

Effects of meteorological factors on airborne bracken (*Pteridium aquilinum* (L.) Kuhn.) spores in Salamanca (middle-west Spain)

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Abstract Temporal variation of airborne bracken (*Pteridium aquilinum*) spores concentration in Salamanca during 10 years from January 1998 to December 2007 were studied by using a Burkard spore trap, and correlations with some meteorological parameters were analyzed. The number of spores that were counted was very low, due probably to the distance between the spore trap and the main bracken populations which were located 70 km away from the city. Long-range transport caused by winds coming from the Second Quadrant (IIQ) is supposed to be responsible for the appearance of bracken spores in Salamanca. The season period from August to late October shows the most intense spore dispersal process, with an early morning distribution along the day. Years 2002 and 2007 with a low quantity of airborne spores were also characterized by low mean temperatures, always under 18°C from May to June. Daily spore concentration shows positive correlation with temperature and sun hours but negative with IVQ winds and with relative humidity. No correlation between daily spore concentration and rainfall was found. Also, a positive correlation between number of spores and IIQ winds was observed during the main spore season (MSS) and prepeak period (PRE).

Keywords Bracken · Meteorology · *Pteridium* · Spores · Salamanca · Spain

Introduction

Bracken (*Pteridium aquilinum* (L.) Kuhn.) is a sub-cosmopolitan fern which occurs worldwide in thickets of different forests and their serial stages, or even belonging to open temperate grassland. In the Mediterranean region, it is located in mountainous zones and in steep riverbanks and other shady ravines (Muñoz-Garmendia 1986). Bracken usually spreads by rhizomes, though it produces many millions of spores (Marrs and Watt 2006) that are able to turn into gametophytes and later into young sporelings. Despite this staggering amount of spores, its incidence in cases of allergy is low (Hyde 1972; Chew et al. 2000), even in tropical areas, and nor have cases of cross-sensitivity among other airspora, included fungal spores been reported (Newson et al. 2000).

It is well known that bracken has high toxicity that may induce pathological effects if ingested by domestic livestock (Yamada et al. 2007), and therefore it might become a risk to human health (Evans et al. 1971). Moreover, in recent years, some studies concerning the carcinogenic properties of bracken spores (Povey et al. 1996; Freitas et al. 2001) have indicated that humans might be exposed to this risk due to the inhalation and subsequent ingestion of the spores (Wilson et al. 1998).

Pteridophyte spores are rarely airborne (Kasprzyk 2004). There are few papers reporting data about airborne fern spores (Hurtado and Riegler-Goihman 1986; Yasmeen Singh and Devi 1988; Noetinger et al. 1994); in the case of bracken airspora, some analyses in Great Britain (Lacey and McCartney 1994; Caulton et al. 2000) have been carried out with volumetric spore traps that are located near to the sporing stands.

The aim of this study was to analyze the occurrence of bracken spores in the atmosphere of Salamanca in

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relation to meteorological variables. The lack of studies on airborne bracken spore behavior across continental Europe, especially in places located far away from high density areas of this fern, makes this kind of study necessary in order to show the influence of temperature on the development of bracken sporangia and subsequent spore production.

Materials and methods

Analysis of the spore counts and spore fall distribution were performed on the basis of the data collected from 1 January 1998 to 31 December 2007. The volumetric measurement unit was located on a central historical building at a height of 20 m above ground level. The spore count was monitored by the volumetric method with a Burkard 7-day spore trap, and is expressed as the number of spores in a cubic meter of air per day. The samples were mounted and counted by the method proposed by the Spanish Aerobiology Network (Domínguez et al. 1991; Galán et al. 2007). *Pteridium* spores were counted and evaluated according to their known morphological differences (Andrew 1984; Muñoz-Garmendia 1986; Renault-Miskovsky and Petzold 1989; Tyron and Lugardon

1991), with a Nikon Optiphot microscope (40×, 0.70 objective).

The city of Salamanca lies in the mid-west of the Iberian Peninsula (40°58'N, 5°40'W) at 800 m a.s.l (Fig. 1) with a typical Mediterranean continental climate. The annual mean temperature is 11.7°C, with an average maximum temperature of 17.9°C and a minimum of 5.5°C. The yearly precipitation average is about 382 mm, with a typical pattern throughout the year: a rainy period in spring and autumn and a dry season in the summer months.

The main surrounding forests are represented by pastures with holm oaks (*Quercus ilex* L. subsp. *ballota* (Desf.) Samp.) and the largest bracken density is located forming thickets in forests with oak trees (*Quercus pyrenaica* Willd.). These forests are present in the mountainous region located about 70 km to the south of the city. In phytosociologic aspect, they belong to the *Holco mollis-Quercetum pyrenaicae* sigmetum and to the *Genisto falcatae-Quercetum pyrenaicae* sigmetum (Rivas-Martínez et al. 2002).

The main spore season (MMS) was estimated from the period that includes 95% of the total of annual airborne spores. Therefore, the initial period where up to 2.5% of total spores are obtained as well as the final one, where more than 97.5% of the spores have been captured, are both

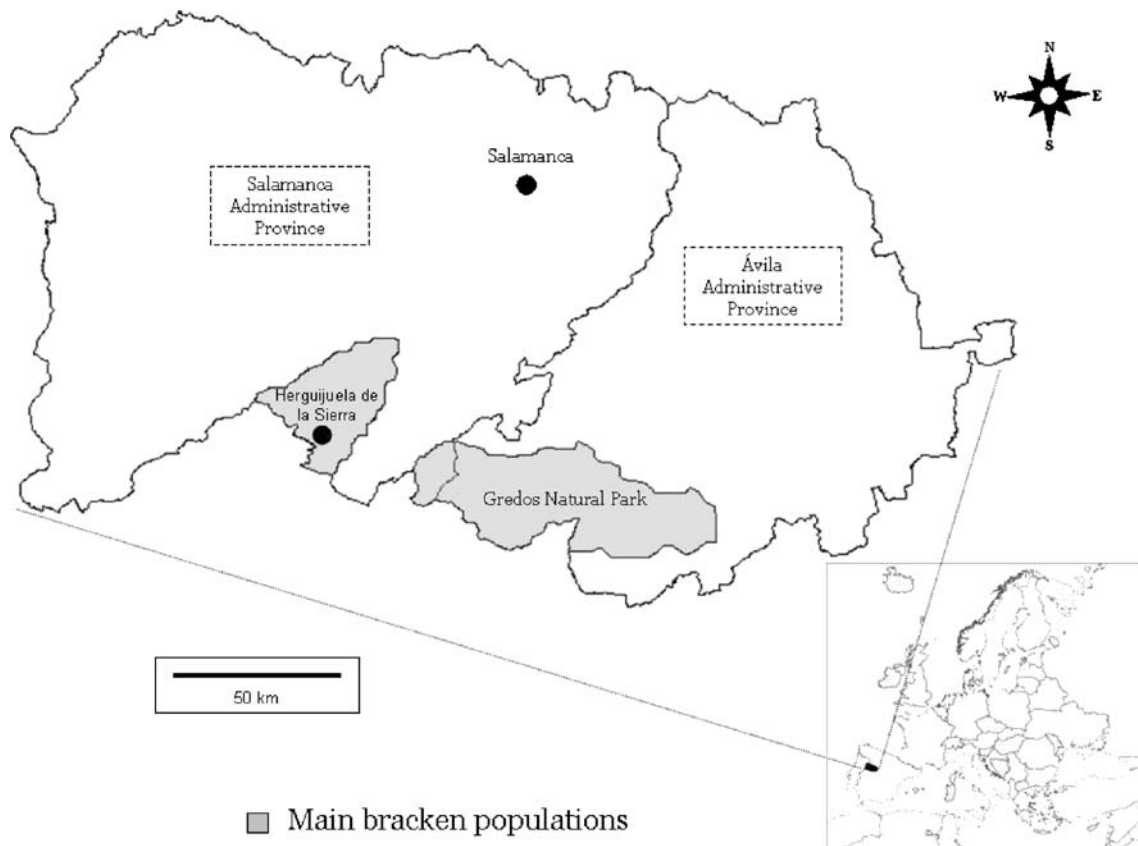


Fig. 1 Geographical location of Administrative Provinces of Salamanca and Ávila, showing areas with high density of bracken (*Pteridium aquilinum*) thickets (grey) and the location of meteorological stations (black dots)

Table 1 Aerobiological parameters for airborne bracken (*Pteridium aquilinum*) spores during the studied period

<i>Pteridium</i>	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	Mean
Annual total	111	83	39	63	12	85	89	131	56	4	67
Date of peak-day (peak value)	29 Sep (20)	9 Sep (8)	25 Sep (4)	4 Oct (4)	3 Aug (4)	6 Sep (19)	12 Aug (15)	4 Sep (20)	23 Aug (7)	-	9 Sep (4)
Starting–ending date of MPS	31 Aug – 12 Oct	12 Aug – 27 Sep	15 Aug – 30 Sep	18 Aug – 13 Oct	-	14 Aug – 4 Oct	10 Aug – 22 Sep	24 Aug – 4 Oct	14 Aug – 6 Oct	-	17 Aug – 5 Oct
Length of MSS (days)	43	47	47	57	-	52	44	42	54	-	48

Years 2002 and 2007 had no MSS due to the low number

eliminated (Andersen 1991). The prepeak period (PRE) includes the period between the starting date of the MSS and the date on which the highest concentration is reached. Daily variations are shown from August to October in order to get a clear picture of the average seasonal distribution.

Since the airborne spores did not seem to display normal distribution, Spearman's non-parametric correlation test was used. This method produces possible correlations between bracken spore concentrations and the main meteorological variables, such as mean, maximum and minimum temperatures (°C), sunshine hours, rainfall (mm), relative humidity (%), mean wind speed (m/s), wind direction (%), and wind calm (%). Correlations took into account both the main spore season and the time of bracken spores dispersal (from August to October).

Meteorological data for the period 1998–2007 were supplied by the Spanish State Meteorological Agency from the meteorological station (Matacán) located 10 km east of the sampling site. We also analysed the data from the meteorological station of Herguijuela de la Sierra (648 m a.s.l.; 40°26'N, 6°04'W), located in Batuecas-Sierra de Francia Natural Park, very close to bracken populations (Fig. 1), to determine the influence of temperature in the

development of sporangia. The SPSS v.13 software package was used for the statistical analysis.

Results

The annual totals of *Pteridium aquilinum* spores in the atmosphere of the city of Salamanca are shown in Table 1. As can be seen, they were rather variable in the studied period. No other types of Pteridophyta spores were found. The total annual spore count fluctuates from a maximum of 131 spores in 2005 to a minimum of 4 spores in 2007. Years 1998 and 2002 also show rather high and low annual amount of spores, with 111 and 12, respectively. Although the numbers of spores counted were low, the particular distribution of these spores in Salamanca during late summer and early to mid-autumn, when the presence of pollen grains in the atmosphere has decreased considerably, implies an important percentage of the total of pollen grains and pteridophyte spores. In fact, the highest numbers were generally recorded in September, and the maximum daily concentrations were observed on 29 September 1998 and 4 September 2005 (20 spores/m³).

Fig. 2 Daily variation of the average of the daily spore counts of bracken in Salamanca for the studied period (in percentage over total spore counts average for the 10-years). The line shows the 5-day running means

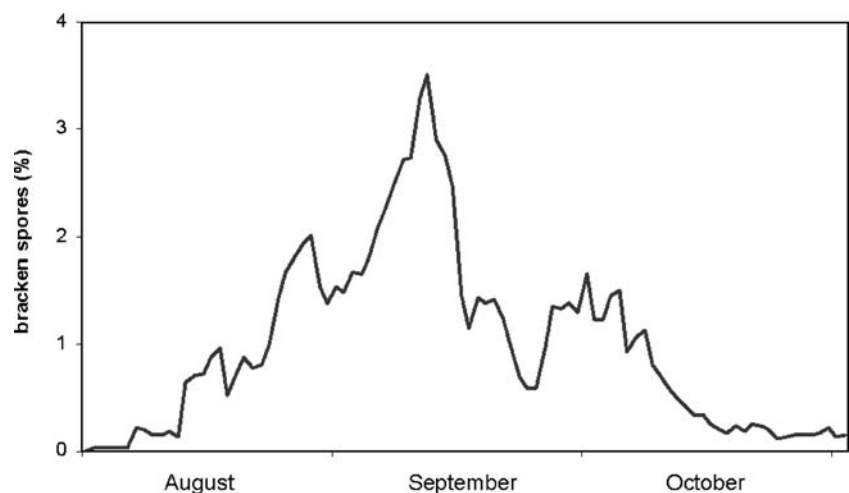
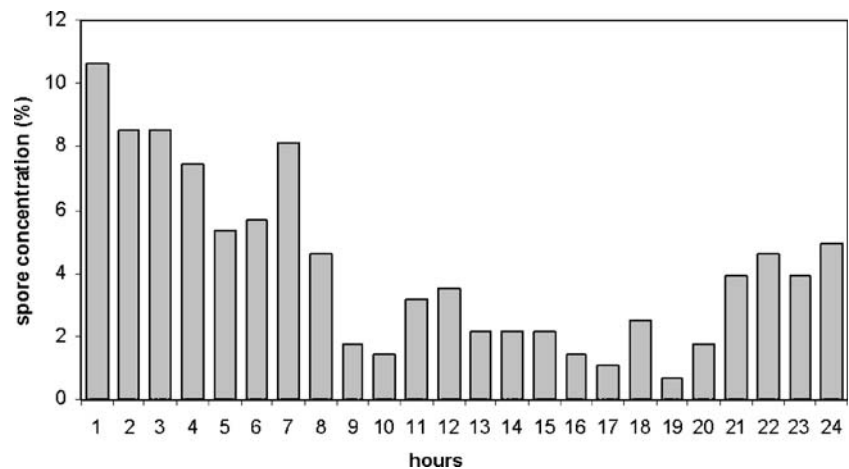


Fig. 3 Intra-daily variation of bracken spore concentrations, mean daily counts (%)



Seasonal variations in the daily average of bracken spore concentrations from 1 August to 31 October is shown in Fig. 2, together with an average of the 5-day running means. From late August, the daily levels of spore increases gradually until the second week of September (9 September), when they reach the highest mean daily value (4 spores/m³). On average, the MSS starts on 17 August, and ends on 5 October. The period of the MSS ranged between 42 and 57 days and its average length was 48 days.

A study of the intra-daily variation of bracken spores (Fig. 3) revealed that the highest values were recorded during the first hours of the day, specifically between 0000 and 0700 hours, reaching, on average, more than half the total of daily spores. Subsequently, these values decrease and remain low during the morning and afternoon, becoming higher again at night.

The percentage of *Pteridium* spores with respect to the total pollen and pteridophytes spore counts was very low in the studied period. Nevertheless, this ratio was significantly high during the 3 months August–October in which bracken spores were present in the atmosphere varying

from 0.5% in 2007 to 24% in 1999, with an average value of 13%. The weekly average of the studied years (Fig. 4) shows that, in the period between the last week of August and the first week of October, the bracken spores have important percentages with regard to the total airborne elements of vascular plants.

During the years 1998–2007 and from May to July, the effects of temperature over the airborne spore content were analyzed (Fig. 5). In years 2002 and 2007, the number of atmospheric spores that were counted was lower than in the other years of the studied period. This was closely related with the average temperatures of May, June and July of those years, always with values below 18°C. In fact, year 2000 had the third lowest annual total and an average temperature of 18.1°C.

Finally, correlations between daily spore counts and the main meteorological parameters were calculated with the Spearman's non-parametric test (Table 2). Data for the whole studied period, for the MSS and for the previous days to the peak (PRE) were used to this purpose. Correlations with a statistically significant positive correla-

Fig. 4 Weekly mean totals of pollen and bracken spores from August to October during the period 1998–2007

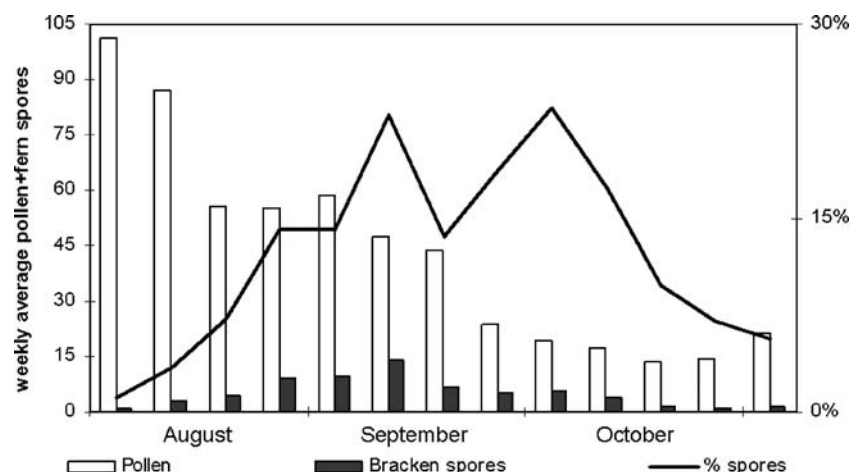
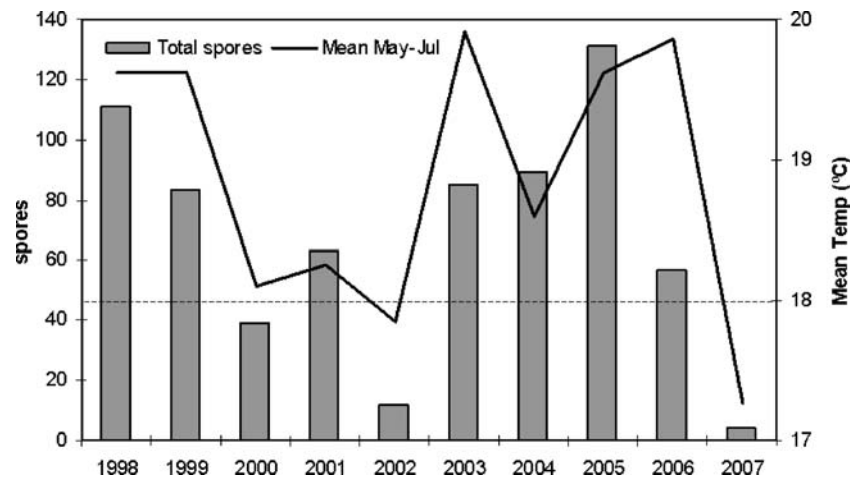


Fig. 5 Mean temperatures from May to July in the meteorological station of Herguijuela de la Sierra and total spore concentrations recorded in Salamanca during the studied period



tion ($P < 0.01$) were found in a large number of cases: average temperature, maximum and minimum temperature, sunshine hours, calm, and only if the analysis is limited to the MSS during the years of the studied period, with winds coming from Second Quadrant (IIQ). On the other hand, a highly significant negative correlation was observed with winds coming from the Fourth Quadrant (IVQ) with relative humidity and with wind speed. All these correlations shown during MSS can also be found in PRE, but also including a significant positive correlation with minimum temperature.

Discussion

The shedding process of bracken spores in Salamanca seems to be similar to that already described in Great Britain (Lacey and McCartney 1994; Caulton et al. 2000). The same behavior has also been referred to in different places in Poland, regarding the spores of other pteridophytes such as *Equisetum* and *Lycopodium* (Kasprzyk 2004). In this case, the number of spores counted was lower than observed in rural areas of Scotland or the city of Edinburgh, both near to important bracken thickets (Lacey and McCartney 1994; Caulton et al. 2000). Rantio-Lehtimäki (1994) determined three different ways in which airborne particles are transported: short, when the space scale is not above 1 km; medium, when the distance limits range from 1 to 100 km; and long-range transport, controlled by large-scale and very difficult to predict events. The study developed by Lacey and McCartney (1994) in an experimental farm with bracken shrubs, pointed to short-range transport as the mechanism that disperses the spores. In our study, the nearest important bracken formations are located 70 km south, far away from the city of Salamanca. Therefore, the spores would get to the trap by means of medium-range transport with

the help of IIQ winds, which would also explain its positive correlation with the spore concentrations during MSS and PRE periods (Table 2). Nevertheless, the presence of important topographical barriers, such as mountains, and the great size and ornamentation of this kind of spore showed that these aerobiological particles had considerable difficulties in traveling medium distances. Consequently, very large volumes of air may be needed to collect these spores from medium distances with an air sampler and allow us to take into account long-range transport as the most probable type of transport. In the same way, large-scale transport was considered for

Table 2 Spearman's correlation coefficients for *Pteridium* daily spore counts in relation to the meteorological parameters during the studied period

	1998–2007	MSS	PRE
Tmean	0.255**	0.119*	0.182*
Tmax	0.249**	0.090	0.131
Tmin	0.245**	0.098	0.168*
Sunshine	0.103**	0.062	0.080
R	−0.033	−0.095	−0.069
RHum	−0.116**	−0.107*	−0.161*
Wind speed	−0.066**	−0.041	−0.108
IQ	0.063	−0.019	−0.121
IIQ	0.041	0.242**	0.299**
IIIQ	0.082	0.011	0.092
IVQ	−0.467**	−0.399	−0.492
Wind Calm	0.114**	0.067	0.083

MSS Main spore season, PRE prepeak period, Tmean mean daily temperature, Tmax maximum daily temperature, Tmin minimum daily temperature, Sunshine sunshine hours, R daily rainfall, RH relative humidity, Wind speed mean wind speed, IQ winds coming from the First Quadrant, IIQ winds coming from the Second Quadrant, IIIQ winds coming from the Third Quadrant, IVQ winds coming from the Fourth Quadrant, Calm frequency of wind calm

* $P = 0.05$

** $P = 0.01$

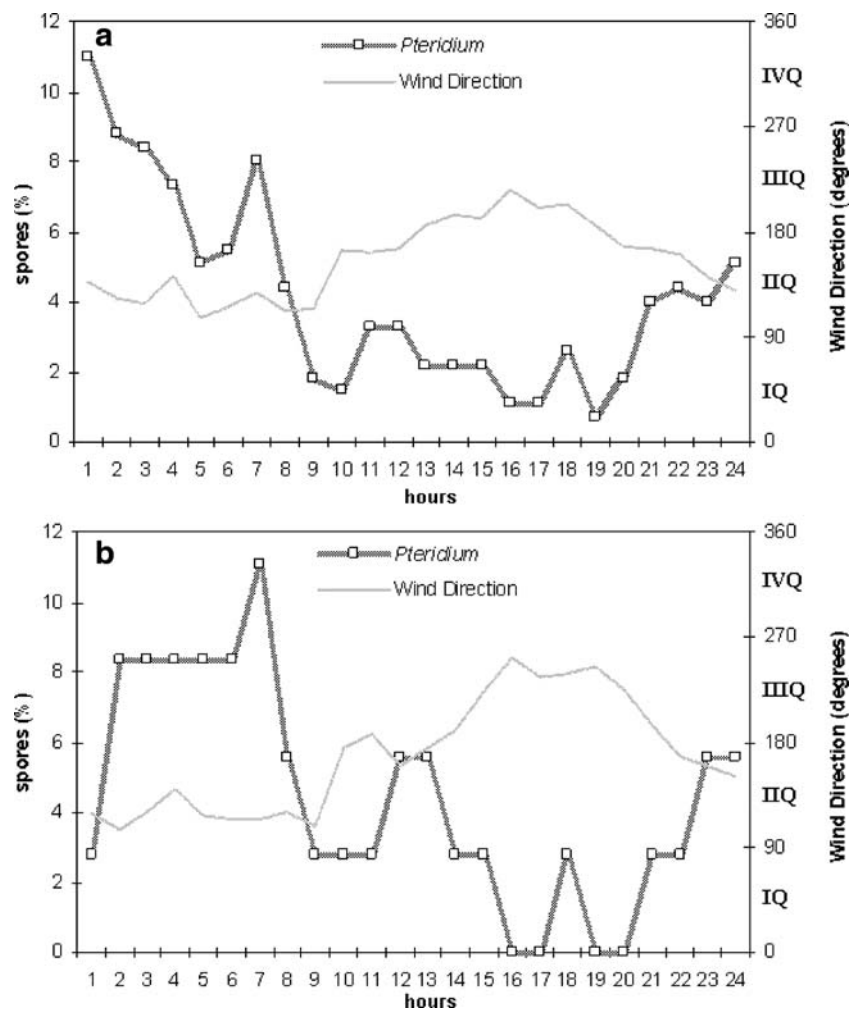
Lycopodium spores (Di-Giovanni et al. 1995), owing to their settling velocities, very similar to those measured in some anemophilous pollens.

The spore behavior throughout the day in the city of Salamanca, with a marked nocturnal periodicity (Fig. 3), seems very different to the hourly distribution reported by Lacey and McCartney (1994), in which bracken spores were mainly observed around 1200 hours. As was said before, this study was developed with the help of a spore trap that was located near to bracken formations (this was useful to find out that bracken airspora were closely related with the production of sporangia). In addition, the effects of wind direction may explain the particular intradaily concentrations (Fig. 6), since in MSS and PRE, the hourly concentrations of spores were higher when winds were coming from the Second Quadrant. Thus, since there are different areas with bracken populations surrounding Salamanca, every one of them with different times of spore liberation from sporangia, wind direction plays an important role in the rhythm of capture of spores by the spore trap.

The absence of negative correlations between airborne spores and rainfall could be related with the storms that frequently occur in late summer, and also with the particular hourly distribution of local precipitations in the city of Salamanca (Rodríguez et al. 2008). These circumstances could decrease the spore concentration during a few hours of the day, but they should not affect neither the number of spores in the remaining hours nor the total spore concentration of the day. Rainfall usually takes place only during a few hours, while spore concentrations can be observed in the remaining hours once the rainfall has finished. In fact, relative humidity, one of the most invariant parameter across the day and closely related to rainfall, showed negative significant correlations with bracken airspora.

Conway (1957) observed the critical importance of meteorological conditions in the 3 months that precede the sporing process when sporangia are developed. As was already said, the years 2002 and 2007 show the lowest number of spores in the atmosphere. These low concentrations could be related to the mean temperature of May,

Fig. 6 Effects of wind hourly direction in hourly spore distribution during MSS (a) and PRE (b) of the studied period. IQ: Winds coming from the First Quadrant, IIQ winds coming from the Second Quadrant, IIIQ winds coming from the Third Quadrant, IVQ winds coming from the Fourth Quadrant



June and July in the analyzed years, always below 18°C. Likewise, year 2001, with the third lowest concentration, had an average temperature of 18.1°C. The effects of mean temperature in the 3 months prior to the sporing process might be related to a threshold of 18°C, below which the development of bracken sporangia is reduced and subsequently there is only a minor production of spores (Fig. 5). The effects of maximum temperatures were not as significant as Caulton et al. (2000) reported for Edinburgh, probably due to the large differences in climate between Scotland and the centre of the Iberian Peninsula. Further studies are needed for a better understanding about bracken sporangia development in relation to temperature in May, June and July. Nevertheless, the observed positive and significant correlations between bracken spores concentrations and temperature during the whole analyzed period may indicate an important influence of this meteorological factor in the development of sporangia. In this manner, Pakeman and Marrs (1996) developed a model to predict that bracken biomass would increase its presence in some areas of Great Britain due to future climate change scenarios. The increase of bracken biomass could be related to a greater spore production and subsequently a greater number of health hazards.

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