Effect of Leaf Wetness Duration and Temperature on the Infectivity of Guignardia bidwellii on Grape Leaves

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ABSTRACT

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Leaves of potted grape plants were sprayed with suspensions of *Guignardia bidwellii* conidia (50,000/ml) to study the effects of selected combinations of leaf-wetness duration and temperature on infection of American bunch grapes and French hybrid cultivars. Plants then were exposed to postinoculation treatments in which leaf-wetness duration and temperature were variables. Infection occurred after 6 hr leaf wetness at 26.5 C but 24 and 12 hr of leaf wetness were required at 10 and 32 C, respectively. Disease was significantly less severe with either increasing or decreasing temperature regimes during the infection period than with constant temperature. Infection occurred after rewetting inoculated leaves that had been kept dry for up to 2 days, although a 1-day postinoculation dry period caused a significant reduction in severity. This information on effects of leaf wetness duration and temperature on infection was reliable for determination of when foliar-infection periods had occurred during two seasons in a commercial vineyard.

Additional key words: epidemiology, *Vitis* spp.

Black rot, which is caused by *Guignardia bidwellii* (Ellis), Viala & Ravaz, is one of the most serious grape diseases in the eastern United States (3). Although several protectant fungicides may provide satisfactory control if applied correctly and at the proper time, no currently registered grape fungicide has significant eradicative capability (7). Moreover, we know little about the judicious scheduling of fungicidal sprays.

A quantitative relationship between black rot and weather conditions has been observed (4, 5), but researchers have failed to quantify the relationship. Caltrider (1) reported that the optimum temperature for growth and conidial production was 25 C, whereas the optimum temperature for spore germination was 30 C.

Objectives of this study were to determine: (i) the combinations of leaf wetness duration and temperature which are necessary for infection of American and French hybrid grape cultivars, (ii) the effect of constant and variable temperatures on infection, and (iii) the effect of various dry periods of inoculated leaves on subsequent infection. A preliminary report has been published (6).

MATERIALS AND METHODS

Dormant cuttings from *Vitis labrusca* L. 'Catawba' and 'Concord' and *Vitis vinifera* L. × *Vitis labrusca* L. 'Aurore' and 'Baco Noir' were rooted in a greenhouse mist bed. Rooted cuttings, trained to single shoots, were planted in 10-cm diameter polystyrene pots in Wooster silt loam and maintained as described previously (7).

Guignardia bidwellii was isolated and cultured as described previously (7). Conidia were harvested from 10- to 12-day-old cultures. Water suspensions containing 5×10^4 conidia/ml were sprayed to runoff with an artist's airbrush operated at 1.4 kg/cm^2 onto the three terminal leaves of each plant. Because control plants sprayed with water without conidia and kept in a moist chamber for 24 hr produced no symptoms, controls were not always included in every test.

Inoculated plants were placed in dark growth chambers lined with several layers of wet cheesecloth at temperatures from 10 to 32 C (Table 1). Chamber temperature was determined with calibrated mercury thermometers. Thermocouples throughout the chambers, connected to a multipoint recorder, indicated acceptable temperature uniformity.

After 5 hr, groups of plants were removed at 30-min intervals to determine the minimum duration of leaf wetness required for infection at each temperature. Plants were dried in separate growth chambers at temperatures equivalent to those used during incubation. Total leaf wetness duration included time in the moist chamber plus drying time after removal from the chamber (approximately 30 min). After drying, plants were kept in the greenhouse at 21±5 C and 34±15% RH. Disease severity was determined visually 2-3 wk after inoculation. Each cultivar was replicated five times, and the experiment was repeated at least twice for each wetness duration-temperature combination.

Because constant temperature in the vineyard seldom occurs, the effect of variable temperature on black rot infection was studied. Moist chamber temperature was manually increased or decreased hourly in equal increments during an 8-hr period. Control plants were

exposed to a constant temperature equal to the average of the corresponding variable temperature regime.

To determine the effect of a postinoculation dry period on infection, plants were air-dried immediately after inoculation. Following periods of 1, 2, 3, and 4 days, plants were rewetted and placed in the moisture chamber for 24 hr at 24 C. Inoculated control plants were rewetted and placed in the chamber immediately after leaves had air-dried.

Cultivars and number of replicates used in specific experiments are indicated in Tables 1-3. A completely random experimental design was used. Data were subjected to analysis of variance and Duncan's multiple range test. In addition, variable temperature data were analyzed with a factorial treatment design with cultivar and temperature regime as factors.

Vineyard studies.—In 1975 and 1976 the following parameters were monitored in a commercial Aurore vineyard at Chesterville, Ohio: vine growth, disease incidence, air temperature, relative humidity (RH), and leaf wetness. During each season, 30 buds were marked, and the number of leaves per shoot was determined weekly from bud burst (in May) through August. The oldest leaf (shoot base) was designated Leaf #1, and increasingly higher numbers were assigned to each distal leaf. Unfolded leaves and leaves smaller than 2 cm width were not numbered. The locations of diseased leaves on each shoot were recorded weekly. Temperature and RH were monitored with a 7-day recording hygrothermograph situated 1.5 m above the ground in a weather-instrument shelter. Leaf wetness was monitored with a 7-day recording leaf wetness meter (M. DeWit, Hengelo, The Netherlands). Rainfall data were obtained from the permanent Federal station 'Fredericktown 4S' located 19 km from the vineyard. Duration of leaf wetness caused by dew was determined from leaf wetness meter data.

TABLE 1. Leaf wetness duration-temperature combinations necessary for grape foliar infection by *Guignardia bidwellii* conidia

Temperature (C)	Minimum leaf wetness duration for light infection (hr)[a]
10.0	24
13.0	12
15.5	9
18.5	8
21.0	7
24.0	7
26.5	6
29.0	9
32.0	12

[a]Data represent a compilation from several experiments with cultivars Concord, Catawba, Aurore, and Baco Noir. Each cultivar was replicated five times per experiment.

RESULTS

Infection occurred after 6 hr of leaf wetness at 26.5 C, but required 24 and 12 hr at 10 and 32 C, respectively (Table 1). Temperatures higher than 32 C were not tested; and at 7 C, no infection occurred on any cultivar after a 48-hr leaf wetness period.

Disease severity on plants exposed to variable temperatures during infection was significantly less than that in plants infected at constant temperature, in two specific comparisons (Table 2). When temperature regime was considered as a main factor, overall disease severity was significantly less ($P = 0.01$) if plants were maintained in a variable rather than constant temperature during infection. This effect was significant ($P = 0.01$) in both increasing and decreasing temperature regimes.

Infection occurred after rewetting inoculated leaves of Baco Noir and Catawba that had been kept dry for up to 2 days, although a 1-day, postinoculation dry period caused a significant reduction in severity ($P = 0.05$) (Table 3).

To determine whether the effect of postinoculation drying on development of infection resulted from increased leaf resistance with aging or from a direct effect of drying of conidia, two experiments were performed. First, to study leaf resistance factors, a droplet containing 5×10^4 conidia/ml of fresh inoculum was placed daily for 4 consecutive days on the most recently unfolded leaf (average initial size 35 mm × 45 mm) of a Baco Noir shoot

TABLE 2. Effect of variable and constant temperature on the severity of grape foliar infection by *Guignardia bidwellii*

	Lesions per plant (avg. no.)[a,b,c]		
Temperature regime	Catawba	Baco Noir A	Baco Noir B
Experiment A			
Constant[d]	9 x	8 x	12 x
Variable (increasing)[d]	2 x	4 x	6 y
	Concord	Baco Noir A	Baco Noir B
Experiment B			
Constant[e]	3 x	35 x	4 x
Variable (decreasing)[e]	1 x	3 y	3 x

[a]Each value represents the mean of 12 replications in Experiment A; 18 replications in Experiment B.

[b]Numbers followed by the same letter within columns are not significantly different at $P = 0.05$ according to Duncan's New Multiple Range Test. Each experiment was analyzed independently.

[c]Baco Noir A and B refer only to differences in infection conditions, not cultivar.

[d]Constant temperature = 24 C for Catawba and Baco Noir A, 18.5 C for Baco Noir B; variable temperature = 18.5 increasing to 29.5 C for Catawba and Baco Noir A, 13.0 increasing to 24 C for Baco Noir B.

[e]Constant temperature = 24 C for Concord and Baco Noir A, 18.5 C for Baco Noir B; variable temperature = 29.5 decreasing to 18.5 C for Concord and Baco Noir A, 24 decreasing to 13 C for Baco Noir B.

in the greenhouse. The number of infections per six replications was 4, 2, 3, and 0 for 0, 1, 2, and 3 days, respectively, after initiation of the experiment. Thus, leaves that had aged 2 days during the experiment were still susceptible, but leaves which had aged 3 days were resistant.

To study the effect of drying on germination of *G. bidwellii* conidia, four drops of conidial suspension (each containing 5×10^4 conidia/ml) were placed on a leaf. These were sampled immediately and after 1, 2, and 3 days of drying by removing leaf disks which included the entire area initially occupied by the drop of inoculum and rewetting with distilled water. Leaf disks were incubated in a petri dish moist chamber at 24 C for 24 hr. Treatments were replicated six times, and the experiment was repeated twice. Germination of conidia that had been dried for 0, 1, 2, and 3 days was 13, 8, 2, and 0.3%, respectively.

Vineyard studies.—The duration of leaf wetness and temperature information (Table 1) were applied to vineyard weather data to identify periods suitable for infection. Average temperature was calculated for each wetness period, and decisions concerning infection were based on Table 1 information. Although infection is dependent on a minimum wetness duration that varies with temperature, infection was not altered by the source of wetness (rain or dew).

From 22 to 26 May, four infection periods (IP) occurred (Fig. 1). During this time, leaves 2 and 5 were the most susceptible to infection. Symptoms appeared on these four leaves by 10 June when nine leaves were present on this shoot. Thus, the incubation period was approximately 2 wk. A similar pattern relating foliar symptoms to leaf susceptibility during a preceding IP was repeated throughout the growing season (Fig. 1). Infection often could not be assigned to a specific IP when several had occurred in a short time because disease was evaluated weekly and length of the incubation period was influenced by environmental conditions.

Because of the 2-wk incubation period, only infections that occurred before 26 August 1975 and 10 August 1976 could be confirmed. The total number of IP prior to the above dates was 54 and 34 in 1975 and 1976, respectively (Table 4).

In 1975, eight fungicide applications were made from 28 May to 11 July. These reduced the number of effective IP by 13. Lack of rain-related spore dissemination prior to dew-related IP further reduced the number by 21, leaving 20 effective IP in 1975 (Fig. 1 and Table 4). In addition, six of these 20 IP occurred after berry color change (on 10 August), the time when berries become resistant to black rot infection.

In 1975, all 20 effective IP were followed by foliar infection. Similarly, all infections were associated with calculated IP (Fig. 1).

In 1976, 12 fungicide applications were made from 27 April to 17 July. These reduced the number of effective IP by 13. Lack of dissemination conditions prior to dew-related IP further reduced the number by 13, leaving eight effective IP in 1976 (Table 4). One of these IP occurred after berry color change on 5 August.

In 1976, black rot foliar symptoms appeared within 2 to 3 wk after all eight IP. In a portion of the vineyard which was not sprayed with fungicides, black rot symptoms were associated with all 21 IP (the 13 IP nullified by fungicides were effective in this portion of the vineyard). As in 1975, infection was always linked with a calculated IP.

TABLE 3. Effect of postinoculation dry periods on the severity of grape black rot foliar infection

Post-inoculation dry period (days)[a]	Leaf infection per plant (Avg. %) [b,c]	
	Baco Noir	Catawba
0	40 w	61 y
1	15 x	18 z
2	4 x	3 z
3	0 x	0 z
4	0 x	0 z

[a]Dry period followed by infection period of 24 hr leaf wetness at 24 C.

[b]Each value represents the mean of six replications.

[c]Numbers followed by the same letter within columns are not significantly different at $P = 0.05$ according to Duncan's new multiple range test.

DISCUSSION

In this study, the optimum temperature for foliar infection by *G. bidwellii* conidia was about 26.5 C. The duration of leaf wetness and temperature requirements for infection established herein closely parallel the effects of temperature on growth and conidial germination determined by Caltrider (1).

At the extreme temperatures (10 and 32 C) Aurore and

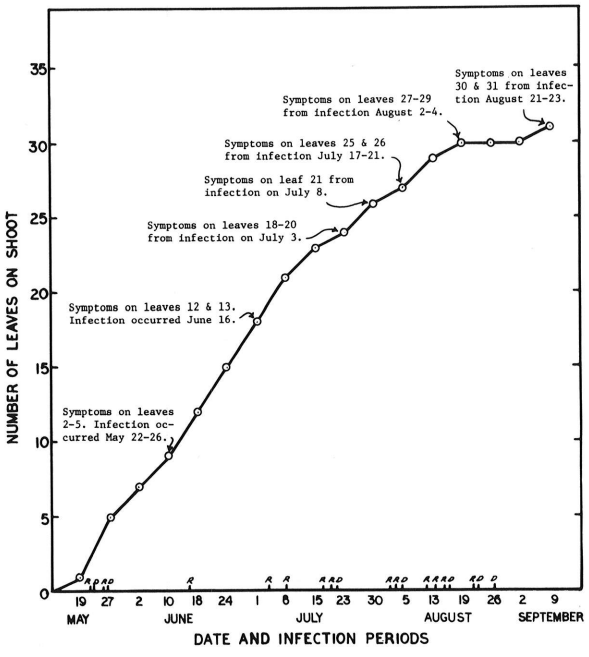

Fig. 1. Grape shoot growth, foliar symptom location and time of appearance, and black rot infection periods of typical shoot of cultivar Aurore during the 1975 growing season. Midbloom occurred 18 June, berry color change on 10 August. Wetness for infection from rain (R) or dew (D). Leaves numbered starting at shoot base.

TABLE 4. Number of grape black rot foliar infection periods occurring during 1975 and 1976 growing seasons in a commercial vineyard

	1975	1976
Total number infection periods[a]		
Rain	23	19
Dew	31	15
Number ineffective dew infection periods[b]	21	13
Number ineffective infection periods involving fungicide protection[c]		
Rain	10	12
Dew	3	1
Net effective infection periods[d]		
Rain	13	7
Dew	7	1

[a]Conditions necessary for infection based on data in Table 1.
[b]Infection period considered ineffective if not preceded within 24 hr by 1 or more hr of rain for conidial dissemination.
[c]Effective fungicide residue length considered 7 days.
[d]Number of effective infection periods = (total number) − ineffective number due to fungicide protection and lack of dissemination prior to dew.

Baco Noir appeared more susceptible than Catawba and Concord. Although this cultivar variation was observed, differences did not justify construction of individual curves for each cultivar in this study.

Variable temperature regimes were used to simulate morning to mid-day (increasing) and afternoon to evening (decreasing) temperature patterns. Although the variable temperature during infection resulted in decreased disease severity, the fact that infection did occur contributes to the general applicability of the wetness duration-temperature guidelines established herein.

In the conidial droplet experiments, leaves became resistant 3 days after inoculation. Because leaves were 4 days old when the study began, grape foliage under these conditions was susceptible for approximately 1 wk. The significant reduction of infection resulting from a 1-day postinoculation dry period is thus attributed to a direct effect of dryness on leaf surface conidia. However, the conidial germination decrease did not fully account for the decreased disease severity, and additional factors apparently are involved.

When the leaf wetness duration-temperature combinations from growth chamber experiments were used as guidelines to determine when foliar infection occurred in the vineyard, all infections were associated with calculated IP in both 1975 and 1976. Furthermore, all calculated effective IP resulted in actual infection of susceptible leaves during the IP. It must be emphasized that only the terminal leaves on a shoot were susceptible and resistance developed in about 1 wk. Dew-IP, not preceded by rain dissemination of conidia, and rain-, and dew-IP nullified by fungicides did not cause infection. Furthermore, many wetness periods were recorded which were 1 or 2 hr less than that required for infection (based on Table 1 guidelines), and no infection resulted from these wetness periods. Usually, 2 to 3 wk elapsed between infection and completion of lesion development. Initial symptoms were observed 10 to 12 days after infection.

Although eight fungicide applications were made in 1975, 62% berry infection occurred and the crop was not harvested. In 1976, 12 fungicide applications were made, and 12% berry infection was observed. If fungicide was applied only when an IP occurred, and a minimum spray interval of 7 days was imposed, 10 applications in 1975 and eight in 1976 would have been required for acceptable black rot control. Thus, use of IP guidelines to schedule fungicide applications may not result in fewer fungicide applications but disease control should be improved. It should be noted that currently registered fungicides for black rot control are not eradicants, and effective post-infection control has been achieved only with experimental fungicides (7).

The temperature and leaf wetness duration relationships reported here were not developed as part of a rigid mathematical model but rather to serve as guidelines for use in a practical disease-forecasting system. These guidelines were reliable for determination of infection periods under vineyard conditions. Based on our studies, conidia appear to function as the major type of primary inoculum in Ohio (Spotts, *unpublished*), and this observation agrees with a previous report (5). It has not been definitely established whether the leaf wetness-temperature combinations for conidial infection also apply to ascospore infection, but Ferrin (2) reported optimum infection by ascospores at 27 C and no infection occurred at 32 C. Similarly, quantitative environmental relationships necessary for berry infection are not yet available.

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