Influence of Conidia Dispersal and Environment on Infection of Grape by Guignardia bidwellii

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ABSTRACT

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Artificial field inoculations with suspensions of conidia (5×10^4/ml) of *Guignardia bidwellii* were carried out in a black-rot-free *Vitis labrusca* 'Concord' vineyard at East Lansing, MI at various times during the 1975 growing season. Maximum leaf and berry infection resulted from inoculations made at mid-bloom and at 1-cm berry-diameter stages. Inoculations made after berry color change were not successful. Maximum conidia catches in rainwater run-off from leaf lesions bearing pycnidia occurred during the weeks of 10 July 1974 (4.9×10^5 conidia/ml of rainwater collected) and 2 July 1975 (6.1×10^5 conidia/ml of rainwater collected). Conidia also were trapped in rainwater run-off from newly rotted berries, with a maximum catch of 2.2×10^5 conidia/ml of rainwater collected the week of 3 September 1975. Conidia were trapped from overwintered rotted berries in 1976, with a maximum catch of 4.4×10^5 conidia/ml of rainwater, which occurred the week of 23 July. Healthy potted Concord grape 'trap' plants placed in the field for 1-wk periods, became infected only during weeks with rainfall. Environmental factors that correlated positively with infection were number of hours of leaf wetness following rainfall, number of rain events, duration of rain ($P = 0.01$ for all three factors) and amount of rain (cm) was positively correlated with infection ($P = 0.075$).

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

We have reported previously on an epidemiological study dealing with the ascigerous stage of the grape black rot fungus, *Guignardia bidwellii* (Ellis) Viala and Ravaz (3, 4). Ascospore inoculum was shown to be airborne and as a result of rainfall. Ascospores are liberated from previous years mummified berries, beginning at the onset of vine growth in the spring and continuing throughout the growing season. Ascospore inoculum is at relatively low levels in the air. Ascospores cause primary infection of the leaves. Pycnidia that develop in the resulting leaf lesions contain conidia, which are liberated and spread by rain. Conidia are liberated in vast quantities and are responsible for rapid disease increase. No field epidemiological information dealing with the imperfect stage has been published. Conidia germinate readily in water in 3 to 4 hr (5, 6). Caltrider (1) found the optimum temperature for growth and production of pycinidia in culture was 25 C, and the optimum temperature for germination of conidia was 30 C. Spotts (7) reported infection of leaves by conidia required a wetting period of 6 hr at 25.6 C and 24 and 12 hr at 10 and 32 C, respectively. Spotts (7) also reported that fluctuating temperatures during the wetting period, as well as alternate wetting and drying following inoculation, reduced the amount of infection (7, 8). The purposes of the present study were: (i) to determine levels of conidia found in rainwater run-off from infected vines throughout the growing season, (ii) to relate host susceptibility to vine phenology, and (iii) to determine the environmental factors that influence disease development in the field. This information will be used in developing a computer-based black rot disease-warning system.

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MATERIALS AND METHODS

Conidia dispersal.—Conidia produced in the pycnidia of leaf lesions were trapped in rainwater run-off from pycnidia-bearing grape leaves or infected berries by the use of funnel and jug water traps (2). Six, 4-liter plastic milk jugs were connected by Tygon tubing to plastic funnels positioned beneath leaves wtih pycnidia-bearing lesions or beneath rotted berries. Evaporation from the jugs was prevented by inserting the tubing through a hole in the cap of each jug and sealing it with epoxy resin. The jugs were changed weekly and the water collected was examined for conidia.

Suspensions of conidia from the traps were prepared for examination by removing 20 ml of rainwater from each jug, and centrifuging the suspension for 20 min at 4.5×10^3 revs/min in a Lourdes Model AA-C clinical centrifuge (Venitron Medical Products, Inc., Carlstadt, NJ 07072). The pellet was resuspended in 10 ml of distilled water using a vortex mixer and conidia were counted with

the aid of a hemacytometer. Conidia of *G. bidwellii* were identified on the basis of size, shape, and physical appearance. The conidia are globose, ovoid, or sometimes oblong, measure 6.5 - 8.5 - 11.5 μm, and are unicellular and hyaline with a granular appearance (5).

Conidia were trapped from leaf lesions in a Concord vineyard at Paw Paw, MI from 14 June to 3 October 1974, and in a vineyard (cultivar Niagara) at Scottdale, MI from 11 June to 16 September 1975. Conidia also were trapped from newly rotted berries from 30 July to 16 September 1975, and from rotted berries which had overwintered and were serving as primary inoculum from 23 April to 30 July, 1976.

The following weather-monitoring instruments were located in the vineyards: (i) A sheltered 7-day recording hygrothermograph (Bendix Corp., Baltimore, MD 21204); (ii) a 7-day recording rain gauge (Weather Measure Corp., Sacramento, CA 95841); and (iii) a 7-day recording leaf-wetness meter (M. DeWit, Hengelo, Holland). All three instruments were located 1 m above the ground.

Host susceptibility in relation to vine phenology.—Inoculations with conidia were carried out in a vineyard free of black rot at East Lansing, MI at intervals of several days to 2 wk, corresponding to critical vine phenology stages, during the 1975 growing season. Naturally infected leaves were gathered from the field and tissue that contained lesions was excised with a razor blade. The tissue sections were placed in distilled water in a petri dish, and the pycnidia were teased apart and rubbed with a dissecting needle to release the conidia. The resulting suspension of conidia was filtered through several layers of cheesecloth and adjusted to 5.0×10^4 conidia/ml. A DeVilbiss atomizer was used to spray the spore suspension onto five shoots of Concord vines in the field. Inoculated shoots were resprayed with distilled water after 12 hr and covered with transparent polyethylene bags for 24 hr. Five shoots, sprayed only with distilled water, were bagged and served as controls at each inoculation date. The inoculated shoots were labeled and the stage of growth and number of inoculated leaves per shoot was recorded. Lesions were counted weekly for 4 wk after inoculation and the average number of lesions per leaf per inoculation date was calculated.

Periods of natural infection.—Rooted healthy Concord grape cuttings placed in the field for a 1-wk period, served as 'trap' plants to determine the environmental conditions necessary for infection during the exposure period. Five, rooted, healthy Concord grape cuttings growing in 4-liter cans were placed beneath infected vines in a Niagara vineyard at Scottdale, MI during 1975 and 1976. Each set of five plants was replaced at 1-wk intervals, returned to East Lansing, and maintained in isolation in a cold frame. Symptoms were allowed to develop and lesion counts were made at 1-wk intervals for 4 wk from the date the plants were brought from the field. Nonexposed Concord grape cuttings that were kept in the cold frame throughout the season served as controls. Weather parameters were measured as described previously (3, 4). The environmental and 'trap' plant infection data were subjected to complete multiple regression analysis using the campus CDC 3600 computer (Control Data Corp., Southfield, MI 48075).

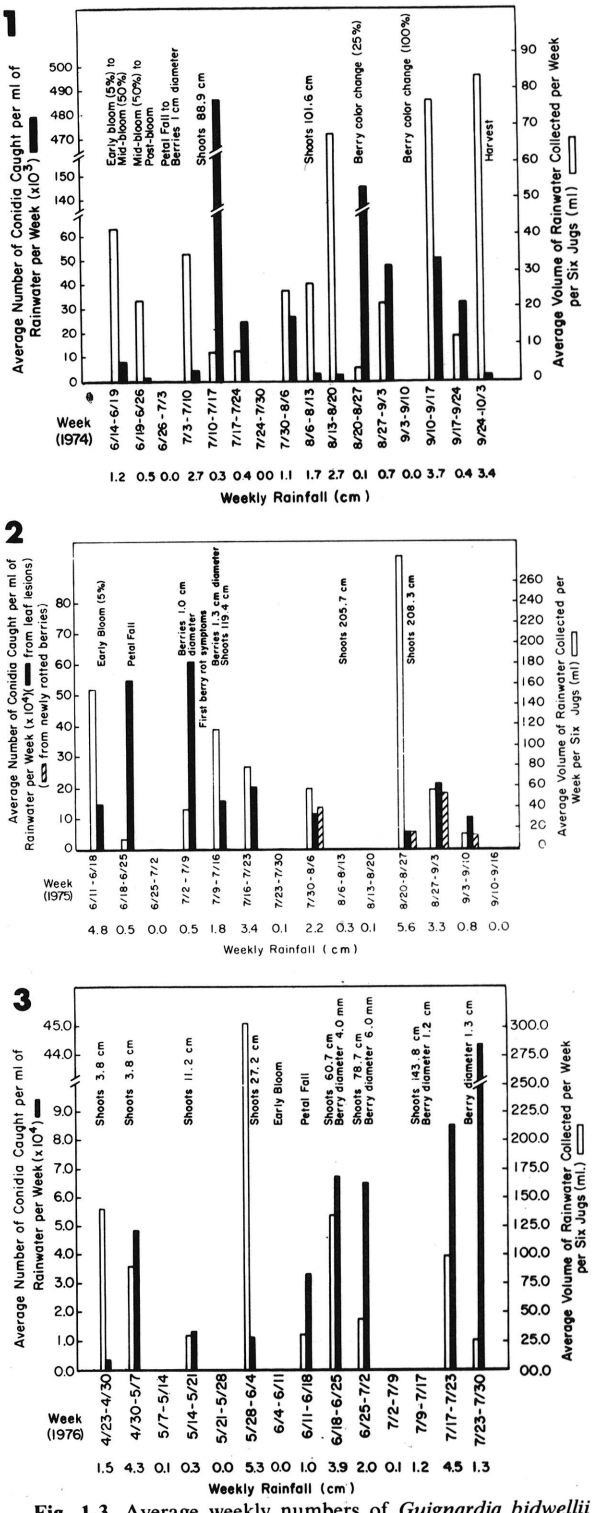

Fig. 1-3. Average weekly numbers of *Guignardia bidwellii* conidia trapped in rainwater run-off from infected Concord or Niagara grape leaves or rotted berries relative to the average weekly volume of rainwater collected: **1)** from Concord grape leaves during 1974 at Paw Paw, MI; **2)** from Niagara grape leaves or newly rotted berries during 1975 at Scottdale, MI; and **3)** from overwintered black-rotted berries during spring 1976 at Scottdale, MI.

RESULTS

Dispersal of conidia.—Conidia trapped from overwintered rotted berries serving as a primary inoculum source were trapped first during the week of 23 April 1976 (Fig. 3). The numbers trapped were relatively low from 23 April to 18 June, but increased to a maximum of 4.4×10^5 conidia/ml of rainwater collected during the week of 23 July at which time berry diameter was about 1 cm.

Conidia were trapped first in rainwater run-off from leaf lesions during the weeks of 14 June 1974, (Fig. 1) and 11 June 1975 (Fig. 2). These weeks corresponded to the times immediately after leaf lesions had appeared in the field. As little as 0.1 cm of rain induced dispersal of conidia (Fig. 1). Peak catches of conidia occurred during the week of 10 July 1974 (4.9×10^5 conidia/ml of rainwater) when berry diameter was about 1 cm. Large numbers of conidia were caught during each rainy period throughout the season until harvest. Peak catches occurred during the week of 2 July 1975 (6.1×10^5 conidia/ml of rainwater), when berries were about 1-cm in diameter. Numbers of conidia trapped from newly rotted berries were similar to those trapped from leaf lesions during the same weeks (Fig. 2). Catches of conidia averaged 1×10^5/ml of rainwater collected per week from newly rotted berries. Peak catches of conidia from newly rotted berries occurred during the week of 27 August (2.2×10^5 conidia/ml of rainwater).

Susceptibility in relation to vine phenology.—Leaf lesions developed 14 days after inoculation with suspensions of conidia on the terminal four or five leaves.

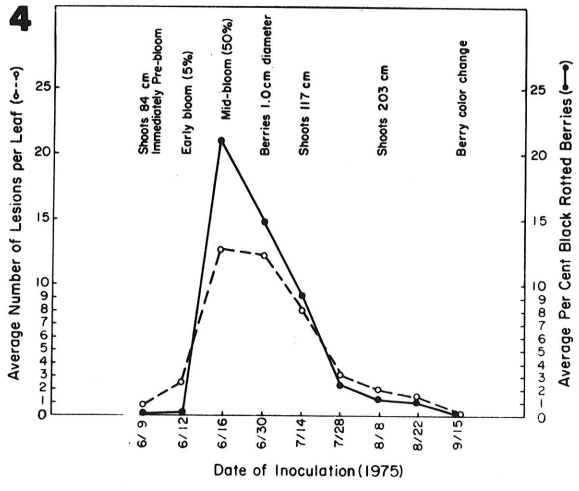

Fig. 4. Infection of Concord grape leaves (o-o) and berries (•-•) by *Guignardia bidwellii* conidia (5×10^4/ml) following artificial inoculations made in a blackrot-free vineyard on various dates during the 1975 growing season at East Lansing, MI.

TABLE 1. Complete multiple regression analysis of infection of healthy potted Concord grape'trap'plants by *Guignardia bidwellii* (dependent variable) as affected by environmental factors at Scottdale, MI in 1975 and 1976[a]

Independent variables	Regression coefficient[b]	Standard error of regression coefficient	Level of significance of regression coefficient	Simple correlation coefficients[c]
Number of rain events	−0.278	0.263	0.300	0.412[d]
Amount of rain	−0.428	0.378	0.266	0.255[e]
Duration of rain	0.055	0.061	0.378	0.381[d]
Hours of leaf wetness after rain	0.083	0.041	0.053	0.504[d]
Average temperature during leaf wetness	0.098	0.046	0.040	0.69[f]
				R = 0.638 R^2 = 0.407

[a]Data inputs are on weekly basis, but only for weeks in which infection occurred. Weekly infection levels varied from 0.0 to 7.9 lesions/leaf.

[b]Computational formula for the regression coefficient is:

$$r = \frac{\sum_{i=1}^{N} X_i Y_i - \left(\sum_{i=1}^{N} X_i\right)\left(\sum_{i=1}^{N} Y_i\right)/N}{\left[\sum_{i=1}^{N} X^2 - \left(\sum_{i=1}^{N} X_i\right)^2/N\right]\left[\sum_{i=1}^{N} Y_i\right)^2/N\right]^{1/2}}$$

[c]General form is the regression is $Y = A + B_1X_1 + B_2X_2 + \ldots + B_kX_k$

[d]Indicates highly significant difference, $P = 0.01$.

[e]Indicates significant difference, $P = 0.075$.

[f]Indicates significant difference, $P = 0.10$.

Lesion numbers were greatest on plants that were inoculated from midbloom to berry size of 1-cm diameter; 13 and 12.5 lesions per leaf respectively were formed (Fig. 4). Berry infection also was greatest during this stage of development, resulting in 21% and 15% infection, respectively. Both leaf and berry infection declined from this point with no new infections resulting from inoculations made after 22 August when the berries were 1.5 cm in diameter.

Periods of natural infection.— Computer analysis of data from 2-yr 'trap' plant infection (lesions/leaf) vs. weather parameters for 1-wk intervals is expressed as simple correlation coefficients (Table 1). The greatest effect on infection was shown by the highly significant positive correlation coefficient between infection and the number of hours of leaf wetness following a rain; 12 hr or more was sufficient for infection, depending upon temperature, the number or rain events, and the duration of rainfall. The amount of rain (cm) also was positively correlated with infection. Rainfall releases conidia inoculum and helps to provide the necessary length of leaf wetness for infection to occur.

DISCUSSION

It is now possible to understand more clearly the influence of environmental factors on the disease cycle of grape black rot. Rainfall is the most important factor, since as little as 0.3 cm of rain is sufficient to induce ascospore release (3, 4) and conidia can be released by even lesser amounts of rain. Limited infection has been observed in the field as a result of less than 0.25 cm of rain, followed by the requisite leaf-wetness period (3), which is caused by high humidity or dew following the rain.

Ascospore dispersal is maximal just prior to and during bloom (3, 4), with decreasing numbers being released as a result of rains up to harvest. Conidia become available coincident with full bloom and are maximal shortly after bloom when the small berries are developing rapidly. Peak periods of conidia dispersal coincide with the time when vines are most susceptible to infection.

Inoculations in the field with ascospores (3, 4) and with conidia have shown that infection of leaves and berries does not occur at economically important levels after late August. Host tissues no longer are susceptible at this time and there is no need for further fungicide treatments.

Numbers of trapped conidia in rainwater can range as high as 44×10^4/ml for a 1-wk period, whereas, the number of ascospores trapped from air in the same vineyard over a 24-hr period ranged in the hundreds (the Burkard spore trap was drawing 10 liters of air per min). Ascospores, which serve as an early inoculum in the spring, are windborne and thus are more widely dispersed than are conidia. Also, ascospores are an important source of genetic variability for the pathogen. Once primary infection is established and pycnidia have formed, conidia in great numbers are responsible for rapid disease progression.

Once spore dispersal has occurred, the requisite period of leaf wetness following rain, in conjunction with the average temperature during this time, become prime factors influencing infection. A 6-hr, leaf-wetness period at 27 C is sufficient for slight infection, by ascospores and for 10, 16, and 21 C, a 12-hr leaf-wetness period is necessary for slight infection (3, 4). A 6-hr leaf-wetness period at 26.5 C is sufficient for slight infection by conidia and at 10, 15.5, 21, and 32 C, the periods of leaf wetness necessary for slight infection are 24, 9, 7, and 12 hr, respectively (7, 8). Both ascospores and conidia are sensitive to desiccation. If leaf-wetness duration sufficient for infection is not present within 24 hr after dispersal, additional leaf wetness later will result in greatly reduced levels of infection (3, 4, 7, 8).

We have incorporated these findings into a FORTRAN IV computer program which forecasts infection periods based on weather data, vine phenology, and time elapsed since the application of fungicide. This program is being field-tested in comparison with a standard 'calendar date' spray schedule. Preliminary results (Ferrin and Ramsdell, *unpublished*) indicate that by use of this program, the number of fungicide sprays can be reduced by 30 to 40% and economically acceptable control of black rot still can be attained.

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