Ecology and Epidemiology

The Effects of Wind Speed, Temperature, and Relative Humidity on Development of Aerial Mycelium and Conidia of *Botrytis cinerea* on Grape

C. S. Thomas, J. J. Marois, and J. T. English

Department of Plant Pathology, University of California, Davis 95616.
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ABSTRACT

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Development of aerial mycelium and conidia of *Botrytis cinerea* on surfaces of infected grape berries was evaluated under conditions of controlled wind speed, temperature, and relative humidity. Inoculated berries were incubated under three wind speeds (0, 0.3, and 0.6 m/sec), three relative humidities (69, 90, and 94%), and four temperatures (16, 21, 26, and 30 C) in a complete factorial design. Numbers of berries with aerial mycelium were determined daily for 6 days. Numbers of conidia produced per berry also were determined. Aerial mycelium developed most quickly at 21 C, 94% relative humidity, and 0 m/sec wind speed. Aerial mycelium did not develop on berries exposed to 69% relative humidity and wind. The greatest number of conidia were produced at 21 C, 94% relative humidity, and 0.6 m/sec wind speed. An estimate of evaporative potential of the air was made for each combination of wind speed, temperature, and relative humidity. Aerial mycelium and conidia developed if evaporative potential was below a threshold level that was related to temperature. Similar results were obtained under field conditions. Within the ranges tested, wind speed and relative humidity had a greater effect on evaporative potential than did temperature.

Additional key words: aerobiology, dehydration, evaporation, ventilation, water stress.

Botrytis cinerea Pers., which causes Botrytis bunch rot of grape berries, can be severe in cool, wet climates and on varieties with dense canopies and/or tight clusters. All varieties can be affected during wet years, especially if rains occur in the fall before harvest. However, disease can be reduced by altering the canopy microclimate. Gubler et al (1) reported that removal of leaves immediately adjacent to grape clusters 2 wk after bloom effectively controlled the disease.

Several studies have investigated the role of climatic factors on the development of *B. cinerea*. The effects of relative humidity and temperature were determined for development (2,3,7,8), germination (7–9), release (3), and dispersal (3) of conidia, growth (3,11,13) and survival of mycelium (11), and infection of plants (2,4,6,12). The effect of wind speed on conidium dispersal (3) and infection of plants (12) also was investigated. However, little is known about the influence of wind speed on development of mycelia and conidia on surfaces of infected plant tissues. The purpose of this study was to investigate the effect of wind speed, in combination with temperature and relative humidity, on growth and reproduction of *B. cinerea* at the surface of the host under laboratory and field conditions. A portion of this study was reported previously (10).

MATERIALS AND METHODS

Grape berries of the cultivars Emperor or Thompson Seedless were obtained with 0.5 cm of the rachis intact. Each rachis was dipped in 0.5% (v/v) sodium hypochlorite to control *Aspergillus niger* v. Tiegh. and *Rhizopus* spp., which would otherwise infect the berry during experiments. Each berry was then injected with 0.1 ml of a suspension of 5×10^4 conidia of *B. cinerea* per milliliter washed from 10-day-old cultures growing on potato-

dextrose agar in the dark at room temperature. The isolate of *B. cinerea* used in all experiments was originally isolated from a naturally infected Red emperor grape berry.

Five inoculated berries were placed in an acrylic tube 20.3 cm in length and 2.2 cm in diameter. Wind speed was generated at 0, 0.3, or 0.6 m/sec with a fan attached to one end of the tube (Fan type V2 41L 6V, serial no. 908305, Micronel US, Hudson, MA). Each tube was placed on a wax-coated wire rack in a plastic humid chamber that contained 400 ml of double-distilled water, a supersaturated solution of potassium nitrate, or a supersaturated solution of sodium nitrite to establish relative humidities of 94, 90, or 69%, respectively. Temperatures within chambers were maintained at 16, 21, 26, or 30 C.

Temperature and relative humidity were monitored in each humid chamber with a temperature and relative humidity probe (type 207, Campbell Scientific, Logan, UT). Temperature and relative humidity probes were inserted at the downwind end of each tube.

Air movement was monitored with a dual resistance temperature detector (RTD) anemometer (series 690, Sierra Instruments, Inc., Carmel Valley, CA). This anemometer is not biased by wind direction and accurately measures wind speed in the presence of turbulence. The RTD anemometer is sturdier than a hot wire anemometer. It also does not have the zero drift problems that hot wire anemometers have. Wind speeds reported are an average of the readings taken from the airspace between tube walls and the grape surfaces nearest to the walls.

The analog outputs from all probes were recorded on a Campbell Scientific 21X micrologger. All laboratory experiments were conducted under 8 hr of 32 μE/m^2/sec illumination and 16 hr of darkness per day for 6 days.

Each day the berries were inspected for the presence of aerial mycelium. After 6 days conidia produced on each berry were counted. To quantify conidia production, berries were vortexed in a 0.004% (w/v) thimerosol solution. The number of conidia per milliliter of solution was estimated using a hemacytometer with six determinations per sample. This value was converted to an average number of conidia per grape. Also, glass slides were coated with petroleum jelly and placed 1 cm from the mouth of each acrylic tube perpendicular to the tube direction to quantify release of conidia. At the end of six days, slides were removed and the number of conidia determined for each slide.

An estimate of the evaporative potential of ambient air was developed for each treatment. Sponge (plastic foam stoppers, Fisher Scientific, Santa Clara, CA) was formed to the approximate dimensions of a grape berry. The sponges were then saturated with distilled water and weighed. Five of them were placed in an acrylic tube in the same manner as were berries in the previous experiments. The sponges were subjected to each of the treatments. The amount of water evaporated was determined by reweighing the sponges either after 7 hr for the treatments that had 0.6 m/sec wind speed or after 23 hr for treatments that had 0 or 0.3 m/sec wind speeds. Evaporative potential was determined as the grams of water evaporated per hour in each tube.

A complete factorial design was used, and each laboratory experiment was replicated three times. Thirty-six humid chambers were randomly selected for each of the temperature-relative humidity-wind speed treatment combinations. Three tubes provided three samples per humid chamber per replicate. Each observation consisted of a mean value for five berries or sponges. The data were analyzed using analysis of variance and multiple regression.

The relationship among wind speed, relative humidity, and temperature and development of aerial mycelium and conidia also was tested in commercial vineyards. The microclimate of the leafed vines was altered by removing leaves adjacent to grape berry clusters 2 wk after bloom as described previously (1). Leaf removal canopies are defined as canopies that received the leaf removal treatment. Nonleaf removal canopies are defined as those in which the leaves were left intact. The experiments were conducted in two locations, one with Zinfandel, a red wine grape, and the other with Chenin blanc, a white wine grape, with two replications per treatment. Each replication consisted of four rows of vines. Campbell 21X microloggers were used to record temperature, relative humidity, and wind speed hourly throughout the 1986 season in the leaf removal and nonleaf removal canopies. Two of each of the meteorological probes described earlier were placed at cluster level within each canopy type. These probes were selected for their small size, great durability, and high accuracy. Calibrations at the beginning and end of the season indicated no drift in accuracy. At harvest, 50 clusters with infected berries were chosen randomly from each of two replicate plots of both leaf removal and nonleaf removal canopies. Clusters were rated for presence of mycelium and/or conidia of *B. cinerea* on the surfaces of berries.

RESULTS

Relative humidity and temperature were similar for the inside of the tube and ambient moist chamber conditions. The range of wind speeds measured in the three wind speed treatments did not overlap.

The development of aerial mycelium was affected by time and climatic factors. Neither aerial mycelium nor conidia were observed in any of the treatments one day after inoculation. In addition, no aerial mycelium or conidia were observed in any treatment combination at 30 C. These data were not included in the analyses. Data from days 2 through 6 were analyzed separately to determine if the significance of main effects and their interactions changed over time. After 6 days of incubation at any temperature, aerial mycelium developed on the surfaces of most of the berries maintained at 90 or 94% relative humidity and any wind speed (Fig. 1). In contrast, aerial mycelium did not develop on any berries maintained at 69% relative humidity and 0.3 or 0.6 m/sec wind speed. On each day, the main effects of wind speed, temperature, and relative humidity were significant (Table 1). The significance of interactions among these factors varied, according to the period of incubation. Each day as relative humidity increased, the number of berries with aerial mycelium increased (Fig. 1). In general, when wind speed was increased, the number of berries with aerial mycelium decreased. The forms of the response surfaces for aerial mycelium development were similar at each temperature. Also,

TABLE 1. Significance level (P) of main effects and interactions in five analyses of variance on number of grapes with aerial mycelium and one analysis of variance on number of conidia per grape

	Significance level for main effects and interactions						
ANOVA for	WS[a]	RH	T	WS*RH	WS*T	T*RH	WS*RH*T
Aerial mycelium							
After 2 days	0.0001	0.0001	0.0001	0.35	0.0008	0.0001	0.44
After 3 days	0.0001	0.0001	0.0001	0.0001	0.0008	0.0001	0.0008
After 4 days	0.0001	0.0001	0.0001	0.0001	0.008	0.02	0.03
After 5 days	0.0001	0.0001	0.0001	0.0001	0.05	0.92	0.15
After 6 days	0.0001	0.0001	0.007	0.0001	0.23	0.55	0.23
Number of conidia per grape	0.0001	0.0001	0.0001	0.0001	0.0001	0.0014	0.0001

[a] WS = Wind speed m/sec, T = Temperature C, RH = % relative humidity.

aerial mycelium appeared earliest at 21 C and latest at 16 C. The number of berries with mycelium increased fastest at 21 C and slowest at 26 C.

The growth habit of the aerial mycelium at all temperatures was affected by relative humidity and wind speed. At all relative humidities, mycelium that developed at greater wind speeds had more compact mycelium than that which had developed at smaller wind speeds (Fig. 2).

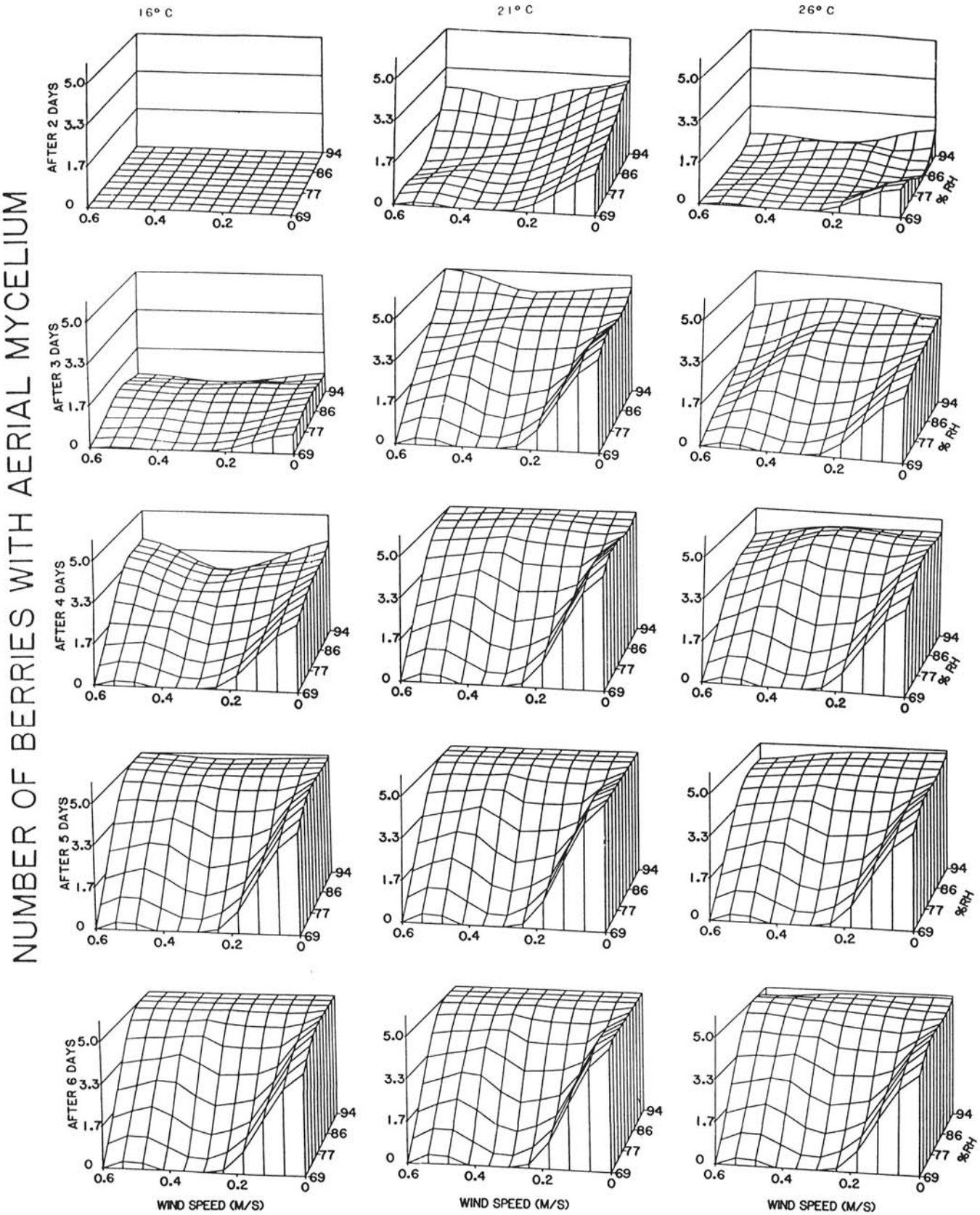

Fig. 1. The effect of wind speed, temperature, and relative humidity on *Botrytis cinerea* aerial mycelium development on berries during six days. Five berries = 100% aerial mycelium. Response surfaces each represent nine replications ($P = 0.001$).

Development of conidia also was affected by climatic factors. Conidium development was greatest at 21 C, 94% relative humidity, and 0.6 m/sec wind speed (Fig. 3). Few conidia developed at 16 C except at 94% relative humidity and 0.6 m/sec wind speed. At 26 C, conidium development was greatest at 90 or 94% relative humidity and 0.3 m/sec wind speed. Overall, more conidia developed at 21 C than at 16 or 26 C. The main effects, two-way interactions, and three-way interactions were highly significant (Table 1).

Because of the significant interactions, the response surfaces for conidium formation were very complex. For example, at 21 C the number of conidia produced at 94% relative humidity increased as wind speed increased (Fig. 3). In contrast, the greatest number of conidia produced at 90% relative humidity was at 0.3 m/sec wind speed. At 69% relative humidity, only the berries at 0 m/sec wind speed produced conidia, because aerial mycelium had not developed at the other wind speeds.

Relative humidity, temperature, and wind speed influenced water loss from the sponges. Evaporative potential ranged from 0 to 0.8 g of water per hour. A stepwise linear regression was conducted using relative humidity, temperature, and wind speed as independent variables and grams of water evaporated from the sponges per hour as the dependent variable to develop an estimate of evaporative potential. The same regression equation resulted from forward and backward stepwise procedures:

$$EP = 0.2565 - 0.0028 \times (\%\,RH) + 5.667 \times (WS)^2 - 0.055 \times ([WS]^2 \times [\%\,RH]),$$

where EP = evaporative potential (grams of water per hour), RH = relative humidity (percent), and WS = wind speed (meters/second). This equation explained 96% of the variation in evaporative potential ($P = 0.0001$). Temperature was not included as a variable because it was not significant ($P = 0.05$) as a main effect over the levels of the variables tested.

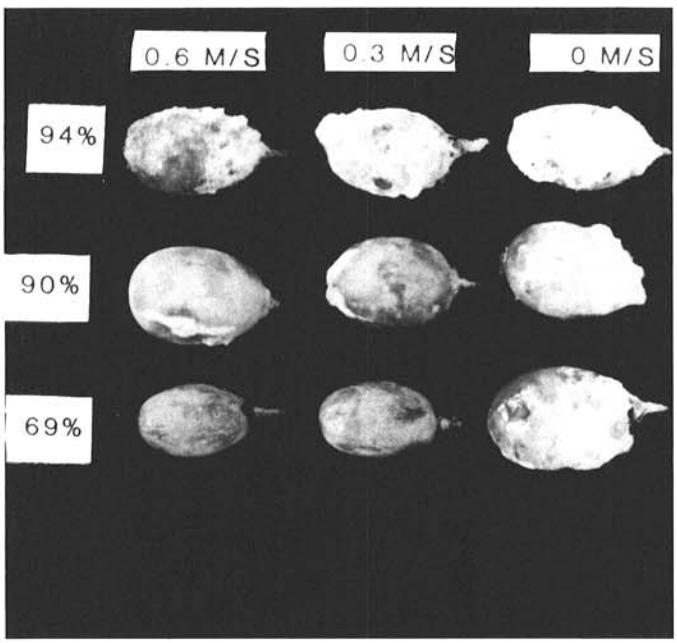

Fig. 2. Aerial mycelium morphology of *Botrytis cinerea* on Thompson seedless berries subjected to three relative humidities and three wind speeds at 21 C. Mycelium became more compact as wind speed increased and was absent in the presence of wind speed at 69% relative humidity. Morphologies similar to these occurred for each wind speed-relative humidity combination at 26 and 16 C.

Development of aerial mycelium on the surfaces of berries was correlated with the evaporative potential of the air passing through the tube (Fig. 4). After 2 days at 21 or 26 C, the number of berries with aerial mycelium decreased linearly as the evaporative potential of the air increased to 0.25 g of water per hour. After 3 days, a similar relationship occurred at 16 C. Even after 6 days of incubation, aerial mycelium did not develop on any berry when the evaporative potential of the air was greater than 0.25 g of water per hour. The evaporative potential threshold at which berries did not develop aerial mycelium was affected by temperature. Mycelium did not develop on berries at evaporative potentials greater than 0.25, 0.20, or 0.15 g of water per hour when air temperatures were 26, 21, or 16 C, respectively.

Conidium development also was affected by the evaporative potential of the air. The greatest number of conidia developed on berries exposed to evaporative potentials between 0.05 and 0.15 g of water per hour (Fig. 5). The evaporative potential associated with the greatest conidium production increased within this range as temperature decreased.

The release of conidia was affected by wind speed. Up to 300 conidia were trapped on slides at the end of the tubes with winds of 0.6 m/sec and 94% relative humidity. Evaporative potentials in these tubes were greater than 0.1 g of water per hour. No conidia were observed on slides for other treatments.

In the field experiments, mycelium and conidia were observed more commonly on surfaces of berries of infected clusters from nonleaf removal than from leaf removal canopies. In the vineyard with Chenin blanc, 90% of infected clusters supported aerial mycelium and conidiation of *B. cinerea* in nonleaf removal canopies. In leaf removal canopies, 5% of the clusters infected with *B. cinerea* supported aerial mycelium and conidiation. In the vineyard with Zinfandel, aerial mycelium and conidia were observed on berry surfaces of 50% of infected clusters from nonleaf removal and 1% of infected clusters from leaf removal canopies.

Estimates of evaporative potential in the vineyard were derived from hourly measurements of relative humidity and wind speed by using the regression equation developed from the laboratory experiments (Fig. 6). In leaf removal canopies evaporative potential increased from 0 g of water per hour in the early morning to 2.3 g of water per hour late in the afternoon and then declined through the evening. In nonleaf removal canopies evaporative

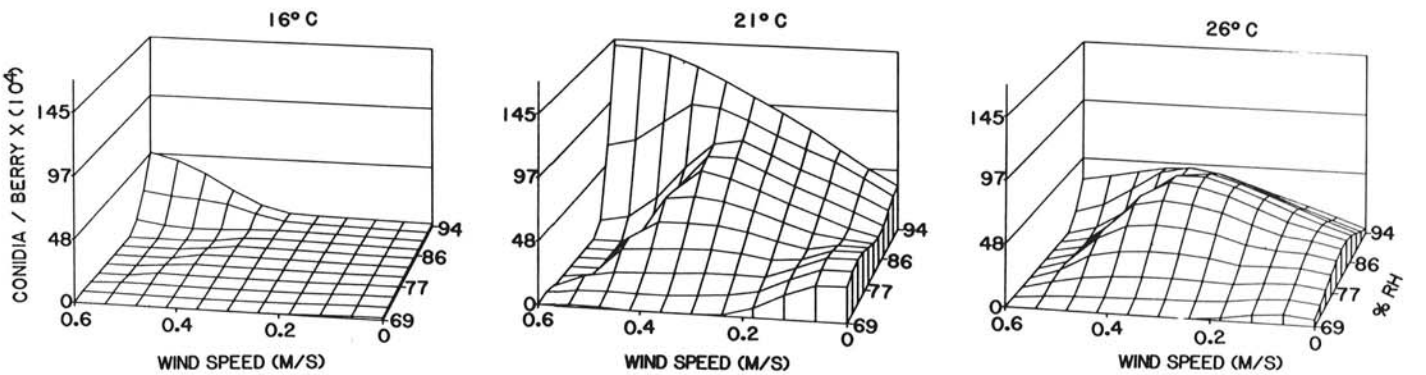

Fig. 3. The effect of wind speed, temperature, and relative humidity on *Botrytis cinerea* conidia development on berries after six days. Response surfaces each represent nine replications ($P = 0.001$).

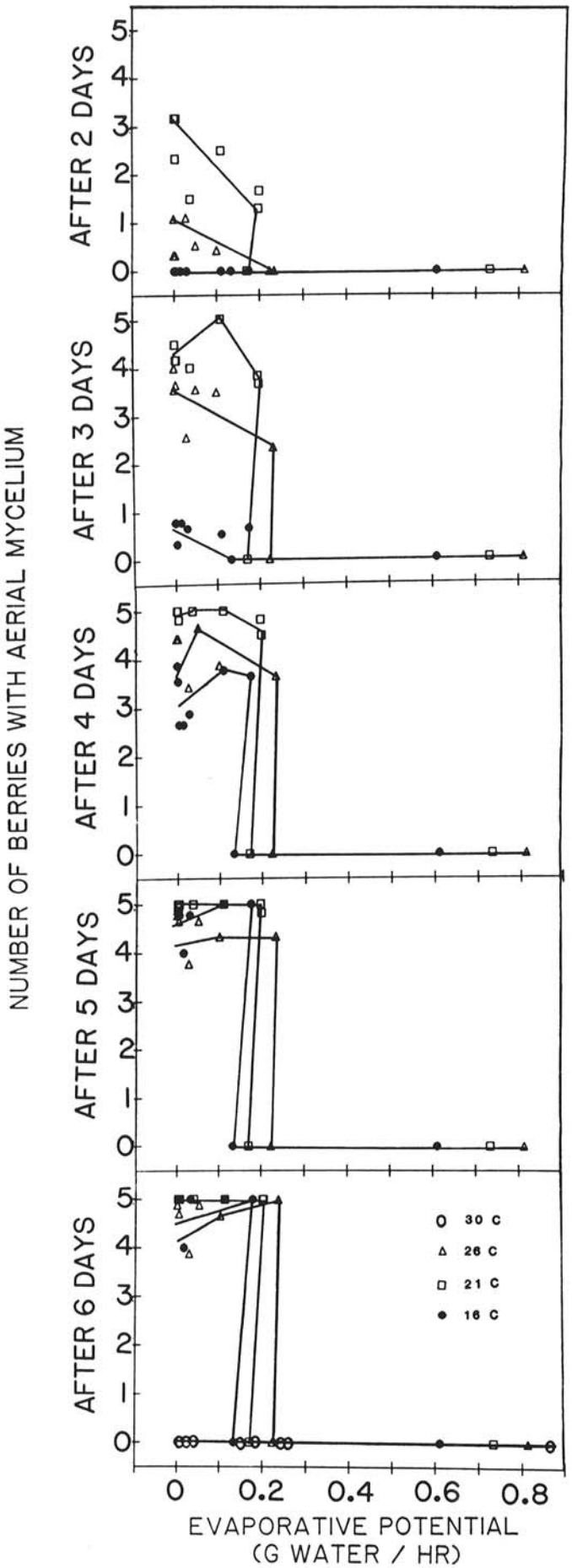

Fig. 4. The effect of evaporative potential of air on *Botrytis cinerea* aerial mycelium development on berries during six days at four temperatures ($n = 9$, $P = 0.001$).

potential fluctuated in a similar diurnal fashion; however, maximum values of only 0.8 g of water per hour were observed in the afternoon. Changes in evaporative potential during the course of the day followed closely the fluctuations in hourly average wind speeds (Fig. 7). Maximum wind speeds recorded were 0.58 and 0.28 m/sec in leaf removal and nonleaf removal canopies, respectively.

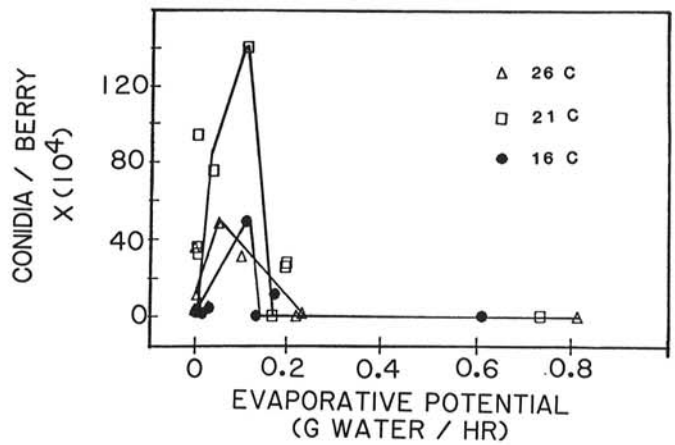

Fig. 5. The effect of evaporative potential of air on *Botrytis cinerea* conidium development on berries after six days ($n = 9$, $P = 0.001$).

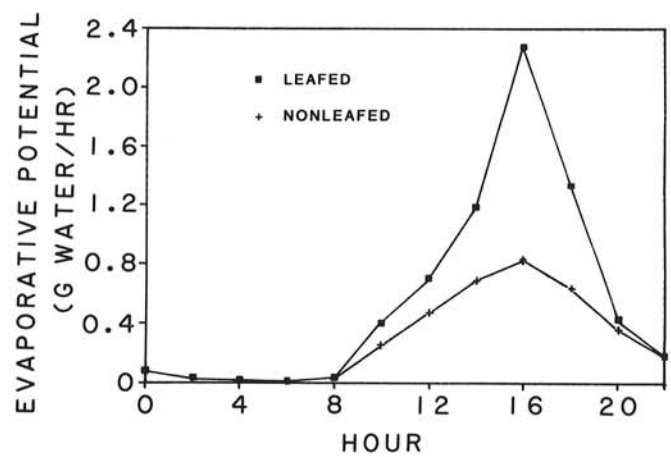

Fig. 6. Diurnal fluctuation of evaporative potential in nonleafed and leafed Zinfandel grapevine canopies.

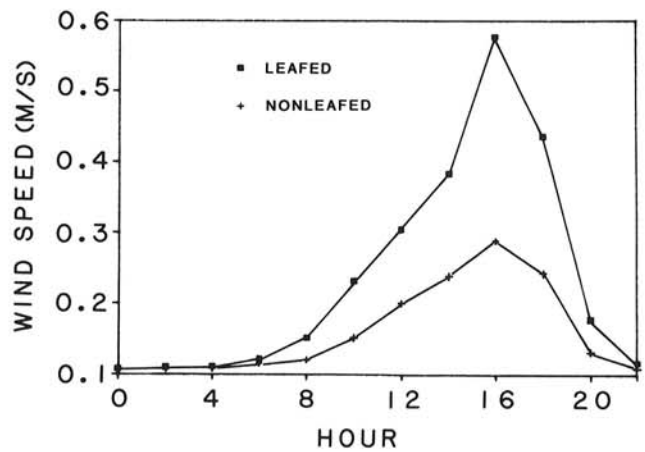

Fig. 7. Diurnal fluctuation of wind speed in nonleafed and leafed Zinfandel grapevine canopies.

DISCUSSION

The development of *B. cinerea* on the surfaces of inoculated grape berries was affected by temperature, relative humidity, and wind speed. The relationships between temperature and relative humidity and the development of mycelium and conidia of *B. cinerea* were similar to those reported by others (2,3,7,8,11). Temperature and relative humidity also may be important factors influencing conidium survival, germination, and mycelial growth (11).

The role of wind speed in the growth and reproduction of *B. cinerea* has not been reported. Leach (5) found that saturated air moving at 0.5–1.5 m/sec decreased the number of conidia produced by *Drechslera turcica* and *Peronospora destructor*. Wilson (12) studied the effect of wind speed, temperature, and relative humidity on infection of bean by *B. cinerea*. He determined that when other conditions were equivalent, wind reduced infection levels. The present report is the first to investigate the effect of wind speed on aerial mycelium growth and conidiation of *B. cinerea*.

The interactions among wind speed, relative humidity, and temperature can be limiting factors in the development of aerial mycelium by *B. cinerea* on grape berries. For example, at 69% relative humidity, wind speed was the limiting factor as aerial mycelium developed only at 0 m/sec wind speed. The interactions of wind speed, relative humidity, and temperature were integrated into a value that reflects the evaporative potential of the air. When evaporative potential exceeded 0.25 g of water per hour, no aerial mycelium developed.

The interactions among wind speed, relative humidity, and temperature can affect the number of conidia produced by *B. cinerea*. For example, at 16 and 21 C, the greatest number of conidia were produced at 94% relative humidity and 0.6 m/sec wind speed. At 26 C, the maximum number of conidia produced was at 90% relative humidity and 0.3 m/sec wind speed. The complexity of these interactions was simplified by measurements of evaporative potential. Maximum numbers of conidia were produced within a range of evaporative potentials between 0.05 and 0.15 g of water per hour and increased as temperature decreased.

Wind speed and evaporative potential of ambient air may be limiting factors in development of aerial mycelium and conidia of *B. cinerea* under field conditions as well. In two vineyards, higher evaporative potentials in leaf removal canopies were associated with lower percentages of infected grape clusters with aerial mycelium.

A role for wind speed in reducing growth and reproduction of *B. cinerea* on berry surfaces may exist through its influence on evaporative potential. A strong empirical relationship between wind speed and evaporative potential was observed in growth chamber studies. When this relationship was applied to climatic data from the field, diurnal fluctuation in evaporative potential followed closely the fluctuation in wind speed in both canopies. It remains to be determined whether estimates of evaporative potential based on regression reflect completely the actual potentials in grapevine canopies. Relative humidities and temperatures outside the range used to develop the regression equation describing evaporative potential, as well as factors that were not measured, such as solar radiation, may accentuate or diminish differences between estimated potentials of leaf removal and nonleaf removal canopies. Further studies are needed in various climate and canopy types to test the applicability of the laboratory data to field situations.

The results of this study indicate that wind speed has a significant effect on the development of *B. cinerea*. This is very relevant to the application of cultural control measures for plant disease. Wind speed is a variable that can be manipulated in the greenhouse with fans or in the field through canopy management, planting orientation, and planting density. Although relative humidity and temperature are more difficult variables to control in the field, these have been the parameters investigated most often by plant pathologists. Although their importance in our understanding of plant disease development cannot be overestimated, investigations that also include wind speed as a parameter may lead to further understanding and control of plant disease.

The concept of evaporative potential as a parameter integrating the influences of wind speed, relative humidity, and temperature on growth and reproduction of *B. cinerea* has great appeal. Such a parameter offers insight into the complex interrelations between these environmental factors and their influence on the behavior and possible control of this pathogen. The experiments performed to date provide evidence for a limiting role of evaporative potential on pathogen development. Further insight into control of *B. cinerea* and other pathogens may be attained by evaluating the influence of evaporative potential on other aspects of epidemic development.

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