Ascospore Dispersal and Infection of Grapes by Guignardia bidwellii, the Causal Agent of Grape Black Rot Disease

Donald M. Ferrin and D. C. Ramsdell

Graduate Research Assistant and Associate Professor of Plant Pathology, respectively, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824. Present address of senior author: Yoder Bros. Inc., Ft. Myers, FL 33901. This publication is partial fulfillment of the M.S. degree. The authors thank Jack Bilger, Felix Dongvillo, and Richard Ketelhut for the use of their vineyards for this research. Graphic art work by Tia Maxwell is gratefully acknowledged.

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ABSTRACT

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Airborne ascospores of *Guignardia bidwellii* were trapped with a Burkard 7-day recording volumetric spore trap located in two Michigan vineyards during the 1974 and 1975 growing seasons. Ascospore discharge was best correlated with duration of rainfall ($r = 0.6738$), and they were trapped continuously from within 1 hr of the onset of rain to 8 hr after the rain ceased. As little as 0.03 cm of precipitation caused ascospore release. Peak ascospore catches were obtained from prebloom (late May) to midbloom stages (mid-June). Artificial field inoculations with ascospore suspensions (5×10^4 spores/ml) resulted in maximum leaf infection beginning when shoots were 10-20 cm long and continuing through early berry development. Maximum berry infection occurred at midbloom. Ascospores did not germinate in atmospheres of 98 or 100% relative humidity, but did germinate in free water on Concord grape leaves within 6 hr at 10, 20, and 30 C. By 24 hr, percentage germination was identical at all three temperatures. When ascospores were incubated on dry leaves, percentage germination decreased with increasing incubation period. No germination occurred after incubation for 48 hr on dry leaves. Infection of Concord grape leaves developed most rapidly at 27 C, but less so at 10, 16, and 21 C; no symptoms developed at 32 C.

Additional key words: epidemiology.

The black rot disease of American grapes (*Vitis labrusca* L.) caused by the fungus, *Guignardia bidwellii* (Ellis) Viala and Ravaz, is among the most economically destructive vine diseases in the northeastern U.S. today. Prior to the development of protectant fungicides, a loss of 25% of the crop was tolerated (1). Although damage to the vines is minimal, fruit losses of from 70 to 100% were not uncommon in years favorable to the disease. Today, black rot of grape is found in all commercial production areas except California where the dry climate prevents disease development (2, 6, 7). Owing to the importance of this disease, most spray recommendations for grapes focus on black rot, particularly in Michigan (3).

Early studies dealt with control practices and breeding for disease resistance (4, 5, 7, 10). It was not until 1911 that Reddick (6) published a clarification of the pathogen cycle. With the availability of improved epidemiological methods, a quantitative study of the pathogen cycle under growing conditions in Michigan was conducted by the authors. The purpose of this research was to gather the necessary biological data which ultimately could be used in a computer-based, disease-warning system.

MATERIALS AND METHODS

Ascospore dispersal.—Airborne ascospores of *G. bidwellii* were trapped with a Burkard 7-day recording, volumetric spore trap [Burkard Scientific (Sales) Ltd., Rickmansworth, Hertfordshire, England] Weather parameters were measured with a 7-day recording rain gauge (Weather Measure, Inc., Sacramento, CA 95841), leaf-wetness meter (M. DeWit, Hengelo, The Netherlands), and a sheltered hygrothermograph (Bendix Corp.. Baltimore, MD 21204); all instruments were positioned 1 m above ground level.

Ascospores were trapped in two locations; a Concord vineyard near Paw Paw, Michigan in 1974, and a Niagara vineyard near Scottdale, Michigan in 1975. The spore traps were adjusted to draw air at about 10 liters/min and were run continuously from 9 May to 1 October 1974, and from 14 May to 16 September 1975.

Ascospores were counted after cutting the Melinex [Burkard Scientific (Sales) Ltd., Rickmansworth, Hertfordshire, England] trap tapes into 48-mm long strips (representing 24 hr), staining the strips in 0.5% (w/v) aqueous safranin, rinsing them twice in glass-distilled water (GDW), and mounting them on glass slides. Ascospores were identified on the basis of size ($5\text{-}7 \times 12\text{-}17$ μm), shape (subovoid to elliptical), and the presence of two highly refractive guttules. A "standard" spore trap tape containing ascospores was used for reference. Spore counts were recorded for each hour of trap operation using a compound microscope at X200 magnification.

Artificial inoculations.—Ascospores were collected from mummified berries soaked in GDW for 30 min and placed on glass slides. After a 1-hr discharge period, ascospore suspensions were adjusted in GDW to 5

× 10^4 spores/ml, using a hemacytometer. Shoots of rooted Concord grape cuttings in the greenhouse or disease-free Concord vines in a vineyard at East Lansing, Michigan, were inoculated with the spore suspension using a DeVilbiss #15 atomizer, to the point of run-off. The potted greenhouse vines were incubated in a mist chamber for 48 hr after inoculation; inoculated shoots in the field were sprayed with GDW and covered with transparent polyethylene bags for 24 hr. Inoculations were made at various times throughout the growing season, corresponding to different phenological stages of the vine. Three greenhouse plants or three shoots in the field were inoculated at each time. Three plants or shoots inoculated with GDW at each time served as controls. Lesions were counted weekly for 1 mo after inoculation.

Germination.—Ascospores were discharged from wetted, mummified berries, onto 1-cm^2 pieces of Concord grape leaves on glass slides. After a discharge period of 1 hr, GDW was added to the leaf pieces and the slide was placed on top of a stack of slides in a glass petri plate. GDW was then added to the bottom of the plates, which were covered to prevent evaporation from the leaf surfaces. Three leaf pieces were placed in each petri plate to provide three replications per time-temperature regime to be studied. The plates then were incubated at 10, 20, and 30 C for 6, 12, and 24 hr. At the end of each incubation period, plates were removed and the leaf pieces were cleared in a glacial acetic acid-ethanol mixture (1:1, v/v) for 24 hr followed by 85% lactic acid for another 24 hr (9). The ascospores were stained with lactophenol-cotton blue for 6 hr, rinsed in lactophenol, and mounted in lactophenol. Percentage germination was obtained by counting 100 spores at random per replication and observing for the presence of germ tubes. Previous tests (Ferrin and Ramsdell, *unpublished*) indicated that ascospores do not germinate at 98% or 100% relative humidity, therefore only free water was used in this experiment.

Spore longevity.—The first and second terminal leaves of 1-yr-old rooted Concord grape cuttings in the greenhouse were spray-inoculated with an ascospore suspension (3.5 × 10^4 spores/ml) until water run-off occurred. The leaves were allowed to dry and were appropriately labeled. Ascospores were incubated on dry leaves in the greenhouse for 0, 3, 6, 12, 24, 48, 72, and 402 hr after which time individual leaves were removed and three 1-cm^2 diam leaf disks were excised adjacent to the midvein. The ascospores on the leaf pieces then were germinated in GDW sprayed onto the vines. Test plants were incubated in a mist chamber for 24 hr at 30 C, prior to counting percentage germination.

In addition, two plants were incubated dry for 12 hr, placed under mist for 6 and 12 hr, respectively, and then placed in dry conditions for another 12 hr prior to germinating the spores in free water at 30 C.

Leaf infection.—Suspensions containing 5 × 10^4 ascospores/ml were sprayed to the point of run-off onto the leaves of 66, 2- and 3-yr-old potted Concord grape cuttings. The plants then were resprayed with GDW, covered with transparent polyethylene bags and placed in growth chambers (Sherer-Gillett Co., Marshall, MI 49068) maintained at 10, 18, 21, 26, or 32 C (constant air temperature) for 6, 12, 18, or 24 hr. One plant was used per treatment with three replications. High relative humidity was maintined with cold water, power humidifiers. At the end of each incubation period, the bags were removed from the plants to permit drying. Leaves dried off within 20 to 30 min. Inoculated plants then were incubated in growth chambers at 24 C and checked weekly for lesion development. Lesion counts were made up to 1 mo after inoculation. Six plants sprayed with GDW, bagged, and incubated for 24 hr at 21 and 26 C (three plants per temperature) served as controls.

RESULTS

Ascospore dispersal.—Ascospores were trapped only on days with measurable rainfall (Fig. 1-A, 1-B, and 2). As little as 0.08 (19 May 1974) and 0.10 cm (31 May 1974) of rain caused catches of four and 28 ascospores per day, respectively (Fig. 1-A). As little as 0.03 cm of rain on 29 May, 12 June, and 10 August 1975 triggered catches of five, four, and three ascospores per day, respectively (Fig. 2). At the beginning of the seasons ascospores initially were trapped on 14 May 1974 and 20 May 1975; the last ascospore catches occured on 21 September 1974 and 5 September 1975. Peak ascospore catches occurred on 7 and 10 June 1974 (84 and 82 ascospores per day, respectively) and on 30 May and 15 June 1975 (63 and 65 ascospores per day, respectively). These peaks occurred

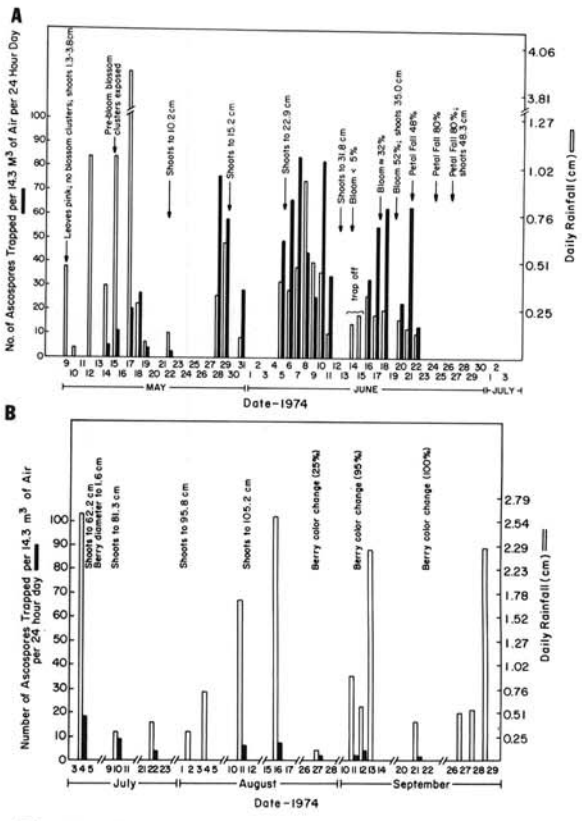


Fig. 1-(A, B). *Guignardia bidwellii* ascospore catches (Burkard sport trap) from a *Vitis labrusca* 'Concord' vineyard near Paw Paw, Michigan in 1974. **A)** Prebloom through petal fall. **B)** Petal fall through harvest.

when shoot lengths were from 23 to 32 cm (about 1 wk prior to bloom) in 1974 and when shoot lengths were 30.5 cm to midbloom stage in 1975. Generally ascospore release began about mid-May, reached a peak prior to and into the full bloom stage, and finally decreased to low levels after mid-July.

A correlation between rainfall and ascospore release on an hourly basis was evident (Fig. 3). Largest numbers of ascospores were trapped during the 2 hr immediately after rainfall ceased; fewer ascospores were detected during the next 6 hr.

Pearson correlation coefficients were calculated on a daily basis for: (i) the mean temperature, (ii) high temperature, (iii) low temperature, (iv) number of hours of 100% RH, (v) number of hours of leaf wetness, and (vi) amount and duration of rainfall. The Pearson correlation coefficients were calculated on a CDC 6500 Computer using the SPSS program [Statistical Package for the Social Sciences, Version 6 (Vogelback Computing Center, Northwestern University, Evanston, IL 60201)]. Highest correlations with numbers of ascospores trapped were obtained with duration of rainfall, $r = 0.6738$ ($P = 0.001$) and amount of rainfall, $r = 0.6500$ ($P = 0.001$). The number of hours of 100% RH had a lower correlation coefficient, $r = 0.2798$ ($P = 0.002$) and so did the number of hours of leaf wetness, $r = 0.2008$ ($P = 0.020$). All remaining climatological factor correlation coefficients were not significant at $P = 0.134$ or greater, and therefore were not considered important.

Artificial inoculations. — In 1974 the greatest amount of leaf infection (16 lesions/leaf) resulting from inoculations made in the field, occurred when shoots were 15 to 20 cm long, 2 to 3 wk before bloom (Fig. 4-A). Successful infection continued through bloom with a high of 12 lesions/leaf during late bloom (about 80% petal fall). Inoculations made during August or September were not successful. In 1975, the greatest amount of leaf infection occurred (19 lesions/leaf) when shoots were 33 cm long (2 to 3 wk before bloom) (Fig. 4-B). Successful infection continued through bloom with 16 lesions/leaf resulting from midbloom inoculations.

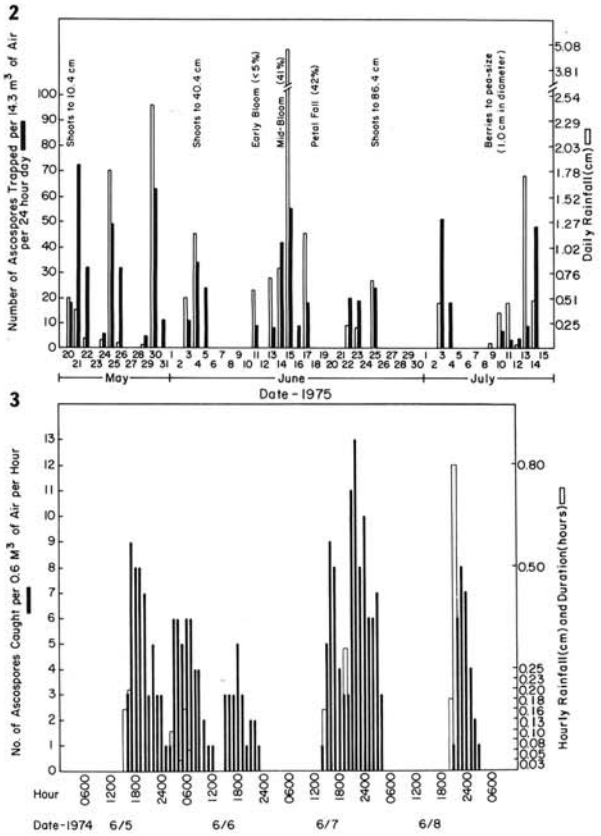


Fig. 2-3. 2) *Guignardia bidwellii* ascospore catches (Burkard spore trap) from a *Vitis labrusca* 'Niagara' vineyard near Scottdale, Michigan, from prebloom through early berry development in 1975. **3)** The effect of rainfall on *G. bidwellii* ascospore dispersal on an hourly basis as measured by a Burkard spore trap in a *V. labrusca* 'Concord' vineyard near Paw Paw, Michigan in 1974.

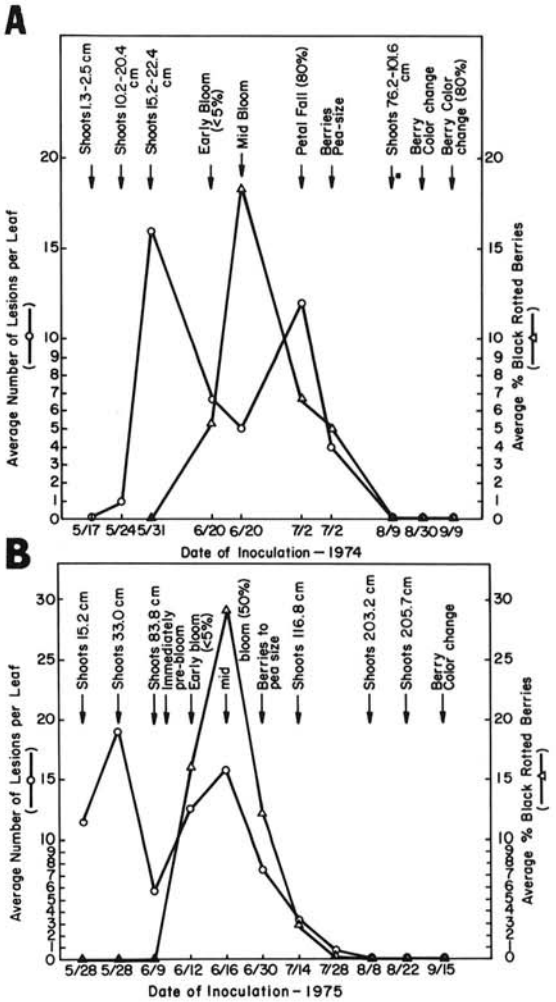
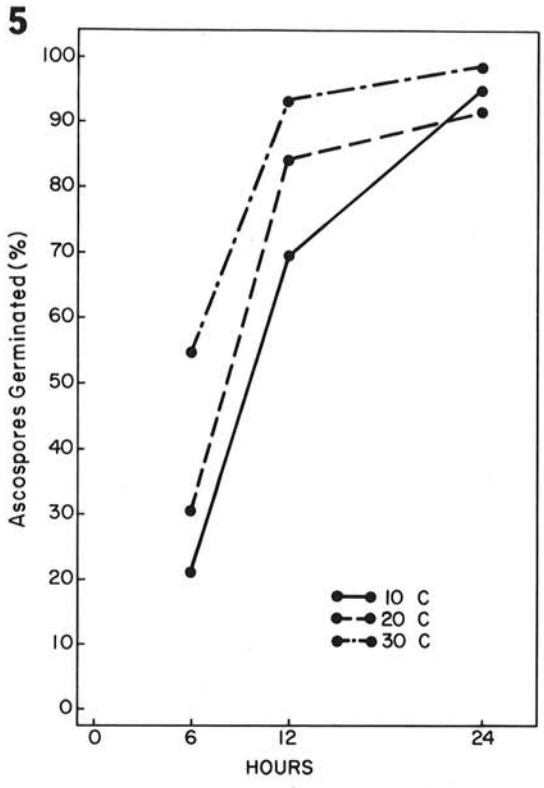


Fig. 4-(A, B). Infection of *Vitis labrusca* 'Concord' leaves and fruit by ascospores of *Guignardia bidwellii* resulting from artificial inoculations (5×10^4 spores/ml) made in the field at East Lansing, Michigan on various dates in **A)** 1974 and **B)** 1975.



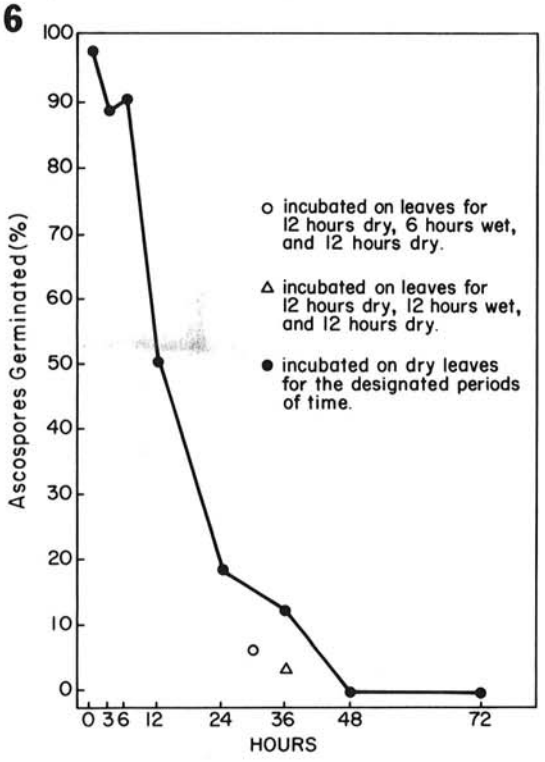


Fig. 5-6. 5) Germination of ascospores of *Guignardia bidwellii* in free water on *Vitis labrusca* 'Concord' leaf pieces placed in petri dishes and incubated at several temperatures. 6) Germination of ascospores of *G. bidwellii* in free water for 24 hr at 30 C on *V. labrusca* 'Concord' leaf pieces following incubation on dry leaves for various times.

Berry infection initially resulted from early bloom inoculations in both years (5.3% and 16% of the berries infected in 1974 and 1975, respectively). Maximum berry infection (18.5% and 29% in 1974 and 1975, respectively) resulted from midbloom inoculations. Berry infection then decreased, and no new infections resulted from inoculations made during August or September.

Germinations.—Ascospore germination occurred within 6 hr at all temperatures; percentage germination was directly proportional to temperature (Fig. 5). Over 90% germination occurred within 24 hr at all temperatures tested. Germination was greatest at 30 C and least at 10 C.

Longevity. — Germination of ascospores incubated for various periods on dry leaves decreased as the period of incubation increased prior to rewetting the leaves to allow germination (Fig. 6). Maximum germination (97%) was obtained with no dry period following inoculation. Germination was less after 6, 12, and 24 hr of dryness (90.7%, 50.3%, and 18.4%, respectively). No germination occurred when the leaves were dry for 48 hr or more, prior to rewetting.

Ascospores incubated on leaves that were alternately dry (12 hr), wet (6 hr), and dry (12 hr) germinated at 6.7%. Ascospores on leaves under the same conditions for 12 hr dry, 12 hr wet, and 12 hr dry germinated at 3.3%.

Leaf infection. — In growth chamber studies, leaf infection occurred most rapidly and reached highest levels (9.8 and 16 lesions per leaf after 12 and 24 hr, respectively) at 27 C (Fig. 7). Infection occurred more slowly and at a lower level at 10, 16, and 21 C. No symptoms developed at 32 C. The aberrant reversal resulting from the 21 C incubation cannot be explained.

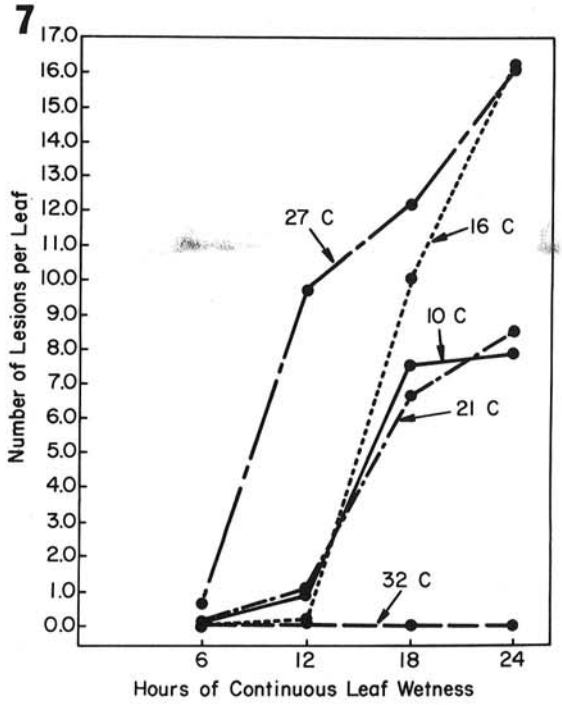


Fig. 7. The effect of temperature on infection of *Vitis labrusca* 'Concord' leaves in free water following inoculation with 5×10^4 ascospores of *Guignardia bidwellii* per milliliter and incubation in growth chambers for times shown at temperatures indicated.

DISCUSSION

Previous workers have suggested that black rot of grapes is most devastating during years with frequent rains and extended periods of fog or high humidity because the vines were prevented from drying out (4, 5, 6, 7). Although the necessity of free water for ascospore dispersal and infection (1, 6, 7, 8, 10) was assumed, little quantitative evidence supported this observation.

Our results confirm the assumption that rainfall is important in ascospore dispersal and infection by *G. bidwellii*. Reddick (6) described the effect of rainfall on ascospore discharge as follows: "With each succeeding rain during the summer, mummies are moistened, the mature asci absorb water, swell, protrude beyond the perethecial wall, and discharge ascospores into the air." We have found that as little as 0.03 cm of rain was capable of triggering ascospore release. That ascospore release correlates best with duration of rainfall is not surprising then, for the longer rainfall continues, the more likely the mummies are to be wetted.

Once ascospores have been discharged into the air, they may be picked up by air currents and deposited on vines, germinate, and initiate infection. Reddick (6) observed that "this is a most effectual means of dissemination, and at the appropriate time, too, since the moisture, as will be seen later, affords opportunity for the germination of the spores." However, he found that ascospore germination proceeded slowly, and obtained no germination in less than 36 hr under any conditions. In the present study, ascospores germinated in as few as 6 hr on wet leaves at all temperatures tested by us. However, when ascospores were subjected to periods of dryness following their inoculation onto leaves, but prior to rewetting, germination was reduced.

Although final percentage ascospore germination was not affected by temperature when leaves were wet for 24 hr, infection was shown to be temperature-dependent under the same conditions. This suggests that appressoria and infection peg formation are the dependent factors which limit infection.

Maximum vine susceptibility corresponded to the stage of vine development, with the vines being most susceptible to infection just prior to bloom through midbloom. This was also the time at which maximum ascospore catches were obtained in the field.

In this same study, infectious conidia were trapped in rain water from leaf lesions. Levels of trapped conidia were many magnitudes higher than ascospores trapped from the air. Results of conidial studies in the field will be published in the near future. Although ascospore numbers were relatively small, their importance in initiating the disease cycle in the spring, and serving as a source of genetic change in the population, must not be overlooked.

In developing a disease forecasting system to aid in control of the grape black rot disease, factors which must be considered are: (i) ascospore release is triggered by rainfall, with the duration and the amount of rainfall being limiting factors; (ii) continuous leaf wetness following rainfall provides the necessary conditions for infection to occur; (iii) the infection process itself is also dependent on the temperature during the period of leaf wetness following rainfall; and (iv) maximum vine susceptibility corresponds to periods of greatest ascospore dispersal provided that conditions are favorable and we suggest that it is this period, just prior to and through bloom, when protective sprays would be most beneficial in preventing disease development.

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