Infection of Grape by *Guignardia bidwellii*—Factors Affecting Lesion Development, Conidial Dispersal, and Conidial Populations on Leaves

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ABSTRACT

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Experiments were conducted to evaluate the effects of temperature and relative humidity on incubation time and pycnidia formation of *Guignardia bidwellii* on grape leaves and to study factors affecting conidial release and adherence to and populations on leaf surfaces. Incubation time was approximately 1 wk at 21 and 26.5 C and 2 wk at 15 C. After lesions appeared, pycnidia formed within 3 (21 C) to 5.5 (15 C) days. Rainfall duration of 1–3 hr provided optimal conidia dispersal. Conidia were readily washed from inoculated leaves except when they were dry for 60 min or longer preceding washing. Conidial populations on leaves in the vineyard were closely related to conditions favoring conidial dispersal, retention on leaves, and disease severity.

Additional key words: epidemiology, *Vitis* spp., grape black rot.

Several studies contributed important epidemiological information necessary for developing control programs for grape black rot, caused by *Guignardia bidwellii* (Ellis) Viala and Ravaz (2,3,7,8). Ascospore and conidia dispersal occur throughout the growing season in the north central United States with peak ascospore and conidia release in June and in July and August, respectively (2,3). Release of ascospores and conidia is water-dependent, and the optimal temperature for germination is 30 C (1,2). Whereas conidia survive 48 hr on dry leaves (8), ascospores survive 24 hr (2). Maximal leaf infection occurs at 26.5 C with conidia (8) and 27 C with ascospores (2). Leaf infection by conidia occurs at 32 C (8), but ascospores fail to infect at 32 C (2).

This study evaluated the effects of temperature and relative humidity (RH) on incubation time and pycnidia formation, the factors affecting conidia release from pycnidia and adherence to leaf surfaces, and the factors affecting conidia populations on leaf surfaces in the vineyard.

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MATERIALS AND METHODS

Incubation and pycnidia formation. Rooted cuttings of *Vitis labrusca* L. 'Catawba' and 'Niagara' and *V. vinifera* L. × *V. labrusca* L. 'Aurore' and 'Baco Noir' were planted in polystyrene pots (10 cm diameter) in Wooster silt loam and maintained in the greenhouse as described previously (8,9).

G. bidwellii was cultured on glucose-maltose-yeast extract agar as described previously (9). Conidia from 10-day-old cultures were harvested, adjusted to 5.0×10^4 conidia per milliliter, and sprayed to runoff with an artist's airbrush onto grape plants as described previously (8). Inoculated plants were placed in a dark growth chamber, lined with several layers of wet cheesecloth, at 15, 21, and 26.5 ±2 C for 16 hr. Plants were transferred to growth chambers at the same temperature as during infection. At each temperature, 10

plants of each cultivar were maintained at 50% ± 10%, 70% ± 10%, and 90% ± 10% RH. Maximal light intensity at median plant height was 18.0 klux programmed for 12 hr of light per day. Plants were observed frequently for appearance of initial lesions and pycnidia. Incubation time was defined as the number of days from inoculation to appearance of visible symptoms.

In a Wooster, OH vineyard of 3-yr-old vines, the terminal three leaves of shoots were inoculated to runoff four times during 1978 with 5.0×10^4 conidia/ml. Inoculated shoots were covered overnight with polyethylene bags overlaid with aluminum foil. Following inoculation, leaves were observed frequently for lesions and pycnidia. Temperature and RH were monitored with a 7-day recording hygrothermograph (Bendix Corp., Baltimore, MD 21204) situated 1.5 m above the ground in a standard weather instrument shelter. Rainfall was monitored with a 7-day recording rain gauge (Weather Measure Corp., Sacramento, CA 95841) located 1.0 m above the ground.

Conidia release during rain. Runoff water was collected in a petri dish from a severely infected, attached Catawba leaf during natural rainfall. Separate aliquots were collected at 15-min intervals during the first hour and at 30-min intervals during hours 2 through 4. Conidia were counted with a hemacytometer. Total water volume in each aliquot was measured and pycnidia on the leaf were counted. The experiment was performed twice.

Removal of conidia from leaf surfaces. Water suspensions containing 1.9×10^5 conidia/ml were sprayed onto leaves of potted *V. vinifera* Riesling plants and dryed for 0, 0.5, 0.75, 1.0, and 24.0 hr. After each dryness period, leaves were sprinkler-washed with 225 ml of water per minute for 5, 15, 30, and 60 min. Leaf disks, 6 mm diameter, were removed before and after washing, covered with a drop of rose bengal to stain conidia (5), and conidia were counted with a microscope.

Conidia on leaf surfaces. Aurore, Baco Noir, Concord, and Ives plants were inoculated with 0, 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0×10^4 conidia/ml. Plants were placed in a moist chamber at 21 C for 24 hr, then maintained in a greenhouse at 21 ± 5 C and 35 ± 15% RH. Leaf disks, 6 mm in diameter, were removed 25 hr after inoculation, stained with rose bengal (5), and conidia were counted. The percentage of diseased leaf area was estimated 3 wk after inoculation.

Naturally occurring conidia on leaf surfaces were counted weekly from May to September 1976, 1977, and 1978 in a research Aurore vineyard at Wooster. Fungicides were applied according to commercial recommendations (4) in a section of the vineyard. Two 6-mm disks were removed from the third-youngest leaf on selected shoots, and conidia were stained and counted. Conidia of *G. bidwellii* were identified according to physical appearance, shape, and size (6). Leaf infection was recorded weekly, berry infection at harvest.

RESULTS

Incubation and pycnidia formation. Incubation time in growth chambers at 21 C did not differ from that at 26.5 C, but was

TABLE 1. Effect of temperature on incubation time and pycnidia formation of *Guignardia bidwellii* on grape leaves

Temperature (C)	Incubation period (days)	Pyncidia formation (days)
Growth chamber[a]		
15.0	13.5	19.0
21.0	7.5	10.5
26.5	7.5	12.0
Vineyard[b]		
19.0	12.0	16.5
22.0	8.5	13.0
23.0	8.0	12.0
24.0	8.0	12.0

[a]Values represent a compilation from several experiments with cultivars Aurore, Baco Noir, Catawba, and Niagara. Each cultivar was replicated 30 times at each temperature.
[b]Values determined from weekly inoculation of 10 to 21 Aurore shoots.

TABLE 2. Effect of *Guignardia bidwellii* inoculum concentration on grape leaf surface conidial population and leaf disease severity

(Conidia/ml × 10^4)	Conidia/cm^2 of leaf[a]	Average diseased leaf area/plant[b] (%)
0	0	0
0.5	20	15
1.0	39	20
2.0	63	19
4.0	112	30
8.0	314	35
16.0	614	49

[a]Each value represents the mean of 18 replicate disks, 10 observations at × 160 per disk. Correlation coefficient (r) of conidia/cm^2 as affected by inoculum concentration = 0.997 (significant at $P = 0.01$).
[b]Each value represents the mean of 16 replications determined 3 wk after inoculation. Correlation coefficient (r) of percentage of diseased area as affected by inoculum concentration = 0.908 (significant at $P = 0.01$).

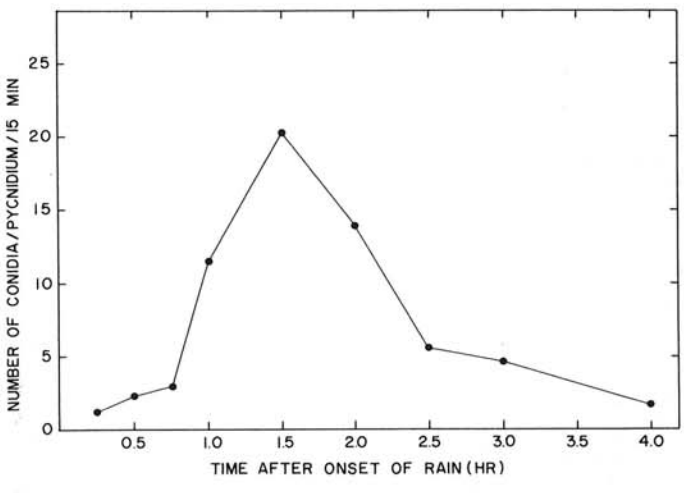

Fig. 1. Number of *Guignardia bidwellii* conidia collected in Catawba grape leaf runoff water at various times after the onset of rain. Each point represents the average of two experiments.

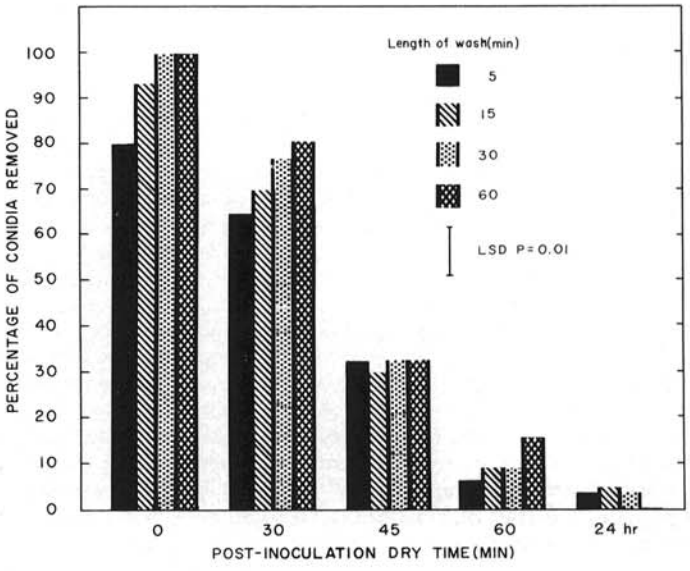

Fig. 2. Effects of postinoculation dryness and washing durations on removal of *Guignardia bidwellii* conidia from Riesling grape leaf surfaces.

TABLE 3. Relationship between amount and duration of rainfall for *Guignardia bidwellii* conidia dissemination and retention, leaf infection, and leaf surface conidia concentration in a Wooster, OH vineyard—1978

Date	Average conidia/cm² of leaf[a]	Rainfall during previous 7 days		Condition for conidia dissemination[c]	Diseased leaves/shoot[d] (no.)
		Amount (cm)	Duration (hr)[b]		
July					
6	0.4	1.8	18	poor	0.4
13	11.5	0.3	1,1	poor	0.6
20	7.1	1.6	1,1,1	poor	0.7
27	16.9	1.0	1,4	poor	2.4
August					
3	28.0	1.5	7,3	good	2.5
10	29.8	6.9	10,2,1	good	2.5
17	15.3	0.1	1	poor	3.1
24	6.3	0.2	1	poor	3.1
31	49.3	1.8	12,3,1	good	3.5

[a]Values represent the average of 52 replicate disks, 10 microscope field counts (160 ×) per disk from Aurore leaves.
[b]Individual rain events are separated by commas.
[c]Conidial dissemination and retention rating derived from conidia release during rain (Fig. 1) and removal of conidia from leaf surfaces (Fig. 2).
[d]Each value represents the mean of 26 replications.

TABLE 4. Seasonal average leaf surface conidia levels, leaf infection of, and berry infection by, *Guignardia bidwellii* in a Wooster, OH vineyard—1976 to 1978

Date	Fungicides[a]	Leaf infection[b] (%)	Berry infection[b] (%)	Conidia/cm² of leaf[c]
1976	−	43 w[d]	58 w	46 w
	+	12 x	12 x	26 x
1977	−	13 y	31 y	25 y
	+	7 y	1 z	7 z
1978	−	8	5	19

[a]Fungicides applied according to commercial recommendations for control of grape black rot. 12, 9, and 9 applications were made in 1976, 1977, and 1978, respectively.
[b]Values represent the mean of 15, 6, and 26 replicate Aurore vines for 1976, 1977, and 1978, respectively.
[c]Values represent the mean of 34, 24, and 28 disks, 10 microscope fields (160 ×) per disk for 1976, 1977, and 1978, respectively.
[d]Numbers followed by the same letter within columns for each season are not significantly different at $P = 0.05$, according to Duncan's new multiple range test. Each season's data were analyzed independently.

lengthened by 6 days at 15 C (Table 1). Considerable variation in time of pycnidia formation was observed. At 15 C, pycnidia appeared in 21 ±2 days. At 21 and 26.5 C, pycnidia appeared in 13 ±3 days (Table 1). No effect of RH on incubation time or pycnidia formation was observed, nor were cultivar differences large enough to justify construction of individual curves. Incubation time under field conditions was 8 to 12 days at average temperatures of 24 and 19 C, respectively. Pycnidia appeared in 12 to 16.5 days after inoculation at average vineyard temperatures of 24 and 19 C, respectively (Table 1).

Conidia release during rain. Maximum numbers of conidia in runoff water from severely infected leaves were collected between 60 and 120 min after a rain began (Fig. 1). Few conidia were collected during the first 45 min or after 3 hr of exposure to rain.

Removal of conidia from leaf surfaces. When inoculated leaves were washed without a dryness interval, all conidia were removed after 30 min of washing (Fig. 2). After each successive drying period, lasting up to 60 min, a significant ($P = 0.01$) increase in retention of conidia occurred. Dryness periods longer than 60 min, however, did not result in additional conidia retention. Duration of washing significantly ($P = 0.01$) increased removal of conidia in the 0 and 30 min dry periods, but not after 45 min of dryness (Fig. 2).

Conidia on leaf surfaces. The quantity of conidia adhering to leaf surfaces and the amount of foliar disease were closely related to inoculum concentration (Table 2). Only a small increase in disease resulted with each doubling of inoculum concentration.

Naturally occurring conidia populations on leaf surfaces in the vineyard were highest on August 3, 10, and 31 (Table 3). During the 7 days prior to these dates, rainfall of 2 to 3 hr occurred and more than two diseased leaves per shoot were present. Fungicide application, which resulted in significant disease control, was related to decreased numbers leaf surface conidia (Table 4). During 1977 and 1978, when disease was less severe than in 1976, average leaf surface conidia populations also were less than in 1976 (Table 4).

DISCUSSION

Previous work concerning infectivity of *G. bidwellii* on grape leaves showed that infection occurred after 6 hr of leaf wetness at 26.5 C, but 24 and 12 hr of wetness were required at 10 and 32 C, respectively (8). The data reported here further define black rot disease development in terms of length of incubation period and pycnidia formation. The incubation period was approximately 1 wk at temperatures above 21 C, but could be as long as 2 wk at 15 C. Because average vineyard temperature over several days is seldom less than 15 C or exceeds 26.5 C during the growing season, temperatures outside these limits were not tested. After lesions appeared, pycnidia formed within 3 to 5.5 days at 21 and 15 C, respectively. Thus, even under unfavorable conditions, time from infection to production of conidia did not exceed 3 wk. Incubation time and pycnidia formation periods in the vineyard were slightly longer than in the growth chamber. Several factors, such as fluctuating environmental conditions and altered host resistance, may be involved. Disease severity on plants exposed to variable temperatures during infection was significantly less than that in plants infected at constant temperatures (8).

Conidial dispersal is rain-dependent (3), but no quantitative relationships between spore dispersal and amount and duration of rain were known previously. Rainfall duration longer than 1 hr provided optimal dissemination of conidia. Rainfall longer than 3 hr may deplete pycnidia of conidia and wash conidia from the leaves if no drying occurs. Approximately 93% of conidia applied to leaves were washed off when no drying occurred, but only 10% were removed after 60 min of drying. However, excessive drying may affect conidia adversely since a 24-hr dryness period significantly reduced infection (8).

Naturally occurring conidia populations on grape leaves were closely correlated with the amount of disease in the vineyard. Prior

to dispersal of overwintered conidia in spring, conidia were not found on new, emerging grape leaves, but were present inside pycnidia on canes, tendrils, and fallen leaves (R. A. Spotts, *unpublished*). As the season progressed, conidia populations on leaf surfaces initially depended on proper conditions for dispersal and retention by leaves and were later related to vineyard disease levels. Conidial populations on leaf surfaces in this study seldom exceeded 63 conidia per square centimeter. This quantity corresponded to inoculation with 2.0×10^4 conidia per milliliter. Conidia catches as high as 6.1×10^5/ml of rainwater have been reported (3). During peak release, 2.2×10^5 conidia per milliliter were found in this study.

Elucidation of factors affecting disease development and inoculum dispersal are essential for development of effective disease control programs. Effective inoculum dispersal must occur or disease development will be limited even during conditions favorable for infection. However, infection can occur during an evening, dew-related, infection period in the absence of rain if a rainfall sufficient for conidia dispersal occurred during the previous day. Thus, conidia dispersal data obtained in this study further refine the information base used in existing plant disease forecasting programs.

LITERATURE CITED

1. CALTRIDER, P. G. 1961. Growth and sporulation of *Guignardia bidwellii*. Phytopathology 51:860-863.
2. FERRIN, D. M., and D. C. RAMSDELL. 1977. Ascospore dispersal and infection of grapes by *Guignardia bidwellii*, the causal agent of black rot disease. Phytopathology 67:1501-1505.
3. FERRIN, D. M., and D. C. RAMSDELL. 1978. Influence of conidia dispersal and environment on infection of grape by *Guignardia bidwellii*. Phytopathology 68:892-895.
4. HILL, R. G., R. N. WILLIAMS, R. A. SPOTTS, and J. D. FARLEY. 1979. Ohio commercial fruit guide. CES/OSU Bull. 506. 87 pp.
5. KO, W. H., HSIN-HSIUNG LIN, and R. K. KUNIMOTO. 1975. A simple method for determining efficacy and weatherability of fungicides on foliage. Phytopathology 65:1023-1025.
6. REDDICK, D. 1911. The black rot disease of grape. Cornell Univ. Agric. Exp. Stn. Bull. 293:289-364.
7. SCRIBNER, F. L., and P. VIALA. 1888. Black rot (*Laestadia bidwellii*). U.S. Dept. Agric. (Botanical Div. Section of Vegetable Pathology) Bull. 7. 29 pp.
8. SPOTTS, R. A. 1977. Effect of leaf wetness duration and temperature on the infectivity of *Guignardia bidwellii* on grape leaves. Phytopathology 67:1378-1381.
9. SPOTTS, R. A. 1977. Chemical eradication of grape black rot caused by *Guignardia bidwellii*. Plant Dis. Rep. 61:125-128.