Effect of environmental conditions and phenology in the dispersal of secondary *Erysiphe necator* conidia in a vineyard

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Summary

An integrated powdery mildew management strategy to identify the principal moments of secondary *Erysiphe necator* conidia dispersal in a vineyard based on aerobiological, phenological and meteorological data was developed. An adaptation of the physiological P-days model was conducted to obtain a descriptive equation for the prediction of the advantageous meteorological conditions for *Erysiphe necator* conidia dispersal in the vineyards of the Ribeiro Designation of Origin area. Moreover, a regression model was developed to predict the conidia concentration as a function of the weather and aerobiological variables with the highest influence on airborne *E. necator* spores. Additionally, phenological observations were conducted during the vegetative cycle of *Vitis vinifera*, with the aim to identify the most susceptible phenological stages to powdery mildew infection by relating them with the detected atmospheric spore concentrations. The study was carried out from 2008 to 2018 in an experimental vineyard. The *E. necator* spores were trapped using a Lanzoni VPPS-2000 sampler and the phenological observations were conducted following the BBCH standardized scale. The highest total fungal spore amount per season in the atmosphere of the vineyard was detected in 2013 with 4828 spores m^{-3}, while the lowest amount was recorded in 2009 with 883 spores m^{-3}. In general, the highest daily airborne spore concentrations were detected during the Flowering (stage 6) or in the previous and next stages, whereas the maximum total spore amount by stage was recorded during Development of Fruits (stage 7). The proposed threshold of P-days for potential secondary infections in the Ribeiro D.O. ranges from 120 to 160 P-days. The combination of aerobiological, phenological and meteorological data provides us a useful tool for the knowledge of the *Erysiphe necator* conidia dispersal behaviour bringing agricultural practices closer to a sustainable system.

K e y   w o r d s :   *Erysiphe necator*; P-days, conidia dispersal; grapevine; airborne spores forecast.

Introduction

The wine-making sector embraces important worldwide socio-cultural and environment values. During the last dec-ades, we are witnessing a huge agriculture mechanization and a rapid modernization of the European viticulture sector because of investments in this primary economical area. The biggest vineyard surface at world level was placed in Spain (13 %) been the third largest wine producer country (13 %) just below Italy (18 %) and France (17 %) (Mapama 2016). Diseases and pests of crops affect potential yields and economic profits. A co-evolution of plants and their parasitic fungi was detected, which explains their fitted adaptation to the real changing conditions of the vineyard agrosystem.

The specific bioclimatological conditions of Northwest Spain favour the development of fungal phytopathogenic diseases, which are responsible for remarkable annual yield loss. Powdery mildew, caused by *Erysiphe necator* is one of the main harmful grapevine diseases in most growing areas of the world (Bois *et al.* 2017).

E. necator belongs to the Erysiphaceae family, being an obligate vine parasitic ascomycetous fungi with a bipolar-heterothallic mating system (Evans *et al.* 1997). The disease cycle of this fungus is based on two main sources of primary inoculum, which are the mycelium within dormant buds and the ascospores released by cleistothecia (Halleen and Holz 2001). In areas with mild winters, dormant infected lateral buds cause flag shoots in the following spring (Hill 1990). But in colder viticultural regions flag shoots are not produced, what had been attributed to the reduced winter hardiness of the infected lateral buds (Gadoury *et al.* 2012). The weakening and mortality of hyphal tissues within the active colonies under low temperatures was demonstrated for nascent colonies on leaves (Moyer *et al.* 2010). The overwintering cleistothecia are an additional source of primary inoculum in the areas where the flag shoots are common (Gadoury *et al.* 2012). This resistance structure is the result of the sexual part of the pathogen's life cycle, and it implies the contact between two parent hyphae. In very rainy areas, the emergence of the primary infection from ascospores released by cleistothecia is the prevailing process (Jarvis *et al.* 2002). During late summer and autumn, mature cleistothecia are dispersed by rain to the bark of the vine or to the soil (Gadoury and Pearson 1988), where they overwinter. These structures can be dispersed as well by high winds (Grove 2004). The optimal temperature for infection by ascospores is 20 °C to 25 °C, being significantly reduced at 15 °C or below. The thermal thresholds for ascosporic infections are 5 °C and 31 °C (Jailloux *et al.* 1998). The fastest mildew colony growth and sporulation occurs from 23 °C to 30 °C, with an optimum temperature of 26 °C (Delp

1954). For initiation of asexual sporulation, a previous period of superficial vegetative growth of mildew colonies is necessary. Gadoury *et al.* (2012) found that the stimulation of conidiation in the colony comes from a quorum-sensing signal from the centre of the colony. They demonstrated that the removal of the colony centre before signalling nearly doubled the latent period from hyphae development to sporulation. This latent period can be increased as well by the thermal conditions, from 5 d with constant optimal temperatures to a minimum of 25 d at 9 °C and under field conditions. Dispersal of conidia is very important in polycyclic diseases with aerial spread, such as grape powdery mildew. This factor determines the spatio-temporal extension of epidemics (Aylor 1990). Conidia dispersal is a passive process triggered by weather (Willocquet *et al.* 1998) and crop managing factors. High-pressure sprays against other grape harmful pathogens (like insects or fungal diseases) can enhance powdery mildew epidemics by conidia dispersal, as the high flow rates generated induce high turbulence in the canopy with shaking of infected leaves and high air speeds at the sporulating powdery colonies of lesion sites (Willocquet and Clerjeau 1998). Wind causes instantaneous dispersal of *E. necator* conidia, although the minimum wind speed for dispersal is 2.3 m·s^{-1} (Willocquet *et al.* 1998, Halleen and Holz 2001).

All green organs are susceptible to powdery mildew infection: sprouts, leaves, inflorescences and immature berries (Fernández-González *et al.* 2013). Infection symptoms appears as grey-white dust on both sides of leaves or fruits (represented by superficial mycelium and conidia) and diffuse dark green spots in vine shoots (Bendek *et al.* 2002). Split berries indicate a typical signal of disease presence, due to parasitized epidermic dead tissue in young berries when the fruit is still growing (Carisse *et al.* 2006). Skin lesions represent an important penetration point for other phytopathogens, such as *Botrytis cinerea* (Carisse *et al.* 2006, Fernández-González *et al.* 2012), since the grape skin acts as an effective barrier against conidia penetration (Coertze *et al.* 2001).

The biotrophic nature of *E. necator* exposes the yeast to a special sensitivity to environmental conditions such as temperature, solar radiation or atmospheric humidity (Delp 1954, Schnathorst 1965, Chellemi and Marois 1991a, Austin *et al.* 2009). Disease development is based on a host-pathogen relation. Consequently, host phenology, which determines plant susceptibility to fungal infection, represent a key factor for the fungal infection processes. Furthermore, grapevine training system, crop nutrition and soil conditions influence the disease advance degree due to changes in the vineyard ecosystem properties and their micro-climatic conditions (Zahavi *et al.* 2001, Jarvis *et al.* 2002, Scandellari 2017, Zheng *et al.* 2017, Kraus *et al.* 2018).

Frequently, the most used strategy for winegrowers to fight against fungal crop diseases is the systematic application of phytosanitary products following pre-set calendars based on plant phenology (Martínez-Bracero *et al.* 2019). In Northwestern Spain, most of them are conducted without taking into account the dispersion pattern of the fungus as

treatment decisions only consider meteorological variables as high temperature conditions after rainy days. But the disease events are promoted by a combination of different favourable conditions. Only a treatment application should be necessary when both, a real risk of infection and a potentially economic damage are identified. Excessive use of phytosanitary chemicals causes serious consequences for the environment and human health (Gubler and Thomas 2006). One of the most important is related with the crop ecosystem damage by stimulating the appearance of resistances in the fungus or the beneficial mycological flora reduction (Gubler and Thomas 2006). Most of the Integrated Pest Management strategies are based on a replacement or reduction of the chemical protection techniques with a more eco-friendly purpose (Barzman *et al.* 2015).

As part of these control strategies, several models have been developed to achieve a better phytosanitary control of grape powdery mildew (Bendek *et al.* 2007, Fernández-González *et al.* 2013, Arafat 2015, Oriolani *et al.* 2015). The airborne spore concentration is a great indicator of the phytochemical treatment need, as the inoculum presence can vary within the different development stages of polycyclic diseases (Thiessen *et al.* 2017). Some of the models are focussed on primary ascosporic infection processes (Caffi *et al.* 2011, 2012), and most of them are based on weather related variables (Arafat 2015, Oriolani *et al.* 2015). Among the weather-based models, the Gubler-Thomas risk index (Gubler *et al.* 1999, Gubler and Thomas 2006) is the most widely used as epidemiological model for grapevine powdery mildew as it works with temperature and leaf wetness duration to predict the ascospores release to initiate the risk index. This model, that predicts both ascosporic and secondary conidial stages of the disease, ranges from 0 to 100 acting as an advisory tool for growers, who can use it to adjust the fungicide timing. Moreover, it was originally developed to be used in California, and has been successfully adapted to other bioclimatic areas (Bendek *et al.* 2007, Fernández-González *et al.* 2013), and even to other crops (Grove *et al.* 2000). This shows the ability of a weather-based model to be adapted. Following this, we propose the adaptation of a potato yield prediction model (Sands *et al.* 1979), that relates the crop physiology and the tuber bulking stage development with weather variables, to develop a descriptive equation for the *E. necator* conidial dispersal in a vineyard based on the thermal conditions of the studied area. This crop physiological model (Sands *et al.* 1979) was previously adapted to forecast a fungal disease, by determining the threshold for a detrimental presence of *Alternaria solani* Sorareur, responsible of the potato early blight (Gent and Schwartz 2003). This adaptation was possible as fungal development is strongly related to weather conditions, as well as crop phenology.

The aim of this study was to analyse the conidia dispersal behaviour of *E. necator* in the D.O. Ribeiro vineyards based on all three causal components of the disease triangle: 1. Phenological information (the assessment of the most susceptible infection stages of the plant), 2. Pathogen presence data (by using a bioindicator such as the levels of airborne fungal spores and their prediction by means the

development of a descriptive equation), 3. The prediction of the occurrence of ideal environmental conditions for the conidial dispersal and possible pathogen growth (following the p-days values model). The combination of these variables could help us to achieve a phytosanitary treatment reduction, which involves a more effective and less environmental aggressive crop protection. In addition, several economic profits are obtained as consequence of the culture management costs reduction and the product quality improvement.

Material and Methods

The study was carried out in an experimental vineyard of the "Vitivinicola do Ribeiro S.C.G." company located at Cenlle belonging to the Designation of Origin area Ribeiro enclosed in the Northwest part of Iberian Peninsula (42°18'49.40"N; 8°6'14.60"W). Following the Multicriteria Climatic Classification System (MCC), most of winemaking regions in this area influenced by the Miño river region would be defined as temperate and warm, sub-humid, with very cold nights (Blanco-Ward *et al.* 2007).

The aerobiological study period took place during the active *Vitis vinifera* L. season since 2008 to 2018, from March 1st to the harvest date. Data of the years 2016 and 2107 were not considered as consequence of accidental chemical treatments conducted in the experimental vineyard. A Lanzoni VPPS 2000 volumetric pollen-spore trap (Hirst 1952) calibrated with a flow of 10 L·min⁻¹ was used to monitor the airborne fungal spore concentrations. The sampler was situated in the central part of the vineyard at plant level 2 meters above ground level in order not to difficult spore trapping by plant growth. Inside the device a melinex tape coated with a 2 % silicone solution was used as spore-trapping surface, which spins at 2 mm·h⁻¹ during a seven-day period. The exposed tape was mounted on daily separate glass slides, by using glycerogelatine between slides and covers, for microscopic analysis with a NIKON OPTIPHOT II microscope with a 60x/0.95 resolution lens. *E. necator* conidia spores were counted and identified (Aira *et al.* 2005) following the protocol proposed by the Spanish Aerobiological Network (REA) based on two longitudinal transects along the slides (Galán *et al.* 2007). A correction factor based on the volume of sampled air, the hole sampler entrance air dimensions, the number of the total analysed slide area and the optical characteristics of the used microscope was applied to the total number of observed conidia on daily samples (Galán *et al.* 2007). The result of this multiplication gave us the daily concentrations of airborne spores per m³ of air. To obtain the spores amount per phenological stage, the daily spore atmospheric concentrations recorded during a given phenological stage were added. To obtain the annual total spores per season we summed up the daily spore atmospheric concentrations of the entire season for each year.

Phenological observations were conducted during the active grapevine growing season from 2008 to 2018 following the standardized BBCH scale (Meier 2001). The 'Treixadura' cultivar was considered for this study due to the preferential character of this autochthonous variety in the Ribeiro D.O. Twenty-two plants with a size of 1,5 m in vegetative rest and 20 years old were randomly selected. A total of 15 phenological phases were identified, belonging to five principal stages: Leaf Development (stage 1), Inflorescence Emerge (stage 5), Flowering (stage 6), Development of Fruits (stage 7) and Ripening of Berries (stage 8) (Lorenz *et al.* 1994). Observations were weekly conducted from the beginning of the stage 1 until the harvest date, increasing to twice a week during Flowering.

Meteorological information was obtained by means of a HOBO Micro Station data logger located in the vineyard. The considered parameters were mean temperature (°C), maximum temperature (°C), minimum temperature (°C), relative humidity (%) and dew point (°C). Information about rainfall (mm) and wind speed (km·h⁻¹) was obtained from the MeteoGalicia Agrometeorological Station located at 1 km from the experimental vineyard in Leiro (MeteoGalicia 2018).

A descriptive equation to predict the days with advantageous environmental conditions for *E. necator* conidia dispersal was adapted from the P-days model (Sands *et al.* 1979). This physiological model offers a plant development measure based on cardinal temperatures, considering as minimum, optimum and maximum growth temperatures 7, 21 and 30 °C respectively (Gent and Schwartz 2003, Pscheidt and Stevenson 1986). P-days daily values from the original model are calculated with daily mean, maximum and minimum temperature values and the cardinal temperatures included in the following expression (Eq. 1):

Eq. 1. P-Days = {1/24[5.*P*(*Tmean*).(*Tmin*) + 8.*P*(*Tmean*).(2.*Tmin*/3 + *Tmax*/3) + 8.*P*(*Tmean*).(2.*Tmax*/3 + *Tmin*/3) + 3.*P*(*Tmean*).(*Tmax*)]}

where *P*(*Tmean*) is a coefficient that depends on the mean temperature value for each considered day as follows (Eq. 2):

P(*Tmean*) = 0 if *Tmean* < 7 °C or if *Tmean* ≥ 30 °C
P(*Tmean*) = 10[1 − (*Tmean* −21)²/(21 − 7)²] if 7 °C ≤ *Tmean* < 21 °C
P(*Tmean*) = 10[1 − (*Tmean* − 21)²/(30 − 21)²] if 21 °C ≤ *Tmean* < 30 °C

(Tmean = daily mean temperature in °C; Tmin = daily minimum temperature in °C; Tmax = daily maximum temperature in °C).

This equation accumulates P-days values until a limit value that indicates a close disease development. The model is only applicable for the first appearance of high fungal spore concentration in the crop above 35 spores m⁻³ of air. We propose a modification of the equations regarding the cardinal temperatures included in the equation (adopting development thermal thresholds for *E. necator)* as well as the direct use of the obtained daily values by the model instead of accumulated values. This modification allowed us to relate the conidia dispersal with thermal conditions all over the season by means only use of temperature data, obtaining daily values that indicate possible secondary conidial atmospheric dispersal episodes that we measure by high airborne spore concentrations. In our study the P-days daily values were calculated for the entire studied period, paying special attention to the values obtained during the First Atmospheric

Spore Peak (the first peak above 35 spores per m³ of air) and the Maximum Atmospheric Spore Peak (the date with maximum spore concentrations in the vineyard) for each studied year. The thermal ranges for *E. necator* conidia dispersal in the proposed adapted equation were obtained from different studies (Delp 1954, Peduto *et al.* 2013). Delp (1954) suggested minimum temperatures around 6 °C for the fungus germination, whilst fast germination, infection and growth occurred between 21 and 30 °C (optimum temperature for germination at 25 °C). Peduto *et al.* (2013) analyzed the *E. necator* colony growth, spore production and germination at eight temperatures above 30 °C and at different exposure times. The temperatures that inhibit the activity of the fungus started at 36 to 38 °C. Based on these findings, temperature values of 6, 25 and 35 °C were considered as minimum, optimum and maximum for the fungus dispersal and development in the proposed adapted equation. We define the final modified expression for P-days, with the new cardinal temperatures, as (Eq. 2):

Eq. 2. P-Days = $\{1/24[5.P(Tmean).(Tmin) + 8.P(Tmean).(2.Tmin/3 + Tmax/3) + 8.P(Tmean).(2.Tmax/3 + Tmin/3) + 3.P(Tmean).(Tmax)]\}$

where $P(Tmean)$ is a coefficient that depends on the mean temperature value for each considered day as follows:

$P(Tmean) = 0$ if $Tmean < 6$ °C or if $Tmean \geq 35$ °C
$P(Tmean) = 10[1 - (Tmean - 25)^2/(25 - 6)^2]$ if 6 °C $\leq Tmean < 25$ °C
$P(Tmean) = 10[1 - (Tmean - 25)^2/(35 - 25)^2]$ if 25 °C $\leq Tmean < 35$ °C

(Tmean = daily mean temperature in °C; Tmin = daily minimum temperature in °C; Tmax = daily maximum temperature in °C).

This equation provides daily P-days values, represented in Fig. 2. Paying attention to the First and Maximum *E. necator* spores peaks, we decide to determine the range between 100 and 160 P-days value as the possible threshold for secondary dispersal conidia episodes. We detected that high airborne spore concentrations for First and Maximum spore peaks were detected after this limit P-days values. The obtained information about the pathogen conidial dispersal depending on temperatures can be used as a possible direct secondary infections risk alert. As previously mentioned, under advantageous meteorological conditions, a latent period of 5 d was stated from aerial conidial detection to conidiation growth for plant lesions (Gadoury *et al.* 2012).

For the statistical analysis, a statistical Spearman´s correlation test was applied to determine the association degree between airborne spore concentration and the main meteorological parameters (maximum, minimum and average temperatures, dew point, relative humidity, rainfall and wind speed). Weather conditions may affect spore production directly or indirectly through their effect on the substrates colonised by the fungus. For that reason, this study also determined the correlation between spore counts for a given day and the main weather-parameter values from the previous one to seven days, time gap considered for sporulation to occur (Carisse *et al.* 2008). The correlation coefficients were calculated using data from 2008 to 2015. The objective of this analysis was to determine the parameters with the highest significant influence on the airborne

spore concentration. Significance was calculated for $p \leq 0.01$, $p \leq 0.05$ and $p \leq 0.1$. Based on these statistical results, a multiple linear regression model was developed to predict the spore concentration in the atmosphere as a function of the weather related variables displaying the highest correlation coefficients. For model development, data from 2008 to 2015 were used. The model accuracy was assessed by the comparison between the real *Erysiphe* airborne conidia airborne concentration detected in 2018 (year not included in the conidial regression model development) and the forecast values with the proposed model for the same year. Moreover, its accuracy was statistically assessed by a *t*-test for dependent samples and the leave-one-out cross-validation procedure. The means and the standard deviations of predicted and observed data for 2018 were compared by the *t*-test, what determined if the differences between these two data set are statistically significant or are only consequence of random differences. The Leave-one-out cross-validation (LOOCV) is a technique used to evaluate the results of a statistical analysis and to ensure that the data used for the training of the model are independent from those used to test the model. Each of the eight study years validation interation comprised observed and expected *E. necator* conidia airborne concentrations, leaving the corresponding annual value out of model run. For each of the N iterations an error calculation was performed. STATISTICA software package was used for the statistical analysis.

Results

Aerobiological analysis showed that the highest *E. necator* spores annual total amount was detected in 2013 with 4828 spores m⁻³ during the year, while the lowest was recorded in 2009 with 883 spores m⁻³ sampled all over the grapevine season (Fig. 1). The presence of the *E. necator* spores in the atmosphere of the vineyard was not constant along the studied period. The highest airborne spore daily concentration during all the studied period was detected in 2014 with 417 spores m⁻³ on May 22nd. In general, high spore values were registered between the second fortnight of May and the beginning of August, with the exception of the year 2015, where the highest values started earlier at the beginning of April (Fig. 1). When the spore amount per phenological stage was studied, the maximum values were recorded in 2014 during the Stage 6 of Flowering with 6386 spores m⁻³ (Fig. 2). The maximum spore amount by stage was recorded during Development of Fruits (stage 7) in six of the studied years (2008, 2009, 2010, 2011, 2012 and 2015). In general, high maximum daily spore concentrations occurred during Flowering stage (stage 6) and the previous and next stages, Inflorescence Emerge (stage 5) and Development of Fruits (stage 7).

The adapted physiological P-days descriptive equation for conidia dispersal advantageous meteorological conditions was determined for the First *E. necator* Spores Peak and the Maximum Spore Peak concentrations for each year. In the case of First Spore Peak, the registered P-days index values were lower than the obtained for the Maximum Spore Peak with the exception of 2008 and 2014 (Fig. 3). In two of
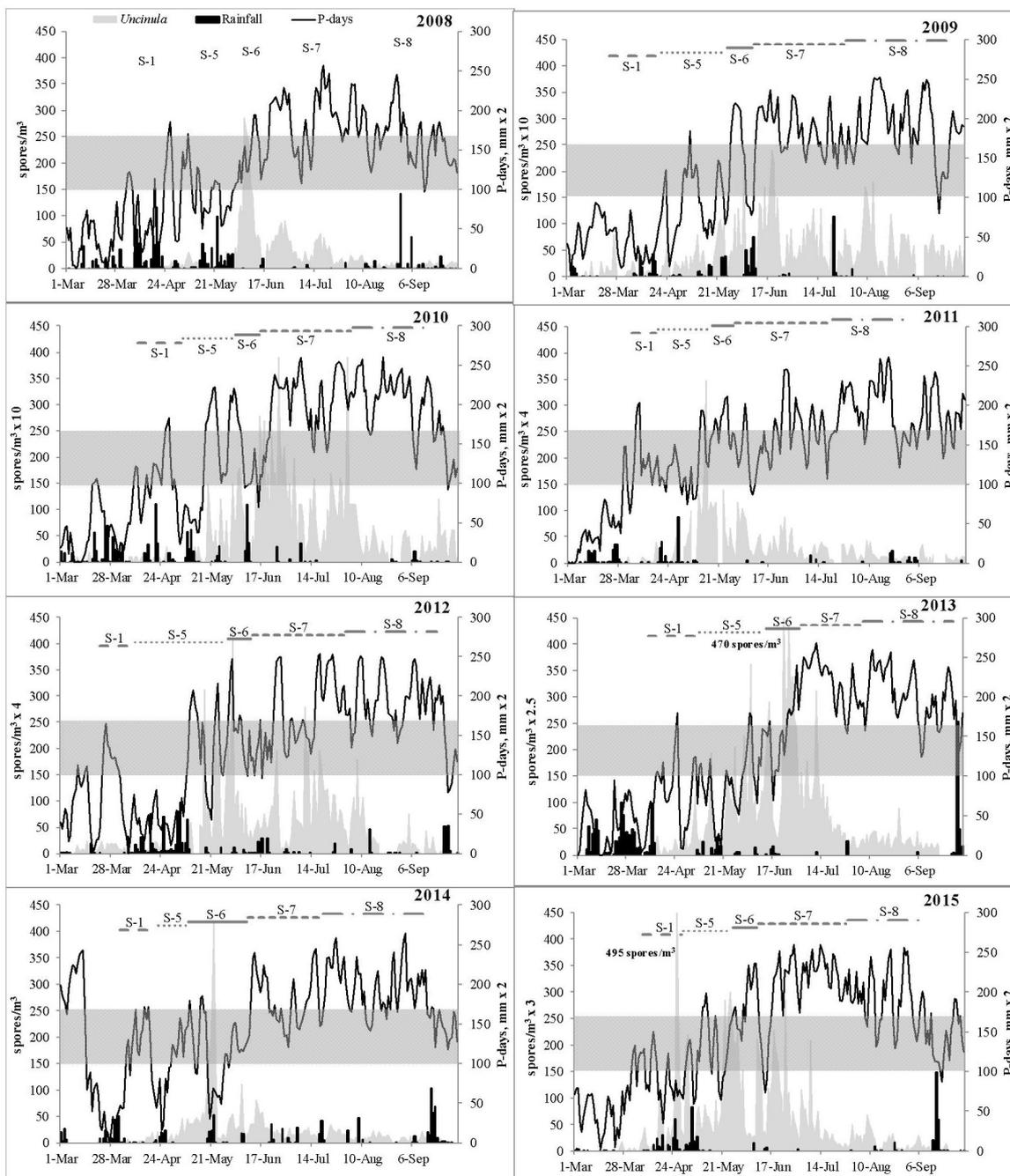
Fig. 1: *Erysiphe* airborne spore concentrations (grey area), rainfall (black bars) and modified P-days equation values (black line) for the studied period, from 2008 to 2015. For a better visualization of the graphs, the values of the spore concentrations were x10 in the years 2009 and 2010, x4 in the years 2011 and 2012, x3 in 2015 and x2.5 in 2013. Rainfall (mm) was x2 for all years. Dark grey horizontal band indicates the identified P-days range between 100 and 160 P-days, what marks risk of conidia dispersal. In the upper part of the graphs the principal phenological stages of the grapevine vegetative cycle are represented by means different kind of horizontal lines: Stage 1 Leaf Development (S-1), Stage 5 Inflorescence Emerge (S-5), Stage 6 Flowering (S-6), Stage 7 Development of Fruits (S-7) and Stage 8 Ripening of Berries (S-8).

the eigth study years (2009 and 2015) the value of 100 P-days was not reached. The Maximum Spore Peak occurs when the P-days index exceeded the value of 100 P-days in most of the study years, with the exception of 2014 and 2015 which did not reach 100 P-days. Moreover, a 150 P-days value was exceeded in four years (2008, 2009, 2010 and 2012). In the area of the study the mean value of the P-days index considering the total data set was 129 P-days for the First Spore Peak and 153 P-days for the Maximum Spore Peak. These results highlighted a threshold of P-days for poten-

tial infections in the Ribeiro D.O. ranges between 120-160 P-days. This boundary marked the presence of the *E. necator* airborne spore peaks for each year. During the first days of the vine active vegetative cycle low spore concentrations were detected. From the end of April to the first days of May the conidial concentration start to rise, coinciding with the increase of the P-days value until stage 5 (Inflorescence Emerge) (Fig. 1). Statistical correlation analysis was carried out between the atmospheric spore concentration and the meteorological variables of the same day and the 1-7 previous
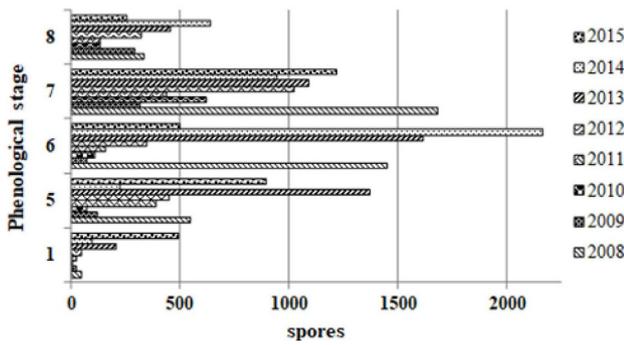
Fig. 2: Total airborne *Erysiphe* spore amount for each phenological stage, Stage 1 (Leaf Development), Stage 5 (Inflorescence Emerge), Stage 6 (Flowering), Stage 7 (Development of Fruits) and Stage 8 (Ripening of Berries), for the studied period.
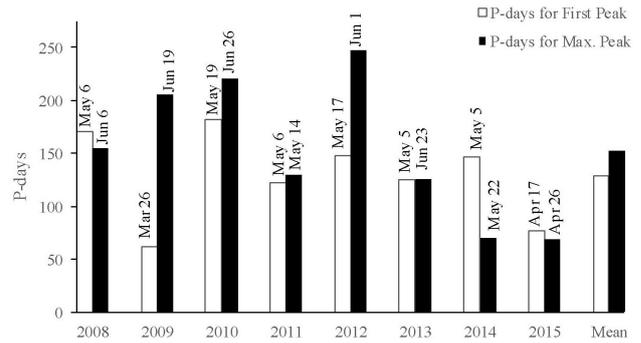

Fig. 3: P-days values for First (white column) and Maximum (black column) *Erysiphe* spores Peak dates, for the studied period. Each date indicates the peak date.

days. The Spearman correlation test using data for the whole study period showed high significant correlation values ($p \leq 0.01$) for all variables with the exception of the relative humidity of 7 d before. The higher Spearman coefficient values were obtained between the spore concentration and the spore concentrations of previous days and the mean,

maximum or minimum temperatures (Tab. 1). Based on these correlation results a multiple linear regression model was developed in order to predict the airborne *E. necator* spore concentration as a function of most influential variables. The obtained model used as independent variables the *Erysiphe* spore concentration and the rainfall of the previous day. The regression equation explained the 66.8 % of the prediction variability with a F-value of 1889.7 (f.d. 2.188) (Tab. 2). The accuracy of the developed model was evaluated by the graphical comparison between the observed *E. necator* spore concentrations during the year 2018 and the values predicted with the model (Fig. 4). The predicted values matched actual spore counts in most cases. This was demonstrated by a dependent samples *t*-test applied to the real and forecasted values for 2018 as no statistically significant difference was found at the 95 % level (p value of 0.778) (Tab. 3). Linear regression analysis (Fig. 5) and Leave-one-out cross-validation procedure (Fig. 6) reinforce the accuracy of the developed model.

Table 1

Spearman's correlation test values ($p \leq 0.01$*** highly significant; $p \leq 0.05$** very significant; $p \leq 0.1$* significant) between *Erysiphe* airborne spores and the main aerobiological and meteorological variables

Spore concentration		RH (%)	-0.244***
Spore concentration -1	0.817***	RH -1	-0.228***
Spore concentration -2	0.767***	RH -2	-0.202***
Spore concentration -3	0.738***	RH -3	-0.153***
Spore concentration -4	0.717***	RH -4	-0.122***
Spore concentration -5	0.700***	RH -5	-0.091***
Spore concentration -6	0.680***	RH -6	-0.062***
Spore concentration -7	0.666***	RH -7	-0.042*
MeanTemp. (ºC)	0.383***	Dew Point (°C)	0.270***
Mean Temp -1	0.363***	Dew Point -1	0.249***
Mean Temp -2	0.332***	Dew Point -2	0.227***
Mean Temp -3	0.301***	Dew Point -3	0.215***
Mean Temp -4	0.282***	Dew Point -4	0.211***
Mean Temp -5	0.260***	Dew Point -5	0.201***
Mean Temp -6	0.239***	Dew Point -6	0.186***
Mean Temp -7	0.219***	Dew Point -7	0.178***
Max. Temp. (ºC)	0.351***	Rainfall (mm)	-0.217***
Max. Temp -1	0.339***	Rainfall -1	-0.235***
Max. Temp -2	0.301***	Rainfall -2	-0.213***
Max. Temp -3	0.263***	Rainfall -3	-0.171***
Max. Temp -4	0.237***	Rainfall -4	-0.146***
Max. Temp -5	0.217***	Rainfall -5	-0.115***
Max. Temp -6	0.192***	Rainfall -6	-0.090***
Max. Temp -7	0.175***	Rainfall -7	-0.070***
Min. Temp. (ºC)	0.309***	Wind Speed (km·h⁻¹)	0.120***
Min. Temp -1	0.280***	Wind Speed -1	0.081***
Min. Temp -2	0.266***	Wind Speed -2	0.084***
Min. Temp -3	0.260***	Wind Speed -3	0.088***
Min. Temp -4	0.256***	Wind Speed -4	0.094***
Min. Temp -5	0.242***	Wind Speed -5	0.087***
Min. Temp -6	0.225***	Wind Speed -6	0.080***
Min. Temp -7	0.211***	Wind Speed -7	0.084***

Discussion

A physiological prediction model (SANDS *et al.* 1979) based on developmental thermal conditions to fungal growth and infection was adapted to identify the risk of powdery mildew secondary conidia dispersal episodes considering the First or the Maximum Spore Peak in the atmosphere of the vineyard. The P-days model, developed by SANDS *et al.* (1979), had already been used to predict the first appearance of potato early blight, caused by *Alternaria solani*, an important disease on these crops (PSCHEIDT and STEVENSON 1986). In this case, many surveys indicated a 300 P-days limit for the appearance of disease symptoms (GENT and SCHWARTZ 2003, PSCHEIDT and STEVENSON 1986). This 300 threshold was effective in US states (Wisconsin and Colorado in these cases), but variations in the limit value for the first disease appearance depending on bioclimatic region were observed
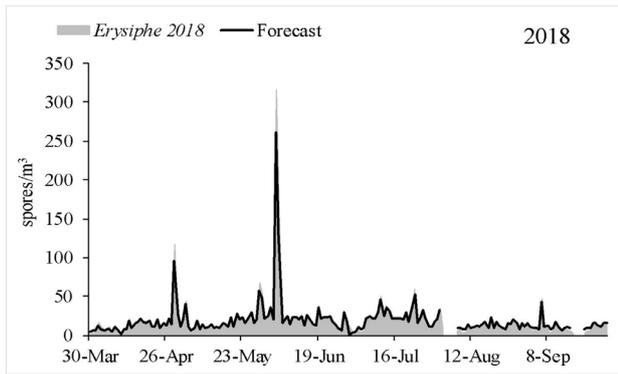
Fig. 4: Real and forecast values of *Erysiphe* airborne spore concentration in 2018.

Fig. 6: Leave-one-out cross-validation procedure between real and forecast values of *Erysiphe* airborne spore concentration along the studied period.
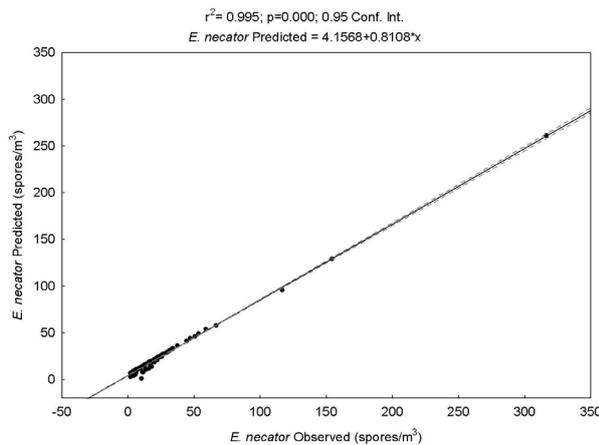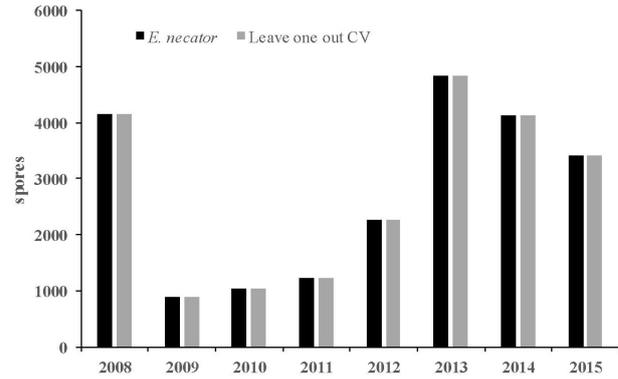

Fig. 5: Linear regression analysis between real and forecast values of *Erysiphe* airborne spore concentration in 2018.

(Gent and Schwartz 2003). Our study showed lower mean values of the P-days index of 129 P-days for the First Spore Peak and 153 P-days for the Maximum Spore Peak in the vineyard. These data are in accordance with other studies developed in Northwest Spain for potato early blight, which

pointed out values between 150 and 175 units of accumulated P-days index for first disease symptoms manifestation (Escuredo *et al.* 2011, Iglesias *et al.* 2007). Our calculations for powdery mildew conidia dispersal in grapevine were not conducted in an accumulative way. They were based on cardinal temperatures due to differences on crop growth between an annual and a perennial plant (Gent and Schwartz 2003, Pscheidt and Stevenson 1986). Early blight disease prediction with this model offered a 2 to 32 d gap to foresee first peak spore value applied as an accumulative expression (Gent and Schwartz 2003). Nevertheless, our modification allows using the model throughout the entire season, indicating the favourable environmental conditions for the successive secondary fungal conidial dispersion development. These characteristics provide different possibilities to execute action plans on crop protection based on weather predictions. Our study noted that high airborne spore concentration values corresponded with high values of the adapted P-days equation. A number of surveys have sought to relate disease levels at a given time with airborne spore concentrations during previous periods (Carisse *et al.*

T a b l e   2

Multiple linear regression model developed to forecast *Erysiphe* airborne spore concentration. In bold the equation coefficients (*Erysiphe*-1: *Erysiphe* airborne spore concentration of the previous day; Rainfall^{-1}: Rainfall of the previous day)

R	R^2	Adjust R^2	F	Freedom degrees (f.d.)	$p <$	Standard error
0.817	0.668	0.668	1889.7	2.188	0.000	28.360

$$Erysiphe = 4.632 + (0.817 \times Erysiphe^{-1}) + (-0.346 \times Rainfall^{-1})$$

	BETA	Std. error of BETA	B	Std. error of B	t (1331)	p-level
Intercepted			**4.632**	0.749	6.180	0.000
Erysiphe^{-1}	0.817	0.013	**0.817**	0.013	61.432	0.000
Rainfall^{-1}	-0.035	0.013	**-0.346**	0.132	-2.631	0.009

T a b l e   3

t-test for dependent samples between real and forecast values for 2018

	Mean	Std. Dv.	N	Diff.	Std. Dv. Diff.	t	df	p
Erysiphe 2018	18.432	28.293						
Forecast	19.054	23.033	176	-0.622	29.247	-0.282	175	0.778

2008). Therefore we observed a direct prediction ability of the adapted P-days equation considering the selected *E. necator* cardinal temperatures, that indicate how near the meteorological conditions of the vineyard are on a given day to the optimal conditions for conidial dispersion and possible fungal growth. Furthermore, this adapted model could trace the possible secondary dispersal conidia periods, although these fungal phenomena also depend on other kinds of variables apart from temperature, such as the phenological host stage or rainfall events.

Previous studies conducted in the same area obtained less fitting results for the prediction of *Erysiphe necator* infection events by the application of models only based on phenology and pathogen presence (Fernández-González *et al.* 2013). Powdery mildew Gubler-Thomas model (Gubler *et al.* 1999) to predict disease pressure on grapevine verified its ability and precision to predict the developmental stage of this fungal disease both during ascosporic and conidial stages. This model offers an index value that indicates the interval of fungicide application as a function of temperature values fluctuation among the grapevine vegetative cycle, predicting secondary infection risk periods (Jarvis *et al.* 2002).

During the studied seasons the highest daily spore concentration values were detected at Flowering-stage 6 (2008, 2012, 2013 and 2014 years) or the previous Inflorescence Emerge stage 5 (2011) and the next Development of Fruits stage 7 (2009 and 2010 years). We propose to administrate a treatment in the previous period to Flowering stage, identified as highly vulnerable for our climatic region by the P-days adapted equation, in order to prevent latent infection and the appearance of high levels of conidia in the atmosphere of the vineyard. During this phenological stage new vegetal organs appear and rapidly vegetative growth takes place, predisposing vines to powdery mildew colonization (Jarvis *et al.* 2002). The same authors pointed out conditions of high photosynthetic vigour in the host as a key point to powdery mildew infection progress. Finally, the water content represents another key factor during the grapevine ontogeny development, as the osmotic pressure in the leaf tissue increases because of the sugar content enrichment in plants (Hidalgo 2011). Goheen and Schnathorst (1963) proved that resistant cultivars to powdery mildew had higher osmotic pressure values (2128-2432 kPa) in their cell sap from leaf tissue than susceptible cultivars, with osmotic pressures of only 1216-1621 kPa. This vital process is comparable to the annual vegetative cycle of grapevines, where plants also gradually increase their sugar content as phenological stages happen. Bunches resistance also increments as they mature (Campbell 2007). This fact can explain the decrease in spore atmospheric concentrations in the final period of the Ripening of Berries (stage 8) detected in the present study.

In addition, we noticed that rain can produce a dual effect on the atmospheric presence of *E. necator* spores. Analysing comparatively weather and aerobiological data, we observed that rainfall events produced an important descent in airborne daily spore concentrations (Fig. 1). Several authors affirmed that colony development of powdery mildew is stopped by rain and water sprays (Yarwood 1939, Ward and Manners 1974, Jarvis and Slingsby 1977, Merchán and Kranz 1986a, b, Chellemi and Marois 1991b, Sivapalan 1993). Nevertheless, we found some cases in which soft rainfall could favour an increase in the spore atmospheric concentrations. The Maximum Spore Peak value in 2010 was registered on June 26th with 39 spores m^{-3}. Within a period without P-days values variations, the atmospheric fungal spore concentration increased significantly after rainfall events occurred the previous day (9.6 mm) and during the spore peak date (0.2 mm). We found a similar behaviour with the Maximum Spore Peak value of 2015, detected on April 26th with 165 spores m^{-3}. In this date the P-days values were lower than 100 during the previous seven days, but a soft rainfall period that occurred during these days seems to promote a great fungal development providing a notable increase in the *E. necator* airborne spores concentration. The increase in grape powdery mildew aerial conidia dispersal promoted by light rainfall was demonstrated by Willocquet and Clerjeau (1998). They found that rainfall events can produce a dual effect on conidial dispersal, depending on their intensity. As consequence of mechanical properties, light rainfall of approximately 2 mm may increase grape powdery mildew conidia dispersal because of the splash effect. Raindrops can disperse dry conidia by mechanical shaking (tap) and radial air movements due to droplet dispersal (puff). By the contrary, continuous rainfall is unfavourable to *E. necator* due to the physical damage on the leaf surface mycelium and conidiophores (Hirst and Stedman 1963). Our statistical results corroborated the importance of temperatures (variable used for the equation of the adapted P-days equation) and rainfall (variable included in the model to predict the atmospheric conidia content in the vineyard) as key developmental conditions to fungal powdery mildew spore dispersion and possible secondary infection episodes.

Studies conducted by Carisse *et al.* (2008) reported a significant correlation between conidia concentration in the atmosphere of the vineyard at a given date and lesion density in plant 1 week later, mainly when both disease intensity and airborne spore concentration were high. The high accuracy of the regression equation model developed in our study for predicting *E. necator* spore concentrations could make possible an 8-day horizon of infection in the vine (Fernández-González *et al.* 2013). Therefore, airborne spore concentrations could be used as bio-indicator of possible pathogen development, increasing the possibility of growers to avoid infections before the symptoms appearance on crops and to reduce treatments. In this sense the combination of the data from the three required causal factors of the disease triangle paradigm, airborne inoculum data, P-days adapted equation and phenological information, provides a valuable tool for establishment of the basis for an accurate Integrated Pest Management strategy in Northwestern Spain vineyards. The proposed model could be extended to areas with the same bioclimatic conditions. The company that provided the experimental vineyard for the study estimates that the proposed strategy avoided the application of 15-25 % of the phytosanitary treatments in the nearest vineyards, depending on the year. This reduction provides a crop costs reduction of 1500 €·ha^{-1} per year (a total of 3 million Euros per year in the entire Ribeiro D.O.).

Conclusions

The proposed threshold of P-days for potential high airborne conidia dispersion in the Ribeiro D.O. ranges from 100 to 160 P-days. The adaptation of the descriptive P-days equation bring us a useful tool to ascertain advantageous meteorological episodes for possible powdery mildew secondary infections in a dynamic way, by combination with the information about the airborne conidia dispersal and the timing for the susceptible phenological stages.

The reduction of the number of phytochemical treatment in the vineyard that can be achieved by applying the obtained equations can diminish the economical crop costs, to improve the quality of the obtained products and to ensure the protection of environment and human health. These are the current important challenges for winegrowers, especially for those related to a wine Designation of Origin area.

Acknowledgements

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