Effects of Wetness Duration and Temperature on Infection of Geranium by *Botrytis cinerea*

C. Sirjusingh, Former Graduate Student, and **J. C. Sutton,** Professor, Department of Environmental Biology, University of Guelph, Guelph, ON N1G 2W1, Canada

ABSTRACT

Sirjusingh, C., and Sutton, J. C. 1996. Effects of wetness duration and temperature on infection of geranium by *Botrytis cinerea*. Plant Dis. 80:160-165.

Duration and temperature of postinoculation wetness periods were investigated in relation to infection of flowers and leaves of geranium by *Botrytis cinerea*. Infection was assessed indirectly by estimating sporulation incidence of the pathogen in inoculated tissues. Sporulation incidence in whole flowers inoculated with conidia increased sharply when wetness duration at 15, 21, 25, and 30°C was increased from 8 to 24 h, 4 to 12 h, 4 to 12 h, and 4 to 6 h, respectively. The pathogen did not sporulate after shorter wetness periods at these temperatures or after 24 h wetness at 5 and 10°C. No sporulation was observed in sepals, petals, and stamens when the wet period at 25°C was ≤4 h, or in pistils and pedicels when wetness lasted ≤6 h, but sporulation increased to 100, 100, 100, 73, and 60% in the respective organs as the wet period was increased to 24, 12, 12, 24, and 24 h. Following conidial inoculations of the foliage, *B. cinerea* sporulated in 1-week-old leaves only when the postinoculation wetness period at 15, 21, 25, and 30°C was ≥6, ≥4, ≥6, and ≥6 h, respectively, and in 10-week-old leaves when wetness at the respective temperatures lasted ≥6, ≥6, ≥8, and ≥8 h. Sporulation was observed in 4-week-old leaves after ≥12, ≥8, and ≥6 h wetness at 15, 21, and 25°C, respectively. The pathogen did not sporulate in leaves of any age group when postinoculation wet periods were 0 to 24 h at 5 or 10°C, or in 4-week-old leaves at 30°C. *Botrytis cinerea* sporulated more frequently in 1- and 10-week-old leaves than in 4-week-old leaves when wetness lasted for 8 to 24 h at 25°C. The pathogen infected leaves more efficiently when conidia were applied to geranium petals that after 24 h were positioned on the leaves, than when conidia were applied directly to the leaves. Logistic regression models were developed that adequately described effects of postinoculation wetness duration and temperature on sporulation incidence and, by inference, infection incidence, in flowers and leaves.

Gray mold or Botrytis blight, caused by *Botrytis cinerea* Pers.:Fr., is a destructive disease of flowers, leaves and stems of geraniums (*Pelargonium* × *hortorum* L. H. Bailey) produced in greenhouses in North America (1,6). The disease can affect geraniums at all stages of production, storage, and shipping (6,20). Management of gray mold depends heavily on fungicides, and more than doubles production costs of geraniums from the standpoint of materials and labor (2). Other concerns are occupational exposure of greenhouse workers to fungicides, residues of fungicides on the crop and in the greenhouse environment, and development of fungicide resistance in populations of *B. cinerea* (12,16,17). Quantitative relationships of greenhouse microclimate and infection cycles of *B. cinerea* in geraniums could form a basis for reducing the need for fungicides, but available information is fragmentary, especially with regard to infection and sporulation of the pathogen. Factors that limit infection may frequently limit rates of increase of gray mold in various greenhouse crops (12) and justify investigation in geranium in order to optimize fungicide timing and to develop biological and other control measures.

Botrytis cinerea infects geraniums by means of aerially dispersed conidia and by mycelium in host tissues, especially in fallen petals of geranium that commonly adhere to healthy leaves and stems (7,9, 14,15,20). Germ tubes from conidia penetrate geraniums through wounds, the cuticle, and probably stomata (8,10,13,27). Age of flowers and leaves markedly influences infection efficiency of conidia (23). Infected petals can function as a food base of *B. cinerea* and increase inoculum potential of the mycelium when the pathogen infects leaves (10,12,23,27). Liquid water increases adherence of petals to plant surfaces (11). Infection from conidia and from mycelium in petals is dependent on a film or droplets of water on the host surface (10–12), but quantitative relationships of wetness duration and temperature of the wetness period to infection have not been reported. The present study was conducted to quantify these relationships with respect to flowers and leaves of geranium, and to explore leaf age as a variable influencing effects of wetness and temperature on leaf infection.

Corresponding author: J. C. Sutton
E-mail: jsutton@evbhort.uoguelph.ca

Accepted for publication 16 November 1995.

Publication no. D-1995-1212-05R

MATERIALS AND METHODS

Host plants. Plants of geranium cv. Americana White (Valk Greenhouses Ltd., Grimsby, ON), a white-flowered tetraploid, were produced from terminal shoot cuttings in a climate-controlled greenhouse as described (23). Plants were inoculated at 5 to 10 weeks after transplanting.

Inoculum and inoculations. Conidia of *B. cinerea* isolate GR-1 from geranium were produced on potato-dextrose agar (PDA), recovered in sterile distilled water plus surfactant (0.1 ml Triton X-100/100 ml), and diluted to desired concentrations as described (23). Germination of conidia on PDA consistently exceeded 96% after 24 h at 21°C. Inoculum suspensions were applied to incipient runoff on all surfaces of geranium inflorescences or leaves using a 200-ml capacity hand sprayer (23). Flowers were inoculated with 10^5 conidia per ml and leaves with 10^6 conidia per ml. Check flowers and leaves were inoculated with sterile distilled water plus the surfactant only. When attached inflorescences or leaves were inoculated, the plants were immediately placed in a clear plastic humidity chamber (1.5 m long × 0.5 m wide × 0.5 m high) (23) with relative humidity (RH) ≥95%. Detached leaves and flowers were inoculated and immediately placed on galvanized wire mesh positioned about 1.5 cm above wet paper towels inside translucent plastic containers (33 cm long × 20 cm wide × 6 cm deep) with tightly fitting lids.

Estimation of infection incidence. Infection was assessed indirectly by estimating sporulation incidence of *B. cinerea* in whole flowers, flower parts, and leaf disks. Detached flowers and leaf disks were surface sterilized as described (23). Whole flowers and flower parts (sepals, petals, stamens, pistils, and pedicels) were placed on water agar (WA), and disks were transferred to paraquat-chloramphenicol agar medium (PCA) (19), incubated at 20 to 22°C for 5 to 7 days, and examined for conidiophores of *B. cinerea* as described (23).

Wetness period, temperature, and flower infection. Attached inflorescences were inoculated with *B. cinerea* and immediately placed in high humidity (RH ≥95%) for 0, 2, 4, 6, 8, 10, 12, and 24 h at 25°C, then in the greenhouse at 22 to

24°C. At 48 h after inoculation, five flowers were removed from each of three replicate inflorescences for a total of 15 flowers per treatment, surface sterilized, and incidence of infection of the flower parts was estimated using five sepals, five petals, eight stamens, the pistil, and the pedicel of each flower.

In a related study, detached inflorescences were inoculated, kept in high humidity (RH ≥95%) for 0, 2, 4, 6, 8, 10, 12, and 24 h at 5, 10, 15, 21, 25, and 30°C, and subsequently in low humidities (about 50 to 70% RH) at the same temperatures, all in darkness. Low humidities were measured at intervals using a sulfonated polystyrene sensor and datalogger (24). At 48 h after inoculation, five flowers from each of three replicate inflorescences for a total of 15 flowers per treatment were surface sterilized and incidence of infection of the whole flowers was estimated.

Wetness period, leaf age, and leaf infection. Age of leaves was measured starting when the leaf bud was 3 to 4 mm long (23). Attached leaves 1, 4, and 10 weeks old were inoculated with a conidial suspension of *B. cinerea* and the plants were placed in the humidity chamber at 25°C. After wetness periods of 0, 2, 4, 6, 8, 10, 12, and 24 h, five plants were transferred to a greenhouse at 22 to 24°C. At 48 h after inoculation, six whole leaves of the 1-week age group were detached from each of the five plants of each postinoculation wetness treatment (total 30 leaves/treatment). At the same time, five 1-cm-diameter disks were cut from each of three 4-week-old and three 10-week-old leaves of the five plants of each treatment (total 75 disks/treatment). The detached leaves and disks were surface sterilized and transferred to PCA for estimation of sporulation incidence of the pathogen.

Attached leaves 1, 4, and 10 weeks old also were inoculated with petals treated with *B. cinerea*. Attached geranium flowers were inoculated 5 days after opening with the pathogen (10^5 conidia per ml) or with sterile distilled water plus surfactant only, and kept in the humidity chamber for 24 h at 25°C. The petals were subsequently detached and positioned on the adaxial surfaces of geranium leaves. One unfolded petal was placed on each of six 1-week-old leaves of five replicate plants per treatment (total of 30 leaves per treatment), and five unfolded petals were placed on each of three 4-week-old and three 10-week-old leaves of the five replicate plants for a total of fifteen petals per treatment. Plants with inoculated leaves were kept in the humidity chamber for 0, 2, 4, 6, 8, 10, 12, and 24 h at 25°C and subsequently transferred to the greenhouse at 22 to 24°C. At 48 h after inoculation, sites of petal adhesion on leaves were marked, the petals were removed, and a 1-cm-diameter disk was cut from within each marked site. Disks were surface sterilized and transferred to PCA for estimation of sporulation incidence of the pathogen.

Wetness period, temperature, leaf age, and leaf infection. Detached leaves 1, 4, and 10 weeks old were inoculated with a conidial suspension of *B. cinerea* and kept in the humidity containers for 0, 2, 4, 6, 8, 10, 12, and 24 h at 5, 10, 15, 21, 25, and 30°C, and subsequently in low humidities at the same temperatures, all in darkness. At 48 h after inoculation five 1-cm-diameter disks were cut from three 4-week-old and three 10-week-old leaves of the five plants of each treatment (total 75 disks/treatment). The disks and six 1-week-old leaves from each of five plants of each humid period–temperature treatment (total 30 leaves/treatment) were surface sterilized and transferred to PCA for estimation of sporulation incidence of the pathogen.

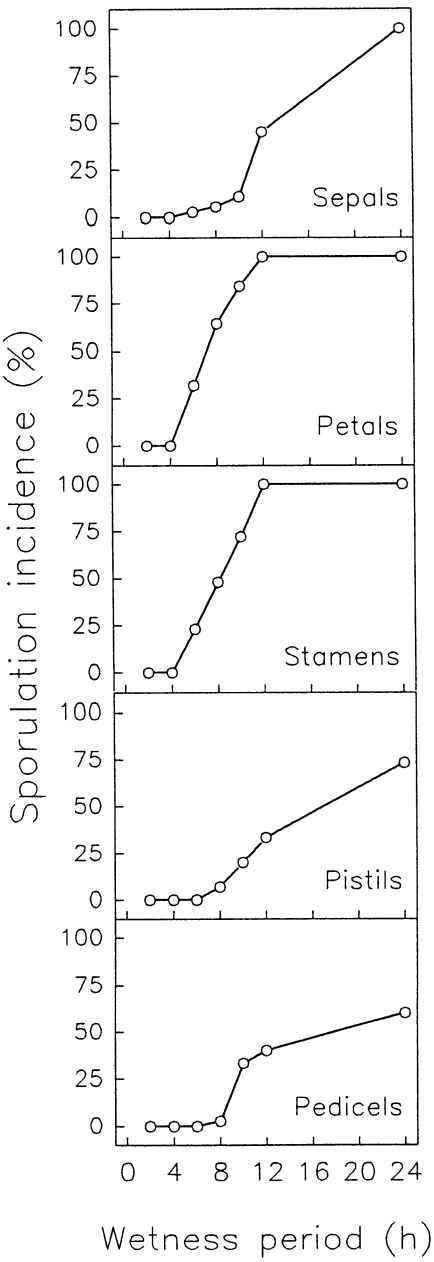

Fig. 1. Effects of postinoculation wetness period at 25°C on sporulation incidence of *Botrytis cinerea* in various parts of geranium flowers.

Experimental design and data analyses. A completely randomized design was used in all experiments, each of which was repeated once. Statistical computations were performed using the Statistical Analysis System (SAS Institute Inc., Cary, NC). Observations of repeated experiments were subjected to analyses of homogeneity of variance and data were pooled when appropriate. All data were examined using analysis of variance (ANOVA) and the treatment means were compared using the Waller-Duncan Bayesian *k*-ratio *t* test (28). Regression analyses were used to determine relationships of postinoculation wetness periods and temperature to incidence of sporulation of *B. cinerea*. Linear, logistic, and monomolecular models were tested for goodness of fit of the regressions (4).

RESULTS

Wetness period and flower infection. Incidence of petals and stamens with sporulation of *B. cinerea* was zero when the postinoculation wetness period at 25°C was ≤4 h, but increased to 100% as wetness duration was increased from 6 to 12 h (Fig. 1). Sporulation incidence in sepals, pistils, and pedicels was zero when postinoculation wetness lasted ≤4, ≤6, and ≤6 h, respectively, but progressively increased after longer wetness periods and reached 100, 73, and 60%, respectively, after 24 h wetness.

Table 1. Estimated parameter values and coefficients of determination (R^2) from logistic regression models describing relationships between postinoculation wetness duration (W) and estimated (%) incidence of sporulation (Y) of *Botrytis cinerea* in different parts of geranium flowers[a]

Flower part	Estimated parameters			R^2
	b_0	b_1[b]	b_2	
Sepal	−4.40	0.20	0.10	0.96
Petal	− 13.48	2.16	−0.05	0.88
Stamen	− 15.73	2.58	−0.06	0.88
Pistil	−2.78	0.14	. . .	0.82
Pedicel	−2.64	0.14	. . .	0.81

[a] Flowers were inoculated with 10^5 conidia of *B. cinerea* per ml and kept in a humidity chamber at 25°C. Data of two independent repetitions of the study did not differ significantly (*F* test) and were combined for analysis. The model used for sepals, petals, and stamens was logit $Y = b_0 + b_1W + b_2W^2 + e_i$, and the model for pistils and pedicels was logit $Y = b_0 + b_1W + e_i$. The logistic transformation of Y was $\ln[(Y + k)/(1 - Y + k)]$, in which k was a constant; b_0 to b_2 were unknown parameters, and e_i was random variability of the models.

[b] All slopes differed significantly from zero ($P \leq 0.05$).

Logistic regression models best described sporulation incidence on different flower parts as a function of postinoculation wetness duration (W) (Table 1). Estimates for unknown parameters (b_0 – b_2) were significant ($P \leq 0.0001$) in all instances. ANOVA and regression analysis indicated that W significantly affected incidence of sporulation (Y), and that there were significant linear and squared components for the petals, sepals, and stamens, and significant linear components for the pistils and pedicels. Slopes of regression lines (b_1 values) were relatively steep for petals and stamens and relatively shallow for sepals, pistils, and pedicels (Table 1). The R^2 values were high for all flower parts evaluated (Table 1). The wetness period required to produce 50% incidence of sporulation of *B. cinerea*, estimated from the regression models, was relatively short for petals and stamens (8.0 and 7.7 h, respectively), but nearly twice as long for sepals, and about three times as long for pistils and pedicels (Table 2).

Table 2. Estimated duration of postinoculation wetness (W) that resulted in 50% incidence of sporulation of *Botrytis cinerea* (EW_{50}) in different parts of geranium flowers that were inoculated with 10^5 conidia of *B. cinerea* per ml and maintained at 25°C

Flower part	EW_{50}(h)[a]	Confidence interval (95%)
Sepal	14.5	13.7 < W < 15.4
Petal	8.0	7.9 < W < 8.1
Stamen	7.7	7.6 < W < 7.8
Pistil	24.3	23.3 < W < 25.4
Pedicel	23.3	22.5 < W < 24.3

[a] Values calculated from the regression models (Table 1).

Wetness period, temperature, and flower infection. *Botrytis cinerea* sporulated on flowers only when the postinoculation wetness period was ≥8 h at 15°C and ≥4 h at 21, 25, and 30°C (Fig. 2). The pathogen did not sporulate when postinoculation wetness lasted 0 to 24 h at 5 and 10°C. Sporulation incidence at 15, 21, 25, and 30°C increased sharply when wetness duration was increased from 8 to 24, 4 to 12, 4 to 12, and 4 to 6 h, respectively. Highest incidences of sporulation at the respective temperatures were 30, 100, 100, and 76%, and were observed after 24, 12, 12, and 6 h of postinoculation wetness. A logistic regression model was moderately effective for describing the observed incidence of sporulation (Y) as a function of postinoculation wetness duration (W) and temperature of the wetness period (T) (Table 3). Terms in the model were significant based on stepwise regression. ANOVA as well as regression analysis indicated that W, T, and their interactions significantly affected sporulation incidence. Relationships between Y and W and Y and T had significant linear and squared components.

Wetness period, leaf age, and leaf infection. Sporulation incidence of *B. cinerea* in 1-, 4-, and 10-week-old leaves inoculated with the conidial suspension was zero when the period of postinoculation wetness at 25°C was 4, 8, and 4 h, respectively, but increased progressively when wetness was increased from 6 to 24, 10 to 24, and 6 to 24 h, respectively (Fig. 3A). Sporulation incidence was significantly higher ($P \leq 0.05$) in 1-, and 10-week-old leaves than in 4-week-old leaves when wetness lasted for 8 to 24 h, and significantly higher ($P \leq 0.05$) in 1-week-old leaves than in 10-week-old leaves when the wetness period was 12 or 24 h.

Sporulation was observed in 1-, 4-, and 10-week-old leaves treated with inoculated petals when the postinoculation wetness period at 25°C was at least 6, 8, and 6 h, respectively, and increased to 100, 79, and 100%, respectively, as wetness was increased to 24 h (Fig. 3B). Sporulation in-

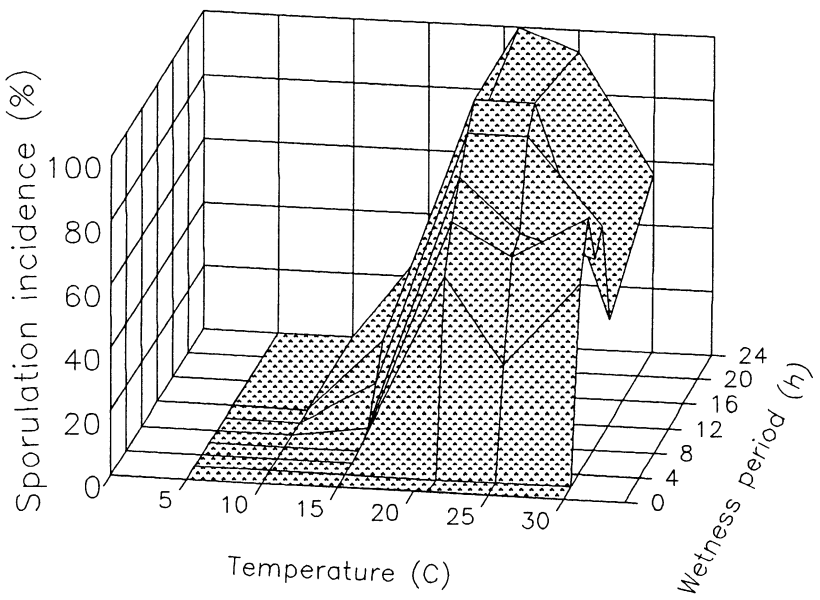

Fig. 2. Effects of duration and temperature of the postinoculation wetness period on sporulation incidence of *Botrytis cinerea* in geranium flowers.

Table 3. Estimated parameter values and coefficients of determination (R^2) for the logistic regression describing relationships of postinoculation wetness duration (W) and temperature of the wetness period (T) to sporulation incidence of *Botrytis cinerea* in geranium flowers that were inoculated with a spore suspension (10^5 conidia per ml) of the pathogen[a]

	Estimated parameters[b]					
Plant part	b_0	b_1W^2	b_2WT^2	b_3W^2T	$b_4W^2T^2$	R^2
Flower	–10.42	–0.04	–0.003	0.01	–0.0003	0.63

[a] Data of two independent repetitions of the study did not differ significantly (F test) and were combined for analysis.

[b] Parameters of the model: $\text{Logit } Y = b_0 + b_1W^2 + b_2WT^2 + b_3W^2T - b_4W^2T^2 + e_i$

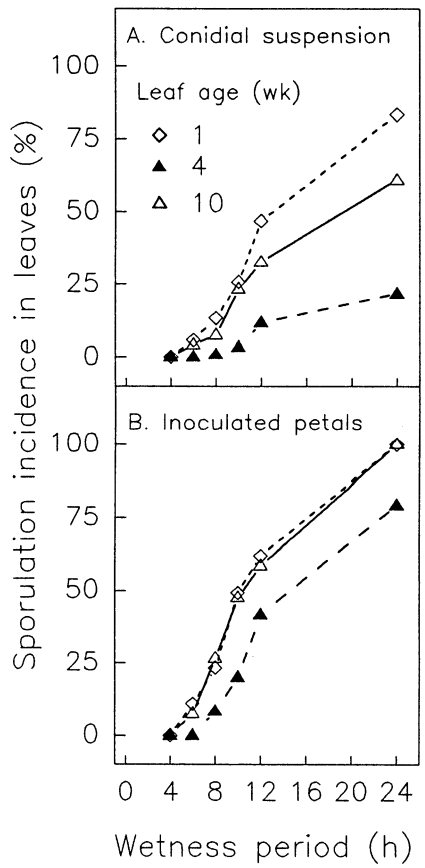

Fig. 3. Effects of postinoculation wetness period at 25°C on sporulation incidence of *Botrytis cinerea* in geranium leaves of various ages that were **(A)** inoculated with a conidial suspension of the pathogen (10^6 spores per ml) or **(B)** treated with geranium petals that had been inoculated with the pathogen (10^5 conidia per ml) 24 h earlier.

cidence in the 1-, 4-, and 10-week-old leaves treated with inoculated petals was higher than in similar leaves inoculated with conidia ($P \leq 0.05$) when postinoculation wetness periods were 6 to 24, 8 to 24, and 6 to 24 h, respectively.

The logistic regression model effectively described sporulation incidence (Y) as a function of postinoculation wetness duration (W) for leaves of each age group inoculated with conidia or with inoculated petals, except 4-week-old leaves inoculated with conidia for which the relationship was best described by a simple linear regression equation (Table 4). Unknown parameters (b_0 to b_2) were in all instances significant ($P \leq 0.0001$). ANOVA and regression analysis indicated that the relationships of logit Y and W had linear components when conidial inoculum was used, and linear and squared components when inoculated petals were used. The R^2 values were high in all instances (Table 4). The duration of postinoculation wetness required to produce 50% incidence of sporulation of *B. cinerea*, estimated from the regression models was 10.2 to 12.8 h when petal inoculum was used, but about twice as long or more (19.3 to 30.0 h) when conidial suspensions were applied (Table 5).

Wetness period, temperature, leaf age, and leaf infection. *Botrytis cinerea* sporulated in 1-week-old leaves only when the postinoculation wetness period at 15, 21, 25, and 30°C was ≥6, ≥4, ≥6, and ≥6 h, respectively, and in 10-week-old leaves when wetness at the respective temperatures lasted ≥6, ≥6, ≥8, and ≥8 h (Fig. 4). In 4-week-old leaves, the pathogen sporulated only when the wetness period at 15, 21, and 25°C was ≥12, ≥8, and ≥6 h, respectively. No sporulation was observed in leaves of any age group when temperature of 0 to 24 h wetness periods was 5 or 10°C, or in 4-week-old leaves kept in the same wetness periods at 30°C. Sporulation incidence progressively increased when wetness periods at 15, 21, and 25°C were increased from the shortest favorable durations to 24 h, and when wetness at 30°C was increased from 6 to 12 h. Sporulation incidence in 1- and 10-week-old leaves decreased ($P \leq 0.05$) when wetness duration at 30°C was increased from 12 h to 24 h. For wetness periods of 8 to 24 h, 21°C was the most favorable temperature for sporulation.

The logistic regression model best fitted the data for the interactive effects of postinoculation wetness period (W) and temperature (T) on sporulation incidence (Y) of *B. cinerea* in the 1-, 4-, and 10-week-old leaves (Table 6). Estimates of the unknown parameters were in all instances significant ($P \leq 0.0001$). ANOVA and regression analysis indicated that W, T, and their interactions significantly affected Y, and that there were linear and squared components ($P \leq 0.0001$) in the relationship of W, T, WT, and Y. The R^2 value was high for 1- and 10-week-old leaves (0.90 and 0.86, respectively) and moderate (0.60) for 4-week-old leaves.

DISCUSSION

Observed effects of postinoculation wetness and temperature on sporulation incidence of *B. cinerea* in flowers and leaves of geranium were indirect and presumably mediated by direct influence on infection and colonization of inoculated tissues. Wetness and temperature have been widely recognized as important variables affecting infection of geranium and other hosts by *B. cinerea* (11,12,27) but few reports detailed quantitative relationships between these variables and infection (3,18,31).

From observations of sporulation incidence, there is a strong implication that moderate postinoculation wetness periods at moderate to warm temperatures are highly conducive to infection of geranium flowers by conidia of *B. cinerea*. A minimum of 4 to 6 h wetness was required for infection at 21 to 30°C and at least 6 to 8 h wetness was required at 15°C. By comparison, the pathogen required 6 to 12 h wetness at 21°C to infect open florets and seed capsules of onion (*Allium cepa* L.) (21), 5 to 7 h of high RH at room temperature to infect gerbera (*Gerbera jamesonii* H. Bolus ex J. D. Hook.) florets (22), and 6 to 12 h at 15 to 30°C to infect flowers and fruit of strawberry (*Fragaria* x *ananassa* Duchesne) (3). Although a single wetness period of greater than 4 to 8 h was not sufficient for *B. cinerea* to infect geranium flowers, this did not preclude possible infection during successive wetness periods of shorter duration. Increase in infection with wetness duration was rapid in open flowers of geranium kept at 15 to

Table 4. Estimated parameter values and coefficients of determination (R^2) from regressions describing relationships between postinoculation wetness duration (W) and estimated (%) incidence of sporulation (Y) of *Botrytis cinerea* in geranium leaves of different ages that were inoculated with a spore suspension of *B. cinerea* (10^6 conidia per ml) or with petals treated with the pathogen (10^5 conidia per ml), and maintained at 25°C[a]

Leaf age (week)	Inoculum type	Regression model[b]	Estimated parameters			
			b_0	b_1[c]	b_2	R^2
1	Conidia	A	−3.35	0.18	...	0.94
	Petals	B	−17.38	2.06	−0.03	0.92
4	Conidia	C	−0.10	0.02	...	0.92
	Petals	B	−4.50	0.35	0.004	0.96
10	Conidia	A	−3.63	0.22	...	0.87
	Petals	B	−16.48	1.98	−0.03	0.93

[a] Data of two independent repetitions of the study did not differ significantly (F test) and were combined for analysis.
[b] Model A, Logit $Y = b_0 + b_1W + e_i$; model B, Logit $Y = b_0 + b_1W + b_2W^2 + e_i$; model C, $Y = b_0 + b_1W + e_i$; in which b_0 to b_2 are estimated parameters, and e_i is the estimated variability associated with the models.
[c] All slopes differed significantly from zero ($P \leq 0.05$).

Table 5. Estimated duration of postinoculuation wetness (W) that resulted in 50% incidence of sporulation of *Botrytis cinerea* (EW_{50}) in geranium leaves of different ages inoculated with a spore suspension of the pathogen (10^6 conidia per ml) or with petals treated with the pathogen (10^5 conidia/ml) and maintained at 25°C

Leaf age (week)	Inoculum type	EW_{50} (h)[a]	Confidence interval (95%)
1	Conidia	22.1	21.5 < W < 22.7
	Petals	10.3	10.0 < W < 12.4
4	Conidia	30.0	29.5 < W < 30.5
	Petals	12.8	12.4 < W < 14.2
10	Conidia	19.3	19.1 < W < 19.5
	Petals	10.2	10.0 < W < 11.7

[a] Values calculated from the regression models (Table 3).

Table 6. Values of estimated parameters (b_0 to b_7) and coefficients of determination (R^2) from a logistic regression equation for sporulation incidence of *Botrytis cinerea* on geranium leaves of different ages as a function of postinoculation wetness duration (W), temperature (T), and WT[a]

Leaf age (week)	Estimated parameters								
	b_0	b_1	b_2	b_3	b_4	b_5	b_6	b_7	R^2
1	10.34	0.15	0.73	0.02	0.02	0.002	0.001	0.0001	0.90
4	3.83	0.05	0.06	0.01	0.001	0.001	0.000	0.00001	0.60
10	5.13	0.00	0.25	0.02	0.01	0.002	0.005	0.0001	0.86

[a] Leaves were inoculated with a suspension of 10^6 conidia of *B. cinerea* per ml. Data of two independent repetitions of the study did not differ significantly (F test) and were combined for analysis. The following model was used: Logit $Y = -b_0 + b_1W + b_2T - b_3W^2 - b_4T^2 + b_5WT^2 + b_6W^2T - b_7W^2T^2$.

30°C. Similar rapid increases were observed in florets of onion and gerbera, and in petals of strawberry (3,21,22). From observations of minimum wetness duration required for infection, slopes of infection incidence in relation to postinoculation wetness periods, and time required for 50% infection, petals and stamens of geranium were considerably more receptive to *B. cinerea* than were sepals, pistils, and pedicels.

Observations also strongly implied that moderate wetness periods and temperatures were highly favorable for infection of geranium leaves by conidia of *B. cinerea*. From response surfaces for sporulation incidence, patterns of relationships of temperature and wetness in relation to infection were similar in leaves of different age groups, but incidence was generally highest, lowest, and intermediate in leaves 1, 4, and 10 weeks old, respectively. The lower receptivity of 4-week-old leaves supported other observations of leaf age in relation to infection (23). Response patterns for leaves were in many respects similar to patterns obtained for flowers. These similarities included the minimum temperature and wetness durations required for infection and the optimum temperature near 21°C. However, infection decline with temperature increase above the optimum was much steeper for leaves than for flowers. Optimum temperatures near 21°C have been reported for infection of several hosts by *B. cinerea* (3,11,21,25,26,31). Low temperature (5 and 10°C.) did not allow infection of leaves or of flowers of geranium in wetness periods of 0 to 24 h, but possibly would do so under longer wetness durations. Conidia of *B. cinerea* are able to germinate at a minimum temperature of 0 to 1°C (10) and were reported to infect gerbera and strawberry flowers at 2 and 4°C, respectively, provided that wetness periods were long (greater than 48 to 72 h) (3,22). Wetness periods required for infection of geranium leaves by conidia of *B cinerea* were shorter, and rate of increase in infection with wetness duration was much higher, than was observed in needles of black spruce (31), the only other host for which quantitative interrelationships of wetness periods and temperature to infection of leaves have, to our knowledge, been reported.

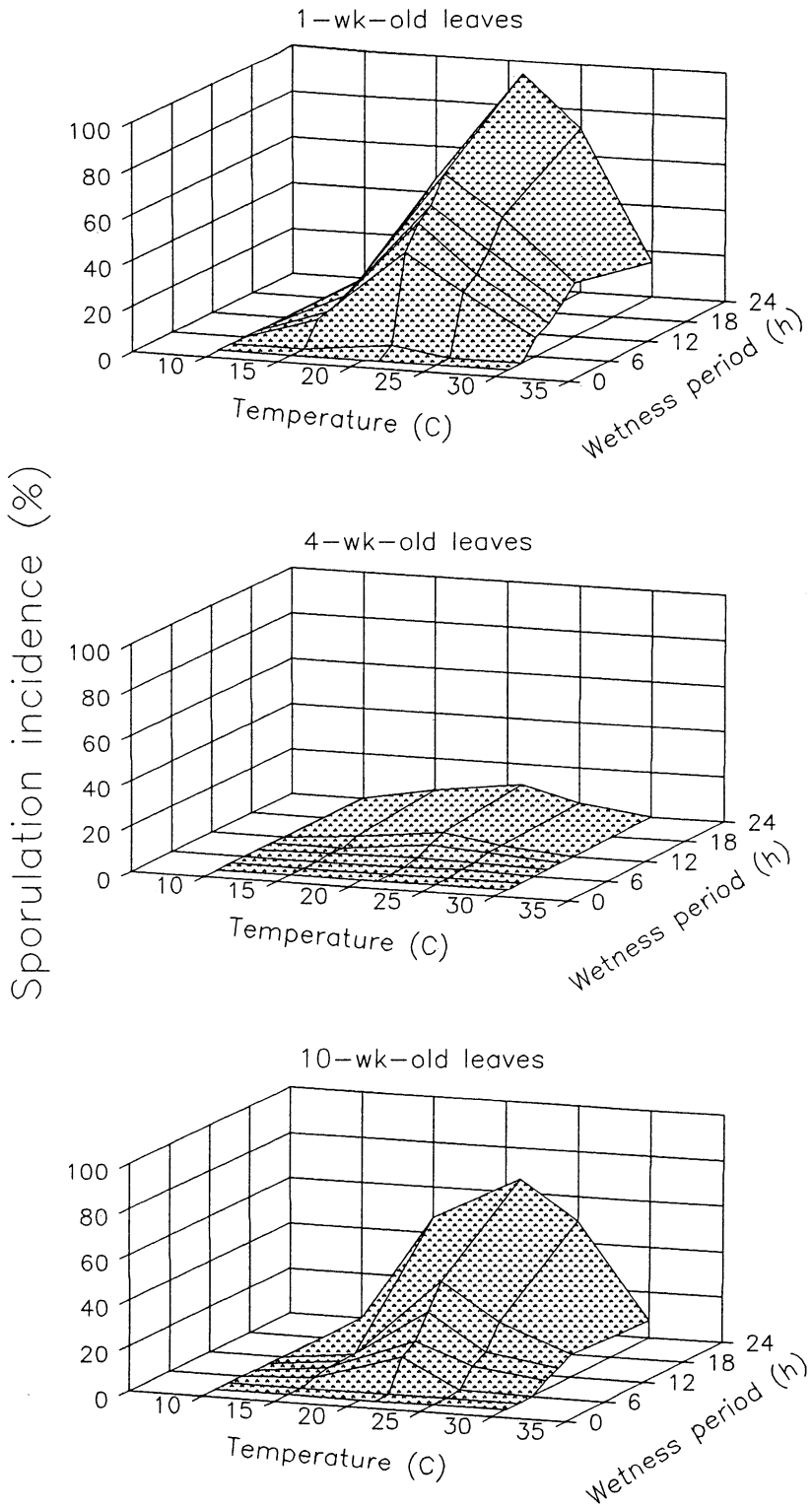

Fig. 4. Effects of duration and temperature of the postinoculation wetness period on sporulation incidence of *Botrytis cinerea* in 1-, 4-, and 10-week-old geranium leaves.

Botrytis cinerea infected geranium leaves more efficiently when conidia of the pathogen were applied to petals that were subsequently placed on leaves than when the conidia were applied directly to leaves, as was found in our other study (23). While minimum wetness periods (at 21°C) required for *B. cinerea* to infect leaves of the three age groups were similar when the leaves were inoculated with treated petals or with conidia, increase in infection incidence with wetness duration was many times more rapid in leaves that were inoculated with petals. This was reflected in slopes of regressions for sporulation incidence as a function of wetness duration, and in estimated wetness periods for 50% infection incidence that were 10.2 to 12.8 h when petal inoculum was used, compared with 19.3 to 30.0 h for conidia. These relationships were found even though the concentration of the conidial suspension applied to petals was 10 times lower than that applied directly on leaves. Petals probably promoted infection of leaves by providing nutrients, serving as a food base for the pathogen, thereby increasing inoculum potential, as has been observed in many other hosts (10,12,23, 27). Leaves could have been infected by conidia on petals or invaded by mycelium of the pathogen in the petals.

Logistic regression models adequately described effects of postinoculation wetness duration and temperature on sporula-

tion incidence and, by inference, infection incidence, in flowers and leaves. The models effectively described wetness duration in relation to infection of sepals, petals, stamens, pistils, and pedicels by conidia of *B. cinerea*, and of leaves by conidia and by the pathogen in inoculated petals. They also adequately described infection incidence of flowers and of young, middle-aged, and old leaves in terms of the duration and temperature of the post-inoculation wetness period. The integrative models for effects of wetness and temperature (Tables 3 and 6) were similar to those used to describe effects of these variables on infection of black spruce by *B. cinerea* (31) and of strawberry flowers and fruits by *Colletotrichum acutatum* J. H. Simmonds and *B. cinerea* (3,30).

Our observations and models have potential application for managing gray mold on geraniums in greenhouses. From the quantitative data, humidity control to avoid wetness periods of ≥4 h is critical to prevent infection. Even more stringent humidity control would be needed should *B. cinerea* be able to infect during successive wetness periods of extremely short duration. Adequate humidity control should often be possible in temperate climates through appropriate heating, ventilation, and air circulation in the greenhouse, and avoidance of overhead irrigation (12). Regimes of low day temperature and high night temperature (5) also may help to avoid reaching the dew point at night. Overlap of temperatures favorable for infection with those optimal for geranium production, which are near 21°C (day) and 16°C (night) for several cultivars (29), precludes temperature regulation as a practical means to directly suppress infection. Used in conjunction with systems to monitor wetness and air temperature in geranium crops (24), the infection models have potential for determining the time of occurrence and severity of infection periods and for predicting when control measures such as fungicide treatments are needed. It would be important, however, to validate the models for prediction accuracy using independent data from greenhouses. The models also could be used to develop rational assays to quantify effectiveness of host cultivars, biological control agents, fungicides, and other means for suppressing infection. Because petals promoted infection of leaves by *B. cinerea*, practices that minimize dispersal of petals onto foliage can be expected to reduce gray mold severity in geranium. Appropriate integration of the epidemiological information into geranium protection programs should substantially improve the management of gray mold.

ACKNOWLEDGMENTS

This research was supported by the Natural Sciences and Engineering Research Council of Canada (grant OGP0006119 to the second author). We thank M. J. Tsujita for valued comments and for making available geranium propagation facilities, and Valk Greenhouses Ltd., Grimsby, Ontario, for providing geranium cuttings.

LITERATURE CITED

1. Berninger, L. M. 1993. Status of the industry. Pages 1-2 in: Geraniums IV. J. W. White, ed. Ball Publishing, Geneva, IL.
2. Brumfield, R. G. 1993. Production costs. Pages 145-156 in: Geraniums IV. J. W. White, ed. Ball Publishing, Geneva, IL.
3. Bulger, M. A., Ellis, M. A., and Madden, L. V. 1987. Influence of temperature and wetness duration on infection of strawberry flowers by *Botrytis cinerea* and disease incidence of fruit originating from infected flowers. Phytopathology 77:1225-1230.
4. Campbell, C. L., and Madden, L. V. 1990. Introduction to Plant Disease Epidemiology. John Wiley and Sons, New York.
5. de Koning, A. N. M. 1988. The effect of different day/night temperature regimes on growth, development, and yield of glasshouse tomatoes. J. Hortic. Sci. 63:465-471.
6. Hausbeck, M. J. 1993. Botrytis blight. Pages 223-228 in: Geraniums IV. J. W. White, ed. Ball Publishing, Geneva, IL.
7. Hausbeck, M. J., and Pennypacker, S. P. 1991. Influence of grower activity and disease incidence on concentrations of airborne conidia of *Botrytis cinerea* among geranium stock plants. Plant Dis. 75:798-803.
8. Hausbeck, M. J., and Pennypacker, S. P. 1991. Influence of time intervals among wounding, inoculation, and incubation on stem blight of geranium caused by *Botrytis cinerea*. Plant Dis. 75:1168-1172.
9. Hausbeck, M. J., and Pennypacker, S. P. 1991. Influence of grower activity on concentrations of airborne conidia of *Botrytis cinerea* among geranium cuttings. Plant Dis. 75:1236-1243.
10. Jarvis, W. R. 1977. *Botryotinia* and *Botrytis* Species: Taxonomy, Physiology, and Pathogenicity. Monograph No. 15. Canada Department of Agriculture, Ottawa, ON.
11. Jarvis, W. R. 1980. Epidemiology. Pages 219-250 in: The Biology of Botrytis. J. R. Coley-Smith, K. Verhoeff, and W. R. Jarvis, eds. Academic Press, London.
12. Jarvis, W. R. 1992. Managing Diseases in Greenhouse Crops. American Phytopathological Society, St. Paul, MN.
13. Louis, D. 1963. Les modalités de la pénétration de *Botrytis cinerea* Pers. dans les plantes. Ann. Epiphyt. (Paris) 14:57-72.
14. Melchers, L. E. 1918. *Botrytis* sp. causing severe injury to flowers and foliage of *Pelargonium hortorum*. Phytopathology 8:76.
15. Melchers, L. E. 1926. Botrytis blossom blight and leaf spot of geranium and its relation to the gray mold of head lettuce. J. Agric. Res. 32:883-894.
16. Moorman, G. W., and Lease, R. J. 1992. Residual efficacy of fungicides used in the management of *Botrytis cinerea* on greenhouse-grown geraniums. Plant Dis. 76:374-376.
17. Moorman, G. W., and Lease, R. J. 1992. Benzimidazole- and dicarboximide-resistant *Botrytis cinerea* from Pennsylvania greenhouses. Plant Dis. 76:477-480.
18. Nelson, K. E. 1951. Factors influencing the infection of table grapes by *Botrytis cinerea* (Pers.). Phytopathology 41:319-326.
19. Peng, G., and Sutton, J. C. 1991. Evaluation of microorganisms for biocontrol of *Botrytis cinerea* in strawberry. Can. J. Plant Pathol. 13: 247-257.
20. Powell, C. C. 1993. Seedling diseases. Pages 215-219 in: Geraniums IV. J. W. White, ed. Ball Publishing, Geneva, IL.
21. Ramsey, G. R., and Lorbeer, J. W. 1986. The role of temperature and free moisture in onion flower blight. Phytopathology 76:612-616.
22. Salinas, J., Glandorf, D. C. M., Picavet, F. D., and Verhoeff, K. 1989. Effects of temperature, relative humidity, and age of conidia on spotting of gerbera flowers caused by *Botrytis cinerea*. Neth. J. Plant Pathol. 95:51-64.
23. Sirjusingh, C., Sutton, J. C., and Tsujita, M. J. 1996. Effects of inoculum concentration and host age on infection of geranium by *Botrytis cinerea*. Plant Dis. 80:154-159.
24. Sutton, J. C., Gillespie, T. J., and James, T. D. W. 1988. Electronic monitoring and use of microprocessors in the field. Page 99-113 in: Experimental Techniques in Plant Disease Epidemiology. J. Kranz and J. Rotem, eds. Springer-Verlag, Berlin.
25. Thomas, C. S., Marois, J. J., and English, J. T. 1988. The effects of wind speed, temperature, and relative humidity on development of aerial mycelium and conidia of *Botrytis cinerea* on grape. Phytopathology 78:260-265.
26. Trolinger, C. J. 1983. Epidemiology of Botrytis blight of greenhouse floral crops. Ph.D. thesis. North Carolina State University, Raleigh, NC.
27. Verhoeff, K. 1980. The infection process and host-pathogen interactions. Pages 153-180 in: The Biology of Botrytis. J. R. Coley-Smith, K. Verhoeff, and W. R. Jarvis, eds. Academic Press, London.
28. Waller, R. A., and Duncan, D. B. 1969. A Bayes rule for the symmetric multiple comparison problem. J. Am. Statist. Assoc. 64: 1484-1499.
29. White, J. W., and Warrington, I. J. 1988. Temperature and light integral effects on growth and flowering of hybrid geraniums. J. Am. Soc. Hortic. Sci. 113:354-359.
30. Wilson, L. L., Madden, L. V., and Ellis, M. A. 1990. Influence of temperature and wetness duration on infection of immature and mature strawberry fruit by *Colletotrichum acutatum*. Phytopathology 80:111-116.
31. Zhang, P. G., and Sutton, J. C. 1994. Effects of wetness duration, temperature, and light on infection of black spruce seedlings by *Botrytis cinerea*. Can. J. For. Res. 24:707-713.