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The risk assessment index in grape powdery mildew control decisions and the effect of temperature and humidity on conidial germination of *Erysiphe necator*

C. E. Bendek, P. A. Campbell, R. Torres, A. Donoso and B. A. Latorre*

Facultad de Agronomía e Ingeniería Forestal. Pontificia Universidad Católica de Chile. Casilla 306. Santiago. Chile

Abstract

Powdery mildew (*Erysiphe necator*) is a major disease of grapevines (*Vitis vinifera*) in Chile. Severe outbreaks have occurred recently despite the use of strict fungicide programs to control it. The objectives of this study were to evaluate the infection risk assessment index (RAI), to predict conditions for *E. necator* infection, and to study the effect of temperature (T), relative humidity (RH) and free moisture (FM) on conidial germination and disease development. Conidial germination was affected by T, RH, and FM. There were significant ($p < 0.001$) interactions between *E. necator* isolates and T and between isolates and RH. Conidial germination was optimal at 25°C. There was no germination at 5°C and 35°C. At 20°C, conidia germinated at a low RH (33-35%). Germination increased at a RH between 47 and 90% but decreased at higher RHs. Powdery mildew development on Carmenere, Chardonnay, and Merlot vines increased linearly from 6°C to 23°C. These grape cultivars were all equally susceptible to *E. necator*. Incubation periods varied. It was 13 to 14 d at 20°C or 23°C, 19 to 24 d at 10°C, and more than 23 d at 6°C. Grape powdery mildew was markedly decreased when inoculated leaves were wet at 1 or 72 h post inoculation. In conclusion, RAI, determined on the basis of air T, was useful to decide on fungicide applications between grape bud burst and veraison in Chile.

Additional key words: disease forecasting, grape diseases, *Uncinula*, *Vitis vinifera.*

Resumen

Índice de riesgo en el control del oídio de la vid y efecto de la temperatura y la humedad sobre la germinación de las conidias de *Erysiphe necator*

El oídio (*Erysiphe necator*) es una de la principales enfermedades de la vid (*Vitis vinifera*) en el centro y norte de Chile. Epidemias severas se han registrado en los últimos años, independientemente del uso de estrictos programas de control químico. Este trabajo tuvo por objetivos evaluar el índice de riesgo de infección (IRI), para predecir condiciones favorables al oídio, y estudiar el efecto de la temperatura (T), humedad relativa (HR) y agua libre (AL) en la germinación de las conidias y en el desarrollo de esta enfermedad. La germinación de las conidias dependió de la T, HR y AL. Se obtuvo una interacción significativa ($p < 0{,}001$) entre aislamientos de *E. necator* y T y entre aislamientos y HR. La germinación conidial fue óptima a 25°C y no germinó a 5°C y 35°C. A 20°C, las conidias germinaron con baja HR (33-35%), aumentando considerablemente con HR entre 47% y 90%, pero diminuyendo con HR superiores. El desarrollo de oídio en vides 'Carmenere', 'Chardonnay', y 'Merlot' aumentó linealmente entre 6°C y 23°C. Sin embargo, estos cultivares fueron igualmente susceptibles a *E. necator*. El periodo de incubación se estimó en 13 a 14 días a 20 y 23°C, 19 a 24 días a 10°C, y sobre 23 días a 6°C. El oídio decreció considerablemente al mojar las hojas 1 ó 72 h después de la inoculación. En conclusión, IRI, determinado según la T del aire, fue una herramienta útil para decidir las aplicaciones fungicidas, entre brotación y envero, en la zona central de Chile.

Palabras claves adicionales: enfermedades de la vid, pronóstico, *Uncinula, Vitis vinifera.*

Introduction

Powdery mildew caused by *Erysiphe necator* Schwein [sin. *Uncinula necator* (Schwein) Burrill] is a major and very destructive disease affecting table and wine grapes (*Vitis vinifera* L.) worldwide (Bulit and Lafon, 1978; Cruz, 2001; Jarvis *et al.*, 2002). The disease is widely distributed throughout Chile; however, severe epidemics most frequently occur in central and northern Chile where very low rainfall and mild temperatures prevail during the growing season (Bendek *et*

* Corresponding author: blatorre@uc.cl
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al., 2002). Powdery mildew reduces yield, and it has been well documented that it can negatively affect quality of table grapes, wine grapes, must and wine (Latorre *et al.*, 1996; Piermattei *et al.*, 1999; Gadoury *et al.*, 2001; Campbell *et al.*, 2003; Calonnec *et al.*, 2004).

Epidemics can develop from mycelia surviving on infected buds and/or over wintering cleistothecia surviving on infected leaves and exfoliating bark of grapevines (Sall and Wrysinski, 1982; Pearson and Gartel, 1985; Cortesi *et al.*, 1997; Steinkellner, 1998; Ypema and Gubler, 2000; Rügner *et al*., 2002; Grove, 2004; Rumbolz and Gubler, 2005).

Cleistothecia have been found in Chilean vineyards (Silva, 2005). However, under the relatively dry environmental conditions of central and northern Chile (Novoa and Villaseca, 1989), it is highly possible that powdery mildew epidemics are mainly initiated from conidia. These conidia are abundantly produced on flag shoots that are the first infected shoots, appearing early in spring, derived from buds infected in the previous season (Rügner *et al.*, 2002; Cortesi *et al.*, 2004; Rumbolz and Gubler, 2005).

Thus, a warning system based on environmental factors affecting conidia could be a useful tool to assist farmers in control decisions. This study focuses on the importance of conidia in the development of powdery mildew epidemics to determine the effect of temperature and humidity on conidia germination, the effect of temperature and humidity on powdery mildew development, and the evaluation of the powdery mildew infection risk assessment index (RAI), developed in California (Gubler *et al.*, 1999; Jarvis *et al.*, 2002) under Chilean conditions, thereby aiming to assist farmers in fungicide application decisions.

Material and Methods

Inoculum

Four isolates of *E. necator,* from the Pontificia Universidad Católica de Chile collection, PUC1, Santiago; PUC2, Coltauco; PUC3, Santiago; and PUC4, Buin, were used. Inoculum was maintained in isolated greenhouse, on potted *V. vinifera* cv. Chardonnay vines. Dry conidia, taken from colonies, developed on young leaves, were used as inoculum. Mass transfer of conidia was done with the aid of a hair brush.

Effect of temperature and humidity on conidial germination

The effect of temperatures of 5, 10, 15, 20, 25, 30, and 35°C on conidial germination was studied *in vitro*. Dry conidia were aseptically transferred with a small brush to a 0.5% water agar emended L^{-1} with ampicillin (150 mg), PCNB (500 mg), pimaricin (5 µg), and rifampicin (10 mg). Plates were incubated for 24 h at the desired temperature in Memmert precision incubators (±1°C). Conidia were considered to have germinated when a visible germ tube emerged, which usually ended in an appressorium at the apex (Green *et al.*, 2002). Unless otherwise stated conidial germination was determined by examining 100 conidia under a light microscope. There was one repetition.

The effect of relative humidity (RH) at 33-35%, 47-52%, 85-90%, and 95-100%, on conidial germination was studied, *in vitro,* at 20°C. Conidia were aseptically transferred with the aid of a small brush onto a glass slide in a Petri dish. The desired range of RH was obtained by embedding a double layer of paper towel, placed at the bottom of each Petri dish, with 5 mL of distilled water (RH: 95-100%) or 5 mL of one of the following saturated salt solutions: $MgCl_2x6H_2O$ (RH: 33-35%), $CaCl_2$ (RH: 47-52%), and NaCl (RH: 85-90%). Plates were sealed with Parafilm (American Can Company, Greenwich, Connecticut). The RH was verified using an RH capture logger (Stow Away RH logger) with sensors placed on the glass slides. Conidia germination was determined after 24 h under a light microscope. The experiment was repeated once.

The effect of alternate wet and dry periods on conidial germination was studied *in vitro*. For this objective, conidia of each *E. necator* isolate were aseptically transferred onto glass slides, placed in Petri dishes at 20°C. In the first experiment, condia were incubated as follows: (1) dry chambers (RH < 50%) for 72 h, (2) humid chambers (RH > 98% with moisture on the slides) for 72 h, (3) dry chamber for the first 24 h followed by 48 h in a humid chamber, or (4) humid chamber for the first 24 h followed by 48 h in a dry chamber. The experiment was repeated with the same objective but conidia were incubated in dry chambers at 20°C for 0, 3, 6, 12, and 24 h followed, respectively, by 24, 21, 18, 12, and 0 h incubation under moist conditions in humid chambers. The proportion of conidia germinated was determined in each of the four replicates. These experiments were repeated once.

The effect of temperature and free moisture on powdery mildew development

The following experiments were conducted on 1 year old grapevines, growing on their own roots, in 1 L containers in isolated greenhouse. Plants with a single 50 cm long shoot were selected for each experiment. Plants were inoculated with dry conidia (approx. 170-180 conidia cm^{-2}) of *E. necator* (PUC3) that were deposited with the aid of a small brush on the adaxial side of five full-expanded newest leaves $plant^{-1}$.

The effect of temperatures of 6, 10, 18, and 20°C on powdery mildew infection was first studied on Chardonnay vines that were incubated for 28 d in growth chambers set at the desired temperature, ±1°C. This experiment was repeated at 6, 10, 18, 20, and 23°C for 23 d. Finally, the effect of temperature of 6, 10, 18, and 20°C on grape cultivars Carmenere, Chardonnay and Merlot was studied. Plants were incubated for 20 d and the number of infected leaves $plant^{-1}$ was recorded every other day after the first powdery mildew colonies appeared.

To study the effect of free moisture, inoculated grapevines were subjected to one of the following treatments: (1) Plants sprayed with 5 mL of sterile water $leaf^{-1}$ 1 h post-inoculation, (2) plants incubated for 72 h before gently spraying them with 5 mL of sterile distilled water $leaf^{-1}$, and (3) plants incubated under continuous dry conditions. Care was taken to avoid run-off that might have washed conidia off the leaves. Plants were then incubated in growth chambers at 20°C and 85 to 90% RH until the first powdery mildew colonies appeared. Results were recorded as above.

Field validation of the infection warning system

The effectiveness of the on-site warning system was evaluated in commercial vineyards of Chardonnay and Cabernet Sauvignon wine grapes, near Santiago during the 2002–2005 growing seasons. At each location, the following spray timings were studied: (1) Model spray program, based on the risk assessment index (RAI) as proposed by Gubler *et al*. (1999). This included the use of 84 g i.a. ha^{-1} of kresoxim methyl (Stroby 50 SG, BASF). Applications started at RAI values of 30 to 50 points. Thereafter, spray applications were repeated at RAI values higher than 50 or at 18 to 20 d intervals if a high risk of infection (RAI > 60) persisted continuously until veraison; (2) a standard spray program using 84 g i.a. ha^{-1} of kresoxim methyl, every 15 to 18 d in 2002-2003 and 2003-2004 and every 10 to 15 d in the 2004-2005 growing seasons, starting when shoots were 15 to 20 cm long until veraison; (3) farmer spray programs were done following farmer's schedule, based on the phenological stage of the grapevines, alternating fungicides with different modes of action and (4) untreated controls. Grapes were always sprayed to run off with a manual sprayer (Solo, Santiago, Chile) with 4 kg cm^{-2} pressure, using approximately 1,200 L ha^{-1}. Disease incidence was determined in a 50 cluster and on 50 leaf samples for each of the four replicates per treatment.

Weather station and risk assessment index

The monitoring weather stations (Davis Instruments, Model RJ 1412HPL, California) continuously measured temperature, rainfall, and RH, at each location, from bud burst (September-October) to veraison (December). The monitoring stations provided average weather information every 30 min. The powdery mildew model initiated calculations of RAI when temperatures between 20°C and 30°C occurred for six consecutive hours. There was a risk of powdery mildew infection if these conditions were met for three consecutive days. Index calculation was started with 30 points (Gubler *et al.*, 1999). Thereafter, 20 points were added for each additional day which met this temperature requirement. Ten points were subtracted for each day when the temperature did not meet the requirements and for each day with a maximum temperature above 30°C for 60 min or 35°C for 15 min. Index values of between 0 to 30, 30 to 60, and 60 to 100 were indicative of low, moderate, and high disease pressure, respectively.

Design and statistical analysis

The effect of temperature and RH on *in vitro* conidial germination was tested by a two-way (isolates and temperature or humidity) analysis of variance using SigmaStat 2.0 (SPSS, Chicago, Illinois) with four replicates. Differences between means were tested according to Tukey ($p < 0.05$). Treatments based on the effect of an initial dry period on *in vitro* conidia germination were distributed according to a complete randomized design. The effect of treatments was tested using the analysis of variance and differences between means were tested using Duncan-Waller k-ratio t tests ($p < 0.05$).

The effect of temperature and free moisture on powdery mildew on Chardonnay vines was tested using

the analysis of variance according to a complete randomized design with six replicates. Each replicated was a single potted grapevine and five leaves were used as experimental units. Differences between means were tested using the Duncan-Waller k ratio t test ($p < 0.05$). Regression analysis was used to determine disease progress curves and apparent infection rate for each incubation temperature, where x = time in days and y = number of infected leaves.

Treatment for the effect of temperature on powdery mildew on Carmenere, Chardonnay and Merlot vines was randomly distributed in a 3×4 factorial design (cultivar × temperature) with five replicates. Each replicate was a single potted vine and five leaves were used as experimental units. Data were subjected to two-way ANOVA using Sigmastat. Regression analysis was again used to determine disease progress curves and apparent infection rate, where x was time and y was disease incidence. Apparent infection rates were statistically compared using the procedure of Zar (1996).

The field experiments to validate the warning system were complete block design with four replicates; each replicate was 10 vines. Data were subjected to ANOVA and differences between means were tested using Duncan-Waller k-ratio t tests ($p < 0$ .05).

Results

Effect of temperature and humidity on conidial germination

Conidia germinated between 10°C and 30°C, except for isolate PUC1 that only germinated from 15°C to 30°C, after 24 h incubation. Germination was completely arrested at 5°C and at 35°C. Regardless of isolate, the optimum germination temperature was 25°C (Fig. 1). The effect of temperature, isolate, and the interaction temperature by *E. necator* isolate interaction were highly significant ($p < 0.001$). Mean differences in conidial germination among isolates were statistically significant ($p = 0.05$) at 15, 20, and 25°C. Isolate PUC2 had the highest germination of 90.5% at 25°C followed by PUC3 (44.5%), PUC1 (41.8%), and PUC4 (37.0%).

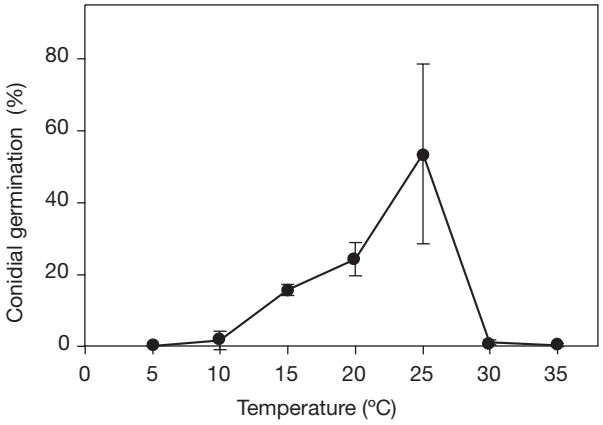

Figure 1. The effect of incubation temperature on conidial germination of *Erysiphe necator.* Means of four isolates replicated four times each. Bars = standard deviation.

Independent of *E. necator* isolate, there was conidial germination at a RH below 35% to above 90% after 24 h incubation at 20°C (Table 1). There was a highly significant ($p < 0.001$) effect of *E. necator* isolate with percent germination ranging from 7.3 to 39%. The interaction isolate by RH was also significant ($p < 0.001$). The highest germination of isolates PUC1 and PUC2 was between 85 and 90% RH and it was between 47

Table 1. The effect of relative humidity (RH), determined *in vitro* at 20°C, on germination of conidia of four *Erysiphe necator* isolates from the Pontificia Universidad Católica de Chile (PUC) collection

Saturated salt solutions	RH[1] (%)	Conidial germination (%)			
		PUC 1	PUC 2	PUC 3	PUC 4
$MgCl_2x6H_2O$	33-35	10.3 b A[2]	12.5 b A	7.3 b A	13.0 c A
$CaCl_2$	47-52	16.5 ab B	18.0 a B	32.0 a A	39.0 a A
NaCl	85-90	24.3 a B	23.8 a B	23.5 a B	34.8 ab A
H_2O	95-100	23.5 a A	17.5 a A	24.5 a A	25.8 bc A

[1] Conidial germination was determined after 24 h incubation on glass slides in sealed Petri dishes containing 5 mL of water or saturated salt solution at 20 ± 1ºC. Isolates PUC 1, 2, 3 and 4 were maintained on potted grapevines under isolation. RH was verified using a relative humidity capture logger (Stow Away RH logger) with sensors placed on the glass slides. One hundred conidia were examined for each replicate. [2] Means followed by the same small letters within a column or capital letters within a row were not significantly different according to Tukey's test ($p < 0.05$).

and 52% for isolates PUC3 and PUC4. Independent of isolate, conidial germination increased significantly ($p = 0.05$) at a RH higher than 33-35%. There were significant ($p = 0.05$) differences among isolates in conidial germination at RHs of 47-52 and 85-90% (Table 1).

In the first experiment, the effect of wet and dry treatment at 20°C on conidial germination was significant ($p < 0.01$). Conidial germination was significantly higher when conidia were kept dry for 24 h, followed by 24 h of wet conditions (Table 2). Germination was considerably decreased when conidia were subjected to dry or wet conditions for 72 h. There was low conidial germination when conidia were exposed to 24 h of wet conditions, followed by 24 h of dry conditions (Table 2).

When the above experiment was repeated, the effect of wet and dry treatment, at 20°C, on conidial germination was again highly significant ($p < 0.01$). Conidial germination increased significantly with 3-12 h of dry conditions followed by 12-21 h of wet conditions. Lowest conidial germination was obtained when conidia were continuously incubated under wet conditions (Table 2). A second-degree polynomial of the form:

$$y = -0.17x^2 + 4.23x + 16.91 \ (R^2 = 0.68, p = 0.006)$$

best explained the relationship between hours of dry conditions (x) and percent conidial germination (y) after 24 h incubation at 20°C (Fig. 2).

Table 2. The effect of wet and dry treatment, at 20°C, on conidial germination of *Erysiphe necator* isolate PUC 3 maintained on potted grapevines under isolation

Incubation treatment[1]	Conidia germination (%)	
72 h dry, 0 h wet	17.3 a[2]	
24 h dry followed by 48 h wet	49.0 b	
24 h wet followed by 48 h dry	11.0 a	
72 h wet, 0 h dry	24.5 a	
	Trial 1	**Trial 2**
0 h dry, 24 h wet	11.3 a	14.0 a
3 h dry followed by 21 h wet	25.0 b	45.0 e
6 h dry followed by 18 h wet	37.0 c	34.0 abc
12 h dry followed by 12 h wet	41.8 c	40.0 bcd
24 h dry, 0 h wet	15.5 a	28.0 ab

[1] Dry, RH near 50% and wet, RH > 98% with evidences of free moisture on the surface of the glass slides. [2] Means of four replicates followed by the same letters were not statistically different according to the Duncan-Waller k ratio t test ($p < 0.05$).

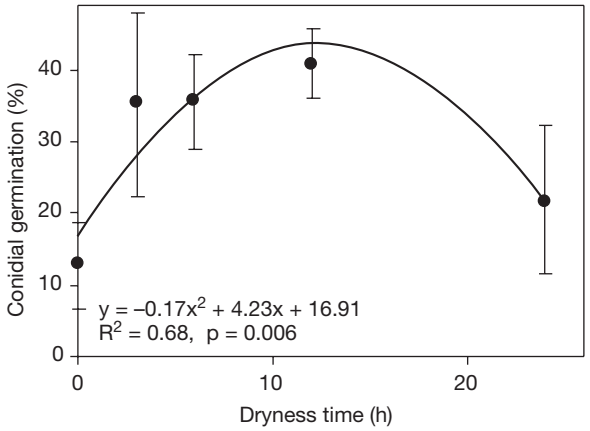

Figure 2. Conidial germination of *Erysiphe necator* in relation to the length of a dry period (about 50% relative humidity) after 72 h incubation at 20°C. Bars = standard deviation.

The effect of temperature and free moisture on powdery mildew development

Temperature significantly ($p < 0.01$) affected powdery mildew development on Chardonnay vines. Disease incidence varied, after 28 d incubation, from 13.4 to 96.6%, and from 3.4 to 83.4%, in the first and second experiments, respectively (Fig. 3). First symptoms appeared after 14-17 d at 18°C and 20°C. Symptoms were delayed to 21 to 28 d at 6°C. The relationship between days of incubation and percentage disease incidence was best explained by a linear regression. The following equations were determined at 6, 10, 18, and 20°C, respectively;

$$y = -16.3 + 0.8x \ (R^2 = 0.41, p = 0.17)$$
$$y = -39.5 + 2.0x \ (R^2 = 0.63, p = 0.06)$$
$$y = 151.2 + 8.8x \ (R^2 = 0.99, p < 0.001)$$
$$y = -151.7 + 8.7x \ (R^2 = 0.99, p < 0.001)$$

When these experiments were repeated the equations were:

$$y = -5.4 + 0.4x \ (R^2 = 0.71, p = 0.035)$$
$$y = -16.1 + 1.1x \ (R^2 = 0.71, p = 0.034)$$
$$y = 13.1 + 0.17x \ (R^2 = 0.96, p < 0.001)$$
$$y = 12.4 + 0.12x \ (R^2 = 0.89, p = 0.005)$$
$$y = 12.0 + 0.1x \ (R^2 = 0.79, p = 0.019)$$

at 6, 10, 18, 20 and 23°C, respectively (Fig. 3).

Powdery mildew was obtained on Carmenere, Chardonnay and Merlot vines continuously kept at 10, 18, and 20°C but there was no sign of powdery mildew after 20 d incubation at 6°C (Fig. 4). The highest mean powdery mildew incidence was 88.0, 84.0, and 68.0%

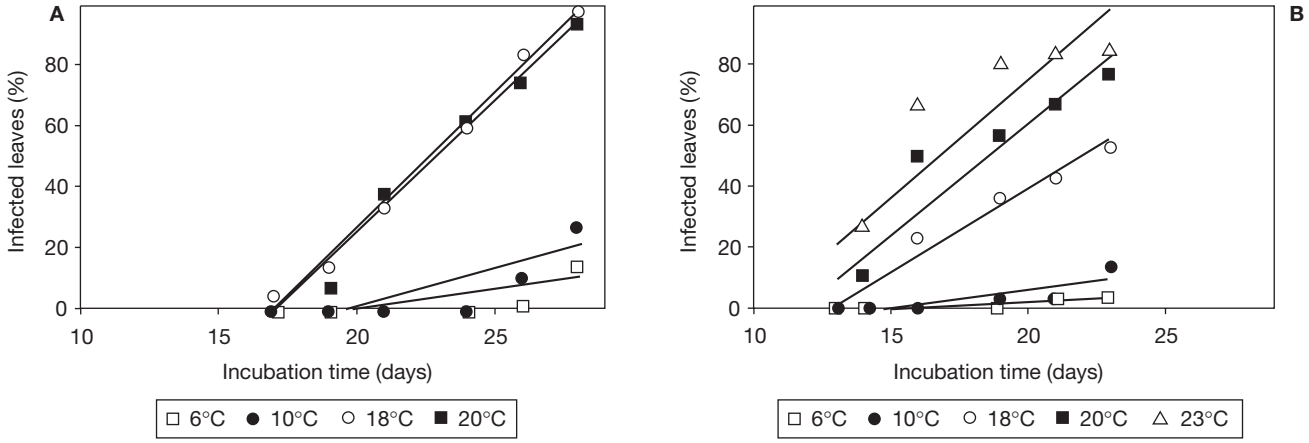

Figure 3. The effect of temperature on the development of grapevine powdery mildew caused by *Erysiphe necator*. A, and B, were results of two experiments with similar objectives using leaves of *Vitis vinifera* cv. Chardonnay after 23 d of incubation.

on Chardonnay, Carmenere, and Merlot vines, respectively when incubated at 18°C. The effect of temperature was highly significant ($p < 0.001$) but cultivar and the interaction incubation temperature by cultivar were not statistically significant. The effect of incubation temperature on disease incidence was best described by a linear relationship of the form:

$$y = -40.03 + 6.08x \ (R^2 = 0.89, p = 0.058)$$
$$y = -34.44 + 6.11x \ (R^2 = 0.97, p = 0.014)$$
$$y = -21.86 + 4.7x \ (R^2 = 0.94, p = 0.03)$$

for vines of Chardonnay, Carmenere, and Merlot, respectively. The slopes of the regressions were not significantly different.

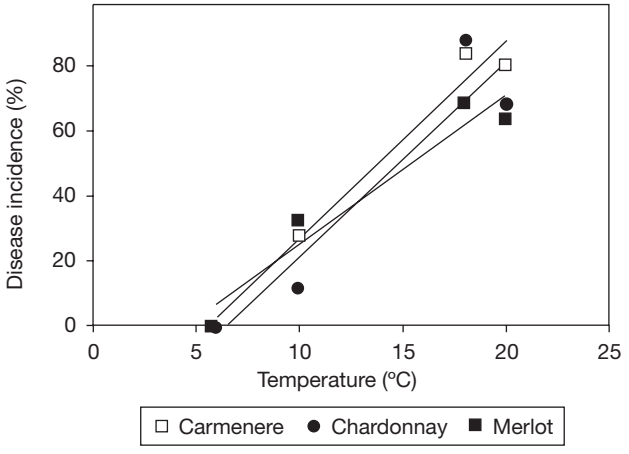

Figure 4. The relationship between incubation temperature and development of grapevine powdery mildew, *Erysiphe necator,* on leaves of:
'Chardonnay': ($y = 6.08x - 40.03$, $R^2 = 0.89$, $p = 0.058$),
'Carmenere': ($y = 6.11x - 34.44$, $R^2 = 0.97$, $p = 0.014$) and
'Merlot': ($y = 4.66x - 21.86$, $R^2 = 0.94$, $p = 0.03$).

A wet period, immediately after inoculation, significantly ($p < 0.01$) reduced the incidence of powdery mildew on Chardonnay vines. The highest incidence was when leaves were dry, after inoculation, and the lowest incidence was when leaves were wet 1 h after inoculation (Table 3). The first powdery mildew colonies were observed after 15 d of incubation. Mean disease incidence between leaves which were not wet and leaves that were wet after 20 d incubation was significant ($p = 0.05$) (Table 3).

Field validation of the infection warning system

Based on the RAI (Fig. 5), fungicide applications significantly ($p < 0.05$) controlled powdery mildew and

Table 3. The effect of free water on powdery mildew development on *Vitis vinifera* cv. Chardonnay inoculated with conidia of *Erysiphe necator*

Water treatment[1]	Infected leaves (no.)	
	15 days	20 days
Dry	2.6 a[2]	4.8 a
Wet, 1 h postinoculation	0.2 b	2.6 b
Wet, 72 h postinoculation	0.4 b	2.8 b

[1] Plants incubated in growth chambers (RH: 85-90% at 20ºC) and sprayed (wet) or nonsprayed (dry). Sprayed plants received a gentle mist equivalent to 5 mL of distilled water leaf^{-1}, 1 h or 72 h postinoculation with conidia of *E. necator* isolate PUC3, maintained on potted grapevines under isolation. [2] Means of five replicates followed by the same letters are not statistically different according to the Duncan-Waller k ratio t test ($p < 0.05$).

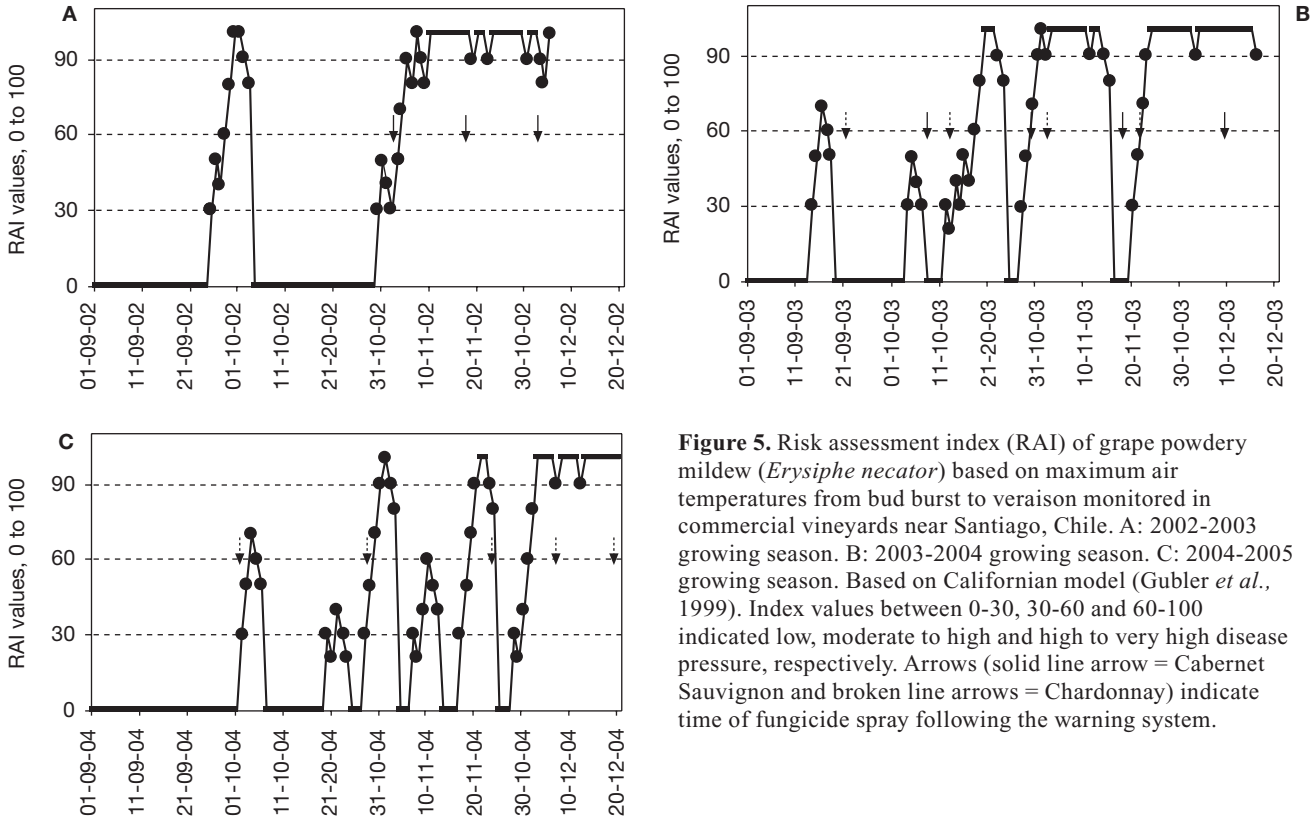

Figure 5. Risk assessment index (RAI) of grape powdery mildew (*Erysiphe necator*) based on maximum air temperatures from bud burst to veraison monitored in commercial vineyards near Santiago, Chile. A: 2002-2003 growing season. B: 2003-2004 growing season. C: 2004-2005 growing season. Based on Californian model (Gubler *et al.,* 1999). Index values between 0-30, 30-60 and 60-100 indicated low, moderate to high and high to very high disease pressure, respectively. Arrows (solid line arrow = Cabernet Sauvignon and broken line arrows = Chardonnay) indicate time of fungicide spray following the warning system.

reduced the number of applications required without negatively affecting the degree of disease control on vine clusters and leaves. For instance, on Cabernet Sauvignon under high disease pressure (87 and 99% leaf and cluster incidence, respectively) in the 2002-2003 growing season, fungicide applications were reduced from five, following the farmer spray program, to three, following the model spray program (Tables 4 and 5).

Discussion

The results showed that the RAI developed to forecast the risk of powdery mildew, due to infection caused by conidia of *E. necator* in California (Gubler *et al.*, 1999), was a very useful tool to determine fungicide applications against grape powdery mildew in Chile. Using the RAI it was possible to maintain or improve powdery mildew control with one to three fungicide applications less per season. Therefore, this warning system will be useful in rationalizing the use of fungicides against *E. necator,* particularly on table grapes, where farmers very often use more than ten fungicide sprays each season without obtaining good control.

Monitoring air temperature from the start of bud burst to veraison would be highly advisable. As previously reported, grapevine stages between the start of flowering and the appearance of small berries (stages 19 through 29) are the most critical stages for powdery mildew development in Central Chile. Nevertheless, early infection periods, which could possibly be detected, using this warning system can occur (Campbell *et al.*, 2007).

Powdery mildew fungus can over winter as cleistothecia which are often considered the main source of primary inoculum (Gadoury and Pearson, 1988; Cortesi *et al.*, 1997; Rügner *et al.*, 2002; Grove, 2004). Cleistothecia have been found in Chilean vineyards (Silva, 2005); however, the relatively dry conditions throughout the year in central and northern Chile (Novoa and Villaseca, 1989) may reduce the importance of infections from ascospores in powdery mildew epidemics. These results enable us to postulate that *E. necator* predominantly survives by perennation of mycelia in infected buds, as has been described under dry weather conditions in California (Ypema and Gubler, 2000). Therefore, the RAI can be a useful index

Table 4. Fungicide applications, environmental data and the infection risk index for powdery mildew (*Erysiphe necator*) development on grapevines in central Chile

Time of fungicide spray[1]				
Model program[2]		Standard program[3]	Farmer programs	
Date	Index		Date	Fungicides, i.a. ha^{-1}
Cabernet Sauvignon, 2002-2003				
31 Oct.	50	14 Oct.	22 Oct.	Wettable sulphur, 864 g
18 Nov.	100	31 Oct.	26 Oct.	Triflumizole, 80 g
06 Dec	100	18 Nov.	11 Nov.	Wettable sulphur, 1,296 g
		06 Dec	20 Nov.	Fenarimol, 30 g
			06 Dec	Fenarimol, 30 g
Cabernet Sauvignon, 2003-2004[4]				
10 Oct.	50	19 Oct.	17 Oct.	Fenarimol, 35 g
31 Oct.	90	28 Oct.	21 Oct.	Wettable sulphur, 864 g
19 Nov.	80	14 Nov.	28 Oct.	Myclobutanil, 84 g
10 Dec.		01 Dec.	06 Nov.	Fenarimol, 30
			28 Nov.	Sulphur dust, 25 kg
			10 Dec.	Sulphur dust, 25 kg
Chardonnay, 2003-2004[4]				
23 Sep.	50	23 Sep.	22 Sep.	Wettable sulphur, 864 g
14 Oct.	70	11 Oct.	04 Oct.	Wettable sulphur, 1,080 g
03 Nov.	100	28 Oct.	15 Oct.	Wettable sulphur, 1,296 g
21 Nov.	50	14 Nov.	25 Oct.	Myclobutanil, 40 g
		01 Dec.	07 Nov.	Fenarimol, 30 g
			28 Nov.	Sulphur dust, 25 kg
			11 Nov.	Sulphur dust, 25 kg
Chardonnay, 2004-2005				
01 Oct.	30	14 Sep.	09 Oct.	Wettable sulphur, 1.3 kg
29 Oct.	50	28 Sep.	18 Oct.	Wettable sulphur, 1.3 kg
22 Nov.	100	12 Oct.	26 Oct.	Myclobutanil, 60 g
07 Dec.	100	22 Oct.	11 Nov.	Kresoxim methyl, 100 g
21 Dec.	100	08 Nov.	18 Nov.	Sulphur dust, 20 kg
		17 Nov.	29 Nov.	Sulphur dust, 20 kg
		30 Nov.	10 Dec.	Fenarimol, 30 g
		14 Dec.		

[1] Except for farmer programs, all other fungicide spray applications were 84 g i.a ha^{-1} of kresoxim methyl (Stroby 50 SG). [2] Model program was based on risk assessment index (RAI values from 0 to 100) according to Gubler *et al.* (1999). [3] Standard program was a spray application every 15 to 18 d in 2002-2003 and 2003-2004 and every 10 to 15 d in the 2004-2005 growing seasons, starting when shoots were 15 to 20 cm long until veraison. [4] Differences in fungicide timing between Chardonnay and Cabernet Sauvignon in the 2003-2004 growing season were mainly due to differences in bud burst. Chardonnay vines were the earliest to burst in the spring.

to predict powdery mildew and adjust spray intervals of fungicides, based on disease pressure. However, fungicide application intervals still need to be studied in Chile.

In contrast to other warning systems (Salt, 1980; Kast *et al.*, 1997; Jarvis *et al.*, 2002; Carroll and Wilcox, 2003), the RAI is based on only monitoring on-site air temperature, assuming that the rate of conidia production is increased by temperatures between 20°C and 30°C. Shorter cycles of conidia production occur as RAI values increase from 0 to 100 (Gubler *et al.*, 1999; Jarvis *et al.*, 2002). Thus, for farmers, it is a simple and economically feasible method to implement and use in their vineyards.

In vitro, conidial germination of Chilean isolates of *E. necator* occurred over a broad temperature range, 10 to 30°C, developing a germ tube ending in a multi-

Table 5. Effectiveness of the risk assessment index developed in California to predict the risk of powdery mildew (*Erysiphe necator*) infections on grapevines (*Vitis vinifera*) under Chilean conditions

Growing season	Grapevine cultivars	Spray[1]		Powdery mildew incidence[2] (%)	
		Program	No.	Clusters	Leaves
2002-2003	Cabernet Sauvignon	Model spray	3	30.0a[3]	34.5a
		Standard	4	26.0a	31.0a
		Farmer spray	5	72.0a	59.5b
		Untreated control	0	99.0b	87.0c
2003-2004	Cabernet Sauvignon	Model spray	4	0.0a	3.5a
		Standard	4	1.0a	3.0a
		Farmer spray	6	1.0a	6.0a
		Untreated control	0	48.0b	37.0b
	Chardonnay	Model spray	4	1.0a	1.0a
		Standard	5	2.5a	1.5a
		Farmer spray	7	3.0a	1.0a
		Untreated control	0	51.0b	38.5b
2004-2005	Chardonnay	Model spray	5	2.0a	nd
		Standard	9	1.0a	nd
		Farmer spray	9	1.5a	nd
		Untreated control	0	34.5b	nd

[1] Model spray program based on risk assessment index (RAI) developed in California (Gubler *et al.,* 1999). Standard spray program was 84 g i.a. ha^{-1} kresoxim methyl (Stroby 50 SG) applied every 15 to 18 d in 2002-2003 and 2003-2004, and every 10 to 15 d in 2004-2005, starting when shoots were 15 to 20 cm long until veraison. [2] Fifty clusters and 50 leaves were sampled for each treatment replicate. [3] Means followed by the different letters within each column and cultivar were statistically different according to the Duncan-Waller k ratio t test ($p = 0.05$). Arcsin square root transformations were used but the table gives pre-transformed values. nd: not determined.

lobed appressorium at the distal end, and a secondary germ tube at the opposite site of the conidium (Braun, 1999; Braun *et al.*, 2002). Germination of conidia developed between 18 and 25°C approximately and was optimum at 25°C (Delp, 1954; Cruz, 2001; Jarvis *et al.*, 2002). This may explain the germination failure observed at 5 and 35°C in this study (Fessler and Kassemeyer, 1995). Depending on *E. necator* isolate conidial germination was either absent or extremely low at 10 and 30°C. Thus, in this study, temperatures above 30°C were considered deleterious for powdery mildew infection. Ten points were subtracted from RAI calculations when there was a temperature higher than 30°C for at least one hour a day. Based on these results, the lower temperature threshold of 20°C appeared accurate for RAI calculations. However, the significant interaction of temperature by *E. necator* isolate suggested that conidial germination was isolate-dependant. Therefore, it would be advisable to study a larger number of isolates of *E. necator* to obtain a better understanding of the effect of temperature on RAI calculations under Chilean conditions.

Vines of Merlot, Carmenere, and Chardonnay appeared to be equally susceptible to *E. necator*. The shortest incubation period was 13 d when vines were incubated at 20°C or 23°C; it was delayed to 19 to 24 d at 10°C and to over 23 d at 6°C. Based on these results, the risk of a severe epidemic is markedly decreased at temperatures below 10°C.

Conidial germination at 20°C was affected by humidity in the range 33-35% to > 95%. There was a significant *E. necator* isolate by RH level interaction. As in previous reports, there was consistent germination at a low RH of 33-35%. It increased at 47 to 90% and decreased at a RH > 95% (Carroll and Wilcox, 2003). This suggests that conidia can initiate powdery mildew epidemics under conditions of very low humidity in the absence of an external water supply, possibly using water from vacuoles in the conidial cell (Bulit and Lafon, 1978; Braun *et al.*, 2002). However, conidia started to shrink after being kept dry for 12 h, resulting in partial dehydration. This may explain why 24 h of dry conditions gave lower conidial germination than 12 h of dry conditions, in this study. Therefore, an ex-

ternal water source was necessary after 12 h. In this study, as in other reports, the presence of free water appeared to restrict powdery mildew development at 20°C (Chellemi and Marois, 1991). However, the significance of this finding in RAI calculations is still to be studied.

In conclusion, we have shown that following the model developed in California, based on *in situ* air temperature, it is possible to estimate the risk of powdery mildew infection of grapes under Chilean conditions. The index can be used to successfully guide fungicide spray applications in vineyards in central Chile. Conidial germination of isolates of *E. necator* from Chile was temperature and humidity dependant but occurred under a broad range of temperatures and humidities, as with isolates previously tested worldwide. Further research is needed for a better understanding of the effect of humidity on the RAI calculation.

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