Effects of Humidity on the Development of Grapevine Powdery Mildew

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ABSTRACT

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The effects of humidity on powdery mildew development on grape seedlings and the germination of *Uncinula necator* conidia in vitro were examined. Studies were conducted at an optimum temperature of $25 \pm 2^\circ\text{C}$. Disease on foliage was markedly affected by humidity levels in the test range of 39 to 98% relative humidity (RH), corresponding to vapor pressure deficits (VPD) of 1,914 to 61 Pa. Incidence and severity increased with increasing humidity to an optimum near 85% RH, and then appeared to plateau or decrease marginally at higher values. Conidial density and chain length also were proportional to humidity, but were

influenced less strongly. There was a strong, positive linear relationship between humidity level and frequency of conidium germination with RH treatments of $\leq 84\%$. However, germination frequency fell sharply at RH levels above a mean of 87%. All measures of humidity were equally accurate in predicting germination responses; however, VPD was slightly more effective than RH in accounting for effects on disease development and pathogen sporulation, and both were more effective than absolute humidity. Humidity appears to play a significant role in grapevine powdery mildew epidemiology, confirming the benefits of management practices to avoid and mitigate high humidity in the vineyard canopy.

Additional keywords: microclimates, *Vitis vinifera*.

Powdery mildew, caused by *Uncinula necator* (Schw.) Burr., is the most ubiquitously damaging disease of grapevines worldwide, reducing yield, vine growth and vigor, and fruit quality (13,24,25). Early reports on grapevine powdery mildew from various international locations included anecdotal observations correlating high disease severity with humid atmospheric conditions, including coastal fog; dull and cloudy weather; poor air drainage in the vineyard, low-lying areas, and areas near river banks; humid weather with little or no rain; and relative humidity (RH) values between 40 and 95% (2,11,14,16,27). However, in the first experimental examination of this relationship, Delp (12) concluded that there was little to no effect of different humidity treatments on either the germination of *U. necator* conidia or the development of primary and secondary hyphae on leaves. In contrast, Mane et al. (21) studied powdery mildew development in irrigated compartments in a vineyard in India and found the rate of disease multiplication to be zero both below 53% RH and at 100% RH. Similarly, Kast (18) developed a system for timing fungicide sprays in German vineyards based, among other things, on a threshold level of 60 to 70% RH for "humid hours" favoring disease development, although he presented no data in support of these specific values. In contrast, the University of California (UC) Davis powdery mildew risk index, recently developed for guiding disease management programs in California vineyards, has not utilized humidity as a determining variable (15). Yarwood (30) considered most powdery mildews to be very tolerant of low humidity and reasoned that high humidity did not favor their development.

Due to the importance of managing powdery mildew in grape production and the sparse yet conflicting data concerning the role of humidity in its epidemiology, the present study was undertaken to determine the effects of this variable on (i) the incidence and

severity of the disease on grape foliage, (ii) pathogen sporulation from infected tissue, and (iii) conidial germination. Brief portions of this work have been published previously (7,8).

MATERIALS AND METHODS

Humidity control and measurement. Saturated salt solutions, prepared as described in Winston and Bates (29) and in Windholz et al. (28), were used to deliver the specific humidity treatments (Table 1), employing a modification of the technique described by Koball et al. (19) (Fig. 1). For each humidity treatment, a Silencer 55R aquarium air pump (Penn-Plax Plastics, Garden City, NY), with 3.2 J/s capacity and set on maximum airflow, was used to move air through the system. Air traveled from the pumps through aquarium air tubing into a 1-liter Erlenmeyer flask containing either distilled water or the appropriate salt solution (for treatments with theoretical humidities above or below the ambient RH of 50%, respectively). Then, the air continued through aquarium air tubing into a 2-liter Erlenmeyer flask containing the appropriate saturated salt solution and, via 9.5-mm-diameter clear tygon tubing, into the inlet hole on the treatment chamber lids. Treatment chambers consisted of clear polypropylene boxes ($30.5 \times 21.6 \times 12.7$ cm [length $\times$ width $\times$ depth]) covered with airtight snap-on lids (Rubbermaid, Wooster, OH), which were fitted with Plexiglas windows (15×25 cm) to allow light entry. Finally, air exited through a 2-cm-diameter outlet hole, covered with one layer of KimWipe (Kimberly-Clark, Roswell, GA), in the opposite corner of the chamber lids. Air was bubbled through all solutions using aquarium air stones. Excess salt was present in each saturated salt solution at approximately 50% of the solution's volume to prevent exhaustion of the solid phase during the experiment.

A HOBO Pro Series Temp/RH datalogger (Onset Computer Corp., Bourne, MA) was fastened to the lid inside each treatment chamber at the start of all experiments. Dataloggers were set to record temperature (T , degrees Celsius), absolute humidity (AH, grams per cubic meter), and relative humidity (RH, percent) at hourly intervals. The accuracy ratings of the dataloggers were

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$\pm 0.2^{\circ}\text{C}$ and $\pm 3\%$ RH ($\pm 4\%$ in condensing environments) and $\pm 3\%$ additional reversible drift when average RH was $>70\%$. Data were downloaded into a computer at the end of each experiment. Vapor pressure deficit (VPD), i.e., saturation vapor pressure (e_s) minus actual vapor pressure (e), was calculated from the T and RH data, where $e_s = 610.78\exp[(17.269T)/(237.3 + T)]$ Pa (23) and $e = (\text{RH} \times e_s)/100$. Treatments in which dataloggers malfunctioned were not included in the results.

All experiments were conducted in a walk-in controlled environment room (Environmental Growth Chambers, Chagrin Falls, OH) set at 50% RH and 25°C . A plant shelf supported the humidity delivery apparatus and the treatment chambers. Lighting was supplied by 30 fluorescent and 10 incandescent lamps in the ceiling and 2 fluorescent lamps approximately 5 cm above each treatment chamber. Lighting was continuous to prevent photo-periodic fluctuations in temperature and related humidity values.

Plant materials and inoculum. Open-pollinated seedlings of *Vitis vinifera* 'Riesling' were used in all experiments. Seeds were germinated in plastic sieve trays under mist in a greenhouse and planted in sterile plastic cell flats filled with a mixture of soilless potting mix and sand (3:1, vol/vol) containing slow-release fertilizer (10-20-5, N-P-K), and flats were covered with plastic domes to prevent desiccation. At the first true leaf stage, seedlings used for growing *U. necator* cultures were transplanted into 5-cm² sterile plastic pots, and seedlings used in humidity experiments were transplanted into individual 50-ml plastic screw-top, conical centrifuge tubes, whose bottom tips were cut off to allow watering. All seedlings were grown in a reach-in growth chamber (Percival, Boone, IA) maintained at 28°C day/ 22°C night (14 h/10 h) in strict isolation from powdery mildew.

Subsequently, seedlings used for growing *U. necator* inoculum were transplanted at the four- to six-leaf stage into 7.5-cm-diameter clay pots and maintained in a greenhouse after inoculation in a spore settling tower (method described below) with a mass culture originally obtained from a severely diseased grape cutting in Geneva, NY. These pots were supported and surrounded by sand inside a 15-cm-diameter clay pot. Plastic sleeves to contain the inoculum and prevent incidental powdery mildew infections of the culture plants were made from the cylindrical portions of two 3-liter soda bottles taped together; the tops were covered tightly with muslin to allow air circulation and the bottoms were sealed by inserting them into the sand encircling each potted plant. The units were placed in saucers filled with water to provide irrigation. Fresh inoculum of this same culture was maintained by reinoculating new seedlings at 2-week intervals.

In planta experiments. At the four- to five-leaf stage, plants growing in tubes were selected for uniformity of growth and simi-

lar sets of plants were grouped for the various humidity treatments. The centrifuge tube caps were securely fastened to the outer surface of each treatment chamber's bottom side, and shoots were inserted into the chambers by passing them through holes drilled through this juncture. Finally, the centrifuge tubes were screwed into the caps so only the shoot portion of each plant was inside the treatment chamber (Fig. 1D), and the holes in the chambers were sealed from the inside with modeling clay around the base of the plants to prevent entry of moisture from the soil and root zone. The centrifuge tubes containing roots and soil stood inside another plastic pan immediately beneath the treatment chambers (Fig. 1F) on a double-layer capillary mat (Fig. 1G) cut from Thermolan Plus cloth (Freudenberg Nonwovens, Durham, NC), which was kept saturated with a layer of distilled water to provide irrigation.

As soon as this process was complete, treatment chambers containing the plants were taken to the greenhouse for inoculation within an enclosed spore-settling tower ($61 \times 76 \times 122$ cm [width $\times$ depth $\times$ height]). Three to four heavily infected leaves from the inoculum seedlings were taped, mildew side down, to the inside of a funnel supported on a bar stand in the center of the tower. The base of the funnel was attached to a source of compressed CO_2 via tygon tubing. A 1-s blast of CO_2 at 345 kPa dislodged conidia from the leaf surfaces into the air inside the tower, providing a near uniform distribution of spores on the plants as they were allowed to settle for 15 min. Treatment chamber lids were removed just prior to inoculating plants and were replaced immediately thereafter. Temperature inside the tower was monitored during each inoculation and never exceeded 27°C . To determine the inoculum density (conidia per square centimeter) in each experiment, five glass microscope slides were placed at arbitrary locations inside the settling tower during inoculation and the number of conidia in 10 views was counted at $\times 50$ magnification under a dissecting microscope. Inoculum densities ranged from 72 to 651 (mean = 299) conidia per cm², depending on the experiment. For each experiment, all plants were inoculated simultaneously to ensure an equivalent inoculum dose on every plant.

Following inoculation, treatment chambers (8 or 10 plants per chamber) were moved to the growth room and attached to the saturated salt solution apparatus. Treatment chambers were checked daily and capillary mats were wetted with distilled water as needed. After 14 days, the experiment was terminated and the chambers were brought to the laboratory. Individual plants were assessed for powdery mildew severity by estimating the percent area infected on each leaf, keeping track of leaf position on the plant. Mean severity ratings were determined from the most severely infected leaf on each of the plants in a treatment. This leaf was carefully

TABLE 1. Theoretical and measured relative humidity (RH) values in experiments examining humidity effects on development of powdery mildew and *Uncinula necator*

Salt	Theoretical RH (%) ^a	In planta		Conidium germination	
		<i>n</i> ^b	Measured RH (%) (CV) ^c	<i>n</i>	Measured RH (%) ^d
None (dH ₂ O)	100.0	... ^e	...	2	93.7 & 96.1
K ₂ SO ₄	97.4	...	...	4	94.0–94.7
CaHPO ₄ ·2H ₂ O	97.0	...	...	1	91.6
KH ₂ PO ₄	96.0	...	...	4	92.8–95.3
KNO ₃	92.5	2	89.6 & 90.1 (1.1 & 1.3)	4	90.3–93.7
KCl	85.0	6	83.0–95.3 (0.5–2.5)	4	83.4–84.1
NaCl	75.5	4	85.1–96.4 (0.9–1.8)	7	73.5–77.4
Ca(NO ₃) ₂ ·4H ₂ O	50.5	7	61.3–98.1 (2.0–9.7)	8	51.8–54.4
MgCl ₂ ·6H ₂ O	32.5	6	39.2–74.3 (2.7–12.0)	4	31.5–33.6
CaCl ₂ ·2H ₂ O	29.5	3	58.1–86.2 (4.9–18.2)	4	28.9–31.4
LiCl·H ₂ O	12.0	2	47.5 & 56.8 (2.3 & 3.5)	6	9.8–10.6

^a Theoretical RH delivered by each saturated salt solution at 25°C .

^b Number of experiments in which this salt was used.

^c Mean values or range of mean values and coefficients of variation measured in experiments assessing powdery mildew development on grape seedlings.

^d Mean values or range of mean values measured in experiments assessing germination of *U. necator* conidia in vitro.

^e Salt not used in these experiments.

detached from the plant so as not to disturb the spores and placed on moist filter paper in a glass petri dish, and the number of conidia per chain was counted for each of 10 chains in the area of the most luxuriant mildew growth. Three 5-mm-diameter disks were cut from these regions with a cork borer, and each disk was frozen in a microfuge tube for later quantification of spores. Spores were quantified by adding 0.5 ml of 0.4 M NaCl plus 0.005% Tween 20 to the tube, rubbing disks with the tip of a glass Pasteur pipette to free conidia, vortexing at maximum speed for 20 s, and counting the conidia in 10 views on a hemacytometer, and from these values, spore density (conidia per square centimeter) was calculated. Subsequently, the number of conidia on each leaf was estimated by assuming a leaf area of 26 cm² (the mean value determined in a separate set of measurements) and then integrating the severity and conidial density data for the individual leaves. The experiment was repeated seven times with three to six humidity treatments per experiment. For each dependent variable (severity, incidence, conidia per square centimeter, conidia per leaf, and conidia per chain), the data points within each experiment were standardized to reflect a percentage of the maximum value in that experiment (Table 2), and then these

adjusted values were expressed as a function of the mean value that was measured for each independent variable (RH, VPD, and AH) throughout that experiment. Scatter plots for disease development and pathogen sporulation were modeled to fit trend lines based on the deterministic portion of the variation in the data sets, point by point, using loess locally weighted polynomial regression analysis (10) and S-PLUS software (3.4 Release 1, 1996, Insightful Corp., Seattle, WA).

Conidium germination experiments. Saturated salt solutions in addition to those employed in the in planta experiments were used to expand the experimental humidity range to include both very low levels and those close to saturation. Four experiments examining the effects on conidial germination were conducted at mean RH levels of 10 to 84%; two were run for 24 h and two for 48 h. Four other experiments were conducted with RH treatments ranging from 10% to a maximum of 94 or 96%; again, two were run for 24 h and two for 48 h. Glass coverslips were dusted with conidia in the spore-settling tower, five coverslips were placed into each of the treatment chambers, and the chambers were moved immediately to the growth room where the humidity treatments were imposed, as described previously. At the same time, an additional set of five coverslips not assigned to any treatment chamber was sprayed with 0.5% cotton blue in lactoglycerine using an artist's airbrush (Preval Model 268; Precision Valve Corp., Yonkers, NY) and each coverslip was inverted onto a microscope slide. These were subsequently examined to determine conidial densities and the background levels of germination present at the start of each experiment. *U. necator* conidia used in these experiments were always derived from colonies initiated 19 to 21 days earlier and were always inoculated onto the coverslips around mid-day.

At the end of the 24- or 48-h experimental periods, coverslips were removed from treatment chambers, sprayed immediately with 0.5% cotton blue in lactoglycerine, and inverted onto a microscope slide. The frequency of spore germination, defined as occurring when the germ tube length was at least equal to the width of the conidium, was determined at $\times 200$ magnification by examining 200 conidia on each of the five coverslips per treatment. The percent germination attributed to the humidity treatment was calculated after adjusting for the number of pregerminated conidia in the control group at the start of the experiment, which represented from 2 to 52% of the 1,000 conidia viewed depending on the individual experiment. Data from the spore germination ex-

Fig. 1. Schematic representation of the apparatus used to deliver individual humidity treatments. Airflow indicated by dotted, curved arrows. **A**, Aquarium air pump; **B**, flask containing either saturated salt solution or distilled water (for treatments below or above the ambient relative humidity [RH] of 50%, respectively); **C**, flask containing saturated salt solution; **D**, treatment chamber, fitted with Plexiglas top, containing shoots of grape seedlings; **E**, HOBO Pro Series Temp/RH datalogger; **F**, screw-top conical centrifuge tubes containing soil and roots; and **G**, double-layer capillary mat for wick irrigation.

TABLE 2. Maximum values of measured variables obtained in the seven experiments assessing grapevine powdery mildew development and *Uncinula necator* sporulation on grape seedlings exposed to different humidity treatments

Exp.	Severity (% leaf area) ^a	Incidence (% leaves) ^b	Conidia/ cm ² ($\times 10^3$) ^c	Conidia/ leaf ($\times 10^3$) ^d	Conidia/ chain ^e
1	91.9	100.0	56.05	1,216.09	7.24
2	50.6	83.3	37.05	537.11	7.06
3	75.7	90.5	46.83	1,053.41	7.61
4	34.6	64.4	54.99	605.54	5.70
5	95.6	91.9	32.59	795.87	7.35
6	46.9	38.3	34.29	469.77	7.31
7	61.9	67.1	31.74	548.57	8.16

^a Mean percent leaf area infected on the most severely diseased leaf of each plant in an individual treatment chamber.

^b Mean percent infected leaves on each plant in an individual treatment chamber.

^c Mean number of conidia per square centimeter of infected leaf surface, based on assays from the most severely infected leaf region on each plant in an individual treatment chamber.

^d Mean number of conidia per standard leaf area (26 cm²) as determined by integrating disease severity and conidial density values for each plant in an individual treatment chamber.

^e Mean number of conidia per chain, determined for 10 chains within the most severely infected leaf region on each plant in an individual treatment chamber.

Fig. 2. Scatter plots of results from all seven experiments assessing powdery mildew development and *Uncinula necator* sporulation on grape seedlings exposed to different humidity levels measured as either relative humidity (RH), vapor pressure deficit (VPD), or absolute humidity (AH). For each measured response, the means within each experiment were standardized to reflect a percentage of the maximum mean value in that experiment, as provided in Table 2. The adjusted values were then plotted as a function of the mean value measured for each independent variable (RH, VPD, and AH) throughout that experiment. Data points were fit to a line using loess locally weighted regression. Dotted lines indicate 95% confidence intervals. The proportion of variance fit to each line is given in Table 3.

periments conducted at $RH \leq 84\%$ were analyzed using linear regression; data from experiments that included humidity levels closer to saturation were analyzed using split-line regression and Genstat 5 software (Release 4.1, 1997, NAG Inc., Downers Grove, IL).

RESULTS

Humidity control. In the in planta experiments, the measured humidity levels were higher than the theoretical levels for virtually all saturated salt solutions used. The magnitude of this deviation and of the variability recorded throughout the 14-day course of each trial was dependent on the salt that was employed. In general, humidities were least variable and closest to theoretical values in treatments designed to produce the highest levels, whereas the converse was true for those intended to produce the lowest levels (Table 1). For instance, for the six experiments utilizing KCl (theoretical $RH = 85.0\%$), the mean RH values ranged from 83.0 to 95.3% with coefficients of variability of 0.5 to 2.5% in each individual run. However, for the six experiments utilizing $MgCl_2$ (theoretical $RH = 32.5\%$), the mean RH values ranged from 39.2 to 74.3% with coefficients of variability of 2.7 to 12.0% (Table 1). In contrast, humidity levels remained virtually constant in every treatment chamber throughout all eight of the spore germination experiments, with the means of the measured values generally deviating from the theoretical by no more than the precision limits of the instrument (Table 1).

In planta experiments. Disease development was strongly influenced by humidity level (Fig. 2). Compared with humidity expressed as RH , VPD accounted for 19 and 17% more of the variance in assessed measures of disease incidence and severity, respectively, whereas AH accounted for 30 and 26% less (Table 3). Conidial density and chain length also were proportional to humidity (or inversely proportional to VPD), with similar relative predictive values among VPD, RH , and AH ; however, these sporulation responses were influenced substantially less by humidity than was disease development (Fig. 2; Table 3). Thus, the

strong influence of humidity on the estimated number of conidia produced per leaf (Fig. 2; Table 3) appears to be more a function of its effect on the sporulating area rather than the sporulation density. Measures of disease development and pathogen sporulation were lowest in the lowest humidity treatments and generally increased proportionally to increasing humidity, although some trend lines plateaued or inflected at the highest humidity levels (Fig. 2). Extrapolating from the asymptotes or points of inflection of the trend lines, the optimum humidity levels for *U. necator* development on grape leaves (at the mean experimental temperature range of 23 to 26°C) were approximately 83.0 to 86.5% RH , 450 to 520 Pa VPD, and 18.5 to ≥ 23.4 g/m³ AH , depending on the measure of disease development or pathogen sporulation (Fig. 2).

Within the range provided, inoculum density had no apparent effect on disease development or pathogen sporulation (data not shown). Average temperature measured throughout the 14-day duration of each individual experiment differed among individual treatment chambers by $<1^\circ\text{C}$ in three experiments, $<2^\circ\text{C}$ in three experiments, and $<3^\circ\text{C}$ in one experiment. The slight variation in temperature among and within the experiments had no significant predictive value for either disease severity or conidia per chain and could account for only 2% of the variance in disease incidence, 10% of the variance in sporulation density, and 5% of the variance in conidia per leaf.

Conidium germination experiments. In experiments in which RH treatments ranged from 10 to 84%, there was a strong and consistent linear relationship between humidity level and the frequency of conidium germination (Fig. 3). When these values were regressed separately against RH , VPD, and AH , the slopes of the lines were virtually identical for each independent variable regardless of the time of assessment or run of the experiment (Table 3). Furthermore, in both experimental runs in which germination was assessed after 48 h, the coefficients of determination were identical for regressions involving either RH , VPD, or AH . They also were identical in one of the two 24-h runs and differed only minimally in the other (Table 3). However, in subsequent experiments that also included humidity levels close to saturation, a

TABLE 3. Regression models and parameters for the response to humidity of powdery mildew development in planta and germination of *Ucinula necator* conidia in vitro

Humidity ^a	In planta experiments		Spore germination experiments						
	Response	Prop. var. fit ^d	$RH = 10$ to $84\%b$			$RH = 10$ to $96\%c$			
			Exp.	Regression equation	r^2	Exp.	s^e	a^f	b_1^g b_2^h
RH (%)	Severity	0.35	24h-1	$y = 0.22x + 21.1$	0.95	48h-3	83.5	8.2	0.225 -1.72
	Incidence	0.37	24h-2	$y = 0.23x + 19.4$	0.63	48h-4	91.2	15.6	0.227 -6.39
	Conidia/cm ²	0.16	48h-1	$y = 0.22x + 11.4$	0.89				
	Conidia/leaf	0.36	48h-2	$y = 0.23x + 22.0$	0.85				
	Conidia/chain	0.28							
VPD (Pa)	Severity	0.41	24h-1	$y = -0.0078x + 43.7$	0.95	48h-3	478	-0.7	0.057 -0.008
	Incidence	0.44	24h-2	$y = -0.0085x + 43.2$	0.64	48h-4	263	-14.1	0.191 -0.008
	Conidia/cm ²	0.18	48h-1	$y = -0.0079x + 33.9$	0.89				
	Conidia/leaf	0.43	48h-2	$y = -0.0084x + 45.5$	0.85				
	Conidia/chain	0.32							
AH (g/m ³)	Severity	0.26	24h-1	$y = 1.02x + 21.4$	0.96	48h-3	17.5	7.44	1.18 -11.2
	Incidence	0.26	24h-2	$y = 1.05x + 19.9$	0.63	48h-4	18.6	15.5	1.14 -36.6
	Conidia/cm ²	0.09	48h-1	$y = 1.01x + 11.7$	0.89				
	Conidia/leaf	0.25	48h-2	$y = 1.06x + 22.3$	0.85				
	Conidia/chain	0.15							

^a Humidity expressed as either relative humidity (RH), vapor pressure deficit (VPD), or absolute humidity (AH).

^b Linear regression equations and r^2 values for the four conidium germination experiments conducted at 10 to 84% RH , as shown in Figure 3. Germination was assessed after 24- or 48-h in each of two experiments, respectively.

^c Split-line regression parameters for germination of *U. necator* conidia in two of the four conidium germination experiments conducted at 10 to 96% RH , as shown in Figure 4. Germination was assessed after 48-h in both experiments.

^d The proportion of variance in the response to humidity fitted by trend lines of a first order polynomial with a 3/4 span using loess locally weighted regression for the in planta experiments shown in Figure 2.

^e The split-point in each line.

^f The intercept for the first part of the split line.

^g The slope of the first part of the split line.

^h The slope of the second part of the split line.

steep drop in germination frequency occurred in the highest humidity treatments in both the 24-h experiments (data not shown) and those extending for 48 h (Fig. 4). No water condensation was observed on any of these slides, ensuring that reduced germination frequencies were due to high atmospheric humidity rather than free water. Data from the two 48-h assessments were analyzed using split-line regression, where $Y = a + (X \leq s)b_1X + (X > s)b_2X$, and parameter s represents the split point, b_1 represents the slope of the first part of the split line, and b_2 represents the slope of the second part of the split line. The b_1 slopes for RH and AH and the

b_2 slopes for VPD were very similar to the linear regression slopes determined in the previous experiments over similar humidity ranges where the highest levels had not been included (Table 3). The split-point parameter s , which gave the theoretical optimum humidity level for spore germination, averaged 87.4% RH, 371 Pa VPD, and 18.1 g/m³ AH for these two experiments (Table 3). Temperature and humidity levels in all treatment chambers remained virtually constant and very close to theoretical throughout the experiments (Table 1).

DISCUSSION

Over a series of experiments conducted at optimal temperatures, the development of powdery mildew on grape leaves was clearly and markedly affected by humidity levels in the test range of 39 to 98% RH (1,914 to 61 Pa VPD). Although infection occurred at all humidities, both the incidence and severity of disease increased with increasing humidity up to an optimum near 85% RH, and then appeared to plateau or decrease marginally at higher values. Although consistent with numerous field observations (2,11,14,16,18,27), our data stand in stark contrast to the experimental results of Delp (12), who concluded that humidity

Fig. 3. In vitro germination of *Uncinula necator* conidia in response to humidity treatments ranging from 10 to 84% relative humidity (RH). Percent germination was assessed after either 24 or 48 h in two experiments each and expressed as a function of either **A**, RH; **B**, vapor pressure deficit (VPD); or **C**, absolute humidity (AH). Data were analyzed using linear regression (Table 3).

Fig. 4. In vitro germination of *Uncinula necator* conidia in response to humidity treatments ranging from 10 to 96% relative humidity (RH). Percent germination was assessed after 48 h in each of two experiments and expressed as a function of either **A**, RH; **B**, vapor pressure deficit (VPD); or **C**, absolute humidity (AH). Data were analyzed using split line regression (Table 3).

had “little or no influence” on the development of *U. necator* on grape foliage and speculated that observations to the contrary more likely reflected the preferential occurrence in humid locations of temperatures that were especially favorable for the disease. However, it seems likely that our conflicting results are substantially due to the different methodologies utilized in the two studies, including (i) the range of humidities investigated, i.e., Delp (12) examined only RH values of 99% and $\leq 25\%$; (ii) the incubation times provided between inoculation and disease assessment, i.e., generally ≤ 5 days for Delp versus our 14 days; and (iii) the techniques used to impose humidity treatments, i.e., Delp utilized a constant source of air at variable temperatures versus our multiple air sources with varying humidities at a near-constant temperature. In addition to the effects on disease development, we also found similar, if less pronounced, effects of humidity on the production of conidia from powdery mildew colonies; however, these were not of the magnitude cited by Bulit and Lafon (5), i.e., a doubling of conidial chain length after 24 h at 90 to 100% RH versus that at 30 to 40% RH. It is unclear whether such apparent differences merely reflect experiments designed to measure the effect of humidity on sporulation rate versus our experiment designed to measure the effect of humidity on sporulation potential (i.e., our assessments of sporulation were not conducted until approximately 7 days after initial colony appearance). Nevertheless, these data suggest that humidity influences the progress of a powdery mildew epidemic through a multiplicative effect involving both the quantity of infected tissue that develops and the density of conidia produced on it.

The frequency of conidium germination also responded to increases in humidity. Germination increased in a modest but distinctly linear manner from the minimum test level of approximately 10% RH to an optimum level of approximately 87% RH, above which germination frequency dropped sharply. In contrast, Bulit and Lafon (5) cited data showing increased germination at 40 versus 20% RH, but no significant effect of humidity at RH levels between 40 and 100%; however, unavailability of their source material precluded analysis of this conflict with our results. Delp (12) also found no significant effect of humidity on conidium germination at or below the optimum temperatures that we utilized. However, Delp expressed germination frequency as a percentage of that in “check” treatments with 400 to 133 Pa VPD at either 24 or 27°C (86 to 95% or 89 to 96% RH), respectively. These humidity levels are in a range in which germination declined precipitously in our experiments, and it is unclear whether such relative data may have been impacted by suboptimal conditions for germination in Delp’s check treatments. In contrast, our results bear some similarity to those of Longre (20), who found suboptimal germination of *Sphaerotheca pannosa* (cause of rose powdery mildew) conidia above 99% and below 95% RH at both 21 and 25°C; however, for *U. necator* conidia the suboptima are lower, i.e., above 91% and below 84% RH.

In our study, we were able to maintain a near-constant temperature while varying the levels of humidity, effectively isolating this variable in repeatable experiments. However, the experimental system also had its limitations. For example, initial attempts to utilize a 12-h light/12-h dark illumination regime caused significant diurnal fluctuations in temperature and associated humidity measures within the treatment chambers, forcing the use of continuous light to overcome this problem. Furthermore, we were unable to provide mean RH levels of $< 39\%$ in the studies examining disease development on grape seedlings, and it was difficult to maintain a constant humidity inside their treatment chambers over the 14-day period of these experiments. The use of seedlings with few relatively small leaves and the separation of moist soil and irrigation water from the treatment chambers were designed to restrict the introduction of extraneous water vapor into them; nevertheless, it appears that the experimental apparatus was unable to fully equilibrate transpirational water vapor, particularly at the

lower intended humidities. This conclusion is further supported by (i) observations of increasing humidity within some treatment chambers as experiments progressed (data not shown), and (ii) the ability of the system to maintain virtually constant RH levels very near all theoretical values throughout the conidial germination experiments when plants were absent. It seems likely that the increased precision in humidity control accounts for at least part of the reduced variability in the results obtained in these experiments compared with the in planta studies. However, examining a single response of the pathogen (i.e., conidium germination) rather than the outcome of interactions between the pathogen and multiple host units also would favor reduced variability.

The mechanistic effects of atmospheric humidity on *U. necator* and other powdery mildew fungi are poorly understood. It is possible that high humidity but not free water favors these organisms because they have evolved to utilize water vapor directly. Brodie and Neufeld (4) observed that the conidial protoplast of *Erysiphe polygoni* shrank only slightly during plasmolysis, suggesting it contains very little free water. Brodie (3) subsequently determined that the apparent osmotic potential of conidial cell sap was $-6,300$ and $-6,800$ kPa for *E. polygoni* and *E. graminis* f. sp. *hordei*, respectively, and postulated that such low values might aid the spores in absorbing water directly from the atmosphere. At very low humidities, Clayton (9) found that germ tubes of *E. polygoni* were short and survived poorly, and Munshi and Singh (22) found that few *U. necator* conidia established successful host–parasite relationships. Kast (18) noted that grapevine powdery mildew is more severe near bodies of water where atmospheric humidities are subject to rapid increases at night, and although this may be related to increased dispersal of conidia under such conditions (6), the effects observed in our experiments occurred in the absence of comparable fluctuations. For biotrophs like the powdery mildew fungi, it also seems plausible that an environmental variable such as atmospheric humidity might affect disease development through an influence on the host and its physiological relationship with the pathogen, although this topic has received little experimental scrutiny. Low humidity favors plant transpiration, which would give rise to a high water vapor flux, favoring directional diffusion of water out of the leaf and out of the powdery mildew mycelium directly (because it is not protected by a cuticle). When interpreting studies of the effects of humidity on powdery mildew development, it should be recognized that humidity in the leaf boundary layer zone inhabited by *U. necator*, i.e., ≤ 500 μm above the surface (17), is likely to be higher than that measured in the bulk air, depending on the bulk air humidity and velocity of air movement (26).

It has been argued (1) that VPD is the most meaningful measure of variation in atmospheric humidity when describing its effects on biological systems, including those involving *U. necator* (12). However, VPD measures were identical to those of RH in accounting for the effect of humidity in our conidium germination experiments and were only modestly superior to RH values when analyzing the in planta experiments. Furthermore, measures of VPD are temperature-specific, thus it often is difficult and cumbersome to utilize them in comparing observations or experimental reports obtained at different temperatures. In contrast, measures of RH allow comparisons of relative water vapor levels across temperatures and are conceptually familiar and easy to interpret. Therefore, to facilitate communication, we have focused our presentation of specific humidity levels in terms of RH measurements; however, we recognize that VPD values may add a modicum of additional precision in some instances and therefore have included these measures for comparison, along with those of AH.

Delp’s (12) data suggested that temperature was the only important environmental variable for predicting the development of grapevine powdery mildew, and the current UC Davis risk index focuses solely on temperature with respect to the forecasting of secondary disease cycles (15). From our results, however, it is

clear that atmospheric humidity also plays a role in multiple phases of this cycle, from the germination of conidia to the development of the resulting colonies on infected tissue and the production of additional conidia therefrom. Our data support Kast's concept of including RH as a predictive variable in the model he developed for scheduling fungicide sprays in German vineyards (18), though not his designation of 60% RH as a minimum for disease development. However, his designation of an optimum level of $\geq 70\%$ RH is consistent with our findings of an optimum RH near 85%. The importance of humidity relative to temperature and the manner in which such information should be applied in disease management programs will most likely need to be elucidated under field conditions in various climatic regions. Nevertheless, viticultural practices designed to minimize humidities within vineyard canopies appear to be prudent components of integrated disease management systems.

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