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

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**ABSTRACT**


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 (R.H.) on incubation time and pycnidia formation, the factors affecting conidial release from pycnidia and adherence to leaf surfaces, and the factors affecting conidial populations on leaf surfaces in the vineyard.

**MATERIALS AND METHODS**

Incubation and pycnidia formation. Rooted cuttings of *Vitis labruscana* L. ‘Catawba’ and ‘Niagara’ and *V. vinifera* L. × *V. labruscana* 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 5.0×10⁴ 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 klx 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 dried 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 counted and stained. 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**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Incubation period (days)</th>
<th>Pycnidia formation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth chamber‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.0</td>
<td>13.5</td>
<td>19.0</td>
</tr>
<tr>
<td>21.0</td>
<td>7.5</td>
<td>10.5</td>
</tr>
<tr>
<td>26.5</td>
<td>7.5</td>
<td>12.0</td>
</tr>
<tr>
<td>Vineyard§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19.0</td>
<td>12.0</td>
<td>16.5</td>
</tr>
<tr>
<td>22.0</td>
<td>8.5</td>
<td>13.0</td>
</tr>
<tr>
<td>23.0</td>
<td>8.0</td>
<td>12.0</td>
</tr>
<tr>
<td>24.0</td>
<td>8.0</td>
<td>12.0</td>
</tr>
</tbody>
</table>

‡Values represent a compilation from several experiments with cultivars Aurore, Baco Noir, Catawba, and Niagara. Each cultivar was replicated 30 times at each temperature.

§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**

<table>
<thead>
<tr>
<th>(Conidia/ml × 10^4)</th>
<th>Conidia/cm² of leaf*</th>
<th>Average diseased leaf area/plant* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16.0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Each value represents the mean of 18 replicate disks, 10 observations at × 160 per disk. Correlation coefficient (r) of conidia/cm² as affected by inoculum concentration = 0.997 (significant at P = 0.01).

†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.](image)

![Fig. 2. Effects of postinoculation dryness and washing durations on removal of Guignardia bidwellii conidia from Riesling grape leaf surfaces.](image)

Vol. 70, No. 3, 1980 253
TABLE 3. Relationship between amount and duration of rainfall for Guignardia bidwellii conidial dissemination and retention, leaf infection, and leaf surface conidia concentration in a Wooster, OH, vineyard—1978

<table>
<thead>
<tr>
<th>Date</th>
<th>Average conidia/cm² of leaf*</th>
<th>Rainfall during previous 7 days</th>
<th>Condition for conidia dissemination³</th>
<th>Diseased leaves/shoot² (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Amount (cm)</td>
<td>Duration (hr)</td>
<td></td>
</tr>
<tr>
<td>July</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.4</td>
<td>1.8</td>
<td>18</td>
<td>poor</td>
</tr>
<tr>
<td>13</td>
<td>11.5</td>
<td>0.3</td>
<td>1.1</td>
<td>poor</td>
</tr>
<tr>
<td>20</td>
<td>7.1</td>
<td>1.6</td>
<td>11.1</td>
<td>poor</td>
</tr>
<tr>
<td>27</td>
<td>16.9</td>
<td>1.4</td>
<td></td>
<td>poor</td>
</tr>
<tr>
<td>August</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>28.0</td>
<td>1.5</td>
<td>7.3</td>
<td>good</td>
</tr>
<tr>
<td>10</td>
<td>29.8</td>
<td>6.9</td>
<td>10.21</td>
<td>good</td>
</tr>
<tr>
<td>17</td>
<td>15.3</td>
<td>0.1</td>
<td>1</td>
<td>poor</td>
</tr>
<tr>
<td>24</td>
<td>6.3</td>
<td>0.2</td>
<td>1</td>
<td>poor</td>
</tr>
<tr>
<td>31</td>
<td>40.3</td>
<td>1.8</td>
<td>12.31</td>
<td>good</td>
</tr>
</tbody>
</table>

*Values represent the average of 52 replicate disks, 10 microscope field counts (160 X) per disk from Aurore leaves.

1Individual rain events are separated by commas.

2Conidial dissemination and retention rating derived from conidia release during rain (Fig. 1) and removal of conidia from leaf surfaces (Fig. 2).

3Each 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

<table>
<thead>
<tr>
<th>Date</th>
<th>Fungicides¹</th>
<th>Leaf infection² (%)</th>
<th>Berry infection³ (%)</th>
<th>Conidia/cm² of leaf⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>1976</td>
<td>—</td>
<td>43 w</td>
<td>58 w</td>
<td>46 w</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>12 x</td>
<td>12 x</td>
<td>26 x</td>
</tr>
<tr>
<td>1977</td>
<td>—</td>
<td>13 y</td>
<td>31 y</td>
<td>25 y</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>7 y</td>
<td>1 z</td>
<td>7 z</td>
</tr>
<tr>
<td>1978</td>
<td>—</td>
<td>8</td>
<td>5</td>
<td>19</td>
</tr>
</tbody>
</table>

¹Fungicides applied according to commercial recommendations for control of grape black rot. 12, 9, and 9 applications were made in 1976, 1977, and 1978, respectively.

²Values represent the mean of 15, 6, and 2 replicate Aurore vines for 1976, 1977, and 1978, respectively.

³Values represent the mean of 1976, 1977, and 1978, respectively.

⁴Values represent the mean of 1976, 1977, and 1978, respectively.

⁵Numbers 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.

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 of rainfall 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 drying 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 $2.0 \times 10^6$ conidia per milliliter. Conidia catches as high as $6.1 \times 10^7$/ml of rainwater have been reported (3). During peak release, $2.2 \times 10^5$ 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