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Influence of environmental conditions in a glasshouse on conidia of *Botrytis cinerea* and on post-harvest infection of rose flowers

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Abstract

Quantification and horizontal distribution of air-borne inoculum 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 (RH) 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 amount 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.

Introduction

The fungus *Botrytis cinerea* Pers.:Fr., the imperfect stage of *Botryotinia fuckeliana* [Ellis and Waller, 1974], is a pathogen to a wide variety of economically important plants, such as vegetables, ornamentals, bulbs and fruits and a saprophyt on senescing and dead plant material. Infection takes place through wounds, via decaying or dead plant tissue, and by direct penetration of the undamaged host [Verhoeff, 1980]. Conidia of *B. cinerea* are dispersed by air currents, water droplets and insects. *B. cinerea* is a major air-borne pathogen in ornamentals grown under glass and causes damage to cut flowers such as gerbera, rose, chrysanthemum and pot plants [De Jong, 1985,

1986]. In the Netherlands, in 1992, 900 ha of roses were grown of which 340 ha of roses on artificial substrate (e.g. rockwool).

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 (e.g. rose flowers). Deposition of conidia of *B. cinerea* on flowers mainly occurs in the glasshouse, during production. Flowers can function as spore traps. After deposition on the flowers, conidia remain dormant until a thin water film is available for germination. Necrotic lesions (spotting), caused by young colonies, occurring on cut flower buds and petals during the post-harvest period. These symptoms occur at 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, dicarboximates) in the glasshouse may increase the risk of fungicide resistance developing [Gullino and Garibaldi, 1987; Witt and 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 is high [Dirkse, 1980].

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 *et al.* [1987] suggest that the patterns of air movement in glasshouses differ according to the spatial arrangement of the crop canopy. 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.

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.

Little is known about the factors influencing the number of viable spores of *B. cinerea* in ornamentals grown in glasshouses. Hausbeck and Pennypacker [1991] showed that grower activity in a greenhouse with potted geraniums resulted in peak conidial concentrations in the greenhouse air. Frinking and Scholte [1983] showed that the complex dispersal process involves aspects of pathogen, host, environment and human activity. Studies on the number of viable conidia and on the horizontal and vertical distributions of conidia of *B. cinerea* in a gerbera crop growing under glass and the effects of environmental factors on infection of gerbera flowers during postharvest have been published [Kerssies, 1993a, b]. The number of viable conidia of *B. cinerea* over time in relation to environmental factors and the distribution of conidia in a glasshouse probably differs between a gerbera and a rose crop. The physical structure of a rose crop is totally different from a gerbera crop. If the results in a gerbera and a rose crop are comparable some general conclusions can be drawn about the epidemiology of Botrytis 'spotting' on cut-flowers grown under glass, and probably, answers on questions of growers can be given on how to control this fungus in the best way and how to give a reliable prediction for the severity of *B. cinerea* spotting of flowers in the post-harvest stage. In this study, the influence of environmental conditions in a rose

glasshouse on conidia of *B. cinerea* and on post-harvest infection of rose flowers were observed. With the aim to improve the efficiency of aerobiological studies in glasshouses the horizontal distribution of conidia of *B. cinerea* in a rose glasshouse were studied. The results of this study and those observed in a gerbera crop [Kerssies, 1993a, b] were compared. Some observations were made on the dispersal of *B. cinerea* conidia outside glasshouses.

Materials and methods

Measurement of environmental conditions. Dry and wet bulb temperatures were measured continuously using 9 psychrometers, distributed within the crop in a regular spatial pattern, 0.5 m above the ground, coupled to a data logger. The temperature in the crop was measured every 10 min and averaged over 1 h periods. Similarly, relative humidity was calculated every 10 min 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 every 10 min and averaged over 1 h periods using a tachometer 8.5 m above the ground [De Jong, 1990].

Conidia of Botrytis cinerea. Experiments were conducted to examine 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 (Ø 9cm) containing 20–25 ml of a selective medium for *B. cinerea* [Kerssies, 1990] to the four sides and the bottom of a wooden cube (9*9*9cm). The traps were custom made. Forty-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 with the selective medium 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 placed for 24 h 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 meters south-west from the glasshouse; prevailing wind direction is from this spore trap to glasshouse E4) each week in 1991 and 1992. In the vicinity of two spore traps (one at a distance of 1 meter and one 40 meters 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. Linear regression analysis is common used in epidemiological studies with airborne fungi by fitting models to data. In this study two dependent variables were used in regressions against environmental variables [Madden and Ellis, 1988]: the mean numbers of colonies per spore trap (five plates per trap) per exposure date (48 trapping locations) on 50 trapping dates in glasshouse E4 over a period of 365 days (1991) and the mean numbers of lesions on rose flowers (144 flowers) in 41 samples (the first 9 weeks the rose-plants did not produce flowers) in glasshouse E4 over a period of 365 days (1991). 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, 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)) counted 1 week later (only for the regression analysis of the mean numbers of lesions). The colony counts of one week before the lesions on the flowers were counted were used in regression analysis, because flowers bloom and were open for spores in the glasshouse until harvest-time, approximately 1 week. Daily mean values of RH, V, T, S and W were calculated for each of the 14 days before a harvest day (MRH, MV, MT, MS and MW).

When successive events occur independently the mean numbers of colonies on spore traps (CFU) and the mean numbers of lesions on petals follow the Poisson distribution. Because Poisson means are required to be positive an additive model is unsatisfactory. By taking the exponent of a linear combination of predictor variables this requirement is fulfilled. In this way, the number of colonies and the number of lesions is described by what is called a generalized linear model in which the link function is the logarithmic function and distribution is Poisson. Before fitting the log-linear models, counts were logtransformed and the best subsets of predictor variables were selected according to the following criteria: maximum R^2, the t-test and the chi-square test. 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, respectively, over a period of 366 days.

In search of significant spatial differences in colony and lesion densities through time (independent variable), Poisson regression models (link function = log) were fitted to the number of colonies per trap location and to the number of lesions per harvest location per counting date (dependent variables).

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. Climatic parameters were used in log linear regression analysis and in Table 1 an overview of the climatic conditions in the experimental glasshouse during the experiment is shown. 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.

Colonies. The efficiency of the spore traps used in the experiments was approximately 3–4 times

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	0.5–1.2	65–75	>700 and <2700
Summer	20–25	0.5–0.8	70–80	>700 and <2700
Autumn	17–20	0.4–0.6	70–85	<700
Winter	15–18	0.5–1.0	55–70	<700

lower than a Burkard volumetric spore trap [Kerssies, personal observation]. The colony counts for locations averaged over a year (n = 50 trapping dates in 1991 and n = 42 trapping dates 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, colony counts in the glasshouse between 2.4 and 5.0, mean = 3.1). No horizontal pattern could be observed (e.g. more colony counts in the corners of the glasshouse) in the glasshouse.

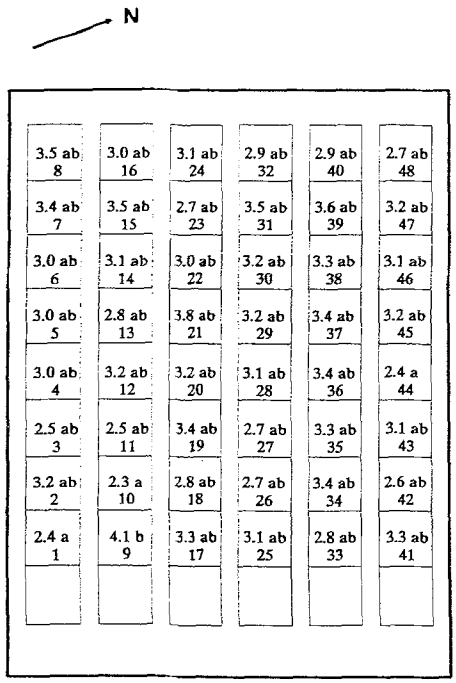

Fig. 1. Spore trap positions in a rose glasshouse. Colony counts for locations averaged (for groups of 5 plates) over 1992 (n = 42 trapping dates). 1–48: location numbers. Colony counts with the same letters are not significantly different ($P \leq 0.05$).

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 pattern in the peaks and valleys could be observed. In 1992 the numbers were high (>5 colonies/spore trap) in August, September and October. On days with high numbers of colonies (>5 colonies/spore trap) and 1–4 days before the exposure date, the relative humidity was high (≥75%), the temperature and the total daily global radiation were variable. The high relative humidity may be related to 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 pattern in the peaks and valleys could be observed, except for the low number of colonies during 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 pattern in the peaks and valleys 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 colony counts for the four locations averaged over a year (n = 35 trapping dates in 1991 and n = 40 trapping dates in 1992) outside glasshouse E4 showed no significant differences (ANOVA; $P \leq 0.05$) between trapping locations, in either year (mean of 6.4 colonies/spore trap in 1991 and 9.4 colonies/spore trap in 1992).

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 were similar to the peaks inside E4 at the same counting dates (Figs. 2 and 4). Most of the peaks in the mean numbers of colonies outside E4 were also similar to peaks inside E3 (Figs. 3 and 4). In 1992 some peaks in the mean numbers of colonies were counted at the same time in E4 and E3, but other peaks were counted in E3 and not in E4 (Figs. 2 and 3). 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; colony counts in the glasshouse

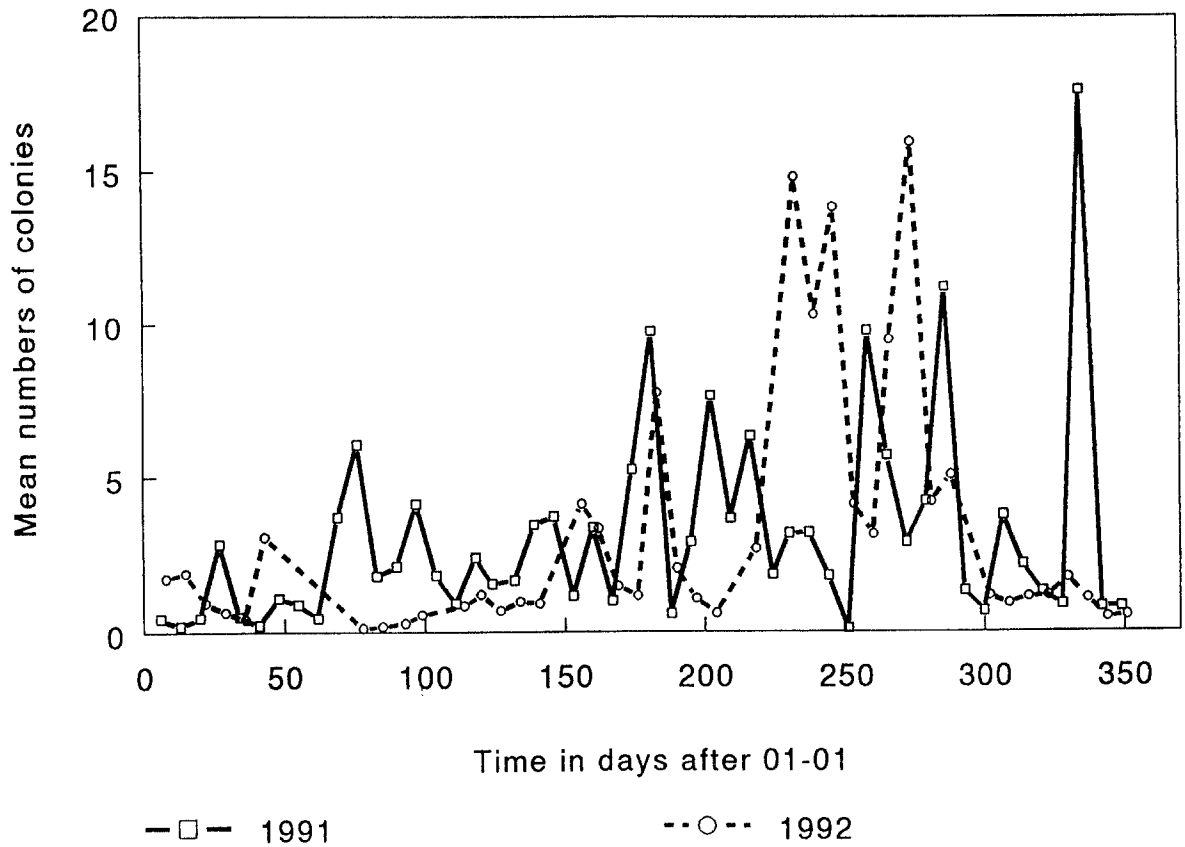

Fig. 2. Evolution over time of colony counts averaged over locations (for groups of 5 plates; n = 48 locations) in glasshouse E4, in 1991 and 1992.

between 1.4 and 4.8, mean = 3.3). No horizontal 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 (Figs. 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. The lesion counts (ln(N+1) for the two locations averaged over counting dates outside glasshouse E4 showed no significant differences (ANOVA; $P \leq 0.05$) between the harvest locations in either year (mean of 47 lesions/flower in 1992).

In 1992 the mean numbers of lesions on rose 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 were similar to peaks inside E4 (Figs. 6 and 8). Most of the peaks in E3 were not similar to peaks in- and outside E4 (Figs. 6, 7 and 8). Peaks in E3 and outside E4 were higher (maximum of 60 lesions/flower in E3 and

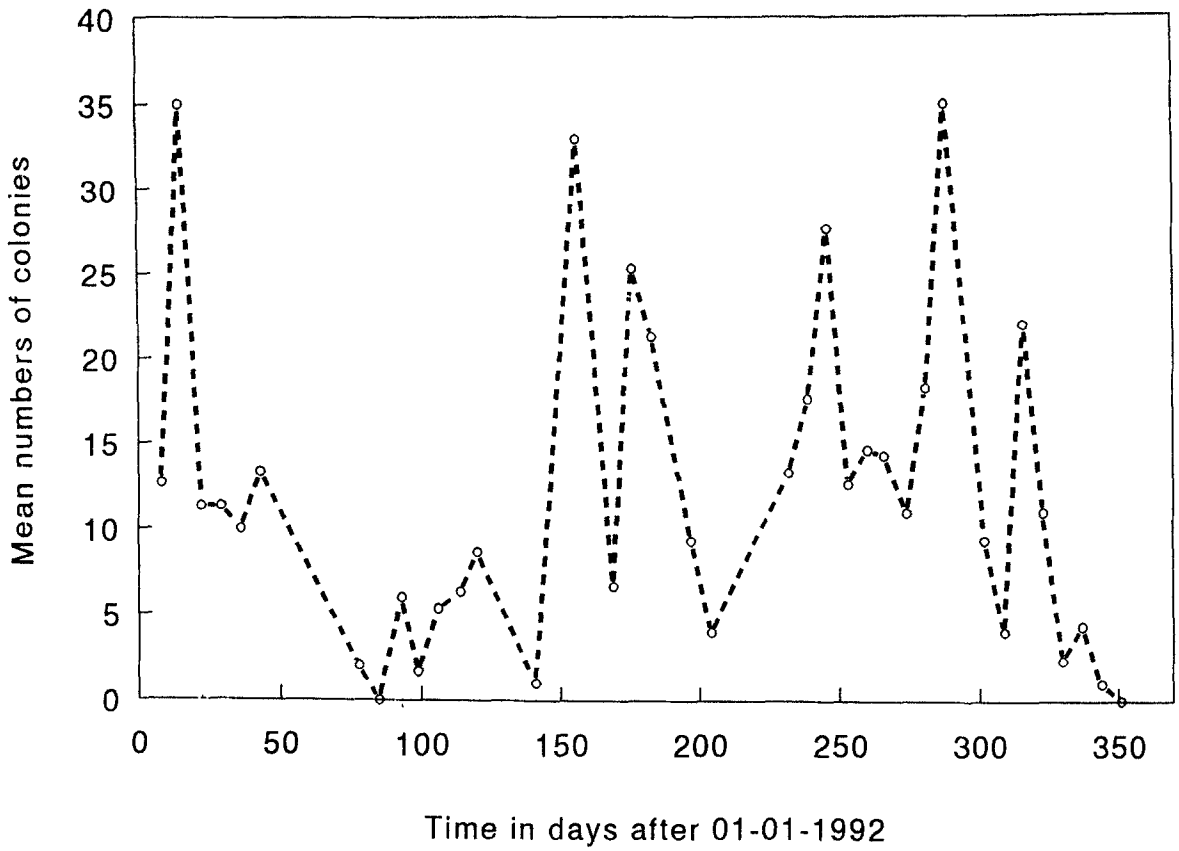

Fig. 3. Evolution over time of colony counts for trapping dates averaged over locations (for groups of 5 plates; n = 3 locations) in glasshouse E3, in 1992.

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 a high relative humidity and the presence of wet tissue on the ground resulting in high numbers of spores in the glasshouse air.

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

Regression analysis. Fluctuations in the number of colonies in glasshouse E4 could not be explained by regressions on any of the independent variables separately and on any combination of these variables. 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 ($P \leq 0.05$):

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

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

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 were significantly different ($P \leq 0.05$) 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), while 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), while the observed number of lesions does (7.1 lesions/flowers). 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 moderate 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 (Figs. 9 and 10; 1991–1992 and 1992–1991, respectively) were significantly correlated ($P \leq 0.005$ at $n = 38$). Figures 9 and 10 illustrate that the partial regression coefficients of the observed and fitted curves were significantly different at $P \leq 0.005$, 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.

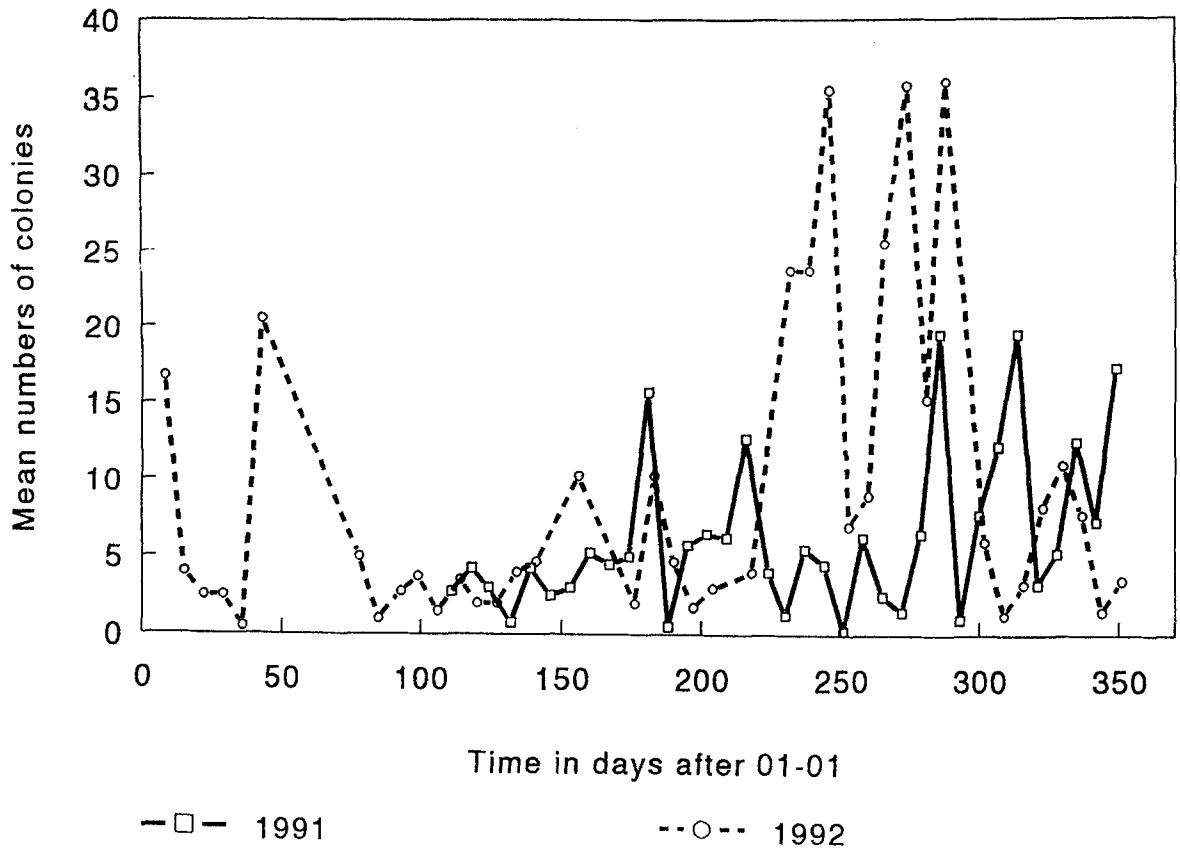

Fig. 4. Evolution over time of colony counts for trapping dates averaged over locations (for groups of 5 plates; n = 4 locations) outside glasshouses, in 1991 and 1992.

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 the relatively small spores (Ø 10 µm) of *B. cinerea* may be dispersed rapidly through the glasshouse by the air movements occurring inside the glasshouse [Frinking *et al.*, 1987]. The spores can be dispersed from a source inside or outside the glasshouse. This pattern may 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 may be sufficient, irrespective of the crop.

Colonies, changes over time. 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 $Jcm^{-2}day^{-1}$). Therefore, the observed changes in the number of colonies on spore traps over time in the rose crop are probably caused by changes in environmental condition, such as radiation, RH and air-movements, in and outside the glasshouse. In the rose glasshouse, the numbers of colonies did not depend on the age of the crop, as with gerbera's,

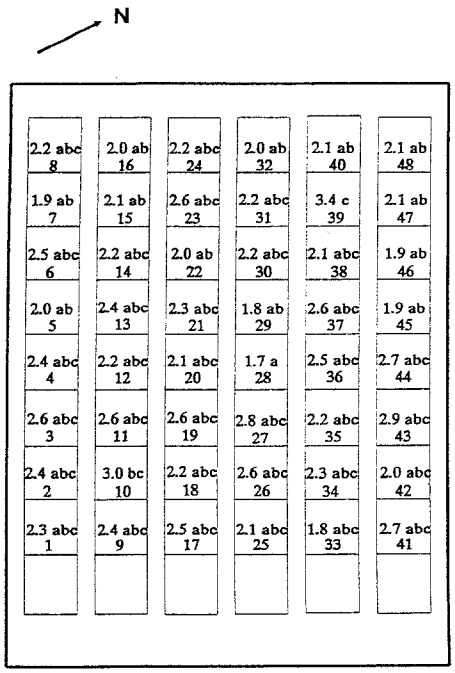

Fig. 5. Spore trap positions in a rose glasshouse. Lesion counts on rose flowers for locations averaged over counting dates in 1992 (n = 48 locations). 1–48: harvest location numbers. Lesion counts with the same letters are not significantly different ($P \leq 0.05$).

where the amount of dead gerbera tissue increases as the gerbera crop ages [Kerssies, 1993a]. The relatively wet, dead leaves in the gerbera crop provided a good substrate for germination, colonization and sporulation of *B. cinerea*. Hausbeck and Pennypacker (1991) showed that a primary factor influencing the occurrence of peak conidial concentrations (PCCs) in a geranium greenhouse was grower activity. The magnitude of the PCCs and the daily conidial concentrations in their potted geranium crop increased during the season, as was observed in the gerbera crop [Kerssies, 1993a] but not in the rose crop, and appeared to be related to the incidences of blighted stems and of stems and necrotic leaves with sporulating lesions. In the rose crop the numbers of colonies appeared to be related to environmental variables. Geraniums are potted plants and the leaves and stems of these plants are more sensitive to *B. cinerea* than the stems and leaves of rose and gerbera cutflowers and therefore much more sporulating spots can be observed in a geranium crop than in a rose or gerbera crop.

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. Abundant sporulaton was often observed on this wet tissue and may have been the source of a higher amount of conidia in the glasshouse air and higher peaks in the numbers of colonies. In glasshouse E4, the dead tissue remained dry most of the time and no sporulation was observed. Outside the glasshouse, more sporulation of *B. cinerea* occurred on dead tissue than in E4 and higher wind speed was recorded than inside. 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 probably more on the spore production outside. Above-mentioned suggestions are in agreement with results or suggestions of others. 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

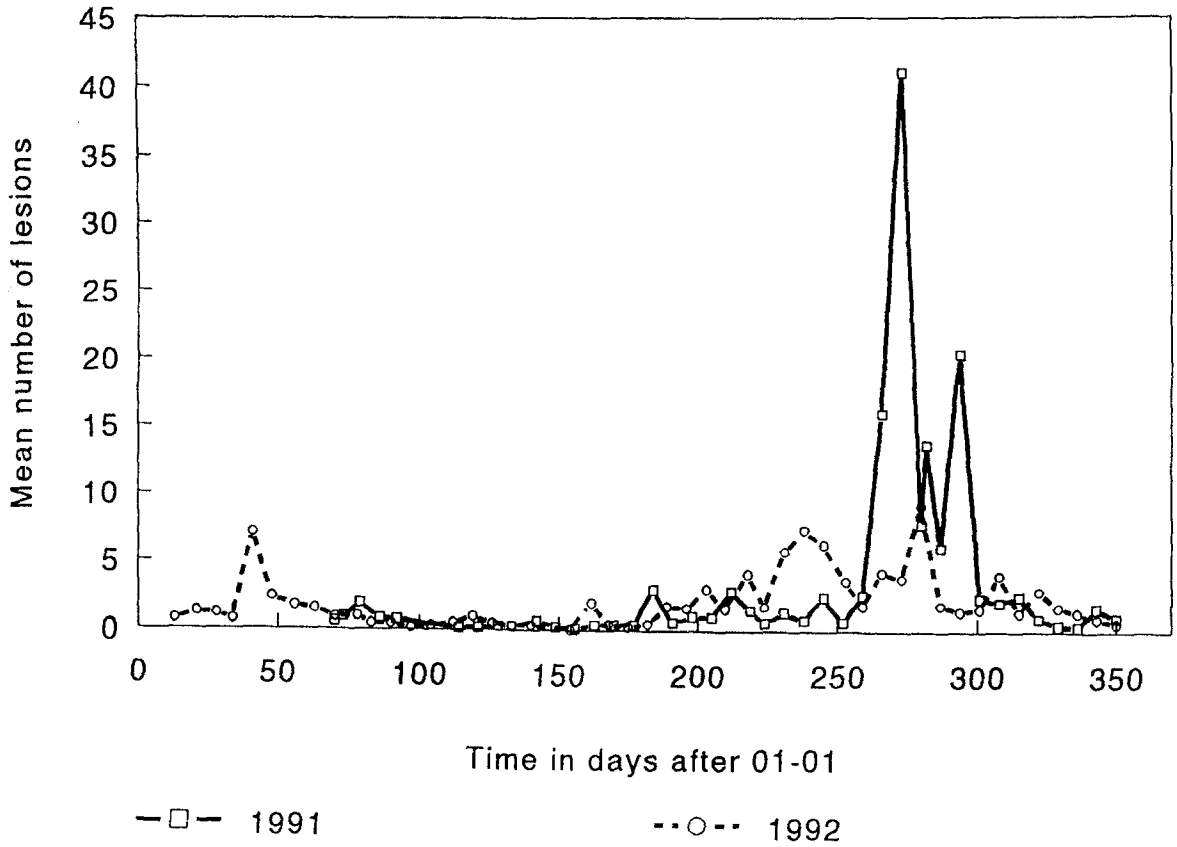

Fig. 6. Evolution over time of lesion counts per rose flower per counting date averaged over locations in glasshouse E4 (n = 144 flowers), in 1991 and 1992.

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. Hausbeck and Pennypacker [1991] stated that increased spacing between geranium plants reduced senescing of the lower leaves and removed potential infection sites. Increased spacing between rose plants have no such an effect on the leaves, because the canopy of a rose crop is already very open and no potential infection sites are present on rose stems.

Lesions, changes over time. As was observed on gerbera, the number of lesions on roses fluctuated less over time than the number of colonies. The exposure time of rose flowers (4–12 days) was longer than that of the spore traps (24 h) 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.

No weekly data were obtained on the relations between sporulation and number of colonies or lesions. In E3 not many sporulating spots could be

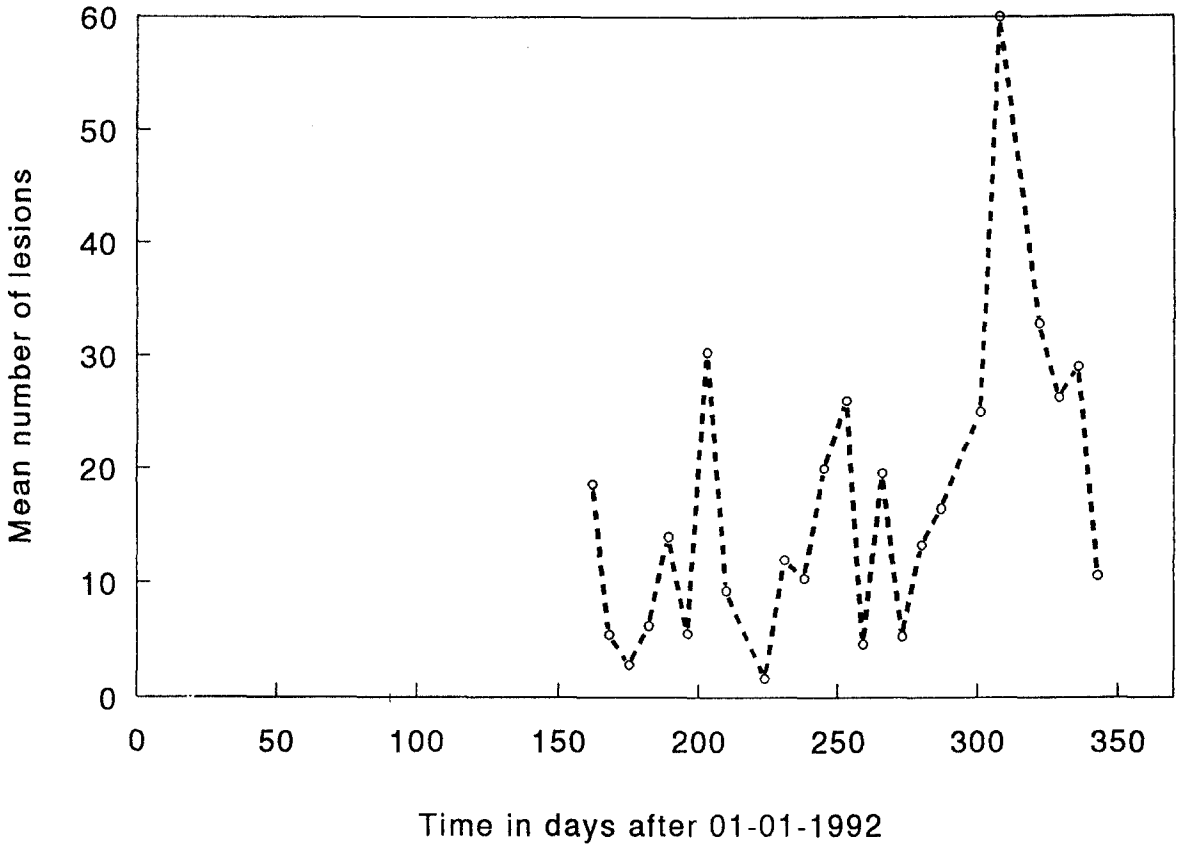

Fig. 7. Evolution over time of lesion counts per rose flower per counting date averaged over locations in glasshouse E3 (n = 5 flowers), in 1992.

counted, but significantly more than in glasshouse E4, were very few spots, mostly none, were counted [Kerssies, personal observation]. One sporulating spot can be enough for infecting the whole glasshouse. If there were sporulating spots, they were not regularly distributed in the glasshouses.

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], exposure to direct radiation under field conditions can accelerate the decline in viability of conidia of *B. cinerea*. Hausbeck and Pennypacker [1991] stated that although RH, temperature, vapor pressure deficit, and irradiance may ultimately determine the magnitude of PCCs in geranium stock plants, the role of grower activity in the occurrence of PCCs is an important consideration in disease control. The difference in conclusions between the study of Hausbeck and Pennypacker and this study can be explained by the absence of sporulating spots in

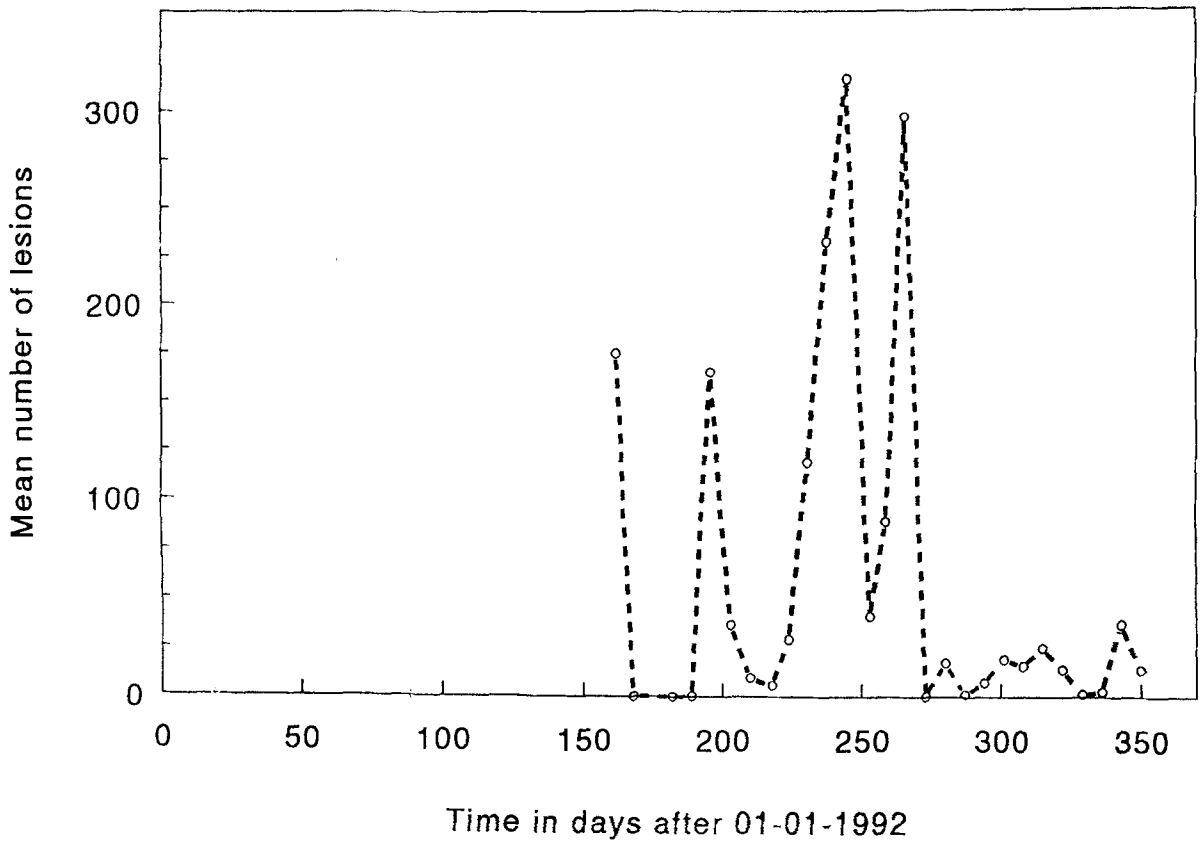

Fig. 8. Evolution over time of lesion counts per rose flower per counting date averaged over locations outside glasshouse (n = 10 flowers), in 1992.

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 ($Jcm^{-1}day^{-1}$)	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

the rose glasshouse. In a glasshouse with potted geraniums many sporulating spots can be observed while in rose glasshouses grown on rockwool very few sporulating spots can be observed. Grower activity in a glasshouse with geraniums can disturb these spots, which can cause spore release. In the rose glasshouse the effect of environmental variables on the spores and on the flowers were important in determining the number of lesions on the flowers in the post-harvest phase, as was shown in the regression equations.

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). To be certain the variable z can be kept at a value of 0.5. 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.

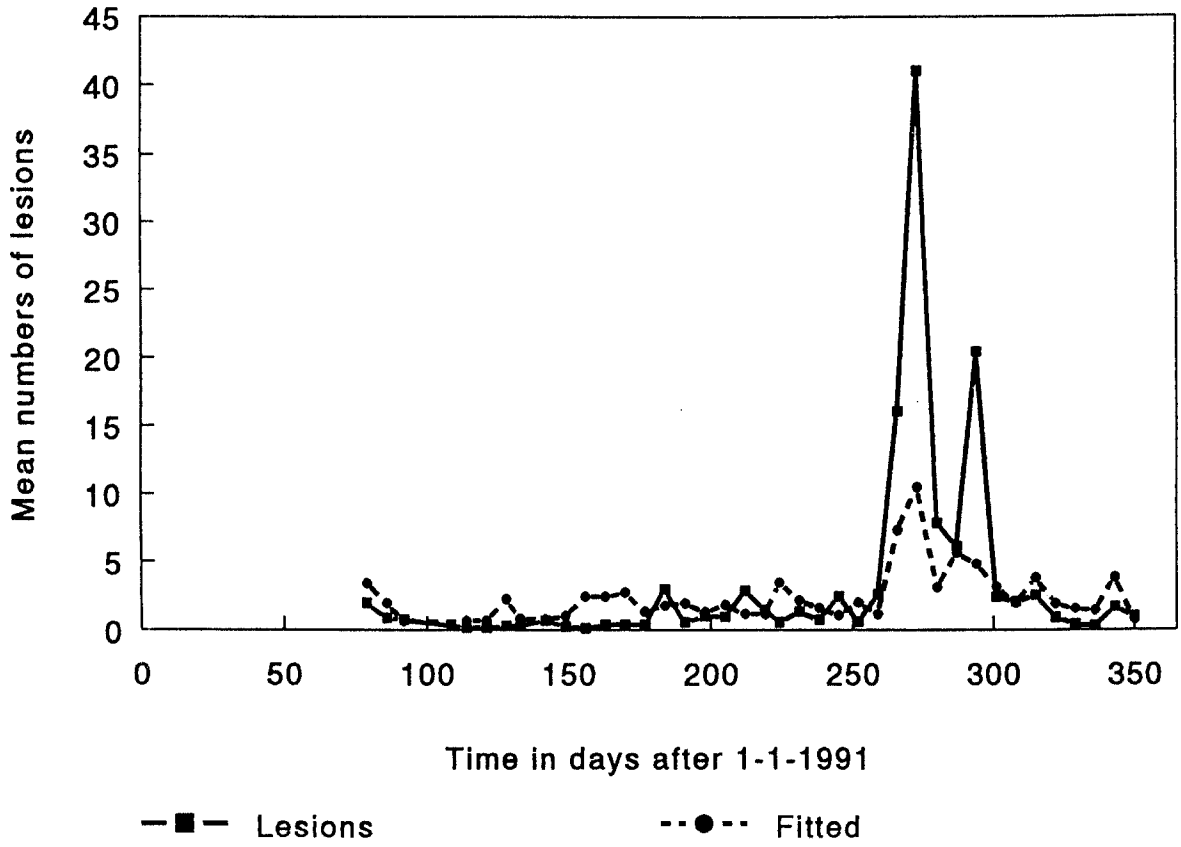

Fig. 9. Observed (1991) and fitted (equation (2) of 1992 used) numbers of lesions per rose flower.

In both years the numbers were high (>4 lesions per flower), when the RH was high and the global incoming radiation was low.

After validation with data from other glasshouses and from other years 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 the air of a glasshouse 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 depend 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.

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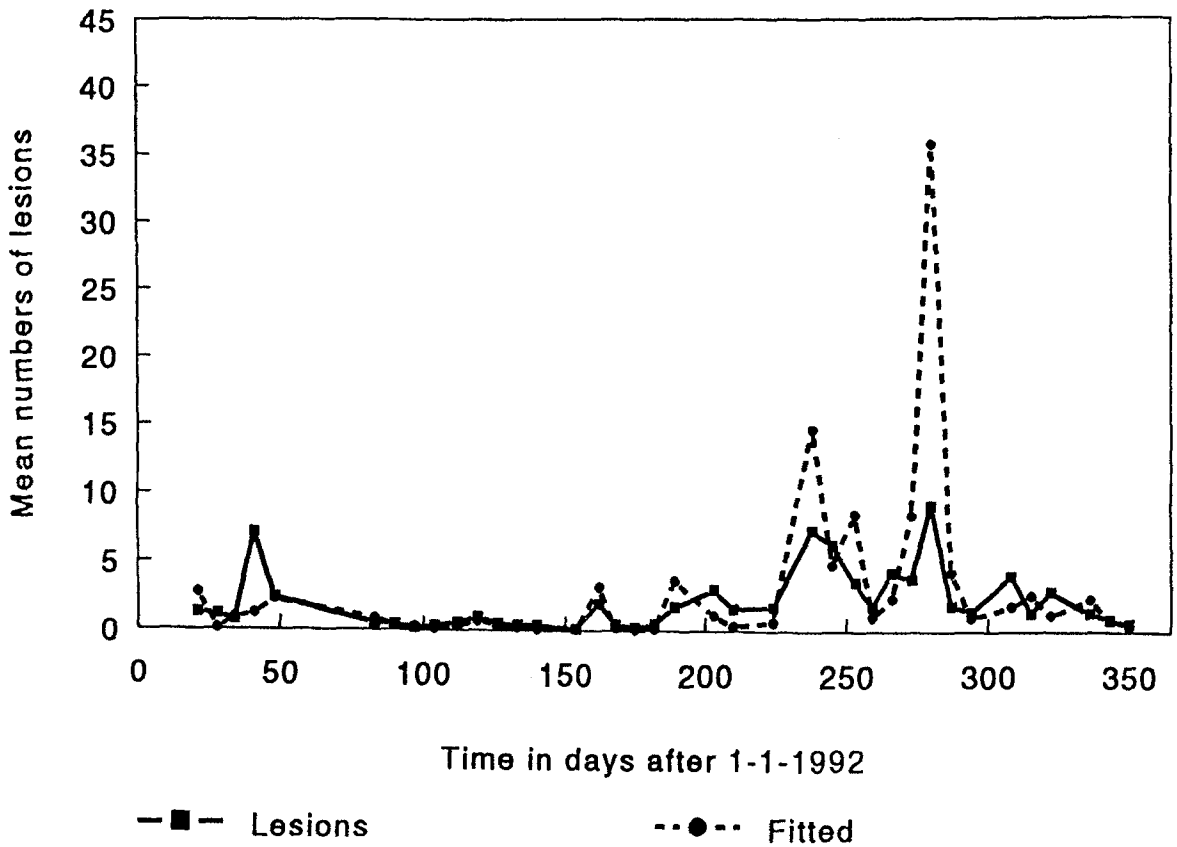

Fig. 10. Observed (1992) and fitted (equation (1) of 1991 used) numbers of lesions per rose flower.

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