

Seasonal Mediterranean pattern for airborne spores of *Alternaria*

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Abstract Airborne *Alternaria* spore presence depends in part on temperature and most studies claim that the highest values are found in summer. Such pattern, however, does not match in Mediterranean countries. The aim of present work is to study the pattern of airborne *Alternaria* in three places in the SW of Spain and to study the influence of meteorological factor in each station. Data of airborne spore concentration for a total of five different years study period, in three cities in the SW of Spain—Badajoz, Cáceres and Mérida—are provided in the present work. Continuous sampling was carried out using a Hirst volumetric spore trap in each location. Results were analyzed taking into account weather parameters regarding temperature, rain and relative humidity. Average concentration

accounting for the complete data set (i.e. considering three locations and full time period) approached 50 spores/m³. Although *Alternaria* spores are present nearly throughout the whole year, monthly data showed that on three occasions October was the month with the highest monthly concentrations up to 342 spores/m³, and January and February those with the lowest concentrations when even no spores were recorded. Daily data showed a concentration peak of 1,380 spores/m³ in Mérida in October. Annual spore concentration showed a pronounced seasonality, with a first maximum concentration in autumn, mainly in October, and a second peak in spring, mainly in May and June. A clear drop was observed in summer, but values remained around the annual average concentration.

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Data of spore concentration showed statistically significant positive correlation with temperature and statistically significant negative correlation with rain and relative humidity. Monthly concentration of *Alternaria* spores was positively affected by temperature and negatively affected by relative humidity and rain; nevertheless, the decrease of relative humidity below 55% showed a drop in spore concentration regardless of any increase in temperature.

Keywords Aerobiology · *Alternaria* · Airborne spores · Seasonal pattern · Hourly pattern · Mediterranean environment

1 Introduction

Alternaria genus is well known as a ubiquitous fungus, a major plant pathogen and a common allergen in humans; nevertheless, many species are only saprophytic. More than 1,100 published names are associated with *Alternaria* although the number of species in the last revision is of 276 (Simmons 2007). They are one of the most frequent agents of decay and decomposition and their club-shaped conidia are airborne widespread. Their optimal development has been shown to be reached at temperature range of 22–28°C, with rarely any growth at 0°C (Hjelmroos 1993). Although spores of *Penicillium* were the first to be suggested that could cause asthma, *Alternaria* spores are the ones that were firstly proven to have this impact (Hyde and Williams 1946), and are currently considered as the genus of molds which most frequently cause allergic symptoms (Bush and Prochnau 2004), nevertheless, it has a lower allergenic threshold (100 spores/m³) than, for example, *Cladosporium* (3,000 spores/m³) (Gravesen 1979).

The first aerobiological studies that specifically dealt with airborne *Alternaria* spores used gravity sampling methods (Durham 1938, 1944; Hyde and Williams 1946). Nowadays only data from volumetric aerobiological sampling are taken into account, as Rooks et al. (1960) early pointed out being one of the first in using this method for *Alternaria*.

It has been known for many years that spores of the genus *Alternaria* cause asthma, being the commonest fungal spore to cause seasonal allergy mainly in the late summer and early autumn (Kilic et al. 2010).

Some aerobiological works focus on the importance of monitoring *Alternaria* in the air as an aid in the fight against phytopathologic problems as blight disease of cotton plants (Bashan et al. 1991) or early blight in potato (Iglesias et al. 2007; Escuredo et al. 2011). These studies revealed that conidia dispersal is mainly local and quite dependent on wind direction and temperature. Nevertheless, they are constantly present in small numbers in the atmosphere throughout the whole year (Bashan et al. 1991; Escuredo et al. 2011).

Alternaria airborne spores are rarely the most common spores in the outdoors, which might be due to the sampling methods, such as passive impact, as results obtained by the device aeroscope coined by Srivastava and Wadhvani (1992). Studies using volumetric sampling showed a great range of variation in airborne spore concentration. Throughout literature consulted average annual concentration has been found to be from less than 10 to more than 100 spores/m³, nevertheless peaks of *Alternaria* spores daily concentration may reach more than 1,000 spores/m³.

There is great agreement that the seasonal pattern of *Alternaria* reaches maximum and minimum concentrations in summer and winter, respectively (Hjelmroos 1993; Herrero and Zaldívar 1997; Bass and Morgan 1997; Stępańska et al. 1999; Mitatakis et al. 2001; Peternel et al. 2004; Konopinska 2004; Sabariego et al. 2004; Rodríguez-Rajo et al. 2005; Iglesias et al. 2007; Grinn-Gofroń and Rapiejko 2009, p.p. in De Linares et al. 2010). Nevertheless there are cases in which the pattern is bimodal, with two peaks, one in spring and another one in fall, and a summer drop in concentration, that occurs always in Mediterranean climates with very hot and dry summers, as in Spain (Angulo et al. 1999; Munuera et al. 2001, p.p. in De Linares et al. 2010) or Sardinia (Cosentino et al. 1995).

Hourly patterns often showed higher concentrations in the afternoon (Angulo et al. 1999; Munuera et al. 2001; Konopinska 2004; Rodríguez-Rajo et al. 2005; Stępańska and Wołek 2009). Nevertheless, they have also been reported before the afternoon (Peternel et al. 2004) or even no daily pattern was found (Sabariego et al. 2004).

The influence of weather parameters in *Alternaria* spore concentration has been widely analyzed. A positive influence of temperature was described by Mitakakis et al. (1997), Herrero and Zaldívar (1997), Angulo et al. (1999), Munuera et al. (2001), Stennett and Beggs (2004), Sabaeriego et al. (2004), Rodríguez-

Rajo et al. (2005), Iglesias et al. (2007), Grinn-Gofron and Rapiejko (2009), De Linares et al. (2010), Escuredo et al. (2011). Negative influence of relative humidity was detected by Timmer et al. (1998) and Rodríguez-Rajo et al. (2005). Negative influence of rain appeared in Mitakakis et al. (1997), Herrero and Zaldívar (1997), Bass and Morgan (1997), Angulo et al. (1999), Sabariego et al. (2004), Rodríguez-Rajo et al. (2005), Grinn-Gofron and Rapiejko (2009) and De Linares et al. (2010). Some studies analyzed airborne spore presence after rain, showing that there was an increase before rains (Hjelmroos 1993), or a decrease during rainy days (Bass and Morgan 1997; Stępańska and Wołek 2009; De Linares et al. 2010). Another environmental factor that has elsewhere been discussed is the harvest of crops, which explains spore liberation in the process (Mitakakis et al. 2001; Corden et al. 2003; Rodríguez-Rajo et al. 2005). Neural networks are also taken into consideration to design models to predict spore concentration of *Alternaria* (Grinn-Gofron and Strzelczak 2008; Tomassetti et al. 2009).

A previous work by our research team (Paredes et al. 1997) in Badajoz revealed a different seasonal pattern for airborne dispersion from the characteristic summer maximum reported in the scientific literature. The present work is aimed at assessing this pattern for other locations—and years—with similar Mediterranean meteorological condition than that in the SW of Spain.

2 Materials and methods

2.1 Sampling sites

Locations under study are located in the SW of the Iberian Peninsula: Badajoz, Cáceres and Mérida (Fig. 1). The respective heights, in meters, above sea level are 184, 459 and 217. The three cities are featured by a stable Mediterranean climate, with an annual mean temperature between 16 and 17°C (Badajoz 16.4°C, Cáceres 16.1°C and Mérida 16.8°C). The highest temperature is reached in the summer, mainly in July and August. Total annual rainfall approaches 500 mm (Badajoz 483.4 mm, Cáceres 509.6 mm, Mérida 510.2 mm), with maximum values between the end of autumn and spring, and with a drought period in the summer, mainly July and August (Fig. 2). Dominant



Fig. 1 Map including place studies

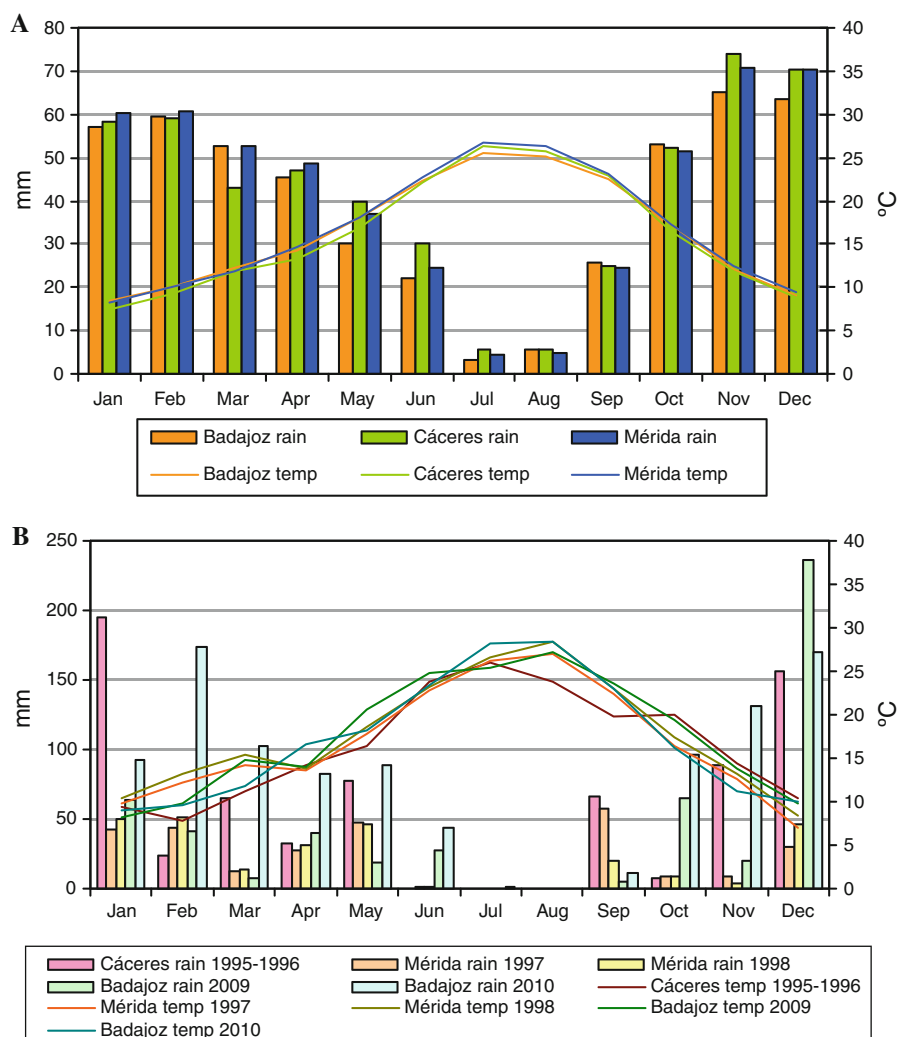
winds in the three places under studied were from the SW throughout the year. Average data provided by the Regional Meteorological Center for 1961–1990 period.

Samplers were located on the outskirts of the abovementioned cities, NW in Badajoz and Cáceres, but SW in Mérida. The most frequent land use around Badajoz and Mérida is irrigated crops (corn, tomato, and fruit trees). However, there is a stronger influence of livestock production and free-range grazing in Cáceres, and less often of cereal, olive and grapevine crops.

2.2 Data analysis

Data of airborne spore concentration were obtained using Burkard 7-day recording volumetric spore traps (Hirst 1952). Air was sampled during 1 year in Cáceres (from 16-10-1995 to 15-10-1996), two in Mérida (1997 and 1998), and two in Badajoz (2009 and 2010). Petrolatum White (CAS number 8009-03-8) was used as an adhesive and two longitudinal scans were counted in each slide sample. Samplers were located on the ground level at 5 m (Cáceres), 25 m (Mérida), and 16 m (Badajoz). Daily data as spores per cubic meter were provided and compared with daily meteorological data of rainfall, temperature and relative humidity. To assess the relationship between rain and spore concentration, daily spore concentration in days with rain days and after rain were analyzed. To assess the relationship between relative humidity and spore concentration, correlation analysis with data of days under a particular percentage of relative humidity was used. In both previous cases all daily data from the three stations were used.

Fig. 2 Monthly average values of temperature and rainfall in the locations under study—period 1961–1990 (a) and for the years studies in each place (b)



2.3 Statistical analysis

Normal distribution of data was tested using Kolmogorov–Smirnov and Shapiro–Wilk tests, since daily data did not follow normal distribution using both statistical tests, even after decimal logarithmic transformation, Spearman correlation coefficient was used. Statistical analysis was performed using the whole daily data set with SPSS 15.0 statistical package.

3 Results

Annual average concentration of airborne spores of *Alternaria* for the three cities and years under study was 49.3 spores/m³, although they ranged from 8.1 to

142.6 spores/m³, depending on the time period and the location. For 4 years, spring was observed to be the season with the highest concentrations, while summer reached that status on 1 year (Mérida, first year). The lowest records always corresponded to the winter. Monthly data showed October as the month with the highest values in 3 years (Fig. 3b, c, d). On another note, May and June achieved the highest values in the two remaining years (Fig. 3a, e). February and January showed the lowest values, including no records on one occasion. A maximum concentration peak of 1,380 spores/m³ was observed on October 9th 1997 in Mérida (Fig. 3b). In such location, concentrations exceeding 100 spores/m³ were achieved in a total of 202 days during the 2 years under study, whereas only 25 days (within the 2 years under study) showed

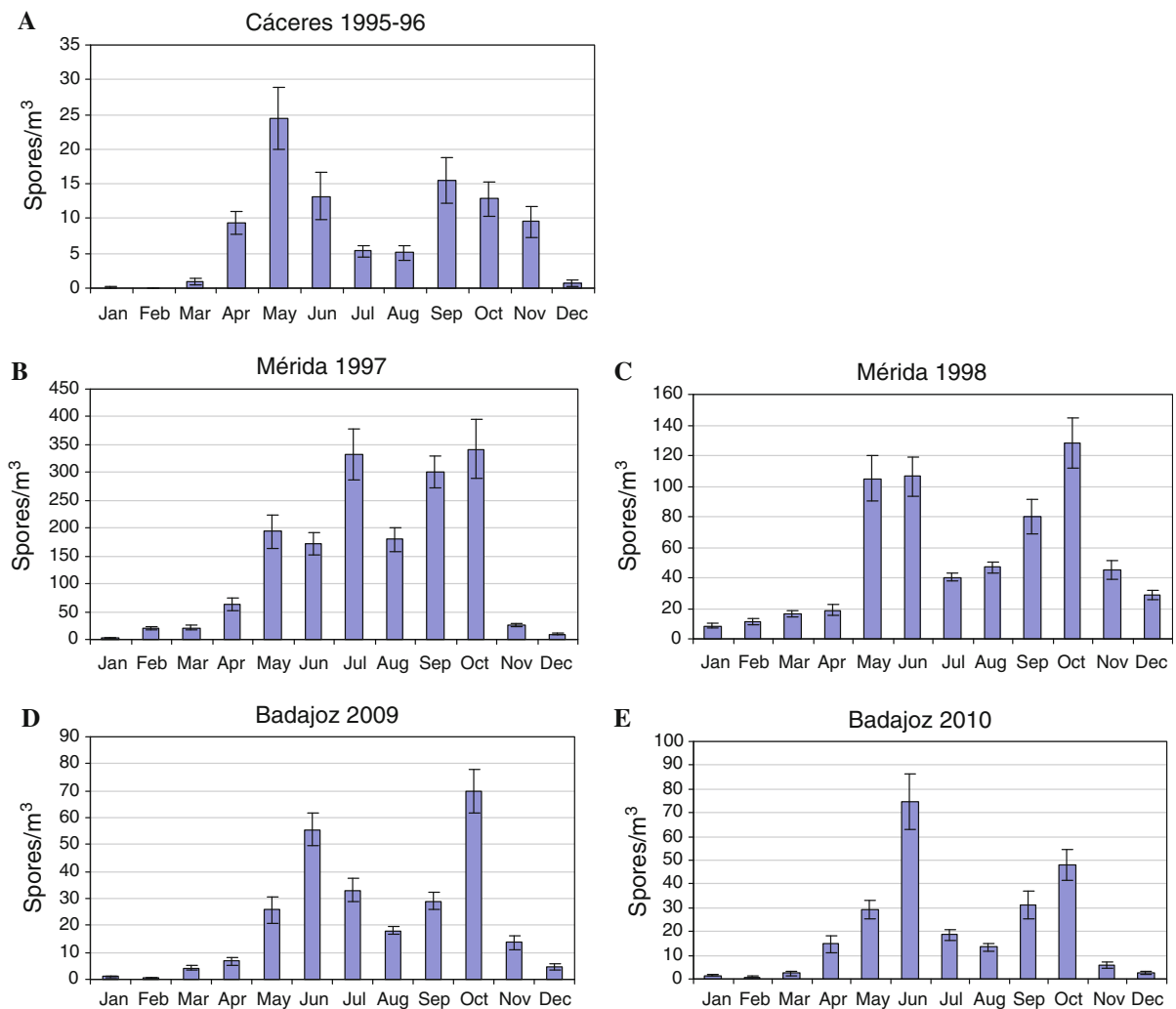


Fig. 3 Monthly average spore concentrations of *Alternaria* in the locations and years under study. Vertical bars represent standard error of the mean

concentrations higher than that value in Badajoz. Finally, the maximum daily peak recorded in Cáceres was 94 spores/m³.

Given that a decrease in concentration between spring and autumn maxima was observed in all cases—during July or August in particular—two annual peaks appeared along the 5 years under study: one in the spring, May–June, or early summer, July, and the other one in autumn, September–October.

Daily data were observed to meet a statistically significant positive correlation with temperature ($r = 0.560$, $p < 0.001$) and a negative correlation with relative humidity ($r = 0.418$, $p < 0.001$) and rainfall ($r = 0.334$, $p < 0.001$). On rainy days,

450 days out of 1,734, the average concentration was 26.8 spores/m³, while a value of 56.5 spores/m³ was achieved for rainless days (1,284 days). A progressive increase in spore concentration was observed after rain had appeared, until the fourth day (Fig. 4a), though not statistically significant. Continuous rainy days achieved a progressive decrease in spore concentration (Fig. 3b). To assess the influence of relative humidity in summer's decrease of spore concentration, data below a particular relative humidity value were correlated with relative humidity. Results are listed in Table 1, showing a positive correlation between spore concentration and relative humidity in days below 55% relative humidity, with a

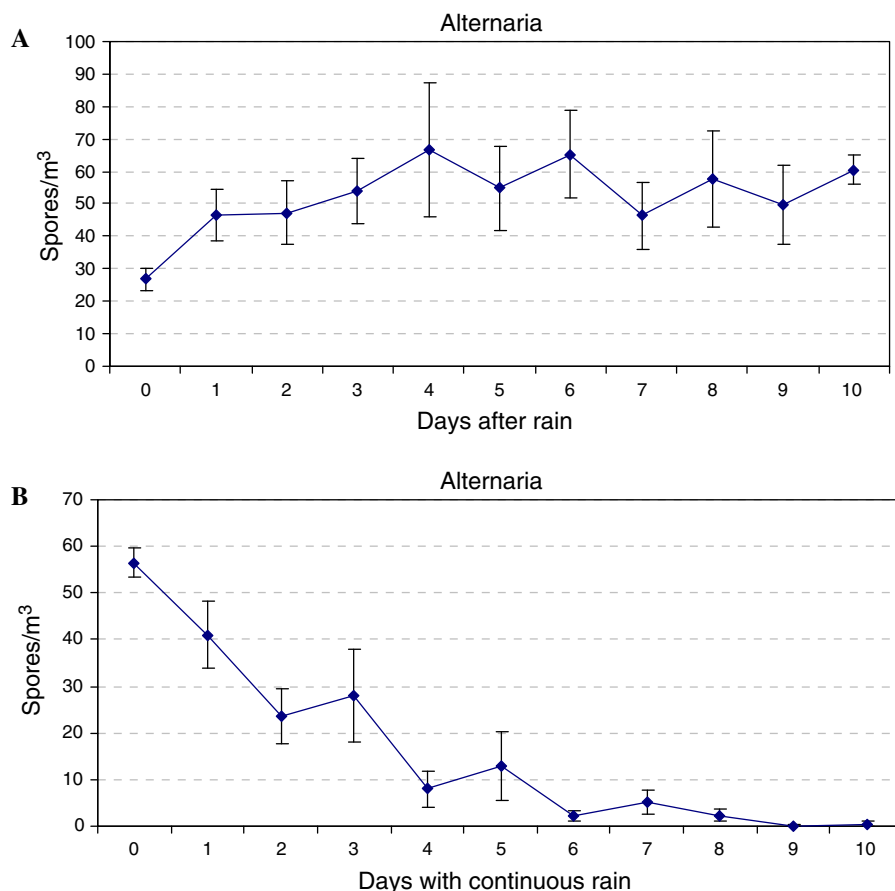


Fig. 4 **a** Average spore concentration of *Alternaria* on rainy days (0) and after-rain days (1–10). **b** Average spore concentration of *Alternaria* in rainless days (0) and consecutive days with rain (1–10)

maximum value at 51% relative humidity. Figure 5 shows the relationship between spore concentration and relative humidity over a period of 10 days in average for Mérida and Cáceres.

Daily patterns are showed in Fig. 6. Cáceres' hourly maximum concentration appeared by the noon; Mérida's maximum concentration was recorded between the afternoon and the early evening, while Badajoz's maximum corresponded to the night or the early morning. Dominant winds in the three locations under study are from the SW. Spore traps in Cáceres and Badajoz were located on the NE of the city. Urban environment might have affected data in both cases. Nevertheless, the spore trap in Mérida was placed on the SW of the city and the crop's environment may have influenced airborne capture much more than the urban environment.

4 Discussion

Average annual concentration of *Alternaria* spores was reported to be very low in some cases, below 10 spores/m³: Stockholm in Sweden (Hjelmroos 1993), Krakow and Zakopane in Poland (Stępańska et al. 1999), Vigo and Ourense in Spain (Rodríguez-Rajo et al. 2005), Vielha, Izaña and Santa Cruz de Tenerife in Spain (De Linares et al. 2010). It was more often observed to meet the range from 10 to 50 spores/m³: Melbourne in Australia (Mitakakis et al. 1997), Sydney in Australia (Bass and Morgan 1997), Moree and Wagga in Australia (Mitakakis and McGee 2000), Murcia in Spain (Munuera et al. 2001), Varsow and Ostrowic in Poland (Stępańska et al. 1999), Barcelona, Bellaterra, Girona, Tarragona, Tortosa, Almería and Granada in Spain (De Linares et al. 2010). Less often between 50

Table 1 Spearman correlation coefficient (r) and probability (p) between relative humidity (RH) and spore concentration, average concentration for each RH in spores/m³, for days below a particular value of RH

RH (%)	Days	r	p	Average
80	1,373	-0.1801	0.0000	58.0
75	1,245	-0.1088	0.0001	61.6
70	1,083	-0.0423	0.1638	64.7
65	913	-0.0208	0.5303	64.4
60	720	0.0421	0.2595	63.7
55	518	0.1672	0.0001	62.6
54	490	0.1844	0.0000	63.9
53	454	0.1965	0.0000	62.4
52	420	0.2000	0.0000	62.3
51	392	0.2587	0.0000	64.9
50	365	0.2475	0.0000	64.4
49	325	0.1929	0.0005	59.4
48	302	0.1751	0.0023	60.0
47	273	0.1952	0.0012	60.8
46	244	0.1904	0.0028	61.1
45	216	0.2210	0.0011	57.7

Days' column includes the number of days with RH below a particular value

and 100 spores/m³: Poznan in Poland (Stępańska et al. 1999), Córdoba in Spain (Angulo et al. 1999), Lublin in Poland (Konopinska 2004), Wagga Wagga in Australia (Mitatakis et al. 2001), Manresa and Lleida in Spain (De Linares et al. 2010). Finally, it was only exceptionally found to exceed 100 spores/m³, as in Moree, Australia (Mitatakis et al. 2001).

The average *Alternaria* spore concentration found in the present work is quite similar to that reported for Murcia (Munuera et al. 2001), Poznan (Stępańska et al. 1999), or even Manresa and Lleida (De Linares et al. 2010). Nevertheless, since the 5-year period under study showed a high degree of variation, it is not possible to state a general rule, although the spore concentration of the first year in Mérida was the highest compared to data from other studies in the literature for other regions—except data from Badajoz in 1995 (Paredes et al. 1997)—notwithstanding the highest values were found in Mérida, where crop and harvest influence may be greater than that in both other cities. This could be in agreement with opinions claimed by other authors (Mitatakis et al. 2001; Corden et al. 2003; Rodríguez-Rajo et al. 2005).

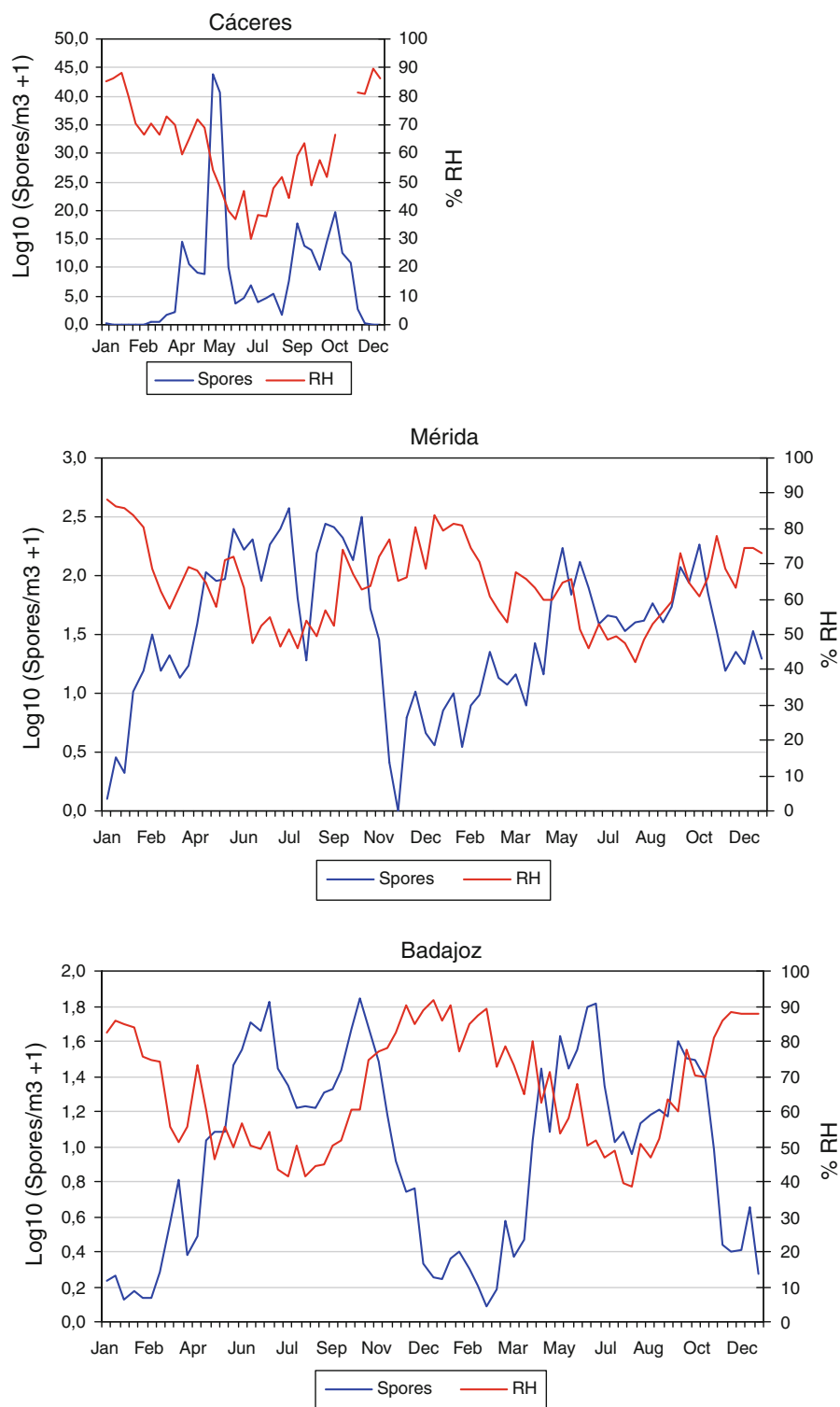
Peaks of *Alternaria* spores daily concentration may reach more than 1,000 spores/m³, but in many cases it depends on the number of sampled years, which allows more opportunities to find those exceptional records. Some of the highest were: 1,125 spores/m³ in Sydney, Australia (Stennett and Beggs 2004), 1,334 spores/m³ in Moree, Australia (Mitatakis et al. 2001), 1,388 spores/m³ in Manresa, and 1,506 spores/m³ in Tarragona, Spain (De Linares et al. 2010), and 1,635 spores/m³ in Badajoz, Spain (Paredes et al. 1997).

The seasonal two-peak pattern found does not agree with the majority of the studies from other authors analyzed so far, for which summer was regarded as the season with the highest concentration values. Similar data have elsewhere been reported for those places studied with similar Mediterranean climatic conditions (Cosentino et al. 1995; Angulo et al. 1999; Munuera et al. 2001; p.p. in De Linares et al. 2010) with very hot and dry summers, when relative humidity reaches the lowest values. The research studies on *Alternaria* colonies in Córdoba, conducted by Nogales et al. (1986), concluded that January was the month with the lowest concentration values, as we found, and peaks were reached in May and June. It would be arguable that land use, including cropping, could affect seasonal pattern, but we have found similar seasonal pattern in spite of differences between Mérida and Badajoz with mainly irrigated crop lands and Cáceres with mainly grazing and cereal crops as land used around the city.

Temperature, relative humidity and rainfall were observed to affect spore concentration in this study in the same way as the vast majority of research works found in the scientific literature. Rainfall clearly washes *Alternaria* spores out of the atmosphere (Langenberg et al. 1977; Timmer et al. 2000; Hill and Hausbeck 2009). Nevertheless, it seemed to be an increase of spore concentration after rain that nearly duplicates concentration. Relative humidity below 60% is not frequent in Europe, except in Mediterranean localities during the summer (SAGE 2002). These low values could be responsible for the decrease in spore concentration during summer, because temperature could be suitable whereas relative humidity might limit growth or spore development, as was showed by Timmer et al. (1998).

The hourly pattern found in Badajoz and Cáceres is in agreement with most studies regarding maximum concentration to be reached in the evening (Angulo

Fig. 5 Ten days average spore concentration of *Alternaria* and relative humidity for the locations under study



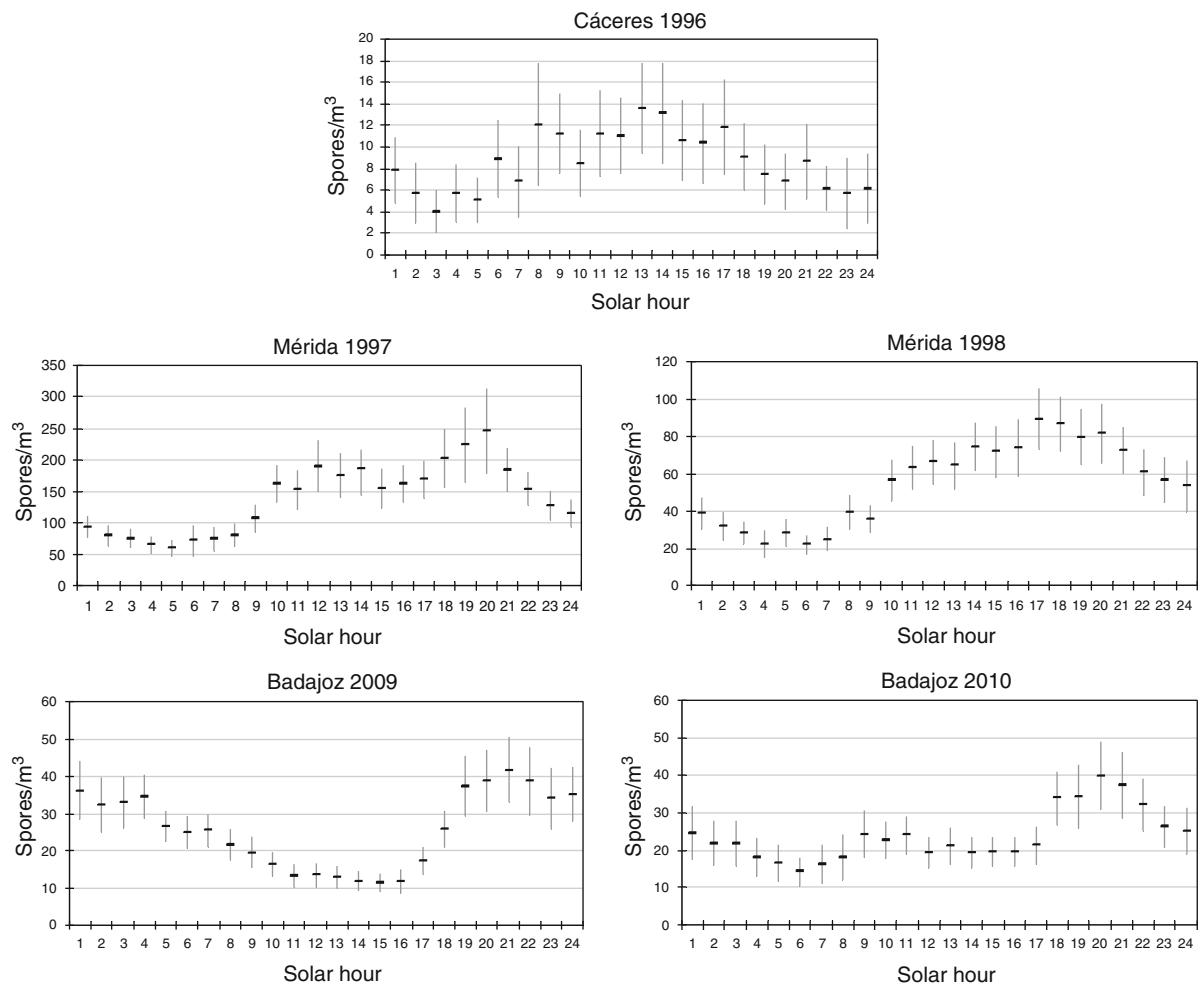


Fig. 6 Average hourly spore concentration of *Alternaria* for the locations and days under study. Vertical bars represent standard error of the mean

et al. 1999; Munuera et al. 2001; Konopinska 2004; Rodríguez-Rajo et al. 2005; Stępańska and Wołek 2009). However, maximum concentration in Cáceres was recorded in the afternoon, notwithstanding data from this locality were the lowest, showing a higher range of variability in hourly data, it is possible that the general hourly pattern did not remain so evident.

5 Conclusions

Alternaria spore presence in the atmosphere follows a seasonal pattern mainly conditioned by temperature and relative humidity. Temperature directly affects their presence, but when relative humidity reaches a

critical value the effect is negatively related. In fact, it seems that below 60% relative humidity airborne spore concentration shows a decrease that is directly affected by this weather factor. The highest correlation was achieved for 51% relative humidity. In Mediterranean locations, summer is regarded as a critical season for *Alternaria* growth. This way, seasonal patterns show two peaks with a maximum in the fall and the spring, and a clear drop in the summer. Finally, the hourly pattern of airborne spore concentration seems to meet that reported by other authors, for which maximum concentration is reached during the evening.

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