

Airborne ascospores in Mérida (SW Spain) and the effect of rain and other meteorological parameters on their concentration

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Abstract Airborne ascospores have been reported to be allergenic or plant pathogens, and their presence has traditionally been associated with rainfall events. The aim of the present study was to analyze the presence of airborne ascospores in relation to weather parameters in a town in SW Spain. A seven-day recording spore trap (Burkard) was used to sample the air over 2 years at 15 m above ground level on the terrace roof of the hospital in Mérida (SW Spain). Fungal spores were identified and counted by means of two longitudinal scans over the slides under $\times 1000$ microscopy. A correlation analysis was made of the daily meteorological data and the airborne ascospore concentrations, and *t*-tests were used to compare data between the 2 years. Nineteen ascospore types were defined, including one-cell ascospores (*Chaetomium*, *Diatrype*, *Helvella*, *Xylaria*), two-cell ascospores (*Diaporthe*, *Mycosphaerella*, *Nectria*, *Venturia*),

transversally septate ascospores (*Melanomma*, *Leptosphaeria*, *Paraphaeosphaeria*, *Sporormiella*, *Mas-saria*), transversally and longitudinally septate ascospores (*Pleospora*), and ascospores within asci (*Sordaria*). *Leptosphaeria* consisted of a group of four types described according to the number of cells, hyaline grade, wall thickness, and ornamentation, and other ascospores comprised one last additional type. The average airborne ascospore concentration was 153 ascospores/m³. One-third each of this total were from the *Leptosphaeria* group, with an average 54 ascospores/m³, and the two-cell ascospores or *Venturia*-like group (*Diaporthe*, *Mycosphaerella*, *Nectria*, *Venturia*) with 51 ascospores/m³ on average. In third position was *Pleospora* with 27 ascospores/m³ on average. The month with highest concentration was September, with 238 ascospores/m³, and the lowest March, with 56 ascospores/m³. By seasons, autumn had the highest concentrations, followed by winter, spring, and summer. The maximum daily concentration reached was 3,371 ascospores/m³. Daily rainfall was significantly correlated with the ascospore types *Diatrype*, *Mycosphaerella*, *Nectria*, two subtypes of *Leptosphaeria*, and *Pleospora*. Relative humidity was positively correlated with those ascospore types and also with *Diaporthe* and *Paraphaeosphaeria*, and negatively with *Chaetomium* and *Melanomma*. The concentration was higher on rainy days than on days without rain for *Pleospora*, *Leptosphaeria* (3 subtypes), *Diatrype*, *Diaporthe*, *Nectria*, *Mycosphaerella*, and *Paraphaeosphaeria*. The daily temperatures

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were in general correlated with the same types as the relative humidity, but with the opposite sign. For the monthly data, there were no statistically significant differences between the 2 years studied.

Keywords Aeromycology · Airborne ascospores · Fungal spores · Meteorology

1 Introduction

The ascomycetes are the largest group of the true fungi. Although most of them produce conidiospores or conidia for asexual reproduction, they are recognized by the formation of meiospores named ascospores (Blackwell and Spatafora 2004). Monitoring of airborne spores has found these ascospores to be present in the outdoor air in very diverse proportions that range 0.59–28.6% (Shaheen 1992; Halwagy 1994; Dames and Cadman 1994; Li and Kendrick 1995b; Lim et al. 1998; Chakraborty et al. 2003; Adhikari et al. 2004; Jothish and Nayar 2004; Ho et al. 2005; Oliveira et al. 2005; Díez-Herrero et al. 2006; De Antoni-Zoppas et al. 2006; Nayar et al. 2007; Nunes et al. 2008; Gillum and Levetin 2008; Das and Gupta-Bhattacharya 2008).

The number of different ascospores identified in those studies varied from one to twenty, with the commonest types found being *Leptosphaeria*, *Pleospora*, and *Venturia*. Since the global number of ascomycetes species that have identified is around 14,000 (Mueller and Schmit 2007), and given the great degree of variation in their ascospore morphology, it is difficult to identify them in aerobiological samples. Moreover, some of the ascospores are hyaline, and particular care is required in their scrutiny under light microscopy, as is well exemplified in the work of Allitt (1986) with *Didymella* ascospores.

The importance of airborne ascospores is because some of them are allergens or plant pathogens and many of them belong to the order Pleosporales that has been reported as important aeroallergens that exacerbate respiratory morbidity (Soeria-Atmadja et al. 2010). Allergy or asthma caused by ascospores has been reported for *Daldinia* (Herxheimer et al. 1966), *Leptosphaeria* (Ganderton 1968; Burge et al.

1985; Hasnain 1993a; Newson et al. 2000; Grinn-Gofroń and Mika 2008), *Didymella* (Frankland and Gregory 1973; Jenkins et al. 1981; Ackroyd and Fry 1983; Harries et al. 1985; Allitt 1986; Richardson 1996; Newson et al. 2000; Grinn-Gofroń and Mika 2008), *Leptosphaerulina*, *Gibberella*, *Gnomonia* and *Chaetomium* (Burge 1986) and *Pleospora* (Newson et al. 2000). Moreover, the presence of ascospores and other fungal spores has been associated with increases in the number of emergency visits and hospital admissions for asthma (Atkinson et al. 2006), and a recent study (Roussel et al. 2010) shows that antigens are more abundant in ascospores than in conidia or hyphae in one species of *Eurotium*.

With respect to their importance as plant pathogens, there has long been recognition of the relationship between the presence in the air of some ascospores and phytopathologies that affect certain crop plants, examples being apple scab (*Venturia inaequalis*), phoma stem canker (*Leptosphaeria maculans*), septoria leaf blotch of wheat (*Mycosphaerella graminicola*), black leaf streak disease of bananas (*Mycosphaerella fijiensis*), *Sclerotinia sclerotiorum* on various hosts, etc. (Meredith et al. 1973; McCartney and Lacey 1991; Aylor and Kiyomoto 1993). In many cases, the severity of these diseases can be related to ascospore concentrations (McCartney and Lacey 1991). There have been attempts to forecast the risk of airborne disease using immunological methods (McCartney et al. 1997) or molecular biology methods such as PCR (West et al. 2008), which have actually identified the diversity of fungal contaminants in house dust and outdoor air using large-scale DNA sequencing, with some advantages over aerobiological methods (Pitkäranta et al. 2008; Freeman et al. 2009). Nevertheless, it is still necessary to make microscopy counts on airborne records to determine the presence of potential ascospore pathogens and their temporal variation in concentration.

Relationships between the airborne presence of ascospores and meteorology have been well documented. There is general agreement that the concentration of airborne ascospores increases during and after rainy spells or even single rainstorms (Bush 1989; Hasnain 1993b; Martínez et al. 1996; Lim et al. 1998; Trouitt and Levetin 2001; Van Osdol et al. 2004). This phenomenon has been extensively studied for the spores of plant pathogens with the aim of developing predictive models of the spread of

diseases, an example being work on *Venturia* (Aylor 1992; Aylor and Kiyomoto 1993; Aylor and Qiu 1996).

The objectives of the present work were to determine the seasonal variation in airborne ascospore concentrations in a town in the SW of the Iberian Peninsula and to analyze the relationship of these concentrations with meteorological parameters in terms of the main spore types usually identified by optical microscopy in aerobiological studies.

2 Materials and methods

The study site was the town of Mérida, Badajoz province (SW Spain, 06°23'W, 38°05'N). The atmosphere was monitored for 2 years (from January 1997 to December 1998) using a Hirst (1952) spore trap (Burkard seven-day) at 15 m above ground level on the terrace roof of the town's Clinic Hospital. White petrolatum was used as adhesive. Two longitudinal scans at $\times 1000$ magnification were analyzed for each slide, both of them in the center of the slide with a 1-mm separation to minimize errors (Tormo et al. 1996).

Meteorological data were provided by the Regional Meteorological Centre. The following daily parameters were analyzed: temperature, rainfall, relative humidity, wind speed, and wind direction. The Spearman correlation coefficient was applied to look for possible association between daily spore concentrations and these meteorological parameters since the daily and weekly data were neither normally nor log-normally distributed. To reduce random effects, only significance levels below 0.01 were taken into account. The log-transformed monthly data were normally distributed, allowing the use of a *t*-test to compare the 2 years. The statistical analyses were performed using the SPSS 15.0 program package.

The influence of rainfall on the ascospore concentrations was studied using the non-parametric Kruskal–Wallis test to compare the mean concentrations on days with and without rain. The concentrations on days during and after rainy spells were also considered. Seasons from winter to autumn were defined as three complete monthly period (January–March, April–June, July–September, and October–December).

Identification of ascospores has been realized using some general references (Grant Smith 1990;

Ellis and Ellis 1997) and specific works (Allitt 1984, 1986; Sierra 2006).

3 Results

Ascospores represented 5.98% of the total fungi propagule count. Table 1 lists the ascospore types identified, together with a brief description and the possible taxa included in each type. These taxa are only meant as a first approximation, with just the most frequent being included. We consulted two principal works in making these assignments—Ellis and Ellis (1997) and Sierra (2006). Three of the types described include hyaline ascospores: *Mycosphaerella*, *Leptosphaeria* type 1, and *Massaria*.

The mean concentration of airborne ascospores for the 2 years studied was 153 ascospores/m³, with no statistically significant differences between years or even between seasons using monthly data, although the summer concentration was the lowest (131 ascospores/m³) and the autumn the highest (182 ascospores/m³) (Fig. 1). The most abundant ascospore group, representing about one-third of the total ascospores (54 ascospores/m³), was *Leptosphaeria*, mainly types 1 and 2 (Fig. 2). This was followed by the *Venturia*-like group at a similar concentration (51 ascospores/m³) with an order of importance of the four types included being *Diaporthe*, *Nectria*, *Venturia*, and *Mycosphaerella*. The third most abundant group was *Pleospora* (27 ascospores/m³). Together, these three ascospore groups represented more than 86% of the total ascospores (Fig. 2). The *Leptosphaeria* group and *Venturia*-like group ascospores showed similar monthly patterns, with maxima in autumn and May, whereas *Pleospora* presented maxima in winter and spring (Fig. 3). The highest monthly concentration corresponded to *Diatrype* with 180 ascospores/m³ in September of the second year (Fig. 7). The highest daily concentration also corresponded to this type (2,697 ascospores/m³), and although this was probably an exceptional situation, there were four successive days with more than 500 ascospore/m³ of this type during a not unusual rainy period, the total amount of ascospores for that day (21 September 1998) was 3,371 ascospores/m³ (Table 2). Apart from these four, the concentrations of the other ascospore types were low with no clear seasonal pattern.

Table 1 Types of ascospores described and possible taxa included (pp means some species of the genus, *pro parte*)

One-cell ascospores		
<i>Chaetomium</i>	Blackish-brown, lemon-shaped	<i>Chaetomium</i>
<i>Diatrype</i>	Pale straw colored en masse, curved allantoid	<i>Diatrypaceae</i>
<i>Helvella</i>	Pink to white, fusiform or elliptic	<i>Helvella</i> , <i>Morchella</i> , <i>Peziza</i> , <i>Verpa</i>
<i>Xylaria</i>	Blackish, fusiform or bean-shaped	<i>Xylariaceae</i>
Two-cell ascospores (Venturia-like ascospores)		
<i>Diaporthe</i>	1-septate, greyish to pale-yellow, broadly ellipsoid or ellipso-fusoid, >10 µm long	<i>Diaporthe</i> , <i>Didymella</i> , <i>Mycomicrothelia</i> , <i>Munkovalsaria</i>
<i>Mycosphaerella</i>	1-septate, septum faint, hyaline, ellipso-fusoid, 4-guttulate, often with hyaline sheath, >10 µm long	<i>Aulographum</i> , <i>Guignardia</i> , <i>Morenoia</i> , <i>Mycosphaerella</i> , <i>Microthyrium</i> , <i>Stomiopeltis</i> , <i>Thyriopsis</i> ,
<i>Nectria</i>	1-septate, greyish to pale-yellow, broadly ellipsoid or ellipso-fusoid, <10 µm long	<i>Lichenopeltella</i> , <i>Nectria</i>
<i>Venturia</i>	Olivaceous to brown, thick walled, broadly ellipsoid or ellipso-fusoid, >10 µm long	<i>Acanthostigma</i> , <i>Arnaudiiella</i> , <i>Didymosphaeria</i> , <i>Didymosphaerella</i>
Transversally septate ascospores		
<i>Melanomma</i>	3-septate, brown to blackish, thick walled, ellipso-fusoid, straight	<i>Capnodium</i> , <i>Capronia</i> , <i>Melanomma</i>
<i>Leptosphaeria</i> 1	3-septate, markedly hyaline, fusiform, lightly curved, weak walled	<i>Leptosphaeria</i> pp
<i>Leptosphaeria</i> 2	3-septate, greyish to pink, fusiform, curved, often one central cell inflated	<i>Leptosphaeria</i> pp
<i>Leptosphaeria</i> 3	5,7-septate, greyish to pink, fusiform, curved, often one central cell inflated, >20 µm long	<i>Keissleriella</i> , <i>Kalmusia</i> , <i>Leptosphaeria</i> pp, <i>Lophiostoma</i> pp, <i>Massariosphaeria</i> pp, <i>Mytilinidion</i> , <i>Nodulosphaeria</i> , <i>Phaeosphaeriopsis</i>
<i>Leptosphaeria</i> 4	3-septate, greyish to brownish, fusiform, straight or curved, warty thick walled, >20 µm long	<i>Leptosphaeria</i> pp, <i>Phaeosphaeria</i>
<i>Paraphaeosphaeria</i>	2-septate, brownish, oblong, one terminal cell larger than others and tapering	<i>Paraphaeosphaeria</i>
<i>Sporormiella</i>	3-septate, brown to blackish	<i>Sporormiella</i>
<i>Massaria</i>	5 to 7-septate, hyaline, fusiform, sharpened ends	<i>Massaria</i>
Transversally and longitudinally septate ascospores		
<i>Pleospora</i>	10-celled or more, brownish to straw colored, straight or curved, elliptic to fusiform, ends tapering or rounded	<i>Chaetoplea</i> , <i>Comoclathris</i> , <i>Cucurbitaria</i> , <i>Leptosphaerulina</i> , <i>Lophiostoma</i> pp, <i>Massariosphaeria</i> pp, <i>Montagnula</i> pp, <i>Platysporoides</i> , <i>Teichospora</i>
Ascospores in asci		
<i>Sordaria</i>	Ascospores in 3 rows, brown to blackish, elliptic, warty	<i>Sordaria</i>

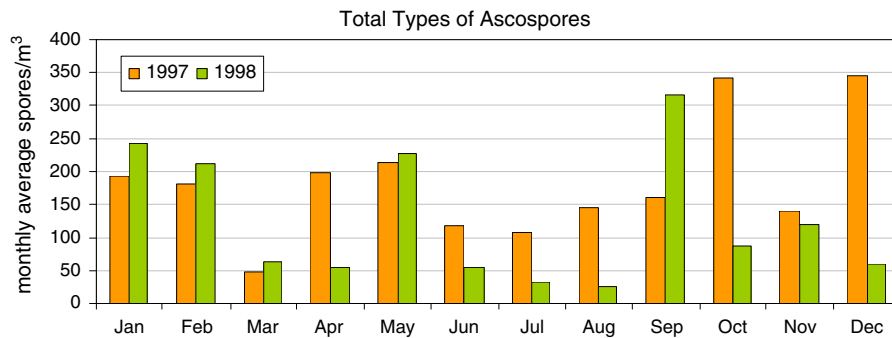


Fig. 1 Monthly average concentration of ascospores during the 2 years studied

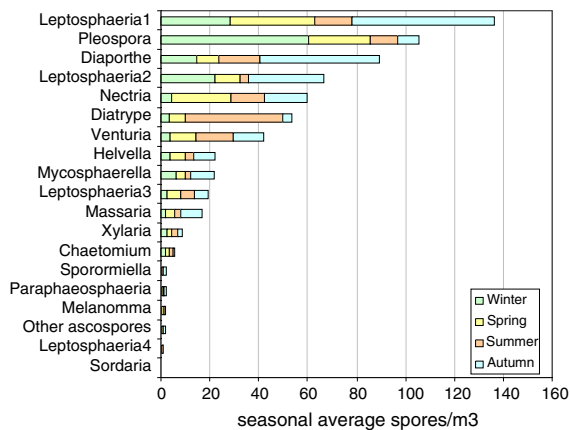


Fig. 2 Seasonal average accumulative concentration for the ascospore types identified

Of the statistically significant correlations between the concentrations and the meteorological parameters, for rainfall these were positive for *Diatrype*, *Diaporthe*, *Mycosphaerella*, *Nectria*, *Venturia*, *Leptosphaeria* types 1 and 2, *Massaria*, and *Pleospora*. With respect to the mean temperatures, there were negative correlations for *Diaporthe*, *Mycosphaerella*, *Leptosphaeria* types 1 and 2, and *Pleospora*, but positive for *Chaetomium*. The relative humidity showed a positive correlation for *Diatrype*, *Diaporthe*, *Mycosphaerella*, *Nectria*, *Leptosphaeria* types 1 and 2, *Massaria*, and *Pleospora*, but negative for *Chaetomium*. Wind speed and direction showed correlations only in a few cases (Table 3).

There were 163 days with rain in the 2 years. For all the ascospores studied except two, *Chaetomium* and *Xylaria*, the airborne concentrations were higher on rainy days than on days without rain. The greatest relative difference corresponded to *Diatrype*, with 8

times more ascospores on rainy days. The differences were significant as determined by the *t*-test for *Diatrype*, *Diaporthe*, *Mycosphaerella*, *Nectria*, *Leptosphaeria* (3 types), *Paraphaeosphaeria*, and *Pleospora* (Fig. 4). For these ascospore types, the airborne concentrations for up to 9 days after a rain event were compared with the rainy day concentration. The variability in the data makes it difficult to confirm whether the differences between the rainy day and the days after the rain event are statistically significant. Nevertheless, one observes in Fig. 5 that for *Leptosphaeria*, *Nectria*, and *Venturia*, the average concentrations remained at similar values for some days after the rain.

The longest rainy spells (of which there were two) lasted 8 days. The distribution of days of rain according to their position in a rainy spell was as follows (given as number of days with the position of the day in a rainy spell in parentheses): 2 (8th day), 2 (7th day), 2 (6th day), 3 (5th day), 9 (4th day), 28 (3rd day), 45 (2nd day), and 72 (1st day including isolated days of just 1 day of rain). The temporal variation in the ascospore concentrations was analyzed during the rainy spells using the Kruskal–Wallis test to compare the values of each day of the spell with that corresponding to the first day. Maximum concentrations that were significantly different from those corresponding to the first day according to the Kruskal–Wallis test were reached on the third day for *Nectria* and on the fourth for *Diaporthe* and *Mycosphaerella* (Fig. 6).

4 Discussion

Assigning airborne ascospores to ascomycetes taxa is a difficult task because of the great number of species

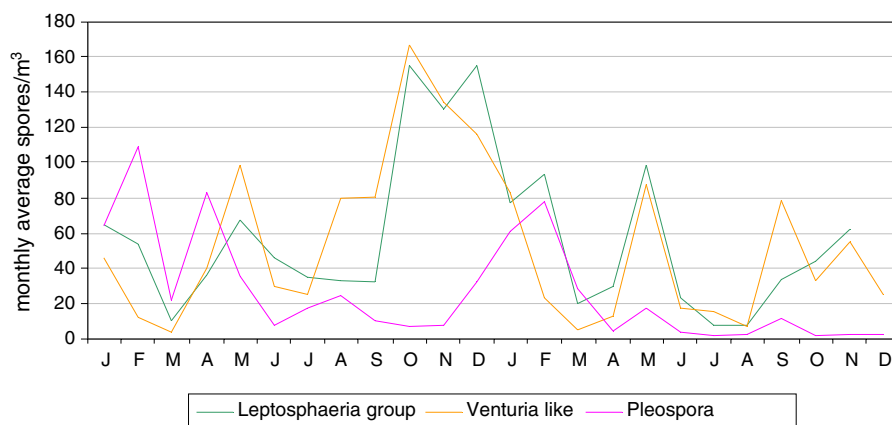


Fig. 3 Monthly average concentration of the most abundant ascospore groups: *Leptosphaeria*-like (4 types), *Venturia*-like (*Diaporthe*, *Mycosphaerella*, *Nectria*, *Venturia*), and *Pleospora*

Table 2 Seasonal maximum (Max) and average (Mean) concentration of ascospores for the whole period studied, values are in spores/m³

	Winter		Spring		Summer		Autumn	
	Max	Mean	Max	Mean	Max	Mean	Max	Mean
<i>Chaetomium</i>	45.6	1.8	36.5	1.6	15.2	1.8	6.1	0.4
<i>Diatrype</i>	69.9	1.0	221.9	2.2	2696.5	13.0	85.1	1.3
<i>Helvella</i>	188.5	1.2	82.1	2.1	185.4	1.2	234.1	2.3
<i>Xylaria</i>	15.2	0.8	12.2	0.5	27.4	0.8	30.4	0.8
<i>Diaporthe</i>	288.8	4.9	139.8	3.0	519.8	5.3	398.2	15.4
<i>Mycosphaerella</i>	100.3	2.1	88.2	1.3	27.4	0.6	112.5	2.9
<i>Nectria</i>	33.4	1.4	370.9	8.2	161.1	4.4	197.6	5.3
<i>Venturia</i>	42.6	1.2	79.0	3.4	486.4	5.0	145.9	3.8
<i>Melanomma</i>	9.1	0.1	12.2	0.3	6.1	0.1	9.1	0.1
<i>Leptosphaeria</i> 1	176.3	9.2	206.7	11.4	112.5	4.9	699.2	17.8
<i>Leptosphaeria</i> 2	291.8	7.2	79.0	3.4	85.1	1.1	401.3	10.4
<i>Leptosphaeria</i> 3	33.4	0.9	54.7	1.7	200.6	1.9	60.8	1.7
<i>Leptosphaeria</i> 4	6.1	0.1	3.0	0.1	18.2	0.1	3.0	0.0
<i>Paraphaeosphaeria</i>	6.1	0.1	9.1	0.1	12.2	0.2	12.2	0.2
<i>Sporormiella</i>	3.0	0.1	3.0	0.1	9.1	0.2	18.2	0.4
<i>Massaria</i>	24.3	0.6	45.6	1.2	54.7	0.8	103.4	2.4
<i>Pleospora</i>	541.1	19.5	617.1	8.3	437.8	3.5	112.5	3.0
<i>Sordaria</i>	24.3	0.0	3.0	0.0	3.0	0.0	0.0	0.0

involved. Some types, such as *Chaetomium*, *Diatrype*, *Xylaria*, *Paraphaeosphaeria*, *Sporormiella*, and *Sordaria*, are relatively clearly defined. But most of the two-cell ascospores and transversally septate ascospores, except *Sporormiella*, are particularly hard to distinguish, and in many cases a given species can have different ascospores as in some

species of *Lophiostoma*, *Montagnula*, *Massariosphaeria*, etc. (Sierra 2006). Furthermore, given the added difficulty of observing hyaline ascospores under optical microscopy, the possibility cannot be discarded that some cases could correspond to immature states, as was noted by Allitt (1984) for some Venturiaceous ascospores. A possible example

Table 3 Correlation analysis of spore concentrations versus meteorological factors: rain (mm), temperature (°C), wind speed (m/s) and direction (hours, calms = no wind), and relative humidity (%)

	Rain	Tmax	Tmin	Tmean	Wind Sp	Calms	NE	SE	SW	NW	Rel.Hum.
<i>Chaetomium</i>											
<i>r</i>	-0.199	0.393	0.259	0.345	0.043	-0.097	-0.188	-0.228	0.085	0.291	-0.455
<i>p</i>	0.045*	<0.001**	0.008**	<0.001**	0.67	0.334	0.058	0.021*	0.396	0.003**	<0.001**
<i>Diatrype</i>											
<i>r</i>	0.528	-0.261	-0.052	-0.170	0.215	-0.183	-0.120	-0.054	0.180	-0.115	0.389
<i>p</i>	<0.001**	0.008**	0.602	0.088	0.03*	0.065	0.229	0.592	0.070	0.249	<0.001**
<i>Xylaria</i>											
<i>r</i>	-0.031	0.109	0.086	0.098	-0.141	0.270	0.072	0.056	-0.047	-0.106	-0.032
<i>p</i>	0.757	0.278	0.393	0.329	0.157	0.006**	0.473	0.579	0.641	0.288	0.752
<i>Diaporthe</i>											
<i>r</i>	0.369	-0.391	-0.223	-0.322	0.019	0.043	0.051	0.124	0.030	-0.182	0.541
<i>p</i>	<0.001**	<0.001**	0.024*	0.001**	0.848	0.668	0.610	0.215	0.764	0.067	<0.001**
<i>Mycosphaerella</i>											
<i>r</i>	0.545	-0.498	-0.290	-0.407	0.152	-0.120	0.032	0.177	-0.031	-0.149	0.671
<i>p</i>	<0.001**	<0.001**	0.003**	<0.001**	0.127	0.231	0.747	0.075	0.756	0.135	<0.001**
<i>Nectria</i>											
<i>r</i>	0.431	-0.056	0.129	0.035	0.280	-0.280	-0.208	-0.199	0.252	0.184	0.291
<i>p</i>	<0.001**	0.575	0.195	0.726	0.004**	0.004**	0.036*	0.045*	0.010*	0.065	0.003**
<i>Venturia</i>											
<i>r</i>	0.333	-0.032	0.121	0.043	0.173	-0.130	-0.112	-0.168	0.192	0.119	0.238
<i>p</i>	0.001**	0.751	0.225	0.668	0.082	0.192	0.260	0.092	0.053	0.233	0.016*
<i>Leptosphaeria_1</i>											
<i>r</i>	0.444	-0.351	-0.164	-0.264	0.144	-0.109	0.079	0.071	-0.081	-0.032	0.559
<i>p</i>	<0.001**	<0.001**	0.099	0.007**	0.147	0.276	0.433	0.478	0.419	0.750	<0.001**
<i>Leptosphaeria_2</i>											
<i>r</i>	0.410	-0.496	-0.405	-0.458	0.008	0.081	0.148	0.288	-0.149	-0.291	0.531
<i>p</i>	<0.001**	<0.001**	<0.001**	<0.001**	0.933	0.419	0.138	0.003**	0.134	0.003**	<0.001**
<i>Massaria</i>											
<i>r</i>	0.273	-0.084	0.078	-0.003	0.158	-0.177	-0.136	-0.152	0.218	0.118	0.278
<i>p</i>	0.005**	0.403	0.434	0.977	0.113	0.075	0.174	0.128	0.028*	0.236	0.005**
<i>Pleospora</i>											
<i>r</i>	0.511	-0.462	-0.308	-0.385	0.195	-0.095	0.211	0.214	-0.199	-0.213	0.509
<i>p</i>	<0.001**	<0.001**	0.002**	<0.001**	0.049*	0.343	0.034*	0.031*	0.045*	0.031*	<0.001**

Only types with statistically significant results have been included (*r*: Spearman correlation coefficient; *p*: *p*-value)* *p*-value < 0.01; ** *p*-value < 0.05

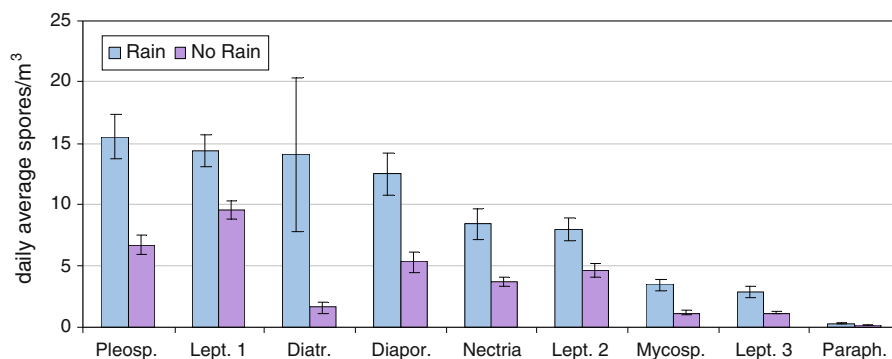


Fig. 4 Daily average ascospore concentration on days with and without rain for types with statistically significant differences (bars represent standard errors). Pleosp.: *Pleospora*, Lept.:

Leptosphaeria, Diatr.: *Diatrype*, Diapor.: *Diaporthe*, Mycosp.: *Mycosphaerella*, Paraph.: *Paraphaeosphaeria*

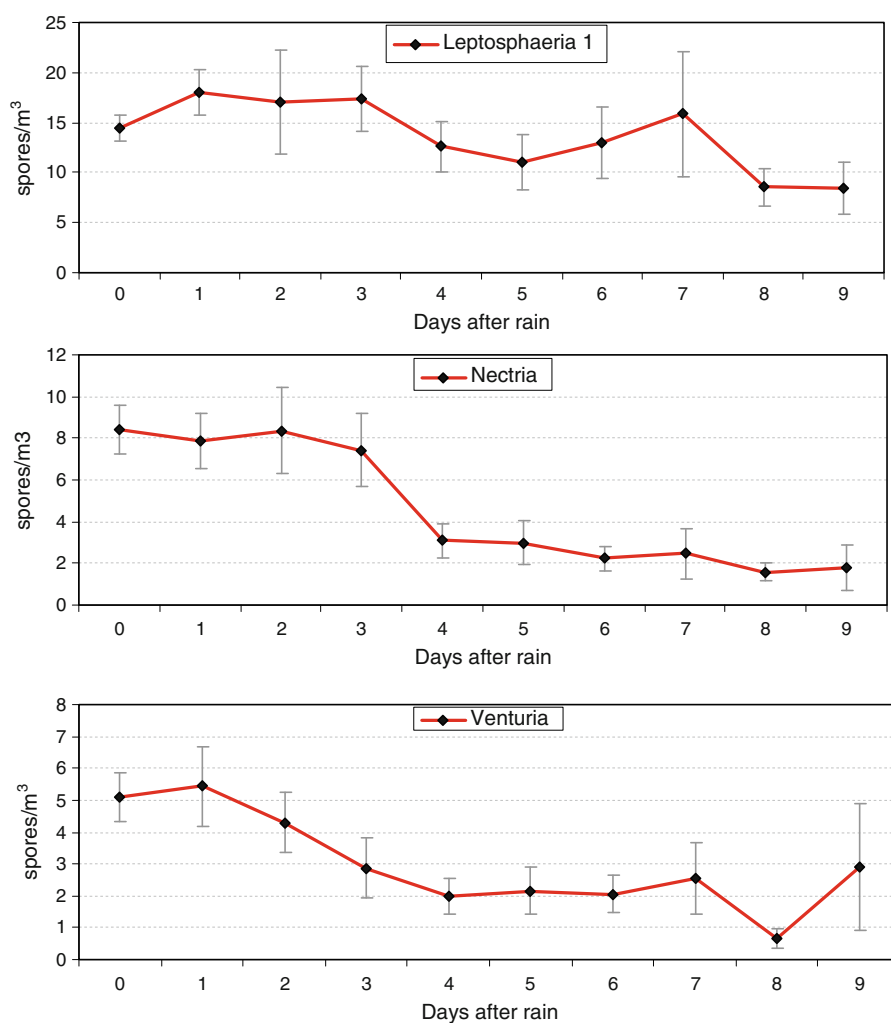


Fig. 5 Average daily concentration on rainy days (0) and on days after rain (1–9) for *Leptosphaeria* (type 1), *Nectria*, and *Venturia* (bars represent standard errors). A number of data are: 0:163, 1:69, 2:46, 3:31, 4:25, 5:20, 6:18, 7:13, 8:12, and 9:10

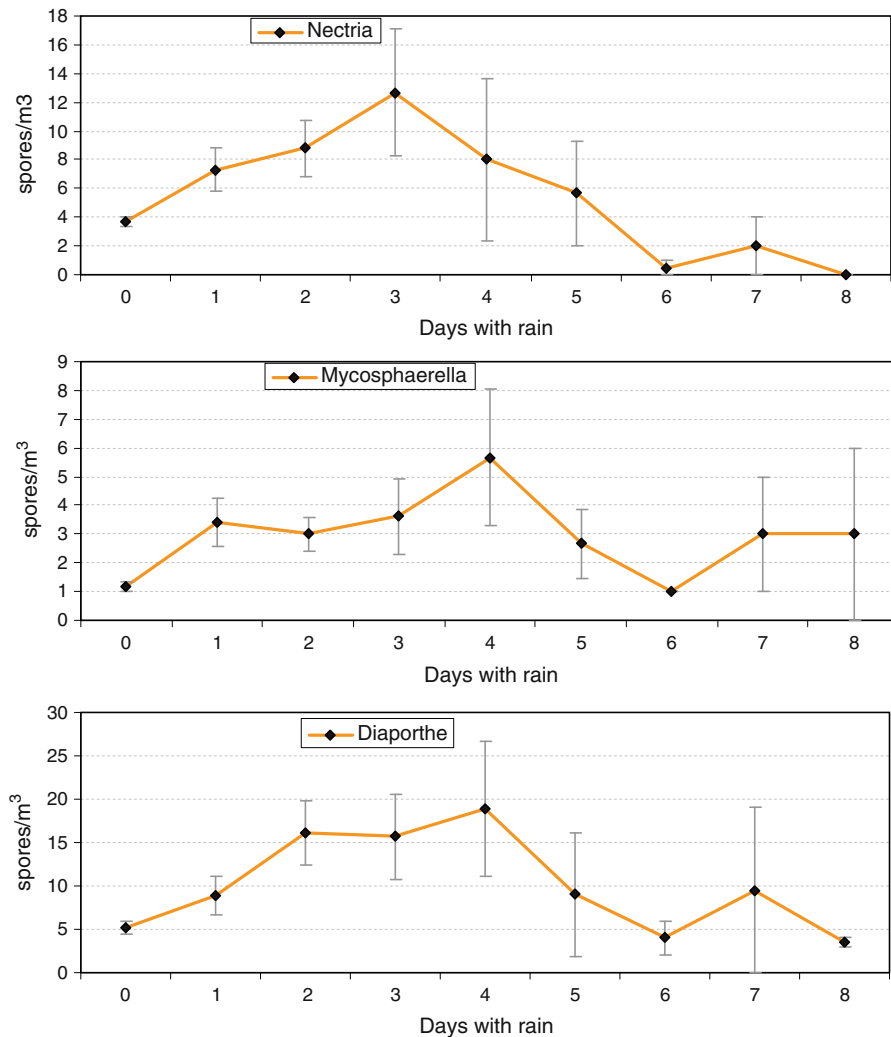


Fig. 6 Average daily concentration during spells of successive rainy days for *Nectria*, *Mycosphaerella*, and *Diaporthe* (bars represent standard errors). 0 means days without rains. A number of data are: 0:540, 1:72, 2:45, 3:28, 4:9, 5:3, 6:2, 7:2, and 8:2

in the present case could be *Leptosphaeria* type 1, although this was one of the most frequent ascospore types found. Indeed, this difficulty in identifying ascospores is reflected in the small number of types identified in the literature: for instance, only one type (Dames and Cadman 1994; Lim et al. 1998; Jothish and Nayar 2004; Ho et al. 2005; Gillum and Levetin 2008), 2 types (Li and Kendrick 1995a, b; Nayar et al. 2007), 3 types (La-Serna et al. 2002; Aira et al. 2002), 4 types (Chakraborty et al. 2003; Adhikari et al. 2004), 5 types (Oliveira et al. 2005), 6 types (De Antoni-Zoppas et al. 2006), 7 types (Shaheen 1992),

8 types (Nunes et al. 2008; Halwagy 1994; Das and Gupta-Bhattacharya 2008), and 20 types (Díez-Herrero et al. 2006).

The annual mean concentration of ascospores found in the present study is comparable with other values reported in the literature: Halwagy (1994) with about 130 ascospores/m³ in Kuwait; Dames and Cadman (1994) with 177 ascospores/m³ in Durban, South Africa; De Antoni-Zoppas et al. (2006) with 136 ascospores/m³ in Caixas do Sul, Brazil; and Gillum and Levetin (2008) with 194 and 250 ascospores/m³ in two places in Oklahoma, USA.

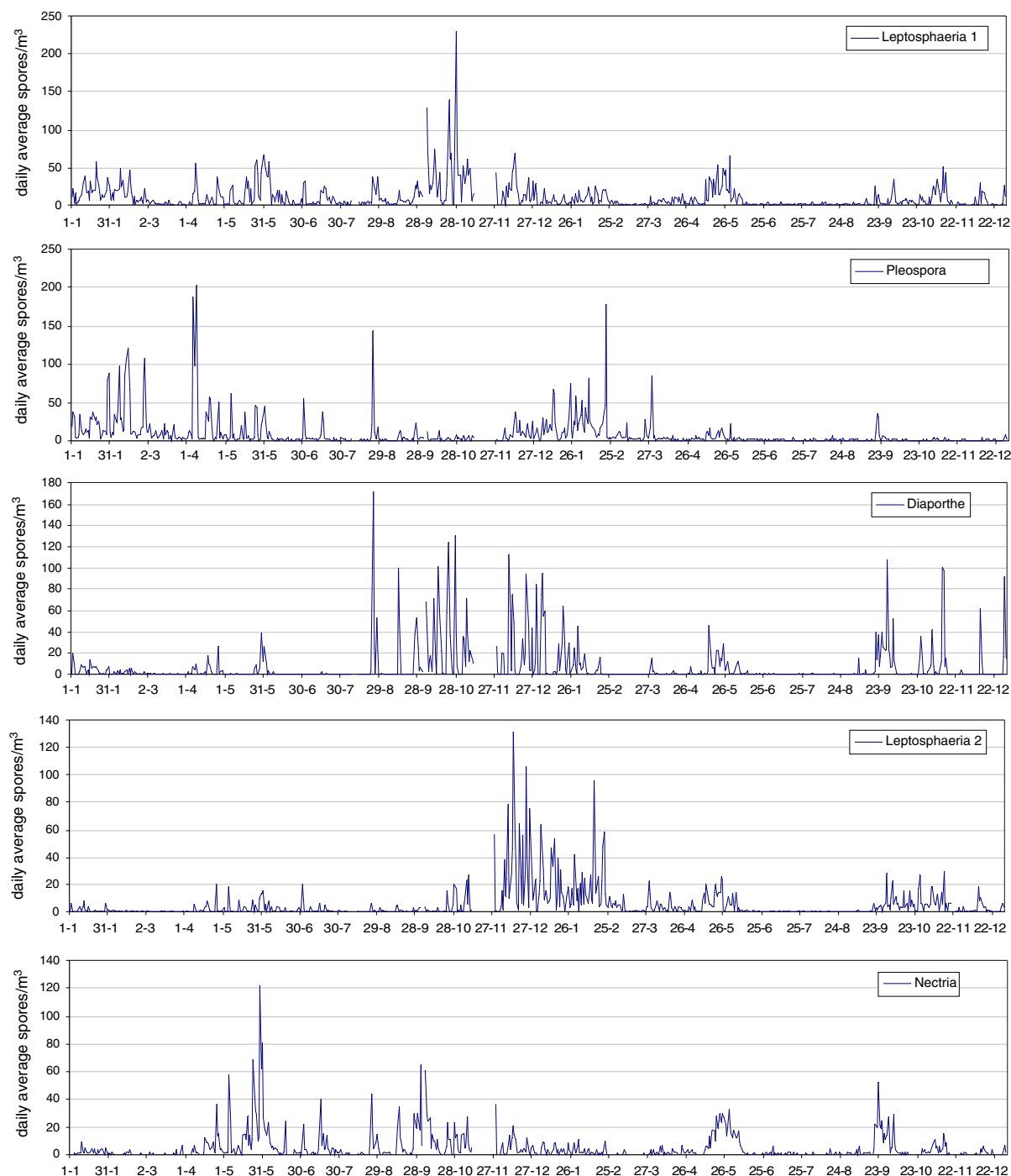


Fig. 7 Daily average concentration for the five more abundant ascospores types for the whole period studied

Other literature studies, however, have reported greater concentrations: Jothish and Nayar (2004) with 740 ascospores/m³ in Kerala, India; and Ho et al. (2005) with 871 ascospores/m³ in Taiwan, with

ascospores in this latter case being the most dominant airborne spore type found. On the contrary, Díez-Herrero et al. (2006) recorded only 48 ascospores/m³ in Madrid.

Autumn and winter were the seasons when ascospores were most frequent, although the actual month with the greatest concentration varied, being September in 1 year and December and October in the other. March was the month with the lowest values. There is no agreement on this aspect in the literature. Halwagy (1994) found the greatest concentrations firstly in March and secondly in August and September; Dames and Cadman (1994) from February to April; Ho et al. (2005) in summer; Das and Gupta-Bhattacharya (2008) in October with 230–240 ascospores/m³; Shaheen (1992) from January to May; De Antoni-Zoppas et al. (2006) in April with up to 800 ascospores/m³ (and minimum values in August); and Das and Gupta-Bhattacharya (2008) in June. This lack of agreement could of course simply be a consequence of different patterns of rainy season distribution depending on climate around the globe. Many ascomycetes are phyloplane fungi that grow on detritus or on leaves following abscission; this could mean that the elevated concentrations of ascospores would be directly associated with increased detritus contamination following leaf abscission (Mishra and Dickinson 1981), so this could be an explanation to their autumn and winter increase.

The most abundant ascospore group in this study was *Leptosphaeria* (4 types), with maxima in autumn and spring (Fig. 7). For this group, Shaheen (1992) found maximum values in March at 1.5 ascospores/m³, Martínez et al. (1996) in May–June at 175 spores/m³, and Oliveira et al. (2005) in December at 13 ascospores/m³. The second most abundant group, the *Venturia*-like ascospores (4 types), also had maxima in autumn and spring. For this group, Shaheen (1992) found a maximum in January with the annual mean concentration being 0.8 ascospores/m³, Martínez et al. (1996) in May at 99 spores/m³, and Oliveira et al. (2005) for *Didymella*, included in the *Venturia*-like types, in December at 13 ascospores/m³. The third group, *Pleospora* type, appeared mainly in winter, with a minimum in summer. For this group, Shaheen (1992) found a maximum in March with the annual mean concentration being 0.1 ascospores/m³, Martínez et al. (1996) in June at 33 spores/m³, and Das and Gupta-Bhattacharya (2008) in spring and autumn at 540–550 ascospores/m³ in April (and a minimum in August). The fourth group, the *Chaetomium* type, had an annual mean concentration of 1.4

ascospores/m³, with maxima first in spring and then in summer. This type has been recorded abundantly in India with concentrations of 30 ascospores/m³ (Jothish and Nayar (2004) and maximum values in June (Das and Gupta-Bhattacharya 2008).

The presence of airborne ascospores is affected by the mechanism of their release from asci, a mechanism that is related to weather conditions. McCartney and Lacey (1991) found that *Sclerotinia sclerotiorum* ascospore release can be triggered by a decrease in relative humidity or an increase in temperature and wind speed, although the triggering conditions may be different in other ascomycetes. According to Corden and Millington (1994), *Didymella* ascospore release is greatly increased when both temperature and rainfall are high, the temperatures triggering this increased release being about 20°C in the period before rainfall. Other mechanisms could be involved depending on ecological conditions. In a study of *Meliolaceae* ascospores in tropical forests, Nayar et al. (1998) indicated that not only rain splash but insect dispersal could be involved. Furthermore, the elongated shape of many ascospores affects their terminal falling velocity, which is often estimated as if the particles were spherical (Gregory et al. 1982). The negative correlation with temperature and the positive correlation with relative humidity found in the present work for many ascospore types are consistent with the findings of other workers (Lim et al. 1998; Ho et al. 2005; Díez-Herrero et al. 2006), and the contrary correlations found for *Chaetomium* are also coherent with the results of Das and Gupta-Bhattacharya (2008). The effect of rain on ascospore concentrations has been studied previously (Díez-Herrero et al. 2006), with increases being found for *Leptosphaeria* (Burge 1986; Hasnain 1993a; Li and Kendrick 1995a, b; Oh et al. 1998) and *Chaetomium* (Davies 1969). The present study, however, also examined the effect on the concentrations on days after the rain event, finding that the influence can last up to even 4 days after the rainfall.

5 Conclusions

Airborne ascospores represent about 6% of total airborne fungi propagules in Mérida (SW Spain). Their concentration shows a clear seasonality, which is greatest in autumn and winter, followed by spring,

and lowest in summer. The annual mean concentration for the period studied was 153 ascospores/m³, although a maximum peak of 3,371 ascospores/m³ was recorded on 1 day. Identification of airborne ascospores can sometimes be difficult because of the presence of hyaline ascospores such as *Mycosphaerella*, some *Leptosphaeria*, and *Massaria*. Nevertheless, nineteen types have been described, and some of them are variations of well-known types. The most abundant groups were *Leptosphaeria*-like and *Venturia*-like ascospores, each group including 4 types and representing about one-third of the total ascospore count. These were followed by the *Pleospora* type. Together, these three groups represented 86% of the total ascospores recorded. Presence of airborne ascospores in the air is greatly affected by weather, especially rain. High relative humidity seems to increase the airborne ascospore concentrations for many types while high temperatures have the opposite effect, although there was a contrary response observed for *Chaetomium*. On rainy days, there was an increase in the airborne ascospore concentrations of *Pleospora*, *Leptosphaeria* (3 types), *Diatrype*, *Diaporthe*, *Nectria*, *Mycosphaerella*, and *Paraphaeosphaeria*, in some cases the increase being by a factor of about eight. The increased concentration may last for up to 3 or 4 days after the start of a rainy spell. The concentrations of *Leptosphaeria*, *Nectria*, and *Venturia* ascospores seem to rise or at least be maintained following the initial increase on a day of rain.

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