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The use of aerobiological methods for forecasting *Botrytis* spore concentrations in a vineyard

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Abstract

Grey mould, caused by *Botrytis cinerea*, is one of the most common diseases in Galician vineyards. Knowledge of local airborne spore concentrations is of great value for developing models to predict fungal propagule concentrations, thus enabling phytosanitary treatments to be applied when a real risk of infection is detected.

In order to develop an accurate model for forecasting airborne *B. cinerea* spore concentrations, a phenological and aerobiological survey was carried out in a vineyard located in Galicia (north-west Spain), from 2004 to 2008. Phenological observations were made on 20 vines of each of three grape varieties (Treixadura, Godello and Loureira) using the BBCH standardised phenological scale. A Hirst-type volumetric spore-trap was used for the aerobiological monitoring. The study sought to chart *Botrytis* spore concentrations as function of grapevine phenological stage.

The highest spore concentrations over the grapevine cycle as a whole were recorded in 2008 (37299 spores), and the lowest in 2005 (1700 spores). In the five study years, the highest concentrations were registered during stage 8 (berry ripening), stage 7 (fruit development) and stage 5 (inflorescence emergence). The weather-related parameters displaying the most significant correlation with spore concentrations were dew point and relative humidity. *ARIMA* (Autoregressive Integrated Model of Running Mean) time-series models was used to forecast daily spore concentrations, considering *B. cinerea* spore concentrations and weather data as predictor variables.

Keywords: Aerobiology, *ARIMA*, *Botrytis*, grapevine, phenology

The general bioclimatological conditions of vineyards located in north-western Spain favour the development of fungal diseases that have a marked impact on the grape harvest. The most common diseases are grey mould (caused by *Botrytis cinerea* Pers.), powdery mildew (*Uncinula necator* (Schw.) Burr.) and downy mildew (*Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni). Fungal diseases are likely to impair future wine quality due to degradation of colorants, destruction of the film containing aromatic substances, reduction in alcohol content, increased fixation of SO₂ and increased volatile acidity (Ribereau-Gayon & Peynaud, 1971). In recent years, new diseases have arisen, including grapevine necrosis, esca disease, *Armillaria* root disease, black

rot and *Eutypa* dieback, which can eventually lead to the death of the plant (Mansilla et al., 1991). Some of these emerging diseases are probably caused by the rise in spring temperatures recorded in various parts of Europe (Frenguelli, 2001).

The strategy most widely adopted by winegrowers to reduce the impact of these fungal diseases is the systematic application of chemical fungicides, generally following preset calendars based on the phenological growth stages of the grapevine (Bugiani et al., 1995). However, since excessive use of these products may cause serious crop damage (e.g. by stimulating the appearance of resistant fungal stumps and eliminating beneficial mycological flora) as well as harming the environment, chemical treatments should be used

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only when there is a real risk of unacceptable economic damage. The integrated management of pests and diseases involves a number of control methods aimed at ensuring effective vineyard protection; preference is given to more natural and less harmful techniques, which enable the use of chemicals to be reduced. Thorough inspection over the whole crop cycle is required in order to detect the appearance of pests and pathogens, to chart their development, and to ascertain both risks and tolerance thresholds. Therefore, crop phenological study, correct identification of the pathogen and its biological cycle, definition and quantification of the damage caused, and description of the affected parts of the plant could be incorporated in the applied methodology in each technical mission. As part of this process, it is essential to determine present and future disease status, not only by tracking weather conditions but also by monitoring airborne phytopathogenic fungal spore levels.

Pathogen biology comprises a number of stages during which interactions between host, pathogen and environment influence disease development. Over recent years, several prediction models providing useful information for plant disease management have been proposed as part of the fight against the major grapevine diseases. The various stages of the disease cycle form the basis of many plant disease prediction models (De Wolf & Isard, 2007); for example, models based on the influence of weather parameters (mainly temperature and moisture duration) have been developed for *Botrytis cinerea* (Nair & Allen, 1993; Broome et al., 1995).

Aerobiological studies provide valuable data on daily and hourly airborne spore concentrations. A number of surveys have sought to relate disease levels at a given time with airborne spore concentrations at that time or previous to it (e.g. Jeger, 1984). In their study of *Botrytis* leaf blight, Carisse et al. (2008) reported a significant correlation between airborne conidium concentrations on a given date and lesion density one week later, particularly when both disease intensity and airborne conidium levels were high. Airborne spore concentrations could serve as an indicator of pathogen development, and may be of particular value when the infection level is determined more by inoculum density than by weather conditions (Jeger, 1984). In these circumstances, reliable monitoring of airborne inoculum is a useful tool for pathogen management (Carisse et al., 2008). A certain threshold level of spore concentration could be utilised as a warning of real risk of disease; from that point onwards, any weather condition favorable to disease development could prompt release of the disease (Bugiani et al., 1995). Implementation of a system of this sort would

clearly lead to a lower number of treatments, and thus to a reduction in both economic costs and environmental damage (Seem et al., 1985).

The present study sought to monitor *Botrytis cinerea* airborne spore concentrations in the Ribeiro Denomination of Origin area. The incidence of this fungus during the various grapevine phenological phases and potential correlations with major weather-related variables were also assessed. Although the most common prediction methods are based on linear regression models, over recent years some attempts have been made to apply time-series analysis – widely used in biomedical research and in air-pollution studies – to aerobiological data sets (Moseholm et al., 1987; Belmonte & Canela, 2002; Galán et al., 2003). The present study aimed to develop an accurate model for forecasting *B. cinerea* airborne spore concentrations.

Materials and methods

The Ribeiro region of north-western Spain covers a total area of 371.4 km² (Figure 1). The study was carried out in a vineyard at Cenlle (altitude 75–400 m.) characterised by fairly steep valleys and hillsides. The main grape varieties grown are Treixadura, Godello and Loureira. The particular Oceanic-Mediterranean transition ecoclimate of this region is favoured by its southern situation in Galicia and by the natural barriers that protect the territory from sub-Atlantic storms. According to the Multicriteria Climatic Classification System (MCC), most wine-making areas in this region watered by the river Miño would be defined as temperate and warm, sub humid, with very cold nights (Blanco-Ward et al., 2007).

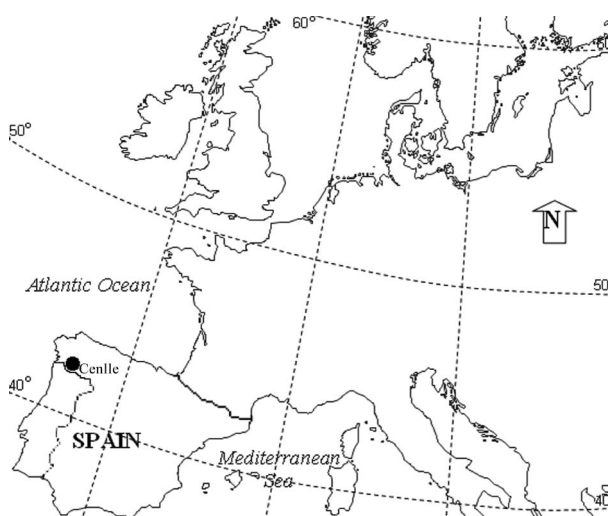


Figure 1. Location of Cenlle in the Ribeiro region of northern Spain.

Sampling was carried out during the active *Vitis* season, from 2004 to 2008. The study period from 2005 to 2008 was 1 April to 14 September; in 2004, sampling started on 20 April and ended on 14 September. Power cuts prevented monitoring on 12/13 May, 22–25 July, and 4–7 September in 2004; on 6 September 2005; from 31 August to 6 September 2006; from 10 to 14 September 2007; and on 30 April 2008.

Spore sampling was performed using a LAN-ZONI VPPS-2000 volumetric trap located in the central part of the vineyard. The sampler was placed 2 m above ground level, so that spore trapping would not be impeded by plant growth. Melinex tape coated with a 2% silicone solution was used as the spore-trapping surface. The exposed tape was cut into seven pieces, which were mounted on separate glass slides. *B. cinerea* conidia were counted following the model proposed by the Spanish Aerobiological Network (REA), based on two longitudinal transects along the slides (Galán et al., 2007). Spores were identified and counted following Aira et al. (2005) and Galán et al. (2007), and daily mean spore counts were expressed as spores/m³ of air.

In order to ascertain spore levels for each phase of plant development, a phenological study was carried out during the active grapevine season from 2004 to 2008 (from 1 April to grape harvest in September); a total of 60 selected plants were monitored, 20 of each of the three varieties grown: Treixadura, Godello and Loureira. Each plant was observed twice a week to determine growth stage and phase, using the scale recommended by Lorenz et al. (1994), adopted by the BBCH (Meier, 2001) for the phenological observation of grapevines. The start date of each phenological stage was taken to be the date by which 50% of studied plants had reached that stage. Fungicide application dates were also recorded.

Spearman's test was used to test for correlations between airborne spores concentration and the main weather-related factors: rainfall (mm), relative humidity (%), hours of sunshine (hours), maximum, minimum and mean temperatures (°C), dew point (°C), wind direction (%), and wind speed (m/s). 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 for the previous one to seven days. Significance was calculated for $p \leq 0.01$ and $p \leq 0.05$, respectively. Data for temperature (maximum, minimum and mean), dew point and relative humidity were obtained from a Hobo H8 Pro Series data logger located in the vineyard itself. Data for rainfall, sunlight and wind speed were recorded on a Davis weather station.

Finally, ARIMA (autoregressive integrated model of running mean) model was used to predict *Botrytis cinerea* spore concentrations. Weather-related variables displaying the highest positive correlation coefficients, and spore concentrations for the previous days, were selected as estimators for the model. Time series are a mixture of several components: T_t or the long trend value, E_t or the fluctuations of the series in periods smaller than one year, C_t or fluctuations of the series in periods longer than one year, and the I_t or random or sporadic factors (Tobías et al., 2004). The equation followed by a time series is an additive model: $Y_t = T_t + C_t + E_t + I_t$.

A model is considered autoregressive if the values of the series depend on, or are related to, previous values of the variable. A multiple linear regression function can be established in which the dependent variable is the observation in the " t " period and the independent variables are those of previous periods that are related to the dependent variable. In the model the three ARIMA parameters were tested: autoregressive (p), differentiation (d) and running mean (q).

p = Number of autoregressive parameters of the model. Each parameter measures the independent effect of the values with a specified delay. A second-order autoregressive means that each value in the series is affected by the two preceding values (independently of each other).

d = Number of times that a time series was transformed calculating the differences between the values of the series and its predecessors.

q = The order of the running mean of the process.

The accuracy ARIMA model developed was tested with data from the 2008 harvest. With the aim of the statistical validation of the ARIMA model proposed predictive ability, a t-test for dependent samples and a Scheffé Anova–Manova test have been carried out (by using an interval of confidence of the 95%). Data from the predicted spore values during the 2008 harvest versus the real spore concentrations registered during this year were considered in both tests. In all statistical analyses, the SPSS 16.0 software package was used.

Results

Over the study period, the highest *Botrytis cinerea* spore concentrations for the grapevine cycle as a whole were recorded in 2008 (37299 spores), and the lowest in 2005 (1700 spores). Intermediate values were recorded in 2007 (15330), 2004 (5022) and 2006 (4881) (Table I).

The presence of airborne *Botrytis cinerea* spores was virtually constant, being recorded on 86–98% of sampling days. In general terms, the highest spore

Table I. Start date, length (days), maximum daily value of *Botrytis cinerea* spores (spores/m³) and the date of the maximum value of the phenological grapevine principal BBCH growth stages (0 – sprouting; 1 – leaf development; 5 – inflorescence emergence; 6 – flowering; 7 – development of fruits; 8 – ripening of berries) during the years studied. The length average of the stages, the average of the total *B. cinerea* spores registered in each stage and the total spores registered in each year during the period 2004–2008 are also displayed.

		Phenological grapevine principal BBCH growth stages						Total annual spores
		0	1	5	6	7	8	
2004	Start date		Apr-18	Apr-27	Jun-02	Jun-08	Aug-9	5022
	Length		9	36	6	62	37	
	Botrytis spores maximum		2	217	97	86	257	
	Date maximum		Apr-26	May-27	Jun-05	Jul-17	Sep-14	
2005	Start date	Apr-01	Apr-04	Apr-27	Jun-02	Jun-11	Aug-12	1700
	Length	3	23	36	9	62	30	
	Botrytis spores maximum	1	23	85	39	37	51	
	Date maximum	Apr-02	Apr-07	May-25	Jun-02	Jun-22	Aug-13	
2006	Start date	Apr-1	Apr-13	Apr-28	May-29	Jun-07	Aug-11	4881
	Length	12	15	31	9	65	35	
	Botrytis spores maximum	91	45	24	41	203	69	
	Date maximum	Apr-09	Apr-19	Apr-28	Jun-03	Jun-16	Aug-29	
2007	Start date	Apr-10	Apr-12	Apr-20	May-25	Jun-07	Aug-02	15330
	Length	11	8	35	13	56	44	
	Botrytis spores maximum		58	415	189	219	408	
	Date maximum		Apr-17	Apr-27	May-29	Jul-27	Sep-04	
2008	Start date	Apr-01	Apr-06	Apr-29	Jun-06	Jun-17	Aug-12	37299
	Length	5	23	38	11	56	34	
	Botrytis spores maximum	67	151	1669	939	711	227	
	Date maximum	Apr-05	Apr-24	May-28	Jun-07	Jun-28	Sep-09	
2004–2008	Stages length		16	35	10	60	40	2034
	Total spores average		377	4127	1272	4919	2034	

counts were observed either in May–June or just before the grape harvest in September (Figure 2).

Since very few phenological differences were observed between the three varieties studied (Treixadura, Godello and Loureira), the average duration of the main growth stages was calculated using data for all 60 plants together. The duration of the cycle from the beginning of stage 1 (leaf development) to harvest was fairly constant, ranging from 157 days in 2004 and 2006 to 168 days in 2008. Nevertheless, considerable year-to-year differences were noted in the duration of individual stages (Table I). The greatest variation was observed for stages 1 (leaf development) and 8 (ripening of berries). The longest-lasting stage for the study as a whole was stage 7 (development of fruits) with a mean duration of 60 days, followed by stage 8 (ripening of berries; 40 days) and stage 5 (inflorescence emergence; 35 days). The shortest stages were flowering (stage 6; mean 10 days) and leaf development (stage 1; 16 days).

In general, total spore counts were higher during the final stages (Table I), especially stage 8 (five-year mean count 2034 spores) and stage 5 (4127 spores), although high levels were also recorded in stage 7 (mean 4919 spores), particularly in 2008 (Figure 3).

In order to ascertain the influence of major weather-related parameters on airborne *Botrytis cinerea* spore counts, Spearman linear correlation analysis was applied, taking *B. cinerea* spore counts and weather parameters for the same day and the seven preceding days as dependent variables (Table II). Correlation-coefficients (calculated using data for each year as well as data for the whole study period) were significant in most of the cases. In 2004, the weather parameter displaying the highest correlation-coefficient was dew-point of two days earlier, while in 2005 the highest values were found for maximum and mean temperature the same day and the dew-point of the previous day. In 2006, the highest value was found for the dew-point of five days earlier and in 2007, for minimum and mean temperature of the previous day. A highly-significant ($p < 0.01$) positive correlation was found for dew-point (Table II) and for local airborne *B. cinerea* spore counts during the previous days (from 0.474 in the year 2005 to 0.765 in 2004).

Similarly, the Spearman correlation test using data for the whole study period (2004–2007) showed that the highly-significant positively-correlated parameters ($p < 0.01$) exerting the most influence on spore

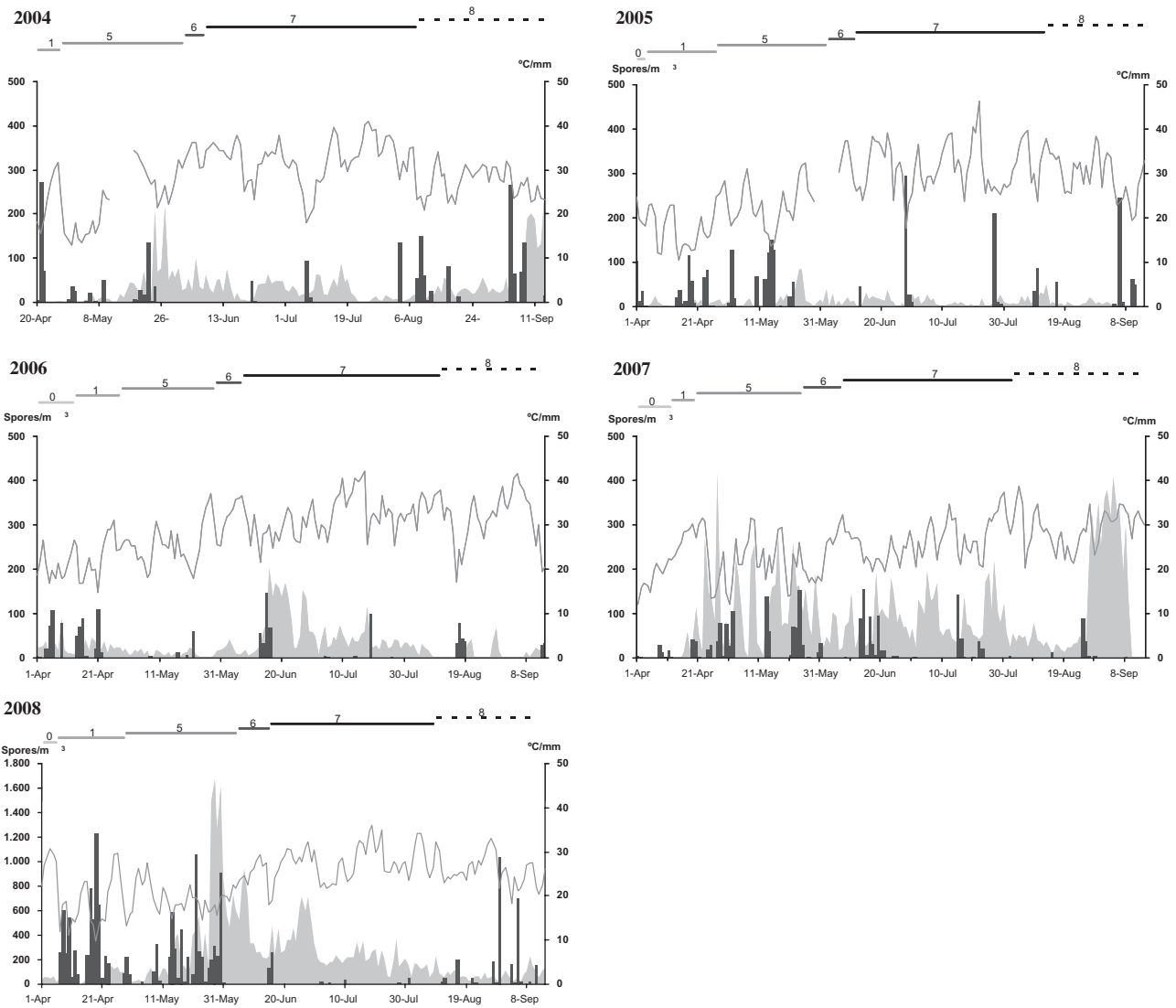


Figure 2. Spore concentrations of *Botrytis cinerea* during the vegetative cycle stages (0 – sprouting; 1 – leaf development; 5 – inflorescence emergence; 6 – flowering; 7 – development of fruits; 8 – ripening of berries) of the grapevine in the studied period. The grey line represents maximum temperature, and rainfall is represented by black bars.

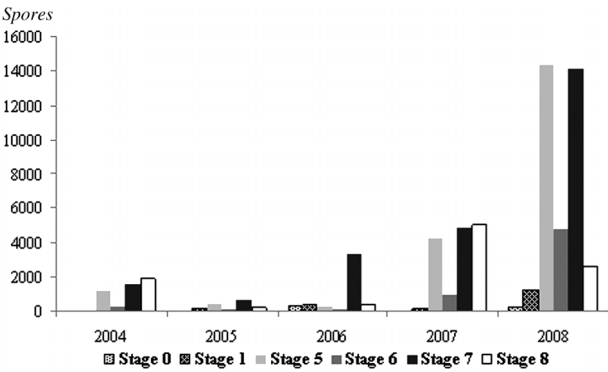


Figure 3. Total spore values of *Botrytis cinerea* during the vegetative cycle stages (0 – sprouting; 1 – leaf development; 5 – inflorescence emergence; 6 – flowering; 7 – development of fruits; 8 – ripening of berries) of the grapevine in the studied period.

counts, were *Botrytis cinerea* spore concentrations over previous days, relative humidity three days earlier, and dew-point two days earlier.

On the basis of these correlation results, the predictive capacity of each of these variables, and several combinations thereof, was evaluated in order to obtain ARIMA time-series models to predict the *Botrytis cinerea* airborne spores in the vineyard (Table III). The most accurate forecasting model obtained was an ARIMA (2,0,1) including only the dew-point two days earlier as independent weather variable, presenting a high R^2 value of 0.737. The model is able to forecast the *B. cinerea* airborne spores concentrations with a prediction horizon of 24 hours.

The prediction capacity of the ARIMA model was therefore evaluated by means of the comparison of

Table II. Correlation between the spore concentrations in the study period and the principal meteorological variables applying Spearman's test ($p \leq 0.01$ *** highly significant; $p \leq 0.05$ ** very significant). Values of the seven previous days were also considered.

	2004	2005	2006	2007	2004–2007
<i>B. cinerea</i> -1	0.765***	0.474***	0.681***	0.664***	0.796***
<i>B. cinerea</i> -2	0.659***	0.390***	0.578***	0.471***	0.725***
<i>B. cinerea</i> -3	0.657***	0.167**	0.554***	0.486***	0.691***
<i>B. cinerea</i> -4	0.570***	0.066	0.416***	0.450***	0.640***
<i>B. cinerea</i> -5	0.530***	0.086	0.415***	0.413***	0.627***
<i>B. cinerea</i> -6	0.498***	0.059	0.361***	0.375***	0.596***
<i>B. cinerea</i> -7	0.428***	0.163**	0.349***	0.253***	0.575***
Maximum temperature	0.229***	0.355***	0.126	0.254***	0.131***
Maximum Temp-1	0.160	0.290***	0.090	0.223***	0.086**
Maximum Temp-2	0.095	0.198***	0.011	0.161**	0.017
Maximum Temp-3	0.089	0.135	0.000	0.145	-0.020
Maximum Temp-4	0.014	0.151	0.029	0.174**	-0.027
Maximum Temp-5	0.010	0.100	0.042	0.254***	-0.021
Maximum Temp-6	0.036	0.076	0.130	0.247***	-0.010
Maximum Temp-7	0.048	0.081	0.168**	0.194**	-0.005
Minimum temperature	0.266***	0.281***	0.115	0.209***	0.116***
Minimum Temp-1	0.264***	0.239***	0.047	0.367***	0.119***
Minimum Temp-2	0.288***	0.274***	0.051	0.343***	0.121***
Minimum Temp-3	0.237***	0.246***	0.102	0.285***	0.104**
Minimum Temp-4	0.211**	0.222***	0.112	0.307***	0.100**
Minimum Temp-5	0.215**	0.205***	0.181**	0.301***	0.103**
Minimum Temp-6	0.238***	0.180**	0.204***	0.250***	0.099**
Minimum Temp-7	0.226***	0.104	0.241***	0.180**	0.076
Mean temperature	0.209**	0.336***	0.125	0.280***	0.127***
Mean Temp-1	0.164	0.260***	0.052	0.306***	0.091**
Mean Temp-2	0.126	0.225***	-0.007	0.265***	0.043
Mean Temp-3	0.080	0.181**	0.019	0.211***	0.212***
Mean Temp-4	0.017	0.178**	0.020	0.241***	0.172***
Mean Temp-5	0.009	0.149	0.068	0.268***	0.122***
Mean Temp-6	-0.065	-0.002	0.146	0.162**	0.131***
Mean Temp-7	-0.061	0.016	0.208***	0.091	0.086**
Dew-point	0.416***	0.311***	0.287***	0.014	0.317***
Dew point-1	0.427***	0.329***	0.294***	0.075	0.325***
Dew point-2	0.446***	0.316***	0.342***	0.129	0.332***
Dew point-3	0.411***	0.250***	0.401***	0.149	0.315***
Dew point-4	0.362***	0.198***	0.404***	0.142	0.300***
Dew point-5	0.365***	0.179**	0.429***	0.190**	0.281***
Dew point-6	0.344***	0.139	0.411***	0.206***	0.242***
Dew point-7	0.281***	0.081	0.351***	0.178**	0.178***
Relative humidity	0.158	-0.126	0.090	-0.038	0.098**
Humidity-1	0.244***	0.030	0.174**	0.027	0.169***
Humidity-2	0.305***	0.112	0.260***	0.102	0.235***
Humidity-3	0.313***	0.126	0.245***	0.113	0.242***
Humidity-4	0.317***	0.090	0.220***	0.082	0.232***
Humidity-5	0.339***	0.080	0.165**	0.036	0.212***
Humidity-6	0.304***	0.030	0.066	0.038	0.172***
Humidity-7	0.251***	-0.036	-0.065	0.033	0.122***
Rainfall	-0.190**	-0.255***	0.105	-0.038	-0.033
Rainfall-1	-0.174**	-0.132	0.123	0.022	0.004
Rainfall-2	-0.090	0.020	0.185**	0.150	0.079
Rainfall-3	0.017	0.014	0.178**	0.139	0.093**
Rainfall-4	0.081	0.065	0.150	0.114	0.107***
Rainfall-5	0.136	0.078	0.140	0.124	0.116***
Rainfall-6	0.174**	0.034	0.003	0.062	0.074
Rainfall-7	0.174**	-0.044	-0.052	0.052	0.052

Table III. Time series of the proposed ARIMA model.

Adjusted R ² = 0.737	B	ET	t	Sig.
AR1	1.581	0.079	20.143	0.000
AR2	-0.592	0.073	-8.079	0.000
MA1	0.846	0.060	14.193	0.000
Dew point-2	3.452	1.733	1.992	0.047

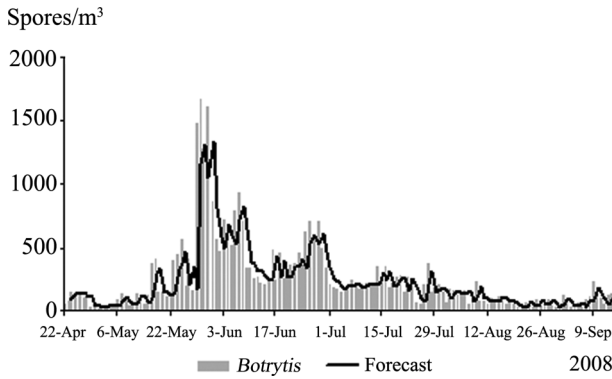


Figure 4. Daily mean *Botrytis cinerea* spore concentration observed and predicted during the year 2008 (which has not been included to develop the model) testing the proposed ARIMA model.

the observed *Botrytis cinerea* spore concentration versus the predicted spore counts of the 2008 harvest (year, which was not taken into account to establish the aforementioned model) once the proposed Time-series equation was applied (Figure 4). The predicted values matched actual spore counts in most cases. Moreover, the good forecast behaviour of the model is also demonstrated statistically as the t-test of dependent samples conducted show that there is not significant difference at the 95% level between predicted *B. cinerea* spore data and observed data during the harvest 2008 (Table IV). Similar results were found by means of the Scheffé Anova–Manova test (*p* value of 0.684).

Discussion

The presence of airborne *Botrytis cinerea* conidia was virtually constant throughout the grapevine growing season, although major variations were recorded between days and also between years. The abundance of these airborne conidia in Galician vineyards has

been reported in previous studies (Díaz et al., 1997, 1998; Rodríguez-Rajo et al., 2002; Albelda et al., 2005).

The synchronism of major grapevine growth stages with airborne *Botrytis cinerea* spore counts has been studied by several authors in different geographical areas. It is widely accepted that the most critical stages for infection are flowering and the period between berry ripening and harvest (Latorre, 1986; Bulit & Dubos, 1988). Here, the highest total spore counts were recorded during growth stages 8 (ripening of berries), 7 (development of fruits) and 5 (inflorescence emergence). Pollen and sugar exudation during flowering favours the colonisation of tissues by this pathogen (Esterio et al., 1996), which might account for increased conidia counts during this phenological stage.

Moreover, the period from the start of ripening of berries to harvest is also considered a vulnerable period for the vine (Latorre, 1986; Bulit & Dubos, 1988). As the here presented results demonstrate, increased *Botrytis cinerea* spore count suggests increased spore load in the atmosphere and/or increased spore deposition; markedly during ripening of berries in 2004 and 2007, with peak daily counts of over 200 and 400 spores/m³, respectively. Similar findings are reported by Díaz et al. (1997) and Díaz (1999) in previous studies in Galician vineyards. There is a particular risk of crop infection during this period, i.e. in the weeks prior to grape harvest, because the susceptibility of the grapevine to *B. cinerea* infection seems to increase as the berries ripen (Kretschmer et al., 1994). Therefore, the influence of favourable weather conditions, and especially of wetness regime, on the establishment of infection will be greater in mature berries (Coertze et al., 2001). Rain, particularly stormy rains with no noticeable temperature drop, favour propagation of the fungus from the first infection focus (Coley-Smith et al., 1980). Here, spore counts increased after a few days of rain in late August and early September. In these conditions, rain could favour wounding; wounds being regarded as major entry sites for the pathogen on grapes (Nair et al., 1988; Coertze & Holz, 1999), and could also favour dispersal of conidia via rain splashes (Jarvis, 1962), thus prompting an increase in airborne conidia levels over the first fortnight of September. Attention has been

Table IV. Results of the t-test. Marked differences are significant at *p* < 0.050.

	Mean	STDV	N	DIFF	STDV DIFF	t	df	p
<i>Botrytis</i> spores 2008	251.7	285.9						
Forecast	237.8	238.7	145	13.9	166.5	1.0	144.0	0.317

drawn to the importance of grape skin lesions in terms of both symptom expression and the epidemiology of the disease by Coertze et al. (2001).

The higher counts registered in stages 5 and 6 in both 2007 and 2008, might also influence conidia concentrations in stages 7 and 8 (in 2008, very high counts were recorded post-harvest, data not used). Latent infections developed during flowering are considered an important inoculum focus for harvest and postharvest infections (Latorre & Vásquez, 1996; Holtz et al., 1997). Given the potential for colonisation of senescent floral debris and aborted berries, practical measures to reduce debris retention may aid disease control (Wolf et al., 1997).

The weather factors displaying the strongest influence on *Botrytis cinerea* mean daily conidia counts were temperature (especially dew-point) and humidity. Both parameters are considered critical for grey mould spore germination and the development of infection (English et al., 1989; Nair & Allen, 1993; Broome et al., 1995). The high counts recorded in 2007 and 2008 may be linked to heavier and more frequent rainfall. In 2007, rainy spells were recorded over much of the growing season, while in 2008 there was frequent heavy rainfall (168 mm over 24 rainy days) during stage 5 (which recorded the highest airborne conidia counts). Moreover, these two years had the highest values for relative humidity during the growing season (74.7% and 78.7%, respectively in 2007 and 2008) with daily values close to 90%, especially at the end of April and May in 2007 and also in September 2008; these values coincided with very high daily airborne conidia counts. A number of authors have noted that high humidity favours adhesion of conidia to the leaf surface (Spotts & Golz, 1996; Coertze et al., 2001) and subsequent germination and infection. Latorre and Rioja (2001) suggest that relative humidities higher than 90% provide the moisture required to initiate germination and possibly to cause severe infection.

Temperature also exerted considerable influence. Increasing *Botrytis cinerea* spore counts to over 200 spores/m³ were frequently recorded in September, corresponding with a mean air temperature of 20°C, widely considered optimal for infection (Latorre et al., 2002). Similarly, increased airborne conidia counts and peak daily mean conidia counts were observed on days preceded by rainy days and maximum temperatures of over 20°C. By contrast, the lowest concentrations, recorded during July and August, coincided with the highest temperatures at the end of July in 2004, or in August in 2006, where temperatures higher than 35°C – considered as the limit for germination of conidia (Thomas et al., 1988) – were frequent (10 days and 9 days, respectively). However, higher airborne conidia counts in stage 7 in

2007 and 2008 coincided with lower mean maximum temperatures in both years (26.5°C and 27.9°C respectively).

The influence of temperature and humidity may account for the marked effect of dew point the same day and on previous days on *Botrytis cinerea* counts, as revealed by correlation tests. High relative humidity values throughout most of the growing season in 2007 and 2008 would lead to the dew point being reached, thus favouring the condensation of water droplets in the form of dew or fog, and contributing to create conditions required for germination of the conidia (Marois et al., 1986).

Linear logistic models are generally used in aerobiological studies to predict spore concentrations. Linear regression models, using only weather-related variables as prediction variables, yield results with a low predictive capacity. Other variables, better reflecting the various factors affecting the conditions on which fungal spore production and release depend, need to be taken into account. Here, these factors were reflected in spore concentrations over preceding days. The proposed ARIMA time-series model (that takes *Botrytis cinerea* counts over the previous days as an autoregressive parameter) present a high accuracy in the forecasting of the *B. cinerea* spore counts (Rodríguez-Rajo et al., 2002; Cotos-Yañez et al., 2004).

The estimated curve accurately described *Botrytis cinerea* spore behaviour throughout the 2008 harvest, despite the application of various phytosanitary chemical treatments during active vine growth. These treatments might account for some lags between predicted and observed curves when the accuracy of the model was checked, since they alter the natural evolution of the pathogenic cycle and therefore the concentration of spores in the air. Over the five study years, two chemical treatments were applied each year: one during the first fortnight in July (stage 7, fruit of development) and the second during August (stage 8, berry ripening). In general, fungicides were applied at critical times for infection, but in future seasons – taking into account both spore counts as forecast using the ARIMA model and the high phytopathogen levels detected during stages 5 (inflorescence emergence) and 6 (flowering) – it may be useful to bring forward the first treatment to these stages in order to avoid further latent grey mould infection.

High airborne *Botrytis cinerea* spore counts appeared be linked to lesion density one week later (Carisse et al., 2008), thus airborne spore counts predicted by time-series regression models are especially suitable as biological indicators for evaluating the short-term effects of pathogen development and infection. This would lead to a lower number of treatments, and thus to a reduction in both economic costs and environmental damage.

Conclusions

Knowledge of grapevine phenology combined with data on airborne pathogenic spore counts provides a valuable tool for developing a precise and modern integrated pest management strategy in vineyards. The practical application of this information may help to increase grape production and improve the quality of the final product. The inclusion of a large number of data in future will help to improve the prediction models proposed.

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References

- Aira, M. J., Jato, V. & Iglesias, I. (2005). *Calidad del aire. Polen y esporas en la comunidad gallega*. Santiago de Compostela: Xunta Galicia.
- Albelda, Y., Rodríguez-Rajo, F. J., Jato, V. & Aira, M. J. (2005). Concentraciones atmosféricas de propágulos fúngicos en viñedos del Ribeiro (Galicia, España). *Bull. Br. Mycol. Soc.*, 20, 1–8.
- Belmonte, J. & Canela, M. (2002). Modelling aerobiological time series: Application to Urticaceae. *Aerobiologia*, 18, 287–295.
- Blanco-Ward, D., Garcia, J. M. & Jones, G. V. (2007). Spatial climate variability and viticulture in the Miño River Valley of Spain. *Vitis*, 46, 63–70.
- Broome, J. C., English, J. T., Marois, J. J., Latorre, B. A. & Avilés, J. C. (1995). Development of an infection model for *Botrytis* bunch rot of grapes based on wetness duration and temperature. *Phytopathology*, 85, 97–102.
- Bugiani, R., Govoni, P., Bottazzi, R., Giannico, P., Montini, B. & 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. & Dubos, B. (1988). *Botrytis* bunch rot and blight. In R. C. Pearson & A. C. Goheen (Eds), *Compendium of grapes diseases* (pp. 13–15). St. Paul, MN: APS Press.
- Coertze, S. & Holtz, G. (1999). Surface colonization, penetration, and lesion formation on grapes inoculated fresh or after cold storage with single airborne conidia of *Botrytis cinerea*. *Plant Dis.*, 83, 917–924.
- Coertze, S., Holtz, G. & Sadie, A. (2001). Germination and establishment of infection on grape berries by single airborne conidia of *Botrytis cinerea*. *Plant Dis.*, 85, 668–677.
- Coley-Smith, J. R., Verhoeff, K. & Jarvis, W. R. (1980). *The biology of Botrytis*. London: Acad. Press.
- Cotos-Yañez, T. R., Rodríguez-Rajo, F. J. & Jato, M. V. (2004). Short-term prediction of *Betula* airborne pollen concentration in Vigo (NW Spain) using logistic additive models and partially linear models. *Int. J. Biometeorol.*, 48, 179–185.
- De Wolf, E. & Isard, A. (2007). Disease cycle approach to plant disease prediction. *Ann. Rev. Phytopathol.*, 45, 203–220.
- Díaz, M. R. (1999). *Aplicación de la Aerobiología en la agricultura. Control de enfermedades fúngicas y producción de Vitis vinifera*. Ourense: Fac. Sci. Vigo Univ. PhD Diss.
- Díaz, M. R., Iglesias, I. & Jato, V. (1997). Airborne concentrations of *Botrytis*, *Uncinula* and *Plasmopara* spores in Leiro-Ourense (NW Spain). *Aerobiologia*, 13, 31–35.
- Díaz, M. R., Iglesias, I. & Jato, V. (1998). Seasonal variation of airborne fungal spore concentrations in a vineyard of North-West Spain. *Aerobiologia*, 14, 221–227.
- English, J. T., Thomas, C. S., Marois, J. J. & Gubler, W. D. (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. & Arroyo, A. (1996). Effectiveness of biological integrated and traditional control programs of *Botrytis cinerea* in grape in the Central Valley of Chile. In J.A.L. van Kan (Ed.), *11th Int. Botrytis Symp., Wageningen 1996 Proc.* (pp. 73). Wageningen: Wageningen Agric. Univ.
- Frenguelli, G. (2001). Interactions between climatic changes and allergenic plants. In G. Moscato (Ed.), *Environment and Allergy. Proc. G. Gherson Symp., Pavia 2001*. (pp. 141–143). Pavia: IRCCS CLR Fond. S. Maugeri. *Monaldi Arch. Chest Dis.* 57.
- Galán, C., Cariñanos, P., Alcázar, P. & Domínguez, E. (2007). *Manual de Calidad y Gestión de la Red Española de Aerobiología*. Córdoba: Univ. Córdoba Serv. Publ.
- Galán, I., Tobías, A., Banegas, J. R. & Aránguez, E. (2003). Short-term effects of air pollution on daily asthma emergency room admissions in Madrid, Spain. *Eur. Resp. J.*, 22, 802–808.
- Holtz, G., Coertze, S. & Basson, E. J. (1997). Latent infection of *Botrytis cinerea* in grape pedicels leads to postharvest decay. *Phytopathology (Abstr.)*, 87, S43.
- Jarvis, W. R. (1962). The dispersal of spores of *Botrytis cinerea* Fr. in a raspberry plantation. *Trans. Br. Mycol. Soc.*, 45, 549–559.
- Jeger, M. J. (1984). Relating disease progress to cumulative numbers of trapped spores: Apple powdery mildew and scab epidemics in sprayed and unsprayed orchard plots. *Plant Pathol.*, 33, 517–523.
- Kretschmer, M., Kassemeyer, H. & Hahn, M. (1994). Age-dependent Grey Mould susceptibility and tissue-specific defence gene activation of grapevine berry skins after infection by *Botrytis cinerea*. *Am. J. Enol. Vitic.*, 45, 133–140.
- Latorre, B. A. (1986). Manejo de *Botrytis cinerea* en uva de mesa. *Rev. Frutícola (Chile)*, 7, 75–88.
- Latorre, B. A. & Rioja, M. E. (2001). Efecto de la temperatura y humedad relativa sobre la germinación de conidias de *Botrytis cinerea*. *Cien. Inv. Agr.*, 29, 67–72.
- Latorre, B. A. & Vázquez, G. (1996). Situación de *Botrytis cinerea* latente en uva de mesa de la zona Central. *Aconex (Chile)*, 52, 16–21.
- Latorre, B. A., Rioja, M. E. & Lillo, C. (2002). Efecto de la temperatura en el desarrollo de la infección producida por *Botrytis cinerea* en flores y bayas de uva de mesa. *Cien. Inv. Agr.*, 29, 145–151.
- Lorenz, D. H., Eichorn, K. W., Bleiholder, H., Klose, R., Meier, U. & Weber, E. (1994). Phänologische Entwicklungsstadien der Weinrebe (*Vitis vinifera* L. ssp. *vinifera*). Codierung und Beschreibung nach der erweiterten BBCH-Skala. *Vitic. Enol. Sci.*, 49, 66–70.
- Mansilla, J. P., Pintos, C. & Abelleira, A. (1991). Problemática fitosanitaria del viñedo en Galicia. *Vitivinicultura*, 6, 42–43.
- Marois, J. J., Nelson, J. K., Morrison, J. C., Lile, L. S. & Bledsoe, A. M. (1986). The influence of berry contact within grape clusters on the development of *Botrytis cinerea* and epicuticular wax. *Am. J. Enol. Vitic.*, 37, 293–296.
- Meier, U. (Ed.). (2001). *Growth stages of mono- and dicotyledonous plants*. 2nd ed. Braunschweig: Fed. Biol. Res. Centre Agric. For. *BBCH Monogr.*
- Moseholm, L., Weeke, E. & Petersen, B. (1987). Forecast of pollen concentrations of Poaceae (grasses) in the air by time series analysis. *Pollen et Spores*, 29, 305–322.
- Nair, N. G. & Allen, R. N. (1993). Infection of grape flowers and berries by *Botrytis cinerea* as a function of time and temperature. *Mycol. Res.*, 97, 1012–1014.

- Nair, N. G., Emmett, R. W. & Parker, F. E. (1988). Some factors predisposing grape berries to infection by *Botrytis cinerea*. *N. Zeal. J. Exp. Agric.*, 16, 257–263.
- Ribereau-Gayon, J. & Peynaud, E. (1971). *Sciences et techniques de la vigne. Vol. 2. Culture, pathologie, défense sanitaire de la vigne*. Paris: Dunod. Trad. Españ. (Montevideo, 1986).
- Rodríguez-Rajo, F. J., Seijo, M. C. & Jato, V. (2002). Estudio de los niveles de fitopatógenos para la optimización de cosechas de *Vitis vinifera* en Valdeorras (1998). *Bot. Complut.*, 26, 121–135.
- Seem, R. C., Magarey, P. A., McCloud, P. I. & Wachtel, M. F. (1985). A sampling to detect grapevine downy mildew. *Phytopathology*, 75, 1252–1257.
- Spotts, R. A. & Holz, G. (1996). Adhesion and removal of conidia of *Botrytis cinerea* and *Penicillium expansum* from grape and plum fruit surfaces. *Plant Dis.*, 80, 688–691.
- Thomas, C. S., Marois, J. J. & English, J. T. (1988). The effects of wind speed, temperature and relative humidity on development of aerial mycelium and conidia of *Botrytis cinerea* on grape. *Phytopathology*, 78, 260–265.
- Tobías, A., Sáez, M. & Galán, I. (2004). Herramientas gráficas para el análisis descriptivo de series temporales en la investigación médica. *Med. Clín.*, 122, 701–706.
- Wolf, T. K., Baudin, A. B. A. M. & Martínez-Ochoa, N. (1997). Effect of floral debris removal from fruit clusters on *Botrytis* bunch rot of Chardonnay grapes. *Vitis*, 36, 27–33.