

First fungal spore calendar of the middle-west of the Iberian Peninsula

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Abstract The aim of this paper was to make a first approximation of the fungal spore airborne content in Valladolid along the year, constructing the first spore calendar for the middle-west of Spain. So that, we monitored the city during 2005 and 2006, being *Cladosporium* the most abundant type, present all over the year (together with *Pleospora*). The greatest atmospheric spore diversity was observed in April in contrast with February. The intra-diurnal pattern for *Alternaria*, *Cladosporium* and *Dreschlera* was very similar with an hourly concentration percentage decreasing along two periods, whereas *Coprinus*, *Ganoderma* and *Periconia* showed a clearly nocturnal pattern. The meteorological parameter that most influenced airborne spore concentrations was temperature, significantly and positively in the case of dry-air spores but negatively for wet-air spores.

Keywords *Cladosporium* · Spore calendar · Meteorological factors · Valladolid

1 Introduction

Palynological studies have an application in different disciplines such as melissopalynology, plant taxonomy, paleopalynology, aeropalynology or even in other areas as forensic palynology, agronomy, biodeterioration.

Nevertheless, its importance has been pointed out not only in the scientific community but also among general population due to its great utility in prevention, diagnostic and treatment of allergic diseases. Through the aerobiological monitoring of multiple sites, it is possible to establish the seasonal behaviour of allergenic particles such as pollen grains and fungal spores, which facilitate the construction of pollen (Rodríguez-de la Cruz et al. 2010) and spores calendars (Mitakakis and Guest 2001; Gioulekas et al. 2004), very useful for patients and allergists in order to take preventive measures.

Nowadays, fungal spore calendars are also included, specially taking into account *Alternaria* and *Cladosporium*, due to the increment in allergic sensitization in the Mediterranean area and the north of Europe, respectively (Sánchez Reyes 2011).

In any way, the number of these researches is still very low at present, since it is not very frequent to find studies which comprise the whole-year data as Mallo et al. (2011) or Almaguer et al. (2014) but temporary (monthly, seasonal, etc.) information of only those spores with allergenic and/or phytopathogenic interest (O'Connor et al. 2014; Pyrri and Kapnasaki-Gotsi

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2015). The reason for that is the time commitment required and the difficulty of identifying fungal spores by non-viable methods as Hernández Trejo et al. (2012) already observed.

In that sense, the Spanish Aerobiology Network (REA, acronym in Spanish) was founded in 1992 (Galán et al. 2007), and its main objective has been to obtain and to spread aerobiological information in Spain. Nevertheless, before the formation of this network, pollen content of Castilla y León had been studied during the 1980s only through temporary assessments, results of which were not even published in international indexed journals but in symposia abstract books. Afterwards, the atmospheric pollen content of the capital city of this region (Valladolid) was not routinely monitored until mid-2006, thanks to the funding from Castilla y León regional government (<http://www.saludcastillayleon.es/ciudadanos/es/polen>).

With respect to fungal spores, the number of studies was even lower, being reduced to specific researches carried out in the cities of León (Fernández et al. 1998), Palencia (Herrero et al. 1996; Herrero and Zaldívar 1997) or Salamanca (Pérez-Gorjón et al. 2003).

Although Castilla y León is the wider region of the European Union (which shows great contrast in vegetation and climate), there is a small number of papers published (some of them more than a decade ago) so that we considered important to improve the knowledge of its territory by monitoring the atmosphere of its capital city, Valladolid, during 2005–2006 period (Sánchez Reyes 2011). The results obtained for fungal spores have been presented in this paper as the first spore calendar for this city which pretends to be a draft guidance to allergists and allergy sufferers, since this type of calendar is a very graphical tool to date the period of main risk for a patient. Furthermore, details of the main spore season of the more abundant taxa are provided together with the intra-daily patterns.

On the other hand, although fungi are ubiquitous and cosmopolite organisms (as they can be found in any site with warm temperature, humidity, oxygen and nutrients), seasonal behaviour of these particles is conditioned by differences in vegetation (De Antoni Zoppas et al. 2006), and also in meteorological parameters which may influence their concentrations and/or their appearance in aerosol significantly

(Hernández Trejo et al. 2012; Maya-Manzano et al. 2012; Recio et al. 2012; O'Connor et al. 2014). For that reason, we decided to include the study of the correlations between daily spore concentrations and main meteorological parameters in order to determine which climate conditions may influence each spore type and in what sense.

2 Materials and methods

Valladolid (41°39'N; 4°43'W) is the largest city in the Castilla y León region, and it is located in the middle of Spain (Fig. 1) at a height of 691 m.a.s.l, surrounded by arable farming lands. From a biogeographical point of view, it is included in the Mediterranean region (Rivas Martínez 2007). According to a 30-year average (1971–2000), the climate is continental (Capel Molina 1981), characterized by a little annual rainfall level (435 mm) which determines a dry season during the summer period. The annual mean temperature corresponds to 12.3 °C with a mean temperature of 21.7 °C during the hottest month. The annual average of fog and chilly days (42 and 61 days, respectively) is quite important, with little snow though (8 days), due to its particular geographic situation.

The aeropalynological monitoring was carried out from 1 February 2005 to 31 January 2007 by means of 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 the ground level. The pollen trap has the air flow rate of 10 L per min similar to the volume of air inhaled by the human lung. The methodology used was the one suggested by the Spanish Aerobiology Network (REA) in regard to the pollen grain and spore count and to samples processing (Galán et al. 2007): four longitudinal traverses of contiguous fields along the length of the slide were performed, starting from the centre and separated by at least 1 mm to avoid oversampling or empty areas and using 40 × 10 magnification in optical microscopy. This method allows us to examine 12–13 % of the surface area. Different keys and morphology works were used for the identification of the moulds (Grant Smith 2000, among others).

The spore calendar construction was based on Spielsma's model (Spielsma 1991), transforming 10-day mean concentrations into eleven classes

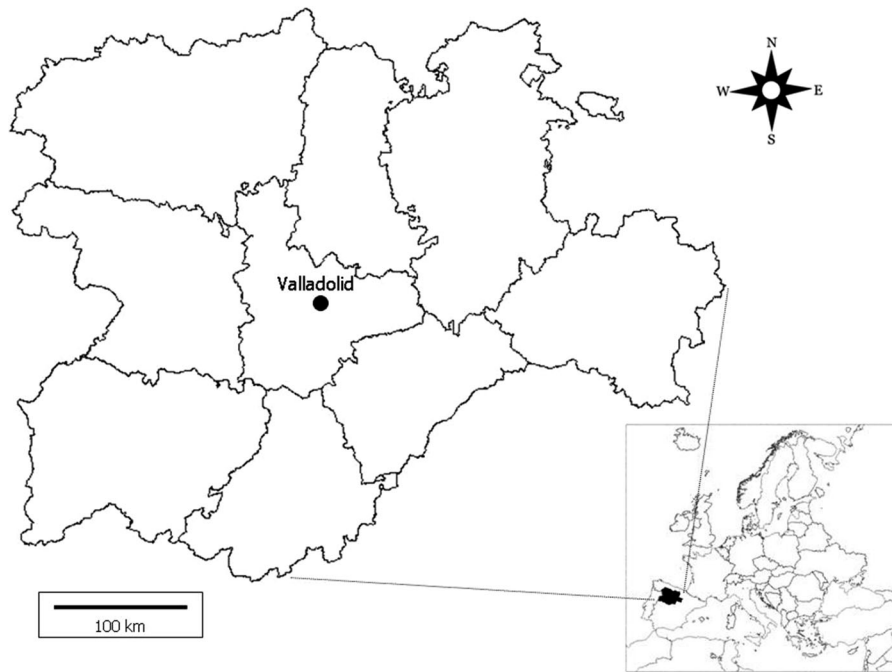


Fig. 1 Map of Castilla y León region showing Valladolid city location

(Fig. 2). Different genera follow the order in which they began to appear in the air spectra, including only those fungi that showed a minimum 10-day mean equal to or greater than 1 spore/m³.

Furthermore, information about the characteristics of the MSS of those spore types that reached a percentage of the annual total count equal to or higher than 1 % (*Alternaria*, *Cladosporium*, *Coprinus*, *Dreschlera*, *Ganoderma*, *Periconia*, *Pleospora* and total spore count) is shown in Table 1, and also intra-daily variations (Fig. 3) and correlations with meteorological parameters (Table 2) were studied.

For intra-daily variations, we have taken into account three different models described by some authors (Galán et al. 1991). Model 1 calculated the value for each hour represented by the sum of the values corresponding to that hour, divided by the total number of days of the MSS. However, in model 2, an ideal day was obtained, dividing this sum of values of each hour only by the number of days of the MSS on which the spore type was present. In model 3, it was important whether rainfall occurred or not. Firstly, the daily average of the spore type was calculated using the total number of days of the MSS period as the denominator. But for the calculations of the hourly

concentrations, we selected just the dry days with a daily value equal to or higher than the average previously calculated. Then, the hourly value was represented by the sum of values corresponding to that hour, divided by the total number of days selected previously. In the graphical representation, values were expressed in percentage rather than in absolute numbers, in order to avoid annual and daily variation and to make three methods comparable. For the graphical representation, we have used a 2-h mean to smooth the tendency.

The main spore season (MSS) has been defined by the method that calculates the start of this period as the date by which the 5 % of the total year spore record was registered, and the end with the 95 % captured (Nilsson and Persson 1981).

The influence of the main meteorological factors on the daily spore concentrations during the MSS was analysed by using the SPSS (version 12) software applying the Spearman nonparametric correlation test since the daily data were neither normally nor log-normally distributed. The meteorological parameters values studied were mean, maximum and minimum daily average temperature (°C), total daily rainfall (mm), daily average relative humidity (%), daily

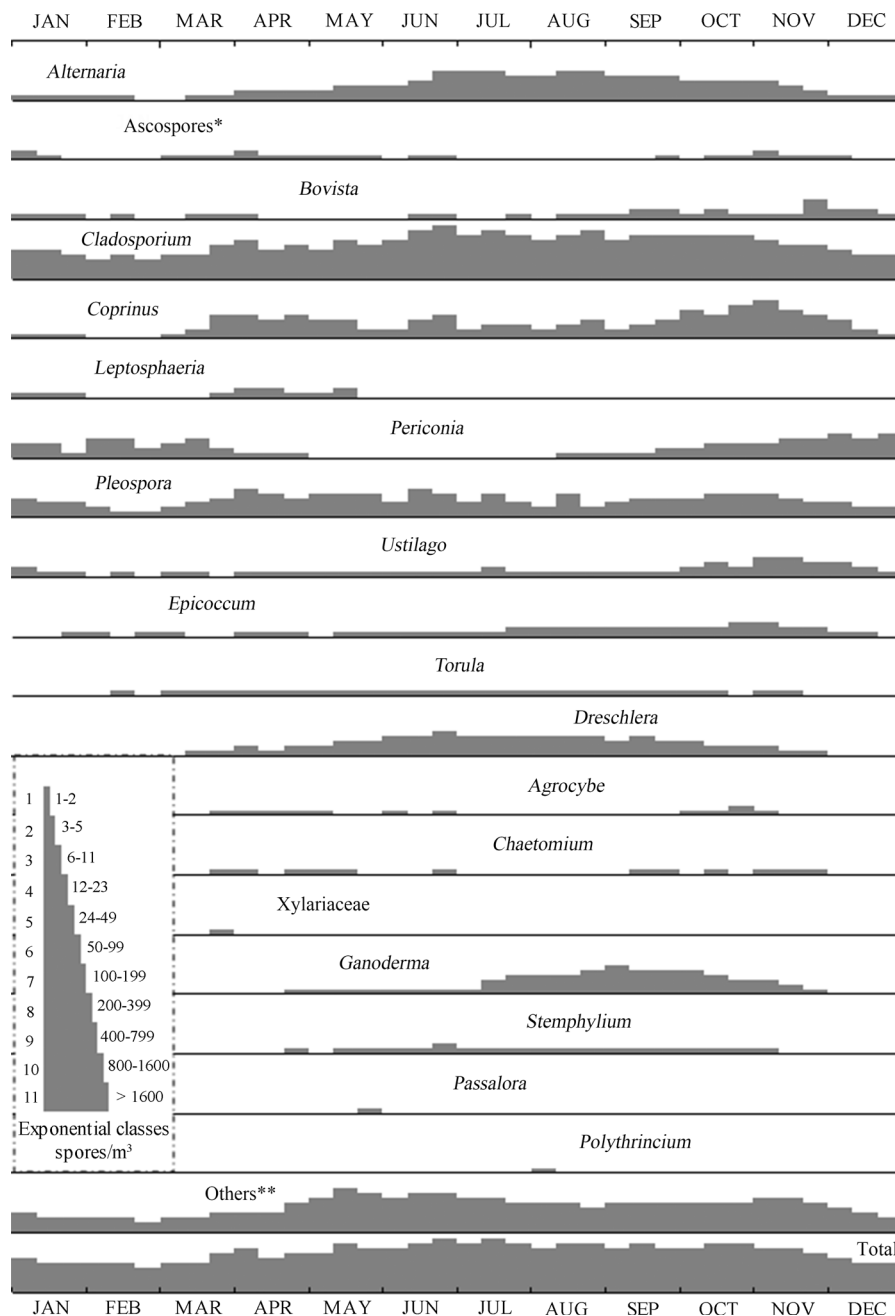


Fig. 2 Spore calendar for Valladolid City. *excluding ascospores identified to a more specific level. **including unidentified spores and those spore types that registered a mean annual percentage lower than 0.01 %

average wind speed (km/h), daily average frequency of north-easterly winds (%), daily average frequency of south-easterly winds (%), daily average frequency of south-westerly winds (%), daily average frequency of north-westerly winds (%), daily average frequency

of calms (%) and daily average sunshine (hours). They have been supported by the Spanish State Meteorological Agency (AEMET, acronym in Spanish), and they were obtained from the station named Valladolid-Observatorio (41°38'N; 4°45'W; 735 m.a.s.l.) located

Table 1 Characteristics of the main spore seasons (MSS) of selected taxa (2005–2006)

	Total MSS (90 %)	Start MSS	End MSS	MSS length (days)	Peak day	Peak value (spores/m ³)
2005						
<i>Alternaria</i>	4500	26-May	27-Oct	155	14-Aug	149
<i>Cladosporium</i>	93744	5-Apr	4-Nov	214	22-Jun	2387
<i>Coprinus</i>	4816	10-Apr	20-Nov	225	3-Nov	383
<i>Dreschlera</i>	2844	30-Apr	16-Oct	170	1-Jun	62
<i>Ganoderma</i>	2100	8-Jul	23-Oct	108	4-Sep	69
<i>Periconia</i>	2795	5-Feb	20-Dec	319	16-Dec	241
<i>Pleospora</i>	6755	3-Apr	16-Nov	228	29-May	386
Total	142617	5-Apr	10-Nov	220	22-Jun	2985
2006						
<i>Alternaria</i>	8385	1-Jun	25-Oct	147	18-Jul	344
<i>Cladosporium</i>	150144	13-May	10-Oct	151	17-Jun	5503
<i>Coprinus</i>	11885	2-Apr	22-Nov	235	27-Oct	444
<i>Dreschlera</i>	2037	3-May	6-Oct	157	26-Jun	90
<i>Ganoderma</i>	3563	16-Jul	28-Oct	105	12-Sep	98
<i>Periconia</i>	1393	4-Jan	22-Dec	353	1-Dec	124
<i>Pleospora</i>	4639	11-Mar	14-Nov	249	9-Apr	184
Total	223179	25-Apr	6-Nov	196	17-Jun	5856

5 km from the trap site, being the observed meteorology representative for the site.

3 Results

3.1 Characteristics of the main spore season of the different taxa

Cladosporium was the most abundant spore which registered an increment in the second year studied (150144 spores) with respect to 2005 (93744) (Table 1). In the same way, it was the spore type that reached the highest daily values (2387 spores/m³ in 2005 and 5503 in 2006) in mid-June, whereas *Dreschlera* (62/90 spores/m³) and *Ganoderma* (69/98 spores/m³) registered the lowest ones (in June and September, respectively). The MSS length of the total count of spores was similar in both 2005 and 2006, being registered from April to November (Table 1) coinciding with *Cladosporium* and *Coprinus* MSS. However, *Alternaria* had a later start date and a previous end date, from late May/beginning of June to October (Table 1). *Periconia* registered the earliest

start date of the MSS coinciding with winter months in both years.

3.2 Spore calendar

Cladosporium spores reached the highest exponential class (class 11; 1600 spores/m³), during the last 10-day mean period of June (Fig. 2). *Coprinus* registered around 200–399 spores/m³ (class 8) in the first 10-day mean period of November, while *Alternaria* (last 10-day mean period of June, first two of July and the last two of August), *Pleospora* (first 10-day mean period of April and the second of June) and *Ganoderma* (first 10-day mean period of September) counted around 50–99 spores/m³ (exponential class 6). *Dreschlera* (in the last of June) and *Periconia* (in the first and the last of December) reached exponential class 5 (24–49 spores/m³). The rest of spore types included in the spore calendar did not registered 10-day mean greater than 49 spores/m³, emphasizing that Ascospores (excluding those ones identified to a more specific level), *Leptosphaeria*, *Torula*, *Agrocybe*, *Chaetomium*, *Stemphylium*, *Passalora*, *Polythrincium* and the Xylariaceae family only

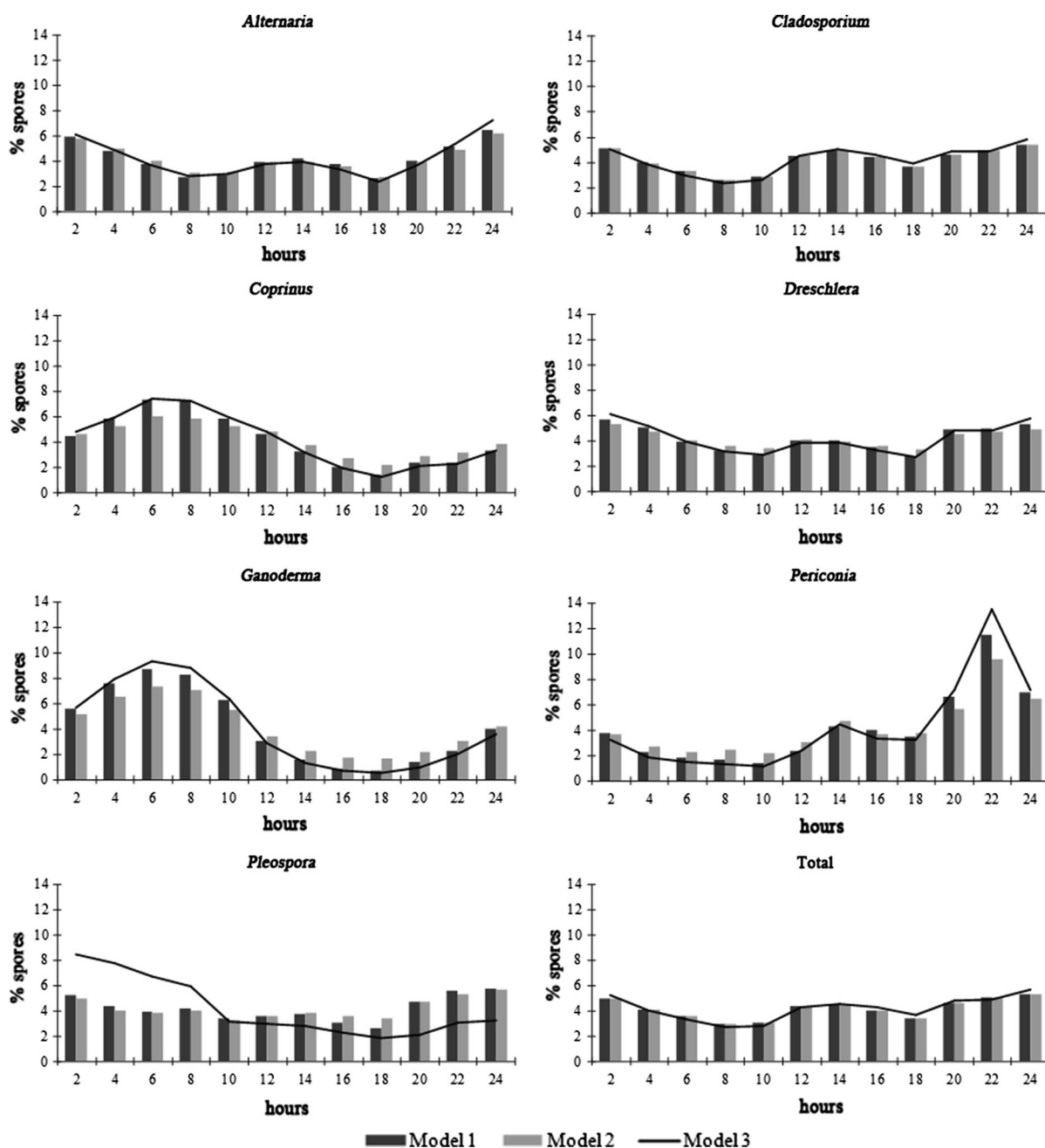


Fig. 3 Fungal spores intra-diurnal patterns (2-h mean)

registered values corresponding to classes 1 (1–2 spores/m³) and/or 2 (3–5 spores/m³).

With respect to the permanence in the atmosphere, it is important to point out that *Alternaria* (34 10-day mean periods), *Coprinus* (33) and *Ustilago* (33) reached 10-day means greater than 1 spore/m³ in

more than 30 10-day mean periods per year (Fig. 2), getting that value for the whole year in the cases of *Cladosporium* and *Pleospora* (36 10-day mean periods). On the other hand, Xylariaceae family, *Passalora* and *Polythrincium* spores registered those records only during a 10-day mean period in the whole year

Table 2 Sign of Spearman correlation coefficients obtained between mean daily spore concentrations and meteorological parameters along the main spore season (MSS)

	T_{mean}	T_{max}	T_{min}	R	RH	WS	Wind NE	Wind SE	Wind SW	Wind NW	CF	Sunshine
<i>Alternaria</i>	.464**	.463**	.409**	-.269**	-.193**	-.166**	.269**	-.122*	-.249**	-.128*	-.128*	.295**
<i>Cladosporium</i>	.260**	.206**	.373**	.185**	.185**	-.166**	.182**	.182**	.114*	.107*	.107*	-.424**
<i>Coprinus</i>	-.412**	-.428**	-.317**	.193**	.623**	-.249**	-.142**	.223**	.114*	-.160**	-.142*	.337**
<i>Dreschlera</i>	.427**	.425**	.364**	-.242**	-.258**	.133*	.303**	-.127*	-.206**	.316**	.243**	.337**
<i>Ganoderma</i>	.211**	.273**	.273**	-.231**	-.231**	-.403**	-.104**	.131**	-.146*	.201**	.201**	-.360**
<i>Periconia</i>	-.433**	-.405**	-.429**	.282**	.282**	-.131**	-.331**	.176**	.269**	-.099*	-.099*	-.467**
<i>Pleospora</i>	-.316**	-.394**	-.093*	.558**	.500**	.140**	-.331**	.173**	.269**	-.099*	-.099*	-.467**
Total	.296**	.231**	.406**	.122*	.122*	.122*	.122*	.122*	.122*	.122*	.122*	.122*

T_{mean} mean daily average temperature (°C), T_{max} maximum daily average temperature (°C), T_{min} minimum daily average temperature (°C), 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 %

(the last one of March, the last one of May and the first one of August, respectively).

The greatest diversity of fungal spores was captured along the summer and autumn seasons (Fig. 2), being April the month that registered the highest number of different spore types (16), followed by March, May, October and November (15). On the other side, we could find the months of February (8), December (9) and January (10) corresponding to winter season.

3.3 Intra-daily variations

Models 1 and 3 showed hourly representation values more similar than with model 2 (Fig. 3), especially in the cases of *Coprinus* and *Ganoderma* (that registered the hourly maximum at 01–08 h), as with *Periconia* (17–24 h).

Models 1 and 2 registered similar results, but very different to those obtained with model 3, in the case of *Pleospora*, establishing the highest hourly values at 17–24 h.

Alternaria, *Cladosporium* and the total spore count registered minor differences among the three models during 17–24 h period as *Dreschlera* did throughout 17–08 h period.

3.4 Correlations with meteorological parameters

The analysis of the correlation coefficients obtained revealed that, in general, the increase in airspora levels is related to an increment in temperatures (Table 2). However, *Coprinus*, *Periconia* and *Pleospora* obtained negative correlation coefficients. Similar behaviour was observed for the total hours of sunshine.

Alternaria, *Dreschlera* and *Ganoderma* had a negative correlation with rainfall but positive in the cases of *Coprinus* and *Pleospora* (Table 2). The same positive correlation was observed among *Pleospora* and relative humidity (Table 2). With regard to this last meteorological parameter, a contrary relation was observed for *Alternaria* and *Cladosporium* (Table 2). On the other hand, *Coprinus* and *Periconia* obtained positive results.

During the main spore season, wind speed (*Ganoderma* and *Coprinus*) and frequency of calms (*Alternaria*) achieved negative correlation coefficients. Winds from the south had a significant negative influence on *Alternaria*, while winds from the north showed the contrary effect (Table 2). With respect to

Coprinus and *Ganoderma*, winds from the south (*Coprinus*) and the fourth quadrant (*Ganoderma*) seemed to have a positive influence (Table 2).

4 Discussion

The seasonal distribution of the different spore types occurred during late spring but especially in summer, as a consequence of the increment in temperatures measured during this period (AEMET; Sánchez Reyes 2011) as other studies have pointed out (Oliveira et al. 2005; Grinn-Gofron and Mika 2008). The same tendency was recovered by other cities of Castilla y León, such as León (Fernández et al. 1998) or Palencia (Herrero et al. 1996).

Cladosporium was the largest group captured in the aerobiological samples as occurred in some other Spanish cities (Díez et al. 2006; Recio et al. 2012), in some European and Mediterranean (Oliveira et al. 2005; Erkara et al. 2008; Pyrri and Kapnasaki-Gotsi 2015), but also in South America (De Antoni Zoppas et al. 2006; Mallo et al. 2011), in the USA (Levetin and Dorsey 2006), or even in South Africa (Dames and Cadman 1994), in Asia (Abu-Dieyeh et al. 2010) and in Australia (Rutherford et al. 1997), so that overall spores pattern was conditioning by *Cladosporium* results.

In that sense, Lima and Gadelha already established in 1983 that Deuteromycotina fungi (*Alternaria*, *Cladosporium*, *Dreschlera*, *Periconia*, etc.) have some characteristics (a low substrate demand, a high capacity to produce and release round spores of low density to the environment, etc.) which make them more abundant worldwide. The presence of *Cladosporium* is more related to urban sites (Awad 2005; Kasprzyk and Worek 2006) which could have been the reason for its great abundance in our study since it was conducted in a medium-sized city. However, we could detect that *Alternaria* annual pattern was also dependent on the proximity of crop fields, as Corden et al. (2003) observed, that are very abundant in the surroundings, since the release of these spores was produced between June and October coinciding with crop harvest season as Awad (2005) already established. Peak daily values were dated in July and August, and afterwards, there was a progressive reduction in its amount in Valladolid airspore, coinciding with the end of this harvest season, conversely

to cities located to the south that registered peaks in October (Maya-Manzano et al. 2012). Nevertheless, *Alternaria* and *Cladosporium* both presented a similar intra-daily pattern, having a greater representation towards the central hours of the day, as Fernández et al. (1998), Peternel et al. (2004) and ÓConnor et al. (2014) had already detected. With respect to *Cladosporium*, Fernández et al. (1998) determined an increment in its airborne concentrations with minimum temperatures above 13 °C, while *Alternaria* would need temperatures around 22–28 °C for an optimal growth, which reveals the adaptation of these spores to be dispersed in warm periods (Hjelmroos 1993), reporting negative correlations with rainfall for both genera (Aira et al. 2008). Our findings were similar in the case of temperatures, but only *Alternaria* correlated significantly and negatively with rainfall. Conversely, winds from the south direction had a significant negative influence on *Alternaria*, whereas northern winds showed a positive relation. Consulting the information provided by the Ministry of Environment and Rural and Marine Affairs (MAGRAMA, acronym in Spanish) through the Map of Crops and Land Use in Spain (2000–2010), this result should 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 other types of moulds associated such as *Botrytis*. On the other hand, south-easterly winds correlated strongly with *Cladosporium*. This could be explained by the fact that warmer temperatures registered in southern areas could have favoured the production of these spores which would be transported by air currents. In that sense, Fernández et al. (1998) and Hjelmroos (1993) determined minimum temperatures above 13 or 15 °C, respectively, for an optimal growth of this spore type, which are easily detected south to Valladolid.

The high representation of *Periconia* during winter months, together with the negative correlations coefficients observed with temperature and total hours of sunshine, could be related to this spore-type affinity for temperatures lower than 10 °C, as Pérez-Gorjón et al. (2003) have already proved in Salamanca. In fact, *Periconia* showed at Valladolid an intra-daily pattern with maximum values registered along the second half of the day, while in previous studies, carried out in India, where daily temperatures are

higher, these values were detected at the first hours of the day (Atluri et al. 1988).

However, the higher presence of *Dreschlera* during the summer time (which has a higher number of sun hours and less rainfall) could have conditioned the obtaining of positive and negative correlation coefficients with these parameters, respectively (Sánchez Reyes 2011).

Growth and liberation of basidiospores (as *Coprinus*, *Ganoderma*, etc.) is reduced in urban (Kasprzyk and Worek 2006) inland (Hasnain et al. 2004) environments, such as the monitored city, which could be the reason for the low records of these spore types detected in Valladolid. Other reason might be that spore traps were designed to capture higher size particles (Calderón et al. 1995), so their efficiency could decrease in the detection of basidiospores. *Ganoderma* showed a seasonal summer–autumn pattern coinciding with the one observed in others researches carried out in the northern hemisphere (Oliveira et al. 2005) and the southern hemisphere (De Antoni Zoppas et al. 2006) countries. Low count of these spores in the Valladolid airspora could be due to the adequate substrate scarcity of fungus in the spore trap surroundings and the low wind velocity, which limited the spore contribution from far away, although winds from the second and the fourth quadrant showed to have a positive influence. In that sense, at the south-east and the north-west of the Valladolid province (Map of Crops and Land Use in Spain 2000–2010, MAGRAMA), there are woody areas where *Ganoderma* is frequently found as De Antoni Zoppas et al. (2006) already observed, while in the south-west, there are areas with ecological characteristics less adequate for the development of this genus. *Coprinus* registered representation percentages higher each time during autumn season, as the substrate availability increased with the fallen leaves. It was not frequent to find *Ganoderma* or *Coprinus* basidiospores during the hottest hours of the day, as Aira et al. (2003) already observed, in order to avoid draining, being observed a daily periodicity in hourly concentrations which increase at night coinciding their behaviour with the daily pattern of relative humidity and temperature (AEMET), which could be the reason for the fact that their capture in the air is negatively correlated with the daily total sunshine. A similar intra-day distribution pattern with high *Coprinus*/*Ganoderma* spore counts at night has been reported by Hasnain et al. (2005) and

Almaguer et al. (2014) and a progressive drop until late afternoon by Stępańska and Wołek (2009).

The presence of Ascomycota spores (*Chaetomium*, *Leptosphaeria*, *Pleospora*, *Xylariaceae*, etc.) is frequent, but in low number, in aerobiological samples (Gonianakis et al. 2005), as we observed in the present paper, since spore concentrations decrease with the height at which the monitoring is carried out (Li et al. 2010). *Pleospora* had an irregular seasonal behaviour, together with significant and positive correlation coefficients with rainfall. Furthermore, a clearly different daily pattern was obtained with the model that removed rainy days. These results support the observations made by Angelosante Bruno et al. (2007) and Hernández Trejo et al. (2012) about the affinity of these spores for rainfalls and relative humidity, being important factors to take into account when studying them.

In that sense, Stępańska and Wołek (2009) have recently established the possibility that intra-daily patterns of some spore types such as *Alternaria* or *Ganoderma* could be different in dry or wet periods, at least in studies that comprise more than 1-year data.

5 Conclusions

The highest capture of fungal spores mainly occurred during summer and autumn months as a consequence of the abundance of *Cladosporium* spores. On the contrary, low temperatures, typical of continental winters, may have conditioned the lowest fungal spore registration along this season.

With respect to the daily patterns study, we propose the general use of model 2 since models 1 and 3 could introduce mistakes by including in the calculation days in which fungal spores were not detected (model 1) or eliminating those spores registered after rainfalls (model 3), which behaviour would not be reflected in the pattern obtained as it seems to occur with *Pleospora*.

“Dry-air” spores (*Alternaria*, *Cladosporium* and *Periconia*) registered their maximum hourly percentages coinciding with the central hours of the day or at night, while the presence of “wet-air” spores (ascospores and basidiospores) was related to high relative humidity conditions or even rainfalls.

“Dry-air” spores daily concentrations correlated positively with temperatures and sun hours as with the

absence of precipitations, while rainfalls and relative humidity had a positive influence on “wet-air” spores. The effect of wind velocity was conditioned by the spore’s size, mainly pointing out the influence of winds from the North on *Alternaria*, for the abundance of cereal crops, and winds from the second and the fourth quadrant on *Ganoderma* and *Coprinus*, for the presence of woody areas.

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References

- Abu-Dieyeh, M. H., Barham, R., Abu-Elteen, K., Al-Rashidi, R., & Shaheen, I. (2010). Seasonal variation of fungal spore populations in the atmosphere of Zarqa area, Jordan. *Aerobiologia*, 26, 263–276.
- Aira, M. J., La-Serna, I., & Dopazo, A. (2003). Identification of fungal spores in the atmosphere of Santiago de Compostela (NW Spain) in the winter period. *Polen*, 12, 65–76.
- Aira, M. J., Rodríguez-Rajo, F., & Jato, V. (2008). 47 annual records of allergenic fungi spore: Predictive models from the NW Iberian Peninsula. *Annals of Agricultural and Environmental Medicine* 15, 91–98.
- Almaguer, M., Rojas-Flores, T. I., Rodríguez-Rajo, F. J., & Aira, M. J. (2014). Airborne basidiospores of *Coprinus* and *Ganoderma* in a Caribbean región. *Aerobiologia*, 30, 197–204.
- Angelosante Bruno, A., Pace, L., Tomassetti, B., Coppola, E., Verdecchia, M., Pacioni, G., & Visconti, G. (2007). Estimation of fungal spore concentrations associated to meteorological parameters. *Aerobiologia*, 23, 221–228.
- Atluri, J. B., Varma, K. V., & Subba Reddi, C. (1988). Circadian periodicity in some airborne fungi over a rice crop. *Grana*, 27, 71–76.
- Awad, A. H. A. (2005). Vegetation: A source of air fungal bio-contaminant. *Aerobiologia*, 21, 53–61.
- Calderón, C., Lacey, J., McCartney, H. A., & Rosas, I. (1995). Seasonal and diurnal variation of airborne basidiomycete spore concentrations in Mexico City. *Grana*, 34, 260–268.
- Capel Molina, J. (1981). *Los climas de España*. Ed.Oikos-tau, S.A. Barcelona.
- Corden, J. M., Millington, W. M., & Mullins, J. (2003). Long-term trends and regional variation in the aeroallergen *Alternaria* in Cardiff and Derby UK—are differences in climate and cereal production having an effect? *Aerobiologia*, 19, 191–199.
- Dames, J. F., & Cadman, A. (1994). Airspora of Durban: A subtropical, coastal South African city. *Grana*, 33, 346–348.
- De Antoni Zoppas, B. C., Valencia-Barrera, R. M., Vergamini Duso, S. M., & Fernández-González, D. (2006). Fungal spores prevalent in the aerosol of the city of Caixas do Sul, Rio Grande do Sul, Brazil, over a 2-year. *Aerobiologia*, 22, 119–126.
- Díez, A., Sabariego, S., Gutiérrez, M., & Cervigón, P. (2006). Study of the airborne fungal spores in Madrid, Spain. *Aerobiologia*, 22, 135–142.
- Erkara, I. P., Asan, A., Yilmaz, V., Pehlivan, S., & Okten, S. S. (2008). Airborne *Alternaria* and *Cladosporium* species and relationship with meteorological conditions in Eskisehir City, Turkey. *Environmental Monitoring and Assessment*, 144, 31–41.
- Fernández, D., Valencia, R. M., Molnár, T., Vega, A., & Sagüés, E. (1998). Daily and seasonal variations of *Alternaria* and *Cladosporium* airborne spores in León (North-West, Spain). *Aerobiologia*, 14, 215–220.
- Galán, C., Cariñanos, P., Alcázar, P., & Domínguez, E. (2007). *Spanish aerobiology network (REA): Management and quality manual*. Servicio de Publicaciones de la Universidad de Córdoba 2007. http://www.uco.es/rea/infor_real/manual_eng.pdf.
- Galán, C., Tormo, R., Cuevas, J., Infante, F., & Domínguez, E. (1991). Theoretical daily variations patterns of airborne pollen in South-West of Spain. *Grana*, 30, 201–209.
- Gioulekas, D., Damialis, A., Papakosta, D., Spietsma, F., Giouleka, P., & Patakas, D. (2004). Allergenic fungi spore records (15 years) and sensitization in patients with respiratory allergy in Thessaloniki-Greece. *Journal of Investigational Allergology and Clinical Immunology*, 14(3), 225–231.
- Gonianakis, M., Neonakis, I., Darivianaki, E., Gonianakis, I., Bouros, D., & Kontou-Fili, K. (2005). Airborne Ascomycotina on the island of Crete: Seasonal patterns based on an 8-year volumetric survey. *Aerobiologia*, 21, 69–74.
- Grant Smith, E. (2000). *Sampling and identifying allergenic pollens and molds*. San Antonio, TX: Blewstone Press.
- Grinn-Gofroń, A., & Mika, A. (2008). Selected airborne allergenic fungal spores and meteorological factors in Szczecin, Poland, 2004–2006. *Aerobiologia*, 24, 89–97.
- Hasnain, S. M., Al-Frayh, A., Khatija, F., & Al-Sedairy, S. (2004). Airborne *Ganoderma* basidiospores in a country with desert environment. *Grana*, 43, 111–115.
- Hasnain, S. M., Khatija, F., Al-Frayh, A., & Al-Sedairy, S. (2005). Prevalence of airborne basidiospores in three coastal sites of Saudi Arabia. *Aerobiologia*, 21, 139–145.
- Hernández Trejo, F., Muñoz Rodríguez, A. F., Tormo Molina, R., & Silva Palacios, I. (2012). Airborne ascospores in Mérida (SW Spain) and the effect of rain and other meteorological parameters on their concentration. *Aerobiologia*, 28, 13–26.
- Herrero, B., Fombella, M. A., Fernández-González, D., & Valencia, R. M. (1996). Aerobiological study of fungal spores from Palencia (Spain). *Aerobiologia*, 12, 27–35.
- Herrero, B., & Zaldivar, P. (1997). Effects of meteorological factors on the levels of *Alternaria* and *Cladosporium* spores in the atmosphere of Palencia, 1990–92. *Grana*, 36, 180–184.
- Hjelmroos, M. (1993). Relationship between airborne fungal spore presence and weather variables. *Cladosporium* and *Alternaria*. *Grana*, 32, 40–47.
- Kasprzyk, I., & Worek, M. (2006). Airborne fungal spores in urban and rural environments in Poland. *Aerobiologia*, 22, 169–176.

- Levetin, E., & Dorsey, K. (2006). Contribution of leaf surface fungi to the air spora. *Aerobiologia*, 22, 3–12.
- Li, K., Dong, S., Wu, Y., & Yao, M. (2010). Comparison of the biological content of air samples collected at ground level and at higher elevation. *Aerobiologia*, 26, 233–244.
- Lima, J. A., & Gadelha, W. (1983). Contaminación de hongos del aire atmosférico en la ciudad de Recife (Pernambuco-Brasil). *Revista Latinoamericana de Microbiología*, 25, 243–251.
- Mallo, A. C., Nitiu, D. S., & Gardella Sambeth, M. C. (2011). Airborne fungal spore content in the atmosphere of the city of La Plata, Argentina. *Aerobiologia*, 27, 77–84.
- Maya-Manzano, J. M., Fernández-Rodríguez, S., Hernández-Trejo, F., Díaz-Pérez, G., Gonzalo-Garijo, A., Silva-Palacios, I., et al. (2012). Seasonal Mediterranean pattern for airborne spores of *Alternaria*. *Aerobiologia*, 28(4), 515–525.
- Ministry of Environment and Rural and Marine Affairs (MAGRAMA). Map of Crops and Land Use in Spain at 1: 50,000 years 2000–2010.
- Mitakakis, T. Z., & Guest, D. I. (2001). A fungal spore calendar for the atmosphere of Melbourne, Australia, for the year 1993. *Aerobiologia*, 17, 171–176.
- Nilsson, S., & Persson, S. (1981). Tree pollen spectra in the Stockholm region (Sweden) 1973–1980. *Grana*, 20, 179–182.
- O'Connor, D. J., Sadyś, M., Skjøth, C. A., Healy, D. A., Kennedy, R., & Sodeau, J. R. (2014). Atmospheric concentrations of *Alternaria*, *Cladosporium*, *Ganoderma* and *Didymella* spores monitored in Cork (Ireland) and Worcester (England) during the summer of 2010. *Aerobiologia*, 30, 397–411.
- Oliveira, M., Ribeiro, H., & Abreu, I. (2005). Annual variation of fungal spores in atmosphere of Porto. *Annals of Agricultural and Environmental Medicine*, 12, 309–315.
- Pérez-Gorjón, S., Rodríguez-de la Cruz, D., González-Suárez, R., & Sánchez-Sánchez, J. (2003). Variación anual de esporas en la atmósfera de Salamanca durante los años 1995 y 2000. *Polen*, 13, 289–297.
- Peternel, R., Čulig, J., & Hrga, I. (2004). Atmospheric concentrations of *Cladosporium* spp. and *Alternaria* spp. spores in Zagreb (Croatia) and effects of some meteorological factors. *Annals of Agricultural and Environmental Medicine*, 11, 303–307.
- Pyrri, I., & Kapnasaki-Gotsi, E. (2015). Evaluation of the fungal aerosol in Athens, Greece, based on spore analysis. *Aerobiologia*, 31, 179–190.
- Recio, M., Trigo, M. M., Docampo, S., Melgar, M., García-Sánchez, J., Bootello, L., & Cabezudo, B. (2012). Analysis of the predicting variables for daily and weekly fluctuations of two airborne fungal spores: *Alternaria* and *Cladosporium*. *International Journal of Biometeorology*, 56, 983–991.
- Rivas Martínez, S. (2007). Mapa de series, geoserias y geopermaseries de vegetación de España [Memoria del Mapa de Vegetación Potencial de España. Parte I]. *Itinera Geobotánica*, 17(1), 1–436.
- Rodríguez-de la Cruz, D., Sánchez-Reyes, E., Dávila-González, I., Lorente-Toledano, F., & Sánchez-Sánchez, J. (2010). Airborne pollen calendar of Salamanca, Spain, 2000–2007. *Allergologia et Immunopathologia*, 38(6), 307–312.
- Rutherford, S., Owen, J. A. K., & Simpson, R. W. (1997). Survey of airspora in Brisbane, Queensland, Australia. *Grana*, 36, 114–121.
- Sánchez Reyes, E. (2011). *Estudio aeropalínológico de la ciudad de Valladolid durante los años 2005–2006 y su relación con los cuadros clínicos de alergia*. Ph.D. Thesis. University of Salamanca, Spain.
- Spieksma, F. Th. M. (1991). Regional European Pollen Calendars. In G. D'Amato, F. Th. M. Spieksma, & S. Bonini (Eds.), *Allergenic pollen and pollinosis in Europe* (pp. 49–65). Oxford: Blackwell Scientific Publications.
- Stępańska, D., & Wołek, J. (2009). Intradial periodicity of fungal spore concentrations (*Alternaria*, *Botrytis*, *Cladosporium*, *Didymella*, *Ganoderma*) in Cracow, Poland. *Aerobiologia*, 25, 333–340. <http://www.saludcastillayleon.es/ciudadanos/es/polen>.