

Study of airborne fungal spores in Madrid, Spain

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Abstract The concentration of fungal spores in the atmosphere of Madrid was recorded and analyzed for the year 2003. Airborne spores were sampled continuously with a Hirst-type spore trap located on the roof of a building of the School of Pharmacy, at about 8 m above ground level. Correlation between the mean daily spore concentrations and meteorological variables were explored by means of Spearman's correlation analyses. Seventy spore types were identified, of which the most numerous were *Cladosporium*, Aspergillaceae (conidia), *Coprinus*, *Agaricales* (basidiospores), *Ustilago* (teliospores) and *Pleospora* (ascospores). These six types of spores represented more than 70% of the total. *Cladosporium* represented 41% of the total fungal spores, while *Ustilago* spores, the concentrations of which in May and June exceeded 47% of the monthly total spore count, constituted the second most important group. Spores reached their highest concentrations in the spring months, and in the autumn, mainly in October. A positive significant correlation was found between airborne spore counts and temperature and relative

humidity. The results provide a picture of the spectrum of airborne fungal spores present in the atmosphere of Madrid and of the 'peak' periods of their presence. Future studies will provide more detailed information on the seasonal dynamics of the spores most frequently found in the air as well as on the extent to which atmospheric conditions influence their release, dispersion and sedimentation processes.

Keywords Aerobiology · Ambient fungi · Meteorological parameters · Madrid (Spain)

Introduction

The identification and quantification of atmospheric fungal spores is of great interest from a clinical point of view, as many species are a trigger for allergic reactions (Caretta, 1992; D'Amato & Spieksma, 1995; Herxheim, Hyde, & Williams, 1969; Lehrer & Horner, 1990; López, Salvaggio, & Butcher, 1976; Santilli, Rockwell, & Collins, 1985). Only a limited number of studies have been published on airborne mycoflora in the city of Madrid, and in most of these the samples the mycoflora were collected by exposing petri dishes with a suitable culture medium (Canto & Jiménez, 1945; Muñoz, Comino, Rodríguez, Aranda, & De Buen, 1988; Paya Vicens, 1983); only Subiza and Jerez (1983) and Sáenz and Gutiérrez (2003) used volumetric Hirst-type spore traps. The main aim of this paper is to report the spectrum and concentrations of

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airborne fungal spores in Madrid during a 1-year period (2003) as a preliminary study to future research.

Materials and methods

The study was carried out in the city of Madrid during 2003 using a Hirst-type spore trap (Burkard) which was located on the roof of a building of the School of Pharmacy, Complutense University (40°27'N, 3°45'W), Madrid University campus. This area has an abundance of parks (Casa de Campo, Parque del Oeste, Dehesa de la Villa), all of which are relatively near the university campus. The vegetation comprises mainly cultivated gardens with a large number of ornamental trees, hedges and lawns; spontaneous flora consists mostly of therophytes with a predominance of nitrophilous and ruderal plants. During 2003 the mean annual temperature was 14.5°C, and annual rainfall reached 519.9 mm.

The research methodology followed the recommendations of the IAA (International Association for Aerobiology) and the REA (Spanish Aerobiology Network) (Jäger, 1995). Fungal spores were classified by appearance and morphological characteristics (colour, size and shape) and identified, where possible, by comparison with published keys (Barnett & Hunter, 1998; Nilsson, 1983; Smith, 1990) and monographs. The correlation between the mean daily spore concentrations and the meteorological parameters (mean temperature, relative humidity, rainfall, wind direction and wind calms) was explored by means of the Spearman's correlation analyses.

Results

A total of 222,374 spores were registered in the air of Madrid during 2003, with a mean daily spore concentration of 609.2 spores/m³. Seventy spore types were identified: 20 ascospores, 8 basidiospores, 37 conidia, 1 Myxomycete, 1 urediniospore, 1 *Peronospora* sporangium and 2 teliospores (*Tilletia* and *Ustilago*) (Table 1).

Spores reached their highest concentrations during the spring months (24,643 spores were collected in May and 32,832 in June) and in the autumn, mainly in October (48,447 spores collected). The lowest

concentrations occurred in the winter and summer (Fig. 1).

The most abundant spore types were: *Cladosporium*, Aspergillaceae (conidia), *Coprinus*, Agaricales (basidiospores), *Ustilago* (teliospores) and *Pleospora* (ascospores), each of which represented over 1.5% of the total. The *Cladosporium* spore type showed the highest annual concentrations, with 91,458 annual spores detected, representing 41.13% of the total. The presence of *Cladosporium* spores remained constant throughout the year, with a higher incidence in October (22,522 spores), June (12,955 spores), April (9,529 spores) and September (9,448 spores). The lowest concentrations were recorded in the months of January and February. The mean daily concentration was 250 spores/m³, and the maximum mean daily concentration occurred on October 16 when 3,157 spores/m³ were collected. A total of 5,512 Aspergillaceae spores were detected in the sampling during 173 days throughout the year. Although this spore type was present all year long, the greatest concentrations occurred in February, March and October (Fig. 2).

Coprinus spores were the most abundant basidiospores, with an annual total of 19,371 (8.7% of the total annual fungal spores). The mean daily concentration was 53 spores/m³, and the maximum mean daily concentration occurred on October 18 (1,084 spores/m³). October had the highest incidence (6,965 spores), followed by November (5,797) and March (952), (Fig. 2). An annual total of 6,397 Agaricales spores, another type of basidiospore, was present in the atmosphere for 85 days during 2003. The highest concentrations were recorded in October, with a peak on October 15 (884 spores/m³).

An annual total of 39,026 *Ustilago* spores were collected (17.55% of the total). The mean daily concentration of *Ustilago* spores was 107 spores/m³, and their maximum mean daily concentration occurred on April 28 (2,274 spores/m³). Although present in the atmosphere all year long, this spore type was more abundant in the spring and summer. The various species of the genus *Ustilago* are cereal pathogens, consequently the production of *Ustilago* spores directly correlates with the growth of these plants. As a result, the greatest concentrations of these types of spores were recorded in May (12,919 spores) and June (15,684 spores) (Fig. 2). The sum of the mean daily concentrations for May accounted for

Table 1 Annual total, percentage of representation and frequency of airborne fungal spores in the atmosphere of Madrid, 2003

Spore type ^a	Annual count	Percentage of total spores	Frequency (% of days)
<i>Cladosporium</i> (C)	91,458	41.13	100
<i>Ustilago</i> teliospores	39,026	17.55	69
<i>Coprinus</i> (B)	19,371	8.71	82
<i>Agaricales</i> (B)	6,397	2.88	23.3
Aspergillaceae (Incl. <i>Aspergillus</i> and <i>Penicillium</i>)(C)	5,512	2.48	46.8
<i>Pleospora</i> (A)	3,391	1.52	71.2
<i>Leptosphaeria</i> (A)	3,295	1.48	65.2
<i>Bovista</i> (B)	2,983	1.34	64.0
Spores of Myxomycetes	2,541	1.14	55.1
Diatrypaceae (A)	2,472	1.11	3.8
<i>Alternaria</i> (C)	2,049	0.92	66.6
<i>Ganoderma</i> (B)	1,762	0.79	53.1
<i>Gymnopilus</i> (B)	1,215	0.55	11.5
<i>Oidium</i> (C)	830	0.37	42.2
<i>Agrocybe</i> (B)	725	0.33	38.1
<i>Drechslera</i> (C)	675	0.30	38.4
<i>Botrytis</i> (C)	625	0.29	12.3
<i>Torula</i> (C)	582	0.26	40.0
<i>Polythrincium</i> (C)	383	0.17	9.3
<i>Chaetomium</i> (A)	224	0.10	20.8
<i>Monodictis</i> (C)	207	0.09	6.0
<i>Epicoccum</i> (C)	203	0.09	19.5
<i>Caloplaca</i> (A)	194	0.09	6.0
<i>Tilletia</i> teliospores	180	0.08	14.0
<i>Deightonella</i> (C)	176	0.08	14.2
<i>Arthrinium</i> (C)	99	0.04	4.1
<i>Stemphylium</i> (C)	93	0.04	10.1
Xylariaceae (A)	84	0.04	3.8
<i>Peronospora</i> sporangium	54	0.02	4.1
<i>Periconia</i> (C)	53	0.02	3.0
<i>Erysiphe</i> (A)	51	0.02	3.3
<i>Leptosphaerulina</i> (A)	49	0.02	0.8
<i>Amphisphaeria</i> (A)	46	0.02	1.9
<i>Curvularia</i> (C)	45	0.02	4.4
<i>Exosporiella</i> (C)	45	0.02	5.2
<i>Boletus</i> (B)	45	0.02	3.8
<i>Chaetosphaerella</i> (C)	38	0.02	2.5
<i>Fusarium</i> (C)	34	0.02	3.0
<i>Helicomyces</i> (C)	33	0.02	3.6
<i>Ascobolus</i> (C)	27	0.01	1.4
<i>Passalora</i> (C)	26	0.01	3.3
<i>Massaria</i> (C)	25	0.01	2.2
<i>Cercospora</i> (C)	25	0.01	1.6
<i>Capronia</i> (C)	22	0.01	0.5
<i>Sporormiella</i> (A)	22	0.01	2.7
<i>Fusicladium</i> (C)	21	0.01	2.2
<i>Paraphaeosphaeria</i>	17	<0.01	1.9
<i>Glonium</i> (A)	11	<0.01	0.8
<i>Bispora</i> (C)	10	<0.01	0.3
<i>Helminthosporium</i> (C)	10	<0.01	0.3
<i>Bipolaris</i> (C)	9	<0.01	0.6

Table 1 continued

Spore type ^a	Annual count	Percentage of total spores	Frequency (% of days)
<i>Pithomyces</i> (C)	7	<0.01	0.6
<i>Boerlagiomyces</i> (A)	6	<0.01	0.8
<i>Splanchnonema</i> (A)	6	<0.01	0.8
<i>Keissleriella</i> (A)	5	<0.01	0.3
Urediniopores	5	<0.01	0.3
<i>Sordaria</i> (A)	4	<0.01	0.5
<i>Beltrania</i> (C)	4	<0.01	0.3
<i>Ceratosporium</i> (C)	4	<0.01	0.5
<i>Fusichalara</i> (C)	2	<0.01	0.3
<i>Helicogermis</i> (A)	2	<0.01	0.3
<i>Lophiostoma</i> (A)	2	<0.01	0.3
<i>Navicella</i> (A)	2	<0.01	0.3
<i>Nigrospora</i> (C)	2	<0.01	0.3
<i>Pestalotiopsis</i> (C)	2	<0.01	0.3
<i>Pseudocercospora</i> (C)	2	<0.01	0.3
<i>Septonema</i> (C)	2	<0.01	0.3
<i>Ulocladium</i> (C)	2	<0.01	0.3
Unidentified spores	15,397	6.9	53.4
Unidentified basidiospores	11,935	5.4	62.2
Unidentified ascospores	7,530	3.4	88.8
Total	222,374	100	

^aC, conidia; A, ascospores; B, basidiospores

52.4% of the monthly total of spores, and 47.77% for June. The lowest concentrations occurred, quite naturally for these spore types, in the winter months. Among the ascospores, *Pleospora* spores were the sixth most numerous, with an annual total of 3,391 spores, representing 1.52% of the total. These spores were present all year long, with the highest concentrations occurring in April and September, with a peak on September 30 (385 spores/m³) and in October (Fig. 2).

An analysis of the annual concentration of total spore types (Fig. 3) shows that conidiospores were the most abundant in the atmosphere, followed by basidiospores, teliospores, ascospores and, in last place, Myxomycetes spores.

A total of 103,342 conidiospores were collected, and the highest concentrations occurred in May, June and October. Basidiospores were present in the atmosphere of Madrid throughout the year – yearly total of 44,433 – and their only seasonal variation was one period of maximum concentration in autumn (October and November). Autumn rains favoured the growth of basidiocarps and, consequently, the greatest concentrations of basidiospores in the air occurred at this time. Basidiospores showed two periods of

Fig. 1 Monthly variation in the concentrations of total fungal spores

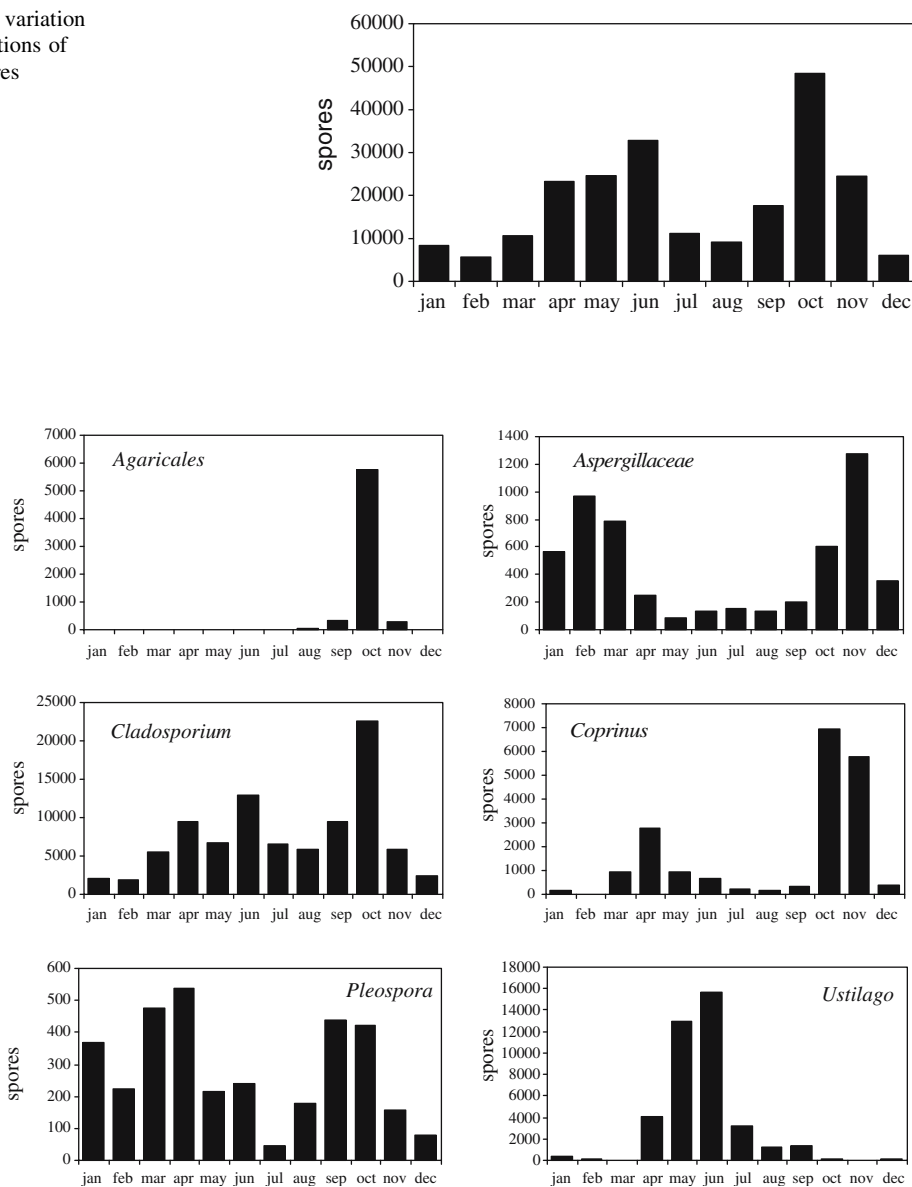
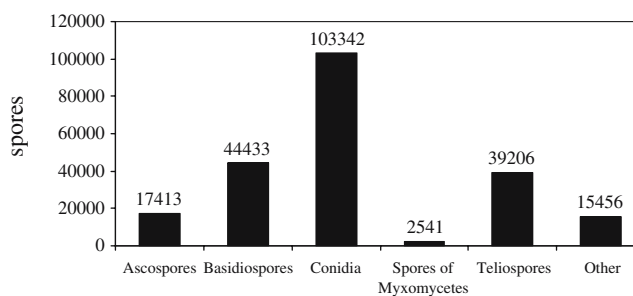


Fig. 2 Monthly variation in the spore concentration of *Agaricales*, *Aspergillaceae*, *Cladosporium*, *Coprinus*, *Ustilago* and *Pleospora* in 2003

Fig. 3 Annual total of the different groups of spores analysed in Madrid, 2003



maximum concentration: spring and September/October. We obtained a total of 39,206 teliospores (Fig. 3), of which 39,026 belong to *Ustilago* and only 180 to *Tilletia*. A total of 17,413 ascospores were recorded, with the majority occurring mainly in April, September and October. Finally, the smallest group was the Myxomycetes spores, whose highest levels occurred in the winter months. The remaining spores were urediniospores (total: 5), *Peronospora* sporangium (total: 54) and unidentified spores (total: 15,397).

The seasonal analysis (Fig. 4) shows that the percentage of conidiospores varied very little in the autumn and winter, with a decrease in the spring and a considerable increase in the summer. The teliospores were the largest group in the spring and the second largest in the summer. Basidiospores were present at a high percentage in the autumn, close to the value recorded for conidia.

Table 2 shows the Spearman correlation coefficients between the daily meteorological parameters

and the daily concentrations of basidiospores, ascospores, teliospores, conidia and their total. The meteorological parameter with the most significant correlation is the average temperature, with a positive correlation for teliospores, conidia and total spores, and a negative correlation for ascospores. In the case of relative humidity and rainfall, the correlation found was positive for ascospores and basidiospores and negative for teliospores. Total spores also showed a positive correlation with rainfall at a 95% significance level. We also observed significant positive correlations between spore concentrations in most groups and the frequency of a southwestern wind.

Discussion

The mean daily fungal spore value recorded in this study – 609.2 spores/m³ – is slightly higher than that obtained by Sáenz and Gutiérrez (2003) at the

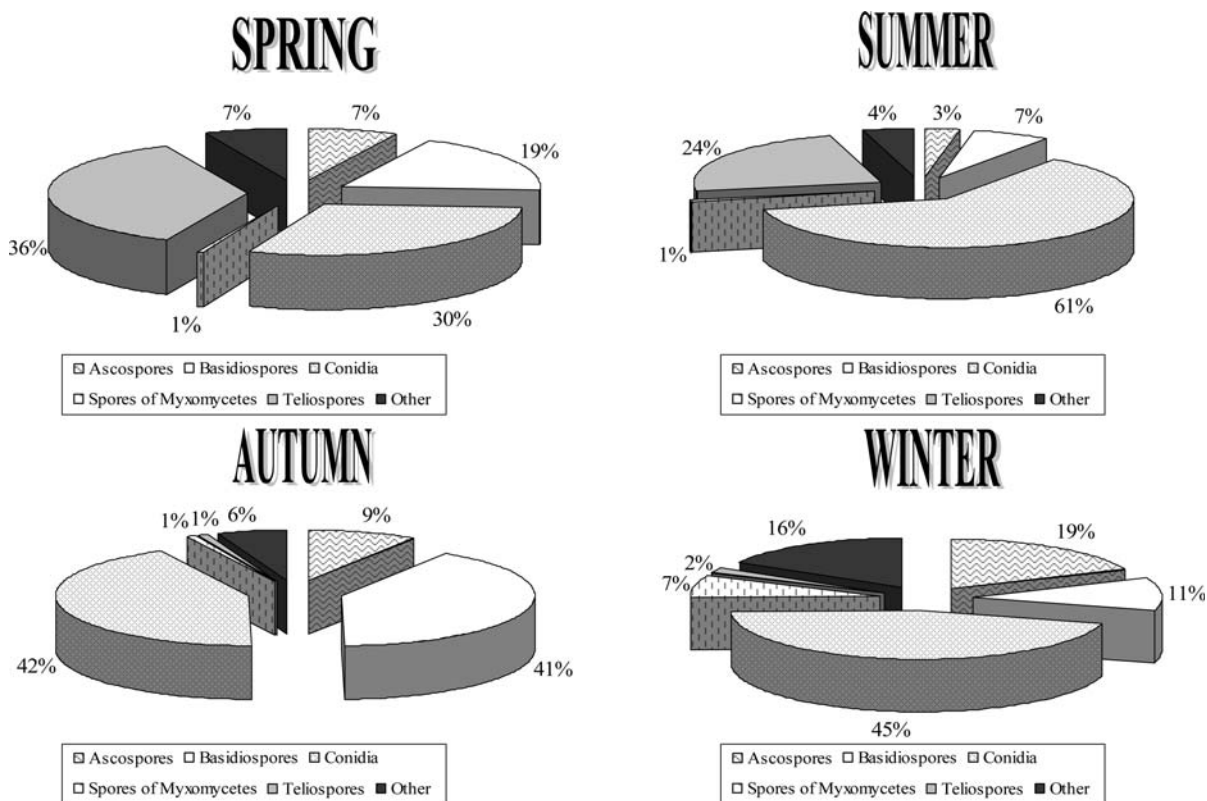


Fig. 4 Percentage of representation of different groups of spores analysed during the seasons of the year

Table 2 Spearman's correlation coefficients between meteorological parameters and spores analysed

	Ascospores	Basidiospores	Teliospores	Conidia	Total spores
Mean temperature	-0.187**	-0.023	0.632**	0.376**	0.307**
Humidity	0.469**	0.319**	-0.619**	-0.091	0.014
Rainfall	0.463**	0.125*	-0.280**	0.056	0.132*
Wind NE	-0.194**	-0.137*	0.065	-0.073	-0.118*
Wind SE	0.021	-0.126*	0.167**	0.058	0.020
Wind SW	0.247**	-0.031	0.185**	0.155**	0.180**
Wind NW	-0.104	-0.182**	0.203**	-0.060	-0.055
Wind calms	-0.222**	0.258**	-0.044	-0.012	-0.013

** denotes 99% significance, $p \leq 0.01$; * denotes 95% significance, $p \leq 0.05$

same location, i.e. the Madrid University campus, in 2000 (355.3 spores/m³). However, it is lower than that recorded in many other cities, such as Badajoz (Paredes, Martínez, Silva, Muñoz, & Tormo, 1996), Melbourne (Mitakakis, Ong, Stevens, Guest, & Knox, 1997), Santiago de Chile (Ibáñez, Rojas, & Roure, 2001) or Sevilla (Morales, 2004), to cite a few examples.

Although, there is considerable variation in the mean daily fungal spore concentration, on the whole, airborne spores appear to present a clear pattern of seasonal prevalence.

This seasonal trend was seen in our study in the two peaks of airborne spore concentration (Fig. 1). The first, the slightly smaller of the two, occurred at the end of spring and the beginning of summer (May and June), and the second, much larger, occurred in the autumn (September and October). This phenomenon of two periods per year of high airborne spore concentration has been reported by various investigators, including Halwagy (1994; Kuwait City), Wahid, Wahid, Moustafa, and Moustafa (1996; Ismailia, Egypt), Herrero, Fombella-Blanco, Fernández González and Valencia-Barrera (1996; Palencia, Spain) and Morales (2004; Sevilla, Spain).

The study reported here is comparable to studies carried out in several cities in various countries in which *Cladosporium* spores were found to be the largest group captured in the aerobiological samplings (Ibáñez et al., 2001; Mediavilla, Angulo, Domínguez, Castro, & Infante, 1997; Mitakakis, 2001; Morales, 2004; Rosas, Calderón, Martínez, Ulloa, & Lacey, 1997; Rutherford, Owen, & Simpson, 1997; Stepalska, Harmata, Kasprzyk, Myszkowska, & Stach, 1999). Consequently, there is an abundance of data confirming the atmospheric relevance of *Ustilago* and

Coprinus spore concentrations (Calderón, Lacey, McCartney, & Rosas, 1995; Mitakakis & Guest, 2001; Morales, 2004; Vittal & Krishnamoorthi, 1988) and of *Leptosphaeria* and *Pleospora* spore types (Mitakakis & Guest, 2001; Vittal & Krishnamoorthi, 1988).

The concentration of fungal spores in the air depends both on internal biological factors and on external factors such as substratum, temperature, relative humidity and rainfall (Larsen, 1981). In Madrid, the highest spore concentrations are detected in the spring and autumn months, when the temperature and humidity provide the optimum conditions for the decomposition of organic matter and the development of fungi. The decrease in spore concentrations in the summer may be related to the absence of rain and the relatively low humidity, which are not conducive conditions for the growth of fungus. Low temperatures in the winter prevent sporulation, which accounts for the low concentrations recorded at this time of year.

The highest concentrations of teliospores appear to be associated with high temperatures and dry meteorological conditions (O'Rourke, Gunyan, Bodour, & Van de Water, 1994). This supports our observation of the positive correlation between the concentration of these spores in the atmosphere and mean temperature, and the negative correlation with humidity and rainfall. We found a positive correlation between three wind directions and teliospore concentrations; this observation can be clarified by the fact that these winds come from areas in the Madrid region with an abundance of cereal cultivation, crops which are commonly parasitized by different species of *Ustilago*. As in with teliospores, basidiospores and ascospores showed a significant positive correlation with precipitation and relative humidity, as both meteorological parameters

favour the production and release of this spore type in the atmosphere (Calderón et al., 1995; Hasnain, 1993; Ingold & Hudson, 1993; Shaheen, 1992). The positive influence of rainfall on ascospore levels is explained by the fact that these spores are hydrofugal and can be dispersed by the impact of raindrops (Lim et al., 1998).

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