A Quantitative Model for Describing the Sporulation of *Plasmopara viticola* on Grape Leaves

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ABSTRACT

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Leaves from potted grape vines of *Vitis lambrusca* 'Catawba' infected with downy mildew were placed in a dark moist chamber maintained at constant temperatures of 10, 15, 20, 25, and 30 C. A humidifier, located within the chamber, maintained atmospheric moisture at or near saturation. Plants were removed for observation after 6, 7.5, 9, 10.5, and 12 hr. The largest number of spores, approximately 300,000 sporangia per square centimeter of lesion area, were produced after 12 hr at 20 C, while no sporulation was observed at any time at 10 or 30 C. When the asymptote, rate, and intercept parameters of the Richards model were represented as quadratic functions of temperature, the model fitted to the pooled data described 90% of the variation in number of sporangia. The model predicted that approximately 50% of the sporangia were formed at 20 C after 8 hr of incubation. Although sporangia were produced after only 7.5 hr of incubation, they failed to germinate, possibly indicating a lack of maturity. However, germination increased as the duration of the sporulation period was increased, until a maximum of 70% was observed for sporangia harvested after 12 hr at 25 C.

Sporulation of *Plasmopara viticola* (Berk. & Curt.) Berl. & de T., causal agent of downy mildew of grape (*Vitis lambrusca*), has been shown to depend on three environmental variables: light, relative humidity, and temperature. After an early investigation by Gregory (4), who reported that sporangiophores were produced most readily in dark, humid conditions, Yarwood (12) found that light actually inhibited sporulation and concluded that alternation of light and dark was necessary for normal nocturnal sporulation. A more recent study by Brook (2) showed that sporangial production was inhibited by near ultraviolet light of 0–400 nm or by green light at 500–560 nm with corresponding intensities of 3 and 3.5 W m^{-2}, respectively.

A high relative humidity (RH), which often occurs during the night, also was shown to be crucial for sporulation. In 1913, Istvanffi and Palinkas (5) reported a minimum RH of 65–75% for production of sporangia; sporangiophores were most dense when formed under 95–100% RH. However, in a recent, more detailed study, Leu and Wu (8) observed that sporangiophores and sporangia were formed at >93% RH, while only the former were produced at 33, 53, and 84% RH.

Unlike light and relative humidity, temperature was not considered to be a limiting factor under field conditions (4,12). Nevertheless, early research by Ravaz (10), which was later reviewed by Kable (6), showed that a minimum of approximately 5 hr at 20 C was necessary for sporulation. These results agreed with those of Gregory (4), who reported an optimum temperature range of 18–20 C, and Leu and Wu (8), who observed most sporulation between 16 and 28 C.

Given these environmental factors, our objective was to develop a mathematical model that would quantitatively describe the sporulation process. The number of sporangia produced per unit area of lesion in darkness would be expressed as a function of temperature and duration of high relative humidity. Such a model would be useful for simulation of sporulation as well as for forecasting purposes. In addition, since spore maturity is critical for germination and infection, we examined the ability of the sporangia to germinate.

MATERIALS AND METHODS

Treatments. During late winter in 1987, hardwood stem cuttings were taken from a Catawba vineyard. After root formation was induced in a mist bench, the vines were transferred to 15-cm pots and placed in a greenhouse. They were planted in a soilless medium of peat moss, Perlite, and Vermiculite (Pro-Mix BX, Premier Brands, Inc., New Rochelle, NY), pruned to a single shoot, and fertilized twice a month (Peters Peat-Lite Special, 15-16-17, W. R. Grace and Co., Fogelsville, PA).

After five to seven leaves were produced on each vine, a suspension of sporangia of *P. viticola* at 10,000 per milliliter was atomized (69 KPa pressure) onto the abaxial surfaces of the youngest, fully expanded leaves. The plants were then placed in a mist chamber located within a larger lighted growth chamber (120 $\mu E\ m^{-2}sec^{-1}$) kept at 20 C. A humidifier (Herrmidifier Company, Inc., Lancaster, PA) maintained liquid water on the leaf surfaces. Temperature was monitored by placing a thermistor, attached to a CR21 micrologger (Campbell Scientific, Inc., Logan, Utah), in the center of the mist chamber at foliage height. After a 24-hr wetting period, the vines were allowed to dry and then moved to a greenhouse kept at 25 ± 5 C. All inoculations were made with a mixture of isolates originally obtained from a Catawba vineyard.

Seven days after inoculation, those plants exhibiting chlorotic leaves were returned to the mist chamber, now dark. Vines were removed for observation after 6, 7.5, 9, 10.5, and 12 hr. A 25 W red incandescent bulb located 9 m from the chamber door provided light for removal. This same procedure was used for constant chamber temperatures of 10, 15, 20, 25, and 30 C. Thus, the experiment consisted of a total of 25 treatment combinations of temperature and duration of high humidity. The entire experiment was conducted three times; because only one chamber was available, the order in which the temperature treatments were performed within each of these three replicates was random.

Estimation of sporulation. The amount of sporulation (S), measured as the number of sporangia produced per unit area of lesion, was estimated for each treatment. A No. 8 cork borer, which has a cross-sectional area of 0.95 cm^2, was used to cut 15 disks from sporulating regions on five leaves. Usually, three disks were taken from each leaf and only chlorotic leaf areas exhibiting sporulation were sampled. The 15 disks, representing a total diseased area of 14.25 cm^2, were then agitated approximately 1 min in 40–50 ml of

cold, distilled water (1 drop of Tween 40/L) at 4 C. The number of sporangia per milliliter of suspension was estimated with a hemacytometer; a total of eight hemacytometer chambers were examined for each treatment. Thus, S could be estimated as follows:

$$S = (\text{no. sporangia/ml}) * (\text{ml suspension})/(14.25\ \text{cm}^2).$$

After the hemacytometer counts were performed, the slide was placed in an incubator at 19 C for 6 hr. The proportion of germinated sporangia was then calculated by dividing the number of empty sporangia by the total number of sporangia. The total number of sporangia counted ranged from 35 to 97 for any given temperature-replicate combination that produced spores. Standard linear regression analysis was used to examine the relationship between germination and duration of the sporulation period.

Model development. The method used for development of a model involved several interrelated steps. First, an equation for predicting the upper asymptote of S, (k) was derived by regressing the maximum S obtained for each temperature (T) against a second-order polynomial of temperature:

$$k = b_0 + b_1 T + b_2 T^2, \quad (1)$$

in which b_0, b_1, and b_2 are regression coefficients. Regressions were performed separately for each replicate (full model) and for the pooled data (reduced model).

In the second step, the linearized version of the Richards function (11) was fitted to the data for each temperature:

$$\ln[1 - (S/k)^{1-m}] = -rH + B, \quad (2)$$

in which H = duration of high relative humidity, k = estimated asymptote from equation 1, and m, r, and B are the shape, rate, and intercept parameters, respectively. A range of values of m were tried to achieve the best possible fit. Values near zero result in a monomolecular-type function, whereas a value of two produces a logistic model. The intercept and rate parameter estimates were then regressed against temperature by fitting a quadratic equation similar to that used to predict k:

$$r = b_0 + b_1 T + b_2 T^2 \quad (3)$$

$$B = b_0 + b_1 T + b_2 T^2 \quad (4)$$

These models were fitted to both the full and reduced data sets.

The final step, modeling S as a function of T and H, consisted of substituting the rate and intercept parameter functions (equation 3 and 4) for r and B, respectively, in the Richards model:

$$\ln[1 - (S/k)^{1-m}] = b_0 + b_1 H + b_2 HT + b_3 HT^2 + b_4 T + b_5 T^2. \quad (5)$$

Coefficients of this function were estimated for each replicate (full model) and for the pooled data (reduced model). Because some S's were greater than the value of k predicted by the quadratic functions, the logarithm of a negative number, an undefined quantity, occurred. To avoid this situation, a constant (50,000) was added to the predicted k's (Eq. 1) derived for the reduced model. The quadratic function developed from the pooled data was also used for predicting k for each of the separate replicate models.

In fitting equations 1, 3, and 4, the individual replication models had a total of five observations (five temperatures), and the models fitted to the pooled data had 15 (five temperatures × three replicates). Because the Richards model in equation 2 was fitted to the data for each temperature, five observations (durations of high RH) were used in each analysis. In the final step, each replicate model had a total of 25 observations (five temperatures × five durations), while the reduced model consisted of 75 observations (all three replicates combined). In each step of the model-building process, a standard F-test was performed to determine if the results for each replication were similar enough to allow pooling of the data (9); i.e., determine if the full model was different from the reduced. All regression analyses were performed by the REGRESS procedure of PRODAS (3).

Model validation. Another set of Catawba cuttings, grown and treated in the same fashion as indicated above, were used to independently test the model. Four chamber temperatures (about 13, 17, 22, and 26 C) and two durations of high relative humidity (7.5 and 10.5 hr) at each temperature were examined. The amount of sporulation was estimated using the same procedures for model development.

The model was also tested for its predictive capabilities under field conditions. During rain periods from mid-June through July 1987, tagged leaves in a Catawba vineyard were inoculated with sporangial suspensions obtained from infected greenhouse plants. Starting 5 days after any given inoculation, the tagged leaves were observed each morning for signs of sporulation. When sporulation did occur, the sporangia density was determined using the techniques described for model development. Temperature (model 107 thermistor) and relative humidity (model 207 RH sensor) were monitored by a 21X micrologger (Campbell Scientific, Inc., Logan, UT).

Predicted values and their 95% confidence interval, based on the model developed, were calculated for each test sporulation period, whether in the chamber or field. The average temperature and duration of RH ≥ 90% were used as inputs for estimating sporulation in the field.

RESULTS

After 6 hr of darkness at relative humidities >95%, no macroscopically visible signs of sporulation were evident at any of the temperatures. However, at 20 C, sporangiophore initials, observed through a dissecting microscope, were often found to be emerging from stomata located within chlorotic regions. After 7.5 hr, sporangiophores bearing small immature sporangia were observed at temperatures of 15, 20, and 25 C. The size and number of sporangia harvested at these temperatures increased dramatically between 7.5 and 9 hr of high RH (Fig. 1). After 9 hr, the rate of sporangia formation declined, particularly at 15 and 20 C; spore production continued at 25 C until leveling-off at 10.5 hr. Neither sporangiophores nor sporangia were observed at any time at the 10 and 30 C temperature extremes.

Estimation of model parameters. When the maximum sporulation observed at each temperature (a measure of the upper asymptote k of the Richards model) was regressed against temperature using a quadratic function (Eq. 1), the replicate 1 and 2 data sets yielded coefficients of determination (R^2) greater than 90% (Table 1). Although the replicate 3 data set produced a relatively high R^2 of 0.78, its F-statistic was much less significant.

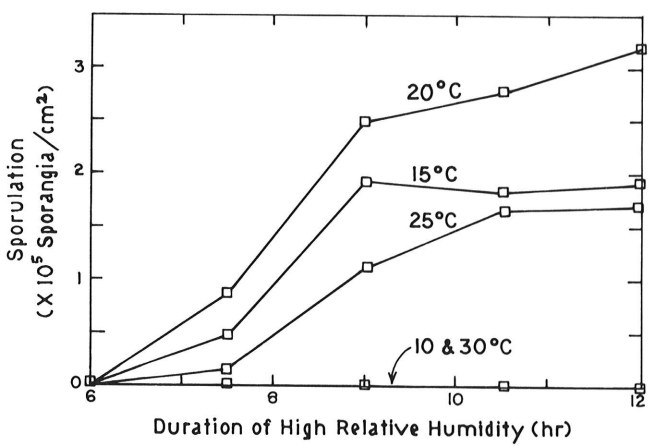

Fig. 1. Effect of incubation time and temperature on the number of sporangia of *Plasmopara viticola* produced per unit area of lesion. Infected plants were placed in a dark growth chamber maintained at a high relative humidity and constant temperature. Each point is an average of three replicates.

However, with only two degrees of freedom, an F-test is not very powerful for determining significant relationships (9). Nevertheless, a comparison of the full and reduced models indicated that the parameters of the separate replication models were not statistically different overall ($F = 0.10$, $P = 0.99$), allowing the data to be pooled. The reduced model fitted to the pooled data set had highly significant parameter estimates and described 94% of the variation in sporulation (Table 1). This model predicted a maximum sporulation of 283,072 sporangia per square centimeter of lesion area at 20.2 C and zero sporulation at approximately 10 and 30 C (Fig. 2A).

The fitting of the Richards model to the data for each temperature and replicate (Eq. 2) resulted in R^2's ranging from 0.68 to 0.99. In general, the t-statistic for the rate parameters tended to be more significant than for the intercepts, but both usually had P-values less than 0.10. Because a slight delay in the increase in sporulation was observed at temperatures of 15–25 C (Fig. 1), values of the shape parameter (m) between 0 and 1 were examined for their effect on the regression results. A value of $m = 0.5$ was eventually chosen because it provided the best overall fit across the different temperatures. The resulting function is intermediate in shape between the monomolecular and Gompertz models.

In the second step of model development, the rates and intercepts from eq. 2 were regressed against temperature. The quadratic functions, eqs. 3 and 4, provided excellent fits for the rate and intercept parameters, respectively (Table 1, Fig. 2B and C). However, as in fitting the asymptote parameter, the replicate 3 data set was not fitted as well by the models and had less significant F-values. Nevertheless, comparisons of the reduced and full models showed that parameter estimates were similar enough to allow pooling of the data; F-values of 0.14 and 0.16 ($P = 0.98$) were calculated for the rate and intercept parameter models, respectively. Both reduced models had highly significant F-statistics and described 91 and 88% of the variation in the rate and intercept, respectively (Table 1). The rate parameter r was predicted to reach a maximum value of 0.5 at 20.2 C, while the intercept parameter B approached maximum predicted value of 2.9 at 20.3 C (Fig. 2B and C). Both parameters were zero at approximately 10 and 30 C.

Sporulation model. Regression models (Eq. 5) fitted to each replicate data set had large coefficients of determination and highly significant F-values and parameter estimates (Table 2). Although the absolute values of the parameters for replicate 3 were somewhat lower than those of the other replicates, an F-test comparing the reduced and full models produced an F-value of 0.50, which was significant only at $P = 0.90$. Thus, the corresponding parameter estimates among all three replicate models were not significantly different, allowing the data to be pooled.

The reduced model fitted to the pooled data described 90% of the variation in sporulation (Table 2). Its parameter estimates, significant at $P < 0.01$, were of intermediate value relative to those of the separate replicate models. Furthermore, note that coefficients b_1, b_2, and b_3 (Table 2) were very close in absolute value to coefficients b_0, b_1, and b_2 estimated for the reduced model which predicted the rate parameter (eq. 3 and Table 1). Similarly, coefficients b_0, b_4, and b_5 (Table 2) were also very close in value to their corresponding coefficients b_0, b_1, and b_2 used to predict the intercept parameter (Eq. 4 and Table 1). This outcome indicated that there was very little redundancy in the terms that constitute eq. 5. For example, if the terms used to describe the rate parameter, namely H, HT, and HT^2, also described some portion of the intercept, then these similarities would not have occurred. In addition, the redundant terms may have been less significant since they would not be explaining any additional variation in sporulation.

Given the results of the linear regression for fitting the reduced model (Table 2), the nonlinear relationship between S and H and T was:

$$S = k(1 - e^{B+\rho})^{1/(1-m)}, \quad (6)$$

in which $k = -873{,}164 + 114{,}369*T - 2{,}828*T^2 + 5{,}000$;

TABLE 1. Estimation of the asymptote, rate, and intercept parameters of the Richards model by fitting quadratic functions of temperature

Model	df Error	SS Error	R^{2a}	F P-value	Estimate/ P-value b_0	b_1	b_2
Asymptote parameter[b]							
Rep 1	2	3.98×10^8	0.99	<0.01	−905069	118615	2927
					<0.01	<0.01	<0.01
Rep 2	2	2.02×10^9	0.95	0.03	−881050	114752	−2827
					0.02	0.01	0.01
Rep 3	2	8.76×10^9	0.78	0.11	−833371	109739	−2730
					0.09	0.06	0.06
Reduced	12	1.23×10^{10}	0.94	<0.01	−873164	114369	−2828
					<0.02	<0.01	<0.01
Rate parameter[c]							
Rep 1	2	0.003	0.98	0.01	−1.60	0.21	−0.0052
					<0.01	<0.01	<0.01
Rep 2	2	0.017	0.87	0.06	−1.60	0.21	−0.0051
					0.05	0.03	0.03
Rep 3	2	0.034	0.72	0.14	−1.44	0.19	−0.0047
					0.11	0.08	0.07
Reduced	12	0.061	0.91	<0.01	−1.55	0.20	−0.0050
					<0.01	<0.01	<0.01
Intercept parameter[d]							
Rep 1	2	0.091	0.98	0.01	−9.69	1.27	−0.032
					0.01	<0.01	<0.01
Rep 2	2	0.76	0.83	0.08	−9.50	1.22	−0.030
					0.06	0.04	0.04
Rep 3	2	1.66	0.61	0.19	−8.53	1.10	−0.027
					0.14	0.10	0.10
Reduced	12	2.90	0.88	<0.01	−9.24	1.20	−0.030
					<0.01	<0.01	<0.01

[a] Coefficient of determination adjusted for number of independent variables.
[b] See text, equation 1.
[c] See text, equation 3.
[d] See text, equation 4.

$$B = -8.96 + 1.16T - 0.029T^2;$$
$$\rho = -\mathrm{rH} = 1.51H - 0.020HT + 0.0049HT^2;$$

and $m = 0.5$.

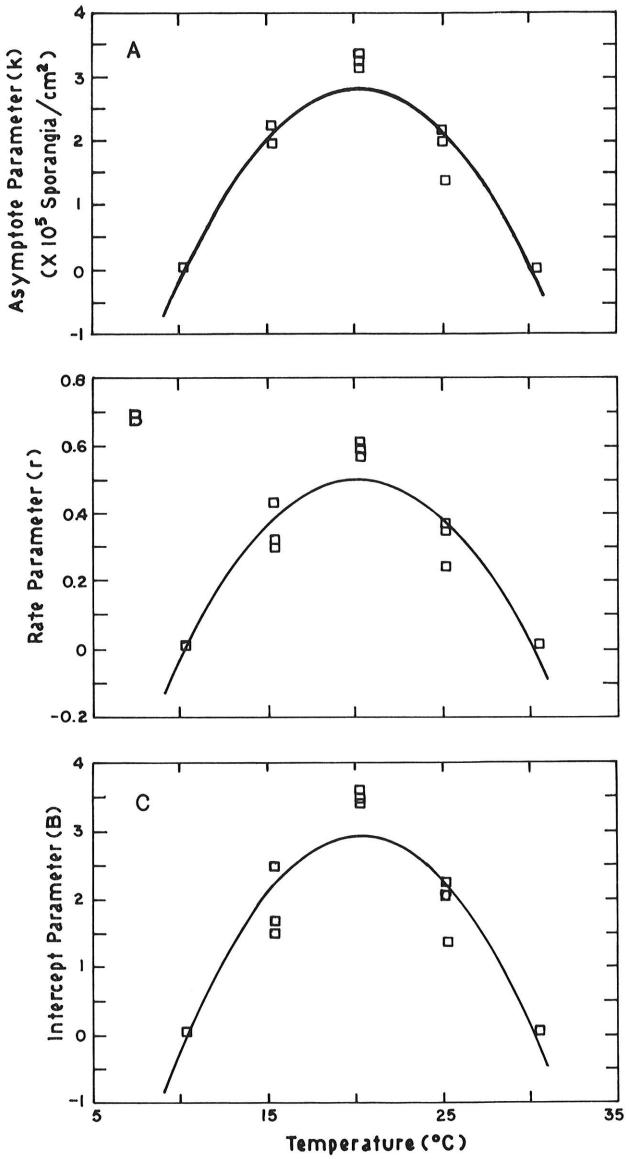

Fig. 2. Relationship between temperature and the asymptote, **A,** rate, **B,** and intercept, **C,** parameters of the Richards function. Solid curves correspond to the predicted values of eqs. 1, 3, and 4, respectively (see text and Table 1). Points represent the observed values (A) or those generated from eq. 2 (B and C) for each of the three replicates.

If solved for various values of H and T, this equation describes a three-dimensional response surface depicting sporulation (Fig. 3). The back-transformed predictions from this equation were highly correlated ($r = 0.97$) with the observed levels of sporulation. At 20 C and 12-hr incubation in darkness and high relative humidity, the model predicts the formation of approximately 309,900 sporangia per square centimeter of lesion area. Predicted levels of sporulation at 10, 15, 20, 25, and 30 C agree closely with the observed amounts (Fig. 4).

Model validation. Seven of the eight observed values recorded for each test were within the 95% confidence interval of the predicted value (Table 3). However, in the chamber test, the model appeared more likely to overestimate the observed values (six overestimated, two underestimated) while in the field, the converse appeared true (three overestimated, five underestimated). Nevertheless, when these test data were combined, the prediction bias was, in general, equal: nine overestimated versus seven underestimated.

Sporangia germination. The proportion of harvested sporangia that germinated in vitro increased as the duration of the sporulation period increased (Fig. 5). The level of germination, which ranged from zero at 7.5 hr to 50–70% at 12 hr, increased in a somewhat linear fashion. Although the duration of the sporulation period explained 80–86% of the variation in germination (Table 4), temperature also had an influence. A comparison of the regression lines using a standard F-test (9) showed that the coefficients of the function fitted to the 15 C data were significantly different from those of the 20 C ($F = 7.2$, $P < 0.01$) and 25 C ($F = 6.8$, $P < 0.01$) data sets. However, the coefficients estimated for the 20 and 25 C data sets were not significantly different ($F = 0.41$, $P = 0.66$).

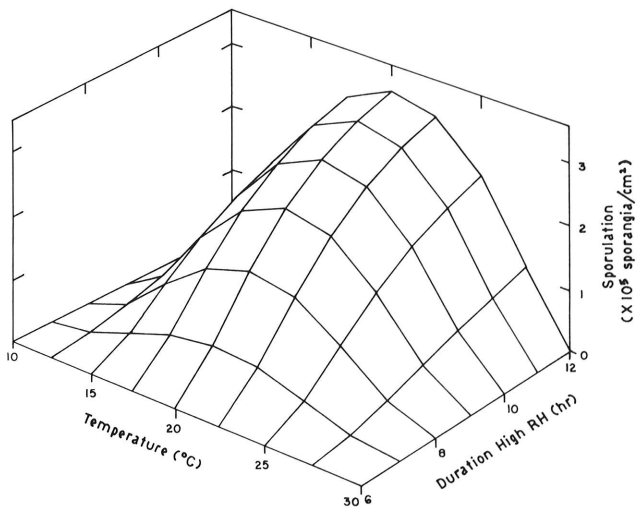

Fig. 3. Three-dimensional representation of the sporulation of *Plasmopra viticola* as a function of temperature and the duration of high relative humidity. The response surface was generated from the nonlinear form of the Richards model, eq. 6, which was derived from the pooled data set.

TABLE 2. Estimation of the sporulation of *Plasmopara viticola* as a function of temperature and duration of high relative humidity by fitting the Richards function[a]

Model	df Error	SS Error	R^{2b}	F *P*-value	Parameter estimate / *P*-value					
					b_0	b_1	b_2	b_3	b_4	b_5
Rep 1	19	1.80	0.92	<0.01	−9.68	1.60	−0.21	0.0052	1.27	−0.032
					<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Rep 2	19	1.85	0.91	<0.01	9.50	1.60	−0.21	0.0051	1.22	−0.030
					<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Rep 3[c]	18	2.18	0.82	<0.01	−6.99	1.24	−0.16	0.0040	0.91	−0.022
					0.02	<0.01	<0.01	<0.01	0.01	0.01
Reduced	68	6.46	0.90	<0.01	−8.96	1.51	−0.20	0.0049	1.16	−0.029
					<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[a] See text, equation 5.
[b] Coefficient of determination adjusted for the number of independent variables.
[c] One observation, an outlier, was excluded from the analysis.

DISCUSSION

Although sporulation of *P. viticola* has not been previously quantified, observations have been reported on the relative amounts of sporangia produced at different temperatures. In general, our results, which indicated maximum sporangial production at 20 C, agree with those of Leu and Wu (8) and Gregory (4) who observed the most sporulation at 16–28 C and 18–20 C, respectively. Leu and Wu, however, also reported that the sporulation process was completed in 4 hr at 24 C and that sporangiophores, but no sporangia, were produced at 4–12 C and at 32 C. Similarly, Ravaz (10) showed that in a dark, humid environment at 20 C, spores will be produced in about 5 hr. These studies were all performed with infected *V. vinifera*, the European grape. We observed no signs of sporulation, including sporangiophores, at either 10 or 30 C, and sporangia were not found until after 7.5 hr at 20 C. This relatively slower rate of sporulation may be due to the higher degree of resistance present in *V. lambrusca* used in our studies.

Once sporangia have been formed and are disseminated, the next crucial step in the infection process is their germination. Although we observed as many as 250,000 sporangia/cm^2 of lesion after 9 hr at 20 C, only about 38% of them germinated. The proportion that germinated, however, increased as the duration of the sporulation period increased. Thus, we suspect that at shorter durations, the majority of sporangia were too immature to germinate. Assuming that only mature sporangia become abscised, then the harvesting technique may have been too vigorous; under natural conditions, many of these spores may not become detached. Furthermore, although the number of sporangia produced approached its maximum after about 12 hr, only 50–70% of the spores were capable of germinating. During routine inoculations, we often observed 90–100% germination for sporangia formed after 16–24 hr. These observations may indicate the existence of a maturation period between sporulation and infection.

Our experiment examined the effect of temperature and duration of high relative humidity on sporulation during a single dark period. In the field, however, sporulation may occur over a series of consecutive nights. Another possibility is that darkness and/or high humidity may only be necessary for the initiation of sporulation. In this case, sporangial production and/or maturation would be occurring during the daytime. Research, however, has shown that the higher temperatures and lower relative humidities often occurring during the day are detrimental to the survival of attached sporangia (1). Thus, it is unlikely that this same environment would also be conducive to spore formation or maturation. Further research is needed to examine the various possible interactions between these environmental variables and the sporulation process.

The infection of *V. lambrusca* by *P. viticola* has been shown to occur at temperatures ranging from 5 to 30 C (7). Maximum infection efficiency, 0.08 lesions per zoospore, occurred after 15 hr of leaf wetness at 15 C. Consequently, the range of temperatures for infection are broader than those for sporulation. Furthermore, the optimum temperature for sporulation is 5 C higher than that for infection: 20 vs. 15 C. Thus, in early to mid-spring, when nights are quite cool, sporulation could limit the epidemic much more than infection. Conversely, during late spring and summer, night temperatures may be optimum for sporulation, but too high for optimum infection; day temperatures would be even higher and more limiting to infection. Additional research is needed to

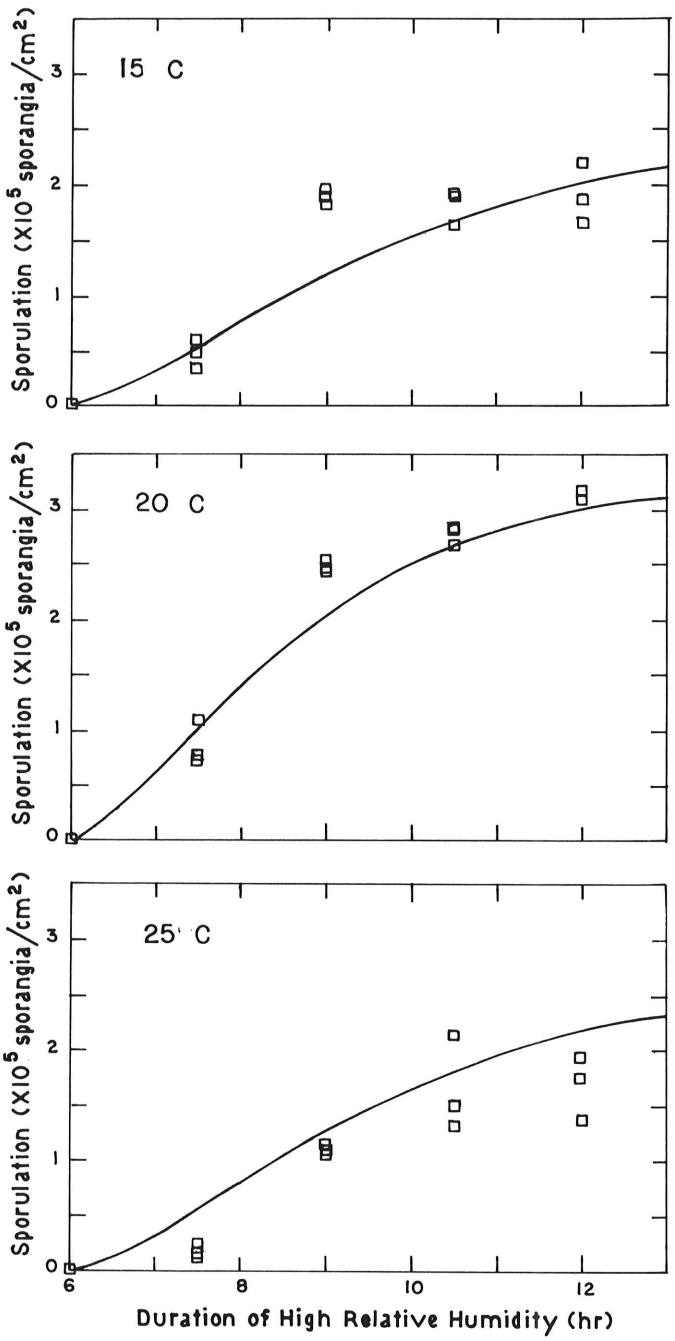

Fig. 4. Comparison of the predicted values of the model fitted to the reduced data set (Eq. 6, solid line) with the observed values. The three observations at each duration correspond to the three replicates performed. At 10 and 30 C, both the predicted and observed values were zero at durations of 6–12 hr.

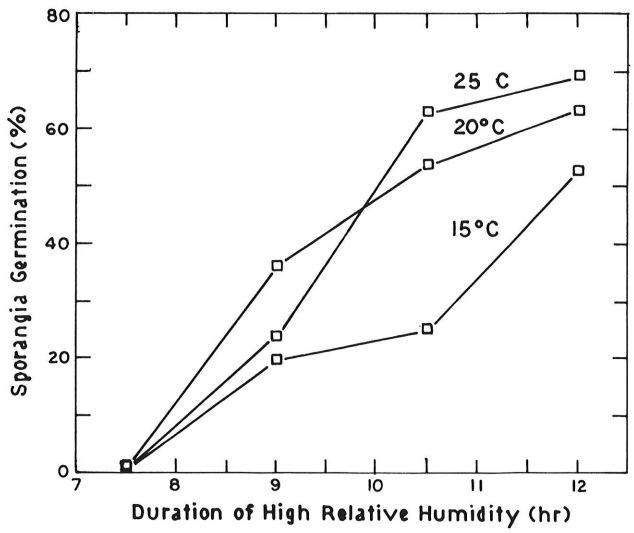

Fig. 5. Germination of sporangia of *Plasmopara viticola* harvested at different times during the sporulation period. No spores were produced at the 10 or 30 C temperatures. Germination was assessed in vitro after 6-hr incubation at 19 C. Each point is an average of the three replicates.

TABLE 3. Comparison of observed and predicted[a] values for sporulation of *Plasmopara viticola*

Validation test	Average temperature (C)	Duration high RH (hr)[b]	Sporulation ($\times 10^3$ sporangia/cm^2 lesion)			
					95% confidence interval	
			Observed	Predicted	Lower	Upper
Chamber	12.5	7.5	28	16	0	65
Chamber	13.0	10.5	80	85	29	127
Chamber	17.4	7.5	66	88	5	175
Chamber	17.5	10.5	315	242	188	274
Chamber	22.1	7.5	64	94	6	184
Chamber	22.3	10.5	240	252	198	283
Chamber	26.5	7.5	6	31	0	98
Chamber	25.1	10.5	147	179	117	217
Field	21.4	9	293	201	115	256
Field	18.1	6	78	2	0	85
Field	18.9	9	208	201	115	256
Field	22.4	7	82	54	0	151
Field	21.9	8	54	133	34	212
Field	20.9	8	121	140	39	219
Field	13.6	8	23	45	0	106
Field	14.6	5	21	20	0	26

[a] Predicted values and confidence limits were calculated from the reduced model (see text, Eq. 5 and Table 2) and then back-transformed to allow comparison to observed data.
[b] For the field test, durations were hours of relative humidity $\geq$90%.

TABLE 4. Linear regression[a] of percent sporangia germination on duration of sporulation at different temperatures

Temperature[b]	df Error	R^{2c}	F *P*-value	Estimate/*P*-value	
				Rate	Intercept
15 C	10	0.80	<0.01	10.8	−81.3
				<0.01	<0.01
20 C	10	0.86	<0.01	13.8	−96.8
				<0.01	<0.01
25 C	10	0.82	<0.01	16.4	−121.1
				<0.01	<0.01

[a] Analysis performed on reduced data (replicates 1, 2, and 3 pooled).
[b] Temperature during the sporulation period; germination was observed after 6 hr at 19 C.
[c] Coefficient of determination adjusted for the number of independent variables.

determine if this difference in temperature optima for these two processes significantly affect development of the epidemic.

Although most of the model predictions for the validation tests were within the 95% confidence interval, the range of those intervals was quite broad (Table 3). This indicated that the precision of the model could be improved, perhaps by employing another growth function. The Richards function, however, was chosen because its shape can be readily altered via the *m* parameter; we believe its fit was acceptable (Fig. 4). Another source of variation could have been the quadratic functions used to estimate the parameters of the Richards model. Each of these functions underestimated the maximum value of the corresponding parameter (Fig. 2). This may have been caused by the arbitrarily chosen temperature extremes of 10 and 30 C. If the true temperature limits were, for example, 11 and 28 C, then the parabolas may have had a smaller width and greater height, possibly intercepting the observed maximums. Whether such an enhancement would augment the predictive precision remains to be examined. Nevertheless, we believe that the quantitative nature of our model should aid in the description, simulation, and forecasting of grape downy mildew epidemics.

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