



Airborne fungal phytopathological spore assessment in three European vineyards from different bioclimatic areas

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Abstract The most common fungal diseases of *Vitis vinifera* are powdery mildew (*Erysiphe necator* (Schw.) Burr.), grey mould (*Botrytis cinerea* Pers.) and downy mildew (*Plasmopara viticola* (Berk. and Curt.) Berk. and de Toni). Knowledge about the concentration of their spores in the atmosphere of the vineyard is important in order to ascertain the fungal load in the vineyard during the reproductive vine cycle. An aerobiological study was conducted in three vineyards across Europe: in North-Western Spain (Cenlle), South Spain (Montilla) and South-Eastern Poland (Rzeszów). During 2016, three Hirst-type spore traps sampled the airborne particles in each vineyard during the reproductive cycle of grapevine. The main goal of this study was the analysis and comparison of the spore concentrations in the three studied viticultural areas using different statistical tools. The most abundant spores in the vineyards

atmosphere were *Erysiphe necator*. The highest fungal spore concentrations belonged to the North-western Spain zone, due to rainfall and temperature conditions were optimal for mould development in this area. In South Spain and South-eastern Poland, the daily spore peaks took place before than North Spain spore peaks. Average temperature, relative humidity and rainfall were the principal factors affecting the spore concentration in all zones. Redundancy Analysis showed high correlation between meteorological parameters and fungal spore concentrations.

Keywords Fungal spores · Vineyards · Diseases · Europe

1 Introduction

Grapevine is one of the most profitable agricultural crop in Europe (67.2% of the world wine production) (Eurostat 2019) that provides high economic yield (25,500 M€ in 2016) (FAO 2019) coming from less than 2% of European arable land (FAO 2019), making it a cost-effective crop in relation to the surface-benefit. However, current management of European vineyards presents important associated agro-environmental problems, such as the high use of external inputs, especially pesticides against fungal diseases. In fact, 20% of pesticides applied in Europe were on vineyards, despite only 2% of the arable land is

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dedicated to this crop. The high use of pesticides in vineyards leads to the subsequent soil pollution together with other soil degradation processes (compaction, erosion, etc.) that may negatively affect the soil biodiversity and its associated ecosystem services.

The most common fungal diseases of *Vitis vinifera* in the European vineyards are powdery mildew (*Erysiphe necator* (Schw.) Burr.), grey mould (*Botrytis cinerea* Pers.) and downy mildew (*Plasmopara viticola* (Berk. and Curt.) Berk. and de Toni) (Fernández-Gonzalez et al. 2009; Rodríguez-Rajo et al. 2010; Rossi et al. 2013; Lisek 2014; Moyer et al. 2014). These fungal pests have caused an important economic loss in all Europe with the largest vineyards area at a world level. Several studies have been focused on these fungal species considering different points of view, as the vineyards aerobiology or phenology (Oliveira et al. 2009; Rodríguez-Rajo et al. 2010; Fernández-González et al. 2012; Holb and Füzi 2016), the presence in the plant (Rossi et al. 2010), direct inoculation (Gadoury et al. 2007; Kiefer et al. 2002) and genetic variations (Rossi et al. 2013; Oliveira and Cunha 2015; Oliveira et al. 2018). All these papers noted the importance of the selected spores for the present study. Airborne spore concentrations may serve as an indicator of pathogen development. Therefore, it can be useful when infection levels are initially determined by the presence of inoculum, rather than prevailing weather (Carisse et al. 2008; Holb and Füzi 2016). Nevertheless, weather parameters are affecting fungal spore concentrations, such as temperature, relative humidity and rainfall. Some studies have shown the importance of low rainfall (< 1.2 mm) as parameters increasing *Hidathe* spore concentrations in Italy and United States (Rossi et al. 2010, 2013; Moyer et al. 2014).

The epidemics produced by *E. necator* can progress rapidly during the entire growing season due to the recurrence of primary (sexual) and secondary (asexual) infections. The ascocarps are able to release ascospores in autumn that survive over winter as a mycelium in buds and release viable spores in the following spring (Gadoury et al. 2012). The development of the powdery mildew disease begins between 5 and 10 °C, with a marked importance above 15 °C, and an optimum between 20 and 25 °C (Jailloux et al. 1998). *P. viticola* epidemics involve primary (sexual) and secondary (asexual) infection cycles. Oospores (sexual stage) overwinter in leaf debris or pruned

canes in soil. In spring, they germinate developing a macrosporangium that releases the zoospores responsible for primary infections on grape leaves and bunches. Successive asexual infection cycles follow these early infections. The required time for this germination is between 40 and 60 min, with an optimum temperature of 22–25 °C (Mansilla et al. 1991). *B. cinerea* overwinter as visible sclerocium mainly in branches and secondarily in buds. In spring, under favourable conditions of humidity and temperature, conidiophores maturation and conidia formation occurs, which are spread by wind or rain. The optimum temperature for germination seems to be around 20–25 °C, although germination can occur since 13 °C at a very slow rate (Hidalgo 2002). The mycelium has its optimal activity at 20 °C and can continue its development until 25 °C, stopping at higher temperature (Latorre 1986). Conidia also need the presence of water droplets or condensation for germination. The incidence of these diseases varies throughout the vine vegetative cycle depending on the meteorological conditions and the susceptibility of each phenological phase.

One of the most important applications of aerobiological data is the model development to predict the main disease risk periods for crops. Previous forecast models were conducted by means the ARIMA methodology (Rodríguez-Rajo et al. 2010; Fernández-González et al. 2012). Other studies showed that different kind of models could help us to understand the relationship between spore concentration and meteorological parameters, such as the multivariable decision trees, canonical correspondence analysis (CCA), detrended correspondence analysis (DCA) and redundancy analysis (RDA) (ter Braak 1986; Sadyś et al. 2015).

The aims of this study were: (1) to analyse the fungal spore concentrations of vineyards in different bioclimatic zones of Europe; (2) to detect similarities in the spore concentrations behaviour and their relationship with weather parameters; (3) to compare different statistical and multidimensional techniques for the study of the relationship between fungal spore concentration and meteorological parameters.

2 Materials and methods

The study was conducted in three European vineyards located at different biogeographic areas, Oceanic Mediterranean (OM) Cenlle, (42.30 N, 8.10 W) (North-western Spain), altitude (75–400) m, belonging to the Designation of Origin area “Ribeiro”, covering a total area of 37,140 Ha; Termo-Mediterranean (TM) Montilla, (37.55 N, 4.55 W) (Southern Spain), altitude (472–496) m, belonging to Designation of Origin “Mointilla-Moriles”, covering a total area of 5134.10 Ha; and finally Warm temperate (Wt) Rzeszów, (49.98 N, 22.02 E) (South-eastern Poland), covering a total area of 1.5 Ha and in altitude from 206 to 280 masl (Fig. 1). In the three study areas, the vineyard plot consisted of grapevines trained on a vertical shoot positioned trellis system with an undivided canopy.

When we analyse the weather variables in each study area, the zone with higher temperatures was Montilla, followed by Cenlle and Rzeszów had the lower ones. Taking into account the rainfall variable, Cenlle has been the zone with higher amount of rainfall distributed in 33 days, 13 of them had a rainfall lower than 1.2 mm (light rainfall) and 20 of them had higher values (high rainfall). Montilla was the second zone regarding the rainfall but the rain was concentrated in 15 days, 6 with low rainfall and 9 of them with high rainfall. Finally, Rzeszów has a lower amount of precipitation with 58 rainy days distrusted in 40 days of low rainfall and 18 days of high rainfall (Table 1, Fig. 2).

B. cinerea spore is unicellular, smooth, ellipsoidal, ovoid, spherical, or subspherical. Colouration varies from hyaline to grey-ash. Size can vary from 10 to 12 µm in length and 8 to 10 µm in thickness. The presence of a slightly protruding hilum is notable,

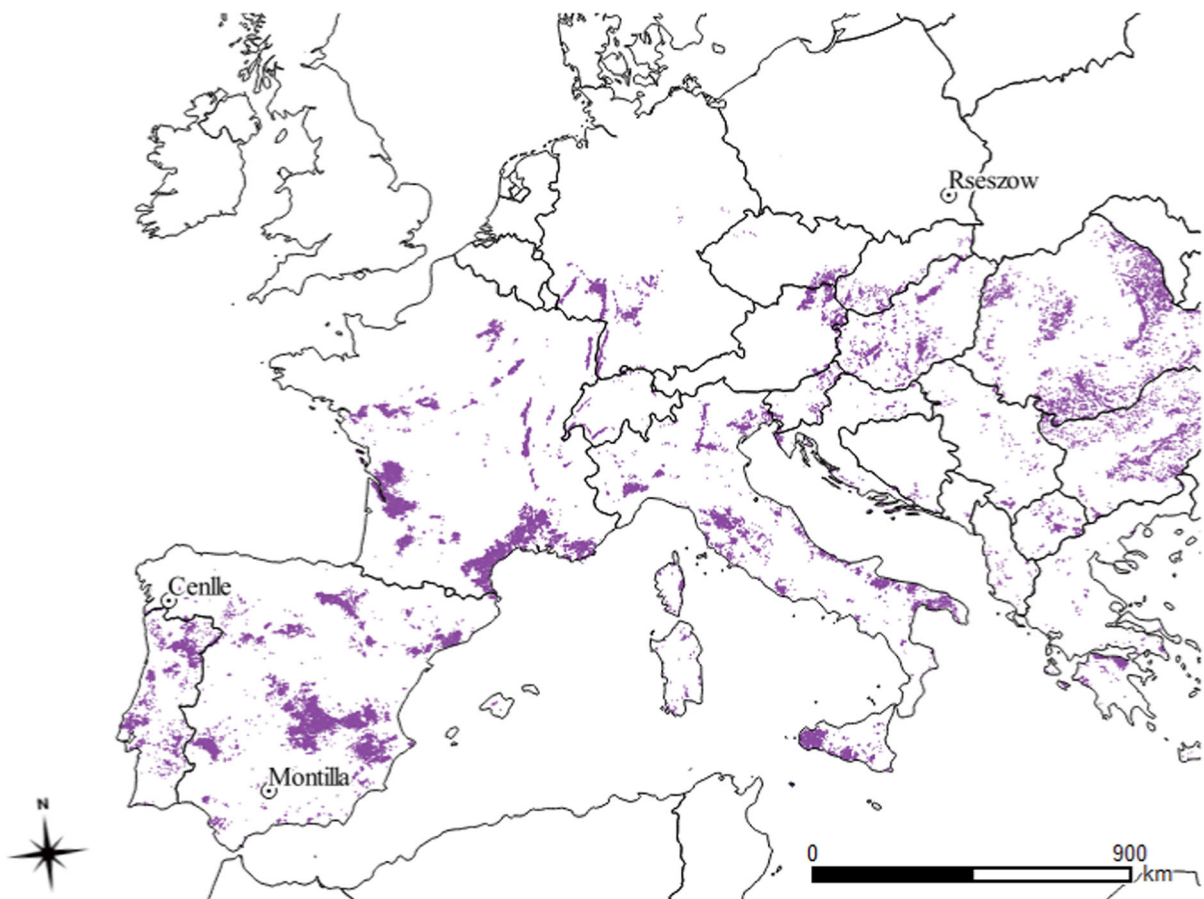


Fig. 1 Sampling location areas (Cenlle, Montilla and Rzeszów), purple zones indicate the vineyard land use by CORINE land cover

Table 1 Main characteristics of fungal spore concentrations and meteorological parameters from May to October 2016 in three study points

	Cenlle	Montilla	Rzeszów
<i>B. cinerea</i> SSIn	16,020	321	488
% of total	46.8	31.4	30.9
Peak (spores/m ³)	637	36	22
Peak day	22-jun	19-may	23-may
<i>E. necator</i> SSIn	16,439	660	955
% of total	48.0	64.5	60.4
Peak (spores/m ³)	399	33	41
Peak day	16-may	11-jun	06-jul
<i>P. viticola</i> SSIn	1785	42	137
%of total	5.2	4.1	8.7
Peak (spores/m ³)	104	11	8
Peak day	08-jun	18-may	22-may
Total Spores SSIn	34,243	1023	1578.95
Total Rainfall (mm)	218.80	110.40	102.80
Max Rainfall (mm)	39.8	29.0	13.2
Low rainfall	13	6	40
High rainfall	20	9	18
Days of rainfall	33	15	58
Average Temp °C	19.7	24.3	17.0
Max Temp °C	40.2	42.9	33.2
Min temp °C	3.4	8.5	0.6

Max: maximum daily concentrations

often seen (Fig. 3a). *E. necator* spore is unicellular, cylindrical, and truncated at the ends. The length varies between 16 and 29 µm and a thickness between 10 and 12 µm. They are characterized by having a hyaline membrane and a granular content (Fig. 3b). *P. viticola* sporangia of are ovoid in shape, being broader at the apex. They are hyaline. Size varies between lengths of 17–40 µm and thicknesses of 10–20 µm (Fig. 3c).

In each vineyard airborne fungal spore concentrations were monitored using a Hirst-type volumetric spore trap (Hirst 1952). We followed the Spanish Aerobiology Network (REA) protocol for sample process and spores count (Galán et al. 2007), as well as the recommendations of the European Aeroallergen Society (EAS) (Galán et al. 2014). The samplers were located at a minimum high of 1.5 m above ground level in the centre of the vineyard, as we considered it enough height to prevent trapping difficulties by plant

growth. The fungal spore concentrations were expressed as spores/m³ of air when referring to daily mean values, or Seasonal Spore Integral (SSIn) when referring to integral over time of spore concentration, it can be obtained by summing the average daily concentration over the given period of time (Galán et al. 2017).

The sampling period was from May to October (both months included) of 2016, covering in all cases flowering and the entire post-flowering period of the vineyard. The daily weather variables taken into account for this study were maximum (Tmax), average (T) and minimum temperature (Tmin), rainfall (Rf), relative humidity (RH), wind speed (WV) and dew point (DP). Moreover, for a better understanding of the role of rainfall in spore concentrations, two additional parameters were tested, considering days were rainfall was higher than 1.2 mm (High rainfall, HRf) or lower (Low rainfall, LRf) (Rossi et al. 2010, 2013; Moyer et al. 2014).

The fungal spore concentration normality has been tested using the Shapiro–Wilk test, followed we carried out a Spearman's correlation test, decision trees and redundancy analysis (RDA) were applied to ascertain the relationship between spore concentrations and weather-related parameters at the same day and with lag time (1–7 days before). In all cases, analysis was separately conducted for each location. For Spearman's correlation, significance was calculated for $p < 0.001$, $p < 0.01$, $p < 0.05$ and $p < 0.1$ by means the R program (R Development Core Team 2008). For a better comparison between viticultural areas, decision trees and RDA analysis were applied in order to standardize fungal spore concentrations. Decision trees have been done to categorize the variables and to show how different variables affected to the spore concentrations; they were constructed using the R Program party package (Hothorn et al. 2012). In the case of RDA, the method explained by Sadyś et al. (2015) was followed, this method requires the Canonical Correspondence Analysis (CCA) for examine relationship and collinearity (redundancy) of independent variables and the detrended correspondence analysis (DCA) to explore the length of axis. CCA is a multivariate method to elucidate the relationships between biological assemblages of species and their environment. The method is designed to extract synthetic environmental gradients from ecological datasets (ter Braak 1986) and, in our case,

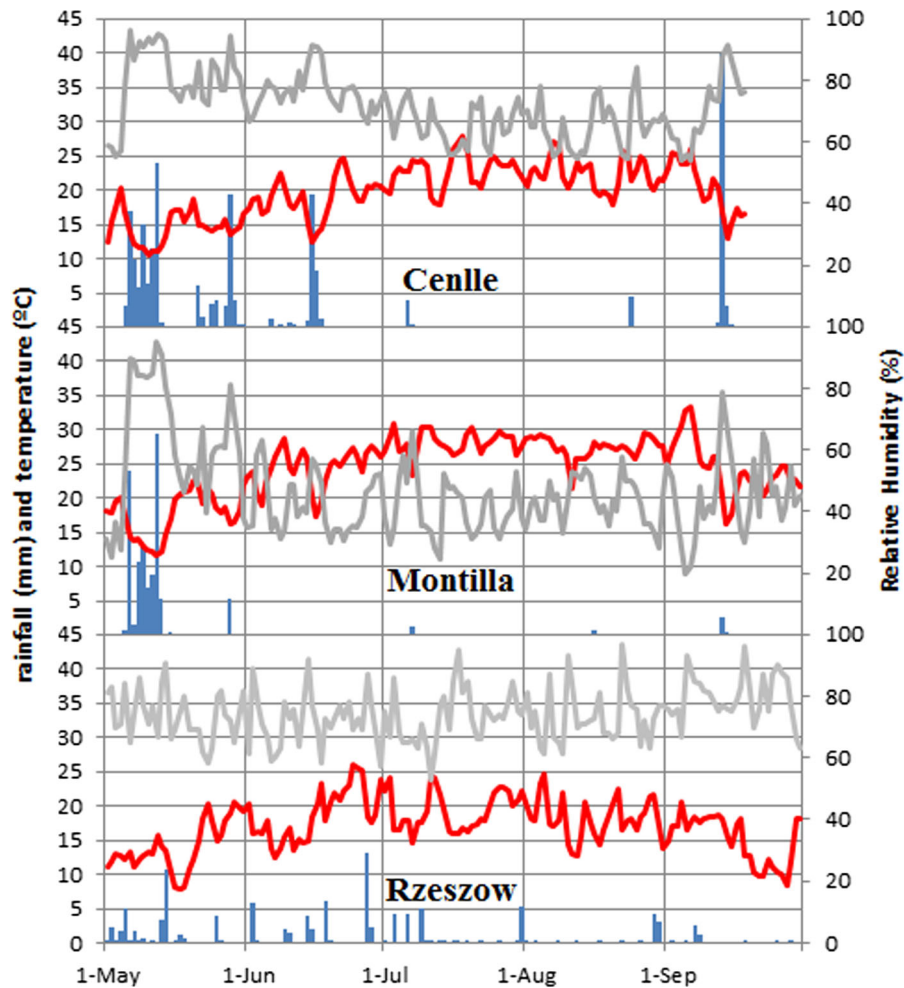


Fig. 2 Main meteorological parameters in the study zone, rainfall (blue bars), Average temperature (red lines) and Average relative humidity (grey lines)



Fig. 3 Microscopic Photographs (400X) of main phytopathological fungus, **a** *B. cinerea*, **b** *E. necator* and **c** *P. viticola*

giving the relationship between environmental independent variables; the character for define the correlation between 2 variables is the variable inflation factors (VIF) which have been under 2 (Sadyś et al.

2015). On the other hand, DCA is another multivariate statistical technique widely used to typify the data matrix in ecological community data. The analysis Of variance (ANOVA) is a collection of statistical models

and their associated estimation procedures used to analyse the differences among group means in a sample and it have been applied to the RDA matrix to denote the significance of the axis. The statistical analysis was conducted by means the vegan package (Dixon 2003). The RDA analysis was used for each geographical region separately, and the results were graphically presented on biplots which illustrated the relationships among the meteorological parameters and fungal spore types. The correlation significance was determined by the vector length, with the longest ones explaining the stronger correlations (ter Braak 1986). The relation of vectors (positively or negatively proportional) was based on the position of spore types with respect to the arrows end.

3 Results

The spore concentrations recorded in the OM area (North-western Spain) was 33 times higher than the observed in the other study areas (Fig. 3, Table 1). In all cases, the most common spores were from *E. necator*, followed by *B. cinerea* and *P. viticola* (Table 1). In the OM area vineyard, the *B. cinerea* and *E. necator* Seasonal Spore Integral (SSIn) were similar, but for the TM and Wt areas the *E. necator* SSIn constituted more than 60% of the total studied spores. In all regions, percentages of *P. viticola* were less than 10%.

Daily peaks of *B. cinerea* and *P. viticola* were observed in May in the Wt and TM, whereas in OM a month later, in June. In the case of *E. necator*, daily peaks were recorded in May in the OM area, June in the TM and Wt areas (Table 1, Fig. 4).

Spearman's correlation coefficients (Table 2) between spore concentration and weather-related parameters showed the statistically significant relationships between relative humidity and rainfall for the airborne *E. necator* spore concentrations, in all cases with a significance higher than 99%. The *B. cinerea* spore concentrations also showed a significant relation, over 99%, with relative humidity and rainfall as well as dew point. *P. viticola* spore concentrations showed a significant relation with rainfall over 99%, but in the case of the South-eastern Poland vineyard the association was not statistically significant. Moreover, temperature was an important factor affecting

spore concentrations in both southern European areas but not significant in the central Europe (Wt area).

The decision tree method, as more accurate than Spearman correlation detected more relationships among spore concentrations and meteorological parameters (Fig. 5). The relative humidity was the most significant parameter for the spore presence in the OM vineyard. In the case of *B. cinerea*, the relative humidity lower than 78.4% on 5 days before, lower than 74.8% on 3 days before and lower than 73.1% 2 days before promoted the lowest daily spore concentrations, whereas the dew point over 13.41 °C on the same day can induce to the highest spore levels. The relative humidity higher of 78.0% on one week before caused the increases on the *E. necator* spore concentrations in the vineyard atmosphere in this study area. *P. viticola* concentrations were related to the temperature of a week before and the presence of low rainfall two days before. In the TM vineyard, temperatures and rainfall were the main factors affecting fungal spore concentrations. The presence of high or low rainfall one week before is the main factor affecting *B. cinerea* concentrations followed by the average temperature down 20.16 °C on the previous day. *E. necator* concentrations decreased when a minimum temperature of 5 days before was lower than 15.07 °C. *P. viticola* concentrations were influenced by rainfall and minimum temperature of 6 days before, respectively, and the minimum temperature of the previous day. In the Wt vineyard, only the minimum temperature of 5 days before affected the *B. cinerea* concentrations. There were no other meteorological factors affecting the concentrations of *E. necator* and *P. viticola* in this area (Fig. 5).

Canonical Correspondence Analysis (CCA) detected a high autocorrelation within the examined meteorological parameters. Hence, it was necessary to remove each time from the data set, as their VIF scored above 5. In this way, different parameters were selected for each area (Table 3).

The relationship between fungal spore concentrations and meteorological variables in each zone were monotonic. The length of detrended correspondence analysis (DCA) first axis analysis resulted in values below 2 showing that RDA is more appropriate than CCA (Table 4). The RDA axis 1 and 2 explained in all cases more than 95% of the variance. RDA1 and RDA2 axes explained, respectively, the 81.72 and 16.53% variability in OM area, 86.78 and 9.26% in

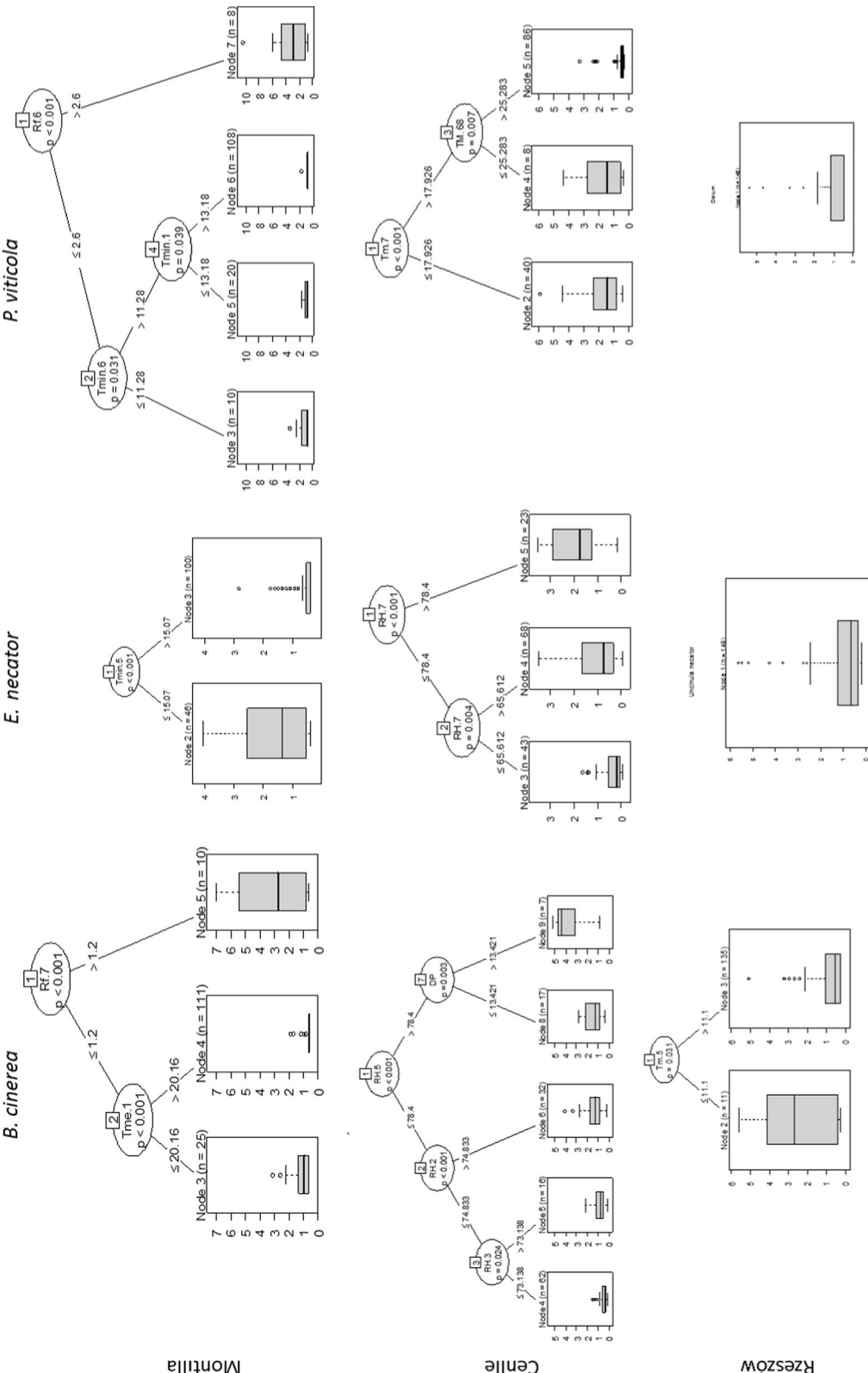


Fig. 4 Decision trees for *B. cinerea*, *E. necator* and *P. viticola* spore standardized concentrations in each study area (Cenlle, Montilla and Rzeszów)

Table 2 Shapiro–Wilk test for three spores (*B. cinerea*, *E. necator* and *P. viticola*) on each study area

	W	p value
Cenlle		
<i>B. cinerea</i>	0.7558	1.19E–13
<i>P. viticola</i>	0.6865	1.15E–15
<i>E. necator</i>	0.8703	1.82E–09
Montilla		
<i>B. cinerea</i>	0.4299	< 2.20E–16
<i>P. viticola</i>	0.2575	< 2.20E–16
<i>E. necator</i>	0.6489	< 2.20E–16
Rzeszów		
<i>B. cinerea</i>	0.7147	6.89E–16
<i>P. viticola</i>	0.5914	2.20E–16
<i>E. necator</i>	0.7351	2.68E–15

TM area as well as 63.89 and 33.15% in Wt zone (Table 5). Results of RDA analysis showed a similar behaviour for *E. necator* and *B. cinerea* within both Mediterranean vineyard's and between *P. viticola* and *B. cinerea* in Wt area. Relative humidity positively influenced all fungal spore concentrations in Mediterranean zones, while in Wt zone the *E. necator* concentrations were positively affected by the relative humidity of 3 and 4 previous days Dew point of 3 days before and minimum temperature of 7 days before negatively affected spore concentrations of all taxa in the Mediterranean areas. Wind speed of 5 and 6 days before positively affected to *B. cinerea* and *P. viticola* spore concentrations in Wt area. Rainfall affected fungal spores in several ways. Low rainfall of 5 and 6 days before in Wt area and 7 days before in Mediterranean areas affected the fungal spore concentrations increase. Low rainfall on the same day promotes the increase of the *P. viticola* concentrations in both Mediterranean areas. Rainfall of 6–7 days before had a stronger effect on *B. cinerea* and *E. necator* spore concentrations (Fig. 6).

Finally, the ANOVA test revealed that in all viticulture areas both two principal RDA axes were statistically significant, with the highest significance for the first axe in all cases ($p < 0.0001$) (Table 6).

4 Discussion

The presence of *B. cinerea*, *E. necator* and *P. viticola* in the vine atmosphere along the grapevine vegetative cycle has been described by several authors all over the Iberian Peninsula and other South European regions (Rossi et al. 2010, 2013), showing the interesting comparison in different biogeographical areas. Our results showed that *E. necator* spores were the most abundant spores in the studied vineyards followed by *B. cinerea* and the last *P. viticola*. The *E. necator* SSIn recorded was higher than the pointed by several authors in different European areas: Szekszárd in South-west Hungary (Holb and Füzi 2016), Amares in Portugal (Oliveira et al. 2009). Also, the *E. necator* values were higher than in other years in some of the studied locations, Cenlle in North-west Spain (Fernández-Gonzalez et al. 2009, 2013, 2016) or Córdoba in South Spain (Martínez-Bracero et al. 2019). This fact could explain the found strongest influence of the meteorological parameters (mainly temperature, relative humidity and rainfall) on *E. necator* spore concentrations. In general, the optimum conditions for spore dispersion are low rain periods followed by a humid spell and temperatures of 25 °C (Gadoury and Pearson 1990). Some researchers noted for asexual reproduction that the most influential weather variable was temperature, with an optimum range between 22 and 27 °C for conidia germination and infection. On the contrary, temperatures higher than 33 °C or lower than 6 °C inhibit the process (Bendek et al. 2002; Jarvis 2002). In addition, the optimal relative humidity for hyphen growth and conidia germination ranges between 76 and 96% (Jarvis 2002). In the studied areas, the highest *E. necator* spore concentrations coincided with favourable meteorological conditions for the development of secondary infection cycles and infection. The highest concentration was registered in the North-western Spain area on May 16 with an average temperature around 18 °C and a high relative humidity of 90% preceded by a rainfall period. On the contrary, the lowest *E. necator* spore concentrations were recorded in the South Spain vineyard with a peak on June 11 coinciding with temperatures around 30 °C, a lower relative humidity of 55% and the absence of rainfall.

The highest *B. cinerea* spore concentrations were recorded in the OM area, followed by the Wt zone and finally the TM area one. Several authors noted in

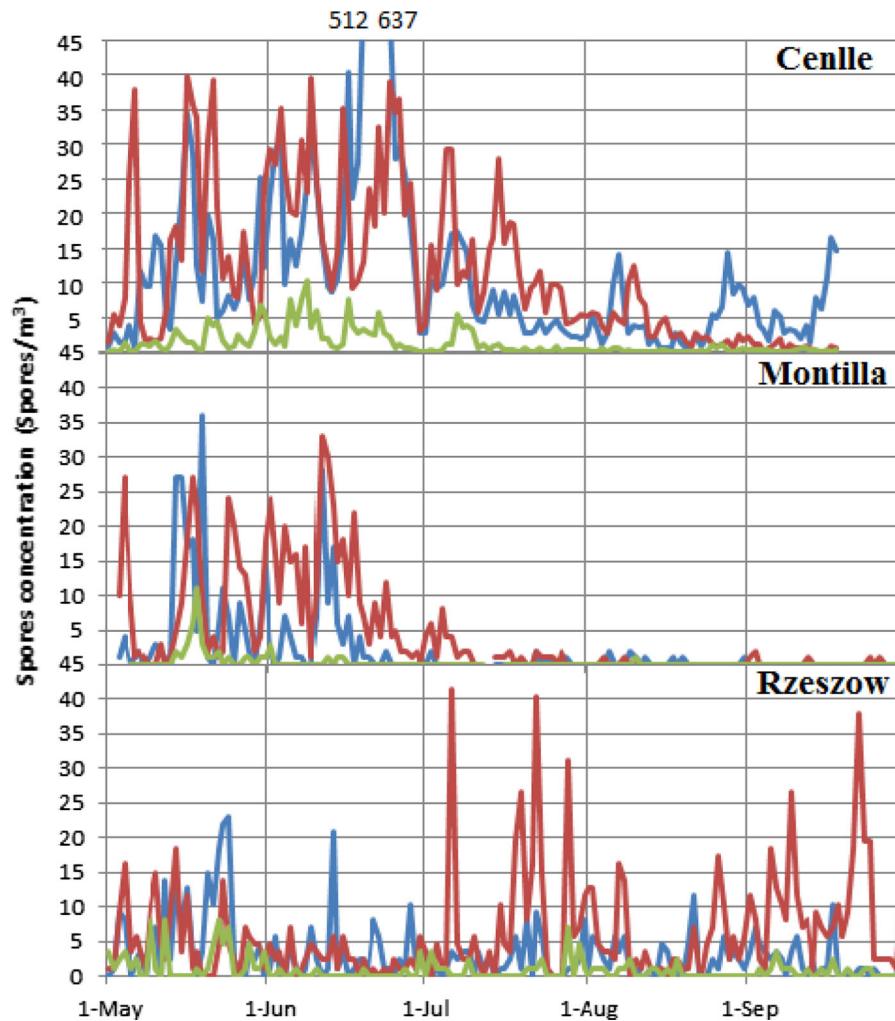


Fig. 5 Daily airborne fungal spore concentrations during the study period in the three sampling areas, Cenlle, Montilla and Rzeszów (blue line: *B. cinerea*; red line: *E. necator* and green line: *P. viticola*)

previous studies similar concentrations in North-west Spain (Fernández-Gonzalez et al. 2009; Rodríguez-Rajo et al. 2010). Higher concentrations than the recorded in Poland and South Spain were noted in Amares-Portugal (Oliveira et al. 2009; Fernández-González et al. 2012), Córdoba-Spain and Rzeszów-Poland (Kasprzyk et al. 2004). The weather variables can markedly influence *B. cinerea* spores dispersion and infection processes, as an average temperature of 20 °C is considered optimal for infection (Latorre 2002), whereas temperatures higher than 35 °C inhibit the conidia germination (Thomas et al. 1988), what probably happened in Montilla (TM), in a very dry climate. The relative humidity and rainfall are important variables for infection, thereby different authors

pointed that relative humidity higher than 90% can initiate germination and cause a severe infection (Latorre and Rioja 2002). In addition, stormy rains without a noticeable temperature drop favour the propagation of the fungus from the first infection focus (Coley-Smith et al. 1980). This type of precipitation can cause wounds, which are important entry sites for the pathogen on grapes (Nair et al. 1988; Coertze and Holz 1999), as well as favour conidia dispersal via rain splashes (Jarvis 1962). In North-west Spain, the *B. cinerea* peak was registered on June 22 coinciding with stormy rains, average temperature around 20 °C and relative humidity around 90%. In Central Europe, the *B. cinerea* maximum concentration coinciding with low rainfall, low average temperatures (15 °C)

Table 3 Spearman's correlation between fungal spore's concentration and weather parameters

	Cenlle			Montilla			Rzeszow		
	<i>B.cinerea</i>	<i>P. viticola</i>	<i>E. necator</i>	<i>B.cinerea</i>	<i>P. viticola</i>	<i>E. necator</i>	<i>B.cinerea</i>	<i>P. viticola</i>	<i>E. necator</i>
DP		− 0.017***		− 0.328***		− 0.327***			
DP.1		− 0.063**		− 0.312***		− 0.325***			
DP.2		− 0.138*		− 0.243*		− 0.377***			
DP.3		− 0.195**		− 0.207*	− 0.149	− 0.403***			
DP.4		− 0.255***	− 0.178	− 0.222*	− 0.143*	− 0.355***			0.165
DP.5		− 0.328***	− 0.208	− 0.268**	− 0.177*	− 0.362***			
DP.6	− 0.278**	− 0.370***	− 0.177	− 0.237*	− 0.194*	− 0.384***			
DP.7	− 0.314**	− 0.389**	− 0.169	− 0.228*	− 0.161*	− 0.370***			
Rf	0.159	0.294**							
Rf.1	0.219*	0.389***		0.159					
Rf.2	0.338***	0.396***		0.202*					
Rf.3	0.379***	0.354***		0.265**	0.142*		0.298**		
Rf.4	0.398***	0.337***		0.310***	0.217**				
Rf.5	0.440***	0.370***	0.220*	0.222*	0.300**				
Rf.6	0.408***	0.381***	0.351***	0.275**	0.285***	0.162			
Rf.7	0.398***	0.412***	0.437***	0.313***	0.376***	0.165	0.210*		
RH	0.354***	0.464***	0.196*	0.191	0.443*		− 0.186		
RH.1	0.466***	0.491***	0.177	0.215*	0.230*		− 0.212*		
RH.2	0.579***	0.536***	0.210*	0.239**	0.222***				0.330***
RH.3	0.608***	0.527***	0.290**	0.245*	0.361***				0.190*
RH.4	0.594***	0.464***	0.357***	0.272**	0.339***				0.194*
RH.5	0.574***	0.477***	0.432***	0.249*	0.356***	0.214*			0.215*
RH.6	0.543***	0.512***	0.533***	0.343***	0.343***	0.239*			
RH.7	0.535***	0.481***	0.564***	0.333***	0.320***	0.298**			
AverT	− 0.221*	− 0.274**		− 0.427***	0.355***	− 0.373***			
AverT.1	− 0.316***	− 0.340***		− 0.425***	− 0.366***	− 0.414***			
AverT.2	− 0.392***	− 0.410***	− 0.214*	− 0.413***	− 0.338***	− 0.410***			− 0.173
AverT.3	− 0.418***	− 0.466***	− 0.301**	− 0.371***	− 0.382***	− 0.380***			
AverT.4	− 0.457***	− 0.462***	− 0.371***	− 0.397***	− 0.370***	− 0.406***			
AverT.5	− 0.496***	− 0.510***	− 0.416***	− 0.422***	− 0.371***	− 0.437***			
AverT.6	− 0.546***	− 0.595***	− 0.437***	− 0.484***	− 0.369***	− 0.458***			
AverT.7	− 0.575***	− 0.594***	− 0.455***	− 0.445***	− 0.355***	− 0.503***			
MaxT	− 0.265**	− 0.317***	− 0.191	− 0.427***	− 0.358***	− 0.345***			
MaxT.1	− 0.374***	− 0.369***	− 0.191	− 0.429***	− 0.374***	− 0.406***			
MaxT.2	− 0.481***	− 0.440***	− 0.249*	− 0.423***	− 0.324***	− 0.402***			− 0.167
MaxT.3	− 0.501***	− 0.507***	− 0.344***	− 0.362***	− 0.354***	− 0.388***			
MaxT.4	− 0.500***	− 0.490***	− 0.399***	− 0.393***	− 0.355***	− 0.396***			
MaxT.5	− 0.510***	− 0.503***	− 0.443***	− 0.402***	− 0.359***	− 0.420***			
MaxT.6	− 0.563***	− 0.589***	− 0.495***	− 0.474***	− 0.351***	− 0.432***			
MaxT.7	− 0.567***	− 0.583***	− 0.503***	− 0.436***	− 0.345***	− 0.478***			
MinT	− 0.186	− 0.192		− 0.430***	− 0.360***	− 0.353***			
MinT.1	− 0.200*	− 0.256*	− 0.128**	− 0.365***	− 0.335***	− 0.373***			
MinT.2	− 0.202*	− 0.291**	− 0.216*	− 0.313***	− 0.390***	− 0.348***			

Table 3 continued

	Cenlle			Montilla			Rzeszow		
	<i>B.cinerea</i>	<i>P. viticola</i>	<i>E. necator</i>	<i>B.cinerea</i>	<i>P. viticola</i>	<i>E. necator</i>	<i>B.cinerea</i>	<i>P. viticola</i>	<i>E. necator</i>
MinT.3	− 0.226*	− 0.340***	− 0.258	− 0.344***	− 0.387***	− 0.370***			
MinT.4	− 0.317**	− 0.373***	− 0.336	− 0.381***	− 0.379***	− 0.441***			
MinT.5	− 0.405***	− 0.457***		− 0.360***	− 0.382***	− 0.492***			
MinT.6	− 0.419***	− 0.492***		− 0.384***	− 0.413***	− 0.505***			
MinT.7	− 0.468***	− 0.482***		− 0.365***	− 0.386***	− 0.571***			
WV.5							0.179		− 0.242**

DP: Dew Point, Rf: Rainfall, RH: Relative Humidity, AverT: Average temperature, MaxT: maximum temperature, MinT: Minimum temperature, WV: wind velocity; Arabic numbers mean previous days. (Signif. codes: 0 ***0.001, **0.01, *0.05 ‘.’ 0.1 ‘ ‘ 1)

Table 4 Variance inflation factors (Vif) or meteorological parameters after reduction

	Cenlle	Montilla	Rzeszów
DP.3	1.219	1.394	
LRf	1.073	1.131	
LRf.1	1.094		
LRf.5			1.099
LRf.6			1.053
LRf.7	1.065	1.153	
Rf.6	1.224	1.363	
Rf.7			1.056
RH	1.242	1.333	1.068
RH.3			1.093
RH.4			1.097
Tmin.7	1.294	1.602	
VV.5			1.171
VV.6			1.271

DP: dew point, LRf: Light rainfall, Rf: rainfall, RH: relative humidity, MinT: minimum temperature, VV: wind velocity, numbers: days before

and relative humidity (60%). Finally, in the South Spain area, the maximum daily peak was preceded by several days with rainfall, temperatures around 20 °C and relative humidity of 55%. The low values registered for this last parameter limited the infection in the South Spain vineyards compared with the other studied viticultural areas.

Table 5 Redundancy analysis (RDA) Eigenvalues in the three study areas (Cenlle, Montilla and Rzeszów)

	RDA1	RDA2
Cenlle		
Eigenvalue	0.690	0.139
Proportion Explained	0.817	0.165
Cumulative Proportion	0.817	0.982
General scaling constant of scores	4.486	
Montilla		
Eigenvalue	0.925	0.098
Proportion Explained	0.867	0.092
Cumulative Proportion	0.867	0.960
General scaling constant of scores	4.389	
Rzeszów		
Eigenvalue	0.290	0.150
Proportion Explained	0.638	0.331
Cumulative Proportion	0.638	0.970
General scaling constant of scores	4.584	

Finally, the *P. viticola* concentrations were higher in the North Spain vineyard and lower in South Spain. Several authors pointed out in previous studies lower concentrations for North-western vineyards (Fernández-Gonzalez et al. 2009, 2019). The main source of *P. viticola* primary infection was overwinter oospores (Salinari et al. 2006; Gessler et al. 2011; Carisse 2016). The primary infection occurs at the beginning of spring, but the most important disease incidence

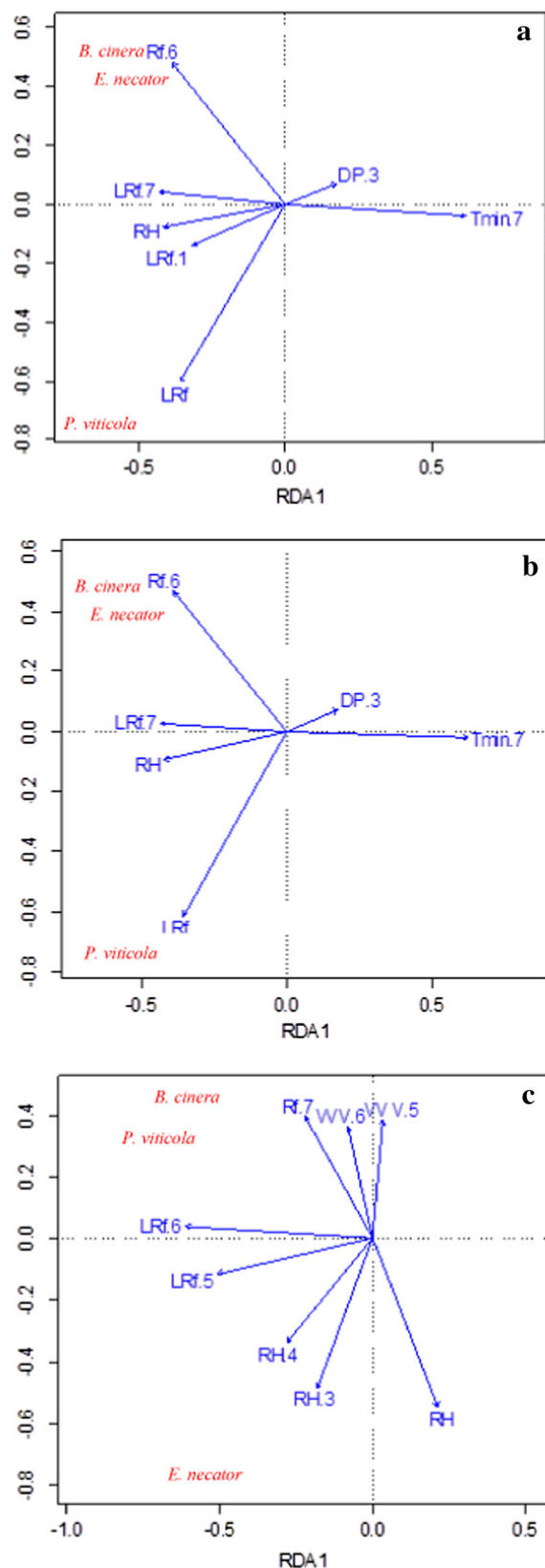


Fig. 6 Redundancy analysis (RDA) between three spore concentrations and the main meteorological parameters (DP: dew point, LRI: Light rainfall, Rf: rainfall, RH: relative humidity, Tmin: minimum temperature, WV: wind velocity, numbers: days before) in the three study areas (**a** Cénlle, **b** Montilla and **c** Rzeszów)

Table 6 Analysis of variance (ANOVA) between RDA1 and RDA2 in each study area (Cénlle, Montilla and Rzeszów)

	Df	Variance	F	Pr(> F)
Cénlle				
RDA1	1	0.690	40.7791	0.001***
RDA2	1	0.139	8.2492	0.055
Residual	130	2.200		
Montilla				
RDA1	1	0.925	87.9959	0.001***
RDA2	1	0.098	9.3926	0.036*
Residual	142	1.493		
Rzeszów				
RDA1	1	0.290	15.9161	0.001***
RDA2	1	0.150	8.2596	0.036*
Residual	142	2.591		

was prompted by secondary infections (Gessler et al. 2011). The optimum conditions for oospores germination take place with temperatures above 10–12 °C and rainfall > 10 mm for 1–2 consecutive days (Laviola et al. 1986; Rossi et al. 2008). Secondary zoospores infections occur under temperatures ranging from 5 to 30 °C with an optimum 20–25 °C (Caffi et al. 2016) and 95–100% of relative humidity (Fernández-González et al. 2019). Our findings showed that in the three study areas the *P. viticola* peak coincided with continuous rainfall episodes, relative humidity values above 60% and average temperatures ranging between 18 and 22 °C, considered as optimum values for infection and disease development. These results accord with the pointed out in different studies which indicate that the primary *P. viticola* infections date ranged between early May to mid-June (Kennelly et al. 2007) since the primary infections depend on temperature and rainfall, while secondary infections are a function of temperature and humidity presence on leaves (Rosa et al. 1995).

As described before, temperature was the main factor affecting the spore concentration of the studied fungus, with optimum temperatures of infection between 15 and 25 °C depending on the spore type (Fernández-Gonzalez et al. 2009; Rodríguez-Rajo et al. 2010; Rossi et al. 2010). Nevertheless, the temperatures ranges were usually lower in Central Europe, and higher in South Spain than in North-western Spain. As a consequence, the CCA analysis has omitted this parameter for the RDA model. Even so, higher concentrations were found earlier in South Spain and Poland than in North Spain for *B. cinerea* and *P. viticola* due to the combination of rainy and high temperature days. The opposite occurred with *E. necator*, which registered the mean daily concentration peaks earlier in Northern Spain than in the other European areas.

In all cases, a strong relationship was detected between the spore concentration values and the water-related parameters, such as relative humidity, rainfall and dew point, as previously noted by other authors (Rossi et al. 2010; Holb and Füzi 2016). In the case of *P. viticola*, low rainfall is the most important factor for higher airborne spore concentration increases in the three viticulture areas, evidencing the effect of drops in leaves and fruits on the dispersion of the zoospores, and the sensibility to dryness that inhibit the secondary infection cycles (Kiefer et al. 2002; Rossi et al. 2013). In warm temperate climate (Poland), the importance of low rainfall was weak.

RDA analysis results reinforced the importance of rainfall in the spore concentration of *B. cinerea* and *E. necator*. This analysis also showed how minimum temperatures negatively affected on the spore concentration inhibiting sporulation (Rodríguez-Rajo et al. 2010; Rossi et al. 2010, 2013; Holb and Füzi 2016). RDA analysis revealed a similar spore behaviour for both Spanish vineyards, showing the importance of the selected parameters. The low number of rainy days detected for Central Europe within the studied period explained the different behaviour detected by the RDA analysis.

5 Conclusions

Biogeographical and bioclimatic conditions are important factors influencing spatial differences of fungal season behaviour in Europe. Fungal spore

season timing and intensity depend not only on the biogeographical regions (Temperate/Mediterranean) but also the type (oceanic/continental). Fungal spore spectra are less dependent on these factors. In all viticulture zones the most common spores in the atmosphere were as follows: *E. necator*, *B. cinerea* and *P. viticola*. The most important meteorological factors are temperature and the water-related parameters, mainly in previous days. The possibility of predicting the risk of infection plants by pathogenic fungi, even several days in advance, may allow an earlier and effective use of fungicides.

In the natural environment, relationships between variables are complicated, difficult to detect and describe by simple statistical methods. To detect them, more and more modern techniques are used. Multidimensional analyses are methods that reveals correlations between variables that are difficult to identify at first. The use of RDA analysis detects the relationship between the influential factors on spore concentration, providing additional information in comparison with the Spearman's correlation test. This analysis must be complemented by CCA/DCA as an instrument for parameter selection. The obtained results in this study show the need to continue with researching in distant vine-growing areas to better explain the mechanisms that favours the airborne spore concentrations increase.

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