European Journal of Plant Pathology **107:** 615–624, 2001.
© 2001 *Kluwer Academic Publishers. Printed in the Netherlands.*

Epidemiology of grey mould in annual waiting-bed production of strawberry

Pedro Boff[1,3,4], Pieter Kastelein[1], Joop de Kraker[2], Matthijs Gerlagh[1] and Jürgen Köhl[1]
[1]*Plant Research International B.V., P.O. Box 16, 6700 AA Wageningen, The Netherlands (Fax: +31317410113; E-mail: pboff@epagri.rct-sc.br, j.kohl@plant.wag-ur.nl);* [2]*Department of Plant Sciences, Wageningen University, P.O. Box 430, 6700 AK Wageningen, The Netherlands;* [3]*Biological Farming Systems – Wageningen University, Marijkeweg 22, 6709 PG Wageningen, The Netherlands;* [4]*Present address: Agricultural Research and Rural Extension Agency of Santa Catarina State (EPAGRI), P.O. Box 121, 88400-000, Ituporanga, Santa Catarina, Brasil*

Accepted 2 May 2001

Key words: Botrytis cinerea, flower colonisation, primary inoculum

Abstract

The epidemiology of *Botrytis cinerea* was studied in five annual strawberry crops using waiting-bed transplants, a system widely adopted in the Netherlands. On dead leaves of transplants the incidence of *B. cinerea* varied from 26.7% to 52.6%, but the leaf area with potential sporulation was low (3.5–15.6%). During each crop cycle, the availability of necrotic leaf substrate for spore production of *B. cinerea* was generally low and varied between seasons and with the quality of transplants. *B. cinerea* sporulated on a maximum of 15.5 cm^2 of leaf area per plant, measured as potential sporulation. The aerial concentration of *B. cinerea* conidia in untreated plots did not differ from the concentration in plots where all dead leaves had been removed, nor from the concentration at 25–50 m distance from the strawberry plots. *B. cinerea* incidence on flowers ranged from 5% to 96%, but no correlation was found with the potential spore production on necrotic leaves. Grey mould at harvest varied from 1.4% to 11.3% and was correlated with the average precipitation during the harvesting period but not with *B. cinerea* incidence on flowers. Post-harvest grey mould ranged from 2.1% to 32.6% and was correlated with petal colonisation by *B. cinerea*. The results suggest that in the annual cropping system with waiting-bed transplants, necrotic leaves are not a significant source of *B. cinerea* inoculum, unlike in other strawberry production systems. Therefore, control measures of grey mould in this annual system should focus on protection of flowers and young developing fruits, and not on the reduction of inoculum production on leaf debris.

Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) can be cultivated in the open field as a perennial or annual crop. Annual crops are grown in spring, summer or autumn (Galletta and Bringhurst, 1990). A particular annual cropping system with waiting-bed transplants is used in Northwest Europe, especially in the Netherlands. In this system, transplants are produced during autumn by planting fresh runners in August into waiting-beds. The transplants are harvested in December and subsequently stored at −2 °C (Rosati, 1991). Such cold-stored dormant transplants can be planted in the production field from April till July. The risk of building up soilborne diseases in the production field is also reduced because of the shorter cropping period. Moreover, the choice of transplanting date allows the grower a flexible planning of crops according to labour load, risks of losses and expected prices.

Fruit rot caused by *B. cinerea* Pers. (Teleomorph: *Botryotinia fuckeliana* (de Bary) Whetzel) (Hennebert, 1973), known as grey mould, is an important world-wide disease in strawberry production systems. According to Powelson (1960), Jarvis (1962a) and

Jarvis and Borecka (1968) grey mould on fruits is mostly due to infection from colonised senescent or dead flower parts by *B. cinerea*. After the development of fruit rot, mycelium of the fungus may invade adjacent strawberry fruit during ripening. Braun and Sutton (1987) demonstrated that the primary inoculum of *B. cinerea* leading to flower infection and fruit rot in perennial strawberry crops is produced mainly on necrotic strawberry tissue inside the crop, present during the flowering period. Several measures have been proposed to control grey mould in perennial and overwintering strawberry, based on suppression of inoculum production on strawberry plant debris, such as sanitation (Sutton et al., 1988), chemical control (Powell, 1952; Jordan and Pappas, 1977) and biological control (Sutton and Peng, 1993).

Quantitative epidemiology of *B. cinerea* in strawberry has been studied in the field and under controlled conditions. In perennial crops, significant correlations were found between flower infection or fruit rot at harvest and weather variables during or after the flowering period (Jarvis, 1964; Wilcox and Seem, 1994; Xu et al., 2000). Under controlled conditions, Bulger et al. (1987) found an increase of flower infection by *B. cinerea* with increasing wetness duration, and could predict the incidence of grey mould as a function of temperature and wetness duration during the flowering period. Sosa-Alvarez et al. (1995) described the effect of temperature and leaf wetness on *B. cinerea* sporulation on dead strawberry leaves.

The epidemiology of grey mould has not yet been studied in the annual cropping system using cold-stored waiting-bed transplants. It may differ from overwintering crops because the oldest leaves of the transplants are mostly removed before planting, so that crops start with few necrotic leaves in spring. The dynamics of necrotic tissue after transplanting and its role as substrate for sporulation of *B. cinerea* may also differ from other cropping systems, because the transplants in the waiting-bed system are artificially maintained in dormancy for 3–6 months at −2 °C.

The objective of this study was to investigate the relationships between the inoculum production of *B. cinerea* on necrotic leaves, colonisation of flowers by *B. cinerea*, and the development of grey mould in the annual waiting-bed production system of strawberry under field conditions. Quantitative knowledge of the relative importance of the different inoculum sources will be helpful to identify the most appropriate targets for control strategies in such a cropping system.

Materials and methods

Field plots

The data for this study were mainly collected in the untreated plots of five field experiments on the control of grey mould of strawberry cv. Elsanta (Boff, 2001). The experiments were located on a sandy soil near Wageningen, the Netherlands. Transplanting dates were (1) 10 April 1997; (2) 27 June 1997; (3) 06 May 1998; (4) 19 June 1998 and (5) 16 April 1999. There were five and four untreated replicate plots in 1997 and 1998, respectively, which were randomised with the other treatment plots within blocks. In 1999, the experiment consisted of a single untreated plot. All plots consisted of 78 plants in an area of 4.5 × 4.33 m, surrounded by at least 10 m grass buffer to reduce inter-plot interference. Cold-stored dormant transplants, kept at −2 °C until the day before planting, were transplanted in double rows with three plants per metre row length. Spacing of rows between double rows was 1 m and within double rows 0.5 m. Sprinkler irrigation (1997) and a mist irrigation system (1998, 1999) were used to ensure water supply for plant development in dry periods from transplanting till beginning of flowering. From beginning of flowering until the first harvest, strawberry plots were irrigated every second night (5 min per 90 min, in total 40 min per night) to stimulate epidemic development of *B. cinerea.*

Primary inoculum on transplants

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

Strawberry phenology and necrotic leaf area

The phenology of the strawberry crops was determined by periodical non-destructive measurements

from transplanting until harvest, on four (1997) or five (1998) plants per plot, arbitrarily selected and labelled. In the single untreated plot in 1999, 10 plants were monitored. The numbers of flowers and fruits per plant were counted on five to eight occasions of which the dates are shown in Figure 1. The area of necrotic leaf tissue was estimated by measuring the length and width of each necrotic leaf part with a millimetre scale ruler and calculating the area as a rectangle. Averages of necrotic leaf area and number of flowers per plant per replicate plot were considered in further data analysis (Figure 1).

Assessment of B. cinerea

Conidial load in the air
The load of *B. cinerea* conidia in the air was monitored using Rotorod samplers Mod. 20 (Sampling Technologies, Minneapolis, USA) with a non-retracting collector. Rotorods were positioned at 0.3 m height in the centre of an untreated plot and 25, 50 and 50 m outside the strawberry plot in the grass buffer for the years 1997, 1998 and 1999, respectively. Spore samples were collected on two, five, three and six different dates in the experiments 2, 3, 4 and 5, respectively. Two to four runs per sampling date with 15 min duration per run were carried out between 8:00 and 13:30 h, the most likely period for spore release of *B. cinerea* (Jarvis, 1980). In experiments 3 and 4, the spore load was also assessed in a plot in which all senescent and necrotic leaflets had been removed twice per week from transplanting till first harvest (sanitation plot). The spores of one rod per run were stained with cotton blue (2 ml lactic acid + 4 ml glycerol + 1.5 ml cotton blue at 1% + 2 ml demineralised water) and conidia of *B. cinerea* were counted on the 22 mm upper part of a rod using a microscope at 200 × magnification, and the number of conidia of *B. cinerea* per cubic metre of air was calculated (Sampling Technologies, 1989).

Colonisation of necrotic leaves
Samples of 10–40 half or whole necrotic leaflets were harvested per plot and incubated under moist conditions to assess the potential sporulation of *B. cinerea* as an estimate of the colonisation of the substrate by the pathogen. In each sample, not more than one leaflet per plant and not more than 5% of the total necrotic leaf area was removed per plot. This limit was considered to be the maximum that could be removed without significantly disturbing the availability of substrate for *B. cinerea* in the field. Six samples of necrotic leaflets were collected per plot in experiment 1 (13, 22 and 30 May, 6 and 20 June and 9 July, 1997), four samples in experiment 2 (22 and 29 July, 5 and 14 August, 1997), three in experiment 3 (7 May, 8 and 16 June, 1998) and three in experiment 4 (20 June, 21 July and 4 August, 1998). No assessment of sporulation on necrotic leaves was done in experiment 5. The leaflets harvested from each plot were put into a plastic tray (50 cm length × 30 cm width × 7 cm height) with wet filter paper at the bottom and closed with a plastic bag and incubated for 14 days at 18 °C in the dark. The percentage area with sporulation of *B. cinerea* was estimated for each leaflet, using a stereomicroscope at 10 × magnification. The area colonised by *B. cinerea* per plant was estimated from the total necrotic area per plant and the proportion of area sporulating after incubation. In the correlation analyses interpolated values of the leaf area were used when the assessment dates were not exactly the same as the dates on which other variables were assessed.

Flower colonisation
Samples of 20–30 flowers per plot were arbitrarily collected, each from different plants, on two (exp. 1, 2 and 5) or three (exp. 3 and 4) sampling dates (Table 2). Only flowers already opened for three to four days with brown anthers and with petals still attached were included in the sample. The flowers were put into plastic boxes (22 cm length × 14 cm width × 5 cm height) with wet filter paper at the bottom and incubated at 18 °C for 14 days in the dark. All flower parts, except peduncles, were examined for the presence of sporulation of *B. cinerea* with a stereomicroscope, at 10–100 × magnification. Flowers were considered colonised by *B. cinerea* when at least one conidiophore with conidia was present on any of the flower parts. The incidence of *B. cinerea* sporulation on flowers was calculated per sampling date per plot.

In addition to the incidence of *B. cinerea* on flowers, the petal area colonised by *B. cinerea* was estimated from separate samples in the experiments 3, 4 and 5. Twenty to 25 senescent petals were arbitrarily collected per sampling date per plot, each petal from a different plant. The number of sampling dates was three (11 and 20 June and 2 July, 1998), four (21 July, 2, 5 and 11 August, 1998) and five (24, 27, 28 and 31 May and 4 June, 1999) for the experiments 3, 4 and 5, respectively. No sampling of petals was done in the experiments 1 and 2. The petals were placed on

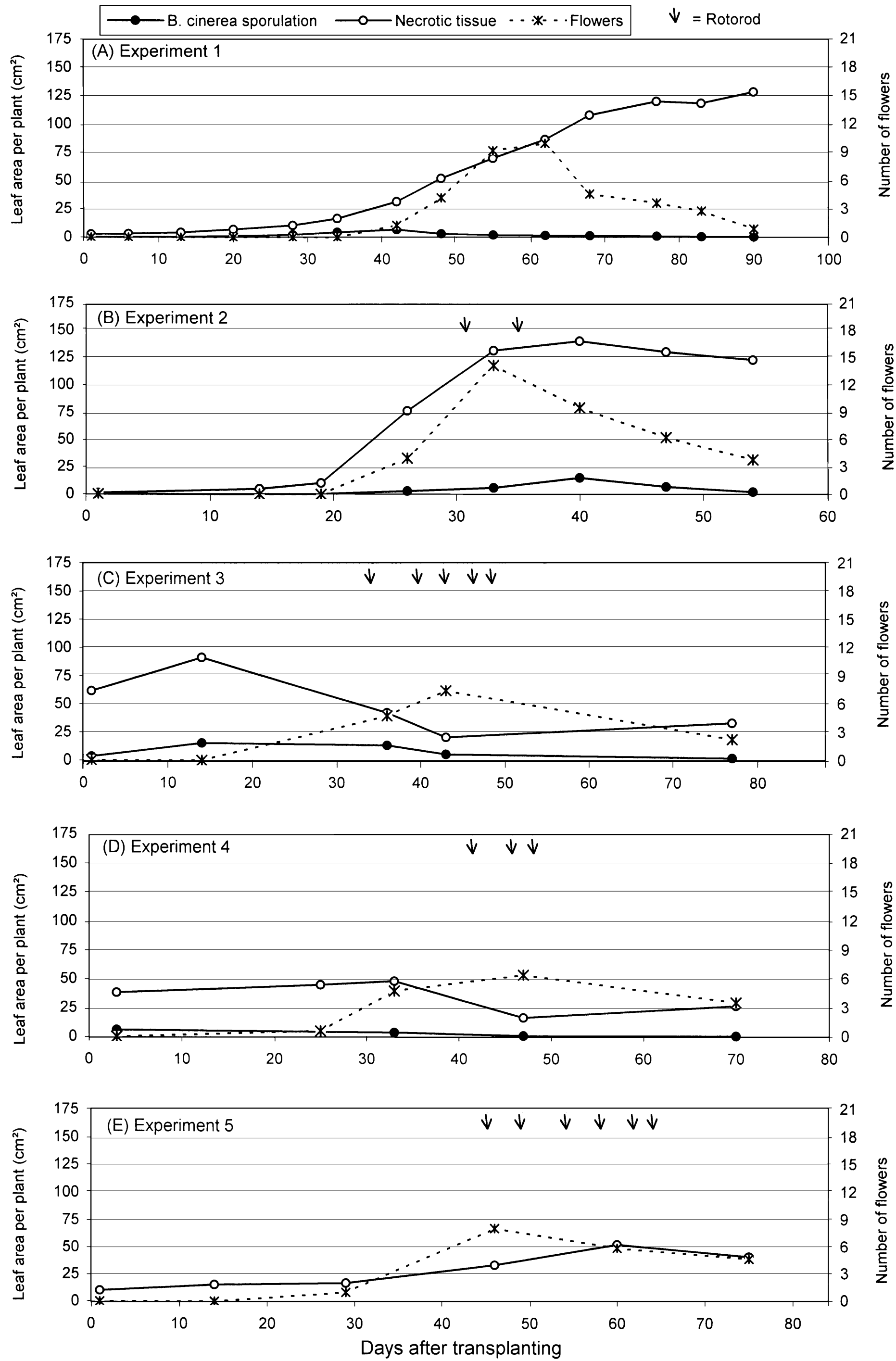

Figure 1. Total necrotic leaf area and area of necrotic leaf tissue with potential sporulation of *Botrytis cinerea* (Y1), and number of flowers (Y2) per plant, in five annual waiting-bed crops of strawberry. No assessment of potential sporulation of *B. cinerea* was done in experiment 5. Crops were transplanted on 10 April 1997, exp. 1 (A), 27 June 1997, exp. 2 (B), 6 May 1998, exp. 3 (C), 19 June 1998, exp. 4 (D), and 16 April 1999, exp. 5 (E). Days at which spores were sampled with Rotorods are indicated.

water agar (1.5% of agar) in sterile plastic petri dishes (14 cm diameter) and incubated for 14 days at 18 °C in the dark. The percentage area with *B. cinerea* sporulation was estimated for each petal using a stereomicroscope, at 20 × magnification.

Fruit rot

Grey mould was determined at harvest by counting the number of diseased fruits with *B. cinerea* symptoms (field fruit rot) and the total number of fruit harvested per plot at each harvesting date. All ripe healthy fruit and all fruit with *B. cinerea* symptoms were picked twice per week. Healthy fruits were always picked separately from diseased fruits to avoid contamination during harvesting. For post-harvest evaluations diseased fruits with non-specific symptoms were placed in moist chambers for 72 h at 18 °C in the dark, to allow further development of symptoms. Post-harvest fruit rot was assessed using a sub-sample of ripe symptomless fruit, consisting of 20 arbitrarily selected fruits per plot. Fruits of each replicate were put into a plastic tray (50 cm length × 30 cm width × 7 cm height) with wet filter paper on the bottom. Each tray was closed within a plastic bag and incubated at 4 °C during five days in the dark and subsequently for three days at 18 °C. After incubation, rotten fruits with *B. cinerea* sporulation on any part of the fruit were counted as post-harvest grey mould.

Microclimatic conditions

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

Data analysis

Statistical analysis was performed with the Genstat 5 version 4.1 package (Numerical Algorithm Group, Inc., Oxford, UK). Correlation analysis among epidemiological and weather variables was conducted by computing Spearman's coefficient of correlation (r_s) (Snedecor and Cochran, 1989). This correlation coefficient was also computed between the observed incidence of *B. cinerea* on flowers and the predicted spore production of *B. cinerea* per strawberry plant in the field over a five-day period before sampling of flowers. Spore production per leaf area was predicted with a descriptive model as a function of observed wetness duration and average temperature during wetness over the five-day period. The model equation used was $\log(Y) = -7.03 + 2.22[3.02 + (0.0026 log(W)T^3) + (-0.00011\ \log(W)T^4)]$, where Y is the number of *B. cinerea* conidia per cm^2 of leaf tissue, W is the duration of leaf wetness in days, and T is the average temperature during the wetness period (equation 7 of Sosa-Alvarez et al., 1995). The spore production of *B. cinerea* per strawberry plant was calculated as the product of predicted spore production per leaf area and the observed necrotic leaf area with potential sporulation per plant. Correlation analysis was also done between observed levels of grey mould in subsequent harvests during a season and predicted values calculated with a descriptive model with observed temperature and wetness duration during the flowering period as inputs. The model equation used was $\ln(Y/(1-Y)) = -3.526 + 0.0228WT - 0.1940W - 1.7 \times 10^{-5}WT^3$, where Y is the proportion of strawberry fruit infected by *B. cinerea*, W is the duration of leaf wetness in hours, and T is the average temperature during the wetness periods (equation 5 of Bulger et al., 1987).

For each experiment separately, mean spore loads of *B. cinerea* in the air monitored at different locations were compared with the paired t-test to detect significant differences. In addition, a confidence interval was calculated for the proportion of days with a higher average spore load of *B. cinerea* inside the untreated plots as compared to outside the strawberry plots.

Results

Conidial load in the air during flowering

The average number of conidia per m^3 of air was always below 25 (Table 1). In only one of the 39 runs the concentration of conidia was higher than 100 per m^3 of air. A higher spore load of *B. cinerea* inside the untreated strawberry plots as compared to outside the plots was found on 10 of 16 days, a proportion of 0.63 (Table 1). This proportion is not significantly different from the expected value of 0.50, given its large 95% confidence interval (0.35, 0.85). Differences between spore loads

Table 1. Estimated air load of conidia of *B. cinerea* as determined by Rotorod sampling during flowering in plots with annual strawberries at Wageningen, the Netherlands

Experiment[1]	Number of spores per m³ of air per run[2]			Proportion of days with spore catch	
	Control	Outside[3]	Sanitation	Control > Outside	Control > Sanitation
Exp. 2, 1997	19.5	10.4	—	0.50	—
Exp. 3, 1998	8.8	7.0	8.3	0.60	0.40
Exp. 4, 1998	23.8	4.7	7.4	0.67	0.33
Exp. 5, 1999	6.3	10.2	—	0.67	—
Pooled data				0.63[4]	

The proportion of days on which more conidia were caught at one location as compared to another is also presented. Control = inside untreated strawberry plots; Outside = outside strawberry plots; Sanitation = inside strawberry plots where all dead leaves were removed.
[1]Rotorod sampling was not conducted in experiment 1.
[2]Means of 5, 12, 10 and 12 runs, in exps. 2, 3, 4 and 5, respectively; paired *t*-tests on log-transformed numbers of spores per run did not show significant differences ($P > 0.05$) in mean air load of conidia between the different sampling locations for any experiment.
[3]Rotorods were located at 25 m (1997) or 50 m (1998, 1999) from strawberry plots.
[4]95%-confidence interval is 0.35–0.85.

inside and outside the strawberry plots were generally less than 10 conidia per m^3 of air. No significant differences between spore loads inside untreated plots, inside sanitation plots and outside the strawberry plots were found in any of the experiments. Daily average and daily peak values of the spore load in the air inside untreated plots as estimated from Rotorod samples, were notcorrelated with the potential spore productions of *B. cinerea* on necrotic leaves, calculated with equation 7 of Sosa-Alvarez et al. (1995) as explained in the materials and methods section.

Substrate availability and inoculum production of B. cinerea

The incidence of *B. cinerea* on cold-stored transplants was 26.7% (exp. 3) and 52.6% (exp. 4) on dead leaves, and 29% (exp. 3) and 32.2% (exp. 4) on stolons. The incidence of *B. cinerea* on senescent leaves was 30.3% (exp. 3) and 75% (exp. 4). The area with potential sporulation of *B. cinerea* on dead leaves was 3.5% and 15.6% for experiments 3 and 4, respectively. The dynamics of necrotic strawberry leaf tissue showed similarity among experiments 1, 2 and 5 and among experiments 3 and 4 (Figure 1). In experiments 1, 2 (1997) and 5 (1999) the amount of necrotic leaf tissue increased steadily after transplanting but was below 25 cm^2 per plant until the beginning of flowering, then increased further during flowering. This trend was not observed in experiments 3 and 4 (1998) in which more necrotic leaf tissue was found during the first three–four weeks after transplanting, whereas the presence of necrotic tissue was lower at flowering. The necrotic leaf area with potential sporulation of *B. cinerea* followed the same dynamics as the total area of necrotic leaf tissue in experiments 2 and 3 but not in experiments 1 and 4. The largest area with potential sporulation of *B. cinerea* per plant (15.5 cm^2) was found in experiment 3, 14 days after transplanting. In relation to strawberry phenology, the area with potential sporulation of *B. cinerea* reached its maximum when the first flower appeared in experiments 1 and 3, at the middle of flowering in experiment 2, and at transplanting time in experiment 4. Among the dates of flower sampling, the total necrotic leaf area per plant varied from 16.5 to 137.9 cm^2, and the leaf area per plant with potential sporulation of *B. cinerea* ranged from 0.7 to 15.4 cm^2 (interpolated values, Table 2).

Flower colonisation

The incidence of *B. cinerea* on flowers varied in the five experiments from 5.1% (exp. 1, sample 1, 1997) to 96% (exp. 2, sample 2, 1998) (Table 2). The incidence of *B. cinerea* on flowers increased during the season in all experiments except in experiment 4. No significant correlation was found between the incidence of

Table 2. Incidence of flower colonisation by *B. cinerea*, cumulative precipitation over the five days preceding sampling of flowers, total area of necrotic leaf tissue, area of necrotic leaf tissue with potential sporulation at the date of flower sampling and predicted number of conidia[1] on necrotic tissue per plant calculated over a period of five days before sampling of flowers of untreated strawberry plots, at Wageningen, the Netherlands

Experiment/ sampling date	Incidence of *Botrytis* on flowers (%)	Cumulative precipitation, −5–0 days (mm)	Necrotic leaf tissue per plant (cm^2)		Predicted number of conidia of *B. cinerea* per plant
			Total	With potential sporulation of *B. cinerea*	
Exp. 1/sample 1 11 June 1997	5.1	89.5	86.3	1.9	25792
Exp. 1/sample 2 17 June 1997	14.9	92.0	108.0	1.7	629
Exp. 2/sample 1 29 July 1997	76.2	11.0	123.6	4.1	162
Exp. 2/sample 2 4 August 1997	96.0	8.0	137.9	14.5	11
Exp. 3/sample 1 8 June 1998	63.7	127.5	49.2	16.5	4192
Exp. 3/sample 2 15 June 1998	70.0	40.5	30.1	8.7	231
Exp. 3/sample 3 2 July 1998	81.3	92.0	26.1	4.9	23656
Exp. 4/sample 1 21 July 1998	83.7	4.5	48.0	3.7	2
Exp. 4/sample 2 5 August 1998	75.0	46.5	16.5	0.8	490
Exp. 4/sample 3 11 August 1998	60.1	109.0	19.2	0.7	88131
Exp. 5/sample 1 6 June 1999	60.2	35.5	88.0	—	—
Exp. 5/sample 2 9 June 1999	65.2	24.5	97.1	—	—

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

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

Grey mould

Fruit rot at harvest ranged from 1.4% (exp. 2) up to 11.3% (exp. 4), and post-harvest grey mould ranged from 2.1% to 32.6% (Table 3, average values per experiment). Post-harvest fruit rot was typically greater than fruit rot at harvest and no correlation was found between these two variables ($r_s = 0.0$, $P > 0.05$). Fruit rot at harvest was significantly correlated with daily average precipitation during the harvest period ($r_s = 0.9$, $P < 0.05$), but post-harvest fruit rot was not ($r_s = 0.1$, $P > 0.05$). The incidence of *B. cinerea* on flowers was better correlated with post-harvest grey mould ($r_s = 0.6$, $P > 0.05$) than with grey mould at harvest ($r_s = -0.1$, $P > 0.05$), but neither of the

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

Experiment	HarvRot (%)[1]	PostRot (%)[1]	LPS (%)[2]	FBI (%)[3]	Petal (%)[4]	Average daily precipitation			
						Flowering		Harvest	
						Period	(mm)	Period	(mm)
Exp. 1, 1997	9.5	13.1	9.4	9.9	—	14 May–23 June	9.6	16 June–17 July	5.5
Exp. 2, 1997	1.4	26.9	5.6	86.1	—	16 July–5 August	2.0	11 August–25 August	3.0
Exp. 3, 1998	10.1	10.4	22.4	71.7	20.3	31 May–9 July	14.6	2 July–3 August	6.2
Exp. 4, 1998	11.3	32.6	9.5	72.9	29.4	8 July–13 August	9.9	13 August–7 September	11.3
Exp. 5, 1999	11.2	2.1	—	62.7	5.8	16 May–17 June	12.5	18 June–12 July	5.6

[1]Average of 10 (exp. 1), 5 (exp. 2), 10 (exp. 3), 8 (exp. 4) and 5 (exp. 5) samples.
[2]Average of 6 (exp. 1), 4 (exp. 2), 3 (exp. 3) and 3 (exp. 4) samples.
[3]Average of 2 (exp. 1), 2 (exp. 2), 3 (exp. 3), 3 (exp. 4) and 2 (exp. 5) samples.
[4]Average of 3 (exp. 3), 4 (exp. 4) and 5 (exp. 5) samples.

correlations were significant. The area with sporulation of *B. cinerea* on petals, assessed in three experiments, was significantly correlated with post-harvest fruit rot ($r_s = 1.0$, $P < 0.05$), but not with fruit rot at harvest (Table 3). A significant correlation between the percentage of fruit rot observed in subsequent harvests in the field and the level of fruit rot predicted with the model of Bulger et al. (1987) was found in one experiment (exp. 3, $r_s = 0.71$, $P < 0.05$), but never for post-harvest fruit rot.

Discussion

Inoculum production on necrotic leaves inside the crop

Necrotic leaf tissue inside the crop is the major source of conidial inoculum for flower infection by *B. cinerea* in perennial strawberry crops (Jordan, 1978; Braun and Sutton, 1987). In our study, the area of necrotic strawberry leaves increased from beginning of flowering till after the peak of flowering in experiments 1, 2 and 5 or from transplanting until beginning of flowering in experiments 3 and 4. These differences were probably due to differences in the quality of transplants and growing conditions after transplanting. In experiments 3 and 4, planting of weak transplants resulted in a relatively large amount of necrotic leaf area soon after transplanting, but these leaves quickly decomposed and almost disappeared by flowering time. Leaves that senesced and died in the five annual strawberry crops were mostly those produced during autumn of the previous year, when the plants were growing in the waiting-beds. This generation of leaves is similar to the one reported by Braun and Sutton (1988) as the major source of conidia of *B. cinerea* during flowering in perennial strawberry crops. However, the amount of necrotic leaf residue produced in the annual strawberry crops in our experiments was much less than in perennial strawberry production systems. The maximum we found was a necrotic leaf area of c. 150 cm^2 per plant, which is equivalent to a dry weight of c. 2 g per plant. In perennial crops, dry weights were found to vary between 10 and 25 g (Sutton et al., 1988).

The trend in the area with potential sporulation of *B. cinerea* differed from the trend in the total necrotic leaf area, except in experiment 2. Sutton et al. (1988) also did not find a relationship between the amount of dead leaf tissue in the crop and the potential sporulation of *B. cinerea* on the tissue. Sporulation on necrotic leaves in the field was rarely observed during our study (Boff, 2001), despite the conducive environmental conditions created by regular irrigation (Sosa-Alvarez et al., 1995). Other factors besides temperature and wetness duration may affect sporulation of *B. cinerea*, such as the quality of leaf substrate. Sporulation of *B. cinerea* was observed more frequently on necrotic tissue of young leaves than on fully expanded necrotic leaves in the field (data not presented).

Despite the considerable incidence of *B. cinerea* on senescent and necrotic leaves of transplants (25–75%), the relatively low amount of necrotic leaf substrate for spore production and the low levels of actual and potential spore production observed, suggest that necrotic leaves were not a major inoculum source of *B. cinerea*

in the annual strawberry crops we studied. This conclusion is supported by the absence of significant differences in air load of conidia inside and outside the crops, and between crops with and without necrotic leaves (Table 1). In this respect the epidemiology of *B. cinerea* in the annual waiting-bed strawberry production system differs from the epidemiology as described for perennial and overwintering strawberry crops.

Colonisation of flowers by B. cinerea

The incidence of *B. cinerea* on sampled flowers was high in almost all experiments. In 10 of 12 samples the incidence of *B. cinerea* was greater than 60%. According to Braun and Sutton (1987, 1988) the inoculum for flower colonisation by *B. cinerea* in spring originates mainly from necrotic leaves inside the crop. We did not find significant correlations between the incidence of *B. cinerea* on flowers and total necrotic leaf area present during flowering, the leaf area with potential sporulation of *B. cinerea*, or predicted amount of sporulation based on colonised leaf area and weather conditions (Sosa-Alvarez et al., 1995). An explanation for the absence of positive correlations is that the amount of conidia produced locally on necrotic leaves in the untreated plots was too low to enhance markedly the inoculum pressure as compared to its background level. The finding that removal of all necrotic leaf tissue from strawberry plots did not affect the incidence of *B. cinerea* on flowers (Boff, 2001), confirms this explanation. Conidia of *B. cinerea* are a common component of the air spores, and the background air load in our experimental area was not higher than reported elsewhere (Jarvis, 1962b). Given the wide host range of this fungus, the inoculum probably came from a variety of sources outside the strawberry plots, and was sufficient to result in an average incidence of *B. cinerea* on 60% of the flowers.

Grey Mould

None of our experiments demonstrated a correlation between grey mould at harvest and the potential production of conidia on necrotic leaves inside the crop or incidence of *B. cinerea* on flowers. Post-harvest fruit rot was significantly correlated with the level of petal colonisation by *B. cinerea*. This is probably due to latent infections of young fruit originating from flower parts (Powelson, 1960; Jarvis, 1962a; Bulger et al., 1987). Fruit rot at harvest was significantly correlated with the daily average precipitation during the harvest period. Jarvis (1964) also found a high correlation between fruit rot and pre-harvest rainfall, and suggested that the transition of latent mycelium in the fruit to an aggressive phase is hastened by increased water content which can be facilitated by rain. Similarly, Wilcox and Seem (1994) found that the rainfall during the green fruit stage plus pre-harvest period was one of the most important variables for predicting grey mould incidence at harvest.

When we applied Bulger's models (Bulger et al., 1987) to predict fruit rot at harvest and post-harvest as a function of leaf wetness and temperature during flowering, we obtained a significant correlation between predicted and observed levels in only one case of fruit rot at harvest, in experiment 3. These results can be partly attributed to the absence of conducive conditions for expression of harvest fruit rot. An additional explanation for the lack of correlation of Bulger's models with observed levels of post-harvest fruit rot, is that the predictions by Bulger's models assume a constant and sufficient presence of inoculum, whereas in our experiments the inoculum load fluctuated daily.

In conclusion, our observations suggest that plant debris inside annual strawberry crops using waiting-bed transplants is a not a major source of inoculum for flower infection. This is confirmed by observations that removal of all necrotic tissue did not affect the incidence of *B. cinerea* on flowers, the incidence of grey mould at harvest, or the incidence of post-harvest grey mould as compared to a control treatment (Boff, 2001). The level of grey mould on fruit in the field depends more on conducive conditions for disease during fruit development and ripening than on the incidence of *B. cinerea* on flowers or inoculum production on necrotic leaves. However, when conditions for symptom expression are suitable, the level of grey mould is determined by the level of flower colonisation by *B. cinerea*, as was demonstrated by the correlation between post-harvest grey mould and petal colonisation. Control strategies for grey mould, in annual strawberry cropping systems using waiting-bed transplants should therefore aim to protect the flowers from colonisation by inoculum of *B. cinerea*.

Acknowledgements

Funding for this research was partly provided by the Brazilian Government – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

(CAPES; Proc. 2959/95-0), which included a full scholarship to the first author. We also acknowledge the European Commission (BIOSPORSUPPRESS; FAIR3 CT96-1898), for partly financial support to the research. We gratefully acknowledge Petra Horsten and Carin Lombaers-van der Plas for their technical assistance, Saskia Burgers for helping in the statistical analysis, Nyckle Fokkema, Ariena van Bruggen, and two anonymous reviewers for comments on the manuscript.

References

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

Braun PG and Sutton JC (1987) Inoculum sources of *Botrytis cinerea* in fruit rot of strawberries in Ontario. Canadian Journal of Plant Pathology 9: 1–5

Braun PG and Sutton JC (1988) Infection cycles and population dynamics of *Botrytis cinerea* in strawberry leaves. Canadian Journal of Plant Pathology 10: 133–141

Bulger MA, Ellis MA and Madden LV (1987) Influence of temperature and wetness duration on infection of strawberry flowers by *Botrytis cinerea* and disease incidence on fruit originating from infected flowers. Phytopathology 77: 1225–1230

Galletta GJ and Bringhurst RS (1990) Strawberry management. In: Galletta GJ and Himelrich DG (eds) Small Fruit Crop Management (pp 83–156) Prentice-Hall Inc., New Jersey, USA

Hennebert GL (1973) *Botrytis* and *Botrytis*-like genera. Persoonia 7: 183–204

Jarvis WR (1962a) The infection of strawberry and raspberry fruits by *Botrytis cinerea* Fr. Annals of Applied Biology 50: 569–575

Jarvis WR (1962b) The dispersal of spores of *Botrytis cinerea* Fr. in a raspberry plantation. Transactions of the British Mycological Society 45: 549–559

Jarvis WR (1964) The effect of some climatic factors on the incidence of grey mould of strawberry and raspberry fruit. Horticultural Research 3: 65–71

Jarvis WR (1980) Epidemiology. In: Coley-Smith JR, Verhoeff K and Jarvis WR (eds) The Biology of *Botrytis* (pp 219–250) Acad. Press, London, UK

Jarvis WR and Borecka H (1968) The susceptibility of strawberry flowers to infection by *Botrytis cinerea* Pers. ex Fr. Horticultural Research 8: 147–154

Jordan VWL (1978) Epidemiologie und Bekämpfung des Grauschimmels (*Botrytis cinerea*) an Erdbeeren. Pflanzenschutz Nachrichten Bayer 31: 1–10

Jordan VWL and Pappas AC (1977) Inoculum suppression and control of strawberry *Botrytis*. In: Proceedings 1977 British Crop Protection Conference – Pest and Diseases, pp 341–348, Brighton, UK

Köhl J, Molhoek WML, van der Plas CH and Fokkema NJ (1995) Effect of *Ulocladium atrum* and other antagonists on sporulation of *Botrytis cinerea* on dead lily exposed to field conditions. Phytopathology 85: 393–401

Powell D (1952) The effect of early spring fungicides on *Botytis cinerea*. Plant Disease Reporter 36: 97–98

Powelson RL (1960) Initiation of strawberry fruit rot caused by *Botrytis cinerea*. Phytopathology 50: 491–494

Rosati P (1991) The strawberry in Europe. In: Dale A and Luby JJ (eds) The Strawberry into the 21st Century (pp 27–35) Timber Press, Oregon, USA

Sampling Technologies, Inc. (1989) Operating instructions for the Rotorod sampler. California, USA

Snedecor GW and Cochran WG (1989) Statistical methods. Iowa State Univ. Press, 8th edn, Iowa, USA

Sosa-Alvarez M, Madden LV and Ellis MA (1995) Effects of temperature and wetness duration on sporulation of *Botrytis cinerea* on strawberry leaf residues. Plant Disease 79: 609–615

Sutton JC, James TDW and Dale A (1988) Harvesting and bedding practices in relation to grey mould of strawberry. Annals of Applied Biology 113: 167–175

Sutton JC and Peng G (1993) Biocontrol of *Botrytis cinerea* in strawberry leaves. Phytopathology 83: 615–621

Wilcox WR and Seem RC (1994) Relationship between strawberry gray mold incidence, environmental variables, and fungicide applications during different periods of the fruiting season. Phytopathology 84: 264–270

Xu XM, Harris DC and Berrie AM (2000) Modelling infection of strawberry flowers by *Botrytis cinerea* using field data. Phytopathology 90: 1367–1374