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METEOROLOGICAL AND AGRICULTURAL EFFECTS ON AIRBORNE *ALTERNARIA* AND *CLADOSPORIUM* SPORES AND CLINICAL ASPECTS IN VALLADOLID (SPAIN)

Estefanía Sánchez Reyes[1], David Rodríguez de la Cruz[1], Mª Eugenia Sanchís Merino[2], José Sánchez Sánchez[1]

[1]Hispano-Luso Agrarian Research Center (C.I.A.L.E.), University of Salamanca, Salamanca, Spain
[2]Immunoallergy Service of Río Hortega University Hospital of Valladolid, Valladolid, Spain

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Abstract: The aeropalynological monitoring was carried out from 1 February 2005–31 January 2007. The total number of spores collected during the main spore season (MSS) in 2005 was 4,500 for *Alternaria* and 93,744 in the case of *Cladosporium*, whereas in 2006 values were increased (8,385 for *Alternaria* and 150,144 for *Cladosporium*), reaching the maximum concentrations on 18 July and 17 June 2006 with 344 and 5,503 spores, respectively. The influence of the main meteorological parameters on spore concentrations was studied, resulting in a positive correlation with temperature. Rainfall, relative humidity and frequency of calms obtained negative correlations in the case of *Alternaria*, and positive for *Cladosporium*, the total daily hours of sunshine having an inverse influence on them. The intra-diurnal pattern was very similar for both genera, with a greater representation towards the central hours of the day and at night. Finally, some clinical aspects for the *Alternaria* spore type were analyzed, with a low percentage of sensitized patients though (9.5%). Only one patient showed positive skin test reaction to *Cladosporium*.

Address for correspondence: MSc Estefanía Sánchez Reyes, Hispano-Luso Agrarian Research Center (C.I.A.L.E.), University of Salamanca, Campus de Villamayor, C/Río Duero No. 12 37185, Villamayor, Spain. E-mail: fani_sanchez@usal.es

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INTRODUCTION

A large amount of fungal spores are present in the atmosphere of many areas around the world although qualitative and quantitative differences can be noted, according to the time of day, the season, meteorology and geographical area in study [28]. In this respect, Hasnain [29] claimed that the knowledge of relations between airspora and climate variables is of vital importance to prevent respiratory allergic diseases. Moreover, since vegetables are the main substrate on which fungi develop their life cycles, any change in plant ecology could have an effect on the airborne spore concentrations [12].

This paper presents the first aeromycota data recorded for Valladolid city based on the identification of their morphological characteristics under an optic microscope. However, in this report, only results obtained for *Alternaria* (extremely common and widespread saprobious on cereal crops) and *Cladosporium* (a dematiaceous mould widely distributed in air and rotten organic material), are presented. The election of these two taxa for this paper relies on the fact that they are the most abundant fungal particles, both ubiquitous, of phytopathogenic and clinical interest, due to its allergenic potential [7].

The presence of pollen and fungal spores may be responsible for the inflammatory process of the upper and lower respiratory tract developed during respiratory allergy disease [24]. In fact, Nyholm *et al.* [44] claimed that Alt-1 was the major allergen of *Alternaria*. On the other hand, as a consequence to the western lifestyle [55], the number

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of inhabitants of developed countries suffering from allergic rhinitis caused by fungi is increasing according to the World Health Organization. Moreover, 15–20% of world population suffers from rhinoconjunctivitis and/or asthma, most of them as a response to fungi spore exposition [6], and it is known that *Alternaria* is a risk factor for triggering asthma in both children and adults [9, 45]. Thus, a threshold level of 50 *Alternaria* spores per cubic metre of air was suggested by Frankland and Davies [20], but other authors have increased this level up to 100 spores or more per cubic metre [26]. More recently, Caretta [7] proposed even higher threshold values up to 1,200 *Alternaria* spores/m^3 and 3,000 spores/m^3 for *Cladosporium*.

According to the Spanish Ministry of Agriculture, cereal is the chief crop in Valladolid 33–50% of the total province area. As *Alternaria* is a common decay fungus which decomposes organic matter, usually abundant in crop fields, this led to the supposition that there is a great abundance of this spore type in the airborne of the capital city. Some researchers [32] have also identified wheat fields as a risk factor for the development of asthma symptoms and this cereal has an important representation on the arable lands of Valladolid.

On other hand, *Cladosporium* can release billions of spores in a day, and is an important aeroallergen associated with asthma, bronchitis, interstitial fibrosis and hypersensitivity pneumonitis [49], mainly in the North of Europe where it is more abundant. As occurred with *Alternaria*, the proximity of open country, especially crop fields, is closely related to greater concentrations of this fungus [21].

The aim of this paper is to determine the airborne concentration of these two genera in Valladolid, and the influence of meteorological parameters on their atmospheric behaviour. In addition, knowledge of the daily and seasonal character is important in order to establish the main season of acute sensitivity in which patients could take personal measures to prevent the development of their symptoms.

MATERIAL AND METHODS

Valladolid (population 316,564) is the largest city in the Castilla-León region, located in the middle of Spain at a height of 691 m above sea level (41º 39´N, 4º 44´O), surrounded by arable farming lands. From a biogeographical point of view, it is included in the Mediterranean region [50]. According to a 30 year average (1971–2000) the climate is continental, characterized by a small annual rainfall level (435 mm) which determines a dry season during the summer period. The annual mean temperature corresponds to 12.3ºC with a mean temperature of 21.7ºC during the hottest month. The annual average of fog (42) and chilly days (61) is quite important, although with little snow (8) due to its particular geographic situation.

The aeropalynological monitoring was carried out from 1 February 2005–31 January 2007. Spore sampling was performed using a Hirst-type trap, Burkard model, placed on the roof of the Río Hortega University Hospital of Valladolid at a height of 24 m above ground level. The methodology used was the one suggested by the Spanish Aerobiology Network (REA) with regard to the pollen grain and spore count and to samples processing [15, 22]. Not only were different keys used for the identification of the moulds, but also aeropalynological and morphology works [25, 37, 53].

An *Alternaria* and *Cladosporium* 5-day running mean and monthly total concentrations were calculated for both annual periods and plotted to assess the seasonal trend. In order to establish the main spore season (MSS) we used the method that defines the start of the season as the date by which the 5% of the total year spore record was registered, and the end with 95% trapped [43]. The prepeak (PRE) includes the period between the start of the MSS and the date on which the highest concentration was reached.

We used the SPSS programme, applying the Spearman non-parametric correlation test in order to establish the influence of the main meteorological factors (temperatures, precipitation, relative humidity, wind speed, winds from the first, second, third and the fourth quadrant, frequency of calms, and total hours of sunshine) on the daily spore concentrations during the main spore season (MSS) and prepeak period (PRE). The meteorological parameters values were provided by the Institute of Meteorology and were obtained from a station located 5 km from the trap site.

We also studied the intra-diurnal variations taking into account three different models described by some authors [1]. The first model calculates the value for each hour represented by the sum of the values corresponding to that hour, while in the second model an ideal day was obtained, dividing this sum of values of each hour by the number of days on which the fungus analyzed was present. In both cases, the values for each hour are expressed as a percentage of the daily total.

In the two models described above all the data recorded was used, in the third model it was important whether or not rainfall was registered. The daily average of the two genera was calculated using the total number of days of the MSS period as the denominator. After that, we selected just the dry days with a daily value equal or higher than the average previously calculated. The value obtained for each hour, represented by the sum of the values corresponding to that hour and expressed as a percentage of the daily total during those selected days, could make possible the comparison with the first and the second models. For the graphical representation we used a 3-hour running mean to smooth the tendency.

For the epidemiological research we examined the clinical history of 767 patients in order to obtain information about different parameters such as age, gender, symptomatology developed, allergy season and diagnostic conclusion. The skin test was performed using intracutaneous injections on the volar surface of the forearm, applying commercial (Alk Abelló) preparations of *Alternaria*

alternata (25 µgr/ml) and *Cladosporium herbarum* (1:20 w/v). Histamine was used as a positive control. The reactions were considered positive with a wheal ≥ 3 mm [24].

RESULTS

Meteorological parameters did not vary significantly during the study, as shown in Table 1. Minimum yearly temperature increased in value in a grade in 2006–2007 period (7.7ºC) with respect to the first sampling period (6.7ºC), probably due to the mild temperatures measured in the winter months. This fact was the cause of the increment of mean yearly temperature (12.4ºC and 13.2ºC, respectively). During the first annual period, the dry season increased in length (May–September) with regard to the reference period. March was also registered a drought season in 2005. According to Table 1, scarcely any rainfall occurred in these months, which determined a lower yearly precipitation value (314 mm). On other hand, between February 2006–January 2007 the dry period was observed during the summer months and in May, with little but present rainfall, with an annual precipitation value (498 mm) similar to that registered during the 1971–2000 reference period. A relative humidity of around 50% was achieved in both years. NE wind direction was predominant (37.4 and 31.4%, respectively) with a higher incidence in the summer season, and a low wind speed (9–10 km/h) and

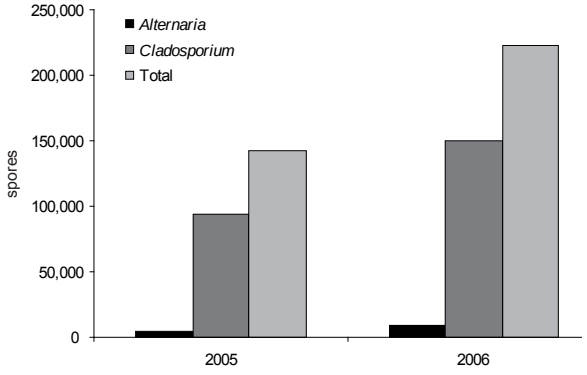

Figure 1. Airborne *Alternaria*, *Cladosporium* and total spores concentrations during 2005 and 2006 Main Spore Seasons (MSS).

Table 1. Meteorological parameters values for the studied period.

2005–2006	F	M	A	M	J	J	A	S	O	N	D	J	Year
T_{mean}	2.3	8.0	11.3	16.1	22.3	22.7	22.2	17.6	13.4	6.4	3.6	2.5	12.4
T_{max}	8.0	15.4	18.1	23.5	31.0	31.1	31.0	25.7	19.1	11.2	8.2	6.0	19.1
T_{min}	-2.1	1.8	5.6	9.4	14.2	14.7	14.0	10.7	8.9	2.7	0.0	-0.3	6.7
R	9	13	39	16	6	0	3	4	112	49	22	41	314
RH	54	46	46	42	28	26	27	32	56	68	69	80	48
WS	10	10	12	10	11	11	9	10	8	9	11	7	10
Wind NE	46.3	31.9	22.3	28.5	36.6	50.8	53.1	42.4	22.5	35.1	40.3	39.7	37.4
Wind SE	9.8	11.5	12.0	8.8	13.1	6.0	6.0	7.8	25.5	10.7	6.5	13.8	11.0
Wind SW	14.5	33.1	38.7	28.5	29.4	25.5	12.9	21.1	36.1	29.0	32.1	18.0	26.6
Wind NW	25.2	23.0	26.4	27.4	20.8	17.6	18.0	28.3	15.4	24.5	20.4	27.4	22.8
CF	0.7	0.5	0.6	0.2	0.2	0.1	0.3	0.4	0.5	0.7	0.6	1.1	0.5
Sunshine	6.5	8.0	8.7	10.2	12.6	13.4	11.8	10.2	5.7	4.3	4.2	2.8	8.2
2006–2007	F	M	A	M	J	J	A	S	O	N	D	J	Year
T_{mean}	3.2	8.9	11.7	16.8	21.0	24.3	20.3	19.3	14.5	10.1	3.6	4.1	13.2
T_{max}	8.8	14.7	18.1	24.5	29.3	32.7	28.5	26.7	20.3	14.5	7.8	7.9	19.5
T_{min}	-0.9	4.1	5.9	9.5	13.4	16.7	13.0	12.8	9.8	6.7	0.2	1.0	7.7
R	42	34	59	10	72	23	26	19	95	83	16	17	498
RH	62	60	51	37	33	31	36	41	60	72	74	81	53
WS	10	12	9	10	9	9	10	8	9	9	8	8	9
Wind NE	37.9	8.8	45.2	32.1	42.8	34.3	56.4	15.6	16.0	30.0	32.3	25.8	31.4
Wind SE	6.7	13.4	9.6	12.0	16.0	12.0	4.0	18.6	20.4	16.4	12.4	16.5	13.2
Wind SW	28.7	57.3	21.7	26.2	16.3	23.9	14.5	36.2	38.8	37.9	28.9	33.1	30.3
Wind NW	26.1	20.3	23.4	29.3	21.3	29.7	21.7	29.1	17.8	15.1	25.6	23.6	23.6
CF	0.5	0.2	0.2	0.4	0.3	0.2	0.1	0.5	0.6	0.5	0.8	0.9	0.4
Sunshine	5.7	6.2	8.8	10.5	10.9	11.2	11.7	7.8	5.5	3.7	3.2	2.7	7.3

T_{mean}: mean daily average temperature (ºC). **T_{max}**: maximum daily average temperature (ºC). **T_{min}**: minimum daily average temperature (ºC). **R**: total monthly rainfall (mm). **RH**: daily average relative humidity. **WS**: daily average wind speed (km/h). **Wind NE**: daily average frequency of north-easterly winds. **Wind SE**: daily average frequency of south-easterly winds. **Wind SW**: daily average frequency of south-westerly winds. **Wind NW**: daily average frequency of north-westerly winds. **CF**: daily average frequency of calms. **Sunshine**: daily average sunshine (hours).

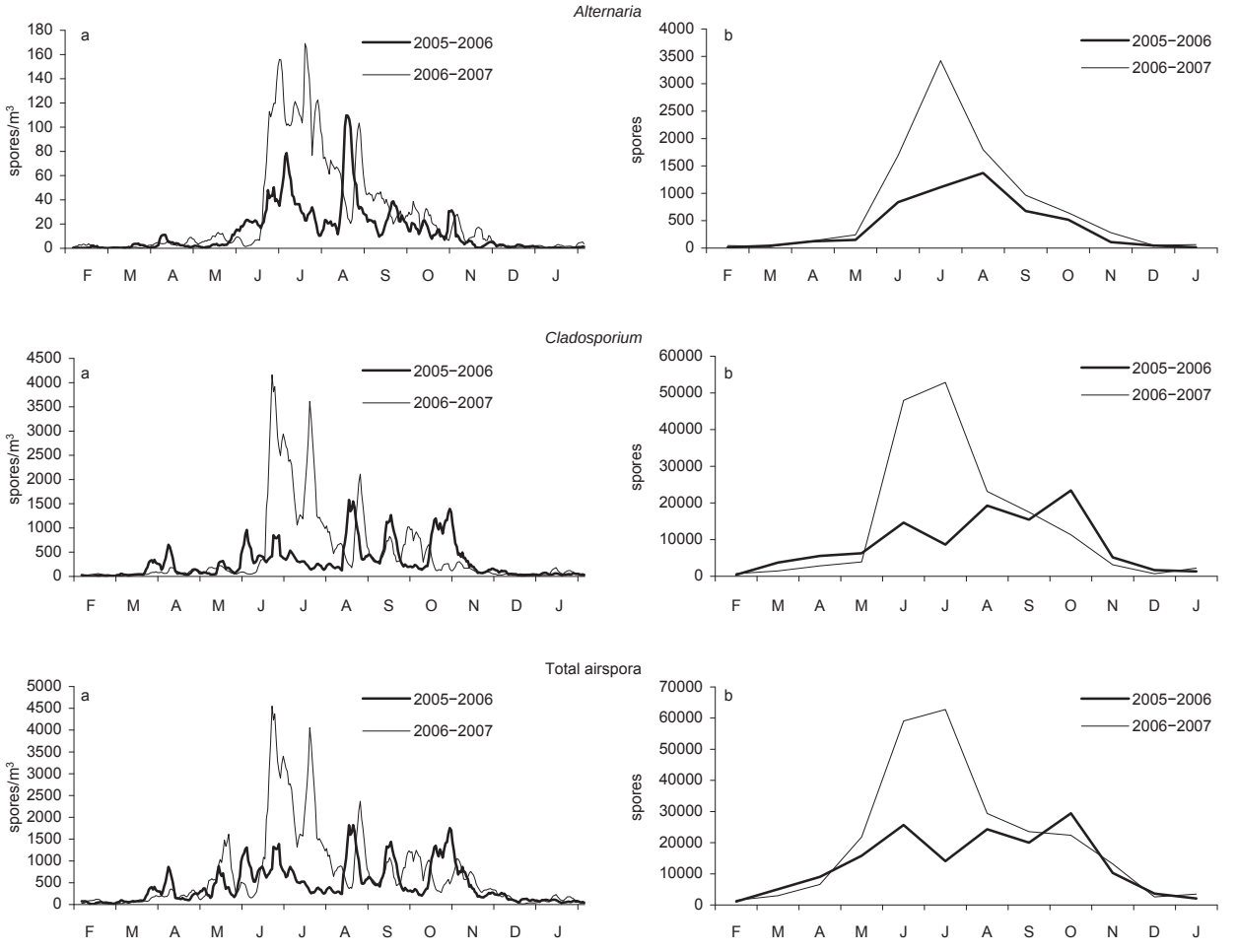

Figure 2. Seasonal variation of *Alternaria*, *Cladosporium* and total airborne spores during both annual periods. a – Daily average (5-day running mean); b – Total monthly concentrations.

a frequency of calms (0.4–0.5%) were reported. The daily average sunshine varied from 8.2 hours in 2005–2006 to 7.3 hours in the second annual period.

The main spore season was established between April–November for both 2005 and 2006, embracing 220 and 196 days, respectively. The start date occurred almost a month late in 2006, although with a similar end date. We recorded a total of 142,617 spores during the MSS in 2005 and 223,179 in 2006, among which *Cladosporium* was the most abundant (93,744 and 150,144, respectively). The highest daily peak values occurred in June (22 June 2005 and 17 June 2006) with 2,985 and 5,856 spores/m^3 trapped. The representation of atmospheric airspora varied throughout both years with the highest values in October 2005 (29,419) and July 2006 (62,744). There was a decrease in July 2005, probably due to the same decline in *Cladosporium* concentration because no precipitations were observed in this month, and the relative humidity registered the lowest percentage of the year. On the contrary, the abundant precipitations registered during October 2005, together with mild temperatures, could be the reason for the highest monthly peak recorded in that year (Figs 1, 2).

In the case of *Alternaria*, the majority of this spore type was trapped in the summer, during the harvesting season, representing a 3.1% of the total amount of spores trapped during the first annual period and 3.8% during the second. The monthly peak was registered in August 2005 (1,371 spores) and in July 2006 (3,422), months in which the daily peaks also occurred (14 August 2005 with 149 spores/m^3 and 18 July 2006 with 344). A total number of 8,385 spores were collected during the MSS of 2006, being almost double that of the 2005 count (4,500), even when the end date of the MSS was the same for both years (27 October), with no significant differences found in the start date (26 May 2005 and 2 June 2006) nor in the MSS length (155 days in 2005 and 148 in 2006).

Cladosporium was the most representative mould (65.7% during the first annual spore count and 67.3% in the second) being present throughout the whole period of study. In both annual periods, the bulk of *Cladosporium* was found during the summer (43,370 spores in 2005 and 93,496 in 2006), followed in descending order by the autumn of 2005 (30,184) and the spring of 2006 (54,693). For this fungus we observed a 38-day start date delay (13 May) of the 2006

MSS compared with 2005 (5 April). On the other hand, the end date of the MSS occurred almost a month before in 2006 (10 October) than in 2005 (4 November). In this way, the MSS length of 2005 lasted 214 days, whereas in 2006 it continued for just 151 days. More similarities were found in the peak day dates registered during the same month (22 and 17 June, respectively), with a higher value in 2006 (5,503 spores/m^3).

The prepeak period varied from 35 days for *Cladosporium* during 2006 to 81 days for *Alternaria* along 2005.

Significant positive correlations were established with the temperatures values for both genera during MSS and PRE periods. Relative humidity and rainfall displayed negative correlation with *Alternaria* MSS and positive with *Cladosporium*. A negative effect of wind speed was recorded during the *Alternaria* PRE and *Cladosporium* MSS. Otherwise, a positive influence of wind direction was found for *Alternaria* with north-easterly (MSS) and north-westerly winds (PRE) whereas south-easterly and south-westerly winds showed an inverse relation only during *Alternaria* MSS. In the case of *Cladosporium*, only south-easterly winds displayed a positive significant correlation with spore concentrations in both MSS and PRE periods. *Cladosporium* spore counts were positively correlated with the frequency of calms in the PRE period, and had an opposite effect on *Alternaria* for both periods. Lastly, daily average hours of sunshine showed a positive influence on *Alternaria* spore counts during the MSS (Tab. 2).

Moreover, we would like to point out that the correlations among total airspora and meteorological parameters were highly influenced by the *Cladosporium* behaviour, since it was the most abundant mould. In this way, correlations signs were similar except for wind direction, although with no significant differences.

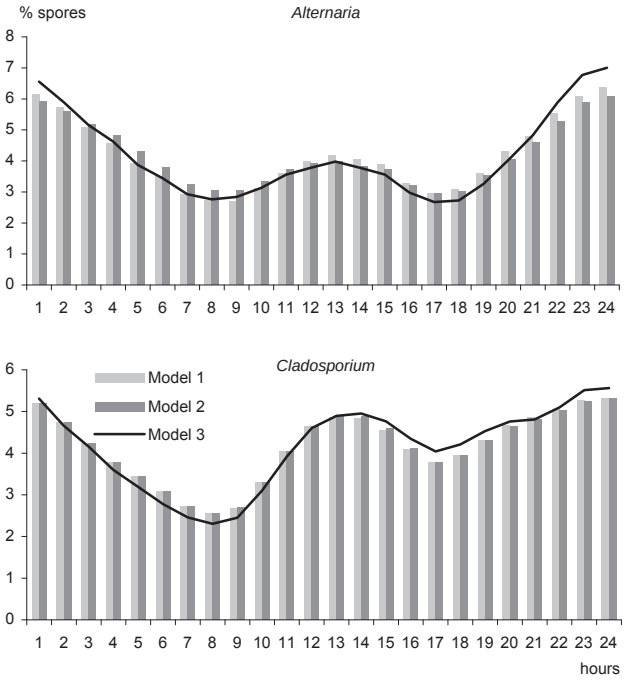

Figure 3. *Alternaria* and *Cladosporium* intra-diurnal patterns (3-hour running mean).

Once the intra-diurnal pattern was plotted, we established similarities between both fungi behaviour and among the three models used. *Cladosporium* and *Alternaria* hourly

Table 2. Spearman's correlation coefficients for *Alternaria*, *Cladosporium* and the total airspora concentration (2-years MSS and prepeak) and meteorological parameters.

	Alternaria		*Cladosporium*		Total	
	MSS (n=303)	PRE (n=128)	MSS (n=365)	PRE (n=114)	MSS (n=416)	PRE (n=133)
T_{mean}	**0.466****	**0.253****	**0.259****	**0.393****	**0.296****	**0.477****
T_{max}	**0.467****	**0.254****	**0.206****	**0.342****	**0.231****	**0.387****
T_{min}	**0.406****	**0.249****	**0.373****	**0.471****	**0.406****	**0.597****
R	**-0.266****	-0.066	0.068	0.022	0.066	0.094
RH	**-0.226****	0.118	**0.151****	-0.037	**0.103***	-0.049
WS	-0.014	**-0.187***	**-0.168****	-0.139	-0.095	-0.082
Wind NE	**0.280****	0.111	0.024	0.076	-0.059	-0.065
Wind SE	**-0.134***	-0.023	**0.183****	**0.345****	**0.173****	**0.304****
Wind SW	**-0.252****	-0.122	-0.013	0.019	0.058	0.097
Wind NW	0.109	**0.191***	0.011	-0.020	0.020	-0.009
CF	**-0.142***	**-0.239****	0.012	**0.202***	0.004	0.116
Sunshine	**0.310****	-0.004	-0.084	-0.022	**-0.099***	**-0.170***

T_{mean}: mean daily average temperature (ºC). **T_{max}**: maximum daily average temperature (ºC). **T_{min}**: minimum daily average temperature (ºC). **R**: total daily rainfall (mm). **RH**: daily average relative humidity. **WS**: daily average wind speed (km/h). **Wind NE**: daily average frequency of north-easterly winds. **Wind SE**: daily average frequency of south-easterly winds. **Wind SW**: daily average frequency of south-westerly winds. **Wind NW**: daily average frequency of north-westerly winds. **CF**: daily average frequency of calms. **Sunshine**: daily average sunshine (hours). **Significance levels**: *, 95%; **, 99% (values in grey indicate not significant correlation).

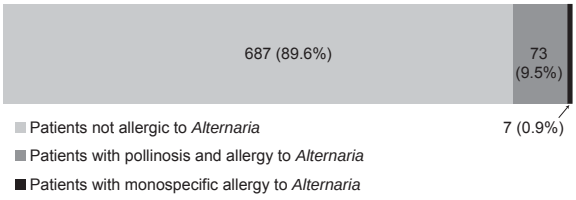

Figure 4. Percentage of patients sensitized to *Alternaria* spores.

concentrations decreased along two periods, (2–9h and 16–18h), without reaching a percentage higher than 7% for *Alternaria* and 6% in the case of *Cladosporium*. In both cases, the maximum hourly concentrations period was registered around midnight. The variation in representation values between the maximum and the minimum peaks was 4% for the two genera. In the same way, differences in the treatment of data used for the three models did not involve variations in the results obtained (Fig. 3).

With regard to the epidemiological research, the clinical histories of 767 patients who showed symptoms of environmental allergy were examined. Only 73 patients (9.5%) were allergic to *Alternaria* but in addition to some pollen allergens and just 7 (0.9%) had a monospecific allergy to *Alternaria* extract. Respiratory allergy symptomatology was gathered, mainly during the second trimester of the year for patients with sensitization to pollen and *Alternaria*, whereas the third trimester was the main season in which patients with monospecific allergy developed their symptoms. 26% of patients allergic to *Alternaria* suffered from rhinoconjunctivitis (RC) and 74% also from asthma, being more frequent in men (59%) than in women (41%), mainly at the ages of between 11–20. On the other hand, only one patient showed positive skin test reaction to *Cladosporium*, which made it impossible to realize a deeper study (Figs 4, 5).

Although the number of monosensitized patients to one or another was really low, there were registered higher daily concentrations than 100 spores/m^3 for *Alternaria* (6 days in 2005 and 29 in 2006) and 3,000 spores/m^3 in the case of *Cladosporium* (13 days in 2006), crossing the threshold values established to develop allergy symptomatology.

DISCUSSION

Lima and Gadelha [39] established that Deuteromycotina fungi have such characteristics as a low substrate demand and a high capacity to produce and release round spores of low density to the environment, among others, which results in them being more abundant. Consequently, airborne spores belonging to this group tend to appear frequently worldwide. In fact, *Cladosporium* presented a frequency range of 66.5%, being the largest group captured in the aerobiological samples which also occurred in some other Spanish cities [4, 13, 17, 41, 47, 52], as well as in some other European cities [24, 27, 46], and South America [12], the USA [14, 38] and even in South Africa [11], Asia [54], and Australia [51], to cite a few examples.

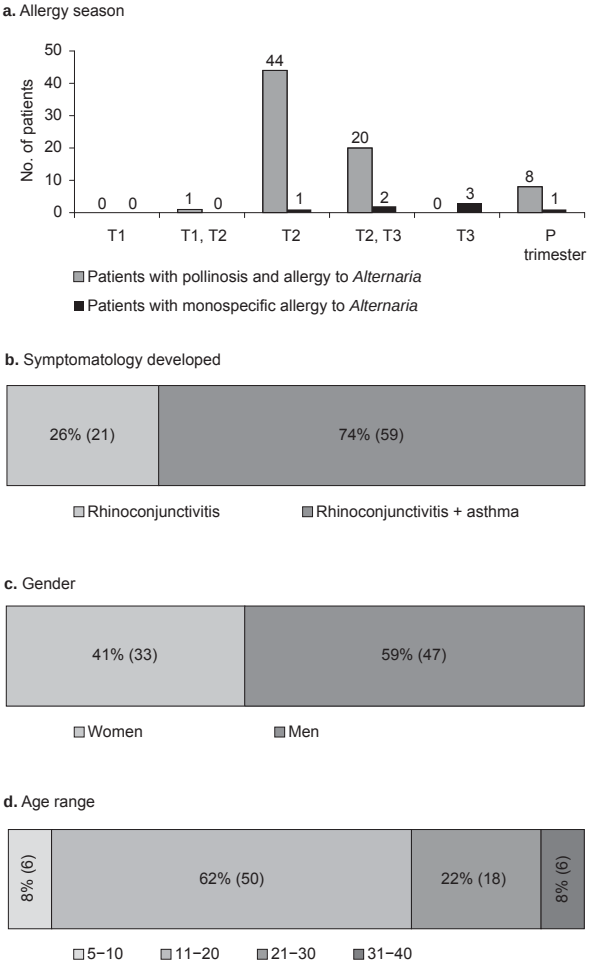

Figure 5. Clinical aspects of patients sensitized to *Alternaria* spores.

On other hand, *Alternaria* had a lower rank order of abundance comprising a 3.4% of the total amount collected. In the same way, *Cladosporium* representation percentage is lower than those obtained in the North of Europe [17, 35], so that its representation decreases at Mediterranean sites with an increment in the *Alternaria* abundance [3, 18, 24], which is more frequent at landlocked sites than in coastal cities [2, 8]. The main spore season occurred during late spring and summer, as other studies have pointed out [23, 38, 58], and during the beginning of autumn [2, 13, 57] in 2005. The maximum values were registered during the summer as a consequence of the increment in temperatures measured during this period. Other cities of Castilla y León, such as León [19] or Palencia [30], reported the same tendency. In general, the increase in airborne spore concentrations was related to the increment in temperature and the absence of precipitations [2, 31, 42, 48], despite which abundant rainfall favoured a late release of the great quantities of conidia [47, 57]. This phenomenon was observed during October 2005, in which *Cladosporium* registered the monthly peak, probably due to a strong rainfall season and mild temperature. On the other hand, the absence of precipitations registered during July maintained spore

concentrations below the counts realized in the same month in 2006. Low temperatures in the winter could be responsible for the lower concentrations recorded at this time of the year. The *Alternaria* annual pattern is also dependent on the proximity of crop fields [8] since the release of these spores is produced between June and October coinciding with the crop harvest season. In fact, Friesen *et al.* [21] had already estimated that an immense amount of these spores are released during wheat harvesting and carried long distances in wind currents. Another factor to take into account is that both taxa have an airborne dispersal mechanism and are abundant on leaf surfaces [33] so that they can release large amount of spores to the atmosphere, coinciding with the moment of leaf expansion of their host [38].

In relation to the influence of meteorological parameters, temperature explained a high percentage of data variability, including total spores as a whole, so that the overall spores pattern was conditioning by *Cladosporium* results. This reveals the fact that these so-called "dry-air spora" were well adapted to dispersal in warm periods [31], reporting negative correlations with rainfall for both genera [2, 40]. However, in our survey rainfall showed an inverse relation [58], probably due to differences in distribution, since *Alternaria* is much more frequent in the Mediterranean area where lower rainfall values are recorded, and Cladoporium is more abundant in the Eurosiberian regions where high relative humidity could favour the dissemination of these spores. Conversely, winds from the south direction had a significant negative influence on *Alternaria*, whereas northern winds showed a positive relation. This result could be explained by the fact that Palencia, located in the north of Valladolid, is also a province with large harvesting areas which might provide adequate conditions to develop and complete its life cycle, whereas vineyards are predominant in the south with another types of associated moulds such as Botrytis. On other hand, south-easterly winds correlated more strongly with *Cladosporium* PRE than MSS concentrations. In this respect, grasslands and minimum temperatures above 13ºC [19] or 15ºC [31], registered at southern areas, were found to favour the production of these spores which would be transported by air currents. In fact, the highest correlation was found with this meteorological parameter, as occurred in Szczecin (Poland) for the same period [27]. At the same time, and since the predominant winds during the summer came from the north-east, a wind direction that did not show significant correlation with *Cladosporium* but with *Alternaria* spores, the frequency of calms had an inverse influence on them. *Alternaria* is also influenced by relatively strong winds [31] which could explain the weak monthly average wind speed of 9–10 km/h measured in our survey correlated negatively with this spore type concentration. The last meteorological factor analyzed, sunshine, has a significant and positive correlation with *Alternaria* MSS. This fact also indicated the southern distribution of this genus due to a greater number of daily sun hours in southern areas.

As occurred in Santiago de Compostela [17], *Alternaria* and *Cladosporium* both presented a similar intra-diurnal pattern, in which hourly spore concentrations changed gradually, having a greater representation towards the central hours of the day [19, 48] and at night [16], with periods of decrease along the morning and during the first hours of the evening. The same behaviour was observed for *Alternaria* in Murcia [42] but without detecting the second hourly-period decrement. Konopińska [36], found the minimum concentrations to be between 00–07 h. However, Srivastava and Wadhwani [56] claimed that *Alternaria* optimal growth conditions occurred mainly in darkness for sporulation, whereas *Cladosporium* concentrations were much higher between 8:00–12:00 according to Hjelmroos [31]. Conversely, in other studies the maximum representation was observed at 18:00 for both *Cladosporium* [1] and *Alternaria* spores [17]. Such variability may be explained by the differences in climate and biogeographical characteristics of the areas under study [34].

Taking into account the clinical aspects, as early as 1976, Buisseret [5] stated that *Alternaria* spores were the main risk for the respiratory problems developed during late summer and early autumn, July, August and September being, the main months for this spore type release and combined with harvesting [8]. For that reason, the third trimester of the year is the main season in which monospecific allergic to *Alternaria* develop their symptoms, bronchial asthma being the main one caused, as already observed by Srivastava and Wadhwani [56]. The age range was very wide (mainly young patients) with a higher male ratio, results also obtained by Thessaloniki [24]. In the same way, threshold values were crossed only on a few days in our study, different to other studies [8], which explains the low number of allergic patients registered. In that sense, *Alternaria* positive skin reactions seem to be related to higher airborne spore concentrations. However, as some researchers have described [24], *Cladosporium* sensitivity seems to be rather low even when its abundance is greater. Other reasons for the low elicitation of positive skin responses could be a poor purification or standardization of commercially available allergen extracts, or the resistance of population to this type of allergy.

Nevertheless, a lower number of people are allergic to fungal spores than pollen. However, in Valladolid, as stated above, the high *Alternaria* and *Cladosporium* spore load was accounted during the harvesting season, since a large amount of them are released during agricultural mechanisms such as threshing, grinding and handling. This is the reason why farmers are potential patients for suffering from professional allergy. Some authors have related the importance of mould spores allergies in cases of acute asthma [59], and in the number of admissions at Emergency Services [10, 45]. As this study has shown, there are significant correlations between spore concentrations and the meteorological parameters. That is the reason why these weather reports should be available to allergic patients in

order for them to know under which conditions *Cladosporium* and *Alternaria* could be more prevalent in the air of Valladolid.

Definitively, a long term monitoring of spore concentrations could provide enough information for the development of predictive models. These are useful tools not only for patients suffering from allergy to fungal spores and clinicians, but also for plant pathologists. Thus, farmers and producers could reduce the pollution of the atmosphere by choosing only the appropriate fungicides during harvesting, thereby avoiding economical losses, since both genera are commonly found as parasites in crop fields of the region.

CONCLUSIONS

The most abundant airborne genus in the Valladolid aerosol was *Cladosporium* which belongs to the Deuteromycotina group, being the maximum value of total spores recorded during the summer.

A positive relation between temperature and spore concentrations was observed, whereas precipitation and relative humidity had a different influence on both genera. Wind direction was an important parameter to explain *Alternaria* spores variability.

The intra-diurnal patterns propose greater concentrations during the central hours of the day and at night, with a regular distribution curve for both *Alternaria* and *Cladosporium* and the three models used.

With regard to the clinical research, 73 of 767 patients suffered from allergy to *Alternaria*, developing rinoconjunctivitis and asthma symptoms during the third trimester of the year, being more frequent in men at ages of between 11–20. Only one patient showed positive skin test reaction to *Cladosporium*.

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