ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 2009, 16, 263–271

INCIDENCE OF FUNGALS IN A VINEYARD OF THE DENOMINATION OF ORIGIN RIBEIRO (OURENSE – NORTH-WESTERN SPAIN)

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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.

Abstract: Knowledge about the fungal spores most abundant in the atmosphere of a vineyard is of great use since it allows development of prediction models of the spore concentration, and therefore application of phytosanitary treatments only when high levels of fungal propaguls are detected. In this study the concentration of phytopathogenic spores is related with the different phenological stages of the vineyard, and a prediction model developed for each fungal type using meteorological, phenological and spore concentrations variables. The study was carried out in a vineyard of the Ribeiro district during the year 2007. For the aerobiological study a volumetric Hirst type trap was used, while phenological observations were carried out on 20 plants of the three varieties monitored (Treixadura, Godello and Loureira) following the phenological scale standardized by the BBCH. *Botrytis* reached the highest annual total value with 16,145 spores, followed by *Plasmopara* with 747 spores and *Uncinula* with 578 spores. In order to forecast the concentration of the phytopathogenic fungal spores, equations of lineal regression were elaborated including as estimators, variables with high correlation coefficient. For *Botrytis* the regression equation explained 42.4% of the variability of spore concentration, 26.1% for *Uncinula* and 24.7% for *Plasmopara*.

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Key words: ***Botrytis*, *Uncinula*, *Plasmopara*, aerobiology, grapevine.**

INTRODUCTION

Wine production in any region is greatly influenced by local climate [40]. The general bioclimatological conditions of vineyards located in north-western Spain favour the development of fungal diseases which have a marked impact on the grape harvest. The most common diseases are grey mould (produced by *Botrytis cinerea* Pers.), powdery mildew (*Uncinula necator* (Schw.) Burr.) and downy mildew (*Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni). In recent years, new diseases have arisen, including grapevine necrosis, yesca, *Armillaria* root disease, black rot and *Eutypa* dieback, which can eventually lead to the death of the plant [36]. These emerging diseases are probably caused by the rise in spring temperatures recorded in various parts of Europe [21].

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 [5]. 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 only when there is a real risk of unacceptable economic damage. It is therefore 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 is represented by different stages during which the interactions among host, pathogen and environment influencing the disease development. During

Received: 23 March 2009
Accepted: 29 October 2009

recent years, forecast models providing useful information for plant disease management have been proposed in order to be used against the most important diseases in the vineyard [28, 40, 43, 46, 47]. Different components of the disease cycles were included in the disease prediction models [15], such as the survival of the pathogen as overwintering *Plasmopara viticola* oospores [48]. Plant disease prediction models, including different aspects of the reproduction of the pathogen, such as the rate of inoculums production, the influence of meteorological conditions (especially temperature and moisture conditions) on their maturation and the time required for that, were fitted also for *Plasmopara viticola* [40, 41, 45, 47, 48] and *Uncinula necator* [28]. The release, transport and survival of the inoculum have also taken into account by different authors [40, 41, 45, 47]. Finally, infection models based on the influence of different meteorological parameters (firstly temperature and wetness duration) were developed for *Botrytis cinerea* [6, 39], *Plasmopara viticola* [9, 14] and *Uncinula necator* [10].

Aerobiological studies provide knowledge of the daily and hourly airborne spore concentrations present in the crop. Some surveys were conducted in an attempt to relate the amount of disease at a given time with the airborne spores concentration at the same or previous time [30]. A significant correlation between aerial conidium concentration at a given date and lesion density one week later of most sampling dates was achieved for *Botrytis* leaf blight [11], especially when both diseases intensity and airborne conidia concentration were high. Airborne spore concentration could be used as an indicator of the pathogen development, and could be useful when the infection level is initially determined by inoculum rather than the weather [30]. In these conditions, the monitoring of airborne inoculums integrated with the use of meteorological data [11] provides a valuable tool for establishing the basis for an accurate, modern, integrated pest-management strategy. A certain threshold level of spore concentration could be utilized as warning of real disease risk, and from that moment every favorable meteorological condition for disease development could provoke release of the disease [5]. This would lead to a lower number of treatments, and thus to a reduction in both economic costs and environmental damage [50].

The present study sought to chart airborne spore concentrations for the major phytopathogenic fungi in northwestern Spain (*Botrytis cinerea* Pers., *Uncinula necator* (Schw.) Burr. and *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni) in the Ribeiro Origin Denomination area. The incidence of these fungi during the various grapevine phenological phases and potential correlations with major weather-related variables were also assessed. The data obtained were used to develop a predictive model of spore concentrations, with a view to optimizing the application of fungicide treatments, thus improving grape quality.

MATERIAL AND METHODS

The Ribeiro region of north-western Spain covers a total area of 371.4 km^2. The study was carried out in a vineyard at Cenlle (altitude 75–400 m) characterized 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 winemaking areas in this region watered by the river Miño would be defined as temperate and warm, sub-humid, with very cold nights [3].

Airborne fungal propagule concentrations were determined using a Lanzoni VPPS 2000 volumetric pollen-spore trap [26] located inside the vineyard. The sampler was placed 2 m above ground level, so that spore trapping would not be impeded by plant growth. Sampling was carried out continuously from 1 April–30 September 2007, except for the period 10–16 September, when energy problems prevented monitoring. The Lanzoni sampler is calibrated to handle a flow of 10 litres of air per minute, and spores are impacted on a cylindrical drum covered by a melinex film coated with a 2% silicon solution as trapping surface. The drum was changed weekly and the exposed tape was cut into seven pieces, which were mounted on separate glass slides. Spore identification was performed using a NIKON OPTIPHOT II microscope equipped with a 40×/0.95 lens. Spore counts were made using the model proposed by Aira *et al.* [1] and Galán *et al.* [25], consisting of two continuous longitudinal lines along the 24-hr slide. *Botrytis* and *Uncinula* conidia and *Plasmopara* sporangium were identified and counted. For the purposes of this study, the generic term "spore", understood as a reproductive mechanism, was used in all cases. Concentrations were expressed as number of spores/m^3 of air.

In order to know the different levels of spores during every phenological phase a phenological study was carried out during the active grapevine season in 2007 (from 1 April to grape harvest in September); 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.* [35], adopted by the BBCH [37] for the phenological observation of grapevines. The date of the start was considered as the date when 50% of the studied plants were observed in every phenological stage. At the same time, the date on which fungicide was applied was also noted.

In order to obtain a model reflecting diurnal fluctuations in spore counts, we selected days on which spore counts were greater than the mean for the sampling period, and including the days without rainfall. The resulting days were used to calculate mean counts every two hours, thereafter expressing the data as percentages.

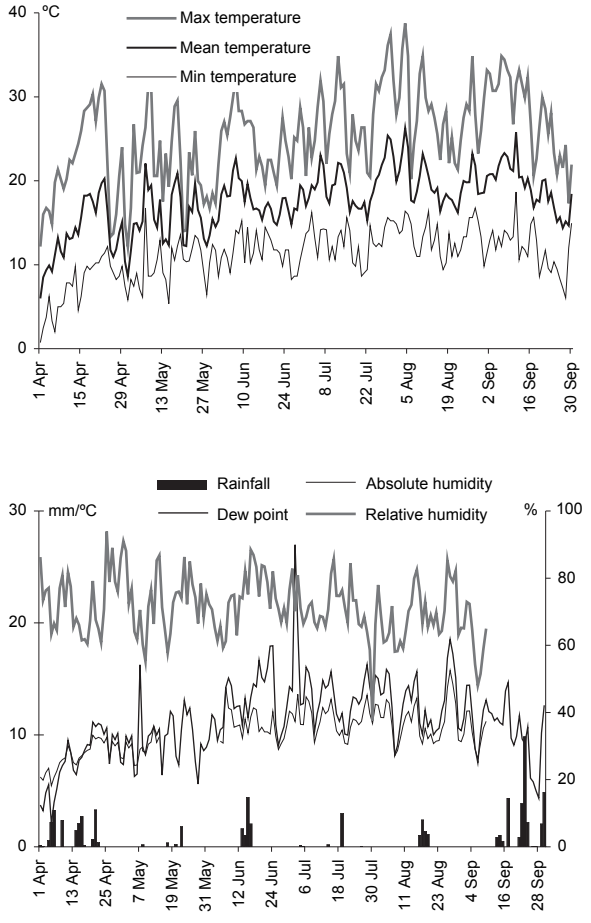

Figure 1. Evolution of the meteorological parameters during the period of study.

Spearman's test was used for correlations between daily mean airborne spore levels and the main weather-related factors: rainfall (mm), relative humidity (%), hours of sunshine (hours), maximum, minimum and mean temperatures (ºC), dew point (ºC), 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 1–5 days. Significance was calculated for $p \leq 0.05$ and $p \leq 0.1$, respectively.

Finally, linear regressions were performed with a view to predicting airborne spore concentrations. Weather-related variables displaying the highest positive correlation coefficients were selected as estimators for the equations. Spore data of the previous days were added to the prediction equation as time series models (such ARIMA models, frequently used in epidemiological studies), are also considered as useful estimators for the median range prediction of spore concentrations for the previous days in order to improve and calculate the forecast models [44, 51]. Model accuracy was tested using data for 2006, which were not used for developing equations due to a shortage of data for some weeks, which might have affected the precision of the models.

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. Figure 1 shows the behaviour of some of these parameters, in order to characterize the climate in the study area.

RESULTS

Since very few phenological differences were observed between the three varieties studied, the average duration of the six principal growth stages was calculated using data for all 60 plants. Stage 7 (development of fruits) was the longest with 55 days, followed by stages 8 (ripening of berries) and 5 (inflorescence emergence) which lasted 43 and 34 days, respectively. The other growth stages were shorter: stage 1 (leaf development) 7 days and stage 6 (flowering) lasted 12 days (Tab. 1).

The most abundant airborne spores in the study area were *Botrytis* (total 16.145 spores), followed by *Plasmopara* (747 spores) and finally *Uncinula* (578 spores) (Tab. 1). *Botrytis* spores were constantly present in the air from 13 April until the end of the sampling period (except on those

Table 1. Average date of the first and end day, and duration of principal phenological growth stages. Average and maximum of daily mean spore concentration values of *Botrytis*, *Uncinula* and *Plasmopara* during principal growth stages.

	Stages	Average date		Duration	*Botrytis*		*Uncinula*		*Plasmopara*	
		start	end	days	average spores/m³	max spores/m³	average spores/m³	max spores/m³	average spores/m³	max spores/m³
0	Bud development	01-Apr	11-Apr		0	0	0	0	0	0
1	Leaf development	12-Apr	19-Apr	7	16	58	0	1	1	3
5	Inflorescence emerge	20-Apr	24-May	34	122	415	2	17	4	28
6	Flowering	25-May	06-Jun	12	78	189	3	15	1	3
7	Development of fruits	07-Jun	01-Aug	55	87	219	5	28	4	18
8	Ripening of Berries	03-Aug	14-Sep	43	130	408	4	14	8	32
	Total year (spores)				16,145		578		747	

days that the sampler failed to work). No *Uncinula* and *Plasmopara* spores were detected on a number of days, mainly in March, April, June and August. Over the 176 days on which the sampler was operative *Botrytis* spores were registered on 93% (163 days), *Uncinula* on 70% (123 days) and *Plasmopara* on only 68% of days (120 days).

Aerobiological and phenological data were integrated in order to ascertain which of the principal grapevine growth stages coincided with the highest airborne pathogen counts, as a means of identifying periods of high spore concentrations which could be considered as plant infection risk periods. The highest *Botrytis* spore counts were recorded during stage 8 (ripening of berries) and stage 5 (inflorescence emerge) with daily average values of 130 and 122 spores/m^3 and maximum daily mean spore concentration of 408 and 415 spores/m^3, respectively. The highest mean daily *Uncinula* counts (5 spores/m^3) and maximum peak values (28 spores/m^3) were recorded during stage 7 (development of fruit), while *Plasmopara* also displayed greater presence during stage 8 (ripening of berries) with a mean daily count of 8 spores/m^3 and a peak spore count of 32 spores/m^3 (Fig. 2).

Table 2. Correlation between concentration of spores in the study period and the main meteorological variables. Spearman test (***p<0.01, ** p<0.05).

	Botrytis	*Uncinula*	*Plasmopara*		*Botrytis*	*Uncinula*	*Plasmopara*
Botrytis		0.425***	0.416***	**Dew point**	0.253***	0.373***	0.307***
Botrytis-1	0.669***	0.346***	0.385***	Dew point-1	0.346***	0.414***	0.363***
Botrytis-2	0.478***	0.327***	0.213***	Dew point-2	0.357***	0.443***	0.392***
Botrytis-3	0.490***	0.288***		Dew point-3	0.303***	0.411***	0.306***
Botrytis-4	0.448***	0.300***		Dew point-4	0.275***	0.325***	0.255***
Botrytis-5	0.405***	0.336***		Dew point-5	0.253***	0.308***	0.237***
Plasmopara	0.416***	0.559***		**Absolute Humidity**	0.306***	0.443***	0.275***
Plasmopara-1	0.252***	0.387***	0.502***	Absolute H-1	0.365***	0.459***	0.327***
Plasmopara-2	0.227***	0.353***	0.395***	Absolute H-2	0.357***	0.487***	0.341***
Plasmopara-3	0.245***	0.284***	0.307***	Absolute H-3	0.298***	0.422***	0.216**
Plasmopara-4	0.304***	0.293***	0.259***	Absolute H-4	0.267***	0.368***	0.193**
Plasmopara-5	0.346***	0.336***	0.286***	Absolute H-5	0.283***	0.366***	0.248***
Uncinula	0.425***		0.559***	**Relative Humidity**		-0.156**	-0.174**
Uncinula-1	0.332***	0.600***	0.428***	Relative H-1			
Uncinula-2	0.240***	0.552***	0.329***	Relative H-2		-0.166**	
Uncinula-3	0.257***	0.395***	0.205***	Relative H-3			
Uncinula-4	0.302***	0.421***	0.261***	Relative H-4			
Uncinula-5	0.381***	0.385***	0.258***	Relative H-5			
Temperature$_{max}$	0.261***	0.362***	0.304***	**Rainfall**	-0.254***	-0.287***	-0.193**
Maximum T-1	0.218***	0.312***	0.264***	Rainfall-1	-0.263***	-0.317***	-0.221***
Maximum T-2		0.319***	0.264***	Rainfall-2	-0.239***	-0.251***	-0.267***
Maximum T-3		0.241***	0.196***	Rainfall-3	-0.156**	-0.338***	-0.214***
Maximum T-4		0.195**		Rainfall-4		-0.276***	-0.188**
Maximum T-5	0.187**			Rainfall-5		-0.274***	-0.196**
Temperature$_{min}$	0.218***	0.399***	0.358***	**Sun hours**		0.306***	0.169**
Minimum T-1	0.374***	0.414***	0.380***	Sun hours-1		0.272***	
Minimum T-2	0.351***	0.406***	0.370***	Sun hours-2		0.309***	
Minimum T-3	0.276***	0.377***	0.263***	Sun hours-3		0.255***	
Minimum T-4	0.281***	0.296***	0.258***	Sun hours-4		0.322***	
Minimum T-5	0.244***	0.299***	0.288***	Sun hours-5		0.212***	
Temperature$_{mean}$	0.288***	0.429***	0.358***	**Wind speed**			
Mean T-1	0.307***	0.429***	0.344***	Wind speed-1			
Mean T-2	0.255***	0.411***	0.364***	Wind speed-2			
Mean T-3	0.176**	0.356***	0.258***	Wind speed-3			
Mean T-4	0.185**	0.282***	0.203***	Wind speed-4		0.169**	
Mean T-5	0.192**	0.221***	0.222***	Wind speed-5		0.278***	0.214***

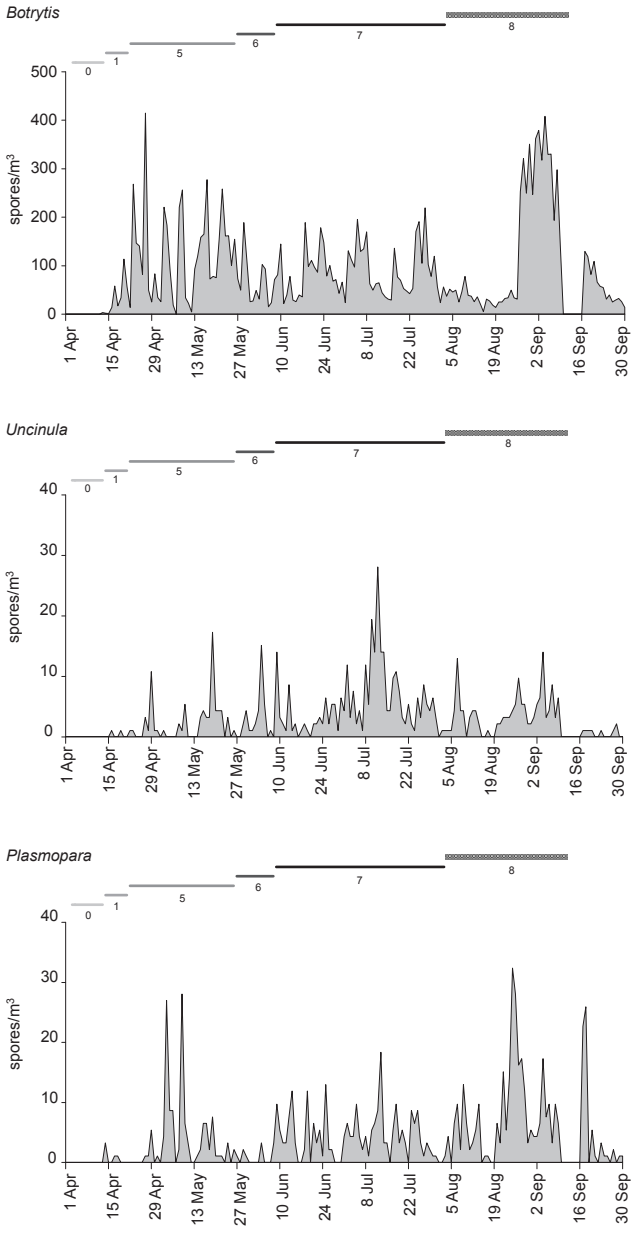

Figure 2. Evolution of the concentration of spores *Botrytis*, *Uncinula* and *Plasmopara*. Stage 0 (Sprouting development), Stage 1 (Leaf development), Stage 5 (Inflorescence emergence), Stage 6 (Flowering), Stage 7 (Development of fruits), Stage 8 (Ripening of Berries).

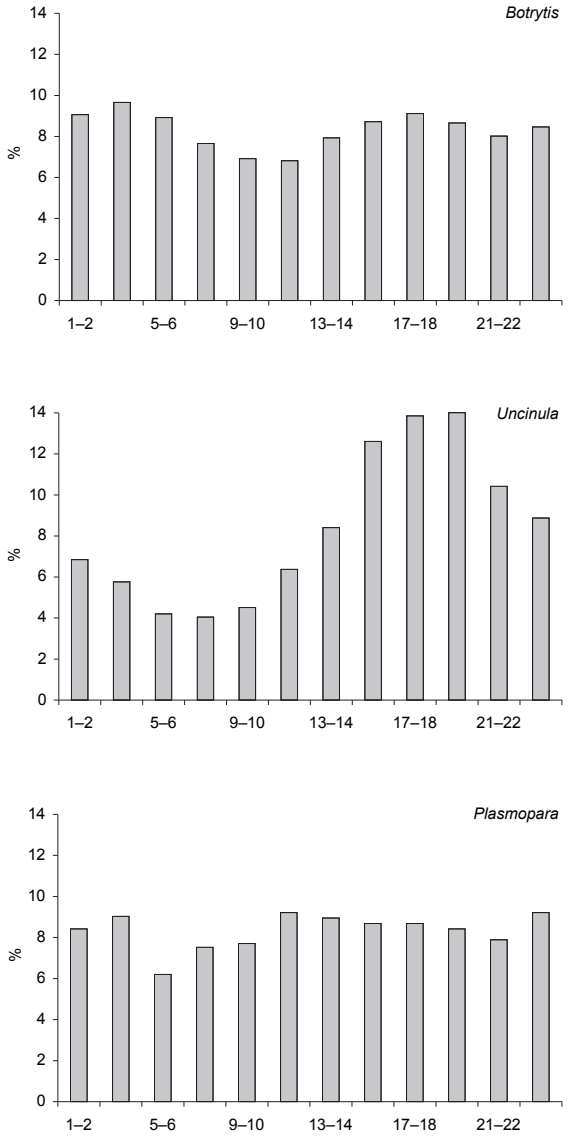

Figure 3. Models of intradiurnal behaviour of spores of *Botrytis*, *Uncinula* and *Plasmopara*.

Curves charting intradiurnal variations in spore counts displayed similar behaviour for *Botrytis* and *Plasmopara*, with no very marked oscillations in the course of the day. *Uncinula* spores were much more abundant during the day, rising in the hours before midnight. Peak daily counts were recorded in the afternoon and early evening. The lowest levels were detected at dawn and in the early morning (Fig. 3).

In order to determine the influence of major weather-related parameters on airborne spore counts for the three fungal phytopathogens studied, an analysis was made of Spearman's linear correlation coefficient (R). Table 2 shows the values obtained, significance being set at 99% ($p>0.01$). A highly-significant positive correlation was recorded between *Botrytis* and the minimum temperature on the previous day, the absolute humidity on the previous day, and minimum temperature, dew point and absolute humidity two days earlier. For *Uncinula*, the most influential parameters were absolute humidity on the previous day and two days before, and dew point on the previous day. Finally, for *Plasmopara*, the chief parameter was the dew point two days earlier, followed by the minimum temperature recorded one and two days earlier. All these correlations were positive, indicating that the parameters in question favoured the presence of airborne spores, and were thus used for linear regression analysis. Better results were obtained when correlations were performed with the pathogenic spore counts for previous days. For all spore types, the highest correlation coefficients were obtained

Table 3. Forecast model of *Botrytis*, *Uncinula* and *Plasmopara* spore concentrations.

	R	R^2	Ajust. R^2	F	Freedom degrees	p<	Error stand.
Botrytis	0.656	0.431	0.424	64.508	2.170	0.000	69.540
Botrytis = 3.161 + (0.622 * *Botrytis*-1) + (2.828 * Dew point-2)							
	Beta	Est. Err. Beta	B	Est. Error. B	t(170)	p	
Intercepted			3.161	18.062	0.175	0.861	
Botrytis-1	0.622	0.059	0.622	0.059	10.453	0.000	
Dew point-2	0.107	0.059	2.828	1.575	1.796	0.074	
Uncinula	0.519	0.269	0.261	31.420	2.170	0.000	3.700
Uncinula = -0.753 + (0.430 * *Uncinula*-1) + (0.236 * Dew point-2)							
	Beta	Est. Err. Beta	B	Est. Error. B	t(170)	p	
Intercepted			-0.753	0.960	-0.784	0.433	
Uncinula-1	0.429	0.068	0.430	0.068	6.262	0.000	
Dew point-2	0.190	0.068	0.236	0.085	2.777	0.006	
Plasmopara	0.505	0.255	0.247	29.217	2.170	0.000	5.057
Plasmopara = -0.699 + (0.419 * *Plasmopara*-1) + (0.298 * Dew point-2)							
	Beta	Est. Err. Beta	B	Est. Error. B	t(170)	P	
Intercepted			-0.699	1.311	-0.737	0.462	
Plasmopara-1	0.431	0.068	0.419	0.066	6.309	0.000	
Dew point-2	0.177	0.068	0.298	0.114	2.599	0.010	

with spore counts for the previous day: $R^2 = 0.669$ for *Botrytis*, $R^2 = 0.600$ for *Uncinula* and $R^2 = 0.502$ for *Plasmopara*.

Linear regression analysis was carried out to predict spore counts using weather parameters with a highly-significant correlation coefficient as independent variables. Those parameters accounting for a higher proportion of variation of the independent variable were selected as dependent variables. For all phytopathogenic fungi studied, the dew point two days before the spore-count was recorded proved to be the best variable for predicting spore counts. For *Botrytis*, results were significant, with an F-value of 64.508 (f.d.=2.170 and p<0.000); the regression equation accounted for 42.4% of variation in predicted spore counts. For *Uncinula*, the F-value was 31.420 (f.d.=2.170 and p <0.000), and the regression equation accounted for 26.1% of variability; in *Plasmopara* the F-value was 29.217 (f.d.=2.170 and p<0.000), and the regression equation accounted for 24.7 % of variability (Tab. 3).

The accuracy of the proposed model was tested by charting spore counts observed during 2006 and comparing them with predicted values. Figure 4 shows spore counts predicted for all three species in general fitted with the real observed data.

DISCUSSION

During 2007, the highest spore concentrations of *Botrytis* were recorded in the studied period. The higher abundance of *Botrytis* than *Uncinula* and *Plasmopara* spores has been noted in previous studies of vineyards in northwestern Spain [2, 16, 17, 43]. As Picco [42] has suggested, a possible reason could be due to the nutritional or toxic antagonistic action exerted by abundant *Botrytis* spores on *Plasmopara*. Also, the minor size of *Botrytis* conidia in relation to *Uncinula* conidia and *Plasmopara* sporangia could also have influence on the different annual total propagules trapped for the three pathogens. Specific fungicide application for every pathogen were applied, two against *Botrytis* and 9 and 11, respectively, were for *Uncinula* and *Plasmopara*, which also influenced the behaviour of the fungus cycle and the production of conidia and sporangia.

The synchronism of major grapevine growth stages with airborne *Botrytis* spore counts has been studied by several authors in different geographical areas. The pollen and sugar exudation during flowering favours the colonisation of the issues by this pathogen [20]. Moreover, from the start of ripening to the harvest is also considered as a vulnerable period for the vine [7, 33]. The results obtained here suggest that since the *Botrytis* spore count increased markedly during the principal growth stage of ripening of berries (maximum mean spore counts for the study as a whole, and maximum average of the daily mean spore concentration with a secondary peak of 408 spores/m^3), there is a particular risk of crop infection during this period, i.e. in the weeks prior to grape harvest. The susceptibility of the grapevine to *Botrytis* infection seems to increase as the berries ripen [31], therefore the influence of the favourable meteorological conditions, specifically wetness periods, on the establishment of infection will be increased on mature

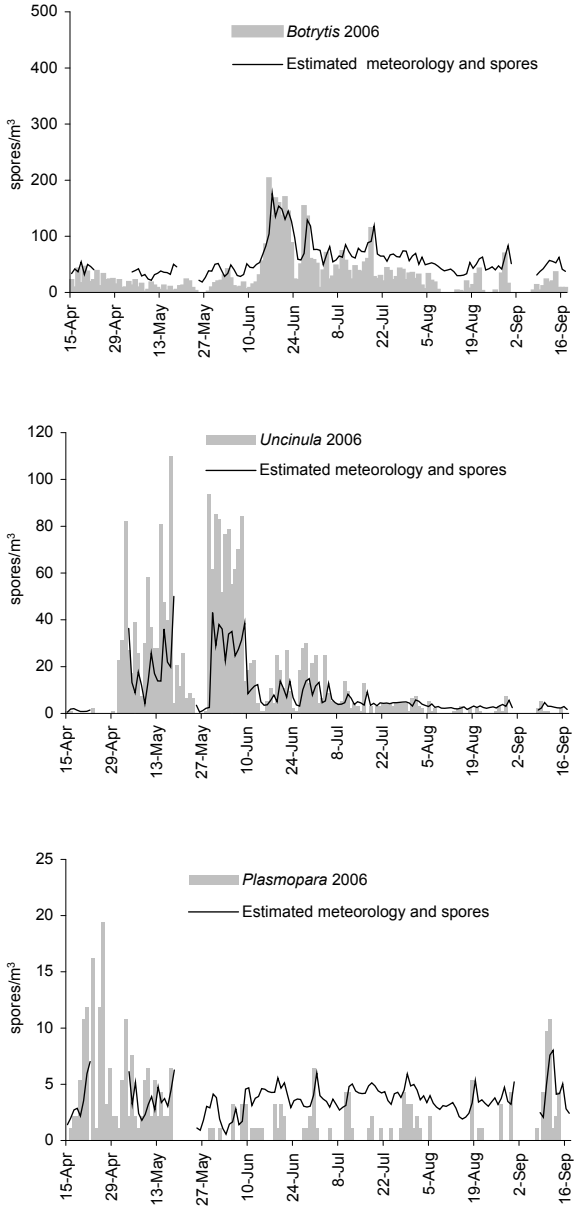

Figure 4. Levels of spores of *Botrytis*, *Uncinula* and *Plasmopara*, observed and predicted in the year 2006.

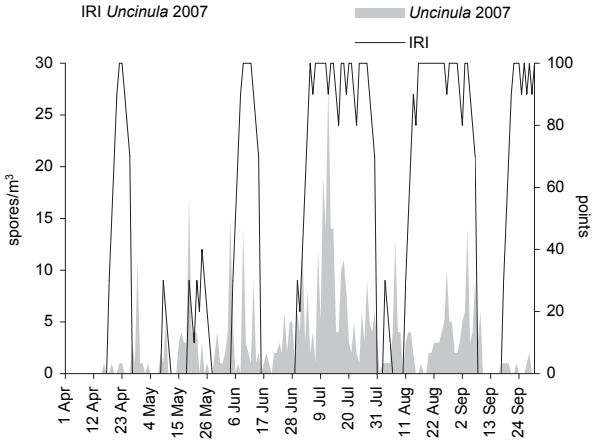

Figure 5. *Uncinula* Infection Risk Index.

berries. This fact has been point out by other authors [21] who stress that *Botrytis* present a peak of concentration in the atmosphere in the month of September when this fungus develops on senescence leaves or ripening fruits. Grape skin lesions play a key role both in the symptom expression and epidemiology of the disease [13]. Wounds are regarded as major entry sites for the pathogen on grapes [12, 38]. In our study, the rainy days registered during the last days of August could favour wounding and therefore the increase of conidia level during the first fortnight of September.

The weather factors displaying the strongest influence on *Botrytis* mean daily spore counts were temperature and humidity. Both parameters are considered as determinant in the grey mould spore germination and infection development [6, 19, 39]. In the present, study increased *Botrytis* spore counts, particularly counts of over 200 spores/m^3, were recorded in September, corresponding with a mean air temperature of 20ºC, widely considered optimal for infection [34]. In the same way, the increase of airborne conidia and the highest daily mean conidia concentration registered at the end of April (stage 5) was preceded by rainy days and maximum temperatures higher than 20ºC. Moreover, the results of correlation tests point to the marked effect of minimum temperature the previous day, and both dew point and absolute humidity one and two days earlier, on *Botrytis* counts.

The highest *Uncinula* counts were recorded during growth stage 7 (development of fruit). This result agrees with the most critical stage, signalled by different authors as the period from the flowering start until the grapes size achieves 7 mm [10, 24]. Even this fungus can persist as mycelia associated with infected buds from the previous season [10, 49], and the rainy conditions in the study area allow the consideration that cleistothecia is the most important primary inoculum [29]. The maturity of the cleistothecia is completed when a threshold of degree day accumulation is achieved [22]. In the study area, 1.100ºC, cited as the threshold and calculated as the sum of mean temperature from November 1 [28], was achieved at the beginning of March.

A number of authors have studied the factors favouring ascospore dispersal in this fungus. Periods of light rain followed by humid spells with temperatures of 25ºC [23] or 15–25ºC [27] were pointed out as optimum conditions, and diminishing at temperatures below 8ºC. Rain is also considered essential, acting as cleistothecia humidifier in order to favour dehiscence [27]. Furthermore, temperature is considered as the most important factor promoting the asexual reproduction of this fungus, within the range of 22–27ºC, the most favourable for conidia production and germination and infection, while temperatures higher than 33ºC or lower than 6ºC inhibit the process [4, 29]. The relative humidity between 76–96% are considered optima for the conidia germination and hyphen growing [29].

In this study, peak *Uncinula* spore counts coincided with weather conditions favouring both cleistothecia dispersal and development and infection caused by spores from secondary infection cycles. The highest counts for 2007 were recorded in mid-July, when mean temperatures were around 20ºC and relative humidity around 75%, preceded by a period of light rainfall.

Maximum temperature can be used to predict a high risk period of infection by this pathogen [29]. Therefore, the Infection Risk Index (IRI) based on this parameter [10] was calculated in this study in order to compare the periods of high airborne spore concentrations with the risk period signalled by that index. The longest risk period signalled by IRI was coincident with the period where the maximum *Uncinula* airborne spores were registered (Fig. 5).

Examination of statistical correlations between spore counts and weather parameters showed that absolute humidity (on the day of the spore count and also either one or two days earlier) and dew point one day earlier were factors favouring the increase of airborne spores.

Sporangia *Plasmopara* levels were low during the whole period, the ripening of berries and the leaf development being the stages in which the highest average daily mean and total count were registered. Flowering and grape ripening stages are considered as the most critics for the disease development [18].

The presence of downy mildew infection depends on spores released after germination of the *Plasmopara* zygote (responsible for primary infection), and the sporangium and spores produced later on by the mycelium generated (secondary infections). Several authors have included temperature and rainfall among the factors most favouring the development of downy mildew. The germination of *Plasmopara* sporangium in spring takes place more quickly with optimum temperatures of around 20–24ºC [36]. Nonetheless, low temperatures during early spring delay this process [48]. Rain is needed to unleash the germination of the sporangium, so that oospores kept in dry conditions cannot germinate [8]. Long dry periods in spring halt zoospore ripening, but the process is renewed once the rains reappear [46, 48]. Weather conditions in the vineyard determine – for primary and secondary infections – both the onset and the duration of fungal propagule incubation. High relative humidity is crucial for sporangium development with a minimum between 65–75% [32]. Some authors report that secondary infections are mainly influenced by the length of the period in which the leaf sustains an appropriate level of humidity and temperature [45]. Therefore, the severity of secondary infections will depend on the number of sporangia formed from the mycelium originated by the primary infection, their release and subsequent sporulation. Ripening of berries was preceded by several periods of non-continuous rain and mean air temperatures of 20ºC, considered optimal for spore germination. Moreover, statistical analysis showed that the dew point two days earlier induced the development of infection.

Finally, regression analysis performed to enable the development of models to predict airborne spore counts, and tested with information from the 2006 harvest, yielded good results, particularly for *Uncinula* and *Plasmopara*. The species were the object of numerous fungicide applications (9 and 11, respectively), which might be thought to hinder model development and verification; yet although these treatments alter the natural pathogen life cycle, and therefore affect airborne spore counts, the proposed models were able to provide a general prediction of airborne spore counts in the vineyard during grapevine growth.

During subsequent years the purpose of our study will be to regulate the specific fungicide application for every pathogen in order to develop a strategy of Integrated Pest Management. At this moment, the chemical control was conducted by means of established calendars which, for the most part are ineffective in spite the high quantity of chemical applications performed. Finally, we would like to test different chemical compounds in order to identify the most accurate for regulating the presence of pathogenic inoculums in the atmosphere of the vineyard.

CONCLUSIONS

Fungal physiology, and such processes as spore emission or fungal infection, is a complex phenomena. Detailed knowledge of grapevine phenology, together with data on airborne pathogenic spore counts, provides a valuable tool for developing a precise, 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. At the same time, it benefits environmental conservation by enabling the regulation and reduction of fungicide applications to meet the real requirements of the vineyard. The inclusion of a large number of data in future will help to improve the prediction models proposed.

Acknowledgements

This study was financed by the project PGIDIT07PXIB2000076PR sponsored by the "Xunta of Galicia".

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