Neth. J. Pl. Path. 99 (1993) 303–311

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

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Accepted 29 July 1993

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 7 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 7 days at 20 °C under fluorescent light (Pope, Ftd 36W/30, 8 μmol m^{-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 3 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; 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

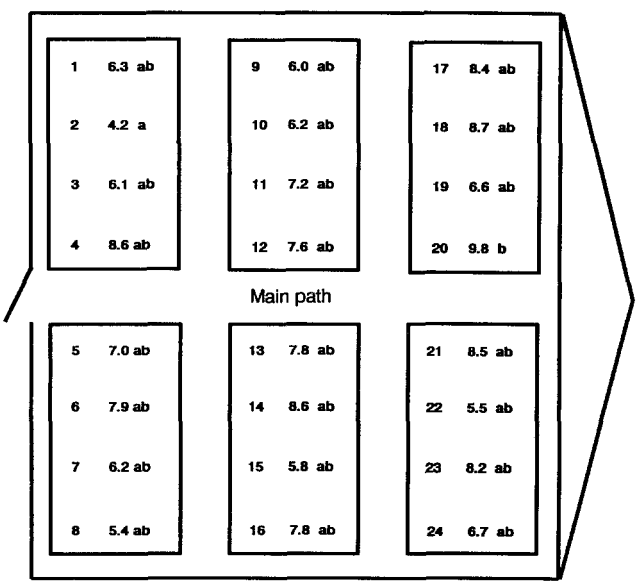

Fig 1A. Mean number of colonies per spore trap ($n = 63$) for the 24 trap locations, within the crop, trapped from 08.30 to 16.30 in glasshouse A. Location numbers refer to traps 1–24. Different letters indicate significant differences ($P \leq 0.05$).

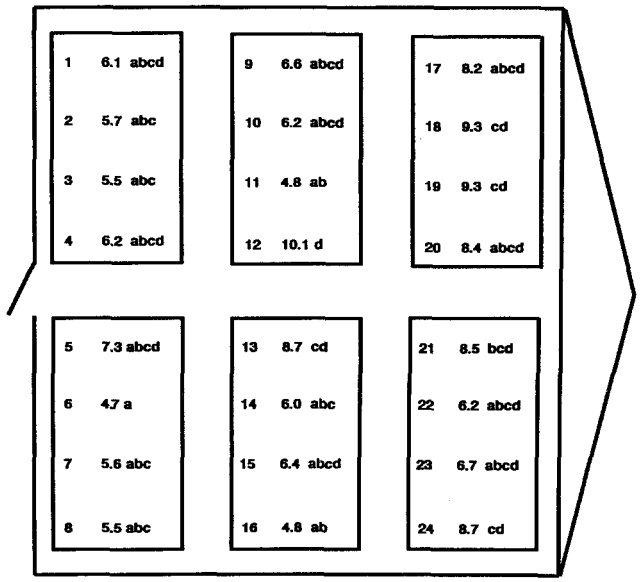

Fig 1B. Mean number of colonies per spore trap ($n = 63$) for the 24 trap locations, 0.5 m above the crop, trapped from 08.30 to 16.30 in glasshouse A. Location numbers refer to traps 1–24. Different letters indicate significant differences ($P \leq 0.05$).

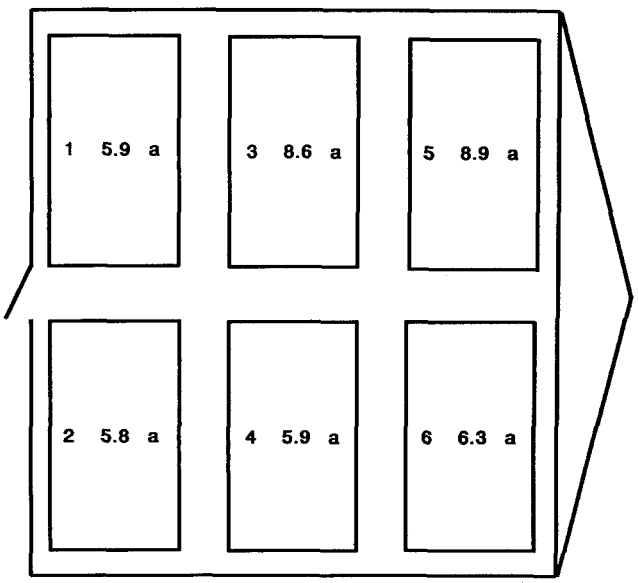

Fig 1C. Mean number of colonies per spore trap ($n = 63$) for the six trap locations, 1.5 m above the crop, trapped from 08.30 to 16.30 in glasshouse A. Location numbers refer to traps 1–6. Different letters indicate significant differences ($P \leq 0.05$).

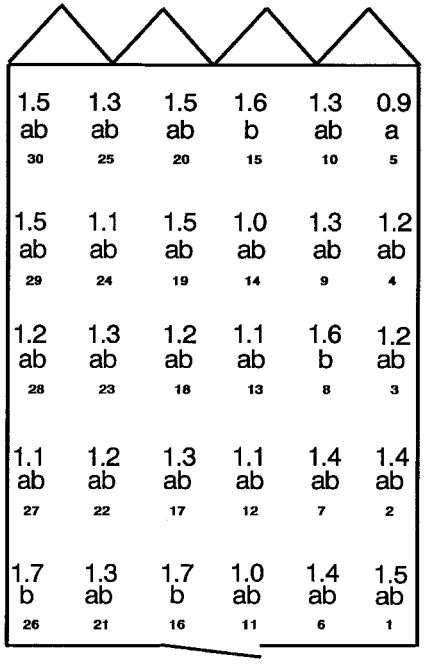

Fig 2A. Mean number of colonies per spore trap ($n = 49$) for the 30 trap locations, within the crop, trapped from 08.30 to 16.30 in glasshouse V. Location numbers refer to traps 1–30. Different letters indicate significant differences ($P \leq 0.05$).

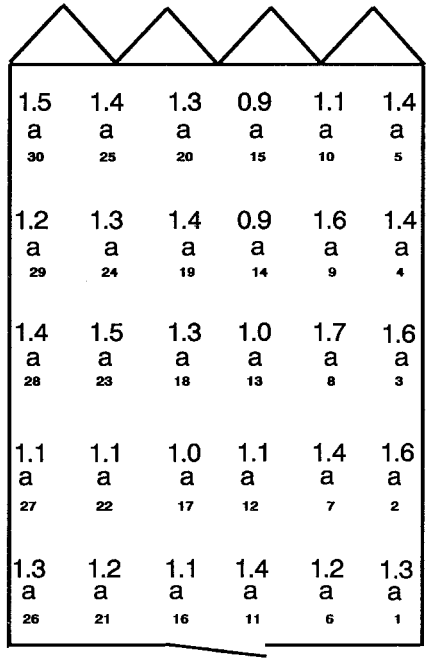

Fig 2B. Mean number of colonies per spore trap ($n = 49$) for the 30 trap locations, 0.5 m above the crop, trapped from 08.30 to 16.30 in glasshouse V. Location numbers refer to traps 1–30. Different letters indicate significant differences ($P \leq 0.05$).

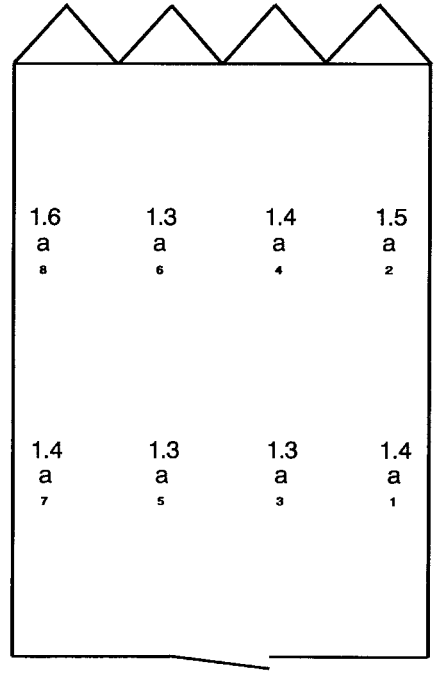

Fig 2C. Mean number of colonies per spore trap ($n = 49$) for the eight trap locations, 1.5 m above the crop, trapped from 08.30 to 16.30 in glasshouse V. Location numbers refer to traps 1–8. Different letters indicate significant differences ($P \leq 0.05$).

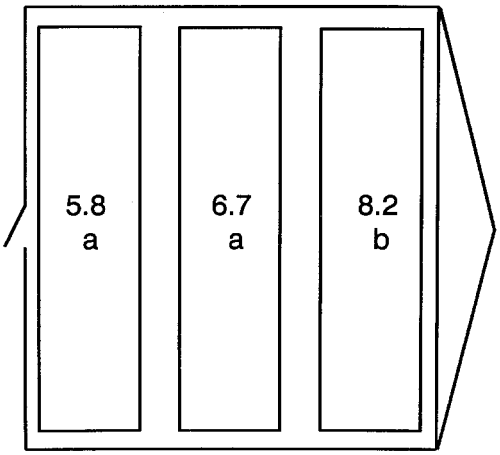

Fig 3. Mean number of colonies per eight spore traps (n = 63) for the trap locations 1–8, 9–16 and 17–24, 0.5 m above the crop, trapped from 08.30 to 16.30 in glasshouse A. Different letters indicate significant differences ($P \leq 0.05$).

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

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).

Lesions. Analysis of variance of the log transformed ($\ln(N + 1)$) numbers of lesions per harvest location per counting date showed few significant differences ($P \leq 0.05$) 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 ($P \leq 0.05$). In glasshouse V no clear pattern could be observed.

Table 2. Mean number of colonies per spore trap height in the day and night periods in glasshouse A and in the day period 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

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

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

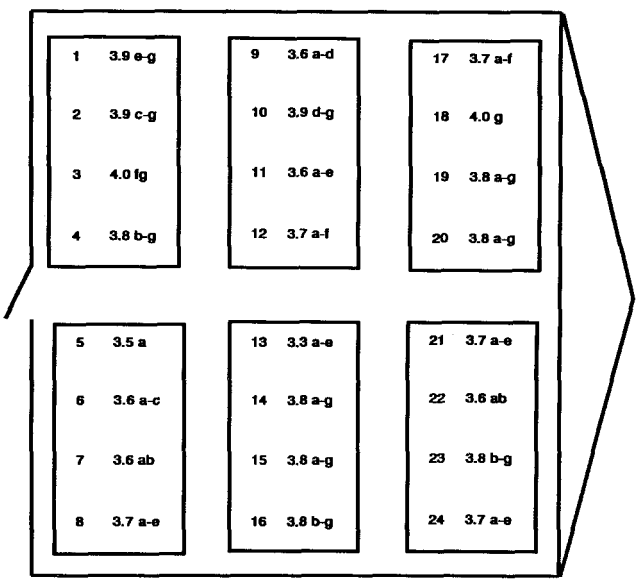

Fig 4A. Log transformed ($\ln(N + 1)$) mean number of lesions on gerbera petals at each harvest location ($n = 46$) in glasshouse A. 1–24: harvest location numbers. Different letters indicate significant differences ($P \leq 0.05$).

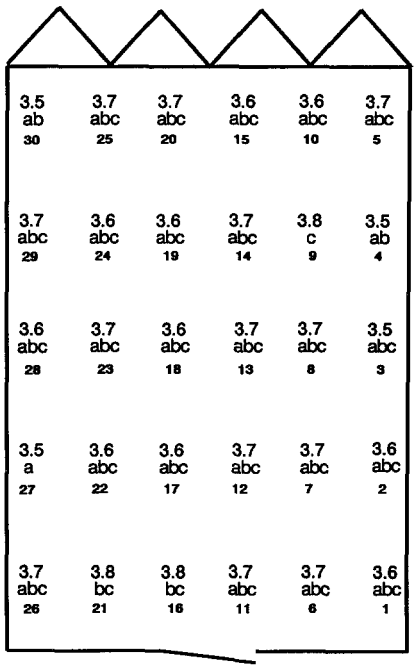

Fig 4B. Log transformed ($\ln(N+1)$) mean number of lesions on gerbera petals at each harvest location ($n = 45$) in glasshouse V. 1–30: harvest location numbers. Different letters indicate significant differences ($P \leq 0.05$).

the distribution of sources of conidia (wet, dead leaves; rarely found) and the distribution of trapped conidia in the glasshouses.

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 6 consecutive weeks. During the first 4

weeks, the numbers of colonies inside and outside decreased, respectively from 11 to 1 and from 18 to 2 colonies per trap, but after 4 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 Prof. Dr J.C. Zadoks, Ir H.D. Frinking, Dr Ir 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.

References

Frinking, H.D., 1991. Aerobiology of 'closed' agricultural systems. Grana 30: 481–485.

Frinking, H.D. & Scholte, B., 1983. Dissemination of mildew spores in a glasshouse. Philosophical Transactions of the Royal Society of London B302: 575–582.

Frinking, H.D., Gorissen, A. & Verheul, M.J., 1987. Dissemination of spores in a glasshouse: Pattern or chaos? International Journal of Biometeorology 31: 147–156.

Hausbeck, M.K. & Pennypacker, S.P., 1991. Influence of grower activity on concentrations of airborne conidia of *Botrytis cinerea* among geranium cuttings. Plant Disease 75: 1236–1243.

Hirst, J.M., 1959. Spore liberation and dispersal. In: Holton, C.S. (Ed.), Plant pathology: problems and progress 1908–1959. University of Wisconsin Press, Madison. p. 529–538.

Jarvis, W.R., 1980. Epidemiology. In: Coley-Smith, J.R., Verhoeff, K. & Jarvis, W.R. (Eds), The biology of *Botrytis*. Academic press, London. p. 219–250.

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

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

Pady, S.M. & Kelly, C.D., 1954. Aerobiological studies of fungi and bacteria over the Atlantic Ocean. Canadian Journal of Botany 32: 202–212.

Richards, M., 1956. A census of mould spores in the air over Britain in 1952. Transactions of the British Mycological Society 39: 431–441.

Schepers, H.T.A.M., 1984. A pattern in the appearance of cucumber powdery mildew in Dutch glasshouses. Netherlands Journal of Plant Pathology 90: 247–256.

Zadoks, J.C., 1967. International dispersal of fungi. Netherlands Journal of Plant Pathology 73, Supplement 1: 61–80.