Influence of Temperature on Apothecial Development and Ascospore Discharge by *Blumeriella jaapii*

S. M. GARCIA and A. L. JONES, Department of Botany and Plant Pathology and the Pesticide Research Center, Michigan State University, East Lansing 48824-1312

ABSTRACT

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Cherry leaves bearing apothecia of *Blumeriella jaapii* with either immature or mature asci were collected from a sweet cherry (*Prunus avium*) orchard near East Lansing, Michigan, for experiments on apothecial development and ascospore discharge, respectively. Disks cut from the leaves were incubated at six temperatures in experiments on apothecial development (4–24 C), and on ascospore discharge (8–30 C). Microscopic examination of crushed apothecia was used to evaluate changes in maturity. The number of asci per apothecium and the percentage of mature plus discharged asci after 7 days of incubation were related to temperature with quadratic regression analyses. The predicted optimum temperature for development of asci was 13.3 C, and the predicted optimum for apothecial development was 16.6 C. Temperatures of 4 and 24 C were unfavorable for apothecial development. The percentage of ascospores discharged from apothecia increased with increasing temperature over a range of 8–30 C.

Cherry leaf spot, caused by *Blumeriella jaapii* (Rehm) Arx, is a major disease of sour and sweet cherry (*Prunus cerasus* L. and *P. avium* (L.) L.) throughout Michigan's cherry-growing region. The fungus overwinters in infected leaves as stroma-like structures that give rise to apothecia in the spring (1,5). The apothecia mature and ascospores are discharged during periods of wet weather beginning with the blossoming stage of cherry tree development. Risk from primary infection continues until the supply of ascospores is exhausted, usually by early summer. Secondary infections are initiated by conidia produced from late spring to early autumn. Cherry leaf spot is effectively controlled with several types of fungicides applied on a 7–10-day schedule (8). Fungicide control programs are initiated when the first susceptible leaf tissue is present (about the petal fall stage of blossom bud development). Five to seven applications of fungicide are applied per season (8,9).

Field observations indicate that temperature and moisture in spring govern the rate of development of the apothecia (1,5,10,11). If temperatures are 15–21 C and the overwintering leaves are wet for 1–2 days, the stroma develops rapidly and apothecia are produced in 24–48 hr (10). Moisture and temperature are also cardinal factors in the maturation of apothecia and the discharge of ascospores. Ascospore discharge begins when mature apothecia become thoroughly wet. Discharge may occur at temperatures ranging from 1 to 36 C, but discharge is greatest at temperatures above 16 C, less rapid at 12 C, and rare at 4–8 C (11).

Precise knowledge of ascospore maturity is important where growers use information on ascospore maturity or discharge to modify the timing of spray applications in calendar-based schedules. Several approaches that are used to evaluate the progress of ascospore maturation in pseudothecia of *Venturia inaequalis* (Cooke) G. Wint., such as microscopic examination of crushed pseudothecia (4,14) and the quantification of ascospores collected in spores traps (13), are also useful for evaluating the progress of ascospore maturation in apothecia of *B. jaapii* (3,6,8). However, these methods are time-consuming and labor-intensive. A better understanding of the effects of temperature on apothecia development and ascospore discharge is needed to devise procedures for predicting ascospore maturity and discharge. The objective of this study was to determine the effects of temperature on apothecial development and ascospore discharge.

MATERIALS AND METHODS

Effect of temperature on apothecial development. Dry sweet cherry leaves with immature apothecia of *B. jaapii* were collected at bud swell (4 April 1991) from beneath trees of the cultivar Hedelfingen in an orchard at the Botany and Plant Pathology Research Farm, Michigan State University, East Lansing. The leaves were divided into two sets. One set was used immediately, the second after 5 days of storage at 4 C.

Disks, 1 cm in diameter, were cut from the leaves with a cork borer. Moist cellulose sponges were placed in the bottom of 12 plastic boxes (12.5 × 8 × 5.5 cm), and 10 leaf disks were placed

Present address of first author: INIA Las Brujas, C. Correo 33085, Las Piedras, Uruguay.

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on the surface of the sponge in each box. Moisture from the sponges was adequate to maintain the leaf disks in a pliable condition without water-soaking throughout the experiment. The boxes were enclosed in plastic bags and placed in incubators in the dark at 4, 8, 12, 16, 20, and 24 C.

At weekly intervals, a piece of leaf tissue bearing apothecia was removed from each of the 10 disks at each temperature and fixed in a mixture of 2-propanol, water, propionic acid, and formaldehyde (45:45:5:5, v/v). The leaf tissue was examined with a dissecting microscope at 25×. Two apothecia near the center of the microscope field were removed with a dissecting needle, transferred to a drop of lactophenol with cotton blue on a glass slide, crushed, and examined with a compound microscope at 200×. Twenty apothecia per replicate were examined for each incubation temperature.

Asci in each apothecium were rated individually based on their stage of maturity as follows: immature = no ascospores present; mature = some to all ascospores delimited; and discharged = ascospores not present and the apex of the ascus open (Fig. 1). The number of asci in each maturity class was determined weekly, and the results were presented as a percentage of the total number of asci per apothecium. Weekly observations were continued until the apothecia disintegrated.

The experiment was conducted in a completely randomized design with two replications for each temperature treatment, and was repeated once. Data from each run were analyzed with regression analysis (12).

Effect of temperature on ascospore discharge. Overwintered leaves with mature apothecia were collected on 26 April and 5 May from the orchard used in the first experiment. Leaf disks were cut with a cork borer, and 20 disks were placed in each box as described above. The boxes enclosed in plastic bags were held at room temperature for 2 hr. The disks were transferred to 250-ml beakers coated with dichlorodimethlysilane to prevent ascospores from sticking to the inside surface of the beakers. The beakers contained 75 ml of distilled water adjusted to 8, 12, 16, 20, 24, and 30 C. Temperature-controlled air from within each incubator was bubbled through the water in the corresponding beaker for 2 hr with a Dinatomic Hi-Tech P-310 pump; the disks were removed from the beakers; and the water along with water used to rinse the beakers was filtered through a 47-mm cellulose acetate grid filter of 0.8-μm pore size. Each filter was cut in half, and the halves were mounted in lactophenol with cotton blue on microscope slides. Ascospores were counted in five grid-squares chosen at random on each half filter. To compare data between experiments, the replication with the highest number of ascospores in each experiment was used to adjust the data for the remaining replications proportionally. The results were expressed as a percentage.

The experiment was completely randomized with two replications for each temperature treatment. It was repeated a total of three times. The data from the three experiments were pooled for analysis (12).

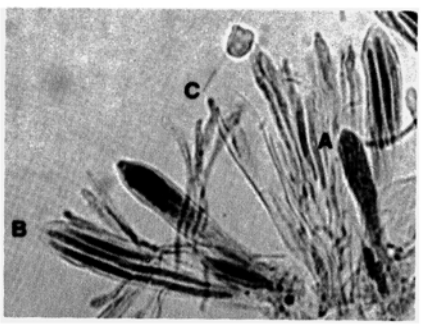

Fig. 1. Maturity classes of asci of *Blumeriella jaapii*. **(A)** immature asci = no ascospores present, **(B)** mature asci = some to all ascospores delimited, and **(C)** discharged asci = ascospores not present and the apex of the ascus open.

RESULTS

Effect of temperature on apothecial development. When the experiment was initiated, there was a mean of 13.7 asci per apothecium and 99.8% of the asci were immature. After 7 days of incubation at 4–24 C, there were 24.1 to 105.8 asci per apothecium in run 1 and 16.2 to 100.3 in run 2. An initial evaluation of the data on the effect of temperature on asci per apothecium by one-way analysis of variance indicated that the coefficients of variability for both runs were low and similar. Also, the F test between the error mean squares of the two runs indicated that the error variance for the two runs was not significantly different ($P > 0.05$). A similar examination of the data for the effect of temperature on changes in maturation of the apothecia indicated that the results from the two runs could be pooled.

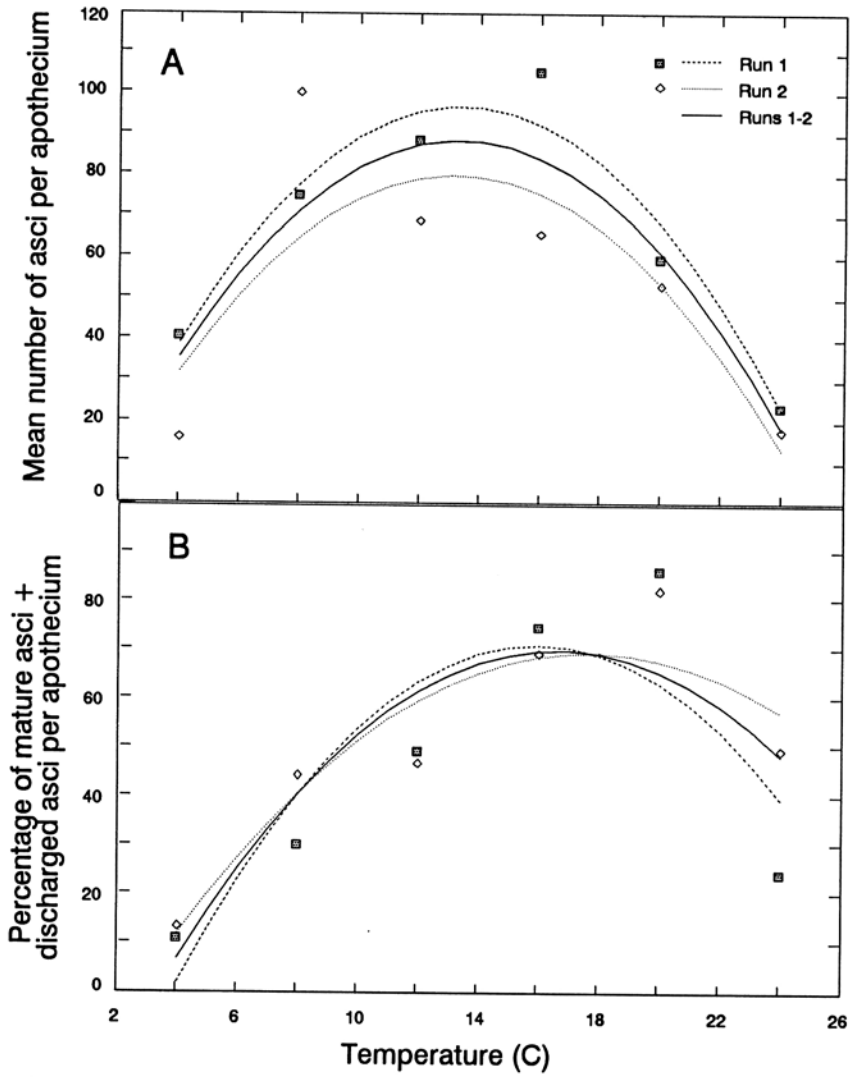

Fig. 2. The effect of temperature after 7 days of incubation on **(A)** the number of asci per apothecium and **(B)** the percentage of mature (including discharged) asci per apothecium of *Blumeriella jaapii*. Values are means of two replications for each of two runs of the experiment.

However, the data were analyzed by individual runs and by the two runs combined to show that the estimated models gave similar curves with similar optimum temperatures (Fig. 2).

The relationship between temperature (x) and apothecial development (y) during the initial 7 days of incubation (Fig. 2) was best described by quadratic regression equations (Table 1). The regressions accounted for 55.1–66.7% of the observed variation in the number of asci per apothecium and for 71.6–79.1% of the observed variation in the percent mature (including discharged) asci per apothecium. Examination of residuals supported the assumption that errors were independent and normally distributed with a mean of zero and a constant variance (*residuals not shown*). The predicted temperature optimum (13.3 C) for the development of asci was nearly identical for the two runs (Table 1). The predicted optimum for maturation and discharge of asci was higher (16.6 C) and slightly more variable than the optimum for development of the asci (Table 1).

When the leaf disks were held at the respective temperatures for an additional 35 days, moisture from the sponges was adequate for ascospore release and eventual exhaustion of the apothecia. Apothecia incubated at 16 or 20 C developed quickly, and most of the ascospores were discharged by day 14 (Fig. 3A). Discharged asci were difficult to count because they failed to stain or were lost in the process of preparing the samples for microscopic examination. Disintegration of the apothecia and the leaf tissue was accelerated at high temperatures and humidity.

Apothecia incubated at 4, 8, and 12 C did not disintegrate for up to 42, 42, and 28 days, respectively (Fig. 3). Productivity of the apothecia increased with temperature from 4 to 12 C. At 4 C, most of the asci failed to mature. There was little or no increase in the size of the asci, nor were ascospores delimited in the asci. By day 21, some asci were distorted and others were observed to contain large vacuoles. Distorted asci were also observed at 8 and 12 C.

Apothecia maintained at 24 C produced very few ascospores, and these apothecia disintegrated in 2 wk (Fig. 3). Many small atypical apothecia were observed among those that formed at 24 C. Some lacked paraphyses; others contained short paraphyses that lacked the enlarged hooked apex typical of the paraphyses observed in apothecia that developed at lower temperatures.

Effect of temperature on ascospore discharge. Ascospore discharge occurred at all temperatures tested, and the number of ascospores discharged increased as the temperature of the water holding the disks was increased (Fig. 4). The analysis of variance procedure indicated that there were differences due to temperature in the percentage of ascospores discharged in each run of the experiment ($P < 0.092$, $P < 0.009$, and $P < 0.0007$ for runs 1–3, respectively). The relationship between percentage of ascospores discharged and temperature during the discharge period was examined with the exponential equation $y = ae^{bx}$, where a and b are estimated regression coefficients (Table 2), y is an estimate of the percentage of ascospores discharged, and x is temperature from 8 to 30 C. Before pooling the three data sets for regression analysis, tests for homogeneity of regression coefficients were performed. The resultant F statistic was significant ($P < 0.05$) for the intercept constant (a) but not for the slope (b), indicating that although the experiments were conducted under different conditions, all three models estimated the same rate of change.

DISCUSSION

It was possible to assess the maturity and discharge of ascospores of *B. jaapii* by microscopically examining crushed apothecia. Although our experiments were conducted to determine the effect of temperature on the maturation of apothecia, the assessment method we used should also be useful for estimating or forecasting ascospore maturity and discharge in advisory programs for the management of cherry leaf spot. Cherry growers could be advised in much the same way that apple growers are advised of the development of pseudothecia of *V. inaequalis* (9,14). Rotorod spore traps are currently used by specialists in Michigan to assess the number and characteristics of the discharge periods (3,6,8).

Our results indicate that under optimum conditions for maturation and discharge of ascospores, primary inoculum from apothecia could be exhausted in as little as 2 wk. Conversely, if temperatures are low, inoculum may be available for about 6 wk. These results are in general agreement with field observations that indicate ascospore discharge occurs over a 6 or 7 wk period (8,11).

Our results on the influence of temperature on the release of ascospores confirm those obtained in Wisconsin by Keitt et al (11). There was considerable variation between replications in the number of ascospores discharged. This variation may have been due to differences in maturation among apothecia on the same leaf, in maturation of asci among apothecia of similar maturity, and in the number of apothecia per disk. Although efforts were made to select leaf disks with similar numbers of apothecia, the exact number of apothecia per disk was not determined.

In addition to temperature and maturity of the apothecia, the relative wetness of the apothecia may affect the release of ascospores. In our experiments, the apothecia were kept in water throughout the 2-hr discharge period. Although Keitt et al (11) reported that ascospore release

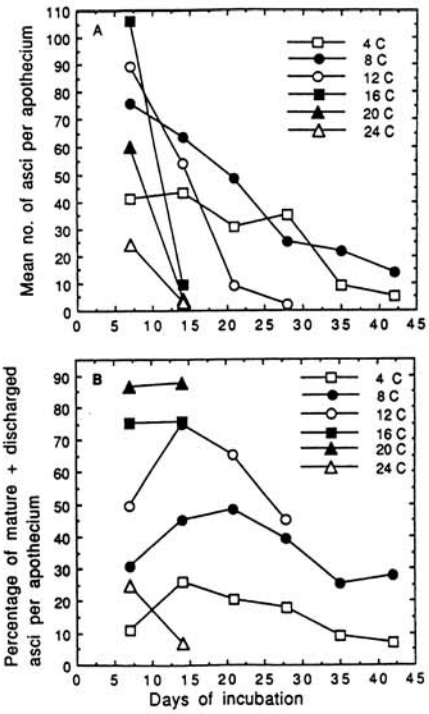

Fig. 3. Influence of temperature over 42 days on the maturation of apothecia of *Blumeriella jaapii*. The apothecia were evaluated by assessing **(A)** the number of asci per apothecium and **(B)** the percentage of mature (including discharged) asci per apothecium. Values are means of two replications with 20 apothecia each.

Table 1. Linear regression equations for apothecial development of *Blumeriella jaapii* after a 7-day incubation period at temperatures (x) of 4–24 C

Value Run (no.)	Regression equations	R^2	Probability ($>F$)	Predicted T_{max} (C)
Mean (y)[y]				
1	$y = -20.385 + 17.542x - 0.656x^2$	0.889	0.0001	13.4
2	$y = -18.415 + 14.970x - 0.569x^2$	0.551	0.0273	13.2
1+2	$y = -19.400 + 16.256x - 0.6125x^2$	0.667	0.0001	13.3
Percentage (y)[z]				
1	$y = -52.005 + 15.437x - 0.484x^2$	0.716	0.0034	15.9
2	$y = -26.615 + 10.881x - 0.308x^2$	0.791	0.0009	17.6
1+2	$y = -39.310 + 13.159x - 0.396x^2$	0.717	0.0001	16.6

[y] Mean number of asci per apothecium.
[z] Percentage of mature plus discharged asci per apothecium.

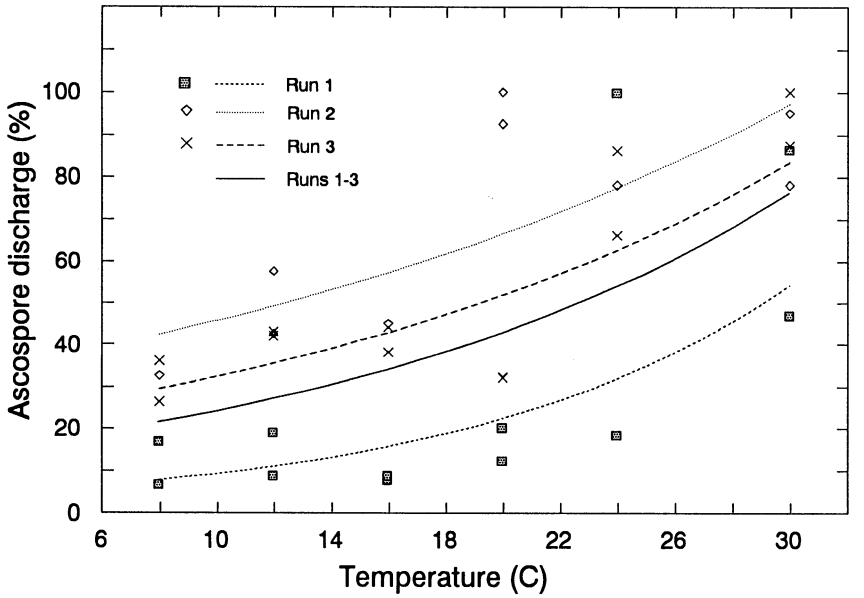

Fig. 4. Relationship of the effect of temperature on discharge of ascospores of *Blumeriella jaapii*. The replicate in each of three runs of the experiment with the highest number of ascospores was set to 100 ascospores, then the data for the other replications in that run were adjusted proportionally.

Table 2. Regression statistics for testing the relationship between temperatures of 8–30 C and the natural logarithm of ascospores of *Blumeriella jaapii* released from apothecia in 2-hr discharge tests

Run (no.)	Intercept	Slope	Standard error of slope	*F* statistic (*P* > *F*)	Coefficient of variation
1	3.7890b[y]	0.0886a	0.0247	12.8 (0.005)	21.26
2	31.1994a	0.0378a	0.0096	15.4 (0.002)	5.92
3	20.0273a	0.0475a	0.0106	19.7 (0.001)	7.02
1–3[z]	13.3284	0.0580	0.0152	14.4 (0.0006)	18.40

[y] Intercept and slope followed by the same letter are not statistically different according to the Student *t* test.
[z] Data from the three runs were pooled.

started once physiologically mature apothecia were thoroughly wet, they also observed that release increased as the apothecia dried. Further experiments are needed to define the effect of drying of apothecia on ascospore release. If ascospore release is accelerated by drying, then interrupted wet periods may be more favorable for ascospore discharge than continuous wet periods.

A weather-driven model for identifying environmental periods suitable for infection of cherry leaves by *B. jaapii* has been developed (2,3). However, the model does not account for variations in inoculum levels. As reported in this paper, low temperatures delay the maturation of the apothecia and reduce the number of ascospores discharged by physiologically mature apothecia. Incorporating the effect of temperature on maturation of apothecia and discharge of ascospores into this model might improve the prediction of infection periods. This would allow management of the disease based on the use of fungicides with postinfection activity (2,7,15).

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