The Journal of Agricultural Science

cambridge.org/ags

Identification and evaluation of the main risk periods of *Botrytis cinerea* infection on grapevine based on phenology, weather conditions and airborne conidia

E. González-Fernández[1,2], A. Piña-Rey[1,2], M. Fernández-González[1,2,3], M. J. Aira[4] and F. J. Rodríguez-Rajo[1,2]

[1]Department of Plant Biology and Soil Sciences, University of Vigo, Vigo, Spain; [2]CITACA, Agri-Food Research and Transfer Cluster, Campus da Auga, University of Vigo, 32004-Ourense, Spain; [3]Earth Sciences Institute (ICT), Pole of the Faculty of Sciences University of Porto, Porto, Portugal and [4]Department of Biology, University of Santiago de Compostela, Santiago de Compostela, Spain

Crops and Soils Research Paper

Cite this article: González-Fernández E, Piña-Rey A, Fernández-González M, Aira MJ, Rodríguez-Rajo FJ (2020). Identification and evaluation of the main risk periods of *Botrytis cinerea* infection on grapevine based on phenology, weather conditions and airborne conidia. *The Journal of Agricultural Science* **158**, 88–98. https://doi.org/10.1017/S0021859620000362

Received: 16 April 2019
Revised: 21 February 2020
Accepted: 7 April 2020
First published online: 8 May 2020

Key words:
Botrytis cinerea; fungicide reduction; Integrated Pest Management; spore threshold; risk periods; *Vitis vinifera*

Author for correspondence:
M. Fernández-González,
E-mail: mfgonzalez@uvigo.es

Abstract

In the present study, a new method for a decision-support system for fungicide administration against the pathogen *Botrytis cinerea* in vineyards was developed based on Integrated Pest Management principles which identified an infection risk before the appearance of disease symptoms. The proposed method is based on the combination of (i) the phenological observations of the main susceptible stages to infection, (ii) the airborne spores monitoring, (iii) the forecasting of the suitable meteorological conditions for *B. cinerea* spore germination during the subsequent 4–6 days after the spore detection. Aerobiological, phenological and meteorological analyses were carried out using data from 2008 to 2015 in a vineyard of Northwestern Spain. Aerobiological spore data were obtained using a Lanzoni VPPS-2000 pollen-spore trap. Phenological observations were conducted on 22 plants of Treixadura cultivar following the BBCH (Biologische Bundesanstalt für Land und Forstwirtschaft, Bundessortenamt und CHemische Industrie) scale. The Magarey generic fungal model was applied for the identification of the main meteorological suitable periods for infection within the susceptible phenological stages of flowering and ripening of berries. Our results showed that climatic conditions favoured fungal development during flowering, although a higher incidence of *B. cinerea* infection risk-periods occurred during the prior-to-harvest stage of ripening of berries, the most susceptible phenological stage to *B. cinerea* infection obtained by the proposed methodology. This approach enables more precise targeting in pesticide spraying and reduction in pesticide application from 4–5 to 2–3 times per year at our commercial study. It also illustrates the real-world benefits of integrated disease risk modelling.

Introduction

Grey mould, caused by *Botrytis cinerea* Pers., is one of the most common vineyard diseases and can cause severe damage. Bioclimatic conditions in Northwestern Spain, with warm temperatures during the day and high humidity during the night, favour the development of this fungal infection, which reduces crop productivity markedly. To prevent this disease and to diminish its impacts, the most common strategy employed by winegrowers is the systematic application of chemical fungicides, generally following preset calendars based on the grapevine phenological growth stages (Bugiani *et al.*, 1995). However, these fungicides should only be applied when a real risk of unacceptable economic damage in the vineyard is detected, in order to avoid the consequences associated with their excessive use, such as the appearance of resistant fungus or the alteration of beneficial mycological flora.

Knowledge of pathogen biology and disease cycles, including interactions between pathogen, environment and host, is essential to avoid or to reduce the consequences of any plant disease (De Wolf and Isard, 2007). Disease appearance is a consequence of the occurrence and interaction of three causal agents, which are (i) a susceptible plant host, (ii) the presence a virulent pathogen, (iii) suitable environmental conditions for the pathogen development. The absence of any of these components prevents plant disease (Stevens, 1960; Agrios, 2005). This natural condition is one of the paradigms of plant pathology known as *disease triangle*, and is the basis for an Integrated Pest Management strategy guided to chemical products reduction in crop managing (Stevens, 1960; Agrios, 2005).

Optimal meteorological conditions for *B. cinerea* development are high relative humidity and warm temperatures during the grapevine reproductive cycle (Broome *et al.*, 1995; Rodríguez-Rajo *et al.*, 2010). Furthermore, microclimatic conditions in the grapevine canopy

CAMBRIDGE UNIVERSITY PRESS

markedly influence disease development because leaf removal near clusters significantly reduces the incidence and severity of *Botrytis* bunch rot (English *et al.*, 1989). From the phenological point of view, the most widely accepted grapevine phenological stages to be susceptible to *B. cinerea* colonization and infection are flowering (stage 6 of BBCH-Biologische Bundesanstalt für Land und Forstwirtschaft, Bundessortenamt und CHemische Industrie scale) and ripening of berries (stage 8 of BBCH) (McClellan and Hewitt, 1973; Esterio *et al.*, 1996). In addition, aerobiological studies can be used to quantify possible pathogen presence in the vineyard using biosensors that measure daily and hourly airborne spore concentrations (Fernández-González *et al.*, 2012). Since many authors have related fungal disease levels at a given time to airborne spore concentrations during previous periods, airborne spore concentrations can be used as bio-indicators of pathogen development (Jeger, 1984; Carisse *et al.*, 2008).

The main objective of the current study was to reduce *Botrytis* fungicide treatment in vineyards of the Ribeiro Designation of Origin area (one of the protected winemaking areas of Spain due to the quality and history of its wines) by detecting the main risk periods of *Botrytis* development and infection. To achieve this, a comprehensive study was conducted of the airborne fungal conidia in the atmosphere of the vineyard, vines phenology and optimal weather conditions for infection. The combination of these factors describes the behaviour of the fungus in relation to the environmental conditions of the vineyard agrosystem, and potentially identifies the optimal time for fungicide application.

Materials and methods

The studied vineyard is located in Cenlle, at 42°18′55.88″N–8°6′3.28″W (Datum WGS84), and 199 m above sea level (SIXPAC, 2018). It belongs to the Ribeiro Designation of Origin region (D.O.), in Northwestern Spain (Fig. 1). Steep valleys and hillsides characterize this area. The particular Oceanic-Mediterranean transitional eco-climate of this region is favoured by its southern situation in Galicia, as well as by natural barriers that protect this territory from sub-Atlantic storms. According to the Multicriteria Climatic Classification System (MCC), most winemaking areas in this region, watered by the Miño River, would be defined as temperate and warm, sub-humid, with very cold nights (Blanco-Ward *et al.*, 2007).

Detection of the pathogen presence in the vineyard

Airborne fungal propagule *Botrytis* concentrations were determined using a Lanzoni VPPS-2000 (Lanzoni s.r.l.) volumetric pollen-spore trap (Hirst, 1952) located in the central part of the vineyard and situated 2 m above ground level in order to avoid confounding spore trap measurements with plant growth. Aerobiological sampling period took place during the active *Vitis vinifera* L. season over the studied years, from 2008 to 2015. The vegetative period started on 20 March and ended at harvest date (usually in the second fortnight of September). A Lanzoni trap was calibrated to sample a constant volume of 10 litres of air per minute; air particles passed through a cylindrical drum covered by a Melinex film impregnated with a 2% silicone solution, as a spore-trapping surface. This drum was changed weekly (daily from June to August), and the exposed tape was cut into seven pieces, which were mounted on separate glass slides. *Botrytis cinerea* spores were counted following the protocol proposed by the Spanish Aerobiological Network (REA) (Galán *et al.*, 2007). For spores identification and count, we analysed two lines along the slides at 400× magnification using a light optical microscope. Results were expressed as spores when referring to total values, or spores/m^3 of air when referring to daily mean values (Galán *et al.*, 2017).

Phenological observations of the main susceptible stages to infection

A phenological study was carried out in order to relate plant developmental stages to the detected spore levels. Field observations were conducted during the active grapevine season for each study year. Among the multiple cultivars grown in the study area, we considered the Treixadura cultivar, which is the preferential autochthonous cultivar of the Ribeiro D.O. area. We randomly selected 22 plants that were observed weekly, except during the flowering stage, when the number of observations was increased to twice a week. Phenological stages were monitored using the scale recommended by Lorenz *et al.* (1994), adopted by the BBCH as a standardized scale for phenological grapevine observations (Meier, 2001). Five main grapevine growth stages were considered: stage 1 – leaf development, stage 5 – inflorescence emergence, stage 6 – flowering, stage 7 – development of fruits and stage 8 – ripening of berries. For grapevine phenological calendar development, we considered the start date of each stage to be the date when 50% of the studied plants had reached that stage.

Suitable meteorological conditions for Botrytis *spore germination*

Meteorological data were obtained from an HOBO (ONSET HOBO® USB Micro Station Data Logger – H21-USB), located in a row of the vineyard at 1.5 m above ground level next to the spore sampler. The monitored parameters were maximum, mean and minimum temperatures, relative humidity and dew point. Information about rainfall and wind speed was obtained from the MeteoGalicia Meteorological Station located in the Viticulture and Enology Station of Galicia (EVEGA) in Leiro, at 5 km from the study vineyard (MeteoGalicia, 2018).

The Magarey generic model was adapted to forecast the suitable meteorological conditions for fungal plant pathogen infection. This model estimates the wetness duration requirement needed to accomplish a critical disease intensity at a given temperature. *Botrytis cinerea* infection is defined as having a 20% disease incidence on an infected part of the plant (Magarey *et al.*, 2005). This model was applied to the study vineyard in order to determine the potential risk of disease development related to weather conditions. It is based on a temperature response function scaled to the minimum and optimum values of the surface wetness duration requirement.

First, possible disease development periods were identified as wet periods, with relative humidity RH ⩾95% within the hourly values of each day. If various wet periods are separated by a dry period of RH <95% in the same day, they can be summed if the dry period (D) is lower than the D_{50} value. This parameter is defined as the duration of a dry period that will result in a 50% reduction in disease compared with a continuous wet period (Magarey *et al.*, 2005). If a dry period (D) is higher than D_{50}, which is 13 h for *B. cinerea* (Bregaglio *et al.*, 2013), the wet

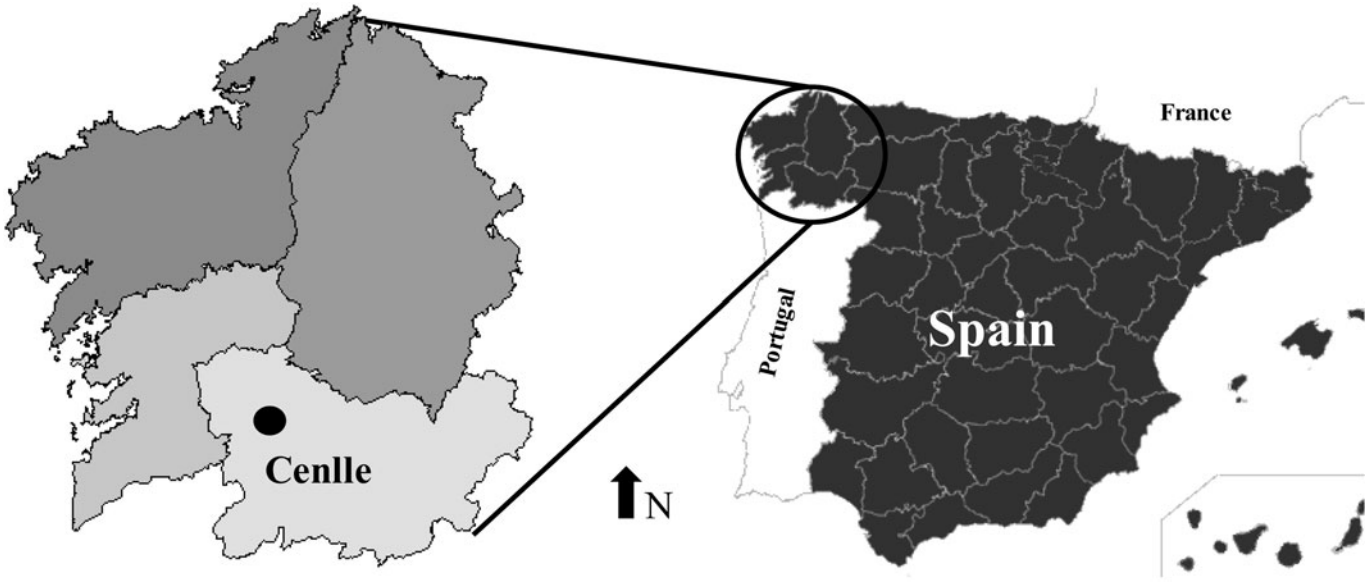

Fig. 1. Location of Ribeiro wine Designation of Origin in Galicia, at Northwestern Spain.

periods cannot be summed and two different infection risk periods are considered for the same date.

After that, a temperature response function (Equation 1) for each wet period was applied:

$$f_{(T)} = \left(\frac{T_{max} - T}{T_{max} - T_{opt}}\right)\left(\frac{T - T_{min}}{T_{opt} - T_{min}}\right)^{(T_{opt} - T_{min})/(T_{max} - T_{opt})} \quad (1)$$

where T: average temperature (°C) during the wetness period, T_{min}: minimum temperature for infection, T_{max}: maximum temperature for infection and T_{opt}: optimum temperature for infection.

The obtained results allow calculation of wetness duration requirement value ($W_{(T)}$), in hours, by the following expression (Equation 2):

$$W_{(T)} = \left(\frac{W_{min}}{W_{min}}\right) \leq W_{max} \quad (2)$$

where $W_{(T)}$: wetness duration requirement for the critical disease threshold at temperature T, W_{min}: minimum value of wetness duration requirement for the critical disease threshold at any temperature and $f_{(T)}$ is the temperature response function. W_{max}: parameter that indicates an upper boundary on the value of $W_{(T)}$.

To develop the original model, Magarey *et al.* used experimental data from 53 published studies on the temperature and moisture responses of different plant pathogens (Magarey *et al.*, 2005). Information on the temperature–wetness combination effect on *B. cinerea* infection of grape flowers and berries comes from Nair and Allen (1993). Based on model parameter values identified in this study (Table 1), we calculated the $W_{(T)}$ values for our study area in two different phenological stages: flowering (stage 6) and ripening of berries (stage 8). The calculated values for this fungal disease indicator offer an indirect measurement of infection risk. To express the potential risk identified in each period, we graphically represented (Fig. 2) the risk periods identified with the Magarey model as the difference between the W_{max} value for flowering (12 h) and the W_{max} value for ripening of berries (10 h). The resulting $W_{(T)}$ values show a direct measure of infection risk during these episodes. We expressed this measurement as *Magarey units* rather than hours of required wetness duration.

Furthermore, in order to determine the statistical relationship between airborne spore concentrations and the main weather parameters altogether, we applied a Principal Component Analysis (PCA) for the 2008–2015 data set. This statistical procedure reduced the dimensionality of the set of weather predictor variables to determine the highest influence on airborne spore concentrations. The considered variables were the *Botrytis* airborne spore concentrations (*Botrytis*), mean temperature (T_{mean}), maximum temperature (T_{max}), minimum temperature (T_{min}), relative humidity (RH), dew point (Dew P), rainfall (Rain) and wind speed (Wind S). The STATGRAPHICS Centurion XVI version 16.1.11 was used for the statistical analysis.

Identification and evaluation of risk infection periods

For the identification of the real infection risk periods caused by *B. cinerea*, we followed the *disease triangle* principle taking into account the three causal agents for plant disease occurrence. First, we developed a phenological calendar for each study year based on field observations to determine the timing of the susceptible stages for *B. cinerea* development: flowering (stage 6 BBCH) and ripening of berries (stage 8 BBCH). From this, we considered the influence of suitable weather conditions within the susceptible phenological stages by applying the Magarey model to identify the main meteorological suitable periods of disease development. As previously stated, this model uses the hourly relative humidity and temperature to obtain a requirement of wetness duration for a critical disease threshold, taking into account meteorological conditions and its proximity to optimal fungal development conditions. Finally, in the suitable meteorological periods detected by the Magarey model during the susceptible phenological stages 6

Table 1. Model parameters for infection model developed by Magarey *et al.* (2005) and D_{50} value for *Botrytis cinerea*

Grapevine phenological stage	T_{min} (°C)[a]	T_{max} (°C)[a]	T_{opt} (°C)[a]	W_{min} (hours)[a]	W_{max} (hours)[a]	D_{50} (hours)[b]
Grape flower (flowering)	1	34	25	1	12	13
Grape (ripening of berries)	10	35	20	4	10	

[a]Model parameters obtained from the Nair and Allen (1993) temperature–wetness combination study.
[b]Reference for D_{50} value in Bregaglio *et al.* (2013).

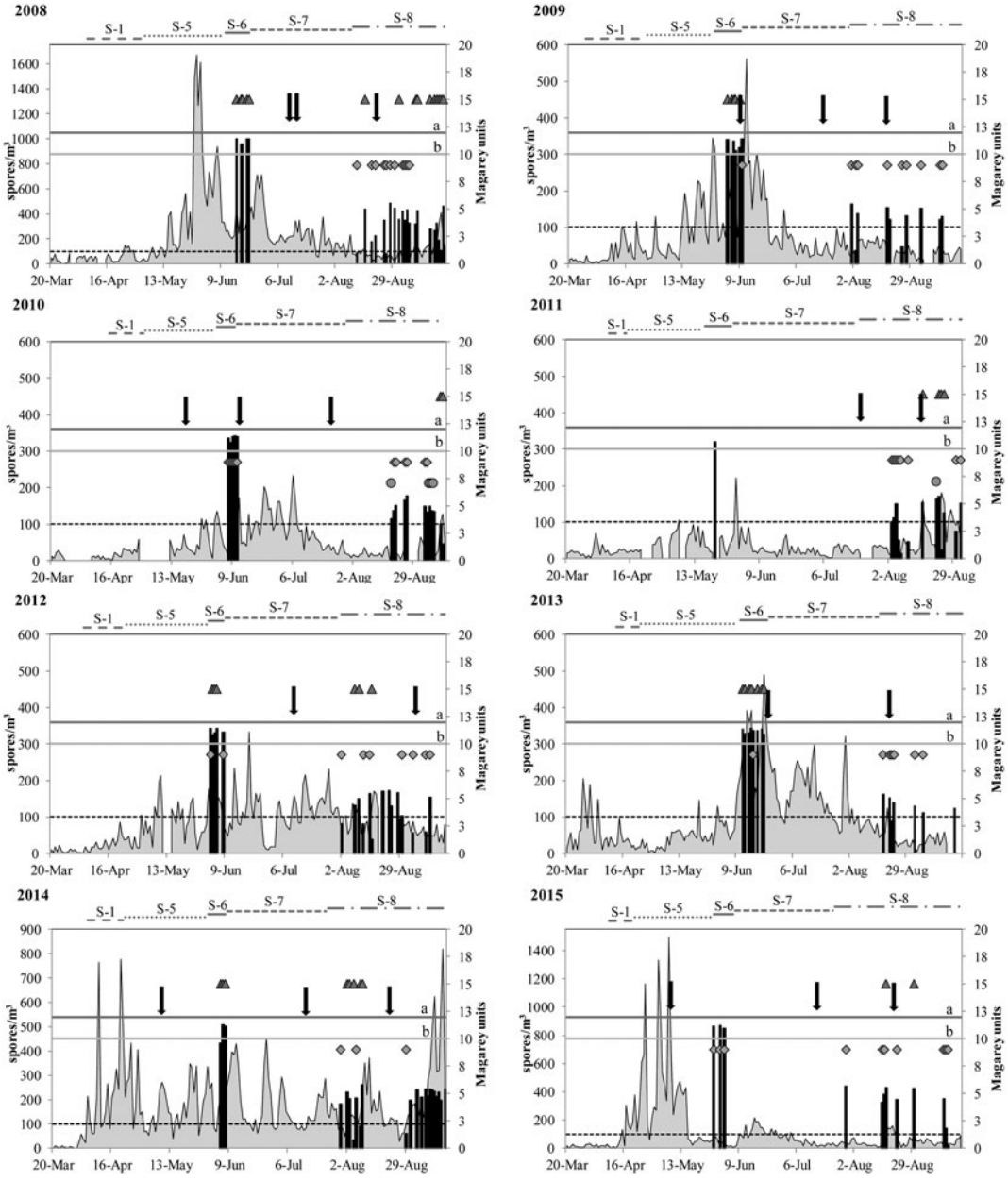

Fig. 2. Airborne *B. cinerea* spore concentrations (light grey area), 100 spores/m^3 threshold (represented by discontinuous line), grapevine phenological stages (upper lines of S-1, S-5, S-6, S-7 and S-8), anti-*Botrytis* treatments (arrows), Magarey suitable meteorological periods (values expressed as Magarey units = W_{max}–$W_{(T)}$ for each phenological stage, in black bars), and the evaluated risk periods. The represented risk periods are the result of the combination of a susceptible phenological stage (flowering-S6 or ripening of berries-S8), one identified Magarey suitable meteorological period and the airborne spore concentrations above 100 spores/m^3 (high-risk period (HR) in red triangle), between 10 and 100 spores/m^3 (moderate-risk period (MR) in orange diamond) or below 10 spores/m^3 (low-risk period (LR) in blue circle). (*a*) W_{max} (Equation 2) for *B. cinerea* in flowering stage risk periods: 12 h (dark grey line). (*b*) W_{max} (Equation 2) for *B. cinerea* in ripening of berries stage risk periods: 10 h (light grey line).

and 8, we checked the recorded *Botrytis* airborne spore concentrations in order to identify the real infection risk periods during the subsequent days.

Moreover, we propose the classification of the real infection risk periods detected into three categories: high risk (HR) at spore concentrations ⩾100 spores/m^3, moderate risk (MR) if spore concentrations are between 10 and 100 spores/m^3 and low risk (LR) with spore concentrations <10 spores/m^3.

The study was conducted in collaboration with the main vine company of D.O. Ribeiro, 'Viña Costeira' S.R.L. Our experimental data were used by the company to regulate the treatments in their vineyards. Decisions concerning spray administration depended on the combination of phenological observation of a susceptible phenophase, the possibility that predicted meteorological conditions would allow spore germination, the consequent infection of the plants during the next 4–6 days and the detection of spore thresholds in the atmosphere of the vineyard. The fungicides applied in the vineyard were Ciprodinil 37.5% +Fludioxonil 25% or Fenhexamida 50% (WG) P/P by means of fogging and farm tractor mechanical application.

Results

Detection of the pathogen presence in the vineyard

The highest spore amount was registered in 2008, with 39 299 spores, followed by 2014 with 32 073 spores. The lowest spore amount was observed in 2011 with 5747 spores. The maximum values of daily spore concentrations were registered on 28 May 2008 and 7 May 2008 with a value of 1669 and 1495 spores/m^3, respectively. In general, the main peak values were observed every year between the end of May and the beginning of July (except for the two latest seasons, where the highest values were detected on 15 September in 2014 and during 2015, on 7 May).

Phenological observations related to pathogen presence in the vineyard

The airborne spore concentrations in the vineyard were analysed in relation to the main phenological growth stages of grapevine. We determined the phenological stage with the highest airborne *B. cinerea* spore concentrations and the highest total annual spore amount per season (Table 2). The maximum values corresponded with a period between stage 5 (inflorescence emergence) and stage 7 (development of fruits). In addition, our study showed that the average spore concentrations per phenological stage were mostly higher during the flowering stage (years 2009, 2012 and 2013) and the previous (stage 5: inflorescence emergence) and later (stage 7: development of fruits) phenological stages (Table 2).

Suitable meteorological conditions for Botrytis spore germination

The Magarey generic fungal prediction model was applied to determine suitable meteorological disease development periods during the phenological stages of flowering (stage 6) and ripening of berries (stage 8), and it detected several episodes of disease development (Fig. 2). The model predicted, for all considered years, the lowest requirements for possible infections during flowering, with a range of values between 0.559 and 2.387 h. This indicates that flowering is the grapevine phenological stage most susceptible to *Botrytis* infection according to the meteorological conditions included in the Magarey model for our bioclimatic region. Considering all years during the flowering stage, the highest number of infection risk periods was identified in 2013, with eight suitable meteorological periods, while the year with the lowest number of suitable meteorological periods was 2011 with only one (Fig. 2).

During ripening of berries stage, the Magarey model also identified possible suitable meteorological periods. Generally, the total number of disease periods detected was higher during this stage, probably because the length of this growth stage is generally longer than flowering (Table 2). During this stage, the maximum number of suitable periods was detected in 2008 (21 suitable meteorological periods) whereas the minimum in 2013 (seven suitable meteorological periods). Nevertheless, the derived wetness requirement values ($W_{(T)}$) were higher than the obtained during flowering, with a range of 4.073–9.772 h.

A PCA analysis was performed to ascertain the meteorological variables that most influenced spore presence in the atmosphere. The PCA analysis for the 2008–2015 data set extracted two principal components that accounted for 66.3% of the data variance. The first component (Cp1) explained 46.5% of the variance, and it grouped the mean, maximum and minimum temperature with dew point. This component clearly clustered temperature-related variables. The second component (Cp2) explained the 19.8% of variance and grouped the airborne spore concentrations with the humidity-related variables of relative humidity and rain with positive correlation coefficients, and wind speed with a negative correlation coefficient (Table 3). This indicates that wind speed has a negative influence on spore concentrations (Table 3).

Identification and evaluation of risk infection periods

The results of the risk infection period evaluation were graphically represented in Fig. 2 through the combination of phenological growth stages, Magarey suitable periods and airborne spores concentrations. Additionally, we noted fungicide treatment dates due to their importance in the presence of spores in the atmosphere. Table 4 shows the chemical fungicides used, the dates of application, the corresponding daily airborne spore concentrations and the reason for each treatment decision. The decision to spray fungicide depended on the combination of the phenological observation of a susceptible phenophase, the spore threshold and the potential for suitable meteorological conditions in the vineyard that would allow spore germination and the consequent infection of the plant during the subsequent 4–6 days. According to our data and the field observations of the agronomic technicians of the company owning the experimental vineyard, we established a general spore threshold of 100 spores/m^3 for a high risk of infection (HR).

Considering the interaction of the three causal agents required for infection, the year with the highest number of high-risk periods (HR) was 2008 with 15 periods during flowering and ripening stages, while the lowest number of HR was obtained for 2010 and 2015, both with two periods during flowering and ripening. The highest number of moderate-risk periods (MR) was obtained for 2008 and 2010, with 11 periods considering flowering and ripening together, while the lowest number was detected in 2014 with just three periods. We obtained HR and MR periods for all the considered seasons, but low-risk periods (LR) were only detected for 2010 and 2011, with four and one periods, respectively, during both flowering and ripening.

https://doi.org/10.1017/S0021859620000362

Table 2. Start date and length (days) of the principal BBCH growth stages (S-1 Leaf development, S-5 Inflorescence emergence, S-6 Flowering, S-7 Development of fruits, S-8 Ripening of berries), with the corresponding average and maximum airborne *Botrytis* spore concentrations for each stage

Phenological stage		S-1	S-5	S-6	S-7	S-8	Total spore
2008	Start date	Apr 07	May 04	Jun 11	Jun 23	Aug 10	39 299
	Length	27	38	12	48	45	
	Max. spores/m^3	151	1669	475	711	408	
	Date max.	Apr 24	May 28	Jun 16	Jun 28	Sep 21	
	Average spores/m^3	57	456	301	249	99	
2009	Start date	Mar 29	Apr 27	May 29	Jun 11	Aug 01	12 960
	Length	29	32	13	51	53	
	Max. spores/m^3	116	346	258	562	75	
	Date max.	Apr 21	May 27	Jun 05	Jun 12	Aug 13	
	Average spores/m^3	33	101	133	102	38	
2010	Start date	Apr 15	May 01	Jun 02	Jun 11	Jul 30	7392
	Length	16	32	9	49	45	
	Max. spores/m^3	59	115	136	233	127	
	Date max.	Apr 28	May 26	Jun 02	Jul 06	Sep 11	
	Average spores/m^3	23	48	61	87	27	
2011	Start date	Apr 07	Apr 15	May 17	May 29	Jul 21	5747
	Length	8	32	12	53	43	
	Max. spores/m^3	27	105	63	222	180	
	Date max.	Apr 11, 13	May 06	May 24	May 30	Aug 24	
	Average spores/m^3	18	50	34	30	54	
2012	Start date	Apr 04	Apr 24	Jun 01	Jun 09	Aug 02	13 781
	Length	20	38	8	54	49	
	Max. spores/m^3	85	213	218	334	170	
	Date max.	Apr 21	May 10	Jun 04	Jun 20	Aug 17	
	Average spores/m^3	25	79	133	114	79	
2013	Start date	Apr 18	Apr 29	Jun 13	Jun 26	Aug 15	17 052
	Length	11	45	13	50	41	
	Max. spores/m^3	41	289	490	321	124	
	Date max.	Apr 18	Jun 12	Jun 22	Jul 31	Aug 20	
	Average spores/m^3	22	64	296	143	37	
2014	Start date	Apr 05	Apr 22	May 30	Jun 08	Jul 24	32 073
	Length	17	38	9	46	55	
	Max. spores/m^3	777	433	336	453	818	
	Date max.	Apr 20	Apr 25	May 30	Jun 26	Sep 15	
	Average spores/m^3	258	181	209	178	192	
2015	Start date	Apr 09	Apr 21	May 28	Jun 07	Jul 24	21 487
	Length	12	37	10	47	60	
	Max. spores/m^3	305	1495	87	215	159	
	Date max.	Apr 17	May 07	May 29	Jun 16	Aug 20	
	Average spores/m^3	87	359	41	77	47	

https://doi.org/10.1017/S0021859620000362

Table 3. Factor loadings of the considered meteorological and aerobiological variables for the 2008–2015 data set

Component	Cp1	Cp2
T_{mean}	**0.514**	0.005
T_{max}	**0.486**	−0.148
T_{min}	**0.438**	0.322
Dew P	**0.430**	0.396
Botrytis	0.027	**0.331**
RH	−0.280	**0.579**
Rain	−0.193	**0.507**
Wind S	−0.080	**−0.122**

In bold the loadings with the largest value for each variable.

Analysing each susceptible phenological stage separately, we observed that the highest number of HR and MR during the stage 8 ripening of berries was detected in 2008, with 10 HR-S8 and 11 MR-S8 periods (Fig. 3). In the case of stage 6 flowering, the highest number of HR was obtained for 2013 with seven HR-S6, and the highest number of MR was registered for 2010 with five MR-S6. An average of five risk periods was found for the studied years during flowering, considering both high- and moderate-risk periods. For the ripening stage, double the number of risk periods was found, with an average of ten high- and moderate-risk periods for the studied years. Within this average, the moderate-risk periods for ripening stand out, with seven MR-S8 as the mean value (Fig. 3).

Discussion

Prediction models based on the main risk factors that lead crops to epidemic diseases are of great importance for the integrated management strategies. Traditionally, the main risk infection factors were associated with agricultural practices (such as crop rotation, planting dates, tillage practices, etc.), environmental conditions (propitious meteorological situations) or different host susceptibility according to the phenological stage of the plant (Rosa *et al.*, 1995; Twengström *et al.*, 1998; Rossi and Giosuè, 2003; Rossi *et al.*, 2003; Manter *et al.*, 2005; Paul and Munkvold, 2005; De Wolf and Isard, 2007; Ciliberti *et al.*, 2015). The *disease triangle* is one of the paradigms in plant pathology (Stevens, 1960) that claims the existence of a plant disease absolutely requires the interaction of a susceptible host, a virulent pathogen and environment-favourable conditions for disease development (Stevens, 1960; Agrios, 2005). Therefore, plant disease is prevented with the absence of any one of these three causal components. Based on this, the combination in our study of (i) the identification of the grapevine phenological stage vulnerable to infection, (ii) the observation of spore levels considered as a pathogen biosensor and (iii) the identification of suitable environmental conditions via the meteorological Magarey model led us to ascertain the optimal moments for phytosanitary treatment, taking into account the environmental characteristics and biological conditions of this agroecological system. The proposed method deals with the need for an effective monitoring system and the establishment of disease thresholds aimed to an appropriate decision-support system for crop management guidance on pest control based on the third principle of '*Decision based on monitoring and thresholds*' included in the eight principles of the Integrated Pest Management (Barzman *et al.*, 2015).

Detection of the pathogen presence in the vineyard

The *Botrytis* spore presence was constant in the atmosphere of the vineyard during the study period, as previously noted by other authors in the Iberian Peninsula (Oliveira *et al.*, 2009; Rodríguez-Rajo *et al.*, 2010). We proposed 100 spores/m^3 as a threshold for high risk of infection caused by *B. cinerea*, based on our field observations and the data of the agronomic technicians of the company owning the experimental vineyard. We observed that lesions in vines started when airborne spore values were above the 100-spore level, indicating that this represents an important disease risk indicator in the vineyard, and a reasonable threshold to justify a fungicide treatment. Furthermore, Carisse and Van der Heyden (2015) found similar results on their study about the influence of airborne conidia concentration on flower and stem-wound infections at three different temperatures of 15, 20 and 25°C. They observed that no infection of stem-wounds occurred under 100 conidia/m^3, and the proportion of infected flowers remained low under 10 conidia/m^3 rising with the increase of the spore concentrations. Based on this, we proposed a second threshold for moderate risk of infection between 10 and 100 spores/m^3.

Phenological observations related to pathogen presence in the vineyard

Several authors have noticed the synchronism between the most vulnerable grapevine growth stages and *B. cinerea* presence in many geographical areas (Esterio *et al.*, 2011; Ciliberti *et al.*, 2016; Carmichael *et al.*, 2018; Hatmi *et al.*, 2018; Martínez-Bracero *et al.*, 2018). The most widely accepted critical stages for grey mould infection are flowering (stage 6) and ripening of berries (stage 8). During the flowering stage, pollen and sugar exudation favour the colonization of tissues by the pathogen (Esterio *et al.*, 1996). This nutritional effect is also evident during ripening, when the presence of sugars, which increases over the stage, has a synergetic effect with the increasing ontogenic susceptibility of grapes as they mature, and propitious meteorological conditions. This effect increases disease intensity in the stage closest to the grape harvest (Latorre, 1986; Bulit and Dubos, 1988; Kretschmer *et al.*, 1994). Frenguelli (2001) noted a *Botrytis* airborne spore concentration peak in September, when the fungus develops on senescence leaves besides on ripening fruits. Furthermore, infection events during flowering have special interest due to fungal colonization of floral debris. This process represents an important inoculum source for late infection episodes, affecting during maturation and before harvest when the berries susceptibility to *B. cinerea* infections increases (Holz *et al.*, 1997; Wolf *et al.*, 1997). Our results support these affirmations as we found that the highest spore levels were registered near flowering stage. These findings agree with the values obtained by the Magarey fungal disease model, as we detected risk periods for critical disease incidence at this phenological stage for the study area. Furthermore, we also found high spore concentrations during the ripening of berries stage, corroborating the results noted by other authors (Fernández-González *et al.*, 2009; Rodríguez-Rajo *et al.*, 2010).

https://doi.org/10.1017/S0021859620000362

Table 4. Date of the fungicide treatments, type of fungicide (C: *Ciprodinil*, FL: *Fludioxinil*, Fh: *Fenhexamida*), spore concentration in the atmosphere, the day of the spray and reason of spray decision (Avoidable*: the Company decided to administrate the spray unilaterally*; Preventive treatment: *spray administration due to suitable meteorological conditions for spore germination*; High spore concentration in the vineyard: *spray administration due to high presence of pathogen in the atmosphere of the vineyard*; Infection risk period during the next 4–6 days: *spray administration due to the detection of high or moderate infection risk periods during the next 4–6 days because of the combination of a propitious phenological stage, exceedance of spore concentration thresholds and suitable Magarey meteorological periods*)

Date		Treatment	Spores/m^3	Reason of spray decision
2008	Jul 11	C + FL	215	High spore concentration in the vineyard
	Jul 16	C + FL	353	High spore concentration in the vineyard
	Aug 21	C + FL	64	Infection risk period during the next 4–6 days
2009	Jun 9	Fh	175	Infection risk period during the next 4–6 days
	Jul 15	C + FL	71	Preventive treatment
	Aug 21	C + FL	75	Infection risk period during the next 4–6 days
2010	May 18	Fh	70	Preventive treatment
	Jun 12	Fh	173	Infection risk period during the next 4–6 days
	Jul 22	Fh	45	Avoidable
2011	Jul 18	C + FL	13	Avoidable
	August 16	C + FL	161	Infection risk period during the next 4–6 days
2012	Jul 10	Fh	117	High spore concentration in the vineyard
	Sep 4	C + FL	90	Infection risk period during the next 4–6 days
2013	Jun 25	Fh	254	Infection risk period during the next 4–6 days
	Aug 21	C + FL	41	Infection risk period during the next 4–6 days
2014	May 9	Fh	270	High spore concentration in the vineyard
	Jul 15	C + FL	131	High spore concentration in the vineyard
	Aug 22	C + FL	125	High spore concentration in the vineyard
2015	May 8	Fh	686	High spore concentration in the vineyard
	Jul 17	C + FL	22	Avoidable
	August 17	C + FL	100	Infection risk period during the next 4–6 days

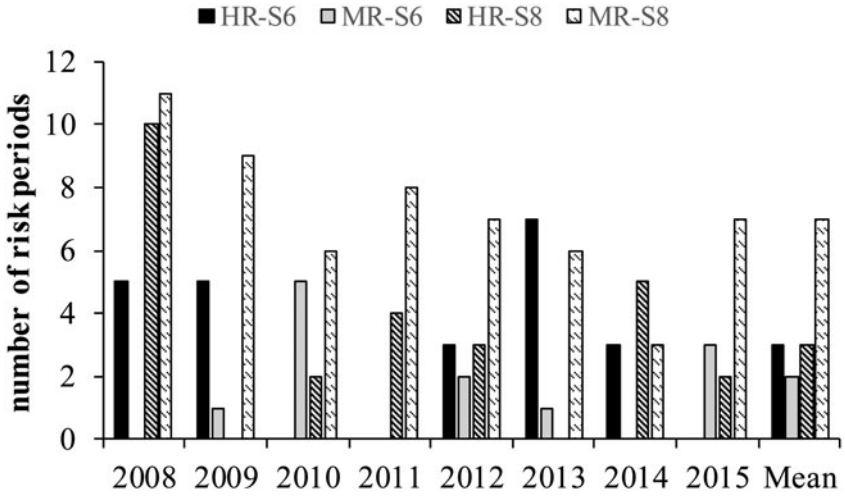

Fig. 3. Number of high-risk (HR) and moderate-risk (MR) periods for *B. cinerea* infection from 2008 to 2015 during the phenological stages of flowering (S6) and ripening (S8).

Suitable meteorological conditions for Botrytis *spore germination*

The Magarey generic fungal infection model applied in this study was one of the first to demonstrate that a single temperature-driven equation can be used to simulate infection response for several vineyard pathogens (Magarey *et al.*, 2005). This model is a simplification of the ecosystem functioning based on meteorological variables, but actually, other parameters have marked influence on disease progress processes. A higher number of infection risk periods was detected during the ripening of berries

https://doi.org/10.1017/S0021859620000362

stage, although with higher model values than those obtained for the flowering stage, which indicates that plants are less susceptible to this fungal disease because of weather conditions. These elevated values of hours of wetness duration requirement indicate that the meteorological conditions were not as favourable for fungal development during the ripening of berries stage as during flowering in the Northwestern Spain region.

The results of the PCA statistical analysis showed the influence of humidity-related variables and wind speed on *Botrytis* airborne spore presence in the atmosphere. These results accurately describe the development and dispersal behaviour of the pathogen as humidity is widely considered as a critical factor for grey mould spore germination and infection (English *et al.*, 1989; Broome *et al.*, 1995). The negative correlation found between airborne spore concentrations and wind speed reflects the predominant dispersion mechanism of propagules in *Botrytis* species, usually dry conidia scattered by wind (Holz *et al.*, 2007). Moreover, this negative association could indicate that the spores detected in the atmosphere of the vineyard are released in the study plot itself, instead of being transported from other areas through long distance transport processes. This circumstance is reflected in a positive correlation between wind and airborne spore concentrations (Moreno-Grau *et al.*, 2016).

Identification and evaluation of risk infection periods related to fungicide application

The proposed method for the identification of the infection risk periods, which account for meteorological conditions, the vines phenological stage and the pathogen's presence, represents a valuable tool for the development of fungicide application schedules. The identification of the main infection risk periods based on spore thresholds makes possible the disease detection between 4 and 6 days before the symptoms appearance, as it is verified in field observations (data not shown). Once the spores are present in the air of the vineyard in higher concentrations than the marked threshold, they still need 4–6 more days (depending on the phenological stage) to develop a new fungus and lesions under propitious meteorological conditions. Carisse *et al.* (2008) also found a significant correlation between airborne spore concentration on a given date and lesion density 1 week later for unmanaged and managed sites on their study.

For the evaluation of risk periods (HR, MR or LR) for disease development, we applied the airborne spore-level thresholds for each category to the identified Magarey suitable periods during the flowering and ripening of berries stages. The obtained results of the evaluation of infection risk periods for the eight studied seasons showed, on average, the same number of three HR infection periods for ripening and flowering. Nevertheless, marked differences were found for the MR periods as we detected two for flowering and seven for ripening. This suggests that the critical grapevine phenological stage for *B. cinerea* infection in our bioclimatic area is the ripening stage.

During the years prior to the present study, 4–5 sprays against grey mould were applied annually in the vineyard following preset calendars based on phenology. The first treatment was conducted annually 2 weeks before flowering, and then during the June to August period, three to four additional treatments were conducted (one in June, another in July and usually one to two in August). The combination of the Magarey model, the aerobiological data and the phenological observations represents a stronger resource for disease risk prediction taking into account both environmental conditions and fungal development, and allowing to reduce the number of phytosanitary treatments on the vineyard. Using the proposed methodology, we either prevented the appearance of lesions or reduced the presence of lesions, as the sprays were applied at a time prior to the visibility of lesions in plants. In the present study, from 2008, annual chemical treatments in the vineyard were reduced to 2–3 depending on the year, achieving a 25–35% reduction in fungicide treatments. The first treatment was conducted during May, but not during all years, and the second and third treatments in July and August. The spray administration depended on the combination of phenological observation of a susceptible phenophase, the exceedance of the 100 spores/m^3 threshold and that the potential suitable meteorological conditions in the vineyard would allow spore germination during the subsequent 4–6 days.

The decision for most of the applied fungicide treatments in the study vineyard from 2008 to 2015 was motivated by high airborne spore concentrations or by high/moderate infection risk period detection (because of the combination of a propitious phenological stage, the rise of the spore concentration and a suitable Magarey meteorological period). Moreover, during the studied period, two preventive treatments were administrated in the years 2009 and 2010, and three avoidable treatments were unilaterally applied by the company on 22 July 2010, 18 July 2011 and 17 July 2015. These treatments were applied during the phenological stage 7 of development of fruits (which is not considered as a susceptible stage for *Botrytis* infections), and under low airborne spore concentrations.

Despite the achieved reduction in treatments in comparison with preset calendars, several authors have demonstrated the effectiveness of plant disease control strategies based on disease forecasting. Madden *et al.* (2000) found similar disease incidence in crops with standard treatments based on preset calendars to those with chemical application based on the identification of environmental conditions which favour sporulation and infection integrated into a warning system. A secure and effective disease control can be implemented by using this kind of models.

Furthermore, we propose to administrate a treatment in the previous period to the flowering stage, identified as highly vulnerable for our climatic region by means of the Magarey model, in order to prevent latent infection and the appearance of disease symptoms. This fact was notable in the last two studied years, 2014 and 2015, where a preventive chemical treatment was applied during the previous phenological stage to flowering (stage 5 inflorescence emergence) coinciding with very high airborne spore concentration levels. The treatment greatly contributed to the control of the latent disease, which would turn into inoculum sources for later infections by colonization of senescent floral debris and aborted berries (Wolf *et al.*, 1997; Rodríguez-Rajo *et al.*, 2010). It was possible to control later infection cycles in 2015 and to reduce spore levels in 2014. Moreover, a possible treatment reduction could be achieved in later stages in 2008, 2009 and 2013 with the application of a preventive treatment during the stage 5, since the highest airborne fungal propagule load coincided near to flowering stages in these years but no treatment was applied during this critical phase.

Finally, it is notable that the proposed method can also act as a crop protection tool against the consequences of climate change because the increase in the variability of climatic conditions may affect vegetal phenology and pathogen biology (Dalla Marta *et al.*, 2010; Lamichhane *et al.*, 2015). This climatic variability and changes are not considered in preset calendars for

https://doi.org/10.1017/S0021859620000362

chemical product applications. The developed evaluation of infection risk can be adapted to other fungal grapevine pathogens, or even for other crops, by considering the specific pathogen requirements, the environmental conditions and the vegetal susceptibility according to the plant phenological stage (Paul and Munkvold, 2005; De Wolf and Isard, 2007). These conditions affect the pathogen–crop relationship at a microscale level because the canopy microclimate regulates fungal growth and development. Several factors, such as wind speed within the canopy, temperature, atmospheric humidity and leaf wetness vary markedly depending on agricultural practices that potentially change canopy architecture, such as leaf removal, plant spacing, cultivar selection or irrigation practice (English *et al.*, 1989).

Conclusions

The proposed method resulted in a useful tool with which to dynamically predict the main grey mould infection risk periods by means of: phenological observations to identify the susceptible stages, exceedance of the 100 spores/m^3 threshold in the atmosphere of the vineyard and the possibility that propitious meteorological conditions enhance spore germination over the subsequent 4–6 days. This supposes a time window enough for winegrowers to apply the required chemical treatments before the *Botrytis* lesions appearance on the crop. A 25–35% reduction in the number of fungicide treatments was achieved following the proposed method in the studied vineyard. This reduction promotes the protection of the environment and human health, and the reduction of economic costs with an added improvement in the products obtained. These are the most important current challenges of winegrowers, especially those related to the wine Designation of the Origin area.

Authorship declaration

The authors have contributed significantly and they are in agreement with the data presented in the present study.

Financial support. This work was funded by Xunta de Galicia CITACA Strategic Partnership (Reference: ED431E 2018/07) and the AGL2014-60412-R Economy and Competence Ministry of Spain Government project. Fernández-González M. was supported by FCT (SFRH/BPD/125686/2016) through HCOP-Human Capital Operational Program, financed by 'Fundo Social Europeu' and 'Fundos Nacionais do MCTES'. González-Fernández E. was supported by the Ministry of Sciences, Innovation and Universities (FPU grant FPU15/03343). Piña-Rey A. was supported by Xunta de Galicia Pre-doctoral Period Support Program (ED481A-2017/xxx).

Conflict of interest. The authors declare that there are no conflicts of interest.

Ethical standards. Not applicable.

References

Agrios GN (2005) *Plant Pathology*, 5th Edn. San Diego, CA, USA: Elsevier-Academic Press.

Barzman M, Bàrberi P, Birch ANE, Boonekamp P, Dachbrodt-Saaydeh S, Graf B, Hommel B, Jensen JE, Kiss J, Kudsk P, Lamichhane JR, Messéan A, Moonen AC, Ratnadass A, Ricci P, Sarah JL and Sattin M (2015) Eight principles of integrated pest management. *Agronomy for Sustainable Development* **35**, 1199–1215.

Blanco-Ward D, Garcia JM and Jones GV (2007) Spatial climate variability and viticulture in the Miño River Valley of Spain. *Vitis* **46**, 63–70.

Bregaglio S, Donatelli M and Confalonieri R (2013) Fungal infections of rice, wheat, and grape in Europe in 2030–2050. *Agronomy for Sustainable Development* **33**, 767–776.

Broome JC, English JT, Marois JJ, Latorre BA and Aviles JC (1995) Development of an infection model for *Botrytis* bunch rot of grape based on wetness duration and temperature. *Phytopathology* **85**, 97–102.

Bugiani R, Govoni P, Bottazzi R, Giannico P, Montini B and Pozza M (1995) Monitoring airborne concentrations of sporangia of *Phytophthora infestans* in relation to tomato late blight in Emilia Romagna, Italy. *Aerobiologia* **11**, 41–46.

Bulit J and Dubos B (1988) *Botrytis* bunch rot and blight. In Pearson RC and Goheen AC (eds), *Compendium of Grapes Diseases*. The American Phytopathological Society, St. Paul, Minnesota, USA: APS Press, pp. 13–15.

Carisse O and Van der Heyden H (2015) Relationship of airborne *Botrytis cinerea* Conidium concentration to tomato flower and stem infections: a threshold for de-leafing operations. *Plant Disease* **99**, 137–142.

Carisse O, Savary S and Willocquet L (2008) Spatiotemporal relationships between disease development and airborne inoculum in unmanaged and managed *Botrytis* leaf blight epidemics. *Phytopathology* **98**, 38–44.

Carmichael PC, Siyoum N, Jongman M and Korsten L (2018) Prevalence of *Botrytis cinerea* at different phenological stages of table grapes grown in the northern region of South Africa. *Scientia Horticulturae* **239**, 57–63.

Ciliberti N, Fermaud M, Languasco L and Rossi V (2015) Influence of fungal strain, temperature, and wetness duration on infection of grapevine inflorescences and young berry clusters by *Botrytis cinerea*. *Phytopathology* **105**, 325–333.

Ciliberti N, Fermaud M, Roudet J, Languasco L and Rossi V (2016) Environmental effects on the production of *Botrytis cinerea* Conidia on different media, grape bunch trash, and mature berries. *Australian Journal of Grape and Wine Research* **22**, 262–270.

Dalla Marta A, Grifoni D, Mancini M, Storchi P, Zipoli G and Orlandini S (2010) Analysis of the relationships between climate variability and grapevine phenology in the Nobile di Montepulciano wine production area. *Journal of Agricultural Science* **148**, 657–666.

De Wolf E and Isard A (2007) Disease cycle approach to plant disease prediction. *Annual Review of Phytopathology* **45**, 203–220.

English JT, Thomas CS, Marois JJ and Gubler WD (1989) Microclimates of grapevine canopies associated with leaf removal and control of *Botrytis* bunch rot. *Phytopathology* **79**, 395–401.

Esterio M, Auger J, Droguett A and Arroyo A (1996) Effectiveness of biological integrated and traditional control programs of *Botrytis cinerea* in table grape in the Central Valley of Chile. *Proceedings of the XIth International Botrytis Symposium,* 23–28 June 1996. Wageningen, The Netherlands. p. 73.

Esterio M, Muñoz G, Ramos C, Cofré G, Estévez R, Salinas A and Auger J (2011) Characterization of *Botrytis cinerea* isolates present in Thompson Seedless table grapes in the Central Valley of Chile. *Plant Disease* **95**, 683–690.

Fernández-González M, Rodríguez-Rajo FJ, Jato V and Aira MJ (2009) Incidence of fungals in a vineyard of the denomination of origin ribeiro (Ourense – north-western Spain). *Annals of Agricultural and Environmental Medicine* **16**, 263–271.

Fernández-González M, Rodríguez-Rajo FJ, Jato V, Aira MJ, Ribeiro H, Oliveira M and Abreu I (2012) Forecasting ARIMA models for atmospheric vineyard pathogens in Galicia and Northern Portugal: *Botrytis cinerea* spores. *Annals of Agricultural and Environmental Medicine* **19**, 255–262.

Frenguelli G (2001) Interactions between climatic changes and allergenic plants. In Moscato G (ed.), *Environment and Allergy*. Proceedings of the Gemma Gherson Symposium; 5–6 October 2001; Pavia, Italy. Pavia: IRCCS CLR Fondazione Salvatore Maugeri, pp. 141–143. Monaldi Archives for Chest Disease, 57.

Galán C, Cariñanos P, Alcázar P and Domínguez E (2007) *Spanish Aerobiology Network (REA): Management and Quality Manual.* Córdoba, Spain: University of Córdoba Publication Service.

Galán C, Ariatti A, Bonini M, Clot B, Crouzy B, Dahl A, Fernandez-González D, Frenguelli G, Gehrig R, Isard S, Levetin E, Li DW,

https://doi.org/10.1017/S0021859620000362

Mandrioli P, Rogers C, Thibaudon M, Sauliene I, Skjoth C, Smith M and Sofiev M (2017) Recommended terminology for aerobiological studies. *Aerobiologia* **33**, 293–295.

Hatmi S, Villaume S, Trotel-Aziz P, Barka EA, Clément C and Aziz A (2018) Osmotic stress and ABA affect immune response and susceptibility of grapevine berries to gray mold by priming polyamine accumulation. *Frontiers in Plant Science* **9**, 1010, 10.3389/fpls.2018.01010.

Hirst JM (1952) An automatic volumetric spore-trap. *Annals of Applied Biology* **36**, 257–265.

Holz G, Coertze S and Basson EJ (1997) Latent infection of *Botrytis cinerea* in grape pedicels leads to postharvest decay. *Phytopathology* **87**(suppl.), S43.

Holz G, Coertze S and Williamson B (2007) The ecology of *Botrytis* on plant surfaces. In Elad Y, Williamson B, Tudzynski P and Delen N (eds), *Botrytis: Biology, Pathology and Control*. Dordrecht, The Netherlands: Springer, pp. 9–28.

Jeger MJ (1984) Relating disease progress to cumulative numbers of trapped spores: apple powdery mildew and scab epidemics in sprayed and unsprayed orchard plots. *Plant Pathology* **33**, 517–523.

Kretschmer M, Kassemeyer H and Hahn M (1994) Age-dependent grey mould susceptibility and tissue-specific deference gene activation of grape vine berry skins after infection by *Botrytis cinerea*. *American Journal of Enology and Viticulture* **45**, 133–140.

Lamichhane JR, Barzman M, Booij K, Boonekamp P, Desneux N, Huber L, Kudsk P, Langrell SRH, Ratnadass A, Ricci P, Sarah JL and Messéan A (2015) Robust cropping systems to tackle pests under climate change. A review. *Agronomy for Sustainable Development* **35**, 443–459.

Latorre BA (1986) Manejo de *Botrytis cinerea* En uva de mesa. *Revista Frutícola* **7**, 75–88.

Lorenz DH, Eichhorn KW, Blei-holder H, Klose R, Meier U and Weber E (1994) Phanologische Entwicklungsstadien der Weinrebe (*Vitis vinifera* L. ssp. vinifera). *Viticulture and Enology Science* **49**, 66–70.

Madden LV, Ellis MA, Lalancette N, Hughes G and Wilson LL (2000) Evaluation of a disease warning system for downy mildew of grapes. *Plant Disease* **84**, 549–554.

Magarey RD, Sutton TB and Thayer CL (2005) A simple generic infection model for foliar fungal plant pathogens. *Phytopathology* **95**, 92–100.

Manter DK, Reeser PW and Stone JK (2005) A climate-based model for predicting geographic variation in Swiss needle cast severity in the Oregon Coast Range. *Phytopathology* **95**, 1256–1265.

Martínez-Bracero M, Alcázar P, Velasco-Jiménez MJ and Galán C (2018) Fungal spores affecting vineyards in Montilla-Moriles Southern Spain. *European Journal of Plant Pathology* **153**, 1–13. doi: 10.1007/s10658-018-1532-6.

McClellan WD and Hewitt WB (1973) Early *Botrytis* rot of grapes: time of infection and latency of *Botrytis cinerea* Pers. in *Vitis vinifera* L. *Phytopathology* **63**, 1151–1157.

Meier U (2001) *Growth Stages of Mono and Dicotyledonous Plants*. The extended BBCH-scale. BBCH Monograph. 2nd Edn. Germany: Federal Biological Research Centre for Agriculture and Forestry.

MeteoGalicia (2018) Galician Institute for Meteorology and Oceanography, Santiago de Compostela, Spain. Environment, Territory and Infrastructure Department of Galician Regional Government. Available at http://www.meteogalicia.gal/web/index.action.

Moreno-Grau S, Aira MJ, Elvira-Rendueles B, Fernández-González M, Fernández-González D, García-Sánchez A, Martínez-García MJ, Moreno JM, Negral L, Vara A and Rodríguez-Rajo FJ (2016) Assessment of Olea pollen and its major allergen Ole e 1 concentrations in the bioaerosol of two biogeographical areas. *Atmospheric Environment* **145**, 264–271.

Nair NG and Allen RN (1993) Infection of grape flowers and berries by *Botrytis cinerea* as a function of time and temperature. *Mycological Research* **97**, 1012–1014.

Oliveira M, Guerner-Moreira J, Mesquita MM and Abreu I (2009) Important phytopathogenic airborne fungal spores in a rural area: incidence of *Botrytis cinerea* and *Oidium* spp. *Annals of Agricultural and Environmental Medicine* **16**, 197–204.

Paul PA and Munkvold GP (2005) Regression and artificial neural network modeling for the prediction of gray leaf spot of maize. *Phytopathology* **95**, 388–396.

Rodríguez-Rajo FJ, Jato V, Fernández-González M and Aira MJ (2010) The use of aerobiological methods for forecasting *Botrytis* spore concentrations in a vineyard. *Grana* **49**, 56–65.

Rosa M, Gozzini B, Orlandini S and Seghi L (1995) A computer program to improve the control of grapevine downy mildew. *Computers and Electronics in Agriculture* **12**, 311–322.

Rossi V and Giosuè S (2003) A dynamic simulation model for powdery mildew epidemics on winter wheat. *European and Mediterranean Plant Protection Organization Bulletin* **33**, 389–396.

Rossi V, Giosuè S, Pattori E, Spanna F and Del Vecchio A (2003) A model estimating the risk of *Fusarium* head blight on wheat. *European and Mediterranean Plant Protection Organization Bulletin* **33**, 421–425.

SIXPAC (2018) Sistema de Información Geográfica de Parcelas Agrícolas. Official reference database for agriculture area identification, of Agriculture, Fishing and Feeding Ministry. Spanish Government. Available at https://www.mapa.gob.es/es/agricultura/temas/sistema-de-informacion-geografica-de-parcelas-agricolas-sigpac-/default.aspx.

Stevens RB (1960) Cultural practices in disease control. In Horsfall JG and Dimond AE (eds), *Plant pathology: an advanced treatise*, Volume 3. NY, USA: Academic Press, pp. 357–429.

Twengström E, Sigvald R, Svensson C and Yuen J (1998) Forecasting *Sclerotinia* stem rot in spring sown oilseed rape. *Crop Protection* **17**, 405–411.

Wolf TK, Baudin ABAM and Martínez-Ochoa N (1997) Effect of floral debris removal from fruit clusters on *Botrytis* bunch rot of Chardonnay grapes. *Vitis* **36**, 27–33.