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47 ANNUAL RECORDS OF ALLERGENIC FUNGI SPORE: PREDICTIVE MODELS FROM THE NW IBERIAN PENINSULA

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Abstract: An analysis was carried out of the atmospheric representivity of *Cladosporium* and *Alternaria* spores in the north-western Iberian Peninsula, registering mean annual concentrations in excess of 300,000 spores/m^3. During the main sporulation period, the highest average daily concentrations corresponded to *Cladosporium herbarum* type (1,197 spores/m^3) while the highest daily value was 7,556 spores/m^3 (*Cladosporium cladosporioides* type). *Alternaria* only represents between 0.1–1% of the total spores identified. In these spore types, the intraday variation was more acute inland than along the coastline due to oceanic influence. In the predictive models proposed that use the meteorological parameters with which a higher correlation was obtained (mean and maximum temperature) as predictive variables, it was seen that the predicted values did not reveal any significant differences as compared to those observed in 2006, data that was only used for verification purposes.

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Key words: ***Cladosporium*, *Alternaria*, regression, predictive models, Spain, allergenic spores.**

INTRODUCTION

Environmental mycology studies have been carried out practically worldwide, in many cases with the aim of discovering the clinical response of populations affected by allergic respiratory illness [16, 35]. In recent decades, the development of the Aerobiology Networks has made a major contribution towards obtaining a more thorough understanding of atmospheric levels of fungal spores in a large number of countries, in both inland [16, 17] and coastal areas [11, 18, 19, 33].

In Spain, this line of research began to be developed in the middle of the last century [10], and since then numerous contributions have been published in the countries different regions of the country [37, 38], highlighting some revisions of types such as *Cladosporium* and *Alternaria* which are the object of this study [21, 22].

In the north-western Iberian Peninsula, since 1993 the Galician Aerobiology Network has created a database on the atmospheric content of pollen and spores (*Cladosporium* and *Alternaria*). This database currently has 47 annual records from samples taken from different spore traps, some of which have been operating for more than 10 years.

Until now, these studies have mainly focused on environmental and agricultural issues [1, 3]; however, they have never been analysed as a whole, and information about their involvement in the production of allergies in this part of the peninsula is very limited, despite their important contribution to pathologies of this type [6]. For this reason, the summary of data included in this study is aimed at determining and evaluating the atmospheric presence of *Cladosporium* and *Alternaria* spores in the north-western Iberian Peninsula, and serving as a point of reference for a subsequent study of the prevalence of these fungi's allergens.

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Thanks to the large number of samples taken, prediction models could be designed, making it possible to precisely predict the atmospheric concentration of the spores of these fungi.

MATERIAL AND METHODS

The study area corresponds to the geographic region of Galicia in the north-western of the Iberian Peninsula, covering an area of 29,575 km^2. It has an oceanic climate, with a mean precipitation of 1,404.8 l/m^2 and a mean annual temperature of 14.4ºC. In biogeographic terms it belongs to the Eurosiberian region, except to the south-east, which forms part of the Mediterranean region [23].

The aerobiological samples, where the identification and counting of *Cladosporium* and *Alternaria* spores was carried out, come from Hirst-type volumetric spore traps (Lanzoni VPPS-2000 model), installed in the centres of Santiago, Coruña, Viveiro, Lugo, Vigo, Ourense, Verin and Trives, with their distribution corresponding to the biogeographical diversity of the region.

The treatment of the samples was carried out using the methodology proposed by the Spanish Aerobiology Network [14]. The criteria of Mediavilla *et al*. [26] has been considered to group the conidia of *Cladosporium* Link ex Fr., according to their morphological features, in 2 types that fundamentally correspond to the most common species in our city. The *cladosporioides* type include species with smooth walled conidia, hyaline to olive coloured, and with a diameter between 3(15) × 2(6) µm. The *herbarum* type include species with thick walled and verrucose conidia, dark olive to brown coloured, and with a diameter between 8(25) × 4(8) µm.

In order to verify the period of the year with the highest spore concentration, the Main Sporulation Period (MSP) was defined, applying the criteria of Nilsson & Persson [30]. This data was used to analyse the intraday behaviour [15] and the correlation of the main meteorological parameters, applying Spearman's non-parametric test using the Statistica programme, and establishing 3 levels of significance ($p<0.1$; $p<0.05$; $p<0.01$).

In order to obtain a model suitable for making predictions, a multiple regression analysis was carried out, using the daily values of the meteorological parameters with which the highest correlation coefficients were obtained as predictive values.

RESULTS

In quantitative terms, the spores of *Cladosporium* and *Alternaria* have their highest presence in the most continental centres (Verín and Trives), reaching values close to 300,000 spores as an average yearly value for the whole of the study period (Fig. 1). On the contrary, the coastal cities of Coruña and Vigo have the lowest levels, with the rest in an intermediate position.

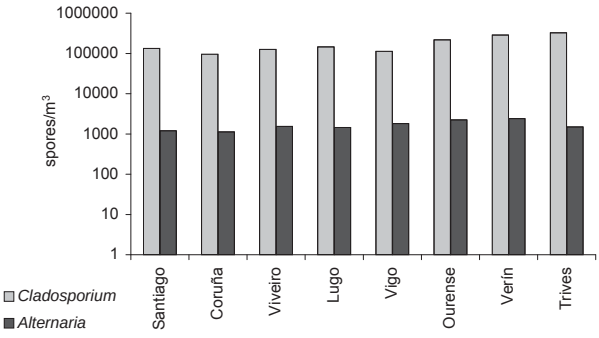

Figure 1. Average values for yearly total of *Cladosporium* and *Alternaria* in the eight centres.

Table 1. Mean data for Main Sporulation Period of *C. cladosporioides*, *C. herbarum* and *Alternaria* (MSP-start: Date on which the MSP starts; MSP-end: Final date of the MSP; MSP-days: Number of days included in the MSP; MSP-mean: Average total spore value during the MSP).

	MSP – start	MSP – end	MSP – days	MSP – mean
Cladosporium cladosporioides type				
Santiago	May, 10	October, 8	152	496
Coruña	April, 20	November, 1	195	343
Viveiro	April, 18	October, 30	195	472
Lugo	April, 22	October, 18	180	334
Vigo	March, 30	October, 25	210	315
Ourense	April, 17	October, 21	189	592
Verín	February, 22	March, 29	258	369
Trives	March, 7	June, 8	229	393
Cladosporium herbarum type				
Santiago	May, 8	September, 23	139	267
Coruña	April, 23	October, 11	172	134
Viveiro	April, 14	October, 27	197	156
Lugo	May, 5	September, 28	147	541
Vigo	April, 4	October, 3	179	263
Ourense	April, 19	October, 9	174	549
Verín	March, 7	October, 2	210	825
Trives	April, 3	October, 4	185	1.197
Alternaria				
Santiago	May, 14	October, 16	154	7
Coruña	May, 22	October, 22	154	7
Viveiro	May, 18	September, 26	126	13
Lugo	May, 20	October, 1	135	11
Vigo	April, 26	October, 23	180	10
Ourense	April, 21	October, 17	183	13
Verín	May, 7	October, 25	172	14
Trives	April, 16	October, 23	193	7

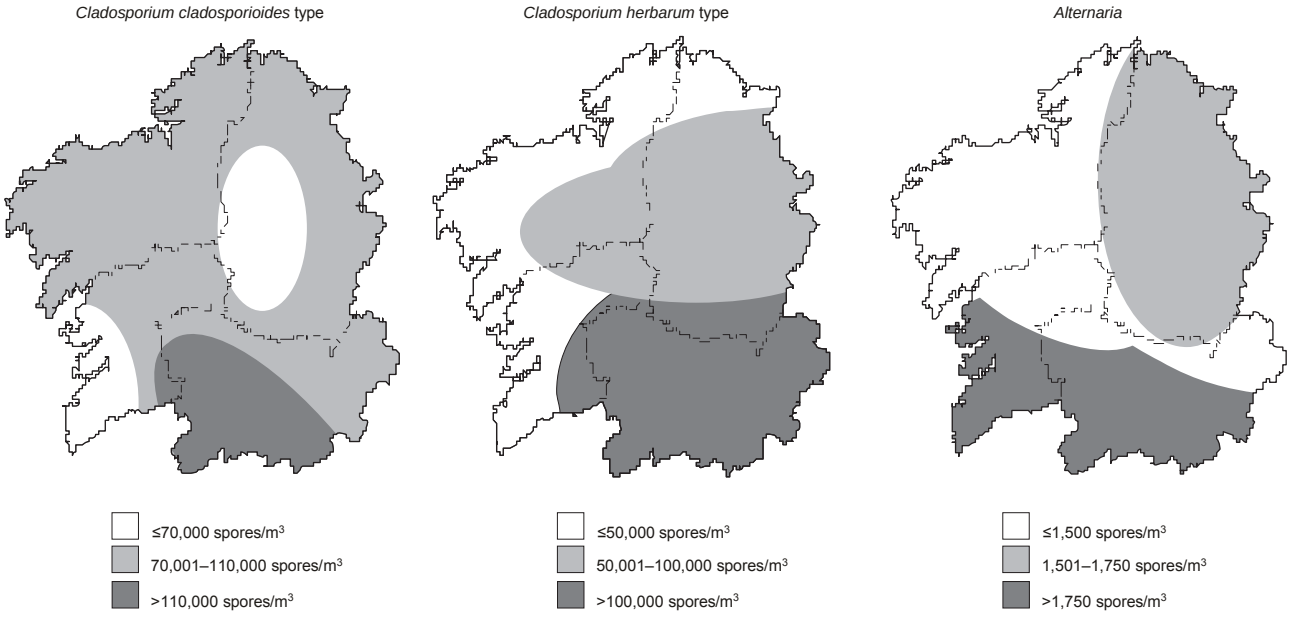

Figure 2. Spatial distribution of spores of *C. cladosporioides* type, *C. herbarum* type and *Alternaria* in north-west Spain according concentrations reached.

Independently from the location of the spore trap, a higher proportion of *Cladosporium* spores was found in relation to those of *Alternaria*, as this type only represents between 0.1–1% of the total number of spores identified.

Figure 2 shows the spatial distribution of the 3 types of spores, with *Cladosporium cladosporioides* type the most abundant in the western half of the region, and those of *Cladosporium herbarum* type predominant in the east.

In terms of temporal distribution, the highest average monthly value for *Cladosporium cladosporioides* spores type, taking into account all the yearly records from all of the spore traps, was in the month of September, with secondary maximums in July and August, while the highest value for *Cladosporium herbarum* type and *Alternaria* was in July (Fig. 3).

Taking into account the data from each yearly record from the whole of the study period, the monthly maximums for *Cladosporium cladosporioides* type varied between 23,739 spores recorded in September 2001 in A Coruña, and 70,934 spores in September 2002 in Ourense. In the case of *Cladosporium herbarum* type the interval was of 7,721 spores (July 2001 in Coruña) and 86,891 spores (July 2003 in Trives), and for *Alternaria* between 355 spores in August 2002 in Coruña, and 1,941 spores in July 1997 in Santiago.

The Main Sporulation Period (MSP) of *C. cladosporioides* type occurred in most of the centres between mid-April and mid-October; its duration varied between 152 days (Santiago) and 258 days (Verín), with its mean concentration in this period varying between 315 spores/m^3 (Vigo) and 592 spores/m^3 in Ourense. The MSP of *C. herbarum* type started in April or May and ended at the end of September or in October; its duration varied between 139 days

Table 2. Maximum mean daily concentration of spores of *C. cladosporioides*, *C. herbarum* and *Alternaria* and date on which they occur in the eight centres during the studied period.

	C. cladosporioides type		*C. herbarum* type		*Alternaria*	
	Maximum	Day	Maximum	Day	Maximum	Day
Santiago	5,951	1/7/01	4,074	10/7/99	653	9/7/97
Coruña	3,944	24/8/02	1,609	11/7/03	114	25/7/01
Viveiro	3,647	11/7/03	3,694	11/7/03	449	31/7/01
Lugo	4,510	9/7/01	4,237	6/7/01	87	26/7/03
Vigo	5,678	15/9/02	6,472	18/8/01	370	11/7/99
Ourense	7,556	8/9/04	7,071	24/5/95	332	27/7/99
Verín	6,694	21/1/02	6,157	10/7/02	149	10/7/02
Trives	4,756	12/9/02	7,130	8/7/03	113	17/5/02

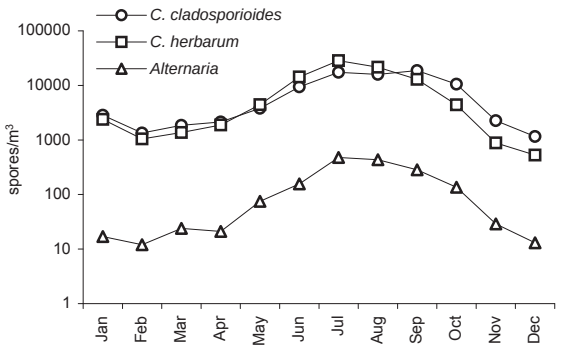

Figure 3. Average monthly values of *C. cladosporioides*, *C. herbarum* and *Alternaria* spores calculated with the data of all monitoring stations.

(Santiago) and 210 days (Verín), and its mean concentration between 134 spores/m³ (Coruña) and 1197 spores/m³ in Trives. In the case of *Alternaria*, its MSP began between mid-April and mid-May, and ended in practically all of the spore traps in October; its duration varied between 135 days (Lugo) and 193 days (Trives) and its concentration between 7 spores/m³ in various locations, and 14 spores/m³ in Verín (Tab. 1).

The maximum mean daily concentrations of *Cladosporium cladosporioides* type were recorded in Ourense (7,556 spores/m³) and Verín (6,694 spores/m³) in the months of September and January respectively: those of *Cladosporium herbarum* type in Trives (7,130 spores/m³) and Ourense (7,071 spores/m³) in July and May, and those of *Alternaria* in Santiago (653 spores/m³) and Viveiro (449 spores/m³) in July in both cases (Tab. 2).

With regard to the intraday distribution of *Cladosporium cladosporioides* spores type as well as those of the *Cladosporium herbarum* type, 3 models were presented, which are repeated in different centres. In Santiago and Lugo the levels are lower during the early morning and at sunrise, increasing progressively from 10–11 a.m. onwards, either gradually (*C. cladosporioides* type) or suddenly (*C. herbarum* type), although in any case the maximum concentrations were obtained in the first few hours of the afternoon, progressively decreasing towards nightfall (Fig. 4). This same tendency is seen in both types of spores in Ourense, Verín and Trives, although the levels started to increase from midday, reaching their maximum levels around 8-9 p.m. However, in Coruña, Viveiro and Vigo the variations throughout the day are much less abrupt (Fig. 4).

With regard to *Alternaria*, the variation model indicating the highest spore levels during the afternoon was repeated in practically all of the centres, while in coastal centres there is a smaller variation in concentrations throughout the day (Fig. 5).

On performing the correlation analysis between the spore concentration and meteorological variables taken as

Table 3. Values for Spearman's Correlation Coefficient between spore concentrations and main meteorological variables.

	Galicia	Santiago	Coruña	Viveiro	Lugo	Vigo	Ourense	Verín	Trives
					Cladosporium cladosporioides type				
Rainfall	-0.109***	-0.137***	-0.141***	-0.091**	-0.098***	-0.179***	-0.043*	ns	ns
Humidity	-0.074***	-0.074***	0.125***	0.153***	-0.053*	-0.192***	ns	ns	ns
Max. temp.	0.376***	0.290***	0.577***	0.245***	0.281***	0.515***	0.313***	0.165***	0.322***
Min. temp.	0.339***	0.311***	0.531***	0.272***	0.356***	0.518***	0.334***	0.287***	0.401***
Mean temp.	0.403***	0.336***	0.586***	0.288***	0.348***	0.541***	0.357***	0.220***	0.377***
					Cladosporium herbarum type				
Rainfall	-0.240***	-0.290***	-0.155***	-0.165***	-0.176***	-0.368***	-0.172***	-0.158***	-0.208***
Humidity	-0.217***	-0.138***	ns	0.122***	-0.157***	-0.294***	-0.145***	-0.139***	-0.184***
Max. temp.	0.503***	0.440***	0.542***	0.341***	0.366***	0.619***	0.403***	0.487***	0.511***
Min. temp.	0.265***	0.414***	0.474***	0.355***	0.330***	0.559***	0.409***	0.526***	0.521***
Mean temp.	0.454***	0.479***	0.540***	0.386***	0.397***	0.630***	0.459***	0.523***	0.549***
					Alternaria				
Rainfall	-0.314***	-0.291***	-0.310***	-0.187***	-0.369***	-0.395***	-0.280***	-0.253***	-0.259***
Humidity	-0.208***	-0.165***	ns	0.093*	-0.220***	-0.253***	-0.238***	-0.150***	-0.139***
Max. temp.	0.429***	0.471***	0.529***	0.390***	0.063*	0.548***	0.458***	0.295***	0.330***
Min. temp.	0.252***	0.384***	0.436***	0.402***	-0.071*	0.405***	0.319***	0.242***	0.295***
Mean temp.	0.411***	0.485***	0.530***	0.469***	ns	0.520***	0.455***	0.295***	0.342***

Levels of significance: (ns – not significant p>0.1; *p<0.1; **p<0.05; ***p<0.01).

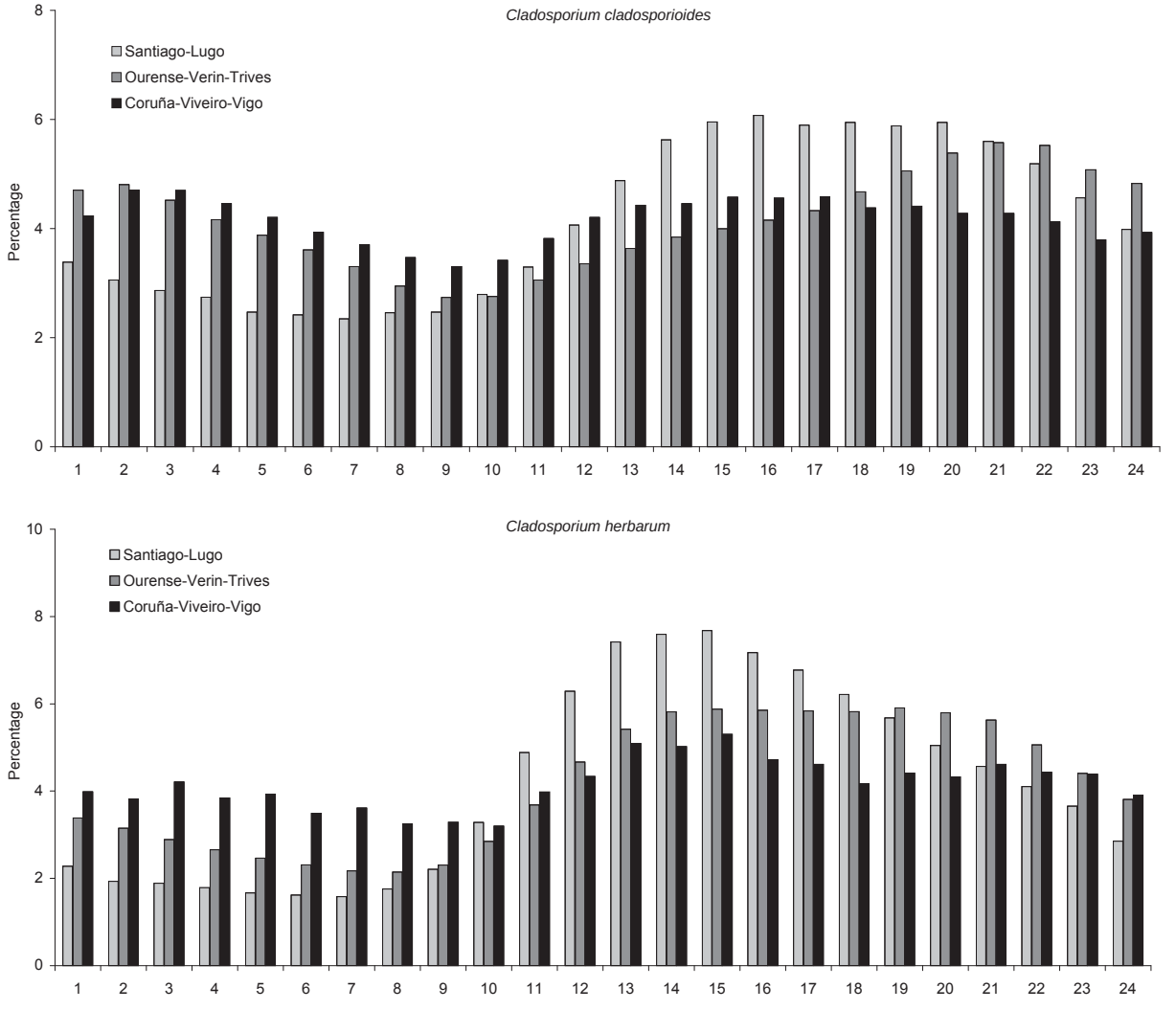

Figure 4. Intraday variation models for *C. cladosporioides* and *C. herbarum* in different zones of Galicia (Inland South areas: Santiago, Lugo; Inland North areas: Santiago, Lugo; Coastal areas: Coruña, Viveiro, Vigo).

a whole (the days included in the MSP of all the years and seasons) (Tab. 3), it was observed that the variables with the highest correlation coefficients were mean temperature for spores of *C. cladosporioides* type and maximum temperature for those of *C. herbarum* type and *Alternaria*, with a high level of confidence in all cases ($p<0.01$).

In the statistical analysis applied to each centre, the same results were maintained, with the exception of the centres of Lugo, Verín and Trives, where the minimum temperature was the variable with highest correlation coefficients more for spores of *C. cladosporioides* type, the same as in Verín for those of *C. herbarum* type, which were positive in all cases, and in Lugo for spores of *Alternaria*, where the negative influence of rainfall over the rest of the variables was an important feature. Regardless of whether the data is analysed jointly or individually, the influence of rainfall was always negative on atmospheric spore concentration, with humidity negative in most cases, and temperature always positive.

In the case of cities with data from a larger number of years (Santiago, Ourense and Vigo), multiple linear regression models were designed which included as predictive variables those which in each case have reached the highest correlation coefficients and significance. Also, the concentrations of spores from the previous day were included in order to improve the predictive capacity.

The models obtained were significant, and the percentage variance explained of the spores was high in all cases, with 51.8% for *C. herbarum* type, 48% for *C. cladosporioides* type and 34.5% for *Alternaria* (Tab. 4).

Verification of the model was carried out using the data from 2006 that was not included in designing the model. The representation of the spore values observed for 2006 and the estimated values are in line with each other; however, in order to verify the presence or absence of significant differences between the data predicted for the models for 2006 (line) and those actually obtained in the recounts (area), the parallel samples test was applied (Tab. 5).

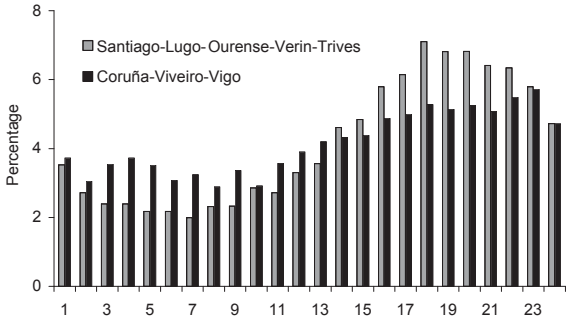

Figure 5. Intraday variation models for *Alternaria* in different zones of Galicia (Inland areas: Santiago, Lugo, Ourense, Verín, Trives; Coastal areas: Coruña, Viveiro, Vigo).

The results obtained (Fig. 6) indicate the presence of significant differences in the case of *Alternaria* ($p<0.014$), while no highly significant differences were found in *C. cladosporioides* type ($p<0.041$), or significant differences in *C. herbarum* type ($p<0.542$).

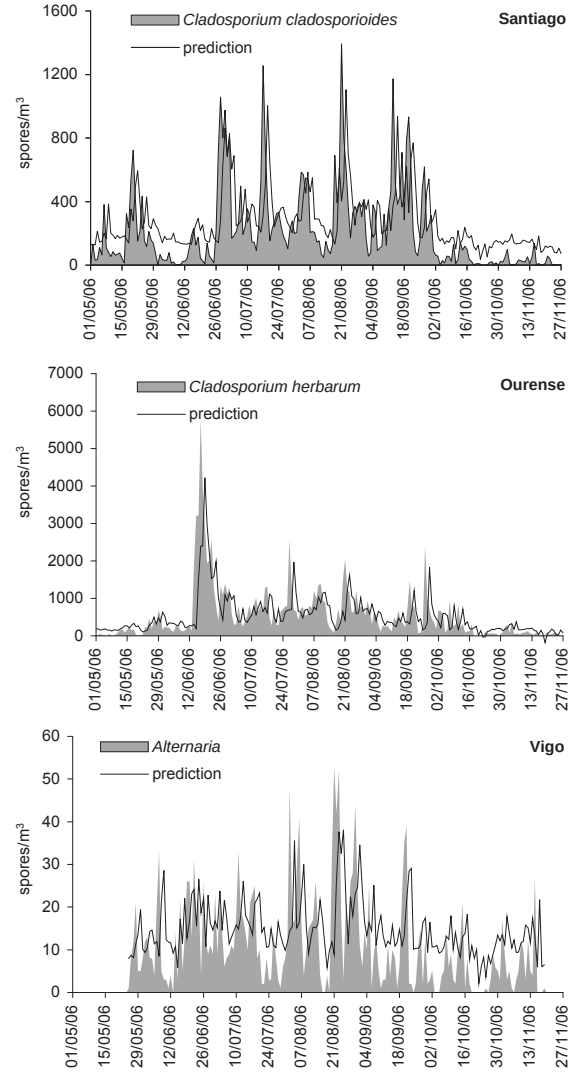

Figure 6. Examples of validation of prediction models by using data from 2006 not included in designing the model. Representation of the spore values observed for 2006 in areas, the predicted values in lines.

Table 4. Multiple regression models proposed for predicting concentrations of different types of spores.

	BETA	SE	B	SE	p
Cladosporium cladosporioides type					
Intercpt			97.335	8.544	0.000
C. cladosporioides-1	0.689	0.008	0.691	0.008	0.000
Mean temperature	0.048	0.011	1.847	0.431	0.000
Rainfall	-0.038	0.011	-1.983	0.580	0.001
SE – standard error; R = 0.693, R^2 = 0.481, adjusted R^2 = 0.480, F(3.770) = 2376.5, p<0.000, Estimated SE: 403.88					
Cladosporium herbarum type					
Intercpt			41.061	11.098	0.000
C. herbarum-1	0.699	0.009	0.700	0.009	0.000
Max. temp.	0.107	0.011	4.181	0.447	0.000
Rainfall	-0.100	0.011	-5.561	0.633	0.000
R = 0.720, R^2 = 0.518, adjusted R^2 = 0.518, F(3.684) = 2450.0, p<0.000, Estimated SE: 453.58					
Alternaria					
Intercpt			1.913	0.370	0.000
Alternaria-1	0.572	0.010	0.567	0.010	0.000
Max. temperature	0.108	0.015	0.105	0.014	0.000
Rainfall	-0.125	0.015	-0.165	0.019	0.000
R = 0.588, R^2 = 0.345, adjusted R^2 = 0.345, F(3.661) = 1163.0, p<0.000, Estimated SE: 15.334					

DISCUSSION

Amongst the factors that influence quantitative variations of fungal spores in bioaerosol in difference centres, the most relevant are fungal sources and climatic differences [9]. This study has shown that these aerobiological particles are more abundant in inland than coastal areas, related to the presence of larger extensions of substrate available for fungal development, and higher temperatures during the main sporulation period.

In some European countries, *Cladosporium* may reach values of 700,000 spores/year, while *Alternaria* varies between 20,000–30,000 spores/year [29]; however, in the Iberian Peninsula the annual representation of both types varies from close to 70,000 spores/year [32, 38] to more than 200,000 [37], only exceeding the levels of 300,000 spores quoted for the north-western Iberian Peninsula in some areas [26]. On the contrary, the levels of *Alternaria* spores, with maximum mean annual levels of 2417 spores as indicated in this study, are clearly surpassed by those from other centres, in excess of 15,000 spores [34].

As in other parts of Europe, the concentration of spores in the north-western Iberian Peninsula increases during the summer. In several Italian cities, high quantities of *Cladosporium* and *Alternaria* are found from May to October, reaching their maximum levels in September [41]. However, in areas at lower latitudes where precipitation and humidity are limiting factors, but not temperature, the spores increase in the months before and after summer [25].

Table 5. Parallel samples test between observed and predicted spores values calculated by means of data for 2006 not included in designing the model.

	Mean	Std. Dev.	N	t	df	p
Cladosporium cladosporioides type						
2006	281	268,991				
Predicted	324	187,131	153	-2,064	152,000	0.041
Cladosporium herbarum type						
2006	559	734,594				
Predicted	535	530,678	207	0,610	206,000	0.542
Alternaria						
2006	14	11,779				
Predicted	17	6,578	122	-2,503	121,000	0.014

Also, the intraday variation models obtained in this study found similarities in other parts of the Iberian Peninsula, as both types have a higher concentration in the afternoon, while in some coastal cities the atmospheric presence of *C. cladosporioides* type, *C. herbarum* type and *Alternaria* is much more homogenous and practically without variations throughout the day [13, 37].

Based on the study of the influence of meteorological factors on the concentration of these spore types in the air, some authors have concluded that in order for them to be liberated into the atmosphere, a certain degree of dryness is required in the atmosphere, which occurs when the temperature rises [28, 31], in line with the results found in this study. In general terms, the concentration of all atmospheric particles is reduced during heavy rainfall, while the rainfall recorded in periods prior to emission considerably increases their concentration, from which it is possible to deduce its positive effect on sporulation, as cited in different geographical areas [2, 5, 36, 40].

The presence of spores in the outside air is related to climatic factors, making it possible to design predictive models for spore concentrations in centres with an extensive database. Different authors have indicated mean temperature as the most influential meteorological factor on these 3 fungal types, meaning that it is the most suitable parameter for designing predictive models of atmospheric concentrations of both *Cladosporium* [27] and *Alternaria* [12], although Angulo *et al*. [4] obtained the best results when they used the mean temperature accumulated over one week. In Tulsa (USA), researchers also indicated temperature as being the most consistent meteorological variable for the prediction of high spore concentrations in the atmosphere [8, 39]. Different Italian cities obtain the best results when they include the mean temperature, maximum temperature and relative humidity as predictive variables [5]. In the case of *Cladosporium*, Damialis & Gioulekas [12], obtained the best results when they used solar radiation in the daily prediction models. Burge [9] indicates temperature and dew point.

In the models proposed in this study, rainfall and maximum temperature, in the case of *Cladosporium herbarum* type and *Alternaria*, or rainfall and mean temperature, in the case of *Cladosporium cladosporioides* type, proved to have the best predictive capacity. However, the percentage variance explained increases considerably when the concentration of spores from the previous day is introduced into the model. It may be considered that this variable reflects the series of conditions that the concentration of spores in the air depends on, both for their formation, release and transportation. The correlation between data recorded on consecutive days is known and frequently used for designing models that use statistical techniques based on autoregression [12].

Despite the fact that the representation of the spore values observed for 2006 and the estimated values are in line with each other, the t-Test for the dependent samples performed to discover significant variations between them indicates the worthiness of the model in the case of *Cladosporium*; however, the model obtained for *Alternaria* does not reveal any statistically significant predictive capacity.

One of the most widely-used systems for measuring allergic response to fungi are prevalence studies, using the skin prick test. One recent study performed in the European Union using this system indicated that 1.7% are positive for *Cladosporium* and 3.3% for *Alternaria* [7]. However, these figures increase considerably in Turkey, with 8.1% allergic to *Cladosporium* and 11.9% to *Alternaria* [6], and in Saudi Arabia the figure for the latter type rises to 21.6% [20]. In Spain, fungi represent the third major cause of allergic respiratory illness, with *Alternaria* as the most frequency involved kind [24].

Although the allergic response of a given population, in this case to fungal allergens, varies depending on the characteristics of the population (age, sex, health condition, etc.), the level of exposure to the antigen is one of the variables that must be taken into account. In this respect, in the north-western Iberian Peninsula, the atmospheric concentrations of *Cladosporium* and *Alternaria* spores, as concluded in this study, are important and similar to those from other geographic areas with a high presence of allergic illnesses, meaning that it is likely that the importance of fungi in the north-western Iberian Peninsula has not been fully appreciated until now.

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