Sporulation of *Uncinula necator* on Grape Leaves as Influenced by Temperature and Cultivar

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ABSTRACT

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Sporulation of *Uncinula necator* on grapevines (*Vitis vinifera* 'Carignane' and 'Chardonnay') was quantified at 19, 22, 26, and 30 C by harvesting conidia at periodic intervals from individual colonies. Temperature was shown to influence the length of the infectious period. Colonies began sporulating 5 days after inoculation, except at 19 C, when sporulation was first observed after 7 days. Sporulation stopped by 35, 35, 25, and 20 days after inoculation at 19, 22, 26, and 30 C, respectively. Sporulation was greater on Carignane than Chardonnay at all temperatures except 19 C. The maximum number of conidia produced per colony was 11,450. The Richards function was used to describe observed cumulative sporulation over time. The time at which the maximum sporulation rate of colonies occurred, as determined by the inflection point, was influenced by temperature and cultivar. Inflection points ranged from 14.7 days at 30 C to 25.5 days at 19 C.

Effects of environmental conditions on sporulation have been quantified for a number of powdery mildew fungi. Maximum sporulation of *Erysiphe graminis* DC f. sp. *hordei* Ém Marchal occurred at 20 C and decreased sharply at higher and lower temperatures (20). Optimum sporulation occurred at 100% relative humidity and decreased with decreasing humidity. Light intensity and photoperiod had little effect on sporulation. Under temperatures ranging from 18 to 25 C, colonies of *E. g. tritici* Ém Marchal were shown to sporulate for 26 days in one study and 19 in another (8,15).

In most studies in which sporulation of powdery mildews has been quantified, inoculations were done with a spore-settling tower or by gently shaking sporulating colonies over host tissue. Result-

ing sporulation was measured as the number of conidia produced per unit area. With this method, it is difficult to determine the length of the infectious period of individual colonies because of the proximity of sporulating colonies to each other (15). The number of conidia per colony produced by *E. g. tritici* has been shown to be related to colony size and to be inversely proportional to the number of colonies per leaf (12,14,17). Thus, the density and pattern of initial inoculum can influence the sporulation potential of powdery mildew fungi.

The purpose of this study was to examine the effects of temperature and cultivar on production of conidia and length of period of infection for individual colonies of grape (*Vitis vinifera* L.) powdery mildew caused by *Uncinula necator* (Schwein.) Burrill. An inoculation technique was developed in which individual colonies from infection by a single conidium could be observed at different time intervals.

MATERIALS AND METHODS

Inoculations. A single-spore isolate of *U. necator*, collected in 1987 from a vineyard in Napa Valley, CA, was maintained on grape seedlings produced from the cultivar Carignane and grown in 1.9-L glass jars in the laboratory. The same isolate was used for all subsequent inoculations. During winter of 1988, hardwood cuttings were taken from the grapevines (*V. vinifera* 'Carignane' and 'Chardonnay') at the University of California at Davis, and were rooted in 10-cm pots of U.C. mix (1). Cuttings were maintained in a greenhouse free of powdery mildew of grape and were fertilized weekly with half-strength Hoagland's solution (10).

Individual conidia were transferred from 7- to 10-day-old colonies to the three youngest, fully expanded leaves on the cuttings with a single camel hair attached to a dissecting needle. Five conidia were placed on the surface of each leaf. Plants were then enclosed in 12- × 30-cm clear plastic cylinders with an open bottom and a 25-cm^2 air vent on the side. Plants were placed inside illuminated growth chambers receiving 14 hr of light at 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ (LI-COR LI200S pyranometer, Lambda Instruments Corp., Lincoln, NE).

Treatments. Temperatures within the plastic cylinders were maintained at approximately 19, 22, 26, and 30 C with a vapor pressure deficit of 1, 1, 1.6, and 2.4 kPa, respectively. Temperature and relative humidity in the plastic cylinders with plants were monitored by placing a temperature and relative humidity sensor, attached to a CR21 micrologger (Campbell Scientific, Logan, UT), in the center of a plastic cylinder at foliage height. Vapor pressure deficit was calculated from the recorded temperature and relative humidity (18).

Each temperature treatment was repeated six times with two growth chambers. Growth chambers and the order of temperature treatments were randomly assigned for each repetition. Within each repetition, seven plants of each cultivar were inoculated. One plant from each cultivar was removed from the chamber 5, 7, 10, 15, 20, 25, or 35 days after inoculation and sampled for conidial production. Because of variation in infection efficiency of *U. necator* (4), accidental dispersal of conidial chains during watering and equipment failure, six repetitions were not always obtained for each sample date.

Data collection and analysis. A destructive-sampling technique was used to find the number of conidia produced per colony. Leaf disks containing colonies were removed with a 1.5-cm cork borer, placed in microcentrifuge tubes, and agitated for 10 sec in a 1-ml solution of 1.5% sodium chloride containing 0.15% sodium dodecyl sulfate. The numbers of conidia in 10 5-ml drops were counted and used to determine the number of conidia produced per colony. The number of conidia produced per colony at each sample date was expressed as the average from colonies sampled on all three leaves. This was done to minimize differences in the physiological age of leaf tissue between repetitions due to nonuniform growth of the cuttings.

A model for estimating sporulation over time was developed with a general equation for density-dependent growth proposed by Richards:

$$\frac{dN}{dt} = \frac{rN[(K/N)^{1-m}-1]}{1-m} \quad (1)$$

in which r = rate of sporulation, N = cumulative number of conidia at time t, K = maximum number of conidia produced per colony, and m = shape parameter (13). A shape parameter near 1 approximates a Gompertz function, while a value of 2 produces a logistic function (13). Integration of equation 1 produces:

$$N = K[1-be^{-rt}]^{1/1-m} \quad \text{if } m<1, \text{ or}$$
$$N = K[1-be^{-rt}]^{1/1-m} \quad \text{if } m<1 \quad (2)$$

in which b is an intercept parameter and constant of integration. Since the variance of conidial numbers increased proportionately to the mean response, a square-root transformation was applied to the model (2). Parameters for the model were obtained with nonlinear regression analysis (16). A test of lack of fit, with the ratio of mean square for lack of fit to the mean square for residual error, was conducted to evaluate how well the model described the observed data (2). The inflection point (i.e., the time at which the maximum sporulation rate occurs) was estimated by:

$$N_i = Km^{(1/1-m)} \quad (3)$$

in which N_i is the cumulative sporulation total at the inflection point (19). Inflection points were used to compare the various temperature and cultivar effects.

RESULTS

Growth of colonies was determinate and did not exceed 1.5 cm in diameter on either cultivar at any temperature. Mortality of established colonies, based on observations of colony appearance at each sample date, was not observed until 20 days after inoculation.

Observed sporulation. Sporulation was observed after 5 days at all temperatures except 19 C, at which it was first observed after 7 days. At 19 C, the mean maximum number of conidia per colony was 4,000 and 5,861 on Carignane and Chardonnay, respectively (Figs. 1 and 2). Sporulation of colonies on Carignane increased rapidly between 10 and 15 days, but began to diminish after 20 days. Host tissues at colony centers became chlorotic by 25 days. At 35 days, senescence of entire leaves was observed. At 19 C, sporulation of colonies on Chardonnay continued to increase up to 25 days after inoculation (Fig. 2). However, by 35 days conidial production had ceased.

At 22 C, the mean maximum number of conidia per colony was 8,198 and 6,344 on Carignane and Chardonnay, respectively. Sporulation on Carignane continued to increase up to 25 days after inoculation. Colonies with hyphae that appeared normal were still observed after 35 days, but few recently produced conidia were evident. Similar trends of sporulation were observed on Chardonnay at 22 C.

At 26 C, the mean maximum number of conidia per colony was 6,788 and 3,686 on Carignane and Chardonnay, respectively. Sporulation began to diminish after 20 days on both varieties. By 25 days, sporulation had ceased and host tissues at colony centers became necrotic. Viable colonies were not observed at 35 days.

At 30 C, the mean maximum number of conidia per colony was 2,354 and 1,643 conidia per colony on Carignane and Chardonnay, respectively. Sporulation diminished rapidly on both varieties after 15 days. By 20 days, chlorotic tissues at colony centers were observed and sporulation had ceased. Viable colonies were not observed at 35 days.

The rapid growth of vines within the limited confines of the plastic cylinders made it increasingly difficult to irrigate vines without dislodging conidia from the colonies. Thus, on occasion,

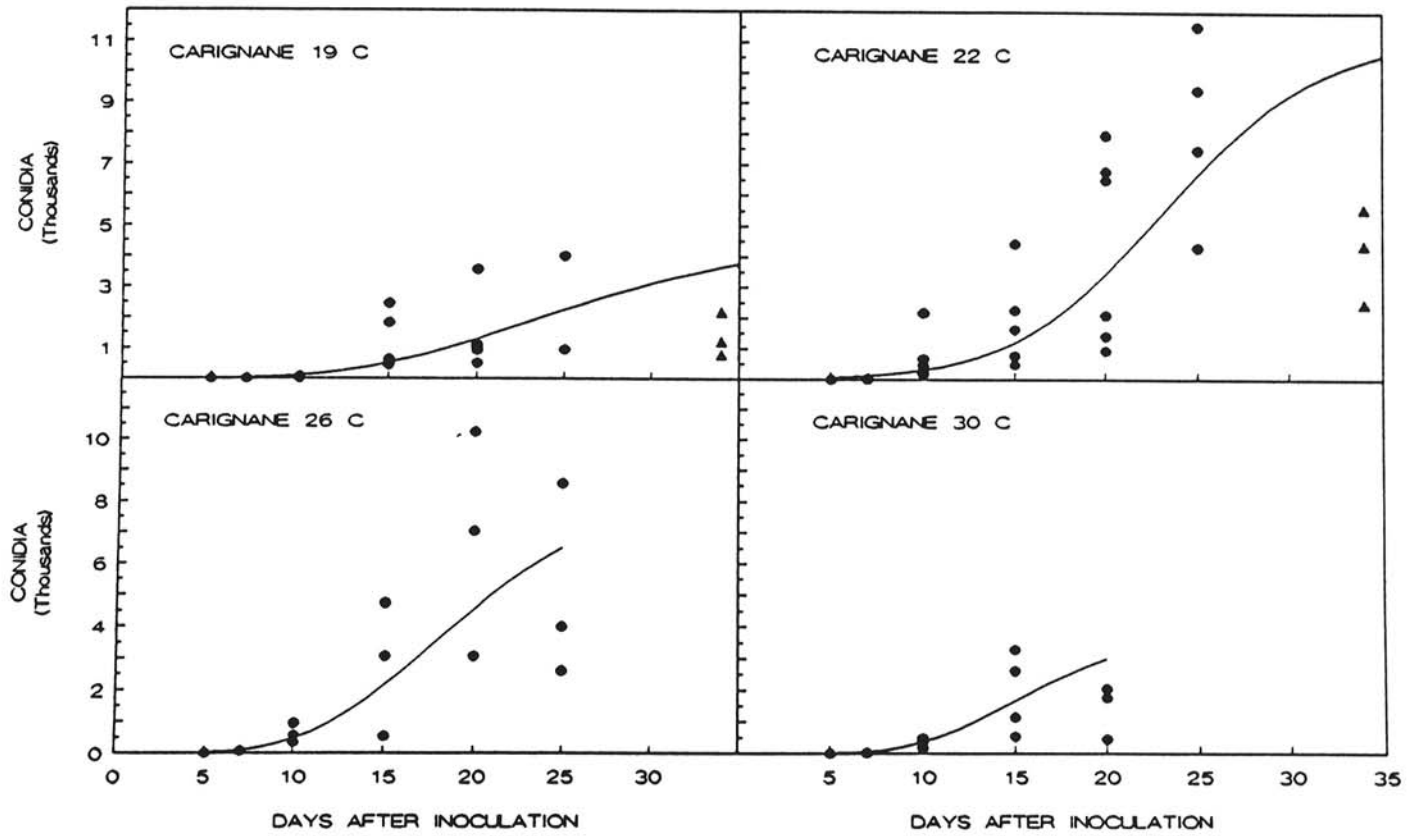

Fig. 1. Observed and predicted values for sporulation of *Uncinula necator* on *Vitis vinifera* 'Carignane.' Values represented by triangles were not included in the analysis.

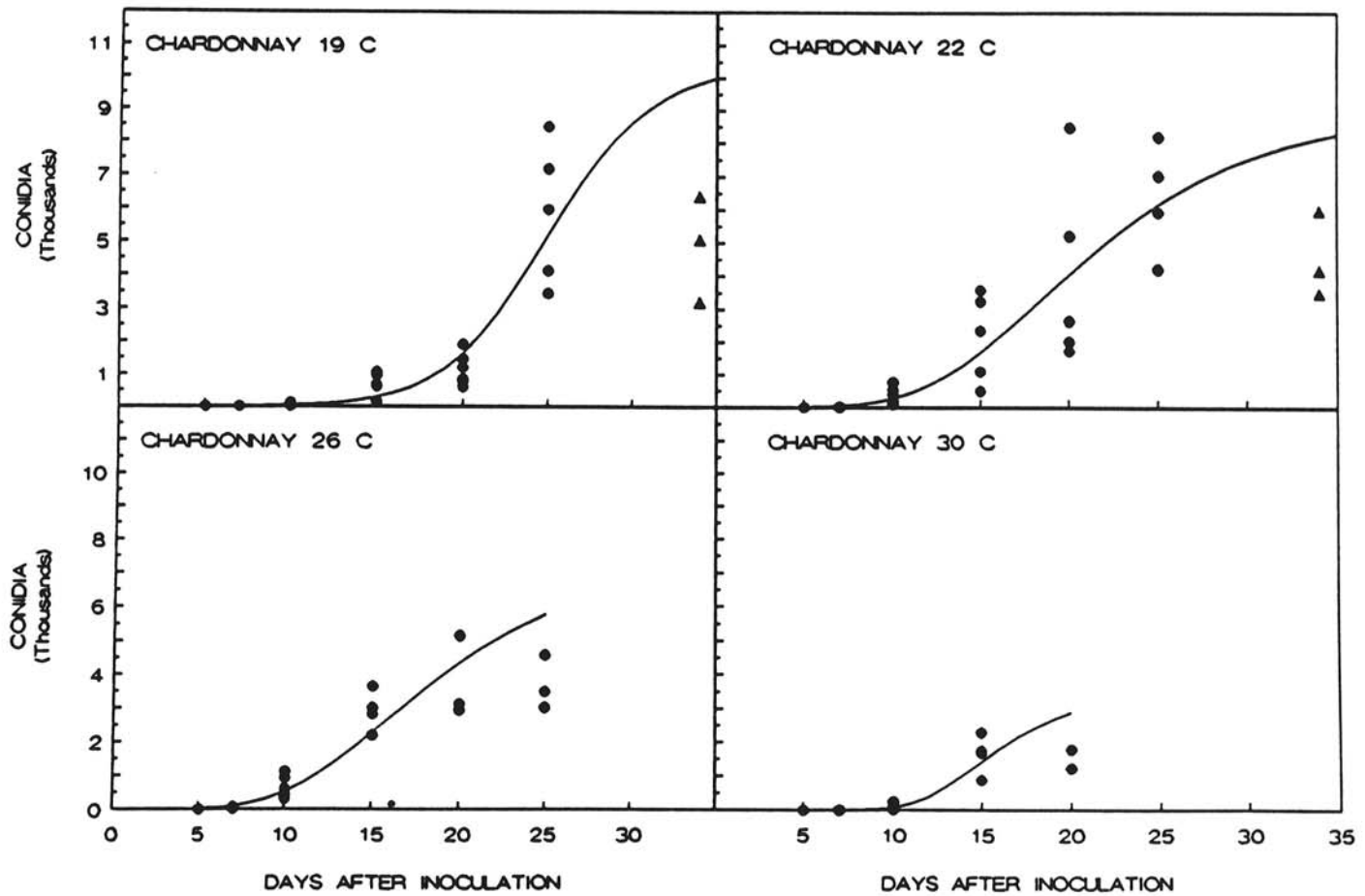

Fig. 2. Observed and predicted values for sporulation of *Uncinula necator* on *Vitis vinifera* 'Chardonnay.' Values represented by triangles were not included in the analysis.

TABLE 1. Estimation of the asymptote, intercept, rate, and shape parameters of the sporulation model and the results from a lack-of-fit test

Cultivar	Temperature (C)	Asymptote	Intercept	Rate	Shape	MSLF/MSPE[a]	$P > F$[b]
Carignane	19	4,900	0.17	0.11	1.0	1.60	0.23
Carignane	22	11,450	78.93	0.21	2.3	0.84	0.49
Carignane	26	9,020	0.30	0.15	1.0	1.10	0.41
Carignane	30	4,220	0.45	0.20	1.0	2.61	0.15
Chardonnay	19	10,490	866.29	0.29	2.4	2.24	0.15
Chardonnay	22	9,020	0.32	0.15	1.0	0.10	0.80
Chardonnay	26	7,190	0.11	0.16	1.0	1.09	0.41
Chardonnay	30	3,600	1.45	0.29	1.0	4.31	0.05

[a] Mean square for lack of fit divided by mean square for pure error.
[b] Probability of obtaining mean square ratios as great or greater.

TABLE 2. Asymptotic standard errors for estimated parameters

Cultivar	Temperature (C)	Intercept	Rate	Shape
Carignane	19	8.98	0.09	0.44
Carignane	22	5,352.22	0.30	0.76
Carignane	26	9.24	0.10	0.39
Carignane	30	1.99	0.07	0.45
Chardonnay	19	3,147.53	0.13	0.70
Chardonnay	22	7.98	0.07	0.38
Chardonnay	26	5.62	0.06	0.35
Chardonnay	30	62.86	0.24	0.41

TABLE 3. Estimates of inflection points

Cultivar	Temperature	Conidia	Time (days)
Carignane	19	1,830	22.8
Carignane	22	6,060	24.0
Carignane	26	3,390	17.6
Carignane	30	1,590	14.7
Chardonnay	19	5,620	25.5
Chardonnay	22	3,390	18.6
Chardonnay	26	2,670	15.9
Chardonnay	30	1,350	14.8

accidental dispersement of conidia occurred between 25 and 35 days after inoculation. When this artifact was combined with a reduction in conidial production, a decrease in the cumulative number of conidia was observed at 35 days. Thus, observations at this sample date were omitted from further analysis.

Sporulation model. Sporulation on both cultivars was adequately described by the Richards function (Figs. 1 and 2). A significant lack of fit was observed only for Chardonnay at 30 C (Table 1). Observations of residual errors revealed no discernible patterns except at 30 C.

Values for the shape parameter ranged from 1.0 on Chardonnay at 26 C to 2.4 on Chardonnay at 19 C (Table 1). Rate parameters ranged from 0.11 per day on Carignane at 19 C to 0.29 per day on Chardonnay at 19 and 30 C. Intercept values ranged from 0.11 on Chardonnay at 26 C to 866.29 on Chardonnay at 19 C. Asymptotic standard errors of the estimated parameters are presented in Table 2.

Inflection points decreased with increasing temperatures (Table 3). Similar inflection points of 14.7 and 14.8 days were observed on Carignane and Chardonnay, respectively, at 30 C. At 26 C, inflection points were reached after 15.9 days on Chardonnay and 17.6 days on Carignane. At lower temperatures, an interaction between cultivar and temperature on the time at which colonies reached their maximum sporulation potential was observed. At 22 C, colonies on Carignane reached their maximum sporulation rate after 24 days while colonies on Chardonnay required only 18.6 days. At 19 C, colonies on Chardonnay and Carignane required 25.5 and 22.8 days, respectively, to reach their maximum sporulation rate.

DISCUSSION

The number of conidia produced by powdery mildew colonies on grape was highly variable and the variability appeared to increase over time. Some variation may be explained by differences in the aging of host tissue. Repeated penetration of epidermal cells and subsequent haustorial formation are adversely affected by maturing tissue (6). Hirata (9) showed a direct relationship between numbers of haustoria and conidial production for *E. g. hordei*. Thus, differences in the age of host tissue between replications may account for some of the observed variability in sporulation. In studies that examine environmental effects on germination and penetration of *U. necator*, a high degree of variation in germination and penetration rates was also observed (4). Thus, variation in vital rates such as germination and sporulation appear to be a phenomenon inherent in *U. necator*.

Variation in sporulation potential may also be common for other powdery mildew fungi, but may be masked because of experimental methods. When large quantities of inoculum are placed onto host tissue, competition among developing colonies for space and substrates negatively affects the reproductive capacity of individual colonies (12,14,17). Multiple infections that result from large spore loads may also cause premature senescence of host tissue (3). In this study, a maximum of five colonies per leaf were permitted to develop and each colony was separated from adjacent colonies by several centimeters, allowing colonies to reach their maximum reproductive potential.

Cumulative sporulation totals for individual colonies of *U. necator* appear to be significantly less than for *E. graminis* (7,8,15,20). However, *U. necator* had longer infectious periods and shorter latent periods than *E. graminis* (15). Infection rates in epidemics of plant disease are governed by latent period, infectious period, sporulation rate, and inoculum efficiency (22). Elucidation of these parameters for other powdery mildew fungi would permit a more direct comparison of the epidemic process in various cropping systems.

The influence of cultivar on the sporulation process was minimal at higher temperatures. Similar inflection points and shape parameters were obtained at 26 and 30 C. The major difference was in the mean maximum number of conidia produced by a colony; more conidia were produced on Carignane than on Chardonnay. It is interesting that Carignane is better adapted for growth in warmer grape-growing regions, while Chardonnay is best suited for growth in the cooler areas of grape production (21).

The type of cultivar had a significant impact on the sporulation process at lower temperatures. More conidia were produced from colonies on Chardonnay at 19 C than on Carignane, but it took 3 days longer for colonies on Chardonnay to reach their maximum rate of sporulation. At 22 C, more conidia were produced on Carignane than on Chardonnay, but it took 5.5 days longer for colonies on Carignane to reach their maximum rate of sporulation. Both cultivars are considered highly susceptible, but field observations indicate that powdery mildew is more severe on Carignane (5).

Preliminary analysis of the observed sporulation suggests that inoculum production is greatest between 22 and 26 C. The mean maximum number of conidia produced by colonies on Carignane and Chardonnay was greatest at 22 C. The longest infection periods occurred at 22 C for both cultivars and decreased significantly with increasing temperature. However, at 10 and 15 days after inoculation, more conidia (absolute production) were produced at 26 C than at other temperatures in the study.

The Richards function provided a reasonable description of cumulative sporulation at all temperatures except 30 C. At 30 C, production of conidia declined rapidly between 15 and 20 days after inoculation. In addition, the viability of existing conidia was affected by high temperature (4). Thus, it is possible that many conidia produced before 15 days were not recovered at the later sample date. This may explain the observed decline in the cumulative number of conidia recovered at 20 days. The Richards function is only appropriate to describe monotonic curves and did not adequately approximate the observed decrease in sporulation at 30 C.

The Richards function was selected to describe the data for several reasons. The observed determinate growth patterns of individual colonies suggested a maximum threshold of colony size and subsequent conidial production. The Richards function includes a parameter to account for density-dependent regulation. The inclusion of a third parameter in the Richards function enabled the description of a variety of different data sets. Finally, the quantitative benefits from use of the Richards function to describe sporulation data of fungi has already been demonstrated (11).

In summary, the development of methods that minimized inhibitory effects from competing colonies enabled a detailed quantification of sporulation potential and infectious periods for *U. necator*. In addition, the deterministic nature of growth exhibited by colonies of *U. necator* on the surfaces of grapeleaf tissue was demonstrated. Finally, use of inflection points as an additional parameter for describing epidemiologic processes showed the pleiotropic effect that temperature can have on the sporulation process. This information along with environmental effects on infection efficiency should provide a clearer understanding of the processes that cause inoculum development in *U. necator*.

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