Estimating Infection Efficiency of *Plasmopara viticola* on Grape

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ABSTRACT

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To estimate the infection efficiency of *Plasmopara viticola*, the number of sporangia applied to a leaf and the number of zoospores they produced were determined. A relationship was developed between leaf area and the volume of inoculum retained without runoff. Given this relationship and the concentration of sporangia, the number of sporangia applied to a leaf could be estimated. Zoospore production from sporangial suspensions was examined in vitro at various temperatures; an average of seven zoospores per sporangium was observed for incubation temperatures of 5–25 C. Finally, potted vines of *Vitis labrusca* cv. Catawba were inoculated with sporangial suspensions of different concentrations. Although disease severity increased with inoculum concentration, the infection efficiency was estimated to be about 6% for each treatment.

Early studies on downy mildew of grape caused by *Plasmopara viticola* (Berk. & Curt.) Bel. & de Toni focused on the influence of climate and phenology on the occurrence of infection periods (2,11,12). Various parts of the infection cycle, such as the germination and sporulation phases, also have been examined (4,6,10,17). Although zoospore activity on the leaf surface has been studied (8,14), little is known about zoospore infection efficiency. Infection efficiency (ratio between the number of lesions produced and the number of spores applied) is an important component of the disease monocycle and a constituent of the corrected basic infection rate (18). This is an attempt to estimate infection efficiency for zoospores of *P. viticola*. Because the enzyme activity and physiology of leaves change when they are detached (5), whole plants were used for the estimation.

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To determine the denominator of the infection efficiency ratio, two preliminary experiments were conducted. First, a relationship was developed between the area of a grape leaf and amount of water it retained when atomized for a specified length of time. Given this relationship and the concentration of sporangia in a suspension, the number of spores applied to a leaf could be estimated from its area. However, because zoospores and not sporangia infect the plant, a second experiment was devised to determine the number of zoospores produced per sporangium. Although such values have been reported (6), this experiment also examined the effect of temperature on zoospore production. Finally, given the above results, the infection efficiency was estimated for a set of potted vines inoculated with sporangial suspensions of different concentrations. It was hypothesized that as the inoculum concentration increased, disease severity should also increase, but the infection efficiency should remain constant.

MATERIALS AND METHODS

Leaf area-water capacity relationship. Twenty-three leaves of varying sizes of *Vitis labrusca* cv. Catawba were collected from a vineyard. Their video images were recorded on VHS tape and later played back on an image analyzer (Imageplus, Dapple Systems, Inc., Sunnyvale, CA) to estimate leaf area. After the fresh weight of each was determined, the abaxial surfaces were sprayed with water at 69 kPa with a DeVilbiss atomizer. Three spray passes were made across each leaf, each pass consisting of a set of parallel, overlapping strokes. This technique produced a uniform distribution of minute droplets on the leaf surface without runoff. The volume of water applied was obtained by calculating the difference between the wet and dry leaf weights. This procedure was repeated five times for each leaf, allowing the leaves to dry between atomizations. An equation relating water volume as a function of leaf area was obtained by performing a linear regression using all 115 observations.

Zoospore production. Potted vines with 10-day-old lesions were placed in a growth chamber without light for 20 hr at 20 C and >95% relative humidity (RH). Sporangia then were harvested by washing the lower leaf surface with cold distilled water (one drop of Tween 40 per liter) at 4 C. Portions of this suspension were placed in incubators at 5, 10, 15, 20, 25, and 30 C. After most sporangia had germinated, a drop of dilute crystal violet (1%) was added to the suspension to arrest zoospore motility. The number of zoospores and germinated sporangia per milliliter was estimated with a hemacytometer. A minimum of 250 of both zoospores and sporangia were counted. The experiment was repeated once. The relationship between the number of zoospores per sporangium and temperature was determined by linear regression. The slope and intercept of the resulting regression function were tested for significance with standard t tests.

Infection efficiency. Twenty-five 1-yr-old Catawba vines were grown in the greenhouse in 15-cm pots and divided into five groups of five plants each. The vines were planted in a soilless medium of peat moss, perlite, and vermiculite (Pro-

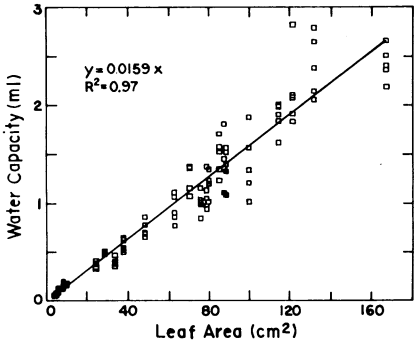

Fig. 1. Water-holding capacity of grape leaves as a function of their area. The abaxial surfaces of 23 Catawba leaves of varying sizes were atomized with water for a specified length of time. Five observations were recorded for each leaf.

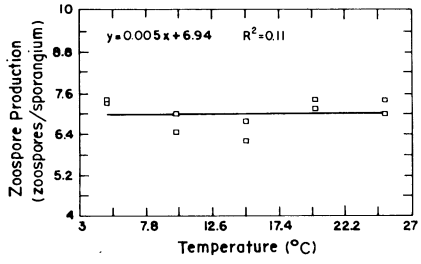

Fig. 2. In vitro zoospore production of *Plasmopara viticola* as a function of incubation temperature. Sporangia in water suspensions were allowed to germinate in incubators at various temperatures. A minimum of 250 spores of each type were counted for each data point.

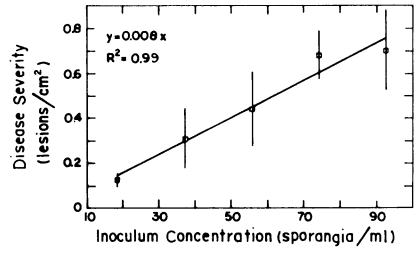

Fig. 3. Relationship between disease severity and inoculum concentration for *Plasmopara viticola* on Catawba grape. Each point represents the average disease severity obtained from observations on five potted vines, three leaves per vine. Bars represent the 95% confidence interval of the mean.

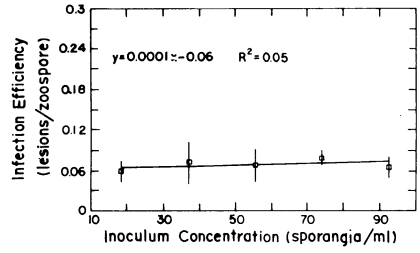

Fig. 4. Relationship between infection efficiency and inoculum concentration for *Plasmopara viticola* on Catawba grape. Each point represents the average infection efficiency obtained from observations on five potted vines, three leaves per vine. Bars represent the 95% confidence interval of the mean.

Mix BX, Premier Brands, Inc., New Rochelle, NY), pruned to a single shoot, and fertilized weekly (Peters Peat-Lite Special, 15-16-17, W. R. Grace & Co., Fogelsville, PA). After about seven to 10 leaves had been produced on each vine, the second, third, and fourth youngest leaves were tagged. Images of these leaves were recorded on VHS tape to determine their area with the image analyzer.

Another set of five infected plants was used to produce inoculum. Sporangia were harvested from 10-day-old lesions and diluted to form suspensions of 20, 40, 60, 80, and 100 sporangia per milliliter. Each plant group then was inoculated with a different suspension by the technique described. Only the lower surface of each of the three tagged leaves on each vine was inoculated. The proportion of viable sporangia was determined by observing their germination in vitro. After inoculation, the plants were placed in a growth chamber at 20 C and >95% RH for 22 hr. They were then moved to the greenhouse for the remainder of the incubation period. After 10 days, total lesions on each leaf were counted.

The number of viable sporangia (N) applied to each leaf was calculated with the following equation:

$$N = V * C * P,$$

where V = volume of inoculum applied as determined from the leaf area-water capacity relationship, C = concentration of sporangia in the inoculum suspension, and P = proportion of sporangia that germinated in vitro. This value was then multiplied by the average number of zoospores per sporangium to estimate the number of zoospores on each leaf.

Both infection efficiency (number of lesions per zoospore) and disease severity (number of lesions per square centimeter of leaf) were modeled as a function of inoculum concentration by linear regression. Regression coefficients were tested for significance by standard t tests. All 75 observations, 15 per treatment, were used in the analysis.

RESULTS

Leaf area-water capacity relationship. The volume of water retained on a leaf increased in a linear fashion with its area (Fig. 1). Leaf area described 97% of the variation in water capacity; the y-intercept was not significantly different from zero ($P = 0.72$). The coefficient of variation of the water capacity for any given leaf ranged from 0.9 to 10.4%, with an average of 5.5%.

Zoospore production. The number of zoospores produced per sporangium was the same at all temperatures between 5 and 25 C (Fig. 2); the slope of the line fitted to the data was not significantly different from zero ($P = 0.82$). Consequently, all 10 data points were averaged to produce an overall estimate of seven zoospores per sporangium. Because sporangia did not germinate at 30 C, the upper threshold must be between 25 and 30 C.

Infection efficiency. Disease severity (lesions per square centimeter) increased with an increase in the concentration of sporangia in the inoculum suspension (Fig. 3). The inoculum concentration accounted for 99% of the variation in the mean disease severity when a linear model was fitted to the data. Because the y-intercept was not significantly different from zero ($P = 0.90$), the model predicted no disease at zero sporangia per milliliter. Within the limits of the experiment, infection efficiency was not a function of inoculum concentration (Fig. 4). The concentration of sporangia accounted for only 5% of the variation in infection efficiency, and the slope of the regression function was not significantly different from zero ($P = 0.44$). The average infection efficiency, calculated across all inoculum concentrations, was estimated to be 0.06 lesions per zoospore.

DISCUSSION

Determination of the number of propagules applied to a plant surface may be one of the most difficult aspects of estimating infection efficiency. Earlier studies focused on more precise techniques for applying the inoculum. Schein (15) developed a "quantitative inoculator" that allowed the application of a known amount of spores to achieve a given deposition density. Lapwood and McKee (9) used a combination of inoculum dilution and a hypodermic syringe to produce the desired number of spores per drop. Our interest was in developing a simple technique to allow rapid inoculation of many leaves. Results indicated that leaf area was an excellent predictor of the volume of inoculum applied and hence the number of sporangia deposited. Even though spray duration consisted of a rather subjective "three spray passes," the relatively low coefficients of variation for any given leaf indicated the repeatability of the technique.

The density of spores deposited on the leaf surface is an important primary factor in infection efficiency. Too high an inoculum density would lead to multiple infections and lesion coalescence, resulting in an underestimation of the parameter's value. Also, interspore competition could reduce the ability of each zoospore to initiate infection. In our experiment, sporangial densities ranged from 0.29/cm^2 at the lowest inoculum concentration to 1.47/cm^2 at the highest. Because the infection efficiency curve was linear, we assumed that these densities were sufficiently low to avoid multiple infections. Thus, Vanderplank's model (16), which is approximately linear at low inoculum densities, was not necessary for correction.

The estimated infection efficiency of 0.06 lesions per zoospore pertains to *V. labrusca* cv. Catawba. Cultivars of *V. vinifera*, which are more susceptible than *V. labrusca* (3,13), may be associated with a much greater infection efficiency. Furthermore, we assumed that the average number of zoospores produced per sporangium on the leaf surface would be the same as in vitro. This assumption may be unjustified; zoospore production may differ among cultivars and could contribute to differences in resistance. Infection efficiency also is expected to vary with temperature and leaf wetness duration. Additional research is needed to quantify the effects of environmental and host factors on infection by *P. viticola*.

Although zoospores are the infective propagules, sporangia are usually considered the primary dispersal agent for downy mildew (1). Thus, in our experiment, the inoculum consisted of sporangia but the calculation of the infection efficiency was based on the number of zoospores. Studies have shown, however, that zoospores of *P. viticola* tend to congregate and encyst around stomata in groups of two to 10, with four or five being the most frequent (7,8,14). This suggests that several zoospores at one infection count may increase the probability of a successful infection. Our experimental method would have facilitated this gregarious behavior, because the zoospores produced by a single sporangium would have been close enough to congregate on the same stomate. The minute size of the inoculum droplets on the leaf surface may have also enhanced this effect by limiting zoospore motility. However, if the inoculum had consisted of a very dilute suspension of zoospores, then the probability of two or more zoospores congregating on the same stomate would have been much lower; the estimated infection efficiency would have been less than 0.06. Conversely, if single zoospores were effective in initiating infection, then the estimated value would have been greater than 0.06. In either case, the importance of this phenomenon would depend on the role, if any, that zoospores might play in the dispersal process.

Sporangia of *P. viticola* were observed to only germinate indirectly by the production of zoospores at temperatures between 5 and 25 C. Furthermore, temperature had no effect on the number of zoospores produced by each sporangium. This outcome agrees with the cytological evidence concerning zoospore production. Gregory (7) observed a single nucleus passing into the sporangium from the sporangiophore. This nucleus then divided to produce a multinucleate sporangium, each nucleus corresponding to a zoospore that eventually formed. Consequently, the lack of a relationship between the number of zoospores per sporangium and temperature seems tenable given that the former is actually predetermined by the number of nuclei produced.

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