Effects of Temperature and Wetness Duration on Sporulation of *Botrytis cinerea* on Strawberry Leaf Residues

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ABSTRACT

Sosa-Alvarez, M., Madden, L. V., and Ellis, M. A. 1995. Effects of temperature and wetness duration on sporulation of *Botrytis cinerea* on strawberry leaf residues. Plant Dis. 79:609-615.

Disks cut from dead strawberry leaves (autoclaved or air-dried) were inoculated with a conidial suspension of *Botrytis cinerea* (10^6 conidia per ml) and incubated at various temperatures (T) ranging from 5 to 30°C. Sporulation (conidia per cm^2) on leaf disks was determined after exposure to wetness durations (W) of 3 to 11 days. Optimum temperature for sporulation was between 17 and 18°C at all wetness durations. Sporulation levels of 10^5 to 10^7 conidia per cm^2 were observed between 15 and 22°C, after 7 days of continuous wetness. As temperature increased or decreased from the optimum, sporulation decreased for the same wetness durations. Very little sporulation was observed at 25°C and no sporulation was observed at 30°C. Logarithmic polynomial models best described the effect of T and W on sporulation of *B. cinerea* on dead strawberry leaf tissue. Coefficients of determination for data from all repetitions of the experiments were at least 0.81. The latent period of *B. cinerea* on dead leaf tissue was longest at the lowest temperature (6 to 7 days at 5°C) and decreased to <3 days as temperature increased to the optimum (15 to 22°C). Interrupted wet and dry periods of 5, 12, and 24 h directly affected sporulation. Total hours of wetness and the duration of individual wet periods had the greatest effect on the amount of inoculum produced at 20°C.

Additional keywords: *Fragaria* × *ananassa*, gray mold, quantitative epidemiology

Gray mold fruit rot, caused by *Botrytis cinerea* Pers.:Fr., is one of the most important diseases of strawberry (*Fragaria* × *ananassa* Duchesne) worldwide. In Ohio, gray mold is the most serious and common fruit rot disease of strawberries. Yield losses of up to 90% have been reported during wet growing seasons (10).

Potential sources of inoculum of *B. cinerea* include mummified fruit, straw mulch, weed residues, and strawberry leaf residues (15). However, research conducted by Braun and Sutton in Ontario (2) demonstrated that nearly all primary inoculum for gray mold infection of strawberry flowers originates from sporulation of the fungus on dead strawberry leaf residue in plant rows. Latent infections of the fungus can become established in apparently healthy strawberry leaves at any time during the growing season. As infected leaves senesce and die during the late summer and fall, the fungus becomes active, colonizes dead tissue, and eventually sporulates the following spring to produce the inoculum for subsequent infections (3). Observation of leaf residues in commercial fields reveals that sporulation occurs only sporadically (M. A. Ellis, *unpublished*). The effects of environmental conditions, such as temperature and wetness duration, on production of conidia of *B. cinerea* in strawberry leaf residues have not been determined. However, effects of these two environmental variables on infection of strawberry flower and fruit are known (5, 6,11).

Disease forecasting for strawberry fruit rots is a potentially beneficial approach to disease control programs (11) that may lead to reduced fungicide applications. A regression model for predicting infection of strawberry fruit and flowers by *B. cinerea*, based on temperature and wetness period, has been developed based on controlled-environment studies (6). This model assumes that inoculum is always present, which could result in an excessive number of fungicide applications if used in forecasting. Linking of sporulation and infection models could lead to the development of an improved forecasting system for gray mold, once models were properly validated in the field.

This study had two objectives: (i) to determine the effects of temperature and wetness duration on sporulation of *B. cinerea* on strawberry leaf residues under controlled environmental conditions; and (ii) to develop empirical equations to describe the initiation of conidial production and the conidial production as a function of temperature and wetness duration.

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Accepted for publication 4 February 1995.

MATERIALS AND METHODS

Inoculum production. An isolate of *B. cinerea* was obtained from an infected strawberry fruit near Wooster, Ohio. Stock cultures were maintained by transferring mycelial plugs from the edge of actively growing cultures onto fresh potato-dextrose agar (PDA) dishes every 2 weeks. Virulence was maintained by inoculating disks cut from immature strawberry fruit placed on PDA. Plugs cut from the edge of 2-week-old cultures of the fungus were placed in the center of each strawberry fruit disk, and the pathogen was reisolated after it had grown through the disk. This procedure was repeated every 2 to 3 weeks.

Inoculum for all experiments was prepared by placing mycelial plugs of the fungus onto PDA in petri dishes (100 × 15 mm). Culture dishes were incubated at 21°C under continuous fluorescent light at 51 $\mu E\ s^{-1}m^{-2}$ for 14 days. Conidial suspensions were prepared by flooding dishes with a solution of 10 ml of deionized water and Tween 40 (one drop per liter). Mycelia and conidia were detached by rubbing the surface with a glass rod. The resulting suspension was filtered through two layers of tissue paper (Kimwipe, Kimberly-Clark Corp., Roswell, Ga.). The remaining conidial suspension was adjusted to 10^6 conidia per ml by using a hemacytometer, and maintained on ice to preserve inoculum viability until used.

Preparation of strawberry leaf tissue. All sporulation experiments were conducted with dead strawberry leaf tissue. Leaves were collected from strawberry (cv. Midway) plants grown in plastic pots (12 × 10.5 × 15 cm) in a mixture of sand, steam disinfested loam, and peat (1:2:2, by volume) in the greenhouse. Plants were irrigated as needed with deionized water only. Water soluble fertilizer, 20-20-20 (N-P-K) (2.6 g/liter), was applied every 2 weeks. Artificial light was supplied to ensure a 14-h photoperiod. Fully expanded, apparently healthy leaves were harvested and transported to the laboratory.

Leaf tissue was prepared in two ways to obtain dead strawberry leaf tissue. In the first approach, disks (1.5 cm diameter, 1.77 cm^2 area) were cut with a cork borer from fully expanded, mature leaves. The tissue was autoclaved after first placing the leaf disks between two layers of moist filter paper on a galvanized metal screen (20

by 20 cm). Screens with leaf disks were then covered by heavy-duty aluminum foil, and autoclaved at 49°C at 0.12 MPa of pressure for 15 min. In the second approach, disks from air-dried strawberry leaves were obtained from detached leaves that had been pressed in a Bio-Quip (Ben Meadows Co., Atlanta, Ga.) plant press (35 by 46 cm) for 4 days.

Sporulation. In separate experiments, autoclaved and air-dried leaf disks were inoculated by soaking them in the conidial suspension for 5 min. Inoculated disks were then placed on top of individual sterile filter paper disks (1.3 cm diameter) (Schleicher & Schuell, Keene, N.H.) that were saturated with sterile water and placed in petri dishes (100 by 15 mm, 10 disks per dish). Leaf disks were placed on the water-saturated paper disks to maintain free moisture on the disks. Dishes were sealed with Parafilm to prevent desiccation. Five dishes (50 disks) were incubated in controlled-temperature chambers under continuous fluorescent light (45 $\mu E\ s^{-1}\ m^{-2}$) at each of six temperatures: 5, 10, 15, 20, 25, and 30°C. Relative humidity (RH) within the dishes, 5 mm above the leaf disks, was determined with wet-bulb and dry-bulb thermistors after correcting for the lack of aspiration. RH was 100% within the sealed dishes at all temperatures tested.

One dish (10 disks) for each temperature regime was removed and examined after 3, 5, 7, 9, and 11 days of incubation (wetness duration). The mean number of conidia produced per cm^2 of tissue was determined for each leaf disk. Leaf disks were removed from the petri dish and individually placed in test tubes containing 10 ml of water and Tween 40 (1 drop Tween 40 per liter). Disks were then macerated using a glass tube and vortexed for 2 min in order to place conidia in suspension. The concentration of conidia was determined using a hemacytometer. Conidial concentration was determined three times and mean concentration (per cm^2 of leaf disk) for the dish was used for data analysis. Each experiment (temperature/incubation combination) was done three times.

In order to simulate more closely naturally infested strawberry leaf residue, a variation of the second leaf tissue preparation method was used in which air-dried leaves were first colonized by the fungus, then exposed to continuous wetness (labeled air-dried colonized). Fully expanded detached strawberry leaves were dried in a plant press and inoculated with a conidial suspension (10^6 per ml) by soaking them for 5 min in a beaker. Inoculated leaves were placed in a moist chamber for 24 h at 20 to 22°C and ≅100% RH. Free moisture (i.e., wet leaf tissue) was maintained by placing moistened tissue paper in the bottom of the chamber. After 24 h incubation, colonized leaves were air-dried at 25°C for 48 h. The purpose of this procedure was to produce dead strawberry leaf tissues that were colonized by *B. cinerea*, but had not yet sporulated. Air-dried colonized leaves were placed in sealed plastic bags and stored at −10°C until used. Leaf disks (1.5 cm diameter, 1.77 cm^2) were then cut from colonized leaves, placed on sterile filter paper disks in petri dishes, sprayed with deionized water, and incubated under selected temperatures and continuous wetness durations as described previously. However, tests were done at 18 and 22°C in addition to the six temperatures in the other experiments. Conidia per cm^2 were determined using the techniques described previously. This experiment was done three times.

Latent period. Latent period is considered here as the time between inoculation of disks and the first observation of sporulation. Inoculated disks were observed using a stereo microscope for the initiation of conidial production on autoclaved, air-dried, and air-dried colonized leaf disks at different temperatures. Studies were done with the leaf disks separately from the sporulation experiments; the procedures were identical, except that number of conidia were not counted. Observations for the presence of conidiophores with spores (≥1 per dish) were made at 12-h intervals after the initiation of incubation for all temperatures tested.

Alternating wet-dry periods and sporulation. In order to determine the influence of interrupted wetness duration (i.e., alternating wet-dry conditions) on sporulation of *B. cinerea*, air-dried colonized leaf disks (prepared as described previously) were exposed to selected wetness intervals that were separated by selected durations of dry time. Sequences consisted of very short wet periods to very long wet periods. The wet-dry sequences were repeated until approximately 7 days elapsed, at which time sporulation was determined.

Previously inoculated and colonized leaf disks were placed on top of individual sterile filter paper disks saturated with deionized water inside plastic petri dishes (10 disks per dish). Dishes were sealed with Parafilm to prevent desiccation and to maintain high RH (100%) and free moisture on the disks within the dishes. Eight wet-dry sequence combinations were tested: 5 h wet–24 h dry; 12 h wet–12 h dry; 12 h wet–24 h dry; 24 h wet–5 h dry; 24 h wet–12 h dry; 24 h wet–24 h dry; 6 days (144 h) dry–24 h wet; and 7 days (168 h) wet (control). To initiate the dry period, lids of petri dishes were removed 1 h before completion of the wet cycle. To initiate the wet cycle, opened dishes with the leaf disks were sprayed with deionized water and sealed with Parafilm. All treatments were incubated under continuous fluorescent light at 45 $\mu E\ s^{-1}\ m^{-2}$ at 20°C for 7 days. After lids were removed from petri dishes, RH decreased from 100% and stabilized at 57% (based on a wet-bulb, dry-bulb sensor). Conidia per cm^2 for each leaf disk were calculated by determining conidial concentration three times with a hemacytometer. All wet-dry sequence treatments were done three times. The mean concentration of conidia per cm^2 of leaf disks in each petri dish for each repetition of each wet-dry combinations was calculated and used for data analysis.

Data analysis. Analysis of variance (ANOVA) was used to determine the effect of temperature (T), wetness duration (W), and their interaction (WT) on conidia per square centimeter (Y) of leaf tissue. The logarithmic transformation was used to stabilize variances of Y. Separate analyses were done for autoclaved, air-dried, and air-dried colonized leaf disks experiments.

Regression analysis was performed to determine the relationships between Y and T and W. The selected regression equation for sporulation had to account for an optimum-type relationship between Y and T, such that Y increases to a maximum (at the optimum temperature), and then decreases, and a relation in which Y increases with increasing W, but predicted Y is constrained to be not less than 0.0, regardless of the value of W. The regression models were evaluated based on four criteria: (i) the randomness and normality of the residuals; (ii) significance of the model coefficients (estimated parameters); (iii) coefficient of determination (R^2); and (iv) coefficient of determination adjusted for degrees of freedom (R_a^2) (7).

A logarithmic model was tested of the general form $\log(Y) = f(T,W)$ (eq. 1) in which $f(T,W)$ represents a function of T and W that consisted of linear combinations of some of the following terms: W, WT, WT^2, WT^3, WT^4, $\log(W)$, $\log(W)T$, $\log(W)T^2$, $\log(W)T^3$, and $\log(W)T^4$. An all-possible-subsets regression was used to find the best combination of terms that met the evaluation criteria. A generalized form of the Analytis Beta model (1) was evaluated also, but the model did not provide an acceptable fit to the data and results are not shown. The acceptable regression models were fitted to the data from each repetition of the experiments as well as the combined data from all repetitions. An F test was used to determine whether the regression results for the repetitions were significantly different (16).

For latent period (L; days), polynomial models were tested to determine the relationship between L and T. The models were of the same type as eq. 1 except that W was not included since, in effect, time was being measured. The criteria for evaluating the models were the same as for sporulation models.

ANOVA was used to determine the effect of alternating wet-dry periods on log-transformed number of conidia per cm^2 of leaf disks [$\log(Y)$]. The least significant

difference (LSD; $P = 0.05$) was used to determine differences among treatments. Also, regression analysis was used to determine the relationship between log(Y) and total hours of wetness over the 7 days.

RESULTS

Sporulation. A general increase in conidial density with increased wetness duration at temperatures less than 25°C was observed for all three experiments (autoclaved, air-dried, and air-dried colonized leaf disks) (Fig. 1). Additionally, at a given wetness duration for all data sets, sporulation increased with increasing temperature up to 15 to 22°C, and then decreased at higher temperatures. Conidia were produced at all temperatures studied except 30°C. At 5 and 10°C, conidia generally were not observed until several days after they were first observed at higher temperatures (also, see latent period section).

ANOVA indicated that temperature, wetness duration, and their interaction all significantly ($P < 0.01$) affected the log-transformed conidial density for the three experiments. The interaction indicated that the effect of temperature on sporulation was dependent on wetness duration, or equivalently, that the effect of wetness duration was dependent on temperature.

Autoclaved leaf disks had the highest observed conidial production at 15°C (Fig.

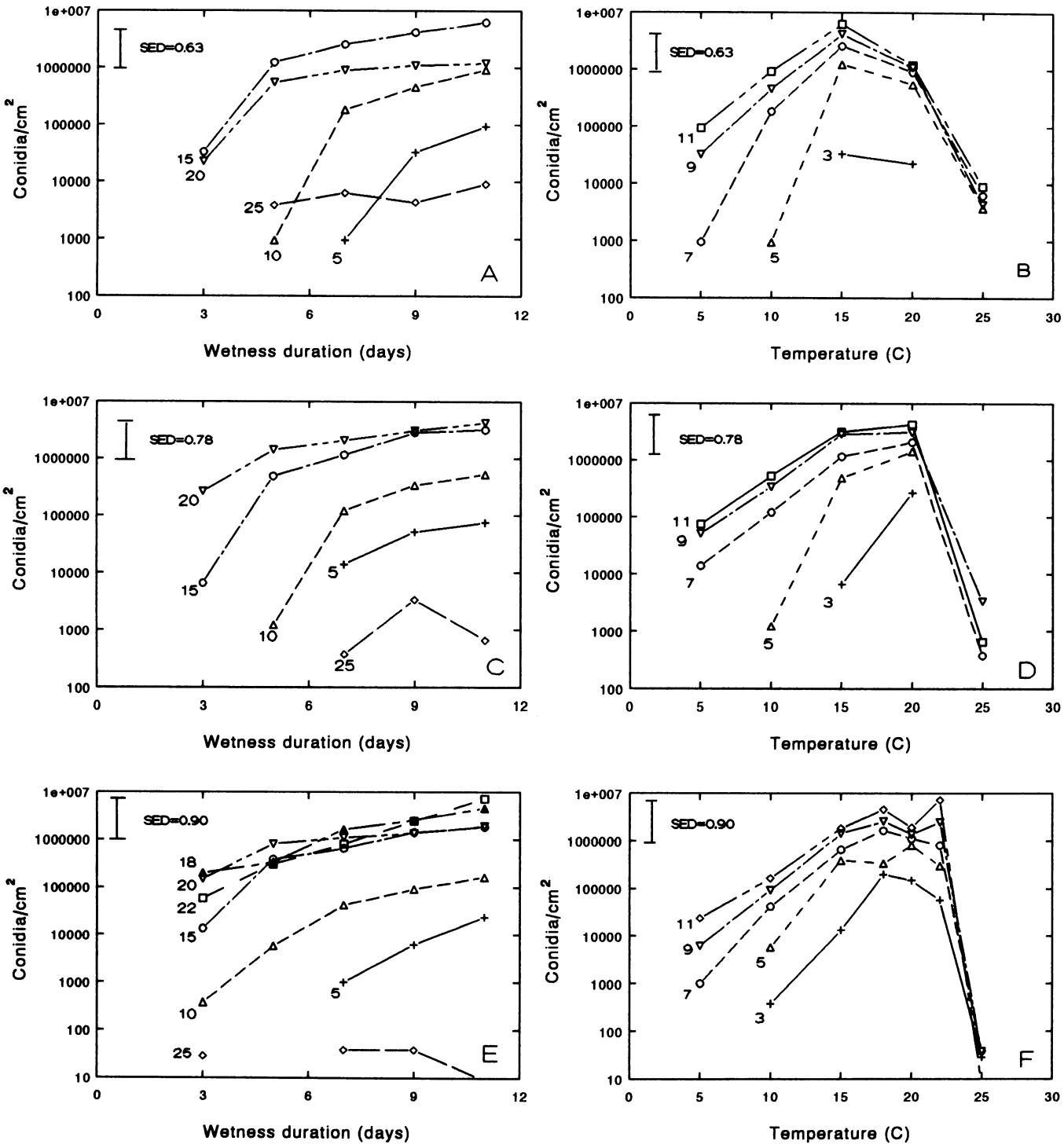

Fig. 1. The effect of wetness duration (incubation time) (**A, C, E**) and temperature (**B, D, F**) on the sporulation of *Botrytis cinerea* on inoculated leaf tissue. Labeled lines represent temperature (Celsius) in the left-hand graphs and wetness duration (days) in the right-hand graphs. Points represent the means from three repetitions of the experiment. Results are for three experiments: (**A, B**) autoclaved leaf tissue; (**C, D**) air-dried; and (**E, F**) air-dried, colonized leaf tissue. (SED = standard error of the difference of means in log units.)

1A), with, for example, an average of 2.6 × 10^6 conidia per cm^2 at 7 days of wetness. There was no sporulation on autoclaved tissue incubated at 5, 10, and 25°C after 3 days of wetness (Fig. 1 A, B). Very low and relatively equal numbers of conidia were produced at 25°C at all wetness durations.

For air-dried leaf disks, the highest level of conidial production was observed at 15 and 20°C (Fig. 1C). Similar to the experiment with the autoclaved leaf disks, no sporulation was observed at 5, 10, or 25°C after 3 days. At 15 and 20°C, about 10^6 conidia per cm^2 were observed after 7 days of wetness duration. At the low temperature of 5°C, sporulation was finally observed after 7 days of wetness. A slow increase in the production of conidia (on a log scale) was observed beyond 7 days of wetness duration at all temperatures tested. As with autoclaved disks, very low numbers of conidia were produced at 25°C at all wetness durations, and no sporulation was observed at 30°C (Fig. 1C, D).

For air-dried leaf disks colonized previously with *B. cinerea* and incubated for 24 h (air-dried colonized), a high level of conidial production was observed between 15 and 22°C at all wetness durations tested (Fig. 1E). No sporulation was observed at 5°C before 7 days of wetness. For temperatures from 10 to 25°C, sporulation was observed after 3 days of wetness (Fig. 1F). At 7 days of wetness, sporulation increased from 10^3 at 5°C to about 10^6 between 18 and 22°C, and decreased to 38 conidia per cm^2 at 25°C (Fig. 1E). There was a slow increase in the number of conidia (on a log-scale) at temperatures of 18 to 22°C between 7 and 11 days of wetness. However, at 25°C conidial production did not increase over time and no sporulation was observed at 30°C. (Fig. 1 E, F).

Regression analysis of sporulation. A logarithmic polynomial model of the form $\log(Y) = b_0 + b_1\log(W)T^2 + b_2\log(W)T^3$ (eq. 2) was found to best describe sporulation (Y = conidia per cm^2) on autoclaved leaf tissue (Fig. 1A, B). Each b is an estimate of an unknown parameter; all estimated parameters were significantly different from zero ($P < 0.01$) (Table 1). The model indicated a quadratic and cubic relationship between T and $\log(Y)$, reflecting the increase in sporulation to a maximum and then a decline as temperature increased. Wetness duration was log-transformed because the rate of increase in sporulation declined as W increased. That is, $\log(Y)$ increased rapidly at first, but the increase was lower at longer wetness durations. Also, there was an interaction between $\log(W)$ and T^2 and between $\log(W)$ and T^3. The R^2 and R_a^2 values for data from all repetitions of the experiment combined were 0.82 and 0.81, respectively. The R^2 values were also high for each repetition of the experiment (Table 1). The F test indicated that there were no differences in the regression results among the repetitions ($P > 0.50$). Equation 2 can be rewritten as $\log(Y) = b_0 + \log(W)[b_1T^2 + b_2T^3]$ (eq. 3), which shows how the rate of increase in $\log(Y)$ with $\log(W)$ was a function of temperature (i.e., a slope of $b_1T^2 + b_2T^3$). Optimum temperature for sporulation is given by $-2b_1/3b_2$, which equals 17°C for the pooled data set.

For air-dried plant tissue (Fig. 1C, D), the best model was of the form $\log(Y) = b_0 + b_1\log(W)T^3 + b_2\log(W)T^4$ (eq. 4). The R^2 and R_a^2 values were about 0.85 (Table 2). Properties of eq. 4 are similar to that of eq. 2, except that the cubic and quartic temperature terms were significant instead of the quadratic and cubic terms. For this equation, the rate of increase in $\log(Y)$ with $\log(W)$, i.e., slope, was $b_1T^3 + b_2T^4$. Optimum temperature for this equation is given by $-3b_1/4b_2$; for the combined data, this was 18°C. Results from individual repetitions were very similar and there was no significant differences among repetitions ($P > 0.50$) (Table 2).

For air-dried plant tissue that was first colonized for 24 h (Fig. 1E, F), the best regression model was the same as eq. 4. The R^2 and R_a^2 values were 0.81 and 0.81, respectively (Table 2). Optimum temperature was 18°C, based on the combined data. As with the other experiments, there were no differences in regression results among the repetitions of the experiment ($P > 0.50$). Predicted levels of sporulation, based on combined data, for this experiment are given Figure 2.

Latent period. In all three experiments, duration of latent period was longest at the lowest temperature tested (5°C) (Fig. 3). At this temperature, *B. cinerea* began sporulating after 6 to 7 days of wetness.

Table 1. Estimated parameters of eq. 2 (see text) for the relationship between log-transformed conidia of *Botrytis cinerea* per cm^2 on inoculated autoclaved strawberry leaf tissue [$\log(Y)$] and temperature (T) and wetness duration (W) during incubation, together with the coefficient of determination (R^2), R^2 adjusted for degrees of freedom (R_a^2), standard error about the regression line (s), and degrees of freedom (df)

	Regression terms						
Data set	Intercept	$\log(W)T^2$	$\log(W)T^3$	R^2	R_a^2	s	df
Repetition 1	3.72	0.0033	–0.0014	0.854	0.837	0.48	17
	(0.277)[a]	(0.0039)	(0.00015)				
Repetition 2	3.12	0.037	–0.0015	0.841	0.821	0.53	17
	(0.306)	(0.0043)	(0.00016)				
Repetition 3	3.10	0.0038	–0.0015	0.803	0.778	0.55	16
	(0.390)	(0.0051)	(0.00019)				
Combined data	3.35	0.036	–0.0014	0.819	0.813	0.51	61
	(0.174)	(0.0023)	(0.00009)				

[a] Numbers in parenthesis are standard error of the estimated parameter.

Table 2. Estimated parameters of eq. 4 (see text) for the relationship between log-transformed conidia of *Botrytis cinerea* per cm^2 on inoculated air-dried[a] and air-dried colonized[b] strawberry leaf tissue [$\log(Y)$] and temperature (T) and wetness duration (W) during incubation, together with the coefficient of determination (R^2), R^2 adjusted for degrees of freedom (R_a^2), standard error about the regression line (s), and degrees of freedom (df)

Experiment/	Regression term						
data set	Intercept	$\log(W)T^3$	$\log(W)T^4$	R^2	R_a^2	s	df
Air-dried							
Repetition 1	4.47	0.0017	–0.000073	0.913	0.898	0.35	12
	(0.189)[c]	(0.00017)	(0.0000069)				
Repetition 2	3.97	0.0018	–0.000075	0.868	0.851	0.47	15
	(0.243)	(0.00021)	(0.0000082)				
Repetition 3	4.36	0.0016	–0.000066	0.813	0.788	0.52	15
	(0.238)	(0.00022)	(0.0000089)				
Combined data	4.26	0.0017	–0.000072	0.854	0.849	0.44	53
	(0.125)	(0.00011)	(0.0000043)				
Colonized							
Repetition 1	3.02	0.0027	–0.00012	0.831	0.820	0.82	30
	(0.307)	(0.00026)	(0.0000010)				
Repetition 2	2.92	0.0025	–0.00010	0.840	0.830	0.66	30
	(0.249)	(0.00021)	(0.0000080)				
Repetition 3	3.11	0.0026	–0.00011	0.800	0.785	0.75	44
	(0.266)	(0.00021)	(0.0000080)				
Combined data	3.02	0.0026	–0.00011	0.812	0.809	0.75	110
	(0.159)	(0.00013)	(0.0000050)				

[a] Leaf disks were cut from leaves that were air-dried in a plant press for 4 days, then inoculated and incubated immediately .

[b] Leaves were air-dried in a plant press for 4 days, then inoculated with a suspension of *B. cinerea* for 24 h, and redried for 48 h. Leaf disks were then cut and incubated.

[c] Numbers in parenthesis are standard error of the estimated parameter.

Generally, there was a decrease in latent period as temperature increased toward the optimum for production of conidia. The shortest latent period was observed at temperatures between 15 and 22°C. Sporulation was observed at these temperatures after 2.5 to 3 days. At 25°C, latent period increased on air-dried and air-dried colonized leaf tissue; however, the latent period remained low for autoclaved tissue. No sporulation ever occurred at 30°C.

The relationship between latent period (L) and temperature (T) between 5 and 25°C was described best by a regression equation of the form $L = b_0 + b_1T + b_2T^3$ (eq. 5). All estimated parameters were significantly different from zero ($P < 0.05$) (Table 3). The coefficients of determination (R^2) were 0.92 to 0.99 on autoclaved, air-dried, and air-dried colonized leaf tissue. Coefficients of determination adjusted for degrees of freedom (R_a^2) were between 0.84 and 0.97. For the data combined for the three treatments, the R^2 and R_a^2 values were 0.85 and 0.83, respectively, indicating that the equation adequately explained the data. However, there were significant differences in regression results among the tissue types ($P = 0.04$), primarily reflecting the differences in latent period at 25°C.

Alternating wet-dry periods and sporulation. Conidial production of *B. cinerea* on air-dried colonized leaf tissue was affected by alternating wet and dry conditions (i.e., interrupted wetness periods) at 20°C. The highest level of sporulation was observed in the continuously wet control (Table 4). Of the other treatments tested, exposure to long periods of wetness (24 h) followed by exposure to short dry periods (5 h) resulted in the highest level of conidial production, next to the continuously wet control. A decrease in the number of conidia produced was observed as dry periods increased in duration to 12 and 24 h. Likewise, increasing the wet period from 5 to 12 to 24 h following 24 h dry periods resulted in a corresponding increase in sporulation at the end of the 7-day experiment. With equal periods of wet and dry hours, more sporulation occurred following 24-h sequences than 12-h sequences, indicating duration of continuous wet periods affects sporulation. No sporulation was observed for the sequence of 5 h wet–24 h dry or 144 h dry–24 h wet, indicating that short periods of wetness were not sufficient for sporulation when temperature was near an optimum.

In general, total hours of wetness during the 7 days (W_T) was positively related to log(Y). For the treatments in which sporulation was observed, conidia production was described by the equation $\log(Y) = 3.25 + 6.1\times10^{-7}W_T^3$ (eq. 6). This equation had an R^2 of 0.86 and the estimated coefficients were significantly different from 0 ($P < 0.01$). There were no significant differences among the repetitions ($P > 0.50$). At a given W_T, deviation of points from eq. 6 was directly proportional to the length of the individual wet and dry periods.

By substituting W_T for the various treatments into eq. 4 (with parameter estimates for pooled data in Table 2), sporulation was predicted by supposing continuous wetness [= $\log(Y)_c$]. The observed sporulation for the wet/dry sequences could be related to this predicted variable with the equation $\log(Y) = -7.03 + 2.22 \log(Y)_c$ (eq. 7), which had an R^2 of 0.90 for the pooled data. Based on this equation, fewer conidia were produced with alternating wet/dry periods than would be expected from the continuous wet conditions, until predicted sporulation reached $10^{-7.03/(1-2.22)} = 7.2 \times 10^5/cm^2$. Based on eq. 6, this would occur at 6.8 days of wetness, about the length of the experiment.

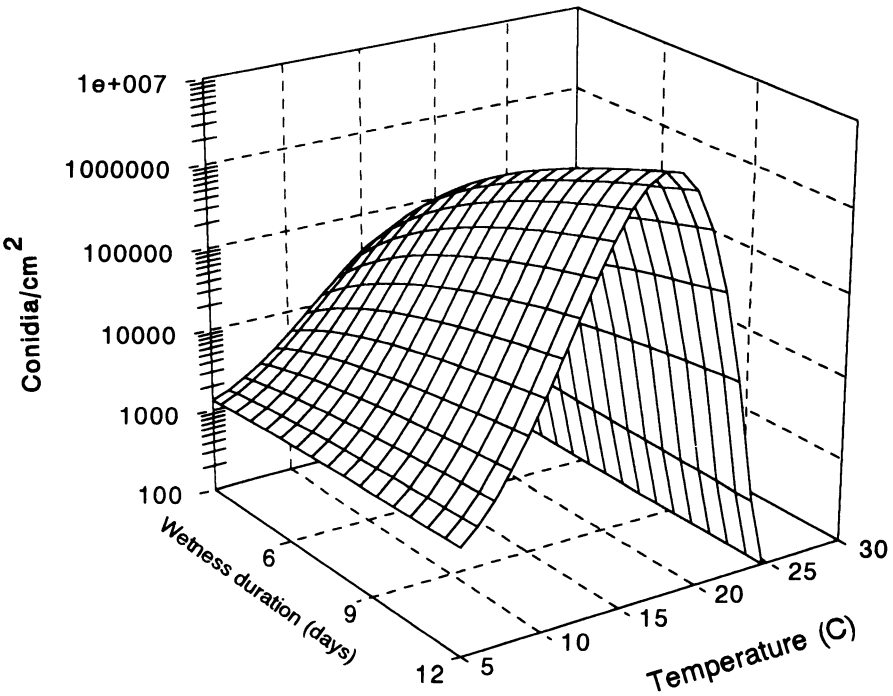

Fig. 2. Effects of wetness duration and temperature on the predicted level of sporulation of *Botrytis cinerea* on inoculated air-dried colonized leaf tissue. Response surface was generated from eq. 4 with the estimated parameters for combined data in Table 2.

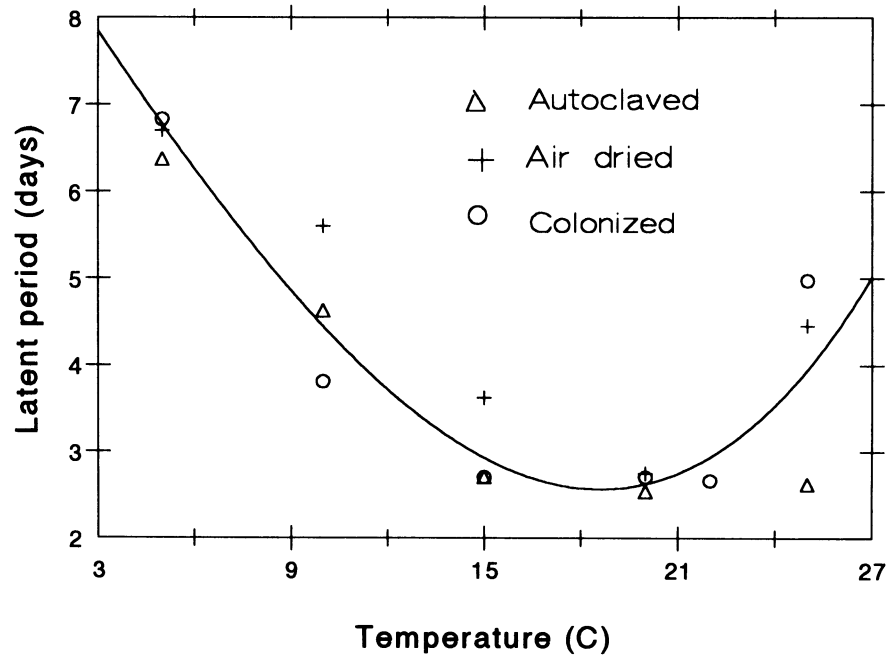

Fig. 3. Relationship between latent period of *Botrytis cinerea* and temperature on inoculated autoclaved, air-dried, and air-dried cololonized leaf tissue. The predictive curve was generated from eq. 5 (see text).

DISCUSSION

It is well established that latent infections by *B. cinerea* occur in strawberry fruit (4,12,13,14,17,22), resulting from flower infections. More recently, Braun and Sutton (3) reported that latent infections by *B. cinerea* commonly occur within strawberry leaves. They observed mycelial fragments of *B. cinerea* within the epidermal cells of apparently healthy strawberry leaves. The fungus then colonizes dead leaf tissue and eventually sporulates with the proper environmental conditions. This sporulation results in primary inoculum for flower and fruit infection. Braun and Sutton (2) estimated the potential inoculum of *B. cinerea* by counting the conidiophores on leaf residues collected from a commercial strawberry field. Assuming that each conidiophore produces 10^3 conidia, they estimated inoculum density of 10^5 to 10^7 conidia per m^2, including the area within fields without colonized leaf residue. However, no quantitative data related to the effects of environmental conditions on sporulation were determined.

Results from our work provide quantitative data on the effect of controlled temperature and wetness duration conditions on the sporulation of *B. cinerea* on dead strawberry leaf tissue. Under temperature conditions near the optimum (15 to 22°C) for sporulation, large numbers of conidia were produced, with 10^4 or more conidia per cm^2 after 3 days of wetness to nearly 10^7 conidia per cm^2 after 11 days. Results found here are in qualitative agreement with Jarvis (13) who reported optimum temperatures for sporulation on PDA as 15 to 20°C. Our results indicate that sporulation did not start until at least 2.5 days of wetness. At some temperatures, sporulation did not occur until a few days later. For all temperatures studied, except 25 and 30°C, conidia production increased as wetness duration increased. Conidia production was observed at temperatures as low as 5°C. At 25°C, no increase in spore production over time was found; no sporulation of *B. cinerea* was observed at 30°C. Although this high temperature inhibited the sporulation of *B. cinerea* on dead leaf tissue, low levels of infection occur on strawberry flowers and fruit at 30°C (5). Moreover, a substantial level of flower infection occurs at 25°C (5,6). Previous studies have been conducted to study the effect of temperature and RH on mycelial growth of *B. cinerea* (13). Other workers have found the optimum temperature for mycelial growth to be in the range of 20 to 25°C (21). Van den Berg et al. (21) reported that the optimum conditions for development of gray mold were 20°C and >93% RH. They also reported mycelial growth of *B. cinerea* in a temperature range of –0.8 to 25°C on PDA under conditions of high RH.

Logarithmic polynomial models (equations 2 and 4) best described the effect of temperature and wetness duration on the sporulation of *B. cinerea* on dead strawberry leaf tissue. R^2 and R_a^2 values for the combined data exceeded 0.80 for autoclaved, air-dried, and air-dried colonized leaf tissue, indicating a substantial proportion of the variation in the log-transformed conidia per cm^2 could be explained by T and W in a controlled environment (16) and that all terms in the models were significant. Similarly, a polynomial model precisely represented latent period as a function of T.

Previously, in controlled-environment studies, the effects of temperature and wetness duration on the infection of strawberry flowers and fruit by *B. cinerea* was quantified (5,6). Bulger et al. (6) developed a polynomial model to estimate or predict infection of inoculated strawberry flowers and fruit by *B. cinerea*, based on T and W. Use of this model or similar ones in a predictive system for disease management requires the assumption that *B. cinerea* inoculum is always present in the field. However, this is clearly not always the case; visual observations in the field reveal that sporulation occurs sporadically (M. A. Ellis, *unpublished*). In the absence of inoculum, forecasting disease increase based on predicted infection periods would be ineffective. Thus, knowledge related to the environmental conditions that coincide with the development of primary inoculum of *B. cinerea* should be useful in understanding the epidemiology of this disease. Although it is well established that high RH and wind speed have a large influence on sporulation of *B. cinera* on other crops (e.g., 20), these factors were not studied here. Little air movement occurs within the crop canopy near the soil surface where the leaf residue occurs. Moreover, sporulation is observed in the field in Ohio only after long wetness periods and rain (M. A. Ellis, *unpublished*). However, based on results from other crops, we expect that RH affects sporulation and feel that more studies are warranted. Recently, Wilcox and Seem (23) developed a regression model, based on field observations, to predict the proportion of harvested fruit that were infected by *B. cinerea*. Their model showed a positive relationship between hours of high RH and surface wetness and gray mold incidence. Presumably, their model combines the effects of inoculum production and infection on disease development, although these individual

Table 3. Estimated parameters of equation 5 (see text) for the relationship of latent period of *Botrytis cinerea* on inoculated autoclaved, air-dried,[a] and air-dried colonized[b] strawberry leaf tissue and temperature (T) and wetness duration (W) during incubation, together with the coefficient of determination (R^2), R^2 adjusted for degrees of freedom (R_a^2), standard error about the regression line (s), and degrees of freedom (df)

	Regression terms						
Data set	Intercept	T	T^3	R^2	R_a^2	s	df
Autoclaved	9.44	–0.490	0.000451	0.920	0.840	0.63	2
	(1.08)[c]	(0.119)	(0.000148)				
Air-dried	8.63	–0.452	0.000341	0.985	0.971	0.29	2
	(0.498)	(0.055)	(0.000068)				
Air-dried colonized	10.22	–0.701	0.000776	0.970	0.955	0.37	3
	(0.596)	(0.062)	(0.000079)				
Combined data	9.509	–0.560	0.000540	0.852	0.831	0.67	13
	(0.648)	(0.070)	(0.000088)				

[a] Leaf disks were cut from leaves that were air-dried in a plant press for 4 days, then inoculated and immediately incubated.
[b] Leaves were air-dried in a plant press for 4 days, then inoculated with a suspension of *B. cinerea* for 24 h, and redried for 48 h. Leaf disks were then cut and incubated.
[c] Standard error of estimated parameter in parenthesis.

Table 4. Mean of the number of *Botrytis cinerea* conidia produced per cm^2 on inoculated air-dried colonized[a] strawberry leaf tissue following alternating wet-dry periods at 20°C for approx. 7 days

Treatment	Mean (conidia per cm^2)[b]	Standard error	Mean [log(conidia+1)]
5 h wet–24 h dry	0	0	0
12 h wet–12 h dry	1.9×10^3	1.2×10^2	3.28
12 h wet–24 h dry	5.4×10^3	2.0×10^3	3.67
24 h wet–5 h dry	2.0×10^5	1.9×10^4	5.30
24 h wet–12 h dry	1.3×10^4	5.4×10^3	4.00
24 h wet–24 h dry	1.6×10^4	7.8×10^3	4.09
144 h dry–24 h wet	0	0	0
168 h wet	1.4×10^6	2.7×10^5	6.13
LSD[c] ($P = 0.05$)			0.37

[a] Leaves were air-dried in a plant press for 4 days, then inoculated with a suspension of *B. cinerea* for 24 h, and redried for 48 h. Leaf disks were then cut and incubated.
[b] Mean number of conidia produced over three repetitions.
[c] Least significant difference.

components were not studied.

Both the frequency and duration of wetness events vary greatly during a growing season, even though controlled-environment studies are generally conducted under conditions of continuous wetness (5, 18). It is anticipated that interrupted wetness periods will affect number of conidia produced (18) even though this has rarely been studied. Results obtained here indicate that although one can generally predict sporulation based on total hours of wetness during the split wet periods (eq. 6), duration of the individual wet periods and dry periods (e.g., 5 h, 12 h) directly affected sporulation at a temperature near the optimum. That is, variation from the prediction line of eq. 6 could be attributed to the length of the individual wet and dry periods. Similar results were found for infection of different plant species by *Cercospora carotae, C. kikuchii,* and *Coccomycetes hiemalis* (8,9, 19). These studies show the complicated relationship between environmental conditions and sporulation.

Our results may be useful in predicting sporulation events of *B. cinerea* or identifying periods when the risk is high. When combined with a predictive model for flower and fruit infection (5,6,11), linked to results for other environmental variables, or used in conjunction with alternative gray mold prediction equations (23), this information could contribute to the development of an effective disease forecasting system. Further studies are needed to validate the effectiveness of these results and models in the field.

ACKNOWLEDGMENTS

Portion of a M.S. thesis submitted to The Ohio State University by the first author. We thank L. L. Wilson for technical assistance. This research was supported by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University, and a gift from the North American Strawberry Growers' Association to the Ohio Agricultural Research and Development Center. Manuscript No.16-95.

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