Effect of Fungicides and Water on Sporulation of *Uncinula necator*

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ABSTRACT

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Five treatments (triadimefon, wettable sulfur, sulfur dust, water, and an unsprayed control) in combination with four temperatures (19, 22, 26, and 30 C) and two grape cultivars (*Vitis vinifera* cvs. Carignane and Chardonnay) were examined for their effect on sporulation of *Uncinula necator*, the cause of grape powdery mildew. Fungicides and water were applied on the seventh day after inoculation of cultivars with individual conidia. The numbers of conidia produced from the developing colonies were determined 10 and 15 days after inoculation. After 10 days, temperature, fungicides, and water had a significant effect on sporulation; after 15 days, significant interactions between temperature and chemical treatments and between cultivar and chemical treatments were also observed. Maximum sporulation after 15 days occurred at 26 C for all treatments and both cultivars. Application of water and fungicides reduced sporulation, but the magnitude of the reduction depended on temperature.

Powdery mildew of grape (*Vitis vinifera* L.), caused by *Uncinula necator* (Schwein.) Burrill, is a disease of major economic importance on cultivated grapevines in California, where repeated applications of fungicides on a regular basis are used to prevent economic loss (10). The fungicides used include sulfur, applied as a dust or a wettable powder, and several sterol biosynthesis inhibitors (SBI), including triadimefon (Bayleton), fenarimol (Rubigan), and myclobutanil (Rally). Despite these regular control measures, powdery mildew can still cause heavy losses on susceptible cultivars in certain years (12). Knowledge of the influence of environmental and physical factors on growth and sporulation of *U. necator* can contribute toward an understanding of the seasonal variability in severity of grape powdery mildew.

The effects of temperature, moisture, fungicides, cultivar resistance, and age of host tissue on conidial germination, penetration, and colonization have been reported (5–7,11). The effects of water or fungicides on sporulation of *U. necator* are not known. Several SBI fungicides and wettable sulfur have been shown to significantly reduce sporulation of *Podosphaera leucotricha* (Ellis & Everh.) E.S. Salmon, the cause of powdery mildew of apple, for up to 20 days (2,4,15), and water has been shown to temporarily decrease sporulation of *P. leucotricha* and of *Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal, the cause of powdery mildew of barley (4,17). However, those studies failed to examine the effect of temperature on efficacy of fungicides or water. The purpose of this study was to examine the effects of several fungicides and water on sporulation of *U. necator* as influenced by temperature and cultivar.

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MATERIALS AND METHODS

Inoculations. A single spore isolate of *U. necator*, collected in 1987 from a vineyard in Napa Valley, California, and maintained on seedlings of the grape cultivar Carignane grown in the laboratory, was used for all inoculations. During the winter of 1988, hardwood cuttings were taken from Carignane and Chardonnay vines at the University of California at Davis and rooted in 10-cm pots containing U.C. mix. Cuttings were maintained in a greenhouse free of grape powdery mildew and fertilized weekly with half-strength Hoagland's solution.

Individual conidia from 7- to 10-day old colonies were transferred to the three youngest, fully expanded leaves of a single shoot using a camel's hair attached to a dissecting needle. Individual conidia were placed at five predesignated locations on the adaxial 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 covered by filter paper (Whatman No. 2) on the side and placed inside illuminated growth chambers receiving 14 hr of light at 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ (LI-COR LI-200S pyranometer, Lambda Instruments Corp., Lincoln, NE).

Treatments. Temperatures within the plastic cylinders were maintained at approximately 19, 22, 26, and 30 C. The relative humidity (RH) and vapor pressure deficit in the plastic cylinders with plants at those temperatures were 60% and 10 mbars, 67% and 10 mbars, 45% and 16 mbars, and 30% and 24 mbars, respectively. Temperature and relative humidity were monitored by placing a temperature and RH sensor, attached to a CR21 Micrologger (Campbell Scientific, Logan, UT), in the center of the plastic cylinders at foliage height. Vapor pressure deficit was calculated from the recorded temperature and relative humidity (14).

Treatments, applied to sporulating colonies 7 days after inoculation, consisted of triadimefon (Bayleton 50WP), 100 ppm; wettable sulfur (Thiolux 80DF), 1,280 ppm; sulfur dust (Lilley-Miller 90%), 0.0056 g per leaf; and deionized water. Unsprayed plants served as controls. Triadimefon, wettable sulfur, and water were applied by misting leaves until just before runoff; an aspirator was used to blow sulfur dust over leaves. Plants were returned to the growth chambers immediately after treatment.

Data collection. A destructive technique was used to count the number of conidia produced 10 and 15 days after inoculation (3 and 8 days after application of fungicides and water). Two disks containing individual colonies were removed from each leaf by means of a 2.5-cm cork borer, placed into separate microcentrifuge tubes, and agitated for 10 sec in a 1-ml solution of 1.5% sodium chloride containing a trace amount of sodium dodecyl sulfate. The numbers of conidia in 10 5-μl drops were counted and used to calculate the number of conidia produced from a single colony.

Experimental design. A 2 × 4 × 5 factorial design with combinations of two cultivars, four temperatures, and five chemical treatments was used. Because colonies from separate plants were sampled destructively at 10 and 15 days after inoculation, a total of 80 plants were used in the experiment. The experiment was repeated three times, with growth chambers randomly assigned for each temperature treatment. The average of the three inoculated leaves per plant was used in the analysis of the data. To help normalize the data and reduce the variance, a natural log transformation was applied to conidial numbers before analysis. An analysis of variance was conducted to determine the significance of main effects or interactions using Proc Glm in SAS (13).

RESULTS

Sporulation after 10 days. Ten days after inoculation and 3 days after

treatments, temperature, fungicides, and water had significant effects on sporulation (Table 1). No significant differences were observed between Carignane and Chardonnay in the number of conidia produced per colony. No significant interactions between main effects were observed. The number of conidia produced in 10 days by a single colony was reduced from 81 to 36 after application of water (Table 2). Greater reductions in sporulation occurred after application of fungicides; no differences in efficacy were observed among fungicides. Production of conidia was maximum at 26 C and minimum at 19 C (Table 2). Temperatures of 22 and 30 C had a similar effect on reducing sporulation.

Sporulation after 15 days. Fifteen days after inoculation and 8 days after treatments, sporulation was significantly affected by cultivar, chemical treatments, and temperature (Table 1). Interactions between chemical treatments and temperature and between chemical treatments and cultivar also were significant. Application of water reduced sporulation from 297 conidia to 25 at 19 C, from 568 conidia to 247 at 22 C, and from 1,351 conidia to 246 at 30 C (Fig. 1). At 26 C, water reduced sporulation slightly, from 1,584 conidia to 1,214.

Application of triadimefon, sulfur dust, and wettable sulfur to colonies growing at 19 and 30 C reduced sporulation to negligible amounts (Fig. 1). At 22 C, sporulation was also negligible after application of wettable sulfur and triadimefon but not of sulfur dust. At 26 C, 18, 33, and 50 conidia were produced after application of wettable sulfur, triadimefon, and sulfur dust, respectively. Sporulation associated with the sulfur dust application varied widely at 26 C.

No significant differences were observed between Carignane and Chardonnay in sporulation of unsprayed control colonies after 15 days (Fig. 2). Application of water had a greater effect on sporulation of colonies growing on Chardonnay than on those growing on Carignane. The effect of fungicides on sporulation was similar for both cultivars.

Table 1. Analysis of variance for numbers of conidia harvested 10 and 15 days after inoculation of two grape cultivars with *Uncinula necator*

		Day 10		Day 15	
Source[z]	df	*F* value	*P*>*F*	*F* value	*P*>*F*
Model	39	8.12	0.001	19.81	0.001
Cv	1	0.74	0.391	8.84	0.003
Chem	4	44.31	0.001	151.57	0.001
Temp	3	35.56	0.001	35.41	0.001
Chem × temp	12	0.91	0.540	2.56	0.003
Cv × temp	3	0.72	0.539	0.42	0.740
Cv × chem	4	0.82	0.516	3.17	0.014
Cv × chem × temp	12	1.30	0.219	0.55	0.882

[z] Cv = cultivar, chem = chemical treatment (sulfur dust, wettable sulfur, triadimefon, or water 7 days after inoculation or unsprayed control), temp = temperature.

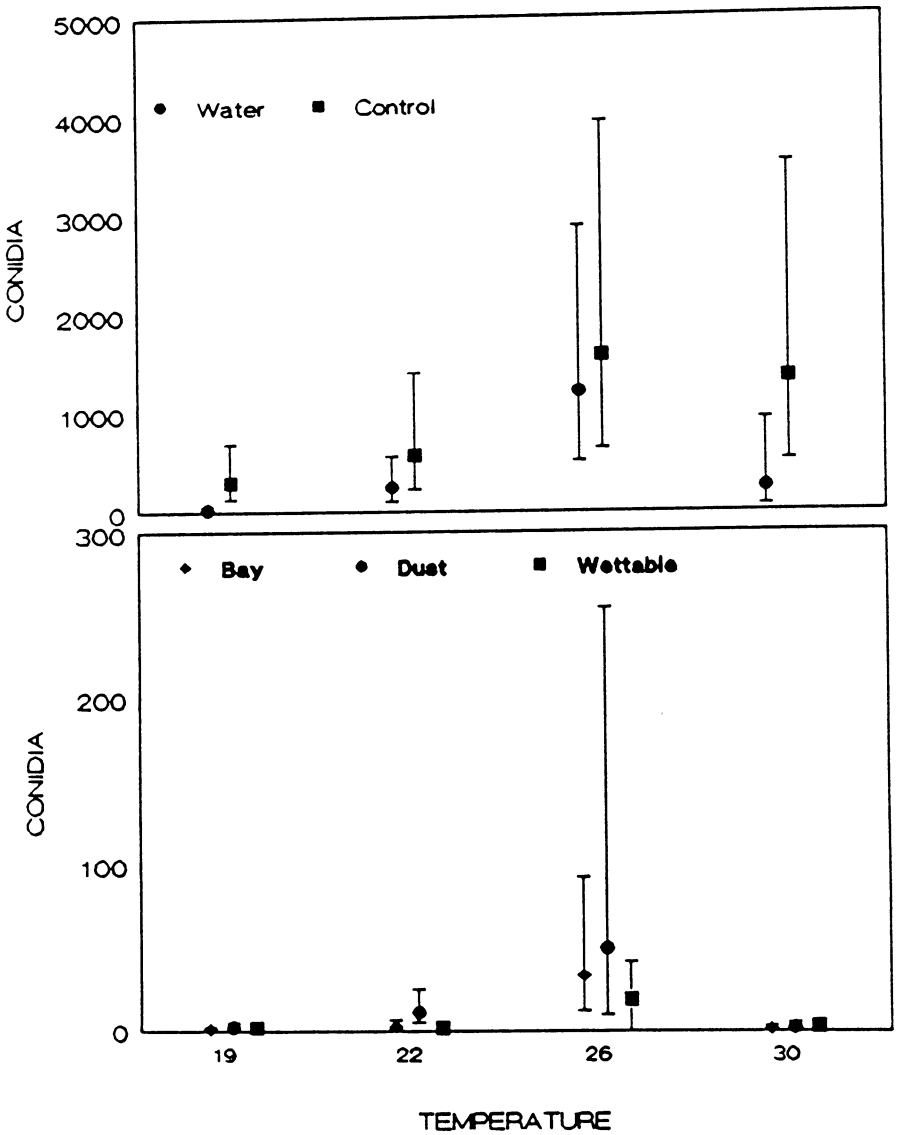

Fig. 1. Number of conidia produced by individual colonies of *Uncinula necator*; 95% comparison intervals for the means of the temperature × treatment interactions, 15 days after inoculation.

DISCUSSION

Application of fungicides to established colonies of *U. necator* significantly reduced sporulation but did not eradicate colonies. In a study by Ogawa et al (11), triadimefon applied 5 days after inoculation at 150 ppm also failed to eradicate colonies. These results support observations in the vineyard that disease

Table 2. Effects of various chemical treatments and temperatures on sporulation of *Uncinula necator* 10 and 15 days after inoculation of two grape cultivars

	Conidia/colony (no.)	
Treatments	Day 10	Day 15
Chemical[x]		
Unsprayed control	80.88 a[y]	760.50[z]
Water	35.98 b	189.80
Sulfur dust	1.71 c	5.06
Triadmefon	1.73 c	3.18
Wettable sulfur	1.22 c	3.11
Temperature (C)		
26	58.26 a	159.77
22	10.47 a	21.30
30	9.19 b	12.15
19	4.22 c	7.65

[x] Applied 7 days after inoculation.
[y] Means followed by the same letter are not significantly different at $P = 0.05$ according to the Waller-Duncan k-ratio t test.
[z] Mean separations are not performed on 15-day data because of significant interactions.

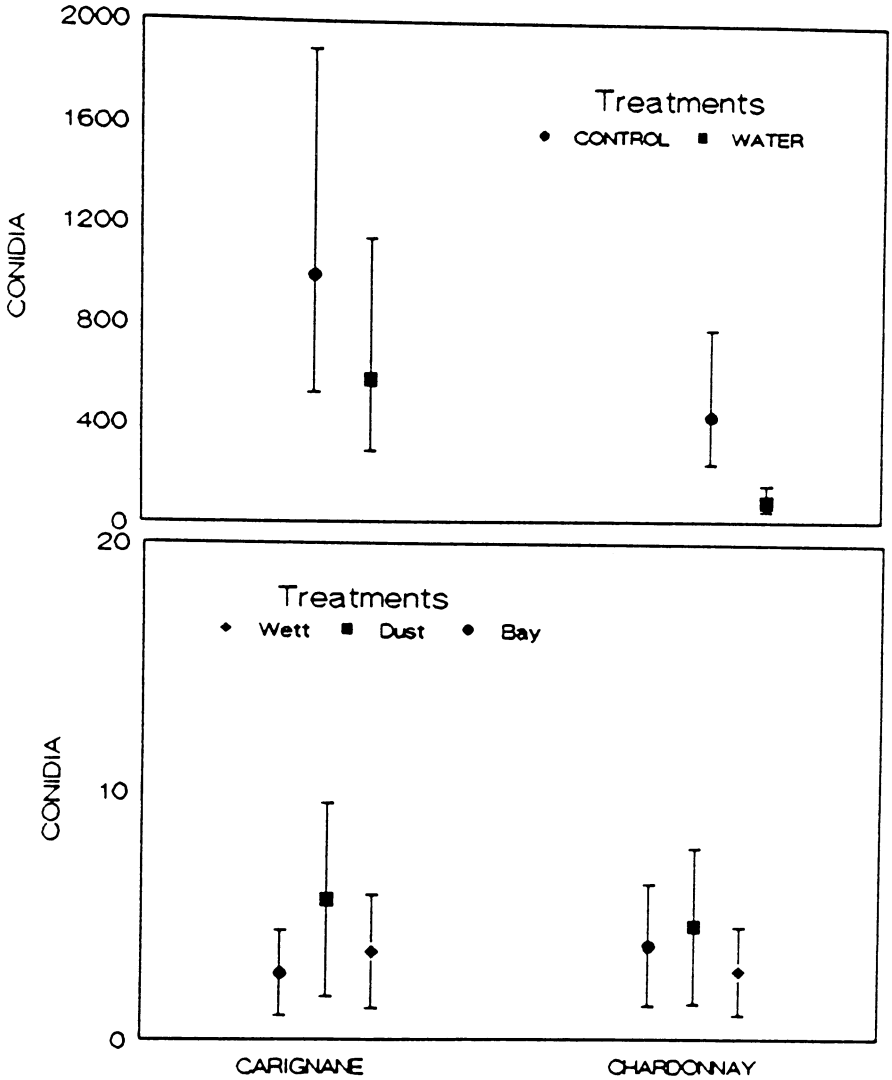

Fig. 2. Number of conidia produced by individual colonies of *Uncinula necator*; 95% comparison intervals for the means of the cultivar × treatment interactions, 15 days after inoculation.

control is improved when sulfur and triadimefon are used as a protectant instead of an eradicant fungicide (3,10,11).

Although colonies were not eradicated, sporulation was reduced. Sulfur, applied as a dust or wettable powder, and triadimefon were equally effective in reducing sporulation at 19 and 30 C. Results were similar for wettable sulfur and triadimefon on powdery mildew of apple (4). At 26 C, however, all three fungicides permitted some sporulation to continue 3 and 8 days after application. Thus, temperature can influence the activity of fungicides on sporulation of *U. necator*. The influence of temperature was not examined in studies on the antisporulant activity of fungicides on other genera of powdery mildew (2,4,15).

Temperature also appeared to have a major impact on the influence of water on sporulation. Large reductions in sporulation occurred when water was applied to colonies growing at 19 and 30 C but not those growing at 22 and 26 C. A large reduction in sporulation was observed after application of water to colonies of the fungi causing powdery mildew of apple and barley (4,17). Those studies, however, did not include water applications over a range of temperatures. In addition, forceful application of water under high pressure may have further reduced sporulation by physically removing mycelium and conidiophores from the colonies. In this study, water was applied to runoff by gently misting leaves.

It is interesting that water reduced sporulation at 19 C but not at the other temperatures used in this study. This may explain why rainfall seems to enhance mildew development in some years and to suppress it in others (1,8,9,16). In the future, such vineyard management practices as overhead sprinkler irrigation may be timed to minimize mildew development.

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