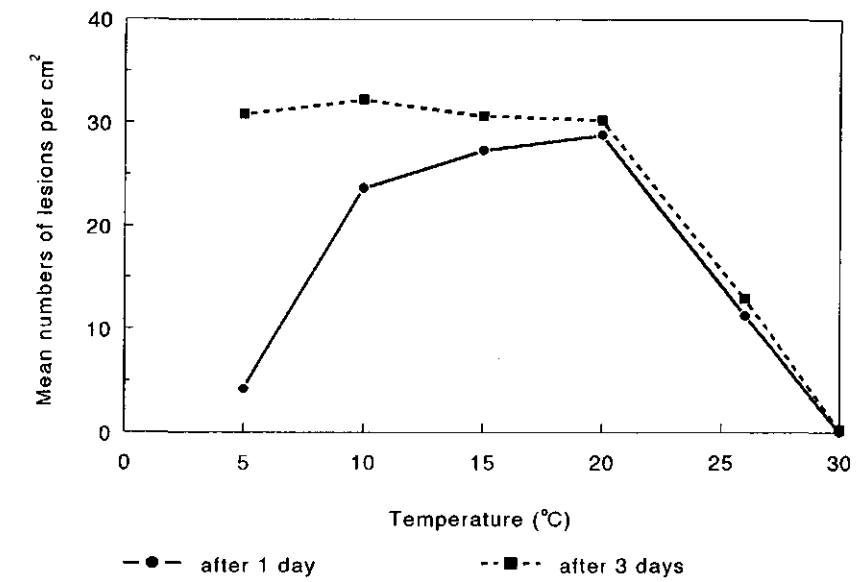

Fig. 5. Experiment 6. Mean numbers of B. cinerea lesions per cm^2 on gerbera petals at different temperatures during incubation at RH>95%.

Discussion

The experiments

Experiments 1 and 2 showed that the temperature range in which *B. cinerea* can germinate and grow *in vitro* is 5-30°C. A similar *in vitro* result was obtained by Ramsey & Lorbeer (1986) and by Shiraishi *et al.* (1970), who found ranges of 3 to 33°C and 5 to 32°C for conidial germination and of 5 to 30°C and 5 to 35°C for mycelial growth of *B. cinerea*, respectively. In heated glasshouses the temperature fluctuates between 17°C in winter and 30°C in summer. *B. cinerea* is therefore able to germinate and grow in Dutch glasshouses during most of the year. After harvest, in cooling chambers and during transportation, when the temperature is between 5 and 20°C, *B. cinerea* is able to germinate and grow.

Experiment 3 showed that at temperatures ≥ 20°C flowers have more lesions of *B. cinerea.* At these temperatures flowers senesce faster or they secrete more nutrients, salts or sugars which favour the infectivity of conidia of *B. cinerea* (Blakeman, 1980), or they evaporate more (= higher RH) which also favours the infectivity of *B. cinerea* conidia. At temperatures ≥ 15°C the infectivity of *B. cinerea* conidia was negatively affected during storage. Conidia on the flower surface probably dry and lose their infectivity faster at higher temperatures, which is in agreement with Coley-Smith (1980). She stated that temperature has a direct effect on the longevity of conidia, high temperatures being more inimical to survival than low. Salinas *et al.* (1989) found that dry, ungerminated conidia of *B. cinerea* on gerbera flowers can remain infective, but their viability decreases as they become older.

The optimum temperature for spore germination and mycelium growth of *B. cinerea in vitro* was between 20-25°C. The optimum temperature *in vivo* is between 18-20°C (Kerssies, personal observation). The temperature extremes *in vitro* and *in vivo* are the same, but the optima are different. Shoemaker and Lorbeer (1971) found for *B. squamosa* also a higher optimum temperature for growth in culture than for germination of conidia on onion leaves.

Experiment 4 showed that at a VPD of 800 Pa flowers have fewer lesions of *B. cinerea* than at 200 Pa. VPD has no significant effect on the susceptibility of gerbera flowers for *B. cinerea* (Table 2), but at a VPD of 800 Pa conidia of *B. cinerea* dry quicker and lose their infectivity faster than at 200 Pa, as found by Blakeman (1980) and Berg & Lentz (1968). The fluid secreted by the flower, containing nutrients, salts and sugars, dries faster at high VPD levels, so that *B. cinerea* conidia cannot use these nutrients for germination. Winspear *et al.* (1970) showed that reducing the relative humidity in glasshouses from 90% to 75% resulted in a decrease of the incidence of *B. cinerea* on tomato plants.

Experiments 7 and 8 showed that high radiation levels in glasshouses negatively

influenced the infectivity of conidia of *B. cinerea* on the flower surface, but had no effect on the susceptibility of gerbera flowers for *B. cinerea*. Hennebert & Giles (1958) found a negative effect on conidia by direct sunlight.

Susceptibility of gerbera flowers

The results of the present study show that the linear regression equation calculated with data obtained from glasshouse experiments (Kerssies, 1993) is based on causal relations. Linear regression accounted for 80% of the variation in the number of lesions on gerbera flowers in glasshouses in terms of relative humidity (positively correlated), global radiation outside the glasshouse (negatively correlated) and age of the crop (positively correlated; not studied in the present paper). The present study shows that the seasonal effect on the numbers of lesions on gerbera petals, observed in glasshouse experiments (Kerssies, 1993), cannot be explained exclusively by a change in the physiology of the flowers. Only temperature had a significant effect on the susceptibility of gerbera flowers (Table 1). In spring and early summer conidia lose their infectivity at high radiation levels, high temperatures and high VPD levels. Therefore, the numbers of lesions can be low compared with the numbers in other seasons, although the numbers of trapped spores are high. Ultraviolet radiation cannot be a factor, because UV radiation could not be detected within glasshouses (Steinbuch, personal observation). In summer, gerbera flowers are more sensitive to *B. cinerea* because of high temperatures in glasshouses, but the negative effect of radiation on the conidia of *B. cinerea* seems to overrule the temperature effect on the flowers.

The effect of temperature on the susceptibility of gerbera flowers can be explained by the change of water relations in the petals. At higher temperatures the numbers of lesions and the turgor in the petals increase. A higher turgor can cause leakage of nutrients, sugars and salts to the flower surface (Meeteren, 1980b). Leakage has a positive effect on germination of *B. cinerea* conidia on the flower surface (Salinas *et al.*, 1989; Blakeman, 1980). This positive effect was corroborated by the relation between the number of lesions at 25°C increasing over time and the increasing turgor of the petals (**Experiment 5**, Fig. 3-4). Coley-Smith (1980) stated that ageing conidia of *B. fabae* contained reserves adequate for germination but insufficient to bring about infection. Infectivity could be restored to old spores by supplementing their reserves with an external source of nutrients.

The effect of temperature on the susceptibility of gerbera flowers can also be explained by a mechanism described by Schnathorst (1959). He showed that a physico-chemical resistance mechanism is involved in the resistance to *Erisyphe cichoracearum* in lettuce. He stated that a difference in osmotic value was one of these mechanisms, because the osmotic value of resistant lettuce plants was more negative than of susceptible plants. He suggested that the role of the osmotic value of host cells in regulating the growth of powdery mildew on lettuce plants might be closely linked with carbohy-

drate metabolism, and that a pathogen cannot succeed if the suction pressure of a host cell contents is higher than that of the parasite. VPD had no significant effect on the water relations in gerbera petals and had no significant effect on the susceptibility of gerbera flowers for *B. cinerea* either. The relation between susceptibility of gerbera petals for *B. cinerea* and their water status (water potential, osmotic potential and turgor) of gerbera petals needs more research.

Lesion formation

The effect of temperature on lesion formation during incubation at RH>95% (**Experiment 6**) was as found by Salinas *et al.* (1989). The result shows that *B. cinerea* can cause big losses of cutflowers during the postharvest period, because this fungus can infect within a wide range of temperatures, as long as the RH>95%.

Conidia of *B. cinerea* survive glasshouse conditions and cause lesions during the postharvest period when the RH>95% (VPD<50 Pa). Gerbera flowers are sensitive for *B. cinerea* conidia during all seasons, under most glasshouse conditions. Therefore it is necessary to decrease the numbers of *B. cinerea* conidia in the glasshouse by keeping the RH as low as possible, especially in autumn and winter and by keeping the radiation as high as possible (clean glass, avoidance of shade), especially in autumn and winter, when the radiation is low in the Netherlands at 52° NL.

The present study shows that flowers with a higher turgor are probably more sensitive to *B. cinerea*. During flower formation the turgor in glasshouse-flowers can be decreased by altering the nutrient solution. During the postharvest period the turgor in flowers can be decresead by lowering the amount of salts or sugars in the storage water.
The temperature during storage has to be as low as possible (<10°C), because at low temperatures the flowers had fewer lesions of *B. cinerea*.

Acknowledgements
The author is indebted to Professor J.C. Zadoks, Ir. H.D. Frinking and Dr. J.J. Fransen for critically reading the manuscript, to Ms. A. Verlind for helping with the statistical analyses and to Ms. A.I. Bosker-van Zessen for assistance in the experimental part of the work.

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Chapter 6

Impaction of conidia of *Botrytis cinerea* on different spore trap types and on gerbera and rose flowers grown in glasshouses

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Kerssies, A., Bosker-van Zessen, A.I. and Frinking, H.D., 1994. Impaction of conidia of *Botrytis cinerea* on different spore trap types and on gerbera and rose flowers grown in glasshouses. Submitted to European Journal of Plant Pathology.

Abstract

The deposition of conidia of *B. cinerea* on spore traps with different trapping angles and on rose and gerbera flowers was studied. Three spore trap types with different angles to the horizontal were used, a horizontal trapping surface, a similar one at an angle of 45°, and two vertical trapping surfaces exposed in opposite directions. The highest spore catches were obtained with the horizontal and slanted spore traps. In general, the horizontal spore trap was the most efficient. The architecture of a flower is one of the factors that influences the number of *B. cinerea* spores trapped on the flower surface. The number of lesions as a result of penetrating spores of *B. cinerea* on a gerbera flower (horizontal) was twenty times higher than on a rose flower (vertical). The flower type determines the type of spore trap that has to be used. These results indicate that sedimentation by gravity is a major factor for the depostion of *B. cinerea* spores in glasshouses.

The number of *B. cinerea* conidia in the air of a glasshouse with a rose and a gerbera crop was studied during several 24 h periods by using a Burkard volumetric spore trap. Spores of *B. cinerea* were always present in the glasshouse air. The numbers of trapped spores were low in the rose crop and high in the gerbera crop. In the rose crop the mean numbers of colonies were significantly higher at day (08.00-20.00 h) than at night (20.00-08.00 h) and in the gerbera crop they were significantly higher at night than at day.

It is surmised that the production system used has great influence on the numbers of spores present in the glasshouse air.

Additional keywords: trapping angles, Burkard volumetric spore trap.

Introduction

The fungus *Botrytis cinerea* Pers. ex Pers., the anamorph stage of *Sclerotinia fuckeliana* (Ellis & Waller, 1974), infects a wide variety of plants. *B. cinerea* causes damage to ornamentals such as gerbera, rose, chrysanthemum and potted plants among which Saintpaulia (De Jong, 1985, 1986). Necrotic lesions ('spotting') occur on flower buds and petals, and are caused by early infections. Infection takes place through wounds, via decaying or dead plant tissue, and by direct penetration of the undamaged host (Verhoeff, 1980). Conidia are important in disease spread in glasshouses. Conidia are dispersed by air currents, water droplets and insects (Jarvis, 1980).

Studies on the dispersal of plant pathogens in glasshouses are scarce (Frinking & Scholte, 1983). Hirst (1959) was among the first to monitor densities of air-borne spores inside and outside glasshouses, and *B. cinerea* conidia were amongst those trapped.

Frinking *et al.* (1987) studied the dissemination of particles in a glasshouse divided into three bays using *Lycopodium* sp.

Little is known about the factors influencing dispersal of spores of *B. cinerea* in ornamentals grown in glasshouses. Frinking and Scholte (1983) showed that the complex dispersal process involves aspects of pathogen, host, environment and activities of man. Studies on the horizontal and vertical distributions of conidia of *B. cinerea* in gerbera and rose crops growing under glass were published (Kerssies, 1993b, Kerssies and Bosker-van Zessen, 1994). In the same glasshouses, the effects of environmental factors on dispersal of conidia and on infection of gerbera and rose flowers during postharvest were studied (Kerssies, 1993a, Kerssies and Bosker-van Zessen, 1994). The aim of the present study was to investigate the differences in impaction of *B. cinerea* conidia on rose and gerbera flowers and on spore traps placed at various angles. The numbers of *B. cinerea* conidia in glasshouse air during a 24 h period in rose and gerbera crops were assessed.

Materials and methods

Trapping situation and conidia of Botrytis cinerea. In a glasshouse of 100 m^2 with a gerbera crop cv. Terrafame, and in a glasshouse of 300 m^2 with a rose crop cv. Sonia three types of spore traps were placed. These types differed in trapping angle, with a horizontal trapping surface, a similar one at an angle of 45°, and two vertical trapping surfaces exposed in opposite directions (Fig. 1). The traps consisted of one Petri dish with a selective medium for *B. cinerea* (Kerssies, 1990). In 1991 and 1992, ten spore traps of each type were placed in the glasshouse 20 cm above the crop in a regular pattern. Fresh Petri dishes on the traps were exposed in the glasshouses for 24 h (08.30 - 08.30), 18 times in the gerbera crop and 32 times in the rose crop. After removal, the Petri dishes were incubated for 7 days at 20°C under fluorescent light (Pope, FTD 36W/30, 8 $\mu molm^{-2}s^{-1}$), after which the number of dark brown colonies were recorded (Kerssies, 1990). Linear correlations over time were calculated between the different trapping angles for the mean numbers of colonies on spore traps.

Results of epidemiological studies in gerbera (Kerssies, 1993a) and rose crops (Kerssies and Bosker-van Zessen, 1994) grown in a glasshouse of 100 and 300 m^2, respectively, were used to study the relation between the trapping situation of flowers and the number of lesions on flowers. The trapping situation of gerbera flowers is approximately horizontal (comparable with trapping situations A and B, Fig. 1) and the trapping situation of rose flowers is approximately vertical (comparable with trapping situation C, Fig. 1).

Numbers of colonies on spore traps and numbers of lesions on flowers were subjected to analysis of variance (ANOVA).

Burkard volumetric spore trap. The Burkard volumetric spore trap is a modification of the trap designed by Hirst (1952). The Burkard spore trap used in the present study was equipped with a seven-day clockwork-driven recording drum, provided with a special sampling tape. Instead of the prescribed sticker a thin layer of Potato Dextrose Agar (PDA) was applied to the sampling tape. Particles are impacted on the agar. In 1991 and 1992, a Burkard volumetric spore trap with its orifice at flower height (1 m above soil level) and with a measured (with a soap-film meter) suction rate of 3 l/min was placed in a glasshouse with a gerbera crop (100 m^2, n=10 periods of 24 h) or a rose crop (300 m^2, n=31 periods of 24 h) for 24 hours. Sections of the exposed tape with PDA, representing periods of 2 h, were placed in 0.5 ml of distilled water. After shaking for 20 minutes, the suspensions were transferred to agar plates containing a selective medium for *Botrytis cinerea* (Kerssies, 1990). The agar plates were incubated for 7 days at 20°C under fluorescent light (Pope, FTD 36W/30, 8 $\mu molm^{-2}s^{-1}$) after which the numbers of dark brown colonies were recorded (Kerssies, 1990). The Burkard spore trap was placed in the gerbera and rose crops used in epidemiological studies reported earlier (Kerssies, 1993a, Kerssies and Bosker-van Zessen, 1994).

Resulting numbers of colonies were subjected to analysis of variance (ANOVA). With this method only the viable dispersal units were counted. Non-linear regression was applied for the mean numbers of colonies (per 2 h periods) in the rose and gerbera crops, with hours of the day as the independent variable.

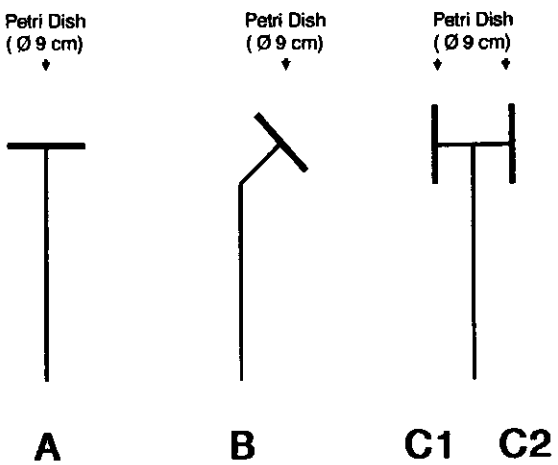

Fig 1. Sketch of the three different trapping positions. A: horizontal, B: slanted at an angle of 45°, C1 and C2: vertical.

Table 1a. Mean numbers of colonies averaged over trapping situation (A, B and C12=mean of C1 and C2) in a gerbera crop. Means followed by the same letter are not significantly different ($P \leq 0.05$, n=18).

Trapping situation	Mean number of colonies
A	2.4 a
B	1.9 a
C12	0.7 b

Table 1b. Mean numbers of colonies averaged over trapping situation (A, B and C12=mean of C1 and C2) in a rose crop. Means followed by the same letter are not significantly different ($P \leq 0.05$, n=32).

Trapping situation	Mean number of colonies
A	6.2 a
B	4.9 a
C12	0.8 b

Table 2a. Linear correlations between the mean numbers of colonies per spore trap (Ln(N+1) transformed) of 3 different trapping situations in a gerbera crop (for $P \leq 0.05$, $r \geq 0.47$ at n=16; C12=mean of C1 and C2)

A	1	1.00		
B	2	0.94	1.00	
C12	3	0.73	0.83	1.00
		1	2	3

Table 2b. Linear correlations between the mean numbers of colonies per spore trap (Ln(N+1) transformed) of 3 different trapping situations in a rose crop (for $P \leq 0.05$, $r \geq 0.35$ at n=30; C12=mean of C1 and C2)

A	1	1.00		
B	2	0.95	1.00	
C1	3	0.86	0.90	1.00
		1	2	3

Results

Trapping situation and conidia of Botrytis cinerea. The mean numbers of colonies on spore traps A (horizontal) and B (at an angle of 45°) were significantly higher than on spore trap C (vertical) in gerbera and rose crops (Table 1a,b). The mean numbers of colonies over time in all 3 trapping situations were significantly correlated ($P \leq 0.05$), in both the gerbera and rose crops (Table 2a,b).

The mean numbers of lesions on gerbera flowers (comparable with trapping situations A and B) was much higher than on rose flowers (comparable with trapping situation C; Table 3), even when the mean numbers of lesions per cm^2 of flower surface was calculated (Table 3).

Burkard volumetric spore trap. The numbers of spores (ln(N+1)) trapped among the roses were more or less constant over the day (Fig. 2a), except for the numbers counted between 16.00 and 18.00 h, which were significanly higher than those between 20.00 and 10.00 h. The numbers of spores among the gerbera flowers showed more variation (Fig. 2b). Between 04.00 and 06.00 h the number of trapped spores was significantly higher than between 12.00-14.00 h, 16.00-18.00 h and 20.00-22.00 h. Between 16.00-18.00 h the numbers were significantly lower than at the other hours. The best fit for the number of spores trapped in the rose crop at different hours of the day was a non-linear model (Fig. 2a):

$$Y=0.43(\pm 0.12)+0.13(\pm 0.08)t-0.023(\pm 0.014)t^2+0.001(\pm 0.0007)t^3 \qquad (R^2=0.53,\ P \leq 0.05),$$

where Y is ln(N) of the mean numbers of spores per 2 h periods and t is the time of the day (2 h periods; t=1-12). The best fit for the number of spores trapped in the gerbera crop at different hours of the day was a non-linear model (Fig. 2b):

$Y=1.7(\pm0.3)-0.44(\pm0.22)t+0.08(\pm0.04)t^2-0.004(\pm0.002)t^3$ ($R^2=0.51$, $P\leq0.05$).

The mean numbers of trapped spores per 12 hours, 8.00 - 20.00 h (=day) and 20.00 - 8.00 h (=night) are significantly different in the rose and gerbera crops (Table 4). In the rose crop more spores were trapped in day-time and in the gerbera crop at night. This corresponds with the regression models (Fig. 2a,b).

The mean numbers of spores per 24 h trapped using the Burkard spore trap in a rose and a gerbera crop was 3.2 and 13.0 spores/m^3 air/hour, respectively. The maximum number of spores trapped within one hour in a rose and gerbera crop was 50 and 56 spores/m^3 air/hour, respectively.

Comparison of the spore trap types. The highest numbers of spores in the rose crop, grown in the glasshouse of 300 m^2 were trapped with the Burkard spore trap, followed by spore traps A, B and C (Table 5). The number of spores trapped by the traps used in the epidemiological studies (Kerssies and Bosker-van Zessen, 1994), wooden cubes with Petri dishes on the four sides and the bottom, was comparable to the number counted on spore trap B (Table 5).

Table 3. Median numbers of lesions per flower and per cm^2 of flower. Means in each column followed by a common letter are not significantly different ($P \leq 0.05$, n=73).

Flower type	area in cm^2 per flower	Median numbers of lesions/flower	Median numbers of lesions/cm^2 flower
Rose	18.5	1.3 a	0.07 a
Gerbera	71.7	100.0 b	1.40 b

Table 4. Mean numbers of spores (ln(N+1)) per 2 h periods at day (8.00-20.00 h) and at night (20.00-8.00 h), trapped with the Burkard volumetric spore trap, in the rose and gerbera crops. Means in each column followed by a different letter are significantly different ($P \leq 0.05$), n=31 for the rose crop and n=10 for the gerbera crop).

Time of the day	Mean numbers of colonies	
	Rose	Gerbera
Day	0.6 b	1.2 a
Night	0.4 a	1.6 b

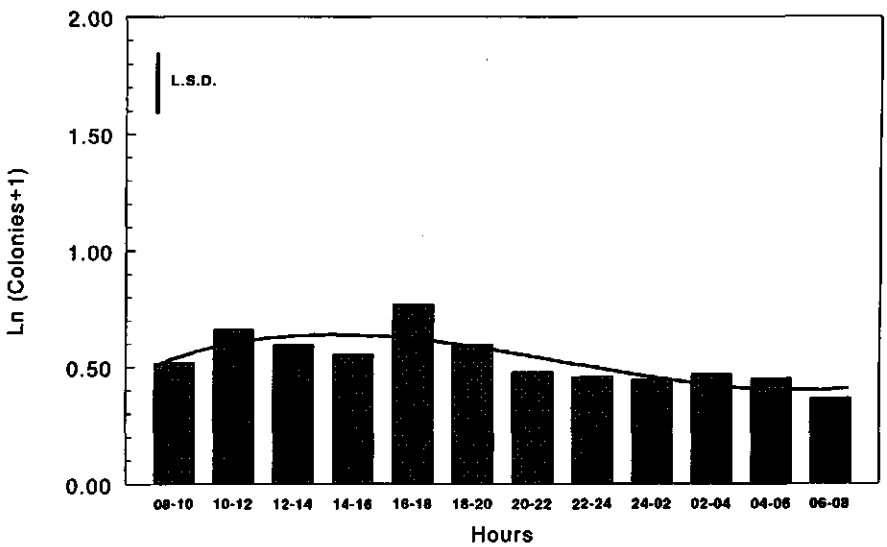

Fig 2a. Mean and fitted numbers of colonies for two hour periods from 08.00 to 08.00 h trapped in a rose crop, at flower height 1 m above the ground, grown in a glasshouse (n=31).

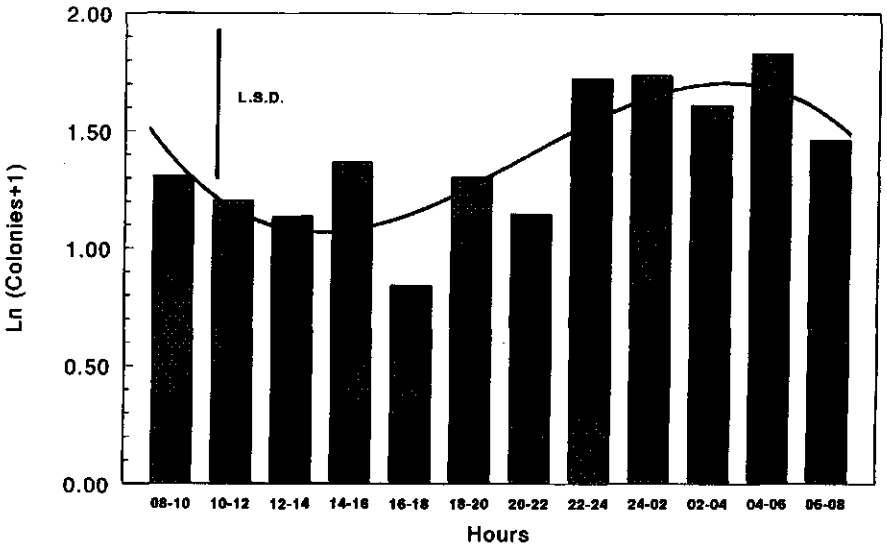

Fig 2b. Mean and fitted numbers of colonies for two hour periods from 08.00 to 08.00 h trapped in a gerbera crop, 1 m above the ground, grown in a glasshouse (n=10 times).

Table 5. Mean numbers of spores caught by different spore trap types, in the rose crop grown in the glasshouse of 300 m², in 1991 and 1992. Mean in each column followed by a common letter are not significantly different ($P \leq 0.05$, n=32).

Spore trap type	Mean numbers of colonies
Burkard (total of 24h)	14.1 a
A (horizontal, one Petri dish)	6.2 b
B (angle of 45°, one Petri dish)	4.9 bc
C (vertical, one Petri dish)	0.8 d
Wooden cube (4 vertical Petri dishes and 1 horizontal at the bottom; Kerssies, 1990, 1994)	3.7 c

Discussion

Trapping situation and conidia of *Botrytis cinerea.*

The highest numbers of spores on spore traps were found with the horizontal and slanted spore traps (types A and B). These results correspond with those of Gregory and Stedman (1953). They found that at low wind-speeds (<1 m/s) the most efficient angles of spore traps are 0° to 45°, for *Lycopodium* spores on glass microscope slides, since the deposition is mainly due to sedimentation under the influence of gravity. Deposition on plane surfaces results from the interaction of several mechanisms: sedimentation, impaction, turbulence and edge effects (Gregory, 1973; Zadoks and Schein, 1979). In still air all spores in the air sink with a constant and characteristic 'terminal fall velocity'. *B. cinerea* has a 'terminal fall velocity' of 0.22-0.45 cm/s. Gregory (1973) concluded that the effect of sedimentation is strong at wind-speeds lower than 2 m/s and slight at wind-speeds of 2 m/s and upwards. Outdoors, the effect of 'terminal fall velocity' is usually masked by the speed and turbulence of the wind, but wind-speed in glasshouses seldom exceeds 1.0 m/s (Frinking, 1991). The results of the present study indicate that sedimentation by gravity is a major factor for spore deposition in glasshouses. Frinking *et al.* (1987) asserted that air movements in a closed glasshouse are sufficient to counteract gravity for dispersal but insufficient for impaction of spores onto plants or trap surfaces. The results of the present study show that not air movement but gravity is the major factor for deposition of *B. cinerea* spores in a glasshouse. Rarely, turbulent deposition of *B. cinerea* spores may be a factor in deposition, when the wind-speed increases above 2 m/s in the glasshouse owing to high wind-speeds outside the glasshouse and open ventilation windows (Zadoks and Schein, 1979).

All three trapping angles used were positively and significantly correlated over time for the mean numbers of trapped spores. Each is suitable for *B. cinerea* spore dispersal studies over time.

Burkard volumetric spore trap.

In the rose and gerbera crops, spores of *B. cinerea* were always present in the glasshouse air, day and night, in approximately constant amounts. The regression models for the trapped spores show a diurnal periodicity in the rose and gerbera crops. In the rose crop the mean numbers of colonies were significantly higher at day (8.00-20.00 h) than at night (20.00-8.00 h) and in the gerbera crop they were significantly higher at night than in daytime.

The number of conidia of *B. cinerea* in the rose and gerbera crops trapped with the Burkard spore trap were low compared to the numbers that Hausbeck and Pennypacker (1991) trapped in a geranium crop grown in a greenhouse, > 2000 conidia/m^3 air/hour. In the present study only the viable spores were counted, whereas Hausbeck and Pennypacker counted the viable and the dead spores. Probably, the relative humidity and

the amount of dead plant tissue in their greenhouse was higher, resulting in higher numbers of sporulating lesions. The suction rate of their Burkard spore trap was 10 l/min, while the suction rate of the Burkard spore trap used in the present study was 3 l/min. The higher suction rate was more efficient in trapping spores of *B. cinerea* (Hirst, 1952). Nevertheless, the suction rate used in the present study was sufficient for trapping spores of *B. cinerea* in glasshouse air.

The ranking order in spore trap efficacy of the different spore trap types (Table 5) was not equal to that observed by Hirst (1959). He also found that with the Burkard spore trap the highest number of spores was trapped outside, 2m above ground at Harpenden, England. But on a vertical trap placed outside he caught a higher number of spores than on a horizontal trap. This difference in ranking order is probably due to the higher windspeeds outside than inside, resulting in different mechanisms of deposition.

Comparison between gerbera and rose

The number of lesions on rose flowers (median was 1.3 lesions/flower, Table 3) was 77 times lower than on gerbera flowers (median was 100 lesions/flower, Table 3), whereas the number of colonies on spore traps was equal to (in comparison with the glasshouse in Vleuten) or 3 times lower (in comparison with the glasshouse in Aalsmeer) than in a gerbera crop (Kerssies, 1993a). The large difference in numbers of lesions in comparison with the small difference in numbers of colonies can be explained by the difference in trapping situation between rose and gerbera flowers, resulting in different accessibilities of the two flower types to spores of *B. cinerea*. The trapping situation of gerbera flowers is approximately horizontal (comparable with trapping situation A and B, Fig. 1) and the trapping situation of rose flowers is approximately vertical (comparable with trapping situation C, Fig. 1). On average 20 times more lesions were counted per cm^2 on a gerbera flower than on a rose flower (Table 3). Spore traps A and B caught on average 5 times more spores than C (Table 1a,b). The period that the flowers were open and accessible to spores was approximately equal for the two flower types. These results suggest that in glasshouses the shape of a flower is one of the factors that influences the number of spores trapped on the flower surface (= accessibility of the infection courts). One lesion of *B. cinerea* in a rose flower can affect a whole petal, but not in a gerbera flower (hypersensitive reaction; Pie and De Leeuw, 1991). With roses 1-3 lesions/flower are enough to colonise and destroy the whole flower, whereas with gerbera's 50-100 lesions are necessary for declassification of the flower. The shape of the flower grown in a glasshouse determines the type of spore trap that has to be used to trap spores at the same level as trapped on the flower surface. When flowers with different shapes are grown in a glasshouse the horizontal type is the most suitable, because on this spore trap the highest numbers of spores were trapped.

The number of spores counted on the Burkard spore trap were low in the rose crop

and high in the gerbera crop. These lower numbers in the rose crop were due to the production system. This production system was fairly similar to the one in glasshouse V (Kerssies, 1993a), which resulted in dry leaves on the ground. Keeping dead tissue on the ground dry, for example by growing plants on gutters, resulted in lower numbers of spores in the glasshouse air. *B. cinerea* needs high relative humidity for germination, colonization of dead leaf tissue and sporulation (Blakeman, 1980). *B. cinerea* can enter senescing leaves as a saprophyte (Blakeman, 1980) and can produce an enormous number of conidia on colonized leaves ($2*10^4$ spores/m^3 air; Jarvis, 1980) if moisture is available.

The results with the Burkard spore trap suggest that the amount of trapped spores of *B. cinerea* depends on the production system (wet or dry, dead plant material on the ground or not), on the activities of man and on the periods that ventilation windows are open. Hausbeck and Pennypacker (1991) found in a geranium crop grown in a greenhouse that maximum densities of conidia typically occurred at midday or in association with grower activity including watering, pesticide application, and harvesting of cuttings. In the gerbera crop high numbers of spores were produced at night in the glasshouse, because of high relative humidity at night. These spores remained in the glasshouse air for a long time owing to closed ventilation windows, resulting in less air movements and higher spore catches at night. In the rose crop very low numbers of spores were produced in the glasshouse. Most of the spores came from outside the glasshouse (Kerssies and Bosker-van Zessen, 1994) and, as the ventilation windows were open during the day only, more spores, coming from the outside environment, were trapped at daytime than at night.

The results of the present study suggest strongly that the production system used and the crop grown in the glasshouse has great influence on the numbers of spores present in the glasshouse air and on the numbers of spores impacted onto flowers.

Acknowledgements

The authors are indebted to Professor J.C. Zadoks and Dr. J.J. Fransen for critically reading the manuscript and to Mr. M. ten Hope for assistance in the experimental part of the work.

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Chapter 7

Relations between physical aspects of gerbera and rose flowers and susceptibility to *Botrytis cinerea*

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Kerssies, A. and Frinking, H.D., 1994. Relations between physical aspects of gerbera and rose flowers and susceptibility to *Botrytis cinerea*. Submitted to Scientia Horticulturæ.

Abstract

The effect of the season, as expressed by certain levels of relative humidity, temperature and total global radiation, on dry weight of petals and weight of wax and cuticle of gerbera and rose petals, on flowering periods of gerbera and rose flowers and on number of lesions on gerbera and rose petals, grown under glass was studied. The differences in dry weight of petals and weight of wax and cuticle of petals between susceptible and less susceptible gerbera and rose cultivars was also investigated.

No clear relation in time was found between dry weight of petals, weight of wax and cuticle of petals and numbers of lesions on the petals. No significant correlation was found between dry weight of petals, weight of wax and cuticle of petals and number of lesions. Except for the gerbera cultivar Delphi, no significant correlation was found between the environmental factors temperature, relative humidity and global radiation and dry weight of petals, weight of wax and cuticle of petals. No relation was found between weight of wax and cuticle of petals and susceptibility of gerbera and rose petals for *B. cinerea*. The thickness of wax and cuticle on flowers seems to be not an important factor for the susceptibility of flowers to *B. cinerea*. The seasonal pattern in number of lesions on flowers is caused by the effect of relative humidity and radiation on the infectivity of conidia present on the flower surface and not by the effect on the susceptibility of flowers.

Additional keywords: dry weight, wax, cuticle, flowering period, lesions, glasshouse.

Introduction

The fungus *Botrytis cinerea* Pers. ex Pers., the anamorph stage of *Sclerotinia fuckeliana* (Ellis & Waller, 1974), infects a wide variety of plants as a saprophyte or as a pathogen. As a pathogen, *B. cinerea* causes damage to ornamentals like gerbera, rose, chrysanthemum and potted plants such as Saintpaulia (De Jong, 1985, 1986).

Kerssies (1993) and Kerssies & Bosker-van Zessen (1994) found that the number of lesions caused by conidial infection of gerbera and rose flowers showed a distinct seasonal pattern. On gerberas grown under glass in spring and early summer few lesions were counted whereas at other times of the year many lesions appeared. On roses grown under glass from August through October many lesions were counted but in the other months few. Gerbera and rose flowers may possess a thicker cuticle and more wax in response to environmental parameters in the periods that the numbers of lesions on the flowers were low. Marois *et al.* (1988) in California showed that the susceptibility of rose flowers to *B. cinerea* was significantly higher in December, January, and February than in October and November, when temperature and radiation were higher. Environ-

mental parameters can affect plant wax and cuticle development (Kolattukudy, 1985). Skoss (1955) found that leaves of *Hedera helix* grown in the sun produced heavier cuticles of greater wax content than leaves grown in the shade. Temperature conditions during plant growth were shown to influence the formation of the cuticle and the deposition of wax. The thickest cuticle was produced at a medium temperature, and the greatest percentage of wax at a high temperature. Plants undergoing water stress produced cuticles containing a greater proportion of waxes than did plants with more favourable moisture conditions. Baker (1974) found that an increase in radiation rate, a decrease in humidity or a decrease in temperature induce large deposits of wax on leaves of *Brassica oleracea* var. *gemmifera*. According to Martin (1964) a high relative humidity induces a thin cuticle. Low humidity and high temperature induce a thick cuticle and cause drying and contraction of the cuticle, thus imposing a barrier to pathogens.

The aim of the present study was to investigate the effect of season (as expressed by certain levels of relative humidity, temperature and total global radiation) on dry weights, weights of wax and cuticle of harvested gerbera and rose petals, on pre-harvest flowering period of gerbera and rose flowers and on number of lesions on harvested gerbera and rose flowers, grown under glass. The differences in dry weights, and in weights of wax and cuticle between susceptible and less susceptible gerbera and rose cultivars were investigated.

Materials and methods

Measurement of environmental conditions. Dry and wet bulb temperatures were measured continuously using 1 psychrometer per glasshouse, placed at 1.5 m above the crop and coupled to a data logger. The temperature 1.5 m above the crop was measured continuously and averaged over 1 h periods. The relative humidity was calculated and averaged over 1 h periods. The total global radiation outside the glasshouses ($Jcm^{-2}day^{-1}$) was measured by a Kipp solarimeter 8 m above the ground at the Research Station for Floriculture in Aalsmeer.

Flowers, inoculation and incubation. Gerbera flowers (cvs. Terrafame, susceptible, and Delphi, resistant) and rose flowers (cv. Sonia, susceptible) were grown on rockwool in glasshouses of 100 and 300 m^2, respectively. Flowers were observed from September 1991 to December 1992. For gerbera flowers this period was from crop establishment to crop removal. For rose flowers this period was from eight months after crop establishment to crop removal. For all experiments isolate Bc-16 of *B. cinerea* was used, obtained from an infected gerbera flower. Cultures were grown on potato dextrose agar under fluorescent light (Pope, FTD 36W/30, 8 $\mu molm^{-2}s^{-1}$) for 7 to 9 days at 20°C

(Salinas *et al.*, 1989). Conidia were freshly harvested in sterilized, distilled water and suspensions were adjusted to a density of $1*10^4$ conidia per ml. Each month, from October 1991 to December 1992, six flowers per cultivar were inoculated with 1 ml conidial suspension in a Potter (1952) spray tower, resulting in approximately 90 conidia/cm^2 petal. The inoculated flowers were air dried for 10 minutes. Afterwards, with gerberas the upper 10 petals of each flower were placed in a plastic box (RH>95%) and with roses the whole flowers were placed in a plastic box. After 1 day of incubation at RH>95%, *B. cinerea* lesions were counted per cm^2 petal under a microscope (10x magnification, Salinas *et al.*, 1989).

Each month, from October 1991 to December 1992, three flowers per flower type were harvested at the commercial stage and used to determine the dry weights per cm^2 of petal and the weights of surface wax and cuticle per cm^2 (one-sided) of petals. For each measurement 16 cm^2 petal per flower, taken from 8 petals, was used.

Dry weight. Eight petal discs (2 cm^2) of each of three flowers (in total 16 cm^2 per flower) were dried at 100 °C and weighed.

Wax. The weight of surface wax was determined according to Silva Fernandez *et al.* (1964), with some modifications. Per flower, eight petal discs of 2 cm^2 each were placed in 4 ml chloroform in a preweighted (20 ml) beaker at room temperature. After 4 minutes the petals were placed on filter paper to dry. The beaker was weighted again after the chloroform in the beaker had evaporated. The difference between the weight of the beaker before and after the chloroform treatment was calculated and divided by the two-sided surface area of the petals ($8*2*2=32$ cm^2) to obtain the weight of wax/cm^2 flower petal (one-sided).

Cuticle. The petals without the surface wax were used to determine the weight of cuticle according to Holloway and Baker (1968), with some modifications. The petal discs (16 cm^2 per flower, without the surface wax) were immersed for 16 h at room temperature in a solution containing 18 g $ZnCl_2$ in 23 ml of concentrated HCl. Subsequently, the petal discs of one flower were placed in 300 ml tap water. After 1 h the cuticles could easily be peeled off from the petal discs, dried at 80 °C and weighted. The weight was divided by 32 cm^2 to obtain the weight of the cuticle/cm^2 petal (one-sided).

Flowering period. Each month, from October 1991 to December 1992, the pre-harvest period over which gerbera and rose flowers bloomed was measured by observing 10 flowers per cultivar from the first day they opened untill the day they were harvested at commercial stage.

Comparison of rose cultivars with varying susceptibility to B. cinerea. The dry weights per cm^2 of petals, the weight of wax and cuticle per cm^2 of petals (one-sided) in 'Sonia' and 'Madelon' (susceptible), and 'Frisco', 'Mercedes' and 'Motrea' (less susceptible), were compared using the methods described above, in January and February, 1992. The flowers for this comparison were obtained from commercial growers.

Statistical analysis. Resulting numbers of lesions per cm^2 on gerbera and rose petals, of dry weights per cm^2 of petals, weights of wax (one-sided) and cuticle (one-sided) per cm^2 of petals and pre-harvest flowering period were subjected to analysis of variance (ANOVA).

Table 1. Daily mean temperature and daily mean relative humidity 1.5 m above the gerbera and rose crop, and daily total global radiation outside the glasshouses, in 1991 and 1992, averaged over three months periods.

Time of year	Gerbera glasshouse		Rose glasshouse		Total global radiation ($Jcm^{-2}day^{-1}$)
	RH (%)	Temp. (°C)	RH (%)	Temp. (°C)	
Oct-Dec 1991	69	18.5	71	17.1	330
Jan-Mar 1992	66	18.7	69	17.6	436
Apr-Jun 1992	67	21.8	69	21.4	1756
Jul-Sep 1992	71	22.3	75	20.9	1418
Oct-Dec 1992	74	18.4	70	17.0	342

Results

Environmental conditions. Relative humidity (daily means of hourly values) and temperature (daily means of hourly values) 1.5 m above the gerbera and rose crop and total global radiation outside the glasshouse (daily values) of 1991 and 1992 were averaged over 3 months periods (Table 1). The mean temperature was high (>20°C) from April-September, due to high irradiation and inadequate ventilation, with larger day/night variations. The mean relative humidity was high (>70%) from July-December

and the mean total global radiation was high (>1400 $Jcm^{-2}day^{-1}$) from April-September.

Lesions. The numbers of lesions per cm^2 petal were averaged over 3 months (n=18; Table 2). The mean numbers of lesions were high (> 30 lesions per cm^2 flower) on flowers of 'Terrafame' from July-December, 1992. The highest densities of lesions on gerbera petals were counted at the end of the experimental period. On flowers of 'Delphi' the numbers of lesions were relatively high (> 5 lesions per cm^2 flower) from July-September. On flowers of 'Sonia' the numbers of lesions were not significantly different between the various three months periods of the experiment (15-19 lesions per cm^2 flower).

Dry Weight. The dry weight per cm^2 petal of gerbera and rose was averaged over 3 months (n=9; Table 2). The dry weight of flowers of 'Terrafame' was significantly higher in the months July-September than in the other three months periods. The dry weight of flowers of 'Delphi' and 'Sonia' was not significantly different between the various three months periods of the experiment.

Wax. The weight of wax per cm^2 petal of gerbera and rose was averaged over 3 months (n=9; Table 2). The lowest weights of wax on flowers of 'Terrafame', 'Delphi' and 'Sonia' were measured from April-June (minima of 7.5, 8.5 and 11.8 $\mu g/cm^2$, respectively).

Cuticle. The cuticle weight per cm^2 petal of gerbera and rose was averaged over 3 months (n=9; Table 2). The lowest cuticle weights of 'Terrafame' and 'Delphi' were measured in fall, at the end of the experimental period. On flowers of 'Sonia' the cuticle weight had a peak in January-March.

Flowering period. The flowering periods of gerbera and rose were averaged over 3 months (n=30; Table 2). The flowering period of 'Terrafame', 'Delphi' and 'Sonia' flowers showed a minimum from April-September and a maximum from October-March, due to differences in glasshouse temperatures between these periods.

The difference between the mean numbers of lesions on the flowers of gerbera cultivars 'Terrafame' and 'Delphi' is large (Table 3, 23.8 and 2.4 lesions per cm^2 of petal, respectively). The mean dry weights per cm^2 of petals and the mean weights of wax per cm^2 of petals did not differ significantly between these cultivars. The mean weights of cuticle (69.2 and 89.2 $\mu g.cm^{-2}$ petals, respectively) and the mean flowering periods (10.0 and 6.3 days, respectively) were significantly different between 'Terrafame' and 'Delphi' (Table 3).

*Table 2. Mean numbers of lesions (n=3*6=18), mean dry weight (n=3*3=9), mean amount of wax (n=3*3=9) and mean amount of cuticular membrane (n=3*3=9), per cm² flower petal and mean flowering period (n=3*10=30), per three months, of gerbera flowers cv. Terrafame and cv. Delphi and of rose flowers cv. Sonia. Means in each collumn followed by a different letter are significantly different (P≤0.05).*

Time of year	Number of lesions per cm²			Dry Weight in mg/cm²			Wax in µg/cm²			Cuticle in µg/cm²			Flowering period in days		
	T	D	S	T	D	S	T	D	S	T	D	S	T	D	S
Oct-Dec 1991	10.0 c	1.6 b	15.7 a	3.0 b	3.1 a	3.6 a	16.3 a	16.0 a	31.3 a	87.2 a	94.4 a	129.0 b	10.9 b	6.9 b	9.1 b
Jan-Mar 1992	22.8 b	2.9 b	16.0 a	3.2 b	3.4 a	3.8 a	11.0 b	10.6 b	23.3 b	72.7 ab	98.6 a	178.3 a	11.0 b	7.1 ab	9.7 ab
Apr-Jun 1992	19.5 b	1.9 b	18.8 a	3.2 b	3.5 a	3.8 a	7.5 c	8.5 b	11.8 c	64.0 bc	101.8 a	149.6 b	8.3 c	6.0 c	4.9 c
Jul-Sep 1992	34.2 a	5.1 a	19.0 a	3.8 a	3.5 a	3.8 a	15.8 a	16.9 a	20.7 b	69.6 b	84.0 ab	135.3 b	7.8 c	5.5 c	5.4 c
Oct-Nov 1992	32.4 a	2.9 b	17.4 a	2.9 b	3.0 b	4.0 a	10.3 bc	11.2 b	24.8 b	52.8 c	67.2 b	118.1 b	11.9 a	7.7 a	10.4 a

No relationship was found between the mean dry weights per cm^2 of petal, the mean weights of wax and cuticle per cm^2 of petal and the mean numbers of lesions on rose petals of different rose cultivars (Table 4). The mean weights of wax and cuticle per cm^2 of petal of the susceptible rose cultivars Sonia and Madelon and of the resistant rose cultivars Mercedes and Motrea were not significantly different (Table 4). The moderately susceptible 'Frisco' had high values for wax and cuticle.

For 'Terrafame', significant linear correlations were only observed between crop age and weight of cuticle, between crop age and number of lesions, between temperature and flowering period, and between radiation and flowering period (Table 5a). For 'Delphi', significant linear correlations were only found between temperature and flowering period and between relative humidity and weight of cuticle (Table 5b). For 'Sonia', significant linear correlations were only found between crop age and weight of petal, between temperature and flowering period, between radiation and flowering periods and between radiation and number of lesions (Table 5c).

Table 3. Mean dry weight (n=5*9=45), mean wax weight (n=5*9=45) and mean cuticle weight per cm^2 petal (n=5*9=45) and mean numbers of lesions (n=5*18=90) per cm^2 petal and mean flowering period (n=5*30=150) of gerbera flowers cv. Terrafame and cv. Delphi and of rose flowers cv. Sonia, over a period of 15 months in 1991 and 1992. Means in each column followed by a different letter are significantly different ($P \leq 0.05$).

Flower type	Dry weight mg/cm^2	Wax $\mu g/cm^2$	Cuticle $\mu g/cm^2$	Number of lesions per cm^2	Flowering period in days
Terrafame	3.2 a	12.2 a	69.2 b	23.8 a	10.0 a
Delphi	3.3 a	12.6 a	89.2 a	2.4 b	6.3 b

*Table 4. Mean dry weight (n=2*2=4), mean wax weight (n=2*2=4) and mean cuticle weight per cm² petal (n=2*2=4) and mean numbers of lesions (n=2*6=12) per cm² petal of rose flowers cv. ' Sonia', 'Madelon', 'Frisco', 'Mercedes' and 'Motrea'. Means in each column followed by a different letter are significantly different (P≤0.05).*

Rose cultivar	Dry Weight mg/cm²	Wax µg/cm²	Cuticle µg/cm²	*Number of lesions per cm²
Sonia	2.7 c	14.0 b	139.3 b	21.8 a
Madelon	3.3 bc	16.5 b	154.8 b	19.7 a
Frisco	4.5 a	30.3 a	301.5 a	12.9 b
Mercedes	3.9 ab	18.5 b	153.0 b	0.3 c
Motrea	3.5 bc	15.5 b	142.5 b	1.0 c

* Experiment performed by A. Hazendonk.

Table 5a. Linear correlations between crop age (A), mean dry weight (DW), mean wax weight (W) and mean cuticle weight (C), mean flowering period (FP), mean numbers of lesions (L), daily mean relative humidity (RH), daily mean temperature (T) and total global radiation (R) in 1991 and 1992, from gerbera cv. Terrafame (For P≤0.05, r≥0.88 at n=5). ns = no significant linear correlation.

		1	2	3	4	5	6	7	8	9
A	1	1.00								
DW	2	ns	1.00							
W	3	ns	ns	1.00						
C	4	-0.90	ns	ns	1.00					
FP	5	ns	ns	ns	ns	1.00				
L	6	0.90	ns	ns	ns	ns	1.00			
RH	7	ns	ns	ns	ns	ns	ns	1.00		
T	8	ns	ns	ns	ns	-0.98	ns	ns	1.00	
R	9	ns	ns	ns	ns	-0.94	ns	ns	0.96	1.00

Table 5b. Linear correlations between crop age (A), mean dry weight (DW), mean wax weight (W) and mean cuticle weight (C), mean flowering period (FP), mean numbers of lesions (L), daily mean relative humidity (RH), daily mean temperature (T) and total global radiation (R) in 1991 and 1992, from gerbera cv. Delphi (For $P \leq 0.05$, $r \geq 0.88$ at $n=5$). ns = no significant linear correlation.

		1	2	3	4	5	6	7	8	9
A	1	1.00								
DW	2	ns	1.00							
W	3	ns	ns	1.00						
C	4	ns	ns	ns	1.00					
FP	5	ns	ns	ns	ns	1.00				
L	6	ns	ns	ns	ns	ns	1.00			
RH	7	ns	ns	ns	-0.96	ns	ns	1.00		
T	8	ns	ns	ns	ns	-0.95	ns	ns	1.00	
R	9	ns	ns	ns	ns	ns	ns	ns	0.96	1.00

Table 5c. Linear correlations between crop age (A), mean dry weight (DW), mean wax weight (W) and mean cuticle weight (C), mean flowering period (FP), mean numbers of lesions (L), daily mean relative humidity (RH), daily mean temperature (T) and total global radiation (R) in 1991 and 1992, from rose cv. Sonia (For $P \leq 0.05$, $r \geq 0.88$ at $n=5$). ns = no significant linear correlation.

		1	2	3	4	5	6	7	8	9
A	1	1.00								
DW	2	0.89	1.00							
W	3	ns	ns	1.00						
C	4	ns	ns	ns	1.00					
FP	5	ns	ns	ns	ns	1.00				
L	6	ns	ns	ns	ns	ns	1.00			
RH	7	ns	ns	ns	ns	ns	ns	1.00		
T	8	ns	ns	ns	ns	-0.98	ns	ns	1.00	
R	9	ns	ns	ns	ns	-0.97	0.88	ns	0.99	1.00

Discussion

The dry weights of gerbera and rose petals were fairly stable during the experimental period. The flowering period was short in summer and longer in falll and winter, but this difference in flowering period had no effect on the dry weights. The wax weight on petals seems to be related to the time of the year. Although in spring low weights of wax and high radiation values were measured, no significant correlation was found between radiation and wax weights. The cuticle weight is related to the age of the crop, as the crop becomes older the cuticle weight per cm^2 petal was lower, particularly on 'Terrafame'. Only on 'Terrafame', the number of lesions on flowers was higher when the crop became older. No significant relation was found between number of lesions and cuticle weight. The dry weight of petals and the weight of wax and cuticle per cm^2 petal depended on the flower type. On rose flowers these amounts were higher than on gerbera flowers. On 'Sonia' flowers (susceptible) the number of lesions was not much lower than on 'Terrafame' flowers (susceptible). The difference in cuticle weight between the susceptible gerbera cultivar Terrafame and the resistant cultivar Delphi was significant. However, the small difference seems not large enough to explain the large difference in number of lesions. The cuticle weight per cm^2 petal of 'Delphi' is only 1.3 times more than that of 'Terrafame', while the number of lesions on flowers of 'Terrafame' were 10 times higher than on flowers of 'Delphi' (Table 3).

The results of the present study show that the distinct seasonal pattern in the number of lesions on gerbera and rose flowers grown under glass cannot be explained by the variation in dry weight of petals and in weight of wax and cuticle per cm^2 petal. No clear seasonal pattern was found in the dry weight of cm^2 petal, weight of wax and cuticle per cm^2 petal. No significant linear correlation was found between the dry weight of cm^2 petal, the amounts of wax and cuticle and the number of lesions. Except for the gerbera cultivar Delphi, no significant correlation was found between the environmental factors temperature, relative humidity and global radiation, and the dry weights of cm^2 petal, and weight of wax and cuticle per cm^2 petal. No relation was found between weight of wax and cuticle per cm^2 petal and susceptibility of gerbera and rose flowers for *B. cinerea*. Kerssies (1994) found that relative humidity and radiation had an effect only on the infectivity of conidia of *B. cinerea* and not on the susceptibility of gerbera flowers for *B. cinerea*. Temperature had an effect on the infectivity of conidia and on the susceptibility of flowers. These results fit with the results of the present study, where no significant correlation was found between environmental factors and susceptibility of gerbera and rose flowers. Therefore, the seasonal pattern in the number of lesions on flowers is caused by the effect of relative humidity and radiation on the infectivity of conidia present on the flower surface and not by any demonstrable effect on the susceptibility of flowers.

The lack of a clear relation between environmental factors (season-dependent) and dry

weight of petals, weight of wax and cuticle per cm^2 petal and susceptibility of flowers to *B. cinerea* could be explained by various hypotheses.

I. According to Kolattukudy (1985) and Köller (1991) the enzymatic hydrolysis of the polyester cutin by cutinase, an enzyme secreted during the initial step of host invasion, was identified as a requirement for perforation of plant cuticles and, therefore, as an essential factor of fungal pathogenicity. Many plant pathogenic fungi gain access to their hosts by penetration of intact plant surfaces (Aist, 1976). These directly penetrating pathogens encounter the cuticle, a noncellular and hydrophobic layer covering the outer walls of epidermal cells, as the first surface barrier to be breached (Martin, 1964; Van den Ende & Linskens, 1974). Stockwell and Hanchey (1984) stated that cuticle thickness plays an important role in the resistance of older plants to *Rhizoctonia solani*. Salinas (1990) treated gerbera flowers, inoculated with conidia of *B. cinerea*, with antibodies raised against purified cutinase of *B. cinerea*. Gerbera flowers treated with these antibodies were completely protected against infection of *B. cinerea*. If the hypothesis of Kolattukudy and Köller is true for cuticles of leaves, cuticles of flowers probably are too thin at any time to be a barrier against penetration of *B. cinerea* conidia. If so, it is not important whether the cuticle is a little thicker in summer than in winter or thicker with resistant than with susceptible cultivars. The present study did not deal with the possible differences in chemical compounds of the wax and cuticle membrane, which could affect the resistance of leaves or flowers against penetration of fungi.

II. According to Nicholson & Epstein (1991) adhesion of fungal spores to the host cuticle is an essential pre-penetration process that determines the success of infection and disease development. They stated that cutinases are involved in adhesion. Adhesion is a fast process, since an ungerminated spore can stick to the plant surface within 2 minutes. When the cutinases were inhibited no infection tooks place. Cutinases are involved in the adhesion process, and are not important in the penetration process. If there is no adhesion the spore pushes itselves up when it tries to penetrate the plant surface. When the hypothesis of Nicholson and Epstein is true the susceptibility of flowers to *B. cinerea* is not influenced by the thickness of wax and cuticle. The hypothesis of Nicholson and Epstein is more in agreement with the results of the present study than the hypothesis of Kolattukudy and others.

After adhesion of a conidium to the flower surface other processes than enzymatic hydrolysis of the cuticle are probably involved in the infection process. Edlich *et al.* (1989) found a positive correlation between the pathogenicity of *B. cinerea* and the intensity of active types of oxygen released. They assumed that these toxins result from the activity of glucose or xylose oxidases, and concluded that active types of oxygen play a decisive role in the infection process of *B. cinerea*.

The difference in susceptibility to *B. cinerea* between various cultivars of gerbera and rose is still not clearly understood. Possibly, differences in chemical compounds of wax and cuticle are important during the infection process, or other processes may be more

important such as secretion of toxins, sugars or salts by flowers.

Further studies on wax and cuticle of cut-flowers are not necessary for epidemiological studies of *Botrytis* spotting on flowers in glasshouses, because no relation was found between season and weights of wax and cuticle and between weights of wax and cuticle and susceptibility of flowers to *B. cinerea*. Differences between the seasons in number of lesions on the flowers were mainly due to the effect of relative humidity and global radiation on the infectivity of conidia of *B. cinerea* and not on the susceptibility of flowers to *B. cinerea*.

Acknowledgements

The authors are indebted to Professor J.C. Zadoks and Dr. R. van Gorsel for critically reading the manuscript.

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General Discussion

Introduction

Botrytis cinerea is a pathogen to a wide variety of economically important plants grown inside and outside glasshouses, such as vegetables, ornamentals, bulbs and fruits and is a saprophyte on senescing and dead plant material. *B. cinerea* is one of the main air-borne pathogens in ornamentals grown under glass. It is obvious that *B. cinerea* is one of the fungi most frequently trapped in air (Paddy & Kelly, 1954; Richards, 1956) and that *B. cinerea* is able to colonize new and clean glasshouses very fast. *B. cinerea* can cause big losses in all kinds of glasshouse crops (De Jong, 1986). The results of chemical control of *B. cinerea* are unpredictable and influenced by the numbers of conidia present on the flower surface. *B. cinerea* on cut flowers grown under glass is a problem during the post-harvest period only. *B. cinerea* in glasshouses with potted plants or vegetables can be a problem during the production stage too. In potted plants *B. cinerea* can grow on senescing leaves on the wet pot soil and subsequently produce many conidia. In vegetable crops *B. cinerea* infects wounds on stems and subsequently invades healthy stems.

The experiments described in this thesis were performed to better understand the epidemiology of *Botrytis* spotting on cut flowers and to answer questions of growers on how to control this fungus in the best way and how to give a reliable prediction for the severity of *B. cinerea* spotting on flowers in the post-harvest stage. A warning system for *B. cinerea* was developed based on the results described in this thesis. In the following sections, different aspects of the epidemiology of *B. cinerea* spotting are discussed and recommendations to growers are outlined.

Sources of *B. cinerea* conidia

In glasshouses, conidia are the only important propagules of *B. cinerea*. Conidia of *B. cinerea* trapped in a glasshouse can originate from sources inside and outside (from neighbouring glasshouses or from the field) the glasshouse. Conidia of *B. cinerea* can enter and escape glasshouses easily, mainly by way of ventilation windows. For glasshouses with a dry production system, resulting in zero or few sporulating *Botrytis* spots, sporulating spots outside the glasshouses are the important sources for *B. cinerea* conidia. In glasshouses with a dry production system the number of conidia are probably higher in seasons with high ventilation rates than in seasons with low ventilation rates. In glasshouses with a wet production system, resulting in many sporulating *Botrytis* spots in the glasshouse, these spots are the important sources for *B. cinerea* conidia. The number of conidia in the air of a glasshouse with a wet production system is much higher than in a glasshouse with a dry production system. In a glasshouse with a wet

production system the number of conidia in a recently planted crop is lower than in an old crop. Controlling diseases by limiting the inoculum is very succesful if it can be reduced to almost zero and the rate of disease is low (Jarvis, 1992). The number of conidia in the glasshouse air originating from sources outside the glasshouse is always much lower than the number of conidia from inside sources. In the Netherlands cut flowers are more and more produced in dry production sytems. The numbers of conidia present in glasshouse air depend more on the production system than on the crop grown. When no sporulation takes place in the glasshouse mostly there is no complete infection cycle in the glasshouse.

Dispersal and distribution of *B. cinerea* conidia

B. cinerea is an air-borne fungus. Dispersal of *B. cinerea* in glasshouses only takes place by means of conidia, mainly by way of the glasshouse air. Conidia of *B. cinerea* are always present in the glasshouse air, day and night, and throughout the year. The numbers of conidia present in glasshouse air can fluctuate rapidly over time. The horizontal and vertical distribution of conidia of *B. cinerea* in glasshouses is fairly uniform, irrespective of the crop grown in the glasshouse. Epidemics of *B. cinerea* spotting of cut flowers are general epidemics (Zadoks & Schein, 1979). Conidia of *B. cinerea* appear all at once, over the whole glasshouse, regularly distributed within the glasshouse.

Spore trapping at one height and at a limited number of locations (e.g. one spore trap per 100 m^2) is sufficient for efficient monitoring of *B. cinerea* in glasshouses. Monitoring of conidia in glasshouses is easy using the spore trap and the selective medium described in Chapter 1. For research purposes, monitoring conidia of *B. cinerea* in glasshouse air using spore traps works adequatly, but for growers it is not the most appropriate method, because it is too time-consuming and too complicated. For growers it is more important to know how many conidia are present on the flower surface and what is the risk of *B. cinerea* damage in the post-harvest stage.

Deposition of *B. cinerea* conidia

Deposition of conidia of *B. cinerea* on cut flowers mainly takes place in the glasshouse during the production stage. Sedimentation by gravity is a major factor for spore deposition in glasshouses, because wind-speeds in glasshouses hardly ever exceed 1.0 m/s (Frinking, 1991). Air movements in a glasshouse are not a major factor influencing deposition of *B. cinerea* conidia. Rarely, turbulent deposition of *B. cinerea* conidia may be a factor in deposition, when the wind-speed increases above 2 m/s in the glasshouse (Zadoks and Schein, 1979).

In glasshouses, the shape of a flower is one of the factors that influences the number of conidia deposited on the flower surface (= accessibility of the infection courts). On flowers with a horizontal architecture (e.g. gerbera flowers) significantly more conidia of

B. cinerea can be trapped than on flowers with a vertical architecture (e.g. rose flowers). Therefore, the shape of the flower grown in a glasshouse determines the type of spore trap that has to be used in epidemiological studies. In glasshouses, the architecture of flowers is horizontal, slanted or vertical.

Germination and penetration of *B. cinerea* conidia

The temperature range in which *B. cinerea* conidia can germinate and germ tubes can grow is 5-30°C. In heated glasshouses the temperature fluctuates generally between 17°C in winter and 30°C in summer. This means that *B. cinerea* is able to germinate and grow in Dutch glasshouses throughout the year, if the relative humidity (RH) ≥95%. In general, conidia on the flower surface in the glasshouse are not able to germinate and penetrate the flowers, because the RH is below 90% most of the time. When the RH >90% (mostly at night), the number of hours of RH >90% is too short for damage to the flowers. On flowers, conidia of *B. cinerea* can survive glasshouse conditions for quite a long time (Blakeman, 1980). After seven days in a climate chamber on the flower surface still more than 50% of the conidia are able to germinate and cause lesions on flowers during the postharvest period when the RH >95%.

Lesion formation

Lesion formation occurs when the RH >95%, and when the temperature is between 5 and 30°C. Therefore, *B. cinerea* on cut flowers grown under glass is strictly a post-harvest problem. The incubation time is temperature-dependent. The optimum temperature for lesion formation is 18-20°C. At these temperatures lesions occur within 24 h after the RH >95%.

Environmental conditions in the glasshouse determine the number of lesions on flowers in the post-harvest stage. Linear regression accounted for 70-80% of the variation in the number of lesions on flowers in the post-harvest stage in terms of RH inside the glasshouse (positively correlated), global radiation outside the glasshouse (negatively correlated) and age of the crop (gerbera crop, positively correlated) or number of *B. cinerea* conidia in the glasshouse air (rose crop, positively correlated). These linear regression equations are based on causal relations.

The seasonal effect on the number of lesions on gerbera and rose flowers, observed in glasshouse experiments, cannot be readily explained by a change in the physiology of the flowers. Only temperature had a significant effect on the susceptibility of gerbera flowers for *B. cinerea*. In spring and early summer conidia lose their infectivity at high radiation levels, high temperatures and high VPD (Vapour Pressure Deficit) levels (= low relative humidities). Therefore, the number of lesions in spring and early summer can be low compared to the number in other seasons, although the numbers of trapped conidia are high. Ultraviolet radiation cannot be a factor, because UV radiation could

not be detected within glasshouses. In summer, flowers are more sensitive to *B. cinerea* because of high temperatures in glasshouses, but the negative effect of global radiation on the conidia of *B. cinerea* seems to overrule the temperature effect on the flowers. In fall the number of lesions is high, due to high levels of RH and low levels of global radiation.

While conidia of *B. cinerea* need high levels of RH for germination and penetration, conidia of the other important air-borne fungus in glasshouses, powdery mildew, can germinate and penetrate when the RH >30%. For example, conidia of *Sphaerotheca pannosa* in a rose glasshouse contain 60% water, while conidia of *B. cinerea* contain only 15% water (Longrée, 1939; Rogers, 1959). Control of powdery mildews by decreasing the relative humidity is not possible.

Susceptibility of flowers to *B. cinerea*

Gerbera and rose flowers are susceptible to *B. cinerea* conidia during all seasons, under most glasshouse conditions. No clear relation was found between season and susceptibility to *B. cinerea* of gerbera and rose flowers. In contrast, Marois *et al.* (1988) found that in California the susceptibility of rose flowers to *B. cinerea* was significantly higher in December, January, and February than in October and November, when temperature and global radiation levels were higher. However, they performed their experiments outside the glasshouse, whereas the experiments described in this thesis were all done inside the glasshouse.

Temperature can influence the susceptibility of flowers to *B. cinerea*. At higher temperatures (≥20°C) flowers are more suceptible then at temperatures ≤15°C. Humidty and radiation only have an effect on the infectivity of *B. cinerea* conidia, but not on the susceptibility of flowers to *B. cinerea*. The cuticle and wax on flowers do not seem to be important factors determining susceptibility of flowers to *B. cinerea*. No relation was found between weights of wax and cuticle of flowers and the susceptibility of gerbera and rose flowers to *B. cinerea*.

For a resistance breeding program in roses, the significant differences in susceptibility to *B. cinerea* between cultivars can provide a good start. Other glasshouse crops such as Poinsettia and Saintpaulia also show differences between cultivars in susceptibility to *B. cinerea*. The factors determining differences in the susceptibility to *B. cinerea* are unknown. For resistance breeding programs it is necessary to obtain more knowledge about the mechanisms controlling susceptibility.

Influence of production system on *B. cinerea*

The production system has a great influence on the number of conidia present in the glasshouse air, wet or dry production system, senescing plant material on the ground or not and the periods during which ventilation windows are open. Keeping dry the senescing plant material on the ground, for example by growing plants on gutters,

resulted in lower numbers of conidia in the glasshouse air. Low windspeeds inside the glasshouse, together with high windspeeds outside cause pressure differences between inside and outside, and in that way a suction force is able to 'clean' the glasshouse from air-borne micro-organisms when the ventilation windows are open, but open windows also allow introductions of new micro-organisms from the outside air (Frinking *et al.*, 1987).

Warning system for *B. cinerea*

To know the periods of high risk of *B. cinerea* damage on cut flowers in the post-harvest stage, it is preferable for growers to use a warning system based on environmental conditions rather than to use spore traps. A warning system is less time consuming and complicated. A warning system will be developed for *B. cinerea* in a gerbera and a rose crop. This warning system will be based on linear regression analysis (Madden & Ellis, 1988), in which the number of lesions on the flowers in the post-harvest stage is the dependent variable and RH inside the glasshouse and radiation outside the glasshouse are the independent variables (Chapters 2 and 4). The seasonal pattern in the number of lesions on flowers is mainly caused by the effects of RH and radiation on the infectivity of conidia present on the flower surface and not by their effect on the susceptibility of flowers to *B. cinerea*. Therefore the equations do not represent the crop grown in the glasshouse. The equations (1) and (2), however, differ in the levels of the variables, because on gerbera flowers with a horizontal architecture significantly more conidia of *B. cinerea* can be trapped per cm^2 than on rose flowers with a vertical architecture. The other variables, crop age and numbers of conidia in glasshouse air, are not included in the equations for the gerbera and rose crops. Therefore, only RH and radiation are used in the general warning system, based on environmental parameters. The equations are:

for gerbera: $Y=-2.5 +0.09*MRH-0.0005*MS$ ($R^2=0.59$ at $P\leq0.05$) (1),

for rose: $Y=-12.0+0.19*MRH-0.0009*MS$ ($R^2=0.49$ at $P\leq0.05$) (2),

where Y is ln(N+1) of the mean number of lesions per flower, MRH is the mean RH for days 6, 7 and 8 before the day of harvesting the flowers and MS is the mean global radiation for days 1, 2 and 3 before the harvest day. The effects of varying values of MRH and MS in equation (1) and (2) on the number of lesions are shown in tables 1 and 2. The **bold** figures in tables 1 and 2 are above the damage threshold. Above this threshold control of *B. cinerea* during the post-harvest stage is necessary (is recommended). On roses 5 lesions/flower are enough to declassify the flower, whereas on gerbera's 50-100 lesions are needed for declassification of the flower. Tables 1 and 2 show that the levels of RH and radiation above which the number of lesions on the

flowers is too high, are equal for both species. Therefore, it is reasonable to use the same thresholds for RH and radiation from which the number of lesions on flowers in the post-harvest stage are not acceptable for cut flowers produced in glasshouses. Tables 1 and 2 show that the RH is the most important factor.

Table 1. Calculated mean numbers of lesions per gerbera flower at different values of MRH and MS using equation Y=-2.53+0.09*MRH-0.0005*MS, where Y is ln(N+1) of the mean numbers of lesions per gerbera flower, MRH is the mean RH for days 6, 7 and 8 before the day of harvesting the gerbera flowers and MS is the mean global radiation for days 1, 2 and 3 before the harvest day (R^2=0.59 at $P \leq 0.05$). The bold figures are above the damage threshold and control of B. cinerea during the post-harvest stage is necessary (is recommended).

Relative humidity (%)	Global Radiation ($Jcm^{-2}day^{-1}$)									
	250	500	750	1000	1250	1500	1750	2000	2250	2500
50	6	5	4	4	3	3	2	2	1	1
55	9	8	7	6	5	5	4	3	3	2
60	15	14	12	10	9	8	7	6	5	4
65	25	22	19	17	15	13	11	10	8	7
70	40	35	31	27	24	21	18	16	14	12
75	**64**	**56**	49	43	38	33	29	25	22	19
80	**101**	**89**	**78**	**68**	**60**	**53**	46	41	36	31
85	**159**	**140**	**123**	**108**	**95**	**84**	**74**	**65**	**57**	**50**
90	**252**	**222**	**195**	**171**	**151**	**133**	**117**	**103**	**90**	**79**
95	**397**	**350**	**308**	**271**	**238**	**210**	**184**	**162**	**143**	**125**

Concluding, the recommendations for cut flower growers are (Table 3):

* First calculate the daily mean RH of days 6, 7 and 8 before the day of harvesting flowers (= MRH = three days average of daily means of hourly observations).
* If the MRH is 70-75%, calculate the mean global radiation for days 1, 2 and

3 before the harvest day (= MS). If the MS <750 $Jcm^{-2}cm^{-1}$, there is a risk that the amount of *B. cinerea* lesions on the flowers is not acceptable for the auction (= *Botrytis Danger* = BD!).

* If the MRH is 75-80%, look at the MS. If the MS <1500 $Jcm^{-2}cm^{-1}$, there is BD!
* If the MRH >80%, MS is not important. There is always BD! at MRH >80%.

If these recommendations are still too complicated for the growers, then a very simple recommendation can be given:

* If the MRH >70%, there is BD!

Table 2. Calculated mean numbers of lesions per rose flower at different values of MRH and MS using equation Y=-12.0+0.19*MRH-0.0009*MS, where Y is ln(N+1) of the mean numbers of lesions per rose flower, MRH is the mean RH for days 6, 7 and 8 before the day of harvesting the rose flowers and MS is the mean global radiation for days 1, 2 and 3 before the harvest day (R^2=0.49 at $P \leq 0.05$). The bold figures are above the damage threshold and control of B. cinerea during the post-harvest stage is necessary (is recommended).

Relative humidity (%)	Global Radiation ($Jcm^{-2}day^{-1}$)									
	250	500	750	1000	1250	1500	1750	2000	2250	2500
50	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0
65	1	1	1	0	0	0	0	0	0	0
70	2	2	2	1	1	1	1	0	0	0
75	**6**	**5**	4	3	2	2	2	1	1	1
80	**16**	**13**	**10**	**6**	**5**	**5**	4	3	2	2
85	**41**	**32**	**25**	**20**	**16**	**12**	**10**	**8**	**6**	**5**
90	**105**	**83**	**65**	**51**	**41**	**32**	**25**	**20**	**16**	**12**
95	**268**	**212**	**167**	**132**	**104**	**82**	**65**	**51**	**40**	**32**

If there is BD!, use a chemical to control *B. cinerea* in the post-harvest stage. Research was started on the possibilities to control *B. cinerea* in the post-harvest stage by means of micro-organisms (Kerssies, 1993). Preliminary experiments show that the yeast *Cryptococcus albidus* and the bacterium *Pseudomonas cepacia* are able to control *B. cinerea* on gerbera flowers as good as current fungicides.

The measurements and calculations of MRH and MS can be programmed in a computer and the decision on BD! conditions can be made by a computer. In general, the BD! conditions of MRH and MS in glasshouses will only be found in September, October and November. Therefore, it is reasonable to recommend the growers to use this warning system in these months only.

Jarvis (1992) stated that the relatively simple microclimatic factors in greenhouses that determine sporulation, spore dispersal, spore germination and many other biological functions in infection and pathogenesis should be relatively easily incorporated into expert systems for many diseases. He stated that grey mold (*B. cinerea*), whose biology is fairly well known, is a universal disease for which an expert system might be constructed for greenhouse crops. A warning system based on environmental parameters is not new. Vincelli and Lorbeer (1988a,b; 1989) found an effect of RH, temperature and calendar date on disease severity of *Botrytis squamosa* in onion. They applied these variables in a weather-based warning system. Yelad *et al.* (1992) developed a model to predict grey mould epidemics in vegetable greenhouses. They recommend growers in Israel to reduce the leaf wetness period to <7 h on nights with expected temperatures between 9 and 21°C.

Recommendations for growers of cut flowers in glasshouse

Using the results of the experiments described in this thesis some recommendations for growers of cut flowers can be formulated:

* Decrease the number of conidia in the glasshouse air as much as possible.
* Use a dry production system, in which senescing plant material on the ground remains dry.
* Keep the RH as low as possible, especially in September, October and November, when the radiation is low, by extra heating the glasshouse during early morning and late afternoon and by ventilation.
* Avoid abrupt changes in RH, because *B. cinerea* has a hygroscopic mechanism of spore release.
* Use a glasshouse with high amount of incoming light and clean glass.
* Monitoring conidia in the glasshouse air is too time consuming.
* Use the warning system described in this thesis. When the RH and radiation are at levels favourable for infection of *B. cinerea* conidia, use a chemical to control *B. cinerea* in the post-harvest stage.

* Use less susceptible cultivars, if available.

Table 3. Schematic representation of the recommendations for cut flower growers. MRH is the mean RH for days 6, 7 and 8 before the day of harvesting flowers and MS is the mean global radiation for days 1, 2 and 3 before the harvest day. BD! is Botrytis Danger, when there is a great risk of such an amount of B. cinerea lesions that flowers are rejected at the auction.

MRH (%)	MS ($Jcm^{-2}day^{-1}$)	BD!
≤70%	not important	NO
70-75%	<750	YES
75-80%	<1750	YES
>80%	not important	YES

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Summary

In the Netherlands most cutflower crops are grown in heated glasshouses, in which the temperature and the relative humidity are controlled by computer. *B. cinerea* is one of the main air-borne pathogens in ornamentals grown under glass. Problems with *B. cinerea* in glasshouses in The Netherlands mainly occur during fall and winter, when the temperature is low (<20°C) and the relative humidity is high (>80%). Infection by *B. cinerea* can take place in the glasshouse during the production stage, mainly on dying and dead plant material. In the post-harvest stage *B. cinerea* mainly is a pathogen on flowers. Use of fungicides (benzimidazoles, dicarboximides) in the glasshouse may increase the risks of fungicide resistance developing, of quality loss of flowers and of chemical residues on the flowers. Quality loss caused by *B. cinerea* during the post-harvest period is difficult to avoid. Chemical control of *B. cinerea* during post-harvest is difficult, especially when the infection pressure on the flowers is high. At this moment, in 1994, *B. cinerea* in ornamentals is still controlled mainly by fungicides. The pesticide use in ornamentals in The Netherlands has to be reduced by 65% in 2000, according to the Multi-Year Crop Protection Plan published by the Dutch government. More knowledge on the epidemiology of *B. cinerea* in glasshouses is needed to take specific measures (e.g. fungicide treatment) at the right time and to reduce the fungicide use.

Experiments described in this thesis were performed to further knowledge on the epidemiology of *B. cinerea* in glasshouses, with gerbera and rose as model systems. The epidemiology of *B. cinerea* in a gerbera (cultivars Terrafame and Delphi) and a rose crop (cultivar Sonia) was studied and compared, because the structure of these crops and their productions systems are quite different.

A selective medium was developed for the use in spore traps to study the dispersal and distribution of *B. cinerea* on gerbera and rose flowers grown in glasshouses (Chapter 1).

The dispersal and horizontal and vertical distribution of *Botrytis cinerea* in gerbera crops grown in two glasshouses 30 km apart was studied over a period of 18 months, in 1988 and 1989 (Chapters 2 and 3). Conidia were caught in spore traps consisting of agar in Petri dishes exposed at different heights in the crop in each glasshouse and counted as colonies, after incubation. No seasonal patterns could be identified in the spore catches, assessed as colonies on the agar traps after incubation. The number of lesions caused by conidial infection of gerbera flowers following incubation, however, showed a distinct seasonal pattern. In spring and early summer few lesions were recorded whereas at other times of the year many lesions appeared. In linear regression analysis, variation in numbers of colonies (spore catches) could not be explained by environmental factors recorded during the experiments. Linear regression accounted for 77 and 81% of the

variation in the number of lesions on flowers in the two glasshouses, in terms of relative humidity (positively correlated), global radiation outside the glasshouse (negatively correlated) and age of the crop (positively correlated). Despite differences in the systems by which the gerbera crop was produced and in the spore catches, the number of lesions on gerbera flowers in the two glasshouses were significantly correlated and not significantly different. The horizontal and vertical distribution of conidia of *B. cinerea* in the gerbera crops grown under glass was fairly uniform in both glasshouses (Chapter 3). Conidia of *B. cinerea* trapped in a glasshouse can originate from sources inside and outside the glasshouse.

In a glasshouse of 300 m^2, dispersal and horizontal distribution of *Botrytis cinerea* in a rose crop was studied in 1991 and 1992 (Chapter 4). The horizontal distribution of *B. cinerea* in a rose crop grown under glass was fairly uniform in both years, as in the gerbera crops. In 1991 no clear seasonal pattern in the number of colonies could be found. In 1992 the numbers of colonies were high in August, September and October. The numbers of lesions on rose flowers showed a distinct pattern in both years. In August, September and October many lesions were counted whereas in the other months few lesions appeared. In linear regression analysis, variation in numbers of colonies (spore catches) could not be explained by environmental factors recorded during the experiments. Linear regression accounted for 76 and 63% of the variation in the number of lesions on rose flowers in 1991 and 1992, in terms of relative humidity (positively correlated), global radiation outside the glasshouse (negatively correlated) and numbers of colonies on spore traps (positively correlated). On roses outside the glasshouse very high numbers of lesions were counted sometimes, mostly during and after rain showers, as a result of rain-deposition of spores onto the flowers.

The results in the gerbera and rose crops suggest that during the production stage RH inside the glasshouse and global radiation outside the glasshouse are the main variables in regulating the number of lesions during storage and transport. Based on these results a warning system has been developed. If the daily mean relative humidity of days 6, 7 and 8 days before the day of harvesting the flowers exceeds 70% and the mean global radiation for days 1, 2 and 3 before the harvest day is below 1500 $Jcm^{-2}day^{-1}$, there is a great risk of *B. cinerea* damage on cut flowers in the post-harvest stage. The results of this study also suggest that spore trapping at one height and at a limited number of locations and dates is adequate to monitor *B. cinerea* in a glasshouse. The numbers of spores in glasshouses depend on the production system. A glasshouse with a system resulting in wet dead tissue on the ground produces higher amounts of spores in the glasshouse air and therewith higher numbers of lesions on flowers.

The effect of vapour pressure deficit, temperature and radiation on the postharvest susceptibility of gerbera flowers to *B. cinerea*, on the water relations of gerbera flowers and on the lesion formation after conidial infection of *B. cinerea* was studied in Chapter

5. The temperature range in which *B. cinerea* can germinate and grow *in vitro* is 5-30°C. In climate chamber experiments flowers had more lesions of *B. cinerea* at temperatures of 20 and 25°C than at 10 and 15°C. At 15, 20 and 25°C the infectivity of *B. cinerea* conidia was negatively affected by a storage-period of 7 days. At a vapour pressure deficit (VPD) of 200 Pa significantly more conidia of *B. cinerea* were infective than at 800 Pa. At VPDs of 800 and 200 Pa the susceptibility of gerbera flowers for *B. cinerea* was not significantly different. High radiation levels in glasshouses in spring and summer negatively influenced the infectivity of conidia of *B. cinerea* on the flower surface, but did not affect the susceptibility of gerbera flowers to *B. cinerea*. In spring and early summer conidia lost their infectivity at high radiation levels, high temperatures and high levels of VPD (= low levels of relative humidity). In summer gerbera flowers could be more susceptible to *B. cinerea* because of high temperatures in glasshouses, but the negative effect of radiation on the conidia of *B. cinerea* seemed to overrule the temperature effect. Thus, the numbers of lesions in spring and summer were low compared with the numbers in other seasons, although the numbers of *B. cinerea* colonies on spore traps were high. The effect of temperature on the susceptibility of gerbera flowers can probably be explained by changes of water status in the petals. At higher temperatures the number of lesions and the turgor (= water potential - osmotic potential) in the petals increased. Temperatures <10°C during lesion formation (RH>95-%) had a temporary negative effect on the number of lesions. After 3 days of incubation the numbers of lesions were about equal (≥30 lesions/cm^2) from 5 to 20°C. At 30°C no lesion formation was observed even after 3 days.

The deposition of conidia of *B. cinerea* on spore traps with different trapping angles and on rose and gerbera flowers was studied in Chapter 6. Three spore trap types with different angles to the horizontal were used, a horizontal trap, a similar trap at an angle of 45°, and two vertical traps with opposite openings. The highest spore catches were obtained with the horizontal and slanted spore traps. In general, the horizontal spore trap was the most efficient one. The architecture of a flower is one of the factors that influences the number of *B. cinerea* spores trapped on the flower surface. The number of lesions per cm^2 of petal as a result of penetrating spores of *B. cinerea* on a gerbera flower (horizontal) was more than ten times higher than on a rose flower (vertical). The flower type determines the type of spore trap that has to be used. These results indicate that sedimentation by gravity is a major mechanism in the deposition of *B. cinerea* spores in glasshouses. The number of *B. cinerea* conidia in the air of a glasshouse with a rose and a gerbera crop was studied during several 24 h periods using a Burkard volumetric spore trap (Chapter 6). Spores of *B. cinerea* were always present in the glasshouse air. The numbers of trapped spores were low in the rose crop and high in the gerbera crop. In the rose crop the mean numbers of colonies were significantly higher at day (08.00-20.00 h) than at night (20.00-08.00 h) and in the gerbera crop they were significantly higher at night than at day. It is surmised that the crop grown in the

glasshouse and the production system used have great influence on the numbers of spores present in glasshouse air.

The effect of the season, as expressed by certain levels of relative humidity, temperature and total global radiation, on weights of petals, wax and cuticle of gerbera and rose flowers, on flowering periods of gerbera and rose flowers and on numbers of lesions on gerbera and rose flowers grown under glass was studied (Chapter 7). The differences in weights of petals, wax and cuticle between susceptible and less susceptible gerbera and rose cultivars was investigated (Chapter 7).

No clear relation in time was found between weights of petals, wax and cuticle, and numbers of lesions on the flowers. No significant correlation was found between weights of petals, wax or cuticle and numbers of lesions. Except for the gerbera cultivar Delphi, no significant correlation was found between the environmental factors temperature, relative humidity and global radiation and weights of petals, wax or cuticle. No relation was found between amount of wax and cuticle and susceptibility of gerbera and rose flowers for *B. cinerea*. The weights of wax and cuticle of flowers do not seem to be important factors for the susceptibility of flowers to *B. cinerea*.

Samenvatting

In Nederland worden veel snijbloemen in verwarmde kassen geteeld. In deze kassen kan het klimaat (temperatuur, relatieve luchtvochtigheid) met een computer worden geregeld. De schimmel *Botrytis cinerea* is een belangrijke luchtverspreide schimmel in de sierteelt en vormt een groot probleem in de snijbloementeelt, meestal in de herfst als de relatieve luchtvochtigheid (RV) hoog (>80%) en de temperatuur laag is (<20°C). Infectie door *B. cinerea* kan in de kas plaatsvinden tijdens de teeltfase, voornamelijk op dood en afstervend plantmateriaal, als dat voldoende vochtig is. Tijdens de naoogst-fase infecteert *B. cinerea* voornamelijk bloemen. Het gebruik van fungiciden (benzimidazolen, dicarboximiden) in de kas tijdens de teeltfase is niet raadzaam vanwege de kans op schade aan het gewas, achterblijvend residu, te hoge arbeids- en behandelingskosten en eventuele resistentieontwikkeling van de schimmel tegen de gebruikte middelen. Chemische bestrijding van deze schimmel in de naoogst-fase is moeilijk, geeft wisselvallige resultaten en is vaak niet afdoende, vooral als het aantal conidiën op het bloemoppervlak groot is. *B. cinerea* wordt momenteel, in 1994, nog voornamelijk bestreden met behulp van fungiciden. Het gebruik van pesticiden in de sierteelt in Nederland moet in het jaar 2000 met 60% zijn verminderd, volgens het Meerjarenplan Gewasbescherming, gepubliceerd door de Nederlandse overheid. Meer kennis van de epidemiologie van *B. cinerea* in kassen is nodig om de juiste maatregelen (bijvoorbeeld fungicide-behandelingen) te kunnen nemen op het juiste tijdstip en om zo het gebruik van fungiciden tegen *B. cinerea* te verminderen.

De experimenten beschreven in dit proefschrift zijn uitgevoerd om meer kennis te verkrijgen van de epidemiologie van pokken veroorzaakt door *B. cinerea* tijdens de teelt- en de naoogst-fase. Gerbera en roos zijn gebruikt als modelsystemen. De epidemiologie van *B. cinerea* in de gewassen Gerbera (cultivar 'Terrafame' en 'Delphi') en roos (cultivar 'Sonia') zijn bestudeerd en vergeleken, omdat de structuur van deze gewassen en de teeltsystemen behoorlijk van elkaar verschillen.

Er is eerst een selectief medium ontwikkeld voor *B. cinerea* om te gebruiken in sporevangers (hoofdstuk 1). Deze sporevangers (met het selectieve medium) werden gebruikt bij het bestuderen van de verspreiding van *B. cinerea* in Gerbera en roos geteeld onder glas (hoofdstukken 2, 3, 4 en 6).

In de hoofdstukken 2 en 3 wordt het onderzoek beschreven naar de verspreiding in de tijd en naar de horizontale en vertikale verdeling in de ruimte van *B. cinerea*, in twee Gerberakassen, gedurende achttien maanden in 1988 en 1989. Deze kassen stonden 30 kilometer van elkaar, in Aalsmeer (100 m^2) en Vleuten (350 m^2). Conidiën, die zich in de kaslucht bevonden, werden op verschillende hoogten in de kas met sporevangers

gevangen zoals beschreven in hoofdstuk 1. De verspreiding van conidiën in de tijd was erg wisselend. De ene week werden erg veel conidiën gevangen en de daarop volgende week erg weinig. Er kon geen seizoengebonden patroon in de aantallen gevangen conidiën worden gevonden. De aantallen lesies (pokken) op de Gerbera-bloemen in de naoogst-fase, als gevolg van infectie door conidiën, vertoonden wel een seizoengebonden patroon. In de lente en de voorzomer werden weinig lesies op de bloemen waargenomen, terwijl in de andere seizoenen veel lesies konden worden geteld. Met behulp van lineaire regressie-analyse kon geen voldoende beschrijving worden gegeven voor de variatie in de aantallen gevangen sporen in de tijd, met de geregistreerde klimaatfactoren. Daarentegen kon de variatie in de aantallen lesies op de bloemen voor ongeveer 80% worden beschreven met behulp van de relatieve luchtvochtigheid in de kas (positief gecorreleerd), de lichtinstralingssom buiten de kas (negatief gecorreleerd) en de ouderdom van het gewas (positief gecorreleerd). In de twee kassen waren de aantallen lesies op de Gerberabloemen significant gecorreleerd en zij verschilden niet significant van elkaar. Dit is opvallend, omdat de produktiesystemen en de aantallen gevangen sporen sterk van elkaar verschilden en de kassen 30 kilometer uit elkaar lagen. De horizontale en vertikale verdeling van *B. cinerea*-conidiën in beide kassen was vrij uniform (Hoofdstuk 3). Conidiën van *B. cinerea* kunnen zowel binnen als buiten de kas gevormd zijn en ook van buiten de kas naar binnen komen (voornamelijk via de ventilatieramen).

In hoofdstuk 4 wordt het onderzoek beschreven naar de variatie in de tijd en de horizontale verdeling in de ruimte van conidiën van *B. cinerea* in een rozenkas van 300 m^2, in 1991 en 1992. De horizontale verdeling van de gevangen sporen en van de aantallen lesies op de bloemen in de ruimte was vrij uniform in beide jaren, net zoals in de Gerberakassen. In 1991 was er geen seizoengebonden patroon te vinden in de variatie van de aantallen conidiën in de tijd. In 1992 waren die aantallen in de maanden augustus, september en oktober veel hoger dan in de andere maanden. De aantallen lesies op de bloemen vertoonden in beide jaren een duidelijk seizoensgebonden patroon. De aantallen lesies waren hoog in augustus, september en oktober, terwijl ze in de andere maanden laag waren. Met behulp van lineaire regressie-analyse kon, net als in de Gerbera-gewassen, geen goede beschrijving worden gegeven voor de variatie in de aantallen gevangen sporen in de tijd, met de geregistreerde klimaatfactoren. De variatie in de aantallen lesies op de bloemen kon voor ongeveer 70% worden beschreven met behulp van de relatieve luchtvochtigheid in de kas (positief gecorreleerd), de lichtinstralingssom buiten de kas (negatief gecorreleerd) en de aantallen conidiën in de kaslucht (positief gecorreleerd). Op rozen buiten de kas konden soms ook grote aantallen lesies worden waargenomen, meestal na een regenbui.

Uit de resultaten in kassen met Gerbera en roos kan worden geconcludeerd dat, tijdens de produktie-fase, de relatieve luchtvochtigheid in de kas en de instralingssom buiten de kas de belangrijkste variabelen zijn die de aantallen lesies op de bloemen in de naoogst-

fase bepalen. Met deze kennis is een waarschuwingssysteem voor de telers ontwikkeld. Als de gemiddelde relatieve luchtvochtigheid in de kas van de dagen 6, 7 en 8 voor de oogstdag van bloemen hoger is dan 70% en de gemiddelde instralingssom buiten de kas van de dagen 1, 2 en 3 voor de oogstdag van bloemen lager is dan 1500 $Jcm^{-2}dag^{-1}$, dan is de kans op een onacceptabele aantasting van *B. cinerea* op de bloemen in de naoogstfase erg groot. Door in de kas sporen van *B. cinerea* op een beperkt aantal plekken en op één hoogte te vangen kan men een betrouwbaar beeld krijgen van de aantallen conidiën die in de kaslucht aanwezig zijn. De aantallen sporen in de kaslucht zijn sterk afhankelijk van het gebruikte produktiesysteem. In een kas met een zogenaamd vochtig produktiesysteem, resulterend in veel nat en dood plantmateriaal, zijn de aantallen conidiën van *B. cinerea* en daardoor de aantallen lesies op de bloemen veel hoger dan in een kas met een droog produktiesysteem.

In hoofdstuk 5 is de invloed van een aantal klimaatfactoren op de gevoeligheid van Gerberabloemen voor *B. cinerea* en op het infectieuze vermogen van conidiën van *B. cinerea* nader uitgewerkt. De invloed van het dampspanningsdeficit (VPD), de temperatuur en de instraling op de gevoeligheid van Gerberabloemen voor *B. cinerea*, op de waterhuishouding van Gerberabloemen en op het infectieuze vermogen van conidiën van *B. cinerea* is onderzocht. *In vitro* kunnen conidiën kiemen en kan mycelium groeien tussen de 5 en 30°C. De aantallen lesies op de bloemen in klimaatkasten was hoger bij 20 en 25°C dan bij 10 en 15°C. Bij 15, 20 en 25°C was het infectieuze vermogen van conidiën van *B. cinerea* significant slechter dan bij 10°C, als de conidiën zeven dagen op Gerberabloemen hadden gelegen in klimaatkasten. Bij een VPD van 200 Pa (hoge relatieve luchtvochtigheid) waren na zeven dagen meer conidiën infectieus dan bij een VPD van 800 Pa. De gevoeligheid van Gerberabloemen was gelijk bij een VPD van 200 en 800 Pa. Een hoge instralingssom in de kas, zoals in lente en zomer, had een negatieve invloed op de infectiviteit van conidiën, maar had geen invloed op de gevoeligheid van Gerberabloemen voor *B. cinerea*. In de lente en in de voorzomer verliezen conidiën hun infectieus vermogen door een hoge instralingssom, een hoge temperatuur en een hoge VPD-waarde (= lage relatieve luchtvochtigheid). Gerberabloemen kunnen in de zomer gevoeliger zijn voor *B. cinerea* door hogere temperaturen in de kas, maar de negatieve invloed van de stralingssom op de infectiviteit van conidiën lijkt belangrijker te zijn dan het temperatuureffect op de bloemen. De lage aantallen lesies op Gerberabloemen en de hoge aantallen sporen in de kaslucht in de lente hoeven dus niet met elkaar in tegenspraak te zijn. Het temperatuureffect op de bloemen zou verklaard kunnen worden door het effect op de waterhuishouding van Gerberabloemen. Zowel het aantal lesies op de bloemen als de turgor (= waterpotentiaal - osmotische potentiaal) van de bloemen was hoger bij hogere temperaturen. Verder hadden temperaturen hoger dan 10°C tijdens het proces van kieming en penetratie (RV>95%) een vertragend effect op de vorming van lesies. Na drie dagen bij RV>95% werden ongeveer evenveel lesies gevormd bij de temperaturen 5 tot 20°C. Bij een temperatuur van 30°C werden geen

lesies waargenomen, ook niet na drie dagen.

In hoofdstuk 6 wordt beschreven onder welke hoek een sporevanger het beste in de kas kan staan om de meeste sporen van *B. cinerea* te kunnen vangen en of er verschil is in aantallen lesies op Gerberabloemen en rozen. De sporevangers werden onder drie verschillende hoeken in de kas neergezet, horizontaal, onder een hoek van 45° en vertikaal. De meeste sporen werden op de horizontale sporevanger en op de sporevanger onder een hoek van 45° gevangen. De stand van een bloem is één van de factoren die bepalen hoeveel sporen er op het bloemoppervlak terecht komen tijdens de teelt. Het aantal lesies veroorzaakt door conidiën van *B. cinerea* op Gerberabloemen (horizontale stand) was meer dan tien keer zo hoog als op rozen (vertikale stand). Dus het type bloem dat in een kas wordt geteeld bepaalt het type sporevanger dat het beste kan worden gebruikt. Deze resultaten geven aan dat in kassen de landing van sporen op bloemen voornamelijk wordt bepaald door de zwaartekracht. De aanwezigheid van conidiën van *B. cinerea* tijdens verschillende 24-uurs perioden in een Gerbera- en rozengewas is bepaald met behulp van een Burkard-sporevanger (hoofdstuk 6). Uit de experimenten met de Burkard-sporevanger blijkt dat conidiën van *B. cinerea* altijd in de kas aanwezig zijn, overdag en 's nachts. De aantallen gevangen sporen waren relatief laag in het rozengewas en hoog in het gerberagewas. In het rozengewas waren de aantallen gevangen sporen overdag (08.00-20.00 uur) significant hoger dan 's nachts (20.00-08.00 uur). In het Gerberagewas was het precies andersom. In deze experimenten bleek ook weer dat het produktiesysteem en het gewas in de kas veel invloed hebben op de aantallen conidiën in de kaslucht.

Het onderzoek naar eventuele seizoenseffecten (verschillen tussen de seizoenen in RV, temperatuur en instralingssom) op het drooggewicht, het waslaaggewicht en het cuticulagewicht van rozen en Gerberabloemen (per cm^2 bloem) is in hoofdstuk 7 beschreven. Er is gekeken naar de invloed van het seizoen op de gevoeligheid van rozen en Gerberabloemen voor *B. cinerea* en op het aantal dagen dat bloemen in de kas stonden voordat ze geoogst werden. Verder is gekeken of er verschillen in drooggewicht, in gewicht van de waslaag en in cuticulagewicht bestonden tussen gevoelige en voor *B. cinerea* minder gevoelige roze- en Gerberacultivars. Er is geen duidelijk verschil gevonden in drooggewicht, waslaaggewicht, cuticulagewicht en gevoeligheid voor *B. cinerea* van rozen en Gerberabloemen tussen de verschillende seizoenen. Er was ook nauwelijks een significante relatie te vinden tussen drooggewicht, gewicht van de waslaag en cuticulagewicht en de gevoeligheid van rozen en Gerberabloemen en tussen drooggewicht, gewicht van de waslaag en cuticulagewicht en de klimaatfactoren RV, temperatuur en instralingssom. De hoeveelheid was en cuticula op bloemen lijkt geen belangrijke factor te zijn voor de gevoeligheid van bloemen voor *B. cinerea.*

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Curriculum vitae

Albert Kerssies werd op 26 april 1961 in Den Helder geboren. In 1977 behaalde hij het MAVO-diploma en in 1980 het VWO-diploma. In 1980 begon hij zijn studie aan de Landbouwhogeschool te Wageningen. In het kader van deze studie heeft hij zes maanden stage gelopen bij 'The New York State Agricultural Experiment Station', Geneva. In 1987 slaagde hij voor het doctoraalexamen in de richting planteziektenkunde met als hoofdvakken fytopathologie en nematologie en als bijvak marktkunde en marktonderzoek. Tijdens deze studie heeft hij één jaar in de hogeschoolraad van de Landbouwhogeschool gezeten als student-raadslid, namens de christen studenten fractie.

Aansluitend aan zijn studie werd hij aangesteld als wetenschappelijk onderzoeker gewasbescherming binnen de afdeling vaktechnische onderzoeksondersteuning (momenteel binnen de afdeling Produktkwaliteit) van het Proefstation voor de Bloemisterij in Nederland. Naast het onderzoek beschreven in dit proefschrift is hij betrokken bij een aantal andere onderzoeksprojecten: de epidemiologie van echte meeldauw bij roos, ontwikkelen van detectietechnieken voor plantpathogene schimmels in siergewassen, biologische bestrijding van *Botrytis cinerea* op gerbera en roos, chemische bestrijding van voetrotveroorzakende *Phytophthora* spp. in potplanten.