European Journal of Plant Pathology **102:** 257–263, 1996.

Relations between glasshouse climate and dry weight of petals, epicuticular wax, cuticle, pre-harvest flowering period and susceptibility to *Botrytis cinerea* of gerbera and rose flowers

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Accepted 4 September 1995

Key words: wax, cuticle, flowering period, lesions

Abstract

Studies were conducted on the effects of seasonal levels of relative humidity, temperature, and total radiation, on dry weight of petals, on fresh weight of epicuticular wax and of cuticle of petals, on numbers of *Botrytis cinerea* lesions in petals, and on preharvest flowering periods in gerbera and rose. No temporal relationships or significant correlations were found among dry weight of petals, weight of wax and cuticle of petals, and numbers of lesions on the petals. Temperature, relative humidity and total radiation did not correlate significantly with dry weight of petals, or with fresh weights of wax and cuticle of petals, except for a positive correlation between relative humidity and cuticle weight in the gerbera cultivar Delphi. No relation was found between weight of epicuticular wax and cuticle of petals and susceptibility of gerbera and rose petals to *B. cinerea*. The thickness of wax and cuticle on flowers did not seem to be an important factor influencing the susceptibility of flowers to *B. cinerea*. The seasonal pattern in number of lesions produced on the flowers by *B. cinerea* was related to the effects of relative humidity and radiation on infectivity of conidia of the pathogen on the flower surface but not to the effects on the susceptibility of flowers.

Introduction

The fungus *Botrytis cinerea* Pers.:Fr., the imperfect stage of *Botryotinia fuckeliana* [Faretra and Antonacci, 1987], is pathogenic to a wide variety of economically important plants and is a saprophyte on senescing and dead plant material. The pathogen frequently damages ornamentals like gerbera, rose, chrysanthemum and Saintpaulia [De Jong, 1985, 1986].

Kerssies [1993] and Kerssies *et al.* [1995] found that the number of lesions produced by conidia of *B. cinerea* in gerbera and rose flowers in the post-harvest stage followed a distinct seasonal pattern. Few lesions were observed on gerberas grown under glass in spring and early summer, whereas many lesions appeared at other times of the year. On roses grown under glass, many lesions were counted from August through October but few in the other months. Marois *et al.* [1988] in California showed that the susceptibility of rose flowers to *B. cinerea* was significantly higher in December, January, and February than in October and November, when temperature and radiation were higher. These and other environmental parameters can affect development of plant wax and cuticles [Kolattukudy, 1985]. Gerbera and rose flowers may possess a thicker cuticle and more epicuticular wax in response to environmental parameters in periods when the numbers of lesions on the flowers are low. Skoss [1955] found that leaves of *Hedera helix* grown in the sun produced heavier cuticles of greater wax content than did leaves grown in the shade. Temperature conditions during plant growth were shown to influence the formation of the cuticle and the deposition of wax. He showed that the thickest cuticle was produced at moderate temperatures (17 °C), but the greatest percentage of wax was observed at a high temperature

(30 °C). Plants stressed by drought produced cuticles containing a greater proportion of waxes than did plants with more favourable moisture conditions. Baker [1974] found that an increase in radiation, a decrease in humidity or a decrease in temperature induce large deposits of wax on leaves of *Brassica oleracea* var. *gemmifera*. According to Martin [1964] a high relative humidity induces a thin cuticle. Low humidity and high temperature cause thickening, drying and contraction of the cuticle, thus imposing a barrier to pathogens. Louis [1963] showed that the degree of penetration of *B. cinerea* was related to cuticle thickness in bean, tomato, and other host plants.

The aim of the present study was to investigate the effect of seasonal levels of relative humidity, temperature and total radiation on dry weight of petals, on fresh weight of epicuticular wax and cuticle of harvested gerbera and rose petals, on numbers of *B. cinerea* lesions in petals inoculated with the pathogen, and on pre-harvest flowering periods of gerbera and rose. The differences in dry weight of petals, and in weights of epicuticular wax and cuticle between highly and moderately susceptible gerbera and rose cultivars were investigated.

Materials and methods

Studies were conducted in glasshouses at the Research Station for Floriculture in Aalsmeer.

Host plants. Gerbera (cvs. Terrafame, susceptible to *B. cinerea*, and Delphi, resistant) and rose (cv. Sonia, susceptible) were grown on rockwool in glasshouses of 100 and 300 m^2, respectively. Flowers were observed from September 1991 to December 1992. For gerbera flowers this period was from crop establishment to crop removal. For rose flowers this period was from eight months after crop establishment to crop removal.

Measurement of environmental conditions. Dry and wet bulb temperatures were measured continuously in each glasshouse by using a psychrometer (TFDL, shielded Pt-100, 2 mm copper-constantane thermocouple, precision 0.2 °C), positioned at 1.5 m above the crop and coupled to a data logger. Hourly values were calculated for temperature and relative humidity. Total radiation was measured outside the glasshouses (Jcm^{-2} day^{-1}) by a Kipp solarimeter positioned 8 m above the ground.

Inoculation and disease assessment. Isolate Bc-16 of *B. cinerea*, obtained from a gerbera flower, was used for all experiments. The pathogen was grown on potato dextrose agar under fluorescent light (Pope, FTD 36W/30, 8 μmol m^{-2} s^{-1}) for 7 to 9 days at 20 °C [Salinas *et al.*, 1989]. Conidia were freshly harvested in sterile distilled water and the suspensions were adjusted to a density of $1*10^4$ conidia per ml by diluting with sterile distilled water. Each month from October 1991 to December 1992, six flowers of each cultivar were inoculated with 1 ml conidial suspension in a Potter [1952] spray tower, which resulted in approximately 90 conidia/cm^2 petal, and air dried for 10 min. The upper 10 petals of each gerbera flower and whole flowers of roses were subsequently placed in plastic boxes with a wet towel on the bottem (RH>95%). After 1 day of incubation lesions were counted on the petals with the aid of a dissecting microscope (10 × magnification), [Salinas *et al.*, 1989].

Estimation of dry weight, epicuticular wax, and cuticle of petals. Each month, from October 1991 to December 1992, three flowers of each cultivar used were harvested at the commercial stage and used to estimate the dry weights of petals and the weights of epicuticular wax and of cuticle of petals. Eight petal discs (2 cm^2 each) of each of three flowers (in total 16 cm^2 per flower) were dried at 100 °C and weighed. The weight of epicuticular wax was determined according to Silva Fernandez *et al.* [1964], with some modifications. Eight discs (each 2 cm^2) from the petals of each flower were placed in 4 ml chloroform in a preweighed (20 ml) beaker at room temperature. After 4 min the petals were placed on filter paper to dry, the chloroform in the beaker was allowed to evaporate and the beaker was reweighed. The difference in weight of the beaker before and after the chloroform treatment was calculated and divided by the two-sided surface area of the petals ($8 * 2 * 2 = 32$ cm^2) to obtain the weight of wax/cm^2 flower petal (one-sided). Petal discs from which epicuticular wax was removed were used to determine the weight of cuticle according to Holloway and Baker [1968], with some modifications. The petal discs (16 cm^2 per flower) were immersed in a solution containing 18 g $ZnCl_2$ in 23 ml of concentrated HCl (37%) for 16 h at room temperature. Subsequently, the petal discs of each flower were placed in 300 ml tap water. After 1 h in the tap water the cuticles were easily peeled off from the petal discs, dried at 80 °C and weighed. The weight was divided by 32 cm^2 to obtain the weight of the cuticle/cm^2 petal (one-sided).

Preharvest flowering period. Each month, from October 1991 to December 1992, the pre-harvest period over which gerbera and rose flowers bloomed was measured by determining from successive groups of 10 flowers per cultivar the number of days from when flowers first opened until they were ready for commercial harvest.

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

Statistical analysis. Statistical analysis was performed by the statistical package Genstat 5 [Payne *et al.*, 1987]. Means of numbers of lesions per cm^2 on gerbera and rose petals, of dry weights per cm^2 of petals, weights of epicuticular wax (one-sided) and cuticle (one-sided) per cm^2 of petals and preharvest flowering period were subjected to analysis of variance (ANOVA). LSD values were calculated for comparing the means.

Results

Environmental conditions. Relative humidity and temperature 1.5 m above the gerbera and rose crop and total radiation outside the glasshouse of 1991 and 1992 were averaged over periods of 3 months (Table 1). The mean temperature was high (>20 °C) from April–September, due to high irradiation and inadequate ventilation, with larger day/night variations than in the other periods. The mean relative humidity was slightly higher (>70%) from July–December than in the other months and the mean total radiation was high (>1400 Jcm^{-2} day^{-1}) from April–September.

Estimation of numbers of lesions, dry weight of petals, epicuticular wax, and cuticle of petals and of preharvest flowering periods. Observations of number of lesions, dry weight of petals, weight of epicuticular wax per cm^2, and cuticle weight per cm^2 of gerbera and rose petals, and preharvest flowering periods of gerbera and rose were averaged over 3 months (Table 2).

The mean density of lesions on gerbera cultivar 'Terrafame' flowers was high (> 30 lesions per cm^2 flower) from July–December, 1992 (Table 2). Density of lesions on gerbera petals was initially low, but high at the end of the experimental period (October–November 1992). Lesion density on flowers of gerbera cultivar 'Delphi' was relatively high (> 5 lesions per cm^2 flower) from July–September, but lesion density on flowers of 'Sonia' did not differ significantly among the various three-month periods of observations (15–19 lesions per cm^2 flower).

Dry weight values for 'Terrafame' petals were significantly higher during July–September than in the other three-month periods (Table 2). The dry weight values of 'Delphi' and 'Sonia' petals were not significantly different among the various three-month periods of the study. The weight of epicuticular wax on petals of 'Terrafame', 'Delphi' and 'Sonia' were lowest from April–June (minima of 7.5, 8.5 and 11.8 $\mu g/cm^2$, respectively). The lowest cuticle weights of 'Terrafame' and 'Delphi' were measured in fall, at the end of the experimental period. On flowers of 'Sonia' the cuticle weight had a peak in January–March.

The preharvest flowering periods of gerbera and rose were short from April–September and long from October–March.

Comparisons of estimations. The mean number of lesions on petals of gerbera cv. 'Terrafame' was about ten times larger than on those of cv. 'Delphi' (Table 3, 23.8 and 2.4 lesions per cm^2 of petal, respectively). The petal dry weight and the weight of epicuticular wax did not differ significantly between these cultivars (Table 3). The weight of cuticle (69.2 and 89.2 $\mu g.cm^{-2}$ petals, respectively) and the preharvest flowering periods (10.0 and 6.3 days, respectively) were significantly different between 'Terrafame' and 'Delphi'.

No relationship was found among petal dry weight, weight of epicuticular wax and cuticle and density of lesions on petals of different rose cultivars (Table 4). The mean weight of epicuticular wax and cuticle of the susceptible rose cultivars Sonia and Madelon and of the resistant rose cultivars Mercedes and Motrea were not significantly different (Table 4). The moderately susceptible 'Frisco' had high values for epicuticular wax and cuticle.

For gerbera cultivar 'Terrafame', significant linear correlations were only observed between crop age and weight of cuticle, between crop age and number of lesions, between temperature and flowering period, and between radiation and flowering period (Table 5a). For 'Delphi', significant linear correlations were only found between temperature and flowering period and

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

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

Table 2. Estimated mean numbers of lesions (n = 3 * 6 = 18), mean dry weight (n = 3 * 3 = 9), mean amount of wax (n = 3 * 3 = 9) and mean amount of cuticular membrane (n = 3 * 3 = 9), per cm^2 petal and mean preharvest flowering period (n = 3 * 10 = 30), during three-month periods of observation of gerbera flowers cv. Terrafame and cv. Delphi and of rose flowers cv. Sonia. Means in each column followed by the same letter are not significantly different ($P \leq 0.05$, ANOVA and Student-test)

Period of observations	Number of lesions per cm^2 petal			Dry weight (mg/cm^2 petal)			Epicuticular wax ($\mu g/cm^2$ petal)			Cuticle ($\mu g/cm^2$ petal)			Preharvest flowering period (days)		
	T	D	S	T	D	S	T	D	S	T	D	S	T	D	S
Oct-Dec 1991	10.0 c	1.6 b	15.7 a	3.0 b	3.1 a	3.6 a	16.3 a	16.0 a	31.3 a	87.2 a	94.4 a	129.0 b	10.9 b	6.9 b	9.1 b
Jan-Mar 1992	22.8 b	2.9 b	16.0 a	3.2 b	3.4 a	3.8 a	11.0 b	10.6 b	23.3 b	72.7 ab	98.6 a	178.3 a	11.0 b	7.1 ab	9.7 ab
Apr-Jun 1992	19.5 b	1.9 b	18.8 a	3.2 b	3.5 a	3.8 a	7.5 c	8.5 b	11.8 c	64.0 bc	101.8 a	149.6 b	8.3 c	6.0 c	4.9 c
Jui-Sep 1992	34.2 a	5.1 a	19.0 a	3.8 a	3.5 a	3.8 a	15.8 a	16.9 a	20.7 b	69.6 b	84.0 ab	135.3 b	7.8 c	5.5 c	5.4 c
Oct-Dec 1992	32.4 a	2.9 b	17.4 a	2.9 b	3.0 a	4.0 a	10.3 bc	11.2 b	24.8 b	52.8 c	67.2 b	118.1 b	11.9 a	7.7 a	10.4 a

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

Discussion

Petal dry weight, epicuticular wax and cuticle of petals, lesions and preharvest flowering period – the experiments. The dry weights of gerbera and rose petals were fairly stable throughout the 15-month period of study. The weight of epicuticular wax on petals apparently was related to the time of the year. In spring the weight of wax was low and radiation values were high, but no significant correlation was found between radiation and wax weights. The cuticle weight was related to the age of the crop; thus the cuticle weight per cm^2 petal declined with age of the crop, particularly on 'Terrafame'. The density of lesions on flowers was higher when the crop became older in 'Terrafame', but not in 'Delphi' and 'Sonia'. No significant relation was found between number of lesions and cuticle weight. The dry weight of petals and the weight of epicuticular wax and cuticle per cm^2 petal depended on the flower type. Values of these variables were higher for rose than for gerbera. Lesion density on 'Sonia' flowers (susceptible) was only slightly lower than on 'Terrafame' flowers (susceptible). Although cuticle weight in susceptible 'Terrafame' was significantly lower than in resistant 'Delphi', the difference does not seem large enough to explain the large difference in number of lesions, however, cuticle thickness could be a threshold phenomenon. Periods of preharvest flowering varied from short in summer to long in fall and winter, but this variation did not influence petal dry weight.

Petal dry weight, epicuticular wax and cuticle of petals, lesions and preharvest flowering period – relations. The results of the present study show that the distinct seasonal pattern in incidence of lesions of *B.*

Table 3. Mean dry weight (n = 5 * 9 = 45), mean wax weight (n = 5 * 9 = 45), mean cuticle weight (n = 5 * 9 = 45) and mean numbers of lesions (n = 5 * 18 = 90) per cm^2 petal and mean preharvest flowering period (n = 5 * 30 = 150) of gerbera flowers cv. Terrafame and cv. Delphi, over a period of 15 months in 1991 and 1992. Means in each column followed by the same letter are not significantly different ($P \leq 0.05$, ANOVA and Student-test)

Gerbera cultivar	Dry weight (mg/cm^2 petal)	Epicuticular wax ($\mu g/cm^2$ petal)	Cutice ($\mu g/cm^2$ petal)	Number of lesions per cm^2 petal	Preharvest flowering period (days)
Terrafame	3.2 a	12.2 a	69.2 b	23.8 a	10.0 a
Delphi	3.3 a	12.6 a	89.2 a	2.4 b	6.3 b

Table 4. Mean dry weight (n = 2 * 2 = 4), mean wax weight (n = 2 * 2 = 4), mean cuticle weight (n = 2 * 2 = 4) and mean numbers of lesions (n = 2 * 6 = 12) per cm^2 petal of rose flowers cv. ' Sonia', 'Madelon', 'Frisco', 'Mercedes' and 'Motrea'. Means in each column followed by the same letter are not significantly different ($P \leq 0.05$, ANOVA and Student-test)

Rose cultivar	Dry weight (mg/cm^2 petal)	Epicuticular wax ($\mu g/cm^2$ petal)	Cuticle ($\mu g/cm^2$ petal)	*Number of lesions per cm^2 petal
Sonia	2.7 c	14.0 b	139.3 b	21.8 a
Madelon	3.3 bc	16.5 b	154.8 b	19.7 a
Frisco	4.5 a	30.3 a	301.5 a	12.9 b
Mercedes	3.9 ab	18.5 b	153.0 b	0.3 c
Motrea	3.5 bc	15.5 b	142.5 b	1.0 c

* Experiment performed by A. Hazendonk.

cinerea on gerbera and rose flowers grown under glass, found in earlier studies [Kerssies, 1993 and Kerssies *et al.*, 1995], cannot be explained by the variation in dry weight of petals and in weight of epicuticular wax and cuticle of the petals. No clear seasonal pattern was found in petal dry weight, weight of epicuticular wax and cuticle of the petals. No significant linear correlation was found between the dry weight of petals, the amounts of epicuticular wax and cuticle and the number of lesions produced by *B. cinerea* on gerbera and rose flowers. Temperature, relative humidity and radiation did not correlate with petal dry weights, and weight of epicuticular wax and cuticle of the petals of gerberas. Probably the low weight of epicuticular petal wax in spring coincided with high radiation values. Kerssies [1994] found that relative humidity and radiation had affected infectivity of conidia of *B. cinerea* but not the susceptibility of gerbera flowers to the pathogen. However, temperature affected both conidial infectivity and flower susceptibility. These results are consistent with observations of the present study that environmental factors did not correlate with numbers of lesions of *B. cinerea* in gerbera and rose flowers. Therefore, the observed seasonal patterns in the number of lesions on the flowers were probably related to the effects of relative humidity and radiation on the infectivity of conidia present on the flower surface and not to differences in susceptibility of flowers.

Hypotheses. The lack of a clear relation between environmental factors (season-dependent) and dry weight of petals, petal weight of epicuticular wax and cuticle and susceptibility of flowers for *B. cinerea* could be explained by various hypotheses.

I. According to Kolattukudy [1985] and Köller [1991] hydrolysis of cutin by cutinase, an enzyme secreted during the initial step of host invasion, is required for pathogens to penetrate plant cuticles. Stockwell and Hanchey [1984] found that increased cuticle thickness of bean leaves of older plants increases the resistance of these leaves to *Rhizoctonia solani*. Salinas [1990] treated gerbera flowers, inoculated with conidia of *B. cinerea*, with antibodies raised against purified cutinase of *B. cinerea*. Gerbera flowers

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

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

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

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

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

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

treated with these antibodies were completely protected against infection of *B. cinerea*. If the hypotheses of Kolattukudy [1985] and Köller [1991] are true for cuticles of leaves, cuticles of flowers probably are too thin at any time to prevent penetration by *B. cinerea* conidia. Thus differences in cuticle thickness in relation to microclimate or cultivar may not be important as variables affecting infection incidence by *B. cinerea*. The present study did not deal with the possible differences in chemical compounds of the wax and cuticle membrane, which also can affect the resistance of leaves or flowers against penetration of fungi [Martin, 1964].

II. According to Nicholson and Epstein [1991] adhesion of fungal spores to the host cuticle is an essential pre-penetration process that determines the success of host penetration by pathogens. According to their experiments with *Erysiphe graminis* on barley leaves and *Colletotrichum graminicola* on cereal leaves cutinases are involved in adhesion, and are not an important factor in the penetration process. Ungerminated spores adhered to the leaf surface within 2 minutes [Nicholson and Epstein 1991]. Inhibition of cutinases resulted in no infection. If there is no adhesion, the spore pushes itself up when it tries to penetrate the plant surface. For a situation in which the hypothesis of Nicholson and Epstein is true, the susceptibility of flowers to *B. cinerea* would not be influenced by the thickness of epicuticular wax and cuticle, but probably by the amount of excreted sugars on the flower surface, as a source of nutrients necessary for germination of conidia of *B. cinerea*. Observations of the present study agreed more closely with the hypothesis of Nicholson and Epstein [1991] than that of Kolattukudy [1985], but the results of the present study do not provide evidence which mechanism(s) determine the number of lesions produced by *B. cinerea*. No significant relation was observed between weights of epicuticular wax and cuticle and susceptibility of gerbera and rose petals to *B. cinerea* (e.g. Table 4). Probably, conidia of *B. cinerea* trapped by flower petals adhere to the surface with cutinase, and after a few hours of high relative humidity and a suitable amount

of sugars the adhered conidia germinate and the hyphae penetrate the flower surface. Therefore throughout the seasons the number of lesions caused by *B. cinerea* is not limited by the amount of epicuticular wax and cuticle of the petals.

The mechanism behind the difference in susceptibility to *B. cinerea* among various cultivars of gerbera and rose remains unclear. Possibly, differences in chemical compounds of wax and cuticle are important during the infection process, or other processes may be more important such as secretion of toxins, sugars or salts by flowers.

Because no relation was found between seasonal climatic conditions and weights of epicuticular wax and cuticle or between weights of epicuticular wax and cuticle and susceptibility of flowers to *B. cinerea*, further studies on epicuticular wax and cuticle in relation to epidemics of *Botrytis* spotting on cut flowers in glasshouses do not seem justified. Seasonal differences in numbers of lesions on the flowers could be attributed to the effect of relative humidity and radiation on the infectivity of conidia of *B. cinerea* as opposed to susceptibility of flowers to *B. cinerea*. The seasonal fluctuations in weights of epicuticular wax and cuticle did not affect the susceptibility of flower petals to *B. cinerea*. Also, Kerssies [1993] and Kerssies *et al.* [1995] could not find any relation between the concentrations of *B. cinerea* spores in glasshouse air and the number of lesions produced by the pathogen on the flowers.

Acknowledgements

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

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