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Forecasting ARIMA models for atmospheric vineyard pathogens in Galicia and Northern Portugal: *Botrytis cinerea* spores

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Abstract

Botrytis cinerea is the cause of the most common disease in the Galician and Portuguese vineyards. Knowledge of the spore levels in the atmosphere of vineyards is a tool for forecasting models of the concentration of spores in order to adjust the phytosanitary treatments to real risk infection periods. The presented study was conducted in two vineyards, one located in Cenlle (Spain) and other in Amares (Portugal), from 2005-2007. A volumetric trap, model Lanzoni VPPS-2000, was used for the aerobiological study. Phenological observations were conducted on 20 vines of three grape varieties in Cenlle (Treixadura, Godello and Loureira) and in Amares (Trajadura, Loureiro and Pedernã), by using the BBCH scale. The highest total spore concentrations during the grapevine cycle were recorded in 2007 in both locations (Cenlle:16,145 spores; Amares:1,858 spores), and the lowest, in 2005 in Cenlle (1,700 spores) and in Amares (800 spores) in 2006. In Cenlle, the best adjusted model was an ARIMA (0,2,2), including the relative humidity four days earlier, while in Amares there was an ARIMA (1,2,3), considering the relative humidity three days earlier and rainfall two days earlier. The t-test showed no significant difference between observed and predicted data by the model.

Key words

Botrytis cinerea, ARIMA, Grapevine, Phenology

INTRODUCTION

Grapevine is the one first crops used by humans for consumption. In Galicia and Northern Portugal it is implemented as a monocrop, being one the most important agricultural resources with a critical role in economic development.

The particular climatological conditions of vineyards located in north-western Spain and northern Portugal favour the development of fungal diseases. Grey mould is a significant disease of grapes (*Vitis vinifera* L.), caused by *Botrytis cinerea* Pers., (Sclerotiniaceae) which is the asexual or anamorphic phase of *Botryotinia fuckleiana* (de Bary) Whetze. Other significant pathogens are *Uncinula necator* (Schw.) Burr. and *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni that cause powdery mildew and downy mildew in our vineyards. The aforementioned fungal diseases are likely to impair wine quality due to degradation of colourants, destruction of the film containing aromatic substances, reduction in alcohol content, and increased fixation of SO_2 and volatile acidity [1].

B. cinerea survives during the overwinter as dormant mycelium (in cracks in the trunks of grapevines or in the buds), or as sclerotia (on branches). In spring and summer, with optimum temperature and humidity conditions, the germination of these propagules take place as well as the conidiophore formation and conidia development, which can be spread by rain or wind. Some authors have indicated that the optimum conditions for these ecological processes are high relative humidity of around 93% [2] and temperatures around 20-25°C, although conidia germination could be slowed down at temperatures of 13°C [3, 4, 5]. It is widely accepted that the most critical stages for *B. cinerea* infection are flowering and the period between berry ripening and harvest [6, 7], although fungus development is possible as a consequence of its penetration in the fruit pulp before the ripening of berries, and under optimum humidity and temperature conditions [8]. Consequently, this disease can produce total crop loss. Therefore, the systematic application of chemical fungicides in the grapevine, generally following pre-set calendars based on the plant phenological growth stages [9], are important to prevent decay development at harvest or during post-harvest [10, 11]. However, the 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, high environmental pressure, and loss of autorregulation capacity, etc.). Therefore, the chemical treatments should be used only when there is a real risk of unacceptable economic damage. The integrated management of pests and diseases involves a number of control methods in order to ensure an effective protection of vineyards. Preference is given to more natural and less harmful techniques which enable a reduced use of chemicals. Thorough inspection over the whole crop cycle is required in order to detect the appearance of pests and pathogens,

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to chart their development, and to ascertain both risks and tolerance thresholds [12].

The establishment of warning meteorological stations could help to predict the high risk episodes from the atmospheric weather conditions. However, these systems present a difficulty, in that the fungal spore amount (on which depends the capacity to produce/extend infection in the crop) is not taken into account [13]. External biological sensors (aerobiologically reliable monitoring of airborne inoculum) are a useful tool for pathogen management [14], completing the agrometeorological stations in order to achieve a better knowledge and estimation of the real risk of disease.

A significant correlation between aerial conidia concentration at a given date and lesion density one week later was achieved for *B. cinerea* leaf blight [14], especially when the disease intensity and airborne conidia concentration are high. Thus, airborne spore concentration can be used as an indicator of the pathogen development and can be useful when the infection level is first determinate by inoculum rather than by weather conditions [15]. In these situations, the monitoring of airborne inoculum integrated with the use of meteorological data [14] provides a valuable tool to establish the basis for an accurate, modern and integrated pest-management strategy.

The objectives of this study were:

1) to ascertain and compare the *B. cinerea* spore concentration in two vineyards – Cenlle (Ourense, Spain) and Amares (Braga, Portugal) – during the grapevine vegetative cycle;
2) to evaluate the influence of the meteorological parameters in the development of this fungus in order to estimate a model to predict the *B. cinerea* spores levels at Cenlle and Amares. Therefore, with this study we expect to develop a useful tool for early-indication of *B. cinerea* attacks, and for decision making of fungicide application calendars.

MATERIAL AND METHODS

Area and period of study. In Spain, the study was carried out in a vineyard located at Cenlle (42°18´ N, 8°6´ W), altitude (75-400) m, belonging to the Designation of Origin area 'Ribeiro', covering a total area of 371.4 Km^2 (Fig. 1). The main grape varieties grown are Treixadura, Godello and Loureira, the size of plants is 1.5m in rest vegetative, and the plants are 11 years old. This site is characterized by fairly steep valleys and hillsides. 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 winemaking areas in this region watered by the river Miño would be defined as temperate and warm, sub-humid, with very cold nights [16]. This area, located near to the city of Ourense, presents an ombrothermic dry and warm climate, an annual level of precipitation of 772mm, and a mean temperature of 14°C [17].

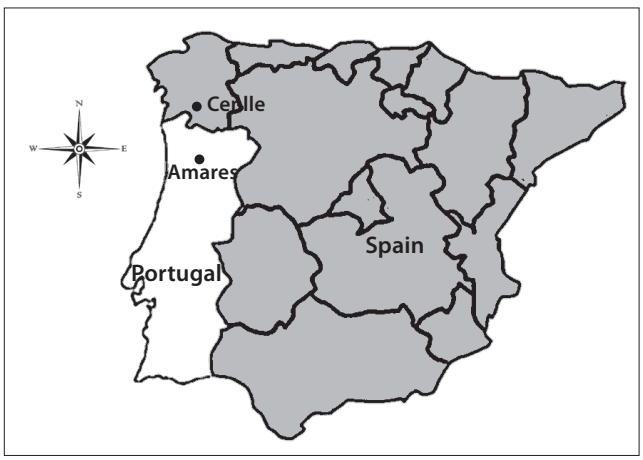

Figure 1. Location of Cenlle in Spain, and Amares in Portugal.

In Portugal, the study was carried out on a farm located at Amares, a rural area near Braga (41°38´N, 8°23´W), altitude (80m), (Fig. 1). The main grape varieties grown are Trajadura, Loureiro and Pedernã, the size of plants is 1.5m in rest vegetative, and the plants are 20 years old. The Braga district, in the 'Vinhos Verdes' Demarcated Region, has an area of 2,637 km^2. The area around the sampler had greenhouses, forest, and field crops [18]. Due to the location of this region between the mountains (Serra Amarela – 1,361m, Serra do Gerês – 1,545m, Serra da Cabreira – 1,262m) and the Atlantic Ocean, the climate is temperate with typically four well defined seasons. The winters are very rainy and cold, with generally moderate west winds. Strong winds can also blow from the north (known as the 'Nortadas'), which usually leads to falling temperatures. This area, located close to the city of Braga, presents a temperate climate with an Atlantic influence, an annual level of precipitation of 1,515mm and a mean temperature of 14°C [19].

In both locations, sampling was carried out during the active *Vitis* season, established from 1 April – 30 September, during 2005-2007.

Aerobiological study. Aerobiological sampling was performed using a LANZONI VPPS-2000 volumetric trap. In Cenlle (Spain), the trap was located in the central part of the vineyard and placed 2.5m above ground level, so that spore trapping would not be impeded by plant growth. In Amares (Portugal), the sampler was located 5m above ground level, so that no natural or architectonical barrier would prevent the natural airflow. 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 [20]. Spores were identified and counted following the Aira *et al.* [21] and Galán *et al.*[20] methods. Results were expressed as spores when the total values or spores/m^3 of air when referring to daily mean values. In Cenlle, the sampling was interrupted due to power cuts on 6 September 2005; from 31 August – 6 September in 2006; and finally, from 10-16 September 2007.

Phenological study. In the two study areas, the phenological sampling was carried out during the active grapevine season from 2005-2007 (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 in Cenlle, and Trajadura, Loureiro and Pedernã in Amares. During the three years of study (2005-2007), a weekly visit to the sampling area was carried out, except during the

flowering stage, in which the number of visits was increased to twice a week. The phenological phases of the selected plants were observed using the scale recommended by Lorenz *et al.* [22], adopted by the BBCH as standardized scale for phenological grapevine observations [23]. The six principal stages were monitored: stage 0 (Bud development), stage 1 (Leaf development), stage 5 (Inflorescence emerge), stage 6 (Flowering), stage 7 (Development of fruits) and stage 8 (Ripening of berries). For elaboration of the grapevine phenological calendar, the start date of each phenological stage was considered when the 50% of studied plants reached that stage.

Meteorological data. In Cenlle, meteorological data were obtained from a Hobo Micro Station data logger, located in the vineyard. The monitored parameters were maximum, minimum, and average temperatures (°C), and relative humidity (%). The information about rainfall (mm) was registered by means of a Davids weather station.

In Amares, meteorological data were obtained from a weather station of the Direcção Regional de Agricultura e Pescas do Norte – Estação de Avisos do Entre Douro e Minho. The monitored parameters were maximum, minimum, and average temperatures (°C), relative humidity (%), and rainfall (mm).

Statistical study. To determine the relationship between the main meteorological parameters (rainfall (mm), relative humidity (%), maximum, minimum and mean temperatures (°C)) in the airborne spore concentration, a Spearman's correlation test was applied. Significance was calculated for $p \leq 0.01$, $p \leq 0.05$ and $p \leq 0.1$. Weather conditions may affect spore production directly or indirectly through their effect on the substrates colonised by the fungus. For that reason, this study also determined the correlation between spore counts for a given day and the main weather-parameter values from the previous one to seven days (time gap considered for sporulation to occur).

Finally, an *ARIMA* (Autoregressive Integrated Model of Running Mean) Time Series model was used to predict daily *B. cinerea* airborne 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.

ARIMA models are a class of models for forecasting a time series. 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 of less than one year, C_t or fluctuations of the series in periods longer than one year, and finally, the I_t or random or sporadic factors [24]. 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. In the presented case, *B. cinerea* sporulation, and consequently its airborne spore concentration, are related to the weather conditions observed during the previous days. 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 ARIMA model 'ARIMA (p,d,q)', three 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 non-seasonal differences. Number of times that a time series was transformed calculating the differences between the values of the series and its predecessors.*

q= *Number of lagged forecast errors in the prediction equation. The order of the running mean of the process.*

The ARIMA model developed was tested with observed data of *B. cinerea* compared with data predicted by the model. With the aim of the statistical validation of the proposed ARIMA model predictive ability, a dependent samples t-test was carried out (by using an interval of confidence of the 95%). Real data was considered as spore concentrations recorded during the study period, compared with data predicted by the model.

In statistical analyses, the SPSS 16.0 software package was used.

RESULTS

Low phenological timing differences were observed between the three varieties studied (Treixadura, Loureira and Godello) in Cenlle and (Trajadura, Loureiro and Pedernã) in Amares. Therefore, the average duration of the main phenological stages for each region was calculated using the average value of the phenological data set for each variety. The duration in days of the phenological cycle (1 April to the vintage) was fairly homogeneous in both areas (Tab. 1). In Cenlle, there were 163, 170 and 172 days in 2005, 2006 and 2007, respectively, whereas in Amares there were 169, 164 and 171 days in 2005, 2006 and 2007, respectively. The highest phenological stage duration differences were recorded in Cenlle, mainly for stage 0 (bud development), stage 1 (leaf development) and stage 8 (ripening of berries). During the study period, in Cenlle and Amares, stage 7 (development of fruits) was the longest with mean duration of 61 and 58 days, respectively, while stage 6 (flowering) was the shortest (with a mean duration of 10 days) (Tab. 1).

B. cinerea spores were constantly present during the study period, both in Cenlle and Amares. The maximum number of spores was recorded in Cenlle in 2007 (16,145 spores), while the lowest concentration was recorded in Amares in 2006 (800 spores) (Tab. 1).

In Cenlle, the highest *B. cinerea* spore concentrations were recorded on 10 May 2005 and 27 April 2007 during stage 5 (inflorescence emerge), with a maximum of 85 and 415 spores/m^3, respectively. In 2006, the maximum values were observed at the beginning of stage 7 (development of fruits), reaching 203 spores/m^3 on 16 June.

In Amares, the highest spore concentration in 2005 was recorded during stage 1 (leaf development) on 22 April (41 spores/m^3) 2006 during stage 0 (bud development), on 9 April (38 spores/m^3), and finally in 2007 the spores peak was observed on 18 June (79 spores/m^3) at the beginning of stage 7 (development of fruits) (Fig. 2, Tab. 1).

Meteorological parameters greatly affect the spore production and dispersal. Generally, in Amares, area the temperatures were milder than in Cenlle. During the study

Table 1. Start date, length of time (days), maximum daily value of *Botrytis cinerea* spores (spores/m^3) and date of maximum value of 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 in Cenlle and Amares. Length average of stages, average of the total *B. cinerea* spores registered in each stage and index of spores registered in each year during 2005–2007.

			Phenological stages						Index (spores)
			0	1	5	6	7	8	
Cenlle	2005	Start date	1-Apr	4-Apr	27-Apr	10-Jun	10-Jun	12-Aug	1,700
		Length of time	3	23	36	9	62	30	
		B. cinerea spores maximum	1	23	85	39	37	51	
		Date maximum	2-Apr	7-Apr	10- May	10-Jun	10-Jun	13-Aug	
		Total stage spores	1	140	478	129	714	237	
	2006	Start date	1-Apr	13-Apr	28-Apr	10- May	10-Jun	11-Aug	4,903
		Length of time	12	15	31	9	65	38	
		B. cinerea spores maximum	91	45	24	41	203	69	
		Date maximum	9-Apr	19-Apr	28-Apr	3-Jun	16-Jun	29-Aug	
		Total stage spores	324	368	306	180	3334	391	
	2007	Start date	1-Apr	12-Apr	20-Apr	25-May	7-Jun	2-Aug	16,145
		Length of time	11	8	35	13	56	49	
		B. cinerea spores maximum		58	415	189	219	408	
		Date maximum		17-Apr	27-Apr	29-May	27-Jul	4-Sep	
		Total stage spores	0	130	4279	1020	4845	5872	
Amares	2005	Start date	1-Apr	10-Apr	28-Apr	8-Jun	19-Jun	13-Aug	1,405
		Length of time	9	18	41	11	55	35	
		B. cinerea spores maximum	38	41	22	15	32	26	
		Date maximum	6-Apr	22-Apr	12-May	16-Jun	31-Jul	13-Sep	
		Total stage spores	224	193	258	43	411	276	
	2006	Start date	1-Apr	14-Apr	25-Apr	2-Jun	12-Jun	4-Aug	800
		Length of time	13	11	38	10	53	39	
		B. cinerea spores maximum	38	10	12	4	22	10	
		Date maximum	9-Apr	19-Apr	7-May	10-Jun	16-Jun	24-Aug	
		Total stage spores	171	44	108	21	235	221	
	2007	Start date	1-Apr	11-Apr	27-Apr	1-Jun	11-Jun	15-Aug	1,858
		Length of time	10	16	35	10	65	35	
		B. cinerea spores maximum	4	8	29	7	79	48	
		Date maximum	10-Apr	18-Apr	30-May	3-Jun	18-Jun	28-Aug	
		Total stage spores	15	54	135	29	1039	587	

period, the minimum temperature registered in Amares was 7.5°C in 2007, observed during stage 0 (bud development); while in Cenlle in the same year, during the phenological stage, it was 2.8°C. However, with regard to maximum temperatures, the values observed in Cenlle were higher than those in Amares. In the Cenlle area, values higher than 30°C were registered in 2005 and 2006 during stage 6 (flowering), and in 2007 during stages 7 (development of fruits) and 8 (ripening of berries). In Amares, maximum temperature values above 30°C were recorded during stage 6 (flowering) and 8 (ripening of berries) in 2006. Regarding to relative humidity conditions, the number of days with values above 90% is higher in Amares in all study years. Finally, in Cenlle there was registered the highest rainfall amount (123.8 mm) in 2007 during the stage 5 (inflorescence emerge) for the entire study period. In the same year in Amares, the highest total study period precipitation (155 mm) was recorded in stage 7 (development of fruits) (Fig. 2).

In order to ascertain the influence of the major weather-related parameters on airborne *B. cinerea* spore counts, a Spearman linear correlation analysis was applied, taking *B. cinerea* spore counts and weather parameters for the same day and for the seven preceding days as dependent variables. Correlation coefficients (calculated using data for each year, as well as data for the whole study period) were significant in most of cases (Tab. 2). A highly-significant ($p<0.01$) positive correlation was found between local airborne *B. cinerea* spore counts for a given day and the previous days in both areas.

Analyzing each studied year separately in Cenlle, it was noted that the temperature was always the weather parameter with the highest correlation coefficient, while in Amares in 2005 and 2006 occurred highest relative humidity and in 2007 the highest temperature (Tab. 2).

The Spearman correlation test using data for the whole study period (2005-2007) showed the highest significant correlation values for relative humidity of the preceding four days in Cenlle, and of the preceding five days in Amares, thus demonstrating the importance that this parameter exerts on the development and infection of the pathogen (Tab. 2).

On the basis of these correlation results, the predictive capacity of each of the aforementioned variables was evaluated in order to obtain an ARIMA time series model to predict the *B. cinerea* spores in each of the study areas (Tab. 3). In Cenlle, the most accurate forecasting model obtained was an ARIMA (0,2,2) including the relative humidity four days earlier as the independent variable , with an R^2 value of 0.69. In Amares, the most accurate forecasting model obtained was an ARIMA (1,2,3) including as independent variables relative humidity three days earlier, and the rainfall two days earlier, with an R^2 value of 0.73.

The accuracy of the *ARIMA* model developed was evaluated comparing the observed *B. cinerea* spore concentration versus the values predicted with the ARIMA models (Fig. 3). The predicted values matched actual spore counts in the most cases. Statistically, this was demonstrated by a dependent samples t-test applied to the good forecast behaviour of the

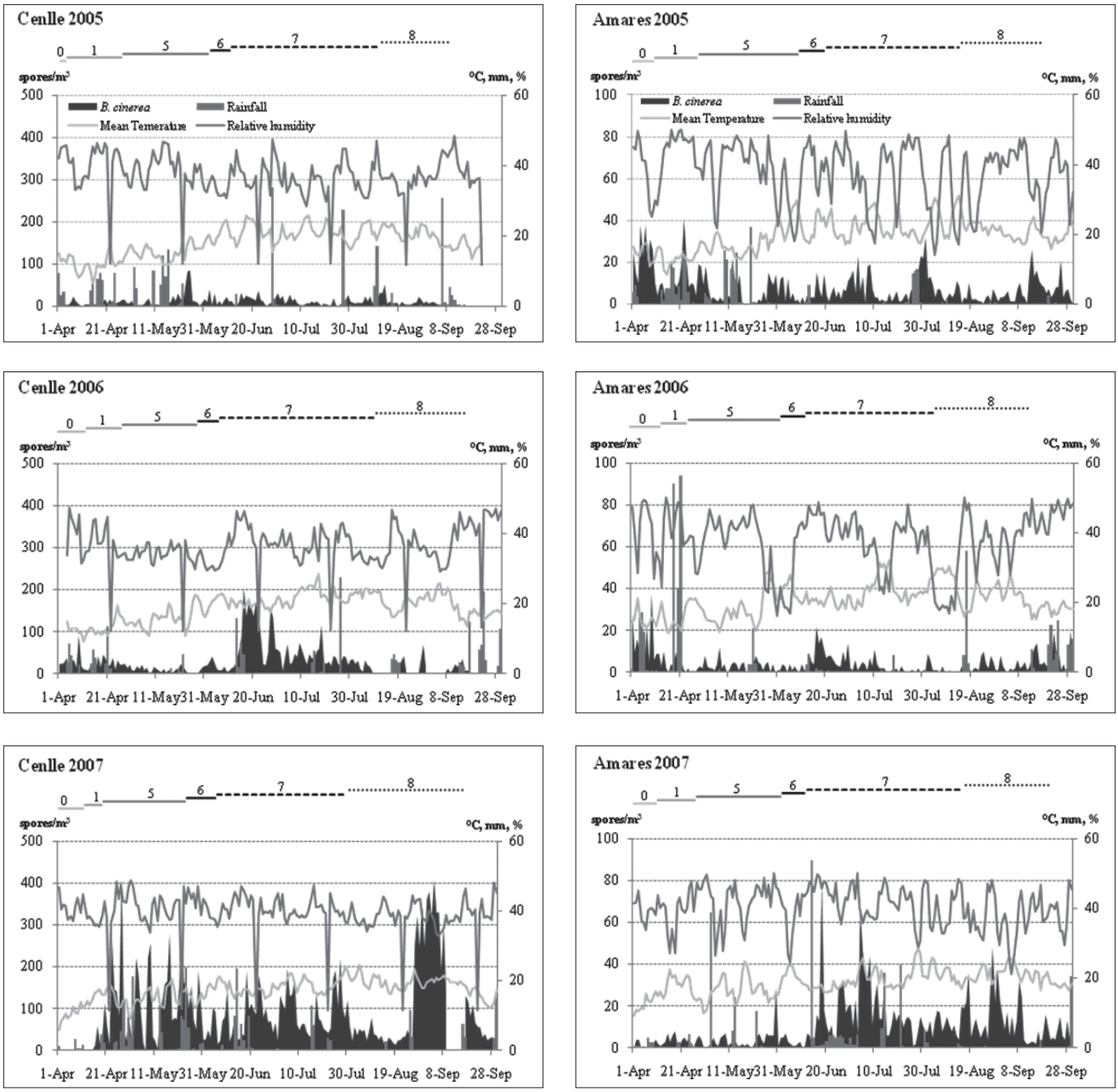

Figure 2. Spore concentrations of *Botrytis cinerea* during the vegetative cycle stages (0 – sprouting; 1 – leaf development; 5 – inflorescence emergence; 6 – flowering; 7 – development of fruits; 8 – ripening of berries) of the grapevine in the studied period. The grey line represents mean temperature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

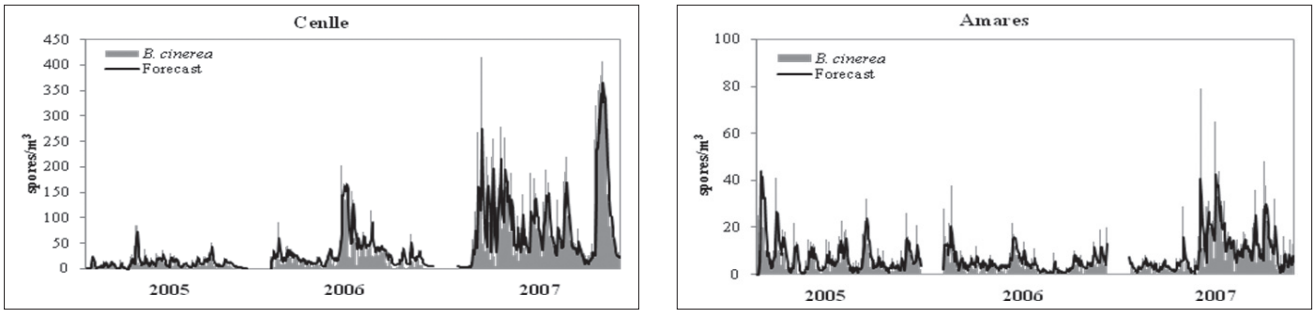

Figure 3. Daily mean *B. cinerea* spore concentration observed and forecasted by the proposed ARIMA model during the study years

Table 2. Correlation between the spores concentration in the period of study and the principal meteorological variables applying Spearman's test (p≤0.01*** highly significant, p≤0.05** very significant, p≤0.1* low significant). Values of the 7 previous days were also considered

	Cenlle				Amares			
	2005	**2006**	**2007**	**2005-2007**	**2005**	**2006**	**2007**	**2005-2007**
B. cinerea-1	0.572***	0.737***	0.669***	0.823***	0.497***	0.399***	0.672***	0.568***
B. cinerea -2	0.503***	0.646***	0.478***	0.762***	0.460***	0.343***	0.530***	0.498***
B. cinerea -3	0.328***	0.615***	0.490***	0.721***	0.367***	0.336***	0.522***	0.454***
B. cinerea -4	0.246***	0.485***	0.448***	0.676***	0.250***	0.228***	0.520***	0.406***
B. cinerea -5	0.263***	0.475***	0.405***	0.667***	0.161**	0.282***	0.458***	0.375***
B. cinerea -6	0.237***	0.425***	0.358***	0.637***	0.106	0.207***	0.480***	0.344***
B. cinerea -7	0.326***	0.409***	0.229***	0.624***	-0.017	0.249***	0.442***	0.293***
Maximum Temperature	0.275***	0.150**	0.254***	0.114***	-0.162**	-0.322***	0.282***	-0.108**
Maximum Temp -1	0.191**	0.131*	0.206***	0.078*	-0.233***	-0.322***	0.233***	-0.150***
Maximum Temp -2	0.084	0.066	0.132*	0.025	-0.256***	-0.308***	0.173**	-0.167***
Maximum Temp -3	-0.011	0.059	0.100	-0.009	-0.307***	-0.314***	0.173**	-0.180***
Maximum Temp -4	-0.010	0.119	0.107	0.009	-0.347***	-0.289***	0.223***	-0.169***
Maximum Temp -5	-0.056	0.141*	0.144*	0.016	-0.329***	-0.255***	0.215***	-0.157***
Maximum Temp -6	-0.052	0.204***	0.130*	0.033	-0.247***	-0.256***	0.203***	-0.132***
Maximum Temp -7	-0.002	0.237***	0.068	0.041	-0.210***	-0.243***	0.181**	-0.131***
Minimum Temperature	0.162**	0.183**	0.249***	0.098**	-0.144*	-0.277***	0.347***	-0.078*
Minimum Temp -1	0.170**	0.183**	0.367***	0.127***	-0.216***	-0.262***	0.314***	-0.107**
Minimum Temp -2	0.232***	0.248***	0.359***	0.156***	-0.181**	-0.251***	0.301***	-0.092**
Minimum Temp -3	0.174**	0.295***	0.335***	0.151***	-0.214***	-0.271***	0.315***	-0.100**
Minimum Temp -4	0.134*	0.281***	0.307***	0.138***	-0.163**	-0.243***	0.382***	-0.054
Minimum Temp -5	0.120	0.282***	0.275***	0.125***	-0.184**	-0.196***	0.363***	-0.056
Minimum Temp -6	0.121	0.235***	0.175**	0.093**	-0.102	-0.188**	0.329***	-0.036
Minimum Temp -7	0.072	0.167**	0.076	0.046	-0.075	-0.158**	0.288***	-0.035
Mean Temperature	0.297***	0.215***	0.321***	0.136***	-0.160**	-0.339***	0.351***	-0.095**
Mean Temp -1	0.217***	0.163**	0.320***	0.110**	-0.232***	-0.314***	0.309***	-0.124***
Mean Temp -2	0.199***	0.143*	0.289***	0.094**	-0.245***	-0.309***	0.248***	-0.141***
Mean Temp -3	0.148*	0.157**	0.241***	0.074*	-0.283***	-0.341***	0.252***	-0.156***
Mean Temp -4	0.123	0.176**	0.223***	0.072	-0.310***	-0.301***	0.301***	-0.135***
Mean Temp -5	0.062	0.216***	0.226***	0.074*	-0.303***	-0.275***	0.289***	-0.131***
Mean Temp -6	0.074	0.232***	0.164**	0.073	-0.223***	-0.253***	0.269***	-0.100**
Mean Temp -7	0.045	0.221***	0.080	0.049	-0.188**	-0.239***	0.246***	-0.099**
Relative humidity	-0.198***	0.052	-0.012	0.104**	0.006	0.300***	-0.045	0.097**
Humidity-1	-0.100	0.104	0.037	0.141***	0.133*	0.293***	0.009	0.153***
Humidity-2	-0.010	0.193**	0.090	0.190***	0.218***	0.271***	0.017	0.181***
Humidity-3	0.015	0.215***	0.074	0.211***	0.281***	0.335***	0.053	0.228***
Humidity-4	0.009	0.182**	0.067	0.212***	0.337***	0.360***	0.073	0.261***
Humidity-5	0.032	0.109	0.012	0.183***	0.342***	0.366***	0.100	0.274***
Humidity-6	-0.006	0.028	0.032	0.164***	0.234***	0.370***	0.059	0.223***
Humidity-7	-0.069	-0.006	0.017	0.141***	0.160**	0.255***	0.083	0.183***
Rainfall	-0.248***	-0.029	-0.054	-0.032	0.167**	0.317***	0.015	0.168***
Rainfall-1	-0.132*	0.010	0.049	0.023	0.124*	0.359***	0.109	0.194***
Rainfall-2	-0.003	0.005	0.176**	0.079*	0.170**	0.300***	0.155**	0.205***
Rainfall-3	0.028	0.010	0.127*	0.076*	0.187**	0.268***	0.156**	0.203***
Rainfall-4	0.031	0.007	0.089	0.065	0.193**	0.205***	0.188**	0.188***
Rainfall-5	0.034	-0.045	0.064	0.031	0.254***	0.193***	0.134*	0.189***
Rainfall-6	-0.013	-0.144*	0.029	-0.007	0.146*	0.165**	0.101	0.133***
Rainfall-7	-0.078	-0.179**	0.035	-0.021	0.110	0.189**	0.122	0.138***

Table 3. Time series ARIMA model proposed

Adjusted R^2= 0.687				Cenlle
	B	ET	t	Sig.
MA1	1.476	0.137	10.755	0.000
MA2	-0.476	0.063	-7.512	0.000
RH-4	-0.009	0.005	-1.952	0.051
Adjusted R^2= 0.730				Amares
	B	ET	t	Sig.
AR1	-0.967	0.047	-20.685	0.000
MA1	0.604	0.071	8.473	0.000
MA2	0.917	0.090	10.153	0.000
MA3	-0.522	0.052	-10.022	0.000
RH-3	-0.002	0.001	-2.095	0.037
Rainfall-2	0.010	0.006	1.760	0.079

model since no significant difference between *B. cinerea* spore data and forecasted data at the 95% level was observed (p value of 0.682 in Cenlle and p value of 0.883 in Amares) (Tab. 4).

DISCUSSION

During the study period, the constant presence of *B. cinerea* spores in the atmosphere of the vineyards was verified, as noted by several authors in north-west Spain [12, 13, 25, 26, 27,] and in Portugal [28]. The synchronism between the *B. cinerea* spores dispersal and the phenological stages has been presented by various authors in different geographical areas [12, 19].

Table 4. Results of the t-test. Marked differences are significant at p<0.050

		Mean	Std.Dv.	N	Diff.	Std.Dv. Diff	t	df	p
Cenlle	*B. cinerea* spores 2005-2007	43.233	67.197						
	Forecast	43.996	58.909	526	-0.762	42.719	-0.409	525	0.682
Amares	*B. cinerea* spores 2005-2007	7.455	9.042						
	Forecast	7.505	7.196	549	-0.049	7.854	-0.147	548	0.883

Some authors have pointed out two critical infection periods by *B. cinerea*, the first during flowering and the second during the time-period between ripening of berries to harvest [6, 7]. Similar results were obtained in the presented study in which the highest *B. cinerea* concentrations were recorded during late phenological stages (7 and 8), in both areas in 2007. During stage 8, the maximum *B. cinerea* spore peaks were recorded in Cenlle with 408 spores/m^3, and 48 spores/m^3 in Amares. This fact can be related to the high disease incidence between stage 5 and stage 7. It is known that latent infections developed during this period are an important source of primary inoculum for harvest and post-harvest infections [29, 30, 31]. *B. cinerea* colonizes senescent floral parts (stamens and calyptras) persisting in latent forms in bunches, and therefore providing the necessary inoculum for late infections during the maturation stage (stage 8) [31, 32]. High concentrations of spores during the ripening of berries stage represent an important risk for the crop, as the *Vitis* susceptibility to infection increases as the degree of ripeness of the berries ripeness progresses, since sugar favours the colonization of plant tissues by *B. cinerea* [33]. During this stage, the main primary points for the pathogen penetration are the stomata and microfissures, or possible wounds in the berry skin [34, 35]. These lesions play an important role on the symptom expression and on the disease epidemiology, because the grape skin is an effective barrier to avoid solitary conidia penetration [2].

Weather conditions affect both grape phenology and fungal disease incidence. Temperature, relative humidity and rainfall have been considered key factors for the *B. cinerea* infection [3, 4, 5]. The interrelationship between these parameters increase the success of spore germination, fungal development and disease virulence. Therefore, peaks that occurred in September 2007 in both areas, were maximized by the special weather conditions registered: mean air temperature values around 20°C, widely considered optimal for infection [36], and high values of relative humidity. It is probable that relative humidity in 2007 shared the main responsibility in the primary inoculum formation observed during stage 5 until stage 7. A higher number of several consecutive days with relative humidity above 90% were registered at both sites, which coincided with higher spore concentrations. Latorre and Rioja [37] have suggested that under high relative humidity conditions, invisible condensation could occur on the host, providing enough water to start the germination, which eventually promotes a severe infection. Some authors have reported that the airborne conidia have the same potential to infect wet and dry surfaces, in spite of the fact that the conidia adhesion should be stronger when the spores are placed on a drop of water or a wet surface than on a dry surface [2, 38]. Studies conducted by Latorre and Rioja [12] suggest that high relative humidity (> 86%) would be insufficient to initiate the germination and cause infection of grey mould in grapes, because the infection in a crop is initiated as a consequence of rain, drizzle or fog. Similar results were obtained in the presented study, since high concentration peaks of spores were preceded by days with precipitation. Moreover, the correlation test confirmed a significant relationship between the airborne spore concentration and the relative humidity four days earlier in Cenlle and five days earlier in Amares. Even though in the Amares area the correlations indicated a significant positive effect of the previous days rainfall on the airborne spore concentration, as described by Latorre and Rioja [37]. Rainfall could favour conidia dispersion throughout rain splashes [39].

Aerobiological studies can be used as an indicator of the pathogen development [15]. The monitoring of airborne inoculums integrated with the use of meteorological data (temperature, relative humidity and rainfall) provide a valuable tool to establish the basis for an accurate, modern, integrated pest-management strategy [14], thereby allowing the development of statistical models to predict the infection risk periods. Generally, forecast models use linear logistic models to predict spore concentrations [13, 40]. Linear regression models usually use only weather-related variables as prediction variables, yielding results with a low predictive capacity. Therefore, in the presented study, an ARIMA time-series model was developed (which takes *B. cinerea* counts over the previous days as an autoregressive parameter), allowing a higher forecast accuracy of the *B. cinerea* spore counts [41]. In Cenlle, the best adjusted ARIMA time-series model was an ARIMA (0,2,2), which included in the proposed model the relative humidity four days earlier. In Amares, the best adjusted ARIMA time-series forecast model was an ARIMA (1,2,3), which considered the relative humidity three days earlier and rainfall two days earlier. Finally, the forecast behaviour of these models was statistically tested using a dependent samples t-test which showed that there was no significant difference (at the 95% level) between the observed data and the data predicted by the model.

CONCLUSIONS

This study shows the ARIMA time series models applied for the prediction of *B. cinerea* spores, presented good results. The combination of meteorological and aerobiological parameters provide useful tools for the development of models for forecasting spore concentrations and therefore risks of infection, and providing a basis for a modern integrated grapevine pest-management strategy.

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REFERENCES

1. Ribereau-Gayon J, Peynaud E. Sciences et techniques de la vigne, Vol. 2. Culture, pathologie, defense sanitaire de la vigne. París: Dunod. Trad. Españ. (Montevideo, 1986) 1971.
2. Coertze S, Holtz G, Sadie A. Germination and establishment of infection on grape berries by single airborne conidia of Botrytis cinerea. Plant Dis. 2001; 85(6): 668-677.
3. Broome JC, English JT, Marois JJ, Latorre BA, Avilés JC. Development of an infection model for Botrytis bunch rot of grapes based on wetness duration and temperature. Phytopathology 1995; 85: 97-102.
4. Hyre RA. Effect of temperature and light on colonization and sporulation of the Botrytis pathogen on geranium. Plant Dis Report. 1972; 56: 126-130.
5. Thomas CS, Marois JJ, English JT. The effects of wind speed, temperature and relative humidity on development of aerial mycelium and conidia of Botrytis cinerea on grape. Phytopathology 1988; 78: 260–265.
6. Bulit J, Dubos B. Botrytis bunch rot and blight. In Pearson RC, Goheen AC (Eds.): Compendium of Grapes Diseases. The American Phytopathological Society, St. Paul., Minnesota 1988.
7. Latorre BA. Manejo de Botrytis cinerea en uva de mesa. Rev Fruti 1986; 7: 75–88.
8. Hidalgo L. Tratado de viticultura general, 3rd. Mundi-Prensa, Madrid 2002.
9. Buggiani R, Govoni P, Bottazzi R, Giannico P, Montini B, Pozza M. Monitoring airborne concentrations of sporangia of Phytophthora infestans in relation to tomato late blight in Emilia Romagna, Italy. Aerobiologia 1995; 11: 41-46.
10. Franck J, Latorre BA, Torres R, Zoffoli JP. The effect of preharvest fungicide and postharvest sulfur dioxide use on postharvest decay of table grapes caused by Penicillium expansum. Postharvest Biology and Tecnology 2005; 37: 20-30.
11. Latorre BA, Lillo C, Rioja ME. Eficacia de los tratamientos fungicidas para el control de Botrytis cinerea de la vid en función de la época de aplicación. Cienc Inv Agr 2001; 28: 61-66.
12. Rodríguez-Rajo FJ, Jato V, Fernández-González M, Aira MJ. The use of aerobiological methods for forecasting Botrytis spore concentrations in a vineyard. Grana 2010; 49: 56-65.
13. Rodríguez-Rajo FJ, Seijo MC, Jato V. Estudio de los niveles de fitopatógenos para la optimización de cosechas de Vitis vinífera en Valdeorras (1998). Botanica Complutensis 2002; 26: 121-135.
14. Carise O, Savary S, Willocquet L. Spatiotemporal relationships between disease development and airborne inoculum in unmanaged and managed Bortytis leaf blight epidemics. Phytopathology 2008; 98(1): 38-44.
15. Jeger MJ. Relating disease progress to cumulative numbers of trapped spores: apple powdery mildew and scab epidemics in sprayed and unsprayed orchard plots. Plant Pathol. 1984; 33: 517-523.
16. Blanco-Ward D, Garcia JM, Jones GV: Spatial climate variability and viticulture in the Miño River Valley of Spain. Vitis. 2007; 46(2): 63-70.
17. Rodríguez-Rajo FJ, Iglesias I, Jato V. Variation assessment of airborne Alternaria and Cladosporium spores at different bioclimatical conditions. Mycol Res. 2005; 109: 497-507.
18. Oliveira M, Ribero H, Delgado JL, Abreu I. Seasonal and intradiurnal variation of allergenic fungal spores in urban and rural areas of the North of Portugal. Aerobiologia 2009; 25: 85-98.
19. Oliveira M, Guerner-Moreira J, Mesquita MM, Abreu I. Import and phytopathogenic airborne fungal spores in a rural area: incidence of Botrytis cinerea and Oidium spp. Ann Agric Environ Med 2009; 16: 197-204.
20. Galán C, Cariñanos P, Alcázar P, Domínguez E. Spanish Aerobiology Network (REA): Management and Quality Manual. Publication Service. University of Córdoba 2007.
21. Aira MJ, Jato V, Iglesias, I. Calidad del aire. Polen y esporas en la comunidad Gallega. Xunta de Galicia 2005.
22. Lorenz DH, Eichhorn KW, Blei-holder H, Klose R, Meier U, Weber E. Phänologische Entwicklungsstadien der Weinrebe (Vitis vinifera L. ssp. vinifera). Vitic Enol Sci. 1994; 49: 66-70.
23. Meier U. Growth stages of mono and dicotyledonous plants. BBCH Monograph. 2nd Edit. Federal Biological Research Centre for Agriculture and Forestry 158, 2001.
24. Tobías A, Sáez M, Galán I. Herramientas gráficas para el análisis descriptivo de series temporales en la investigación médica. Med Clin. (Barc) 2004; 122 (18): 701-706.
25. Albelda Y, Rodríguez-Rajo FJ, Jato V, Aira MJ. Concentraciones atmosféricas de propágulos fúngicos en viñedos del Ribeiro (Galicia. España). Bol Micol. 2005; 20: 1-8.
26. Díaz MR, Iglésias I, Jato V. Airborne concentrations of *Botrytis*, *Uncinula* and *Plasmopara* spores in Leiro-Ourense (NW Spain). Aerobiologia 1997; 13: 31-35.
27. Díaz MR, Iglésias I, Jato V. Seasonal variation of airborne fungal spore concentrations in a vineyard of North-West Spain. Aerobiologia 1998; 14: 221-227.
28. Oliveira M, Ribero H, Delgado JL, Abreu I. Aeromycological profile of indoor and outdoor environments. J Environ Monit. 2009; 11: 1360-1367.
29. Holz G, Coertze S, Basson EJ. Latent infection of *Botrytis cinerea* in grape pedicels leads to postharvest decay. Phytopathology (Abst.) 1997; 87: S43.
30. Latorre BA, Vásquez G. Situación de *Botrytis cinerea* latente en uva de mesa de la zona central. Aconex (Chile) 1996; 52: 16-21.
31. Wolf TK, Baudin ABAM, Martínez-Ochoa N. Effect of floral debris removal from fruit clusters on *Botrytis* bunch rot if Chardonnay grapes. Vitis 1997; 36: 27-33.
32. Jermini M, Jelmini G, Gessler C. La lutte contre le *Botrytis cinerea* du Merlot au Tessin. Le rôle des infections latentes. Revue Suisse de Viticulture, Arboriculture, Horticulture 1986; 18: 161-166.
33. Kretschmer M, Kassemeyer H, Hahn M. Age-dependent Grey Mould susceptibility and tissue-specific defence gene activation of grapevine berry skins after infection by *Botrytis cinerea*. Am J Enol Vitic. 1994; 45: 133-140.
34. Bessis R. Étude de l´évolution des estomates et des tissus péristomatiques du fruit de la vigne. l'Académie des Sciences (Paris) 1972 ; 274 : 2158-2161.
35. Pucheu-Planté B, Mercier M: Étude ultrastructurale de l´interrelation hôte-parasite entre le raisin et le champignon *Botrytis cinerea*: example de la pourriture noble en Sauternais. Canadian Journal of Botany 1983; 61: 1785-1797.
36. Latorre BA, Rioja ME, Lillo C. Efecto de la temperatura en el desarrollo de la infección producida por *Botrytis cinerea* en flores y bayas de uva de mesa. Cienc Inv Agr. 2002; 29(3): 145-151.
37. Latorre BA, Rioja, ME. Efecto de la temperatura y la humedad relativa sobre la germinación de conidias de *Botrytis cinerea*. Cienc Inv Agr. 2002; 29: 67-72.
38. Spotts RA, Holz G. Adhesion and removal of conidia of *Botrytis cinerea* and *Penicillium expansum* from grape and plum fruit surfaces. Plant Dis. 1996; 80: 688-691.
39. Jarvis WR. The dispersal of spores of *Botrytis cinerea* Fr. In a raspberry plantation. Trans Br Mycol Soc. 1962; 45: 549-559.
40. Fernández-González M, Rodríguez-Rajo FJ, Jato V, Aira MJ. Incidence of fungals in a vineyard of the Denomination of Origin Ribeiro (Ourense-North-Western Spain). Ann Agric Environ Med. 2009; 16: 263-271.
41. Cotos-Yañez TR, Rodríguez-Rajo FJ, Jato MV. Short-term prediction of *Betula* airborne pollen concentration in Vigo (NW Spain) using logistic additive models and partially linear models. Int J Biometeorol. 2004; 48: 179-185.