

Effect of temperature and wetness duration on infection by *Plasmopara viticola* and on post-inoculation efficacy of copper

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Abstract A weather-driven model was developed to predict *P. viticola* population dynamics on grape leaf surfaces during a discrete wet period. To develop this model, the relationships between temperature, wetness duration, zoospore release, and infection were investigated with environmentally controlled experiments, and equations were developed that fitted the experimental data. Release of *P. viticola* zoospores from sporangia and infection occurred over a wide range of temperatures, but were highest at 15–20 °C. At these temperatures, zoospore began to be released within 1 h after inoculation, and most infections had already occurred by 3 h after inoculation. β -Gompertz and Weibull equations were used to describe zoospore release and infection, respectively. The post-inoculation efficacy (PIE) of two copper fungicides was also evaluated. For both fungicides PIE decreased rapidly as application time after inoculation increased, and was <20 % after 6 h at the optimal temperature for *P. viticola* development (20 °C). The model proposed here can be used to improve the weather-based models that have been developed for simulating secondary cycles of downy mildew. The model can also be used to predict whether a fungicide application during a discrete infection period will be effective. PIE values for the two copper fungicides

were positively correlated with the proportion of *P. viticola* sporangia on a leaf that had not yet caused infection. Model simulations suggested that the efficacy of a copper treatment increased when these scenarios were less conducive for disease development.

Keywords Downy mildew · Secondary infection · Weather-based model · *Vitis vinifera*

Introduction

Downy mildew, which is caused by the obligate parasite *Plasmopara viticola* (Berk et Curt.) Berl. et de Toni, occurs throughout the world and is considered the most serious grapevine disease in those parts of Europe with warm and wet conditions during the vine growth period (Hewitt and Pearson 1988; Gessler et al. 2011; Rossi et al. 2012). Downy mildew epidemics cause both direct and indirect yield losses, including rotting of inflorescences, shoots, and clusters; reduction in photosynthetic activity of the affected leaves; and premature defoliation of vines (Hewitt and Pearson 1988).

The disease is controlled by regular, calendar-based applications of fungicides (Gessler et al. 2011) or by scheduling fungicides based on warnings provided by mathematical models (Caffi et al. 2011). Treatments mainly involve a variety of synthetic fungicides with high preventative and/or curative efficacy (Gessler et al. 2011).

Control of downy mildew is challenging in organic viticulture. In organically managed vineyards, the

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disease is currently controlled by using copper compounds that lack curative activity and that must be present at a sufficient concentration on plant surfaces to prevent infection (Gessler et al. 2011). Copper compounds are often applied weekly throughout the season to ensure constant plant protection (Pellegrini et al. 2010), and this leads to the distribution of several kilograms of copper per hectare, with negative environmental and toxicological impacts on the vineyard soils (Komarek et al. 2010). To reduce these negative effects, Council Regulation 473/2002/EC introduced limits on the use of copper compounds in agriculture (Gessler et al. 2011). Although effective alternatives to copper fungicides are being investigated (Dagostin et al. 2011; La Torre et al. 2014), organic grape growers still rely mainly on copper fungicides but use them at reduced dosages. To help growers optimize the scheduling and dosages of copper fungicides against downy mildew, decision support systems were developed based on weather data, disease risk, and plant growth (Kuflik et al. 2009; Rossi et al. 2014). However, further efforts are necessary to achieve efficient downy mildew control with reduced copper applications during the grape-growing season.

Organic apple growers use ‘during-infection’ sprays to better target copper applications against scab (caused by *Venturia inaequalis*), i.e., to apply copper only when necessary in order to reduce copper usage during the apple-growing season (Jamar et al. 2008, 2010). This strategy involves spraying of copper fungicides or other fungicides that lack curative properties after ascospores have been deposited on plant surfaces but before germ tubes have penetrated the host tissue (Jamar et al. 2008). Application of the ‘during-infection’ sprays is supported by extensive studies of the underlying biological processes, i.e., ascospore deposition, ascospore germination, germ tube elongation, formation of appressoria, and formation of penetrating hyphae (Machardy 1996), and by the development of mathematical models that predict the dynamics of the ascospore population on plant surfaces as affected by weather conditions (Rossi and Bugiani 2007; Rossi et al. 2006). To our knowledge, ‘during-infection’ sprays with copper have never been evaluated for controlling *P. viticola* in vineyards. For performing this evaluation, preliminary knowledge about *P. viticola* infection and copper efficacy is necessary.

Regarding *P. viticola*, it is known that a downy mildew epidemic is triggered by primary infections from sexual spores (oospores) that have overwintered in the leaf litter and soil; once initiated, the epidemic involves repeated secondary infections from asexual sporangia produced on disease lesions (Gessler et al. 2011). A secondary infection cycle may take 5 to 18 days, depending on environmental conditions and host susceptibility (Lalancette et al. 1988). Biological processes underlying these infections involve: (i) production and dispersal of sporangia (Caffi et al. 2013; Lalancette et al. 1988; Rossi and Caffi 2012; Rumbolz et al. 2002); (ii) deposition and survival of sporangia on plant surfaces (Kast and Stark-Urnau 1999; Kennelly et al. 2007); (iii) exit of zoospores from living sporangia (Williams et al. 2007b); (iv) the swimming of zoospores to stomata; and finally (v) germination of zoospores and stomatal penetration by germ tubes (Unger et al. 2007; Williams et al. 2007a). All of these processes are strongly influenced by environmental conditions. Sporulation of *P. viticola* occurs at night and requires moisture and mild temperatures, while dispersal of sporangia occurs under a wide range of weather conditions (Caffi et al. 2013). Survival of sporangia also depends on temperature and relative humidity (Blaeser and Weltzien 1979; Kast and Stark-Urnau 1999). The release of zoospores and their movement depend on water (Williams et al. 2007a, b) and occur at a faster rate under suitable environmental conditions (Blaeser and Weltzien 1979; Kast and Stark-Urnau 1999; Keil et al. 2006; Williams et al. 2007a, b). The combined effects of temperature and wetness duration on zoospore release by sporangia and on the ability of zoospores to cause infection, however, have not been properly studied with *Vitis vinifera* leaves, and no quantitative data are available for the development of a dynamic population model. Acquiring this knowledge and developing a proper model are then preliminary steps in evaluating the possibility of using ‘during-infection’ sprays for downy mildew control in vineyards.

Regarding the post-infection efficacy of copper, information is available for other plant pathogens, such as *Fusicladium eriobotryae* or *Fusicladium oleagineum* (Obanor et al. 2008b; González-Domínguez et al. 2014), but little knowledge is available for *P. viticola*,

To perform a preliminary evaluation of the ‘during-infection’ copper sprays against grape downy mildew, we: (i) conducted controlled-environment experiments to investigate the effect of wetness duration and

temperature on germination of sporangia and infection by *P. viticola*; (ii) developed a model for the epiphytic population dynamics of *P. viticola* on grape leaves; and (iii) evaluated the post-inoculation efficacy of two copper fungicides to estimate the susceptibility of the epiphytic *P. viticola* population to copper. In addition, we performed a simulation study of *P. viticola* population dynamics under diverse environmental scenarios.

Materials and methods

Fungal material

Plasmopara viticola was maintained on *Vitis vinifera* plants cv. Barbera, which is susceptible to the pathogen (Rossi et al. 2006), in a greenhouse at the University campus of Piacenza (North Italy). The plants were isolated from any external source of *P. viticola*. The original inoculum was a bulk of sporangia collected in commercial vineyards of northern Italy in 2012.

The inoculum suspensions for the three experiments described below were obtained as follows. Freshly sporulating downy mildew lesions were cut from the leaves of the cv. Barbera plants used for inoculum maintenance. The lesions were gently washed with distilled water, and the resulting suspension was adjusted to 10^5 sporangia mL^{-1} . Healthy, young unfolded leaves were excised from other 10-year-old cv. Barbera grapevines grown in isolation. Leaves were placed with their abaxial surfaces up on a layer of wet blotting paper in Petri dishes, and were inoculated with 10 $10\text{-}\mu\text{L}$ drops of the suspension of sporangia prepared as above. Leaves were then kept in a saturated air at $20\text{ }^\circ\text{C}$ with 12 h of light and 12 h of darkness until downy mildew lesions formed and sporangia appeared. These sporangia were finally suspended in distilled water (10^5 sporangia mL^{-1}) and used for the experiments.

Effect of temperature and wetness duration on zoospore release (experiment 1)

The effect of temperature (T) and wetness duration (WD) on zoospore release from *P. viticola* sporangia was evaluated under six constant temperature regimes (T=5, 10, 15, 20, 25, and $30\text{ }^\circ\text{C}$) and seven wetness periods (WD=30 min, 1, 3, 6, 9, 12, and 24 h). For each combination of T×WD, a 2-ml Eppendorf tube containing the suspension of sporangia prepared as previously

described was incubated in darkness. At the end of each WD period, three drops ($10\text{ }\mu\text{L}$ each) were taken from each Eppendorf tube after the suspension was shaken with a vortex mixer. These drops were placed on a glass microscope slide and observed with a microscope ($\times 40$). The percentage of open sporangia (i.e., germinated sporangia) was determined by examining 200 sporangia per drop. The experiment was repeated.

Effect of temperature and wetness duration on leaf infection (experiment 2)

The effect T and WD on infection of grapevine leaves was evaluated under six constant temperature regimes (5, 10, 15, 20, 25, and $30\text{ }^\circ\text{C}$) and six wetness periods (1, 3, 6, 9, 12, and 24 h). For each combination of T×WD, five leaf discs (20 mm in diameter) excised from young, unfolded cv. Barbera plants (managed as previously described) were placed with their abaxial surfaces up on a layer of wet blotting paper in Petri dishes. After the leaf discs were inoculated with five $10\text{-}\mu\text{L}$ drops of sporangial suspension prepared as previously described, they were kept in saturated air at $20\text{ }^\circ\text{C}$ with 12 h of light and 12 h of darkness. At the end of each WD, the inoculation drops were dried with a piece of blotting paper, and the leaf discs were incubated for another 10 days. Disease incidence was assessed as the percentage of inoculation sites (i.e., sites where the inoculum drops had been placed) with typical downy mildew lesions. The experiment was repeated.

Evaluation of the post-inoculation activity of copper-based fungicides (experiment 3)

The post-inoculation activity of two copper-based commercial fungicides: Airone Più (Isagro Spa; Milano; Italy) and Iperion (Siapa; Sumitomo Chemical Italia s.r.l.; Milano; Italy) was evaluated. Airone Più contained 28 % copper oxychloride+28 % copper hydroxide, and Iperion contained 37.5 % copper oxychloride. The fungicides were applied at 1, 3, 6, 12, and 24 h post-inoculation (hpi), and at 2, 3, and 4 (recommended label dose) g L^{-1} of commercial product, which is 1.12, 1.68, and 2.24 g L^{-1} of copper for P1, and 0.75, 1.12, and 1.50 g L^{-1} of copper for P2. Distilled water was applied as a control treatment.

For each combination of product×dosage×hpi, eight leaf discs from cv. Barbera vines were inoculated with a suspension of *P. viticola* sporangia and incubated as

described in the previous section. At 13 days post-inoculation, the percentage of leaf area covered by downy mildew lesions (i.e., disease severity or SEV) was assessed by using the EPPO scale (EPPO 2004). For each combination, post-inoculation efficacy (PIE) was calculated as $PIE = (SEV_u - SEV_t) / SEV_u$, where the subscript u refers to the untreated discs and t refers to the treated discs. The experiment was repeated.

Data analysis

A preliminary analysis of variance showed a lack of significant difference between the trials of the repeated experiments; therefore, the data from the trials of the same experiment were pooled. Different non-linear regression models were fitted to the observed data in experiments 1 to 3. The equation parameters were estimated using the nonlinear regression procedure of SPSS (ver. 21.0, SPSS Inc. Chicago, USA). The different nonlinear regressions were compared based on the Akaike's Information Criterion (AIC). The following models, which provided the smallest AIC values for each experiment (Burnham and Anderson 2002), were selected.

For experiment 1, a combination of a β and Gompertz equation was selected, in the form:

$$Y = \alpha \times \text{Teq}^\beta \times (1 - \text{Teq})^\lambda \times \exp[\delta \times \exp(\varepsilon \times WD)] \quad (1)$$

in which Y is the percentage of sporangia that were open (germinated) relative to the maximum percentage that were opened for the entire experiment; Teq is the temperature equivalent; WD is the wetness duration (in h); and α , β , λ , δ and ε are the equation parameters. Teq is calculated as $\text{Teq} = (T - T_{\min}) / (T_{\max} - T_{\min})$ where T is the temperature regime and T_{\min} and T_{\max} are the minimum and maximum temperatures, respectively, at which sporangia release zoospores.

For experiment 2, a Weibull model (Duthie 1997) was selected, in the form:

$$Y = a \times \left(1 - \exp\left(-f(T) \times (WD - c)^d\right) \right) \quad (2)$$

where Y is the relative infection rate; WD is the wetness duration (in h); a , c , and d are equation parameters; and $f(T)$ describes the response to temperature. The structure of the equation indicates that temperature

affects the intrinsic rate of increase of the response to wetness duration; $f(T)$ is calculated as:

$$f(T) = e \times ((h + 1)/h) \times h^{(1/h+1)} \times (\exp((T-f) \times g/(h + 1)) / (1 + \exp((T-f) \times g))) \quad (2.1)$$

where T is the temperature ($^{\circ}\text{C}$), and e , f , g , and h are equation parameters.

Features of the response characterized by each parameter have been extensively explained by Duthie (1997). Briefly, a ($-\infty < a < +\infty$) represents the upper limits of the response; b ($0 < b < 1$) represents the intrinsic rate of increase in response to wetness; c ($c > 0$) represents the lag period before the response to wetness; d ($d > 0$) is the fraction of the wetness period in which the response decelerates; e ($e > 0$) represents the maximum response at the optimal temperature; f ($-\infty < f < +\infty$) locates the curve on the horizontal axis as a function of the optimal temperature; g ($g > 0$) represents the intrinsic rate of increase in response to temperature; and h ($-\infty < h < +\infty$) characterizes the asymmetry of the curve with respect to the optimal temperature. To reduce over-parameterization, Eqs. (2) and (2.1) can be simplified by fixing values of selected parameters based on prior knowledge of the response (Duthie 1997). In this case, a and e were fixed to 1 (because the results of experiment 2 were rescaled to 1), and c was fixed to 0 (because the infection process began when the wetness period began).

The equation parameters were estimated using the nonlinear regression procedure of SPSS (ver. 19.0; SPSS Inc.). Goodness-of-fit of the data was assessed by the adjusted R^2 , the magnitude of the standard error of the parameters, the root mean square error (RMSE), the coefficient of residual mass (CRM), the concordance correlation coefficient (CCC), and the Akaike's Information Criterion (AIC) (Nash and Sutcliffe 1970; Lin 1989; Motulsky and Christopoulos 2003). Briefly, RMSE represents the average distance of real data from the fitted line, and CRM is a measure of the tendency of the equation to overestimate or underestimate the observed values (a negative CRM indicates a tendency of the model toward overestimation) (Nash and Sutcliffe 1970). CCC is the product of two terms: the Pearson correlation coefficient and the coefficient C_b , which is an indication of the difference between the best fitting line and the perfect agreement line (CCC=1 means perfect agreement) (Madden et al. 2007). AIC values

were used to compare models; the model with the smallest AIC value was considered to have provided the best fit (Madden et al. 2007).

Because different fungicides and dosages were used for experiment 3, a preliminary analysis of covariance (ANCOVA) was performed with the data to test the effect of the covariate (i.e., hpi) and the categorical variable (fungicide × concentration) on the response variable ($\ln(\text{PIE}+1)$). The null hypothesis H_0 of equal slopes within group regressions was tested by determining whether the interaction between the categorical predictor and the continuous predictor was equal to zero (Quinn and Keough 2002).

In a second step, the data were fitted to a logistic equation in the form:

$$Y = 1 / [1 + a \times \exp(-b \times t)] \tag{3}$$

where Y is the PIE, t is hpi when the fungicide was applied, and a and b are the equation parameters.

Modelling *P. viticola* population dynamics on leaves

The population dynamics of *P. viticola* on grape leaf surfaces can be described through the following developmental stages: i) living, ungerminated sporangia; ii) dead, ungerminated sporangia; iii) germinated sporangia, i.e., sporangia that have released zoospores; iv) dead zoospores; v) zoospores swimming in water; and vi) zoospores infecting leaves, i.e., zoospores that have produced a germination tube that has penetrated the leaf tissue through a stoma. These stages are all present at the same time on the leaf surface during a discrete leaf wetness period, and their dynamics are regulated by the following main processes: i) deposition of sporangia on leaves; ii) mortality of sporangia; iii) germination (i.e., opening) of sporangia; iii) infection by zoospores; iv) mortality of zoospores. These processes are represented in Fig. 1, which is explained in the following paragraph.

At the beginning of a wet period, a dose of living, non-germinated sporangia (ST1) is present on leaves. After 1 h of wetness, some of the sporangia that are released from sporulating, downy mildew lesions (ST0) are deposited on leaves at a specific deposition rate (DEP). At the same time, some of the sporangia in ST1 germinate (and release zoospores in water (ST2)), while others do not (they remain in ST1), depending on a specific germination rate (GER); other sporangia can also die at a specific mortality rate (MOR'). After 1 additional hour of wetness, some of

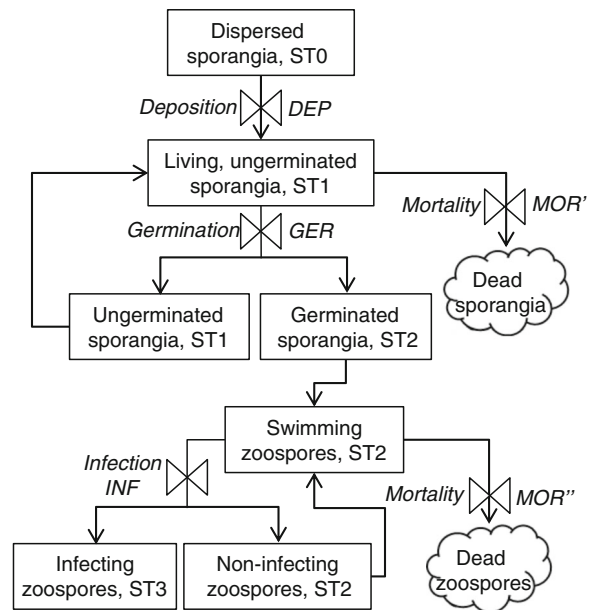


Fig. 1 Relational diagram of the model simulating the population dynamics of *Plasmopara viticola* on grape leaves

the zoospores swimming in water (ST2) produce a germ tube that penetrates a stoma (ST3, infecting zoospores), while others do not (they remain in ST2); some zoospores can also die at a specific mortality rate (MOR''). At the same time, additional sporangia are deposited on leaves at rate DEP , and other sporangia germinate at rate GER . These processes continue until the leaf surface dries; at that time, the swimming zoospores die.

At any time t during the wet period, the above description can be expressed mathematically:

$$ST1_t = ST1_{t-1} + ST0_{t-1} \times DEP_t - ST1_t \times GER_t - ST1_{t-1} \times MOR'_t$$

$$ST2_t = ST2_{t-1} + ST1_t \times GER_t - ST2_t \times INF_t - ST2_{t-1} \times MOR''_t$$

$$ST3_t = ST3_{t-1} + ST2_t \times INF_t$$

To solve this system of equations, the initial value of ST1 must be known, as well as rate values at any time t of a discrete wetness period. In this work, ST1 at time zero was assumed to be insignificant, so that $ST1_{t=0}=0$. DEP , GER , and INF were calculated as functions of the weather variables influencing deposition, germination, and infection, respectively. The mortality rates of both sporangia and zoospores during a discrete infection period with uninterrupted wetness were assumed to be insignificant, so that MOR' and $MOR''=0$.

The equation for the deposition rate of sporangia on leaves (DEP) was derived from a previous report regarding

the production and release of asexual sporangia by *P. viticola* (Caffi et al. 2013). Based on this report, the model assumed that: i) sporulation on downy mildew lesions is triggered in darkness by a wet period of at least 3 h (a period is considered wet when relative humidity (RH) $\geq 80\%$, rain (R) > 0 , and WD > 0.5 h); ii) the production of sporangia follows an asymptotic pattern described by the following equation ($R^2=0.97$): $Y = 1 - (1 - a) \times \exp(-b \times t)$, where Y is the relative cumulative production of sporangia, t is the sporulation period in days, and $a = -0.185$ and $b = 0.018$ are the parameters, with 95 % confidence intervals of -0.069 and -0.302 for a , and 0.022 and 0.014 for b ; and iii) sporangia are released at the same rate as they are produced (Caffi et al. 2013). Therefore, DEP_t was calculated as $Y_t - Y_{t-1}$, where Y is the independent variable of the equation described above.

For the germination rate of sporangia (GER) and the infection rate (INF), the equations developed for fitting the data from experiment 1 and 2 were used. In detail, GER_t was calculated as $Y_t - Y_{t-1}$, where Y is the independent variable of Eq. 1. The infection rate, INF_t , was calculated as $Y_t - Y_{t-1} - GER_t$, where Y is the independent variable of Eq. 2.

Simulation of *P. viticola* population dynamics under three scenarios

The model was operated under three scenarios: (i) WD=24 h, T=20 °C, and a fast deposition rate of sporangia; (ii) WD=24 h, T=16 °C, and an average deposition rate of sporangia; and (iii) WD=24 h, T=12 °C, and a slow deposition rate of sporangia. Medium, fast, and slow deposition rates were simulated by using the average of the parameter estimates of the equation from Caffi et al. (2013) and the lower and upper values of the 95 % confidence intervals of these parameter estimates, respectively (reported above). These scenarios were used to represent severe, intermediate, and mild conditions for *P. viticola* development.

Results

Effect of temperature and wetness duration on zoospore release

P. viticola sporangia opened (i.e., zoospore were released) at all temperatures tested (Fig. 2). Open sporangia were observed after 30 min in water at all

temperatures; $>20\%$ of the sporangia were open at 3 hpi at 15 and 20 °C, and up to 30 % at 6 hpi at 15 and 25 °C. The percentage of open sporangia was highest (42 %) at 24 hpi at 15 °C; at 24 hpi, $>30\%$ of sporangia were open at 10 and 20 °C. At 30 °C, $<20\%$ of the sporangia were open at any wetness duration (Fig. 2).

Equation 1 provided a good fit of the data describing the combined effect of temperature and wetness duration on the opening of sporangia, with $R^2=0.868$, CCC=0.927, and low values of RMSE and CRM (Table 1). The rate at which sporangia opened increased with temperature until 15 to 20 °C and with wetness duration (Fig. 3).

Effect of temperature and wetness duration on infection

No infection was observed at 5 and 30 °C (Fig. 4). At 10 to 25 °C, *P. viticola* sporangia caused some infection at 1 hpi, with 50 % infection at 20 °C. No relevant increase in infection incidence was observed after 3 hpi at 20 and 25 °C, whereas at 10 and 15 °C, infection incidence increased until 9 hpi (Fig. 4).

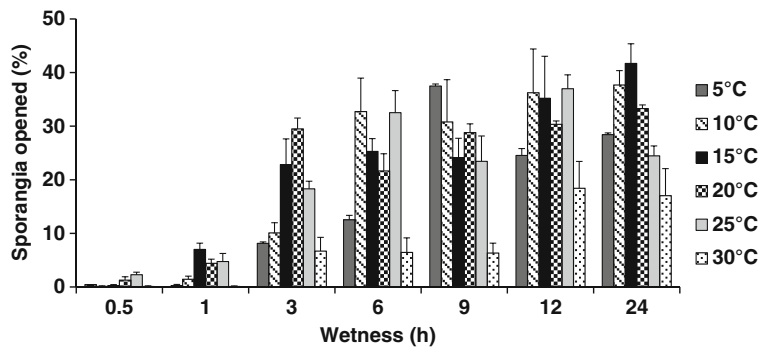
Equation 2 provided a good fit of the combined effects of temperature and wetness duration on infection by sporangia, with $R^2=0.910$ and CCC=0.863 (Table 1). Some overestimation was observed, with CRM=-0.021. The model showed a slow increase of infection incidence with temperature until 22–24 °C and then a fast decrease until 30 °C. The infection incidence increased with wetness duration until 9 h; between 9 and 12 h of wetness, the model indicated an incremental increase in infection incidence when $T < 20$ °C or $T > 25$ °C (Fig. 5).

Equation 2 estimates the proportion of sporangia that caused infection after exposure to a specific combination of wetness duration (WD) and temperature (T) during the wet period, i.e., $Y_{WD,T}$. For instance, after 4 h of leaf wetness at 18 °C, the estimated infection incidence was 0.44. As a consequence, $1 - Y_{WD,T}$ estimates the proportion of sporangia that have not caused infection at a specific combination WD \times T. These sporangia may be not open yet or the zoospores that they released may still be swimming in water on the leaf surface.

Post-inoculation activity of copper-based fungicides

The efficacy of the copper fungicides against *P. viticola* decreased as the time from inoculation to fungicide application increased (Fig. 6). When copper fungicides

Fig. 2 Effect of temperature and wetness duration on the percentage of *Plasmopara viticola* sporangia that have opened (i.e., the percentage that have released zoospores). Values are means+standard errors of two trials with three replicates (200 sporangia per replicate) per trial



were applied at 1 hpi, PIE (post-infection efficacy) was >90 % irrespective of copper type and dosage. At 3 hpi, PIE was higher for the product containing both copper oxychloride and copper hydroxide (Airona) than for that with only copper oxychloride (Iperion) (Fig. 6). PIE was below 10 % when copper fungicides were applied at 12 or 24 hpi, except in one case for P1 (Fig. 6).

The interaction between the six combinations of product and dosage×time of application (hpi) was not significant according to the analysis of covariance ($P=0.591$). Thus, the data were pooled, and the overall fungicide efficacy was described by the following equation: $Y = 1/(1-0.994 \times \exp(-0.005 \times t))$, where Y is the PIE, and t is the number of hours from inoculation to fungicide application, with $R^2=0.965$, RMSE=6.140, and CRM=-0.042.

Values of PIE for the copper fungicides applied at different post-inoculation times were regressed against

the proportions of sporangia that had not caused infection at that time (those proportions were the values of $1-Y_{WD,T}$ from Eq. 2), with $WD=1, 3, 6, 12,$ and 24 h (i.e., hpi to fungicide application) and $T=20$ °C (Fig. 7). The slope and intercept of the regression line did not differ from 1 ($P=0.05$) and 0 ($P=0.76$), respectively, with $R^2=0.98$. This indicated that all of the pathogen structures that had not caused infection at the time of copper application (i.e., all of the sporangia that had not yet opened and all of the swimming zoospores) were killed by the fungicide on the leaf surface.

Simulation of *P. viticola* population dynamics under three scenarios

The three scenarios used to operate the model provided different results (Fig. 8). In scenario (i), >50 and >95 % of the sporangia that had been

Table 1 Parameter estimates and summary statistics of nonlinear regression analyses relating *Plasmopara viticola* zoospore release (experiment 1) and infection incidence (experiment 2) at different temperatures and wetness durations

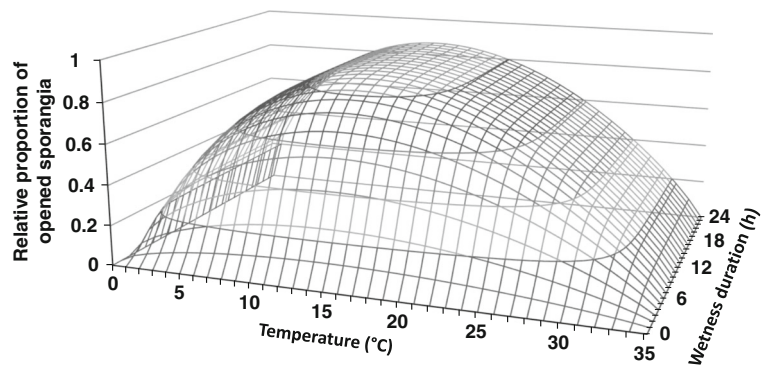
Experiment	Model	Estimated parameters					Goodness of fit ^c			
		α	β	λ	δ	ε	R^2	RMSE	CRM	CCC
1	β -Gompertz ^a	2.434 (0.623)	0.621 (0.151)	0.906 (0.178)	3.353 (0.837)	0.501 (0.104)	0.868	0.122	-0.018	0.927
		h	f	g	d	R^2				
2	Weibull ^b	4.465 (1.949)	24.046 (0.546)	1.475 (0.411)	0.743 (0.114)		0.910	0.110	-0.021	0.863

^aRegression equation is $Y = \alpha \times \text{Teq}^\beta \times (1 - \text{Teq})^\lambda \times \exp[\delta \times \exp(\varepsilon \times \text{WD})]$, where Y is the proportion of sporangia opened relative to the maximum proportion opened; Teq is the temperature equivalent; WD is the wetness duration (in hours); and $\alpha, \beta, \lambda, \delta$ and ε are the equation parameters. Standard errors of the estimated parameters are shown in parentheses. $\text{Teq} = (T - T_{\text{min}})/(T_{\text{max}} - T_{\text{min}})$, being $T_{\text{min}}=0$ °C, and $T_{\text{max}}=35$ °C

^bRegression equation is $Y = a \times (1 - \exp(-f(T) \times (\text{WD} - c)^d))$, where Y is the relative infection rate; WD is the wetness duration (in hours); $a, b, c,$ and d are equation parameters; and $f(T)$ describes the response to temperature. $f(T) = e \times ((h+1)/h) \times h^{(1/h+1)} \times (\exp((T-f) \times g/(h+1)) / (1 + \exp((T-f) \times g)))$, where T is the temperature (°C), and $e, f, g,$ and h are equation parameters. In this case, a and e were fixed to 1, and c was fixed to 0. Standard errors of the estimated parameters are shown in parentheses

^c R^2 , coefficient of determination of the regression line; RMSE, root mean square error; CRM, coefficient of residual mass; CCC, concordance correlation coefficient

Fig. 3 Relationship between temperature, wetness duration, and the relative proportion of opened sporangia of *Plasmopara viticola* as predicted by equation 1. The relative proportion of opened sporangia was calculated by dividing the percentage of opened sporangia at each observation by the maximum percentage in each trial

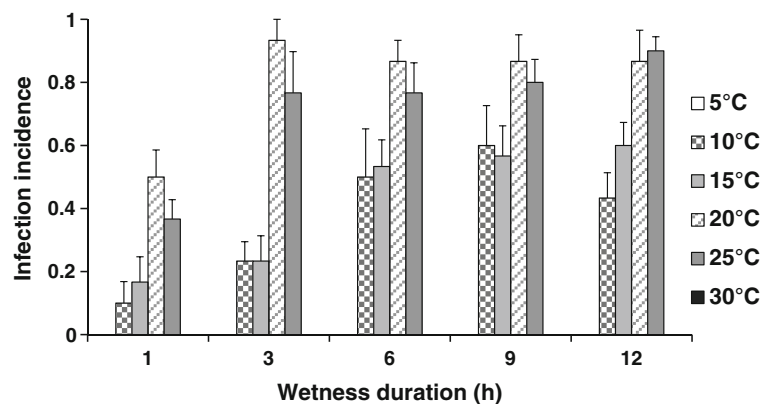


deposited on the leaves had caused infection (i.e., they were in ST3) after 4 and 12 h of wetness, respectively (Fig. 8a). Swimming zoospores (ST2) accounted for <20 % of the *P. viticola* population during the entire wet period (Fig. 8a). The proportion of the copper-exposed, non-infecting population (i.e., ST1+ST2) was <5 % after 12 h of wetness.

In scenario (ii), 22 and 42 % of the sporangia that had been deposited on leaves had caused infection after 6 and 24 h of wetness, respectively (Fig. 8b). Swimming zoospores accounted for 26 % of the population after 12 h of wetness (Fig. 8b). After 24 h of wetness, 50 % of the *P. viticola* population on the leaf surface was non-infecting (Fig. 8b).

In scenario (iii), only 14 and <21 % of the sporangia that had been deposited on leaves had caused infection after 12 and 24 h of wetness, respectively (Fig. 8c). At the end of the 24-h wetness period, 78 % of the sporangia had not caused infection, with 30 % still closed and 48 % opened (Fig. 8c).

Fig. 4 Effect of temperature and wetness duration on the incidence of infection (the proportion of leaf discs showing typical downy mildew lesions) after inoculation with *Plasmopara viticola*. Values are means+standard errors of two trials with five replicates (leaf discs) per trial



Discussion

In this work, a weather-driven model was developed to predict *P. viticola* population dynamics on grape leaf surfaces during a discrete wet period. To acquire the information needed for this model, the relationships between temperature, wetness duration, zoospore release, and infection were investigated with environmentally controlled experiments, and equations were developed that fit the experimental data. To our knowledge, this is the first time that these relationships have been quantified and expressed in equations over a wide range of conditions for *P. viticola* and *Vitis vinifera*. Lalancette et al. (1988) determined the infection efficiency of *P. viticola* on the American grape (*Vitis labrusca*) between 5 and 30 °C and with 1 to 15 h of wetness.

Release of *P. viticola* zoospores from sporangia and infection occurred over a wide temperature range (5 to 30 °C for release and 10 to 25 °C for infection), with the optimum at 20 to 25 °C. At these optimal temperatures, zoospores were released by 1 h post-inoculation, and

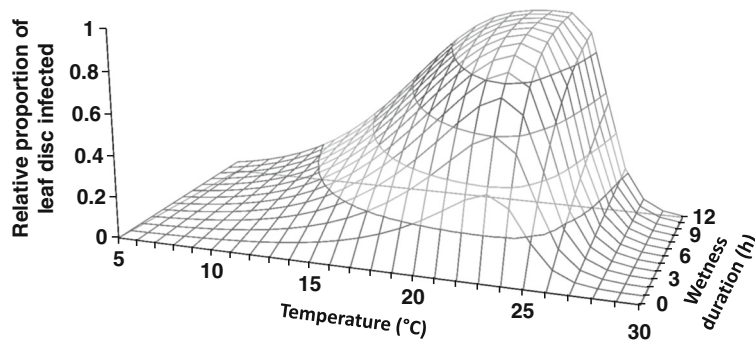


Fig. 5 Relationship between temperature, wetness duration, and relative proportion of leaf discs infected after inoculation with *Plasmopara viticola* sporangia as predicted by equations 2 and

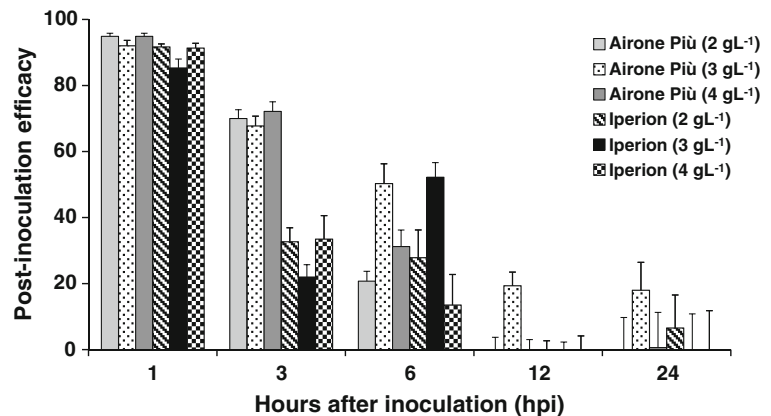
most infections had occurred by 3 h post-inoculation. Our results are consistent with previous reports. Williams et al. (2007b) observed that *P. viticola* sporangia released zoospores at temperatures between 5 and 35 °C, but the authors did not report the percentage of opened sporangia at different temperatures. Williams et al. (2007a) observed leaf penetration at temperatures between 10 and 25 °C, as was the case in the current study. Keil et al. (2006) also observed infection at temperatures between 5 and 30 °C, and Lalancette et al. (1988) reported that infection occurred at 30 °C but not at 5 °C. Keil et al. (2006) and Williams et al. (2007a) observed penetration within 1 h at the optimal temperature. Blaeser and Weltzien (1979) observed that at 6 and 25 °C, 9 and 2 h of wetness were necessary for infection; they also reported that, within the above temperature range, infection occurred when the product of temperature and wetness duration ranged between 44.0 and 59.5. Given the rapid transition between zoospore release, germination, and penetration, it is reasonable that all of these processes would have the

2.1. Relative proportion of leaf discs infected was calculated by dividing the percentage of foliar discs showing typical downy mildew lesions by the maximum percentage in each trial

same or similar environmental requirements, i.e., a mild temperature and free water (Williams et al. 2007a).

The equations developed here to describe zoospore release and infection by *P. viticola* at different temperatures and wetness durations provided a good fit to the data. The β -Gompertz and Weibull equations used in this study have been previously used in plant disease epidemiology (Anco et al. 2013; Arauz et al. 2010; González-Domínguez et al. 2013; Thal et al. 1984). The modified version of the Weibull equation proposed by Duthie (1997) has some advantages: (i) the equation is sufficiently flexible to generate different types of response surfaces, and (ii) all parameters used to describe the response to temperature and wetness duration have some biological significance, which is an advantage of the non-linear models (Madden et al. 2007). To fit data about infection efficiency of *P. viticola*, Lalancette et al. (1988) used a Richard equation in which the asymptotic parameter was derived as a function of temperature using a second-order polynomial. Both equations (i.e., Richard and Weibull) account for

Fig. 6 Post-inoculation efficacy (PIE) of two copper-based fungicides (Airone Più and Iperion) applied at three concentrations (2, 3, and 4 g L⁻¹ of commercial product) and six post-inoculation times (hpi). Values are means + standard errors value of two trials, with eight replicates (inoculated leaf discs) per trial. Airone Più contains 28 % copper oxychloride + 28 % copper hydroxide, and Iperion contains 37.5 % copper oxychloride



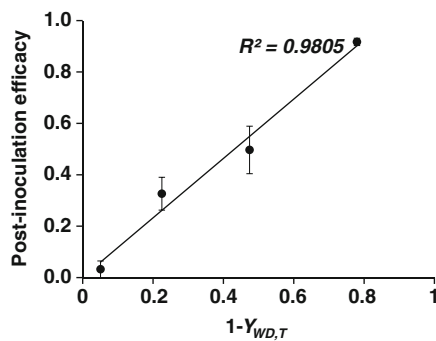


Fig. 7 Regression of post-infection efficacy (PIE) of the copper fungicides on the proportion of sporangia that have not caused infection yet ($1-Y_{WD,T}$) at different wetness periods after inoculation. For each combination of the two fungicides (P1 and P2) and three dosages (2, 3, and 4 g L⁻¹), values are the means+standard errors of two trials with eight replicates per trial. $1-Y_{WD,T}$ was calculated as in experiment 2

asymmetry of the curve that describes the response to leaf wetness duration (Madden et al. 2007). Concerning the effect of temperature, Lalancette et al. (1988) used a polynomial equation to define the asymptote (i.e., temperature affects the maximum infection efficiency only); the Weibull equation includes a nonlinear equation (named $f(T)$ in Eq. 2) that influences both the asymptote and the intrinsic rate of increase of the response to wetness duration, and the biological significance of each parameter of this temperature-dependent equation is clear (Duthie 1997). The latter is a clear advantage of the Weibull equation compared to the equation used by Lalancette et al. (1988) because polynomial models contribute little to the understanding of the underlying processes i.e., the structures of polynomial models do not reflect prior knowledge of the response (Duthie 1997).

The model proposed here uses the above equations to describe, through a mechanistic approach, the rates at which sporangia release zoospores and zoospores cause infection at any combination of temperature and wetness duration in a single infection period; the deposition rate of sporangia was described by an equation previously published (Caffi et al. 2013). Two assumptions were made in this model. The first assumption was that the number of viable sporangia present on leaves when the infection event begins (i.e., when a wet period begins, initiated either by dew deposition or rainfall) is insignificant. This assumption may be reasonable if no or very few living sporangia are present on grape surfaces during the dry period preceding the wet period. Sporulation occurs on downy mildew lesions at night and is

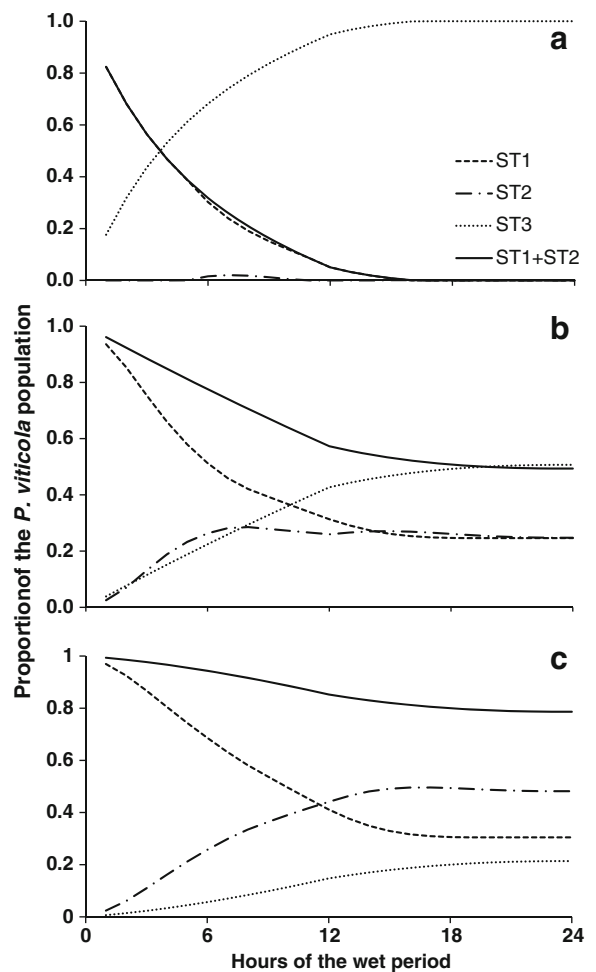


Fig. 8 *Plasmopara viticola* population dynamics predicted by the mechanistic model under three scenarios (a–c). In A, wetness duration (WD)=24 h, temperature (T)=20 °C, and the deposition rate of sporangia is fast. In B, WD=24 h, T=16 °C, and the deposition rate of sporangia is average. In C, WD=24 h, T=12 °C, the deposition rate of sporangia is slow. ST1: rate of living, ungerminated sporangia; ST2: rate of germinated sporangia; ST3: rate of infecting zoospores. ST1+ST2 refers to zoospores that remain in ungerminated sporangia and those that have been released and are swimming

triggered by some hours with rain, wetness, or very high humidity (Caffi et al. 2013). These sporangia can then be released over a wide range of environmental conditions (Caffi et al. 2013). Under vineyard conditions, dispersion of sporangia is associated with rainfall (Blaeser and Weltzien 1979; Kast 1997; Gilles 2004). Data from spore traps showed that the concentration of airborne sporangia during no-rain periods was very small compared to rain periods (Blaeser and Weltzien

1979; Kast 1997); as a consequence, very few sporangia may be deposited on leaves during no-rain periods. In addition, the sporangia deposited under dry conditions are subjected to desiccation. Detached sporangia died in less than 1 day when exposed to a range of temperature \times relative humidity conditions (Blaeser and Weltzien 1979). Kennelly et al. (2007) found that the viability of sporangia on leaves decreased significantly in the first few hours after sunrise under a range of environmental conditions, and that no sporangia were viable after 6 to 8 h of exposure. The above considerations (i.e., dispersal of significant numbers of sporangia is associated with rain, and detached sporangia die rapidly on dry plant surfaces) suggest that no or very few living sporangia may be present on grape leaves during dry periods, and this makes our assumption reasonable.

The second assumption made in the model was that there is no mortality of sporangia or zoospores during a discrete infection period, i.e., in the presence of wetness. No data exist in the literature about the mortality of sporangia or zoospores in water. However, because sporangia open quickly in water and because zoospores are adapted to live in water, it is reasonable to assume that there is little or no mortality in water.

The model proposed here can be used to improve the following weather-based models, which have been developed to simulate downy mildew epidemics resulting from repeated infection cycles driven by asexual sporangia: VINEMILD (Blaise and Gessler 1990), MIL-VIT (Magnien et al. 1991), D-model (Magarey et al. 1991), PRO (Hill 1990), and PLASMO (Orlandini et al. 1993). These models use different approaches to quantify infection severity for any infection period, and all of these approaches would benefit from our results. VINEMILD, which is based on Vanderplank's equation (Blaise and Gessler 1990), uses an infection rate (R_c) that accounts for the susceptibility of the host, aggressiveness of the pathogen, and the favorability of the environment; R_c is empirically estimated for each epidemic. Similarly, the PRO model (Hill 1990) uses an infection rate that was empirically defined based on disease development observations in German vineyards. For these two models, using our equations in place of the empiric values would likely increase model robustness under changing environmental conditions (Rossi et al. 2010). The D-model (Magarey et al. 1991) calculates the severity of any secondary infection by accumulating degree-hours while leaves are continuously wet; in this model,

infection severity ranges between 0 and 1 when degree-hours range between 45 and 71, and this was based on the data of Blaeser and Weltzien (1979). The data of Blaeser and Weltzien (1979), however, define the minimum hours of wetness necessary for a *P. viticola* infection to occur at a given temperature rather than defining infection severity, as our equations do. The PLASMO model (Orlandini et al. 1993) calculates the area of the newly infected tissue generated by an infection period; the calculation uses the equation of Lalancette et al. (1988), which was developed for *V. labrusca* rather than for *V. vinifera*.

In addition to improving these disease models, our equations could be used to evaluate whether a fungicide application during a discrete infection period is likely to be effective. A similar approach has been considered for other plant diseases, such as apple scab, for which the treatment is referred to as a 'during-infection' spray (Jamar et al. 2008). An apple scab model was developed (Rossi et al. 2006) that simulates the dynamics for the following four fungal stages on the plant surface during a discrete infection event triggered by rainfall: non-germinated ascospores, ascospores germinated that have not yet produced an appressorium, ascospores with an appressorium, and ascospores that have successfully completed the infection process. The 'during-infection' spray is applied when that part of the fungal population that is susceptible to a protective fungicide (i.e., that is still on the plant surface and has not yet penetrated) is more prevalent than that part that is not susceptible (i.e., that has already caused infection).

The use of 'during-infection' sprays against *P. viticola* depends on the ability of a fungicide to affect non-germinated sporangia, swimming zoospores, and germinating zoospores of this fungus. In this work, the post-inoculation efficacy of two copper-based fungicides was significantly correlated with the proportion of *P. viticola* sporangia deposited on a leaf that had not yet caused infection. Copper is a multi-site contact fungicide that disrupts cell enzymatic systems (FRAC 2015). Thus, the post-inoculation activity of copper against *P. viticola* may be due to its activity against the pathogen structures on the plant surface, i.e., structures that have not yet participated in penetration of the host tissue.

In the current study, the post-inoculation efficacy of copper at 20 °C (the optimal temperature for *P. viticola* development) diminished rapidly and was <20 % when the copper was applied 6 h after *P. viticola* inoculation.

The effect of time on the post-inoculation activity of copper has been reported for other pathogens. For *Fusicladium eriobotryae* (the causal agent of loquat scab) (González-Domínguez et al. 2014) and *Fusicladium oleagineum* (olive scab) (Obanor et al. 2008b), efficacy was 80 and 50 % when copper-based fungicides were applied 1 and 3 days after inoculation, respectively. For *Diaporthe citri* (citrus melanose) and *Elsinoe fawcettii* (citrus scab), copper compounds had 60 and 25 % efficacy at 1 and 3 days after inoculation, respectively (Mondal et al. 2007). The higher post-infection efficacy documented in the above cases than with *P. viticola* in the current study may be due to differences in the infection processes between the fungi. For instance, *F. eriobotryae* and *F. oleagineum* require >12 h of wetness for infection to begin (González-Domínguez et al. 2013; Obanor et al. 2008a).

Based on the rapid decline in the post-inoculation activity of copper against *P. viticola* documented in this work, one might conclude that a ‘during-infection’ spray is unlikely to be effective against grape downy mildew. However, this rapid decline was observed under optimal conditions for *P. viticola* infection and with a very high concentration of sporangia, all of which were applied at the same time. Under natural conditions, the sporangia are deposited on plant surfaces in lower numbers and over time, and the rate at which these sporangia cause infection depends on environmental conditions. When the model developed in this work was applied to different scenarios involving more realistic sporangia deposition rates and suboptimal environmental conditions, the potential efficacy of a copper treatment increased. For instance, if the temperature after a rainfall that triggered a wet period was set at 12 °C and the deposition rate was low, the efficacy of a copper treatment applied 9 and 24 h after the start of the wet period was estimated to be >90 and >78 %, respectively.

These scenario results should be confirmed under vineyard conditions, where environmental and host conditions change and result in complex interactions. After a robust field validation, the model developed here could help organic viticulturists to estimate the efficacy of a copper treatment applied during a wet period initiated by rain. In apple scab, this strategy has made it possible to reduce the amount of fungicides used in organic orchards by 70 % (Jamar et al. 2008). The same help may be provided to non-organic viticulturists once the post-inoculation efficacy is known for synthetic protectant fungicides, such as mancozeb, thiram and

captan (FRAC 2015). The ‘during-infection’ use of these fungicides may help (i) reduce the number of treatments compared to a calendar-based application, and (ii) reduce the use of systemic fungicides, which frequently have a high risk of resistance (Brent and Hollomon 2007).

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