Effect of Temperature and Relative Humidity on the Latent Period of *Venturia inaequalis* in Apple Leaves

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ABSTRACT

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The latent period for *Venturia inaequalis* was longer in leaves of McIntosh apple seedlings kept at 10 C than at 15 or 20 C in growth chambers. Latent periods were similar on seedlings kept at 15 or 20 C. Latent periods on seedlings kept at 20 or 10 C for 5 days, then at 10 or 20 C, were the same as those on seedlings kept continuously at 20 C. Latent periods on seedlings kept at low relative humidity (RH) (<70%) up to 6 days then at high RH (>70%), were similar to those for seedlings kept continuously at high RH. Lesions failed to develop on inoculated seedlings kept at low RH for up to 30 days, or on those kept at low RH after 3 or 6 days at high RH. Lesions developed on seedlings transferred to high RH after being maintained at low RH for 10–24 days, although latent periods were 4–16 days longer than on seedlings kept continuously at high RH. Latent periods on inoculated trees in the field often were longer than those reported by W. D. Mills. Regression analysis indicated that the duration of the latent period was related to both temperature and RH.

Additional key words: apple scab.

Apple scab, which is caused by *Venturia inaequalis* (Cke.) Wint., occurs in most apple-growing areas of the world. East of the Rocky Mountains, primary scab is generally controlled by applying three to eight fungicidal sprays between the silver tip stage of bud development and about 4 wk after the petal fall stage. Usually, these are protective sprays applied at weekly intervals. The duration of leaf wetness required for establishment of infection by the apple scab fungus has been well characterized (2,4), allowing application of curative or presymptom sprays after predicted infection periods (1). With the advent of sterol-inhibiting compounds, which have effective curative or presymptom control activity (3,11), fungicides might be applied several days after infection is predicted but before symptoms are visible. Knowledge of the factors governing the duration of the latent period is important for timing the application of curative-type fungicides.

For the purpose of timing the application of curative fungicides, the latent period can be considered to start with the onset of a wet period suitable for infection and to end with the appearance of conidia. Nusbaum and Keitt (7) established that for apple scab, conidia appear 5 days before necrosis. In 1946, Mills (5) reported that under orchard conditions, the length of the latent period decreases from 17 days at a mean temperature of 9.5 C for the entire period to 8 days at 18.5 C, with a decrease of 1 day for each 1.11 C (2 F) increase in mean temperature within this range. These conclusions were based on 27 yr of field observations in western New York. He also noted that the mean temperature for the first 5 days after the start of the infection period was the most important factor determining the length of the latent period.

The purpose of this study was to investigate the effects of temperature and relative humidity (RH) on the duration of the latent period under greenhouse conditions and to assess whether the conclusions of Mills (5) were applicable to Michigan conditions.

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MATERIALS AND METHODS

Inoculum. Apple leaves infected with *V. inaequalis* were collected from a research orchard near East Lansing, MI, from 12 June to 2 July 1980. Conidia of *Spilocea pomi* Fr. (conidial stage of *V. inaequalis*) were removed from the leaves by washing with distilled water. Leaf washings were filtered through four layers of cheesecloth and the conidia were concentrated by centrifugation for 5 min. Concentrated suspensions were stored in a freezer until needed. In 1981, inoculum from 1980 was increased on McIntosh seedlings in the greenhouse and on trees in a research orchard. The conidia were removed and processed as in 1980, except that conidia collected in the orchard were used immediately.

Before each inoculation, spore suspensions were adjusted to a concentration of about 10^6 conidia per milliliter. Germinability of the conidia was determined by atomizing a few microliters of the suspension onto a gridded filter (HAWG 04750, Millipore Corp., Bedford, MA 01730) and maintaining the filter in a petri dish at high RH and 18–22 C. Germinated conidia per square millimeter of filter were counted after 12–16 hr. Spore concentrations in all experiments were $2–5 \times 10^5$ viable conidia per milliliter.

Initiation of infection. McIntosh apple seedlings grown in the greenhouse at 18–24 C and 65–95% RH were used in all tests. After misting the seedlings for 1 hr in a moist chamber, the youngest unfolded leaf and three leaves below it were sprayed to runoff with a conidial suspension of *S. pomi.* Seedlings were allowed to dry about 2 hr before the initiation of a 48-hr wet period at 18–24 C, after which seedlings were maintained in environments differing in temperature and RH. Inoculated leaves were examined daily for scab lesions, beginning 8 days after inoculation.

Temperature studies. Several experiments were conducted to determine if the temperature at the start of the latent period influenced the duration of the latent period more than the temperature at the end of the latent period. In the first experiment, seedlings were placed at either high or low temperatures. After 5 days, some seedlings at each temperature were switched to the opposite temperature. Another experiment was also conducted in growth chambers maintained at 20 and 10 C and at 80–85% RH. This experiment was repeated under similar conditions except the seedlings were placed in plastic bags to maintain the RH at or near 100%. In a third experiment, seedlings were placed in plastic bags in growth chambers maintained at 20 and 15 C. A final experiment was conducted in chambers at 26/15 C day/night temperature or at 20/10 C day/night temperature and with a 12-hr light/dark photoperiod and 80–85% RH. Temperature experiments were replicated three or four times with two or four seedlings per replication.

RH studies. A chamber to maintain high RH in the greenhouse was constructed by enclosing a bench with cotton muslin curtains lined on the inside with cheesecloth. A RH of 95–100% (high RH) was maintained in the enclosed area by dripping water onto the side curtains for 1 min every 20 min and by keeping a 5-cm layer of wet sand on the bench. An RH averaging 65% (low RH) existed on a second bench in the same greenhouse. This bench was also enclosed with muslin curtains, but these were not wetted. Hygrothermographs were calibrated weekly with a sling psychrometer and a standardized thermometer.

In the growth chambers, water was continuously dripped onto the cheesecloth lining the walls. Because there was excessive air movement through the chambers, however, RH seldom exceeded 85% and the infection obtained was inconsistent. In later experiments, a RH near 100% was achieved by placing plastic bags containing wet paper towels over each seedling. Temperatures inside the plastic bags were monitored with thermocouples. Seedlings were held at low and high RH for 3, 6, 9, or 12 days, before transferring them to high or low RH, respectively. Control plants were held continuously at low and high RH. Experiments were replicated three or four times, with two or four seedlings per replication.

In two experiments, seedlings were maintained at low RH for 10–24 days before being enclosed in plastic bags. The temperature inside the bag was maintained at 20 C. In one of these experiments, inoculated leaves were cleared and stained by using Preece's method (8), then examined for subcuticular mycelium. Two additional experiments were conducted in the greenhouse. Treatments were high RH, low RH, and high RH for 8–10 hr followed by low RH for 14–16 hr.

Field studies. A portable chamber 5 m long × 3 m wide × 1.8 m high was constructed of aluminum tubing and plastic sheeting. The chamber was enclosed on top with plastic in 1980, but not in 1981. Plastic pipe equipped with mist nozzles was suspended over the trees to be misted. Flexible hose was used to connect the pipe to a water source. The chamber was placed over successive groups of 4- to 5-yr-old apple trees, each group having one tree each of Jonathan, McIntosh, and Golden Delicious, from 21 June to 20 July 1980 and 10 April to 14 July 1981.

On the day of inoculation, the youngest unfolded leaf on 15 terminal shoots per tree was labeled. The misting equipment was operated for about 1 hr before the leaves were sprayed to runoff with a conidial suspension. After the trees had dried for about 2 hr, they were misted according to Mill's (4) criteria for the number of hours required at the prevailing mean temperature required to give a severe infection initiated by ascospores. Beginning 8 days after inoculation, inoculated leaves were examined daily for lesions.

Care was taken to distinguish natural infections from those resulting from inoculations; uninoculated and inoculated leaves were monitored and the data were discarded if infections developed on both sets of leaves. To prevent a buildup of scab before inoculation, trees were sprayed with etaconazole (Vangard 10% W, CIBA-Geigy Corp., Greensboro, NC 27409) after predicted infection periods, except that trees to be inoculated within 2 wk were not sprayed. Etaconazole was used because its residues only persist for about 5 days (3).

Temperature and RH data were recorded in the orchard with a hygrothermograph, which was calibrated weekly with a sling psychrometer and a standardized thermometer. Average daily temperature, daily maximum and minimum temperature, average daily RH, and daily degree days were calculated from hourly data. Degree-day calculations were based on the difference between daily mean temperatures and 0 C. Preliminary analysis indicated that high temperatures delayed disease development, so daily degree-day calculations were based on a maximum of 26 C, even when the maximum temperature for that day was greater than 26 C.

We also calculated the average temperature for the previous week, for the day after inoculation, and for 1–3 and 4–6 days after inoculation, and the cumulative degree days, the adjusted cumulative degree days, and the average RH for 1–3 and 4–6 days after inoculation.

The regressions of latent period on meteorological variables were calculated with a computer program (6). In the regression analysis, data from all tagged infected leaves were averaged and constituted one replication. Only the regression model having the best combination of high coefficients of determination and residuals supporting the assumptions that errors are independent and normally distributed are reported.

TABLE 1. Effect of temperature on the duration (days[r]) of the latent period for apple scab on McIntosh apple seedlings subjected to various temperature treatments starting 48 hr[s] after inoculation with *Venturia inaequalis*

Temperature regime[u]	Temperature (C)[t]			
	20/10[v]	20/15[v]	(26:15)/(20:10)[w,x,y]	20:10[x]
Low/Low	14.7 a	10.2 a	12.2 a	16.1 a
Low/High	12.2 b	10.3 a	NL[z]	13.2 b
High/High	12.0 b	9.2 a	NL	12.8 b
High/Low	12.3 b	9.3 a	13.1 a	13.5 ab

[r] Means within the same column followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.
[s] Wet period required for initiation of infection.
[t] Number to left of slash (/) is the high-temperature and number to right is the low temperature used in each experiment.
[u] Low/Low = low temperature for the duration of the experiment; Low/High = low temperature for 5 days, high temperature for the remainder of the experiment; High/High = high temperature for the duration of the experiment; and High/Low = high temperature for 5 days, low temperature for the remainder of the experiment.
[v] Inoculated plants enclosed in plastic bags in growth chambers.
[w] Day:night temperatures: number to left of colon (:) is high temperature, number to right is the low temperature.
[x] Experiments conducted in growth chamber with walls lined with wet cheesecloth curtains to maintain high relative humidity.
[y] Indicates alternating 12-hr light/dark cycles.
[z] NL = no lesions after 20 days.

TABLE 2. Effect of relative humidity (RH) on the duration of the latent period for apple scab on McIntosh apple seedlings subjected to various temperature treatments starting 48 hr[u] after inoculation with *Venturia inaequalis*

RH regime[v]	Growth chamber[w]	Greenhouse[x]
High RH only	9.5 a[y]	10.3 a
Low RH only	NL[z]	NL
3 days low RH/high RH	9.8 a	10.5 a
6 days low RH/high RH	10.0 a	11.2 a
9 days low RH/high RH	12.2 b	15.0 bc
12 days low RH/high RH	...	18.0 c
3 days high RH/low RH	NL	15.5 bc
6 days high RH/low RH	NL	NL
9 days high RH/low RH	9.5 a	11.0 a
12 days high RH/low RH	...	12.7 ab

[u] Wet period required for initiation of infection.
[v] Low and high RHs were 60–70 and 95–100%, respectively. Growth chamber temperature was 20 ± 1 C, and greenhouse temperatures ranged from 14 to 24 C.
[w] Plants were maintained at high RH by placing them in plastic bags containing wet paper towels.
[x] Plants maintained at high RH were placed in a water-drenched cheesecloth tent on a greenhouse bench.
[y] Means within a column followed by the same letter are not statistically different according to Duncan's multiple range test, $P = 0.05$.
[z] NL = no lesions observed after 20 days of incubation.

RESULTS

Effect of temperature. Apple scab lesions developed 2–3 days earlier on seedlings kept at 20 C than on seedlings kept at 10 C. There was no difference in the time to lesion development on seedlings kept continuously at 20 C; at 20 C for 5 days, followed by maintenance at 10 C; or at 10 C for 5 days, followed by maintenance at 20 C (Table 1). In these experiments, infection on seedlings in growth chambers equipped with cheesecloth curtains was delayed and less consistent than was infection of seedlings in plastic bags. There was no difference in the length of the latent period at 15 and 20 C or at combinations of these temperatures. In the experiment with daily temperature fluctuations, lesions developed on the leaves of seedlings that had received low temperature (20 C day/15 C night), but not in those that received high temperature (26 C day/15 C night), during the latter part of the latent period (Table 1).

Relative humidity. Under greenhouse and growth chamber conditions, the duration of the latent period varied depending on the RH to which the seedlings were exposed to a few days before lesions were expected to appear. Seedlings held at high RH (95–100%) developed symptoms in 9.5 days in the growth chamber and 10.3 days in the greenhouse, but seedlings held at low RH (60–70%) were symptomless after 20 days (Table 2). The latent period for seedlings held 3–6 days at low RH before transfer to high RH did not differ significantly from that for high RH treatment, but the latent period on seedlings held for 9 or 12 days at low RH before transfer to a higher RH was significantly longer ($P = 0.05$). Seedlings transferred to a low RH after 3 or 6 days of high RH either failed to develop lesions or had a long latent period. The latent period for seedlings transferred after 9 or 12 days at high RH was similar to that for plants kept continuously at high RH throughout the experiment.

There was no difference in latent period on seedlings kept at high RH in the greenhouse and those kept at high RH for 8–10 hr then at low RH for 14–16 hr. Lesions did not develop on seedlings kept continuously at low RH.

To determine if prolonged exposure to low RH was lethal, seedlings were maintained for 10–24 days at low RH. Scab lesions developed on these seedlings 1–4 days after transfer to high RH (Table 3). Lesions did not develop on seedlings kept at low RH for 30 days. Microscopic examination of the cleared leaves indicated that subcuticular mycelium development was normal, but that conidia were absent.

TABLE 3. Duration of latent periods for apple scab on McIntosh apple seedlings held under low relative humidity (RH) conditions at 20 ± 1 C for various time periods before humidities were increased by covering the plants with plastic bags, starting 48 hr[v] after inoculation with *Venturia inaequalis*

Dry period (days)	Latent period (days)	
	Experiment one	Experiment two
0[w]	10.0 a[x]	9.9 a
10	14.3 b	13.6 b
11	15.0 bc	14.2 b
12	15.7 bc	15.8 c
13	16.3 c	16.8 c
14	17.7 c	18.0 d
15	18.3 d	…
16	20.3 e	19.9 e
17	20.3 e	…
18	…	21.6 f
20	…	23.4 g
22	…	25.7 h
24	…	27.5 i
30[y]	NL[z]	NL

[v] Wet period required for initiation of infection.
[w] Plants maintained continuously at high RH.
[x] Means in the same column followed by the same letter are not statistically different according to Duncan's multiple range test, $P = 0.01$.
[y] Plants maintained continuously at low RH.
[z] No lesions observed.

Field studies. To compare our field data with those of Mills (5), the average temperature for the first 6 days of each latent period was determined and the data were plotted (Fig. 1). For over one-half of the inoculations observed in 1981, the average temperature

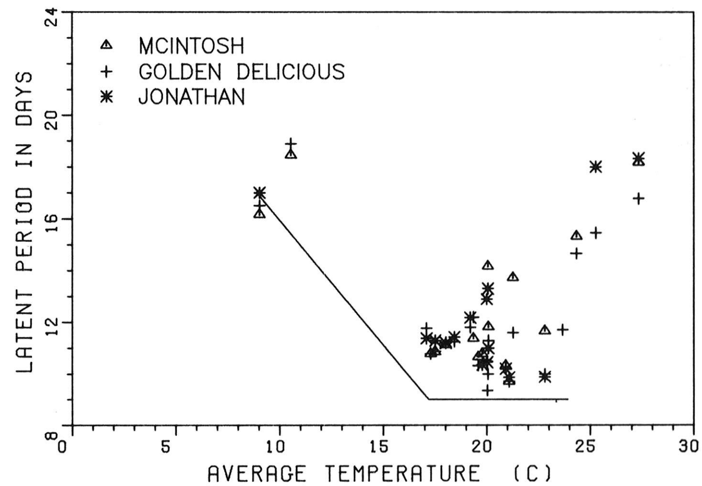

Fig. 1. The relationship of the duration of the latent period for apple scab to average temperature 6 days after inoculation for three cultivars of apple in 1981. The solid line is a plot of latent period as proposed by Mills (5).

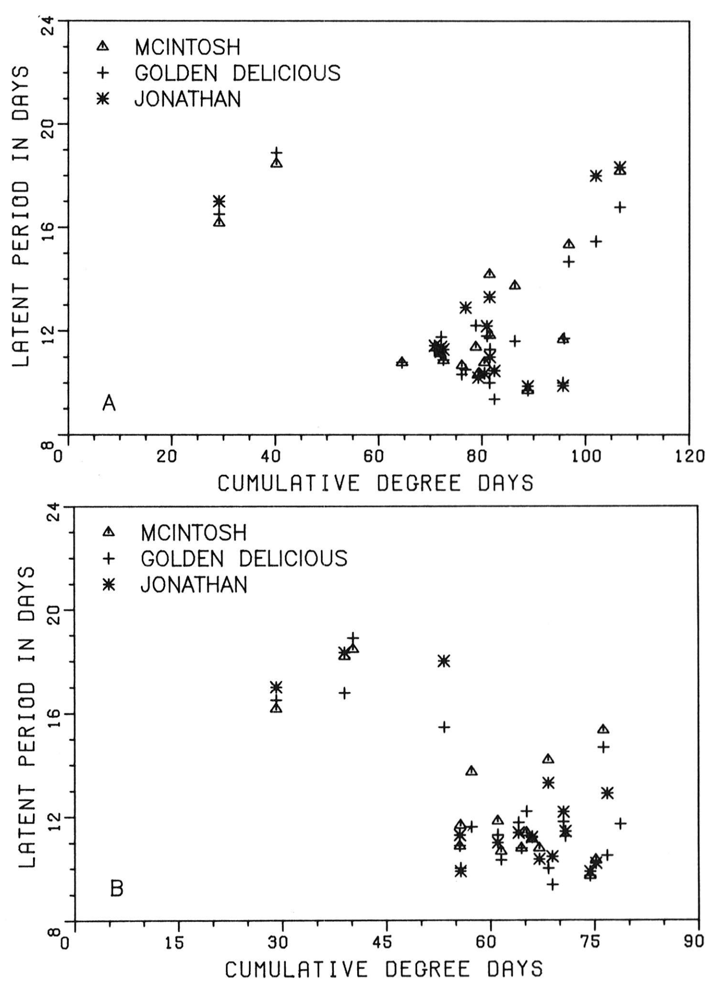

Fig. 2. The relationship of the duration of the latent period for apple scab on leaves of three apple cultivars to **A,** degree days (base, 0 C) accumulated to 6 days after inoculation and **B,** degree-day accumulation based on an upper threshold of 26 C.

for the first 6 days of the latent period was between 17 and 24 C. At these temperatures, Mills (5) indicated symptoms should develop in a minimum of 9 days, compared to the 9.5–15 days observed in our experiments. For an infection period with an average temperature of 11 C, symptoms developed 3 days later than predicted by Mills (5). With an average temperature of 9 C, symptoms developed within 12 days as predicted by Mills (5). At average temperatures above 24 C, the latent periods were 14–18 days. Latent periods did not vary markedly among cultivars.

Duration of latent period was not well correlated with cumulative degree days (base, 0 C). Although most lesions were observed between 70 and 100 degree days (base, 0 C), there were several points outside this range (Fig. 2A). When degree-day accumulation was based on an upper threshold of 26 C, all but three latent periods (when data for cultivars inoculated the same day were combined) were observed between 50 and 80 degree days (Fig. 2B), and the plot appeared more linear.

To evaluate the combined effects of RH and adjusted cumulative degree days on duration of the latent period, several regression analyses were performed. The best model predicts latent period on the basis of adjusted cumulative degree days 1–3 and 4–6 days after inoculation, RH 1–3 and 4–6 days after inoculation, average temperature the week before inoculation, and average temperature the day after inoculation. When this model was used to evaluate the 1981 field data, coefficients of determination on McIntosh, Golden Delicious, and Jonathan were 67, 71, and 81%, respectively. A similar model based on adjusted cumulative degree days and RH from 1–6 days after inoculation had coefficients of determination of 60, 64, and 68%, indicating that degree-day accumulation and average RH in two 3-day intervals is a better predictor of latent period than the same variables evaluated over a 6-day interval. In models combining data from all cultivars, the use of dummy variables to account for cultivar differences increased the coefficients 3–8%. In general, the ability of these models to predict latent period decreased as the size of the data base increased, with the largest decrease in coefficient of determination occurring when data from both years were combined. This large year-to-year variation rendered the model unsuitable for prediction.

DISCUSSION

The time of appearance of apple scab lesions under greenhouse conditions was dependent on temperature and RH. Exposures to low RH of 6 days or less were not detrimental, but longer exposure delayed lesion development. The RH effect was unexpected because Keitt and Jones (2) reported that RHs of 50–90% did not influence the length of the latent period. Our results agree with those of Studt and Weltzien (10) who found that the minimum relative humidity for sporulation was between 60–70%. The effects of suboptimal humidity were not lethal because lesions were evident 1–4 days after infected seedlings were transferred from low to high RH. The important effect of RH on lesion development may explain the difficulty sometimes encountered in inoculating with *V. inaequalis.*

As described by Mills (5), the duration of the latent period of primary infection decreases as mean temperatures increase from 9–18 C. Between 18 and 25 C, the minimum latent period is about 9 days; above 26 C, the latent period lengthens. In growth chamber experiments, lesions failed to develop at 26 C as reported by Keitt and Jones (3). Calculating degree-day accumulation on the basis of an upper threshold of 26 C resulted in a better correlation between cumulative degree days and latent period.

It was not possible for us to determine the suitability of Mills' (5) system for establishing the duration of the latent period at all temperatures. The majority of the infections developed at average temperatures of 17–24 C. In this range, his criteria should give adequate warning of lesion appearance; however, they fail to account for unfavorable conditions of temperature or RH that may extend the latent period.

Many of the latent periods we observed were much longer than would be expected from the data of Mills (5). His results were based on the first important infection period each season, when mean temperatures above 26 C and long periods of low RH would be rare. Most of the infection periods we observed were initiated between mid-May and early-August when high temperatures and low RHs are more common. The effect of temperature and RH probably explains (at least in part) why scab is less of a problem in summer in the southern and western states. Also, leaves develop resistance to infection rapidly as they expand (2,9). Under conditions less favorable for the fungus, the leaf may become resistant and inhibit or prevent the appearance of lesions.

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