



Analysis of the airborne fungal spores present in the atmosphere of Salamanca (MW Spain): a preliminary survey

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Abstract In this paper, we expose a preliminary study of the airborne fungal spore content in the atmosphere of Salamanca City including a fungal spore calendar. Sampling was carried out between February 2014 and February 2016, and along these 2 years of monitoring *Cladosporium* was the most abundant type followed by *Aspergillus/Penicillium*, both present from January to December. The peaks of diversity and abundance were established in two well-defined seasons focused on autumn (especially basidiomycota) and late spring. Others, like *Cladosporium* and *Alternaria*, called the dry spore types, had their maximum during summer. According to the intradiurnal pattern, the results were very similar for the spore types, except for *Agaricus*, *Coprinus* and *Periconia* that showed a nocturnal release pattern. The meteorological factors that had a clearly influence on the spore distribution were temperature, in the case of the dry spore types, and relative humidity in the case

of wet air spores like *Agaricus* and *Coprinus* both basidiomycetes.

Keywords *Cladosporium* · Spore calendar · Aerobiology · Spain · Meteorological parameters

1 Introduction

The study of the airborne content of biological particles is becoming more and more relevant due to the relationship that exists between the science that studies these particles, aerobiology, and other sciences such as medicine (Bush and Portnoy 2001; Tham et al. 2017), agriculture (Frenguelli 1998; Magyar et al. 2006), urban environment (Damialis et al. 2017; Pyri and Kapsanaki Gotsi 2017) and more recently works about the climate change and its influence on other fields (Kaczmarek et al. 2016). In the aerosol, there exist both pollen grains and fungal spores among other biological particles such as viruses, bacteria and other microorganisms and plant material as well as inorganic particles such as air pollutants and dust. The pollen content has been studied for centuries, and there does exist a great number of studies in palynology, including not only about pollen behaviour, quantity or distribution in the atmosphere (Aira et al. 2013), but also about the influence of meteorological factors on their concentrations (Sánchez Reyes et al. 2009a; Skjørth et al. 2016) and the intensity of pollen seasons

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related to clinical aspects (Heinzerling et al. 2009; Rodríguez et al. 2011).

Nevertheless, the fungal spores are not so well known. As Grinn Gofroń and Bosiacka suggest (2015), the aeromycota has been extensively studied, but these pieces of research were carried out in several countries and continents where their importance could be related to specific climatic conditions (Ho et al. 2005), polluted cities (Pyrri and Kapsanaki Gotsi 2017) or medical interests (Chen et al. 2014).

In regard to Europe, several papers exist in different countries like Romania (Ianovici 2016), Austria (Haas et al. 2014), Portugal (Almeida et al. 2018) and Hungary (Magyar et al. 2018), among others. There are certain countries such as Poland (Kasprzyk and Worek 2006; Grinn Gofroń et al. 2016b), France (Sindt et al. 2016) or the UK and Ireland (O'Connor et al. 2014; Sadyś et al. 2016; Sadyś 2017) with a great amount of research in aerobiology, not only in pollen studies but also in fungal spores. Most of these countries have cold climates in which fungal spores can exhibit a different behaviour compared to the Mediterranean region. In that sense, the studies conducted in this area could be interesting for the climate similarities with other countries as Greece (Damialis and Gioulekas 2006), Turkey (Erkara et al. 2008) and Morocco (El Haskouri et al. 2016; Bardei et al. 2017).

In Spain, studies related to the fungal aerosol are very scarce and mostly include data from last several decades (Domínguez Santana and La Serna 1998; Pérez Gorjón et al. 2003; Morales et al. 2006). However, within the last 10 years, new articles have been published in several points of the Iberian Peninsula (Aira et al. 2012) and more specifically in Catalonia (Vélez Pereira et al. 2016), Andalusia (Martínez Bracero et al. 2018) and Castilla–La Mancha (Sabariego et al. 2012) and cities closer to Salamanca with similar weather conditions, such as Valladolid (Sánchez Reyes et al. 2016), Mérida and Plasencia (Hernández Trejo et al. 2012; Maya Manzano et al. 2016).

The difficulty that implies the study of the fungal aerosol is due to the great number of spores present in the atmosphere, added to the ubiquitous fungi distributed widely, induced the low amount of recent research. This is why spore calendars are still rare to find, with only a few studies distributed worldwide, in places like Australia (Mitakakis and Guest 2001),

India (Das and Gupta Bhattacharya 2008), Habana (Almaguer et al. 2015), Poland (Bednarz and Pawlowska 2016) and Spain (Sánchez Reyes et al. 2016). In other surveys, the spore calendar includes one or two different spore types (Katotomichelakis et al. 2016).

This paper aims at the increase in the knowledge of the seasonal behaviour of fungal spores in the middle west of the Iberian Peninsula, through the elaboration of a preliminary spore calendar that could be useful for patients who are sensitive to some types of spores. In the same way, we want to increase the knowledge of their hourly behaviour as well as the influence of meteorological parameters on their atmospheric levels.

2 Materials and methods

The study has been carried out in the city of Salamanca (40°58'N; 5°39'W), located in the middle west of Spain, at a height of 802 m.a.s.l. and belonging, from the biogeographical point of view, to the Mediterranean region (Rivas Martínez 2007). The city is surrounded by arable farming lands to the north-east and by wide expanses of Mediterranean pastures (called *dehesas* in Spanish) to the south and west. The climate is continental cold tempered with a dry season (Capel Molina 2000), characterized by long winters, with several frosts through the year, and summer when a hard drought always takes place. In Salamanca, according to a 30-year average (1981–2010), the annual mean temperature is 12.2 °C with a mean temperature of 18.7 °C during the hottest month and a low rainfall average (372 mm) through the year.

The monitoring was conducted from 17 February 2014 to 16 February 2016, both included. Fungal spores were captured by using a Hirst (1952) spore trap sampler, placed on the roof of the Pharmacy's building at 25 m above ground level. The trap sucked 10 l of air per minute through a small aperture in the front side as it explains the minimum requirements for the aerobiological monitoring (Galán et al. 2014). According to the methodology established by the Spanish Aerobiology Network (Galán et al. 2007), the spores were counted in four longitudinal traverses of contiguous fields along the length of the slide, of equal width, at 400 × magnification in an optical microscope. Daily data are expressed as spores/m³ (Galán

et al. 2017). For spores identification, different keys and texts were used (Grant Smith 2000; Sáenz and Gutiérrez-Bustillo 2003), identifying the spores to the genus level. In that sense, we considered *Aspergillus* and *Penicillium* together as one single category (*Aspergillus/Penicillium* type) because it is difficult to separate both genera (Pyrri and Kapsanaki Gotsi 2007; Almaguer et al. 2015). Also, the category of “others” includes all the unidentified and damaged spores.

The spore calendar was performed according to the Spieksma’s model (Spieksma 1991) but was adapted for spore types (Sánchez Reyes et al. 2016), classifying the 10-day mean concentration into eleven different categories. The order indicated in the calendar was based on their appearing in the air spectra, including only the spore types that showed an annual percentage equal or greater than 1%.

The statistical analysis was performed only for those spore types whose representation percentages reached, together, 90% of the annual total (*Agaricus*, *Alternaria*, *Aspergillus/Penicillium*, *Cladosporium*, *Coprinus*, *Leptosphaeria*, *Periconia* and total spore concentration).

In that sense, we first studied the most relevant characteristics of their main spore season (MSS) (total spore concentration in the MSS—90%, starting and ending date of the MSS, MSS length, peak day and peak values). The MSS was defined with the method that calculates the start of this period when the 5% of the total year spore record was registered, and the end with the 95% captured (Nilsson and Persson 1981). Along with the MSS, we also made a seasonal periodicities study to see the fungal spores behaviour through the months and establish the prevalence in the atmosphere of the aeromycota. For this proposal, we created graphics with the monthly sum of spores.

For the intradiurnal variation study, we chose one model, in which the importance was altered whether it rained or not (Galán et al. 1991; Aira et al. 2003). First, the daily average of the spore types was calculated using the total days of the MSS period through the 2 years of study, and for the hourly concentrations, we only selected the dry days with a daily value equal or higher than the average calculated. Taking into account only these selected days, we calculated the hourly average concentrations. For graphical representation, the concentrations were expressed in terms of 2-h mean percentage.

The influence of the meteorological factors on the spore concentration during the MSS was analysed with the SPSS software (version 23), using the Spearman nonparametric correlation test as the daily data were neither normally nor log-normally distributed. The meteorological factors studied were mean, maximum and minimum daily average temperature (°C), total daily rainfall (mm), daily average wind speed (km/h), daily average frequency of winds from different quadrants (north-easterly, south-easterly, south-west-erly and north-westerly) (%), daily average frequency of calms (%) and daily average sunshine (%). All the meteorological data have been delivered by the Spanish State Meteorological Agency (AEMET), obtained from the station located at Matacán Airport (40°57'N; 5°29'W; 790 m.a.s.l.), 15 km away from Salamanca City.

3 Results

3.1 Main spore season of different taxa

A total of 57 different spore types were identified during the 2 years of study, and all those broken or unidentified spores were included in the others category (Table 1). The total number of spores recorded in the 2 years of the study was similar, although it was lower during the second year (238,935 spores in 2014 and 210,943 spores in 2015). In both years, the season when the spore trap registered the highest quantity was summer (the mean value was around 45% of the total spore concentration), followed by autumn (31%) and spring (17%). Winter was the least abundant season with a 7% mean value of the total spore concentration.

Cladosporium was the most abundant genus (166,748 spores in 2014 and 154,804 in 2015), followed by *Aspergillus/Penicillium* type which decreased in 2015 (13,282 spores) with respect to the previous year (25,987), as it is shown in Table 2. The rest of the spore types analysed did not reach 10,000 spores per year. *Cladosporium* spores also reached the highest daily value (5746 spores/m³ in 2014 and 4514 in 2015) in both years in July (21st and 27th, respectively) in contrast to *Leptosphaeria* with the lowest peak levels (103 and 44 spores/m³) in April and July. *Agaricus*, *Alternaria* and *Coprinus* had similar values in MSS during 2014 (6792, 6073 and 6464 spores, respectively) (Table 2). In addition, *Agaricus*

Table 1 List of all spores identified during the 2 years of study

Spore types identified during 2014–2016	
<i>Agaricus</i>	<i>Leptosphaerulina</i>
<i>Agrocybe</i>	<i>Lophiostoma</i>
<i>Alternaria</i>	<i>Massarina</i>
<i>Ascobolus</i>	<i>Myxomycetes</i>
Ascospores	<i>Nigrospora</i>
<i>Aspergillus/Penicillium</i> type	<i>Octospora</i>
<i>Bovista</i>	<i>Oidium</i>
<i>Caloplaca</i>	<i>Paraphaeosphaeria</i>
<i>Capronia</i>	<i>Passalora</i>
<i>Cercospora</i>	<i>Periconia</i>
<i>Cerebella</i>	<i>Peronospora</i>
<i>Chaetomium</i>	<i>Phaeosphaeria</i>
<i>Cladosporium</i>	<i>Phylacteria</i>
<i>Coprinus</i>	<i>Pleospora</i>
<i>Curvularia</i>	<i>Polythrincium</i>
<i>Deightonella</i>	<i>Pteridium</i>
<i>Diatrypaceae</i>	<i>Puccinia</i>
<i>Deschlera</i>	<i>Pithomyces</i>
<i>Entoloma</i>	<i>Saccobolus</i>
<i>Epicoccum</i>	<i>Spiegazzinia</i>
<i>Exosporium</i>	<i>Spondilocladiella</i>
<i>Fossombronina</i>	<i>Sporormiella</i>
<i>Fusarium</i>	<i>Stemphylium</i>
<i>Ganoderma</i>	<i>Tilletia</i>
<i>Helicoma</i>	<i>Torula</i>
<i>Helminthosporium</i>	<i>Ustilago</i>
<i>Inocybe</i>	<i>Venturia</i>
<i>Isthmospora</i>	<i>Xylariaceae</i>
<i>Leptosphaeria</i>	Others

and *Coprinus*, both belonging to basidiomycota, showed the same peak day in November, during 2014 and 2015 (22nd and 4th, respectively). *Alternaria* showed a late start in the 2 years (May 8 and 4), a similar peak value (286 spores/m³ in 2014 and 291 in 2015) and a similar MSS length (171 days in 2014 and 191 in 2015). *Periconia* spores displayed a winter behaviour reaching the maximum peak value in December (2014) and November (2015). During 2015, the MSS length was 1 month shorter, although in both years the start date was almost the same (March 20 and 19).

3.2 Seasonal periodicities

Seasonal behaviour of the most representative types (*Agaricus*, *Alternaria*, *Aspergillus/Penicillium*, *Cladosporium*, *Coprinus*, *Leptosphaeria*, *Periconia* and total spore concentration) are presented in Fig. 1. *Agaricus* reached the maximum concentration (1530 and 3548 spores) in October and November coinciding with autumn in the 2 years. *Alternaria* showed a calm pattern except in summer when it reached the maximum values in July (3243 spores) and June (1468 spores) for 2014 and 2015, respectively. *Aspergillus/Penicillium* had a low concentration except during May of 2014 (15,719 spores) when they presented their maximum value and the monthly peak in the 2 years of study, although during 2015, they showed a decrease (4214 spores) (Fig. 1). *Cladosporium* exhibited an irregular pattern with several peaks through the year, having the most abundant concentration in summer (63,016 spores in July 2014) and early autumn (22,084 spores in October, 2015). *Coprinus* only had a representative peak in 2014 (2102 and 1949 spores) in October and November, respectively, keeping low values in 2015. *Leptosphaeria* displayed high spore levels in spring (677 spores in April) and winter (626 spores in November) during 2014, while in 2015 a great spore presence was recorded in June (447 spores). *Periconia* showed a winter pattern having the maximum value in 2015 (2082 spores in November), and the total spore concentration had a regular pattern except for July 2014 in which 70,869 spores were registered due to the presence of *Cladosporium* spores (Fig. 1).

3.3 Spore calendar

According to the Spieksma's classification, only the total spore concentration reached the maximum spore level (class 11), with more than 1600 spores/m³ during the first 10 days of June. *Cladosporium*, as the most abundant spore type, showed a class 10 (800–1600 spores/m³) from June to the first 10 days of September. *Aspergillus/Penicillium* had the maximum concentration peak in the first 10-day mean of May (400–799 spores/m³; Class 9), decreasing through the year. *Agaricus* was registered in a class 7 (100–199 spores/m³) in the last 10 days of October and first 10 days of November. Both *Alternaria* and *Coprinus* reached an exponential class 6, with a

Table 2 Main spore season (MSS) of the main spore types studied

	Total MSS (90%)	Start MSS	End MSS	MSS length (days)	Peak day	Peak value (spores/m ³)
2014						
<i>Agaricus</i>	6792	7-Apr	15-Jan	284	22-Nov	139
<i>Alternaria</i>	6073	8-May	25-Oct	171	9-Jul	286
<i>Aspergillus/Penicillium</i>	25,987	19-Apr	18-Nov	214	12-May	2103
<i>Cladosporium</i>	166,748	17-Apr	7-Dec	235	21-Jul	5746
<i>Coprinus</i>	6464	2-Apr	6-Dec	249	22-Nov	209
<i>Leptosphaeria</i>	3958	17-Mar	13-Jan	303	15-Apr	103
<i>Periconia</i>	4050	20-Mar	27-Jan	314	30-Dec	339
Total	238,935	11-Apr	15-Dec	249	21-Jul	5945
2015						
<i>Agaricus</i>	9735	2-May	3-Jan	247	4-Nov	477
<i>Alternaria</i>	5049	4-May	10-Nov	191	17-Jun	291
<i>Aspergillus/Penicillium</i>	13,282	14-Apr	9-Dec	240	4-May	733
<i>Cladosporium</i>	154,804	14-Apr	27-Nov	228	27-Jul	4514
<i>Coprinus</i>	3492	9-Mar	1-Feb	330	4-Nov	59
<i>Leptosphaeria</i>	2966	7-Apr	1-Feb	301	30-Jul	44
<i>Periconia</i>	5861	19-Mar	9-Dec	266	19-Nov	303
Total	210,943	17-Apr	5-Dec	233	1-Aug	4746

number of spores between 50 and 99 spores/m³, but their presence in the atmosphere changed in both cases, finding *Alternaria* spore maximum during the second 10 days of June and all July, while *Coprinus* appeared at its maximum during the second 10 days of November. Other two types that reached an exponential class 6 were *Periconia* and *Pleospora* which reached the maximum values in November and April, respectively. *Bovista* (September), *Epicoccum* (October–November) and *Ganoderma* spores (August–September) showed high concentrations in an exponential class number 5 (24–49 spores/m³). The other spore types (*Leptosphaeria* and *Myxomycetes*) had low values (12–23 spores/m³; class 4), and the category of others revealed 29–49 spores/m³ (exponential class 5) during April (Fig. 2). According to the permanence in the atmosphere, all the spore types reached a 10-day average equal or higher than 1% throughout the year, except for *Ganoderma* spores which only appeared during 26 of the 36 10-day mean periods, from the end of March to the beginning of December.

With regard to the diversity of fungal spore types, maximum values were registered during spring and autumn. In the first year of the study, the spore trap

caught 47 different types in spring and 46 in autumn, while in 2015 the spore types were 43 during spring and 50 in autumn. In both cases, the different spore types constituted a percentage between 75 and 80% of the total spore diversity at these two seasons. Over the course of the 2 years, the quantity of different spores changed according to the meteorological factors. Although in summer the highest values were registered, especially in June–July, due to the major amount of *Cladosporium*, the two main dispersal seasons were well defined in late spring (April–May) and autumn (September–November). On the other hand, the season with the less fungal spore diversity was winter, in which the cold months (January–February) induced a decrease in the presence of several spores.

3.4 Intradaily variations

The different fungal spore types showed a pattern with similar results in the 2 years of the study but with differences between the spore types and the hourly distribution (Fig. 3). In that sense, we classified the aeromycota into three categories, morning, afternoon and night spores, depending on their releasing pattern.

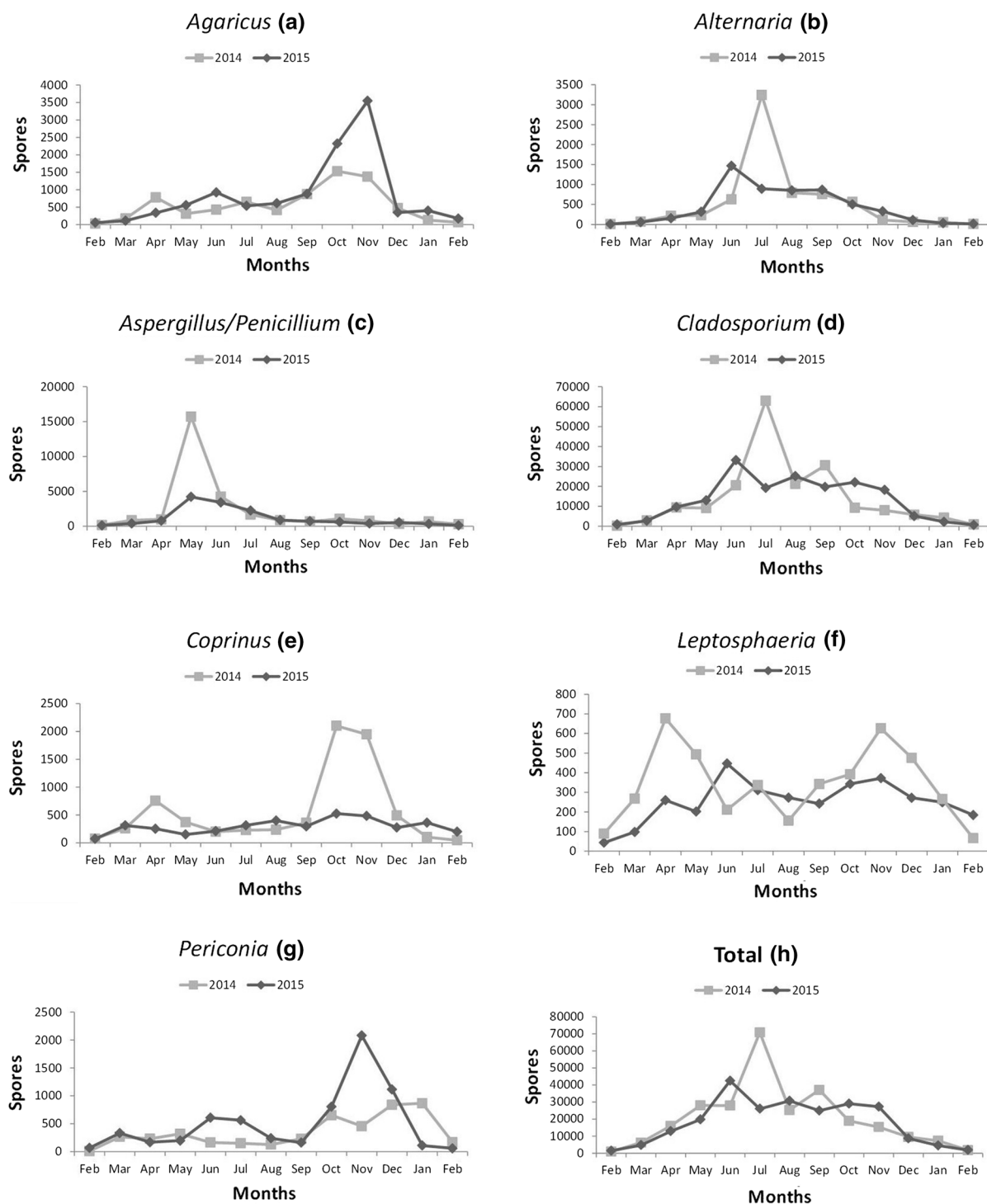


Fig. 1 Seasonal periodicities of the main spore types in the atmosphere of Salamanca

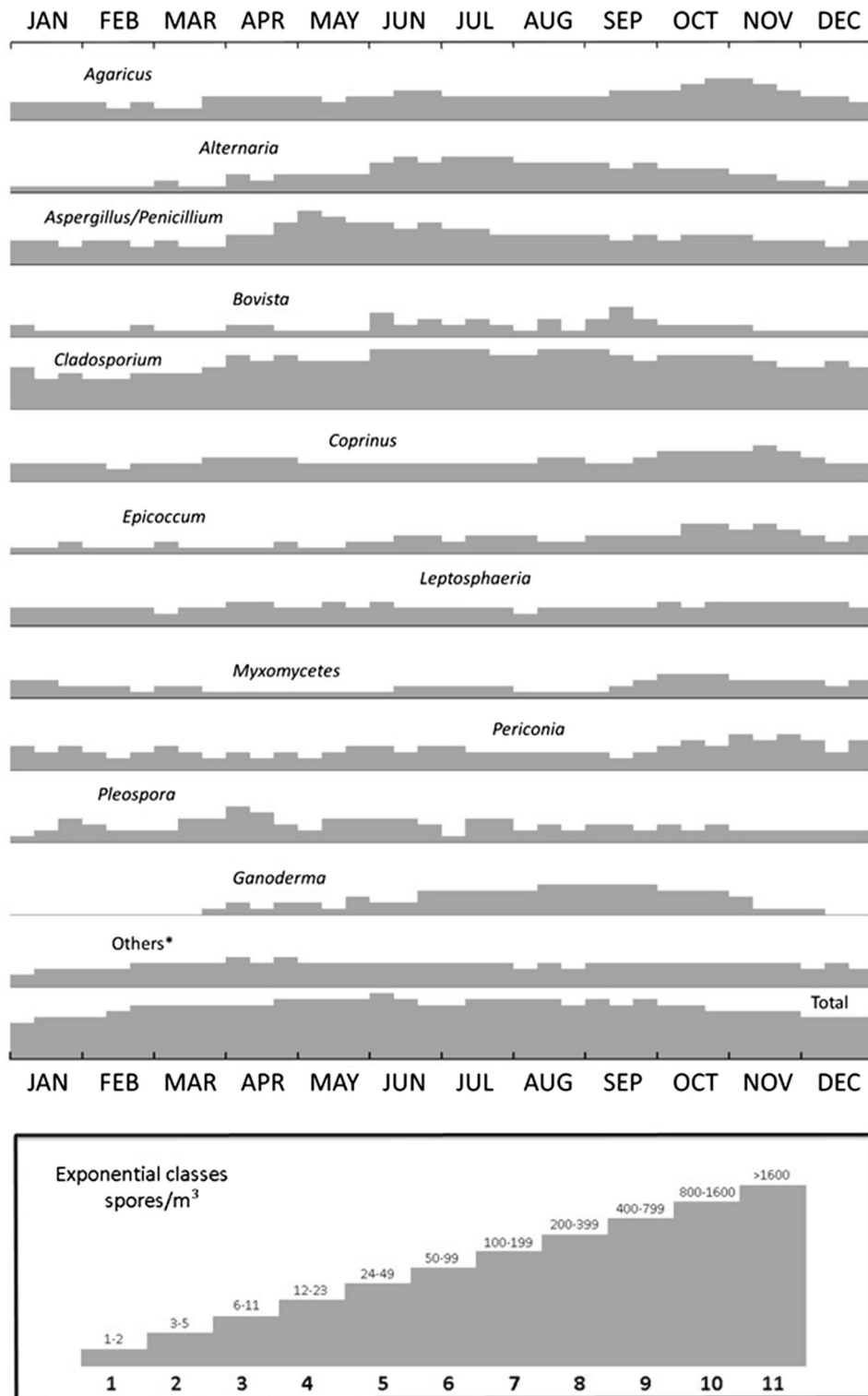


Fig. 2 Spore calendar for Salamanca City. *Including unknown spores and those spores that presented percentage lower than 1%

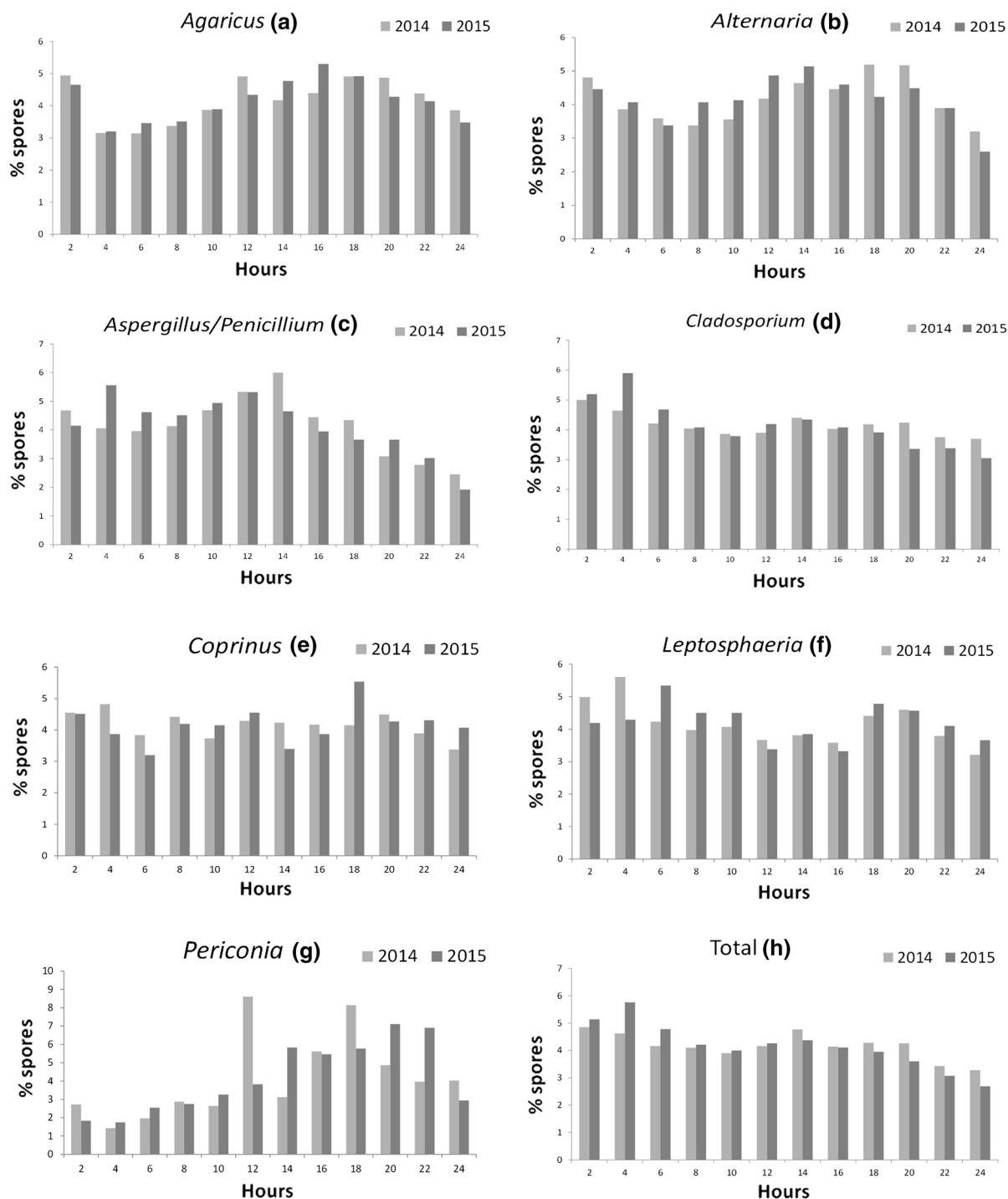


Fig. 3 Fungal spores intradiurnal patterns (2 h mean)

According to the first hours of the morning, *Cladosporium* and *Leptosphaeria* showed peaks between 2 and 4 h, maintaining the spore

concentration stable without peaks in the afternoon and decreasing at 22 h. *Alternaria* and *Aspergillus/Penicillium* spore types showed an increment through

the evening, lasting until the first hours of the night in the case of *Alternaria*. Those spores which showed a nocturnal release pattern were *Agaricus*, *Coprinus* and *Periconia* which revealed increments since 6 h, in the case of *Agaricus*, and 10 h for *Periconia*, with a few hours of delay for *Coprinus*. As Fig. 3 shows, the spore concentration for *Periconia* increases after 12 h but, due to the different concentration values between the 2 years, we cannot confirm a trend in the 12–24-h interval. The total sum of spores showed the same intradiurnal pattern than *Cladosporium* due to the great abundance of this type in the atmosphere.

3.5 Correlations with meteorological parameters

The results of the different correlations with meteorological patterns were represented in Table 3. According to the values obtained, the maximum positive correlation was 0.442, being the rest of the results not very high. Moreover, with only 2 years of data it is not possible to confirm that these results are conclusive and results should be improved including more data in future analysis. Only two spore types obtained negative correlation coefficients with temperature (*Coprinus* and *Leptosphaeria*). Furthermore, *Periconia* showed no significance with the maximum and mean temperature, but it was negatively correlated with the minimum temperature.

Rainfall obtained negative correlation coefficients with all the spore types, except for *Agaricus* and *Leptosphaeria*, whereas *Coprinus* was positively correlated with relative humidity along with *Agaricus* and *Leptosphaeria*.

Only *Aspergillus/Penicillium* displayed a significant positive influence by wind speed (Table 3). *Alternaria* and *Cladosporium* registered a high positive correlation with the north-easterly and south-easterly winds. In the case of south-westerly winds, *Agaricus*, *Coprinus* and *Leptosphaeria* were positively correlated with this parameter. Frequency of calms only showed a positive relationship with *Agaricus*, *Coprinus* and *Periconia* and a negative correlation with *Aspergillus/Penicillium* type. Sunshine is positively correlated with total fungal spore levels, displaying a little negative influence in the case of *Agaricus*.

Table 3 Spearman correlation coefficients obtained between mean daily spore concentration and meteorological factors during the main spore season (MSS)

	$T_{\max}$	$T_{\min}$	T_{mean}	R	RH	WS	Wind NE	Wind SE	Wind SW	Wind NW	CF	Sunshine
<i>Agaricus</i>					.128**	-.127**	-.130**		.093*		.086*	-.092*
<i>Alternaria</i>	.442**	.253**	.413**	-.345**	-.248**	-.216**	.224**	.111*	-.195**			.128*
<i>Aspergillus/Penicillium</i>	.331**	.225**	.315**	-.195**	-.494**	.225**		-.162**		.124**	-.223**	.140**
<i>Cladosporium</i>	.373**	.357**	.396**	-.211**	-.168**	-.209**	.233**	.178**	-.183**			
<i>Coprinus</i>	-.141**	-.143**	-.155**	.386**	.258**	-.130**	-.110**		.100*		.133**	.135**
<i>Leptosphaeria</i>	-.215**		-.151**	-.195**	.328**		-.93*		.127**			
<i>Periconia</i>		-.134**				-.220**		.193**			.139**	
Total	.364**	.339**	.377**	-.140**	-.215**	-.122**	.183**	.134**	-.129**			.102*

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 (%)

Significance levels: *95%; **99%

4 Discussion

Airborne fungal spore diversity and their abundance found in our study match other papers from different areas of the world where *Cladosporium* spores were also the most abundant spore types in the atmosphere (Hasnain et al. 2012; Almaguer et al. 2015; Khan et al. 2016). The different spore types were distributed in the late spring and autumn, but *Alternaria* and *Cladosporium* showed their maxima in July, probably due to the increase in temperature. The high presence of *Alternaria* during June–August was connected to the harvest period, which increased the levels of this spore types in the atmosphere, as indicated by Corden et al. (2003). The distribution of *Cladosporium* occurs during the warm and dry season, the maximum values being obtained in July and June (2014 and 2015, respectively) (Fig. 1). This distribution coincides with other studies from different cities of Spain, such as Valladolid (Sánchez Reyes et al. 2009b) and other countries in the Mediterranean region (Pyrri and Kapsanaki Gotsi 2017). *Aspergillus/Penicillium* spore types had a peculiar behaviour. As Hasnain et al. (2012) already set, these fungal spores do not show a clear seasonal pattern and the spores are distributed over different months in different countries, as pointed out in research carried out in Mexico (Rosas et al. 1993), Ireland (O’Gorman and Fuller 2008) and Jordan (Abu Dieyeh et al. 2010). Recently, Pyrri and Kapsanaki Gotsi (2015) exposed that the absence of a well-defined seasonal pattern was because *Aspergillus* and *Penicillium* spores had overlapping patterns, and the concentration of *Aspergillus* increases during summer, while that of *Penicillium* decreases.

The group of basidiospores, *Agaricus* and *Coprinus*, had a very similar monthly distribution according to their requirements of humidity and organic substrate. The main release season for both spore types was autumn, more specifically in the case of *Coprinus* which needs a high humidity in the soil for the development of its carpophore (Aira et al. 2003). Although *Agaricus* also needs humidity, it can also grow in other seasons like summer and in other countries with high temperatures (Hasnain et al. 2005). In most cases, this could be possible due to the presence of gardens and wet areas within cities.

Leptosphaeria showed high atmospheric levels in June 2015, although its daily peak during that year fell on July 30. This could be explained by the fact that, as

pointed out by Grinn Gofroń et al. (2016a), spores can be blown from crops located 400 km away, and this happens in early July. *Periconia* showed a typical pattern of a wintry spore type, like other surveys pointed out (Sánchez Reyes et al. 2012, 2016). During 2015, the spores had an increase in June–July, probably due to the minimum temperature registered in some days of these months, lower than 10 °C, and within the affinity ranges of *Periconia* (Pérez Gorjón et al. 2003).

In the case of the total spore count, we could verify that the most abundant type of spores (*Cladosporium*) was the one that determined the seasonal distribution of the total sum of airborne spores. Similar results were noted in other studies (Sabariego et al. 2007; Sánchez Reyes et al. 2009b) in which the total spore graphic took the same morphology than *Cladosporium* data. The MSS length of the total concentration was very similar in the 2 years, being registered from April to December, due to the major types of *Alternaria*, *Aspergillus/Penicillium* and *Cladosporium* spores being inside the total MSS.

Taking a look at the spore calendar (Fig. 2), *Cladosporium* representation in the total airborne spectrum is similar to other studies carried out in the Iberian Peninsula (Aira et al. 2012; Almeida et al. 2018) and in other remote geographical areas such as Australia (Irga and Torpy 2016). The abundance of *Cladosporium* spores in the atmosphere was also reported by several authors (Sánchez Reyes et al. 2016; Sousa et al. 2016; Almaguer Chávez et al. 2018), and one of the factors that influences its distribution is the climatic conditions needed for sporulation; optimal temperature for *Cladosporium* development must be between 13 and 21 °C (Sabariego et al. 2000). On the other hand, *Cladosporium* is related to the urban environment, as it was pointed out by Pérez Gorjón et al. (2003). Together with *Cladosporium*, other two spore types (*Aspergillus/Penicillium* type and *Agaricus*) were registered in high values. *Agaricus*, *Aspergillus/Penicillium* and *Cladosporium* appear in the aerobiological bibliography several times as the most abundant spore types (Morales et al. 2006; Hasnain et al. 2012; Haas et al. 2014; Pyrri and Kapsanaki Gotsi 2017). *Aspergillus/Penicillium* spore types were registered with the highest monthly values during May (Table 2), and their values were kept high until August. Adhikari et al. (2006) obtained similar values for these spore

types with the highest concentrations happening between June–August, but these results might be different from country to country as Abu Dieyeh and collaborators pointed out (2010), with the peaks of *Aspergillus* and *Penicillium* spores changing depending on the different regional and local parameters. The presence of *Agaricus* as the third most abundant spore type is not rare because the fact that these fungi produce a great number of spores and are widely distributed (Hasnain et al. 2005). The fruit bodies can be found in different periods of the year, not only in autumn. In this study, the monthly maximum was detected in November, but the main spore season started in April–May and then there were little peaks during June in the first year and July in the second. This is because in the urban parks and gardens, the conditions for the growing of *Agaricus*, both humidity and substrate are the most adequate. Furthermore, in the surrounding area of the city of Salamanca, forest can be found. Here, the increase in dead matter in autumn induces the growing of *Agaricus* in a great number of locations.

On the other hand, *Alternaria* was not one of the most abundant spore types, unlike what other research pointed out (Grinn Gofroñ and Bosiacka 2015; Khan et al. 2016) and the results were different from other similar studies. In the study performed in Sánchez Reyes et al. (2016) found a great quantity of these spores, with it being distributed with maximum values in summer. These high values probably were related to the location of Valladolid in the centre of the Iberian Peninsula and the fact that it was totally surrounded by farming lands and Salamanca only had this situation in the north-east quadrant. The low presence of *Alternaria* spores facing the massive abundance of *Cladosporium* and *Aspergillus/Penicillium* spores can be explained, not only by the distance to the farming lands, but also by the size of the spores. Compared to *Cladosporium*, *Alternaria* spores are heavier (Hasnain et al. 2012) and temperature for their optimal growth is higher than for *Cladosporium* spores (Fernández et al. 1998); hence, the distribution of *Alternaria* spores was established in the summer months. It is important to point out that *Alternaria*, *Aspergillus/Penicillium* and *Cladosporium* spores have been studied for a great number of years due to their relation to human health. Licorish et al. (1985) studied how *Alternaria* and *Penicillium* influence the development of asthma, and most recently it has been established that

Cladosporium spores affect the respiratory process, when daily concentrations of 1500 spores/m³ are observed (Chen et al. 2014). The levels of *Cladosporium* spores exceeded that limit during 33 days in 2014 and during 15 days in 2015. This may affect the allergic population especially during July when these spore types occur in peak values (5746 spores/m³ in 2014 and 4514 spores/m³ in 2015) (Table 2).

Coprinus spores had a relatively regular pattern during 2015, but during the first year of sampling these spores showed two periods with the greater presence in the atmosphere, one in April and another during October–November, although in the 2 years the peak day was detected in November (Table 2), when the optimal conditions of humidity were present (Aira et al. 2003). In the case of *Periconia*, the results obtained were very similar to the study conducted in Valladolid (Sánchez Reyes et al. 2016), which indicated a major presence of these spore types in the winter period. Other study conducted in Salamanca (Pérez Gorjón et al. 2003) established that *Periconia* had affinity for low temperatures (under 10 °C). In other parts of the world, a different behaviour shows increase in the spore levels in the atmosphere with a lower relative humidity and the presence of sandstorms (Ho et al. 2005).

The least abundant spore type in the atmosphere was *Leptosphaeria*. These spores were studied in several occasions due to their implication in agriculture, as it damages the oilseed rape crops worldwide (Magyar et al. 2006; Jedryczka et al. 2008), and also in human health as it causes symptoms of asthma (Tham et al. 2017; Sadyś et al. 2018). In the majority of research, these spores appear in great number and always in humid countries or those with high rainfall levels (Mallo et al. 2011; Almaguer Chávez et al. 2018), but in our study *Leptosphaeria* barely exceeded 1.5% of the total spore count, probably due to the low presence of oilseed rape crops in the outskirts of the city. The other spores that reached at least 1% of representation were *Bovista Epicoccum*, *Ganoderma* the *Myxomycetes* type and *Pleospora*. *Ganoderma* and *Pleospora* reached high concentrations in the study conducted in Valladolid (Sánchez Reyes et al. 2016), but in this survey *Ganoderma* was the spore with less presence during the years in which our study was carried out (registered from the end of March to the first 10 days of December). These results were similar to other study conducted in Valladolid (Sánchez Reyes

et al. 2007) where *Ganoderma* was registered from mid-July to October, but the authors found a smaller amount of spores probably due to the absence of the substrate necessary for their development. On the other hand, the remaining five spore types did not reach the 90% of the total spore count.

In the intradiurnal variations, the spores had a regular pattern of distribution in the atmosphere throughout the day, with moments of the greater abundance in the first hours and at midnight (Fig. 3). The total spore count had almost the same intradiurnal pattern due to the abundance of *Cladosporium*. For the analysis of the rest of the spores, we established a three-type classification according to the hourly pattern obtained. The spores that showed a morning distribution were *Cladosporium* and *Leptosphaeria*. *Cladosporium* spores showed their maximum values from 2 h to 4 h in 2015 decreasing until late afternoon night, while in 2014 decreasing until afternoon. A similar result was registered by Adams (1964) in the UK and other authors who confirmed that the maximum values of *Cladosporium* began at 09:00 am (Sadyś 2017). Then, the values start to increase a little and remain increased through 18 h when they come back down. *Leptosphaeria* had a similar pattern with the maximum value in the first hours of the day decreasing during the afternoon and growing again at 16 h to decrease at 20 h, being similar results to other surveys which registered a diurnal pattern between 03:00 am and 13:00 pm (Huang et al. 2005). In the afternoon pattern, *Alternaria* spores registered a clear release trend from 8 h to 14 h when spore concentrations started to decrease. This pattern was also observed by Bardei et al. (2017) and other authors who established that the risky hours for people with allergy to this particular type were during the evening (Maya Manzano et al. 2016). For *Aspergillus/Penicillium* spores, a greater abundance was recorded in the late hours of the day and a maximum value in the early hours of the morning. These results were the same as Grinn Gofroń (2011) obtained and other studies which confirmed that *Aspergillus/Penicillium* spores had high hourly concentrations in the late afternoon and early morning (Oliveira et al. 2009a, b). The spores with a typical nocturnal dispersal pattern were *Agaricus*, *Coprinus* and *Periconia*. *Agaricus* needs a high humidity for its growth and release, and this is the main cause for a night pattern release, as night temperature was mild and the relative humidity

increased. In addition, during the first hours of the morning the conditions were suitable for the *Agaricus* spores presence (Hasnain et al. 2012). In countries with high temperature, *Agaricus* spores show similar behaviours during the night (Khan et al. 2016). *Coprinus* spores values decreased during the central hours of the day possibly because of its moisture requirements (Aira et al. 2003), maintained stable values during the rest of the day, but increased past midnight, just like other surveys registered (Levetin and Horner 2002). For *Periconia*, the intradiurnal obtained values exhibit an increase that started at midday and were increasing until the first hours of the morning when it decreased, obtaining the same behaviour picture that was registered in Valladolid (Sánchez Reyes et al. 2009b).

Regarding the meteorological factors and their relationship with the spore types, we found that temperature was the predominant factor for the abundance of fungal spores in the atmosphere. Only for *Coprinus*, *Leptosphaeria* and *Periconia* negative correlations were noted due to their needs of humidity and low temperature values (Aira et al. 2003; Pérez Gorjón et al. 2003; Magyar et al. 2006). *Alternaria*, *Aspergillus/Penicillium* and *Cladosporium* spores had a strong positive correlation with this parameter, because *Alternaria* optimal temperature for growing and release is between 22 and 28 °C (Fernández et al. 1998), 13 and 21 °C for *Cladosporium* (Sabariego et al. 2000) and *Aspergillus/Penicillium* had a sporadic behaviour as noted by Oliveira et al. (2009a, b). *Periconia* showed a negative Spearman rank with minimum temperature. This is rare, being a wintry spore type, but in other research carried out in Valladolid (Sánchez Reyes et al. 2012) similar results were explained by the fact that *Periconia* elevates its spore concentration in parallel with the beginning of autumn, when temperature goes down, and this may influence the negative correlations obtained. Rainfall and relative humidity were considered together for the spore presence in the atmosphere as both factors affect the aeromycota. *Agaricus* and *Coprinus*, like the majority of basidiomycota, need high humidity levels and a great quantity of water in the substrate for the development of their carpophores, as Aira and collaborators pointed out (2003). While they obtained a positive correlation with rainfall and humidity, in our survey *Coprinus* only had a positive correlation with relative humidity which seems to be the truly

meteorological factor which affects the growing, sporulation and distribution of *Coprinus* spores in the atmosphere (Almaguer et al. 2014). *Leptosphaeria* spores obtained a strong positive correlation with relative humidity and rainfall, which increased their presence in the atmosphere after rain (Grinn Gofroń et al. 2018), when the relative humidity values were high (Magyar et al. 2006). On the other hand, the day that *Leptosphaeria* reached its peak value was the only day when rainfall occurred in the whole month.

Wind speed was only positively correlated with *Aspergillus/Penicillium* spores. This could be explained by the size of the spores. While other spores are frequently bigger and heavier for aerial transport, *Aspergillus* and *Penicillium* have very small spores (Almaguer et al. 2015) which can be easily transported through the air. Wind directions were separated into two sectors that matched with the dry spores and wet spores. *Alternaria*, *Cladosporium* and *Periconia* were positively correlated with winds from north-easterly and south-easterly directions, while *Agaricus*, *Coprinus* and *Leptosphaeria* had positive correlations with the south-westerly winds. In the case of *Alternaria* and *Cladosporium*, the wind direction was probably related to the great extension of crop fields located in Valladolid (Sánchez Reyes et al. 2016) and the abundance of host plants for *Alternaria* in cities like Toledo (Sabariego et al. 2012). In the case of *Cladosporium*, spores would be distributed in those quadrants as Almeida et al. (2018) already pointed out. For wet spores, the wind from the south-west came from different oak and chestnut forests at “Sierra de Francia y Béjar” Biosphere Reserve, located approximately 70 km away, in which environments are very rich in basidiomycetes. *Aspergillus/Penicillium* spores also showed positive correlation with north-westerly wind. Other surveys registered a high number of these fungal spores at cities located in the north-western part of Spain (Aira et al. 2003). The other wind parameter, calm frequency, influenced positively the presence of wet spores in the atmosphere, as registered by other researches (Díez Herrero et al. 2006). At last, sunshine is a meteorological factor that registered a positive correlation with *Alternaria* and *Aspergillus/Penicillium* spores. The behaviour of these spores confirms the positive correlation with sunshine. The positive correlation of *Alternaria* spores with sunshine also was obtained in nearby cities (Sánchez Reyes et al. 2009b; Sánchez Reyes et al. 2016). In the case of *Coprinus*, the

positive correlation with sunshine could be explained by the values of the sunshine hours percentage instead of the sunshine hours. During October 2014, when the maximum spore concentration of *Coprinus* was registered, the percentage of sunshine was between 80 and 90% during the greater part of the month and this could influence a positive correlation with *Coprinus*.

5 Conclusion

The total content of fungal spores was influenced by a high number of *Cladosporium* spores, and it was registered during summer and early autumn. During the winter months, due to low temperature, the lowest values for all the fungal spores were registered, including *Periconia*, which, despite being a wintry spore type, showed a decrease.

There is a clear separation between the spore types, being divided into two groups: dry air spores and wet air spores. To the first category, *Alternaria*, *Aspergillus/Penicillium*, *Cladosporium* and *Periconia*, which showed their maximum values at midday or at late sunset/night, are included. On the other hand, the wet air spores which include *Agaricus*, *Coprinus* and *Leptosphaeria* had a night distribution pattern and high humidity or rainfall conditions, because their maximum values were recorded during autumn when the conditions were the best for their release.

Meteorological factors had an influence on the distribution of the spores. According to the results, dry spore types showed positive correlation with temperature and the sun hours. However, wet spore types are positively correlated with rainfall and high relative humidity levels. Only the *Aspergillus/Penicillium* spores obtained positive correlation coefficients with wind speed due to their small size. On the other hand, wind from the NE and SE directions had a positive influence on *Alternaria* and *Cladosporium* spores due to the extensions of cereal crops and also winds from SW had an influence on ascospores and basidiospores due to the vicinity of forests being on a wind way.

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