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INFLUENCE OF METEOROLOGICAL PARAMETERS IN HOURLY PATTERNS OF GRASS (POACEAE) POLLEN CONCENTRATIONS

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Abstract: Hourly grass pollen concentrations were recorded over a 10-year study period at an aerobiological station of Badajoz (SW Spain). The record was carried out by means of a Burkard spore trap. Meteorological data were used to find correlation with the hourly patterns in the months of principal concentration. The observed variations were found to be due to independent contributions of patterns corresponding to different groups of species within the family of grasses. Three pollen concentrations peaks were recognized. Peak A, at around 10:00 h, of great importance in April and May, although maintained until July; peak B, at around 15:00 h, important in April, May and June; and peak C, towards the end of the day, dominant in July. Differences with respect to the pollen sources responsible for these peaks are suggested by the influence of the meteorological parameters before and during flowering. The results are directly applicable to the epidemiology of allergies in the zone, since the thresholds of grass pollen concentrations capable of triggering allergic processes shift from the central hours of the day in April and May to the night hours in July. Also, considering the observed patterns of hourly grass pollen concentrations as the result of combining different models could explain the annual and geographical variations found at other locations.

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INTRODUCTION

In palynological studies, there exist groups of species which, because of their stenopalynous character, i.e., the uniformity of their pollen grains, are dealt with together as a single type. In aeropalynology, a differentiated study may be possible because the calendar of appearance of the pollen, together with a knowledge of the flowering phenology of the different species found in the zone, can allow the species to be separated. Most often, however, these types are studied as a unit, yielding overall information about their calendar of appearance, the concentrations attained, the influence of meteorological factors, and the hourly variations of their concentrations over the course of the day.

The majority of these types consist of families, genera or species with so many representatives that it is impossible to separate the characteristics of their pollination processes. Examples are the types that correspond to Quercus, Plantago, Amaranthaceae-Chenopodiaceae, Cupressaceae, Rumex, Urticaceae, and Poaceae, all of great aerobiological importance [26]. It has been observed, however, that some of them – the Amaranthaceae-Chenopodiaceae type [41], and the Plantago type [67] – present a unique hourly pollination pattern which is repeated in different years and

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at different locations. This allows one to construct models of the overall behaviour of these types.

The Poaceae type includes all the grasses, which are represented in the Extremadura region by 174 species [14]. Almost 100 of these can be found in the surroundings of the city of Badajoz, occupying all types of vegetation. Within this family, only a few species can be readily separated in aeropalynological analyses. One such distinguishable species is maize which differs in the large size of its pollen grains. This large size, however, also prevents it from forming part of the usual content of pollen in the air. Some workers also separate the pollen of *Secale cereale* L. [33].

For the Poaceae type, there have been various studies reflecting the existence of different hourly patterns depending on the study site [8, 33, 34], including studies within the Iberian Peninsula [10, 22, 32]. The variations in these patterns are due to differences in the hours of maximum pollination, with the appearance of one or more peaks. In the case of Badajoz, some variations in the pattern of hourly concentrations between years and between the different months of pollination have been found [56]. After 15 years of further study, that work has led to the present analysis of the annual patterns of hourly variations in the concentrations of Poaceae pollen from 1993–2008, considering the monthly and daily patterns, and the influence of meteorological parameters.

Earlier work that has allowed an explanation to be given for these local, annual, and intra-annual variations showed that different grass species release their pollen at different times of the day [36, 62], other work studied the influence of meteorological conditions on pollen concentrations at different times of day [61], and another observed monthly variations in the hourly pattern of grass pollen trapping, relating these variations to the amount of pollen that had accumulated in the atmosphere throughout the pollination period [44].

Analysis of the hourly variations in atmospheric Poaceae pollen concentrations, as with any aspect of their pollination, is of great importance for its application to the study of allergies. In Spain, grass pollens grains, with a wide distribution within the flora (20%) of source species and their allergenic capacity [28], are a major cause of allergies. The zones with the highest percentages of individuals with symptoms of Poaceae sensitized pollinosis correspond to the Iberian Peninsula interior: Madrid and Castilla-La Mancha (75.8%), Castilla-León (73.7%), and Aragón (66.7%), although the figures corresponding to Extremadura and Andalusia (49.1%), the Northern zone (46%), and Murcia (41.4%), are far from negligible. Also, most cases of pollen-caused rhinitis (75%) and bronchial asthma (71%) are due to grass pollens [3].

Hence, a knowledge of the hourly rhythm of the levels of this pollen type at different locations and at different times of its period of pollination could be of great assistance with regard to both prevention and diagnosis. Some authors give a threshold for these pollens of around 50 grains/m^3, beyond which allergic symptoms are triggered [3, 5, 30], although in areas of high air pollution a value of 30 grains m^3 has been argued [51]. This threshold should undoubtedly be taken with a certain degree of caution, since it will vary according to the species and to each individual's susceptibility and prior exposure. For instance, the threshold will generally be lower at the end of the pollen season due to the well-known effect of priming. It will also depend on environmental conditions such as contamination [48, 49, 50], or even rural or urban environment [24, 55]. The aim of this work is to asses the influence of meteorological parameters in the hourly pattern of pollen concentration in grass pollen and to test the hypothesis that this hourly pattern is unique.

MATERIALS AND METHODS

Site description. To describe the context of the station, we shall use the summary of the vegetation cover of the surroundings of the city [38]. This assigns the following percentages of cover to the different units of vegetation [57]: herbaceous crops under irrigation (29.5%), herbaceous crops on unwooded, unirrigated land (24.0%), herbaceous crops on wooded, unirrigated land (8.0%), pasture (5.5%), woody crops, (mainly orchards, olive groves, and vineyards, 4.9%), forestry (eucalyptus, poplar, and pine, 5.9%), scrub (0.8%), holm-oak stands (12.4%), cork-oak stands (0.1%), and urbanized or non-productive lands (9.0%). According to previous work [47], oak forests are the most productive in grass pollen, although in other cases their contribution is similar [1].

There are various types of grasses growing in the area and it could be necessary mention them to explain results. These range from the annual species typical of Mediterranean pastures to the neophytes introduced with irrigation, such as the genera *Echinochloa*, *Paspalum*, etc., including perennial species used as the bases of lawns (e.g., *Cynodon dactylon* (L.) pers. obs.) and those characteristic of riparian vegetation (e.g., *Phragmites australis* subsp. *altissima* (Bentham) W.D. Clayton), and some species grown as ornamentals in the city (e.g., *Cortaderia selloana* (Schultes & Schultes fil.) Ascherson & Graebner).

The trap was located in the Agrarian Engineering School of the University of Extremadura, on the outskirts of the city, at a height of some 6 m, and with no obstacles to the free circulation of the air in its vicinity. The situation is 38º53'N latitude, 6º58'W longitude and 186 meters above sea level. The surroundings of the station can be summarized as follows: quadrant 1 (NE) is occupied almost entirely by irrigated cropland; quadrant 2 (SE) – is principally dryland agriculture, with a small portion of irrigated land including a marked appearance of riparian zones and eucalyptus plantations; quadrant 3 (SW) – corresponds principally to the city; and quadrant 4 (NW) – corresponds to pasture land and dryland crops.

Meteorological data. The meteorological data for the years of the study were provided by the Territorial Meteorological Centre of Extremadura. These were daily data corresponding to the mean, maximum, and minimum temperatures (°C), relative air humidity (%), rainfall (mm), mean wind speed (km/h), calm periods (hours), and duration (hours) of the periods in which the wind was out of each of the following quadrants: 1 (NE), 2 (SE), 3 (SW), and 4 (NW). As well as these daily data, we also used the mean monthly and seasonal mean temperatures (°C) and the monthly and seasonal accumulated rainfall (mm), as given in Table 1. This table lists the data by periods from autumn (October, November and December) to the summer of the following year (July, August and September), under the assumption that the conditions in autumn will influence the following year's vegetation based on preliminary analysis. One observes the remarkable differences in autumn rains, with a clear separation between rainy periods (1994, 1996–1998, 2000–2001, 2003–2007) above the mean of 152.4 mm (1964–1994) from the reference period and dry periods below that value (1995, 1999, 2002, 2008). There were no major differences in temperatures between the years of the study, with only the relatively warm autumn of 1995–1996 and cold winter of 1998–1999 being worth mentioning.

Pollen data. The data used in the present work were recorded in the aerobiological station of Badajoz (SW Spain) during the fifteen-year period 1994–2008. The pollen count data, recorded with a continuously operating Burkard volumetric trap [29], were used to estimate the daily and hourly (solar time) pollen concentrations during the study period. The arithmetical mean hourly concentrations were determined from the counts of every day on which the type appeared in each year, giving the annual patterns of daily pollination. Similarly, but using the monthly hourly means for April–July, we obtained the corresponding patterns for each year given by those hourly means, as well as the curves formed by taking the mean value of each hour with the preceding and the following hour.

Graphical and statistical analysis. In order to try to determine the causes of variations in the daily variations in the patterns we analyzed the monthly patterns corresponding to principal pollination period (April, May, June, and July) [56].

The influence of the annual meteorological conditions on the pollen concentrations at different times of day in each year was studied by means of a correlation analysis using the Pearson coefficient, taking into account also the meteorology of months in advance to pollen the season. The meteorological parameters used in the correlations were the accumulated rainfall, mean temperature and relative air humidity of the months from September of the preceding year to June – a period chosen as presumably having the greatest influence on the flowering and pollination of these species – and the overall seasonal values corresponding to autumn, winter, and spring. The pollination parameters used in the correlations were the mean concentrations at each hour of the day for the period April–July, these being the months in which, at least in some of the years, the hourly means surpassed 50 grains/m^3. The results were analyzed considering only the significance level of the correlation coefficient, distinguishing $p<0.05$ (*), $p<0.01$ (**), and $p<0.001$ (***), as well as the sign of the coefficient.

To determine the daily influence of the meteorological parameters on pollen concentrations, the same type of correlation analysis was made between the daily values of

Table 1. Mean temperature (°C) and rainfall (mm) data in the seasons of the different periods considered (autumn–summer) in Badajoz (Autumn is for the previous year, OND: October–November–December, JFM: January–February–March, AMJ: April–May–June, JAS: July–August–September).

Year	Temperatures				
	Autumn (OND)	Winter (JFM)	Spring (AMJ)	Summer (JAS)	Mean of period
1994	11.72	10.67	18.55	23.99	16.23
1995	13.70	11.38	20.31	24.82	17.55
1996	15.80	10.68	19.14	23.56	17.29
1997	13.45	12.34	18.74	24.32	17.21
1998	14.45	12.04	18.03	25.20	17.43
1999	12.00	9.81	19.49	24.18	16.37
2000	13.49	10.71	18.53	24.42	16.79
2001	13.44	12.26	19.74	24.64	17.52
2002	12.58	11.50	19.08	24.18	16.84
2003	14.56	10.95	20.26	25.61	17.84
2004	11.99	21.99	24.18	26.20	17.32
2005	9.17	20.95	25.26	26.50	17.01
2006	10.21	20.79	26.46	26.41	18.14
2007	11.21	18.79	25.13	24.61	16.96
2008	11.78	18.39	23.61	23.98	16.36
Year	Rainfall				
	Autumn (OND)	Winter (JFM)	Spring (AMJ)	Summer (JAS)	Accumulated in period
1994	170.5	115.6	103.4	1.7	391.2
1995	83.6	56.7	57.7	19.2	217.2
1996	289.4	221.5	109.2	30.9	651.0
1997	238.0	112.1	107.6	84.9	542.6
1998	379.1	98.7	75.8	58.4	612.0
1999	46.6	54.1	91.6	54.4	246.7
2000	173.7	38.5	233.9	8.6	454.7
2001	254.9	190.2	47.0	22.1	514.2
2002	124.0	164.5	69.9	33.1	391.5
2003	191.7	131.7	46.0	28.5	397.9
2004	269.9	65.9	36.2	19.0	304.7
2005	183.6	28.2	32.0	2.1	228.5
2006	166.2	134.9	100.5	47.2	534.7
2007	252.1	89.0	146.0	49.6	375.1
2008	90.5	114.6	98.4	8.6	311.3

those parameters with the pollen concentrations at each hour of the day for the period April–July for the whole period studied. The significance levels considered were the same as those given above.

In this work, we define a peak, in a temporal representation (daily or hourly) of airborne pollen concentration, as the point (the day or the hour) of a maximum concentration with lesser values before and after of this event. As more points before peak have successively greater values and more points after peak has successively lesser values, the peak is best defined.

The correlations of the daily minimum, mean, and maximum temperatures, and of the periods of winds in the different quadrants with the pollen concentrations at each hour of the day were studied independently for the months of April, May, June, and July for the fifteen years studied.

RESULTS

The study begins with the observation of annual patterns of hourly variations of Poaceae pollen during the 10 years of the study (Fig. 1). Apart from the different magnitudes attained in each year, there is a notable difference in the patterns with respect to the number of peaks that they present, the times of day that the peaks appear, and their relative importance. Thus, all the years coincide in presenting the lowest levels of pollination in the early morning hours, and high levels in the afternoon between 14:00 h and 18:00 h. Indeed, the afternoon peak is the only clearly appreciable peak in 1995 and 2000. In other years, there are one or more peaks before noon. In 1996, 1997, 1998, 2001, 2002, 2004, 2006, 2007 and 2008 this peak is observed between 10:00 h–12:00 h, and in 1996, 1998 and 2008 is similar in magnitude to the aforementioned afternoon peak. In 1994, 1999, and 2003, its appearance is earlier, between 08:00 h–10:00 h. Lastly, in many of the years, following the afternoon peak the concentrations fall by stages, this being particularly evident in 1994, 1999 and 2005 when there exists a second evening peak at 18:00 h or 19:00 h, with concentrations higher than those reached at 15:00 h.

Analyzing the monthly patterns corresponding to April, May, June, and July (Fig. 2), the first thing that one observes is the difference between months within a given year, and, *vice versa*, the similarity of each month between

Table 2. Correlations between the various meteorological parameters (accumulated rainfall and mean temperature and relative air humidity from October–June, and in the seasons of autumn, winter, and spring) and the mean Poaceae pollen hourly concentrations for each 15 years studied (n=15). The significant correlations are indicated as * p<0.05, ** p<0.01, and *** p<0.001, together with their sign. Only months and seasons with at least one statistically significant correlation are indicated (Aut: October–November–December, Win: January–February–March).

	Accumulated rainfall					Mean temperature		Relative air humidity			
	Dec	Jan	Feb	Aut	Win	Dec	Aut	Feb	Mar	Apr	Win
1	**+					**+			*+		
2	*+					**+			*+		*+
3	*+					**+			**+		*+
4	**+					**+			*+		*+
5	*+					**+			**+		*+
6						**+			*+	*+	*+
7	*+		*+			**+		*+	*+		*+
8	*+					**+			*+		*+
9	**+			*+	*+	**+	*+		*+		*+
10	***+	*+		*+	*+	**+	*+		*+		
11	**+	*+		*+	*+	**+	*+				
12	**+			*+		**+			*+		*+
13	**+			*+	*+	***+	*+	*+	*+		*+
14	**+			*+		***+	*+	*+	*+		*+
15	*+					**+	*+	*+	*+	*+	*+
16	*+					**+				*+	
17						**+				*+	
18	*+					**+			*+		
19	*+					**+			*+		
20	*+					**+			*+		
21	**+					**+					
22	**+					*+					
23	**+					*+			*+		
24	**+					**+			*+		*+

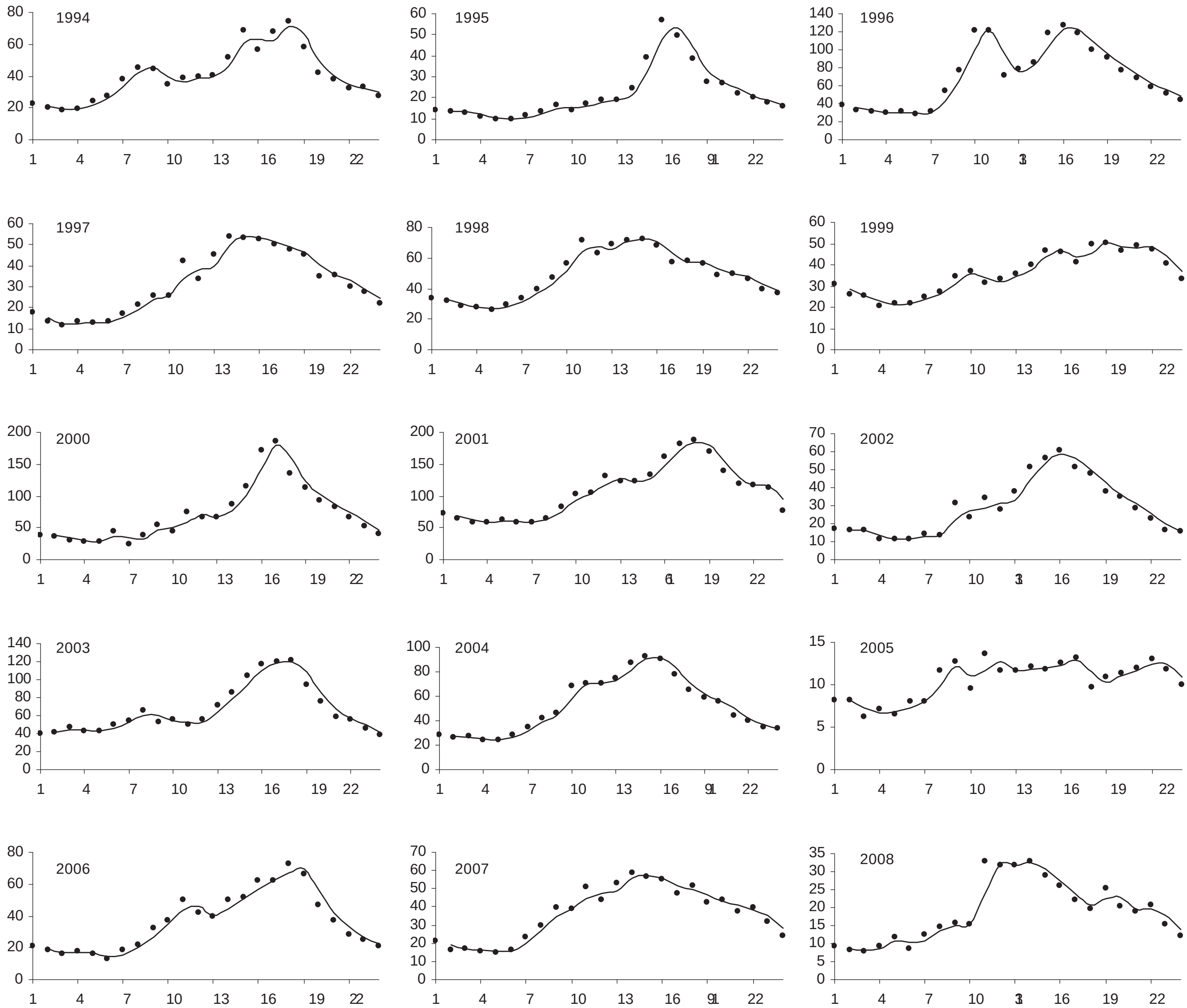


Figure 1. Annual mean concentrations of Poaceae pollen at the different hours of the day in the 10 years studied. Pollen concentrations (grains/m^3 y-axis) are plotted against the solar time (solar hour x-axis).

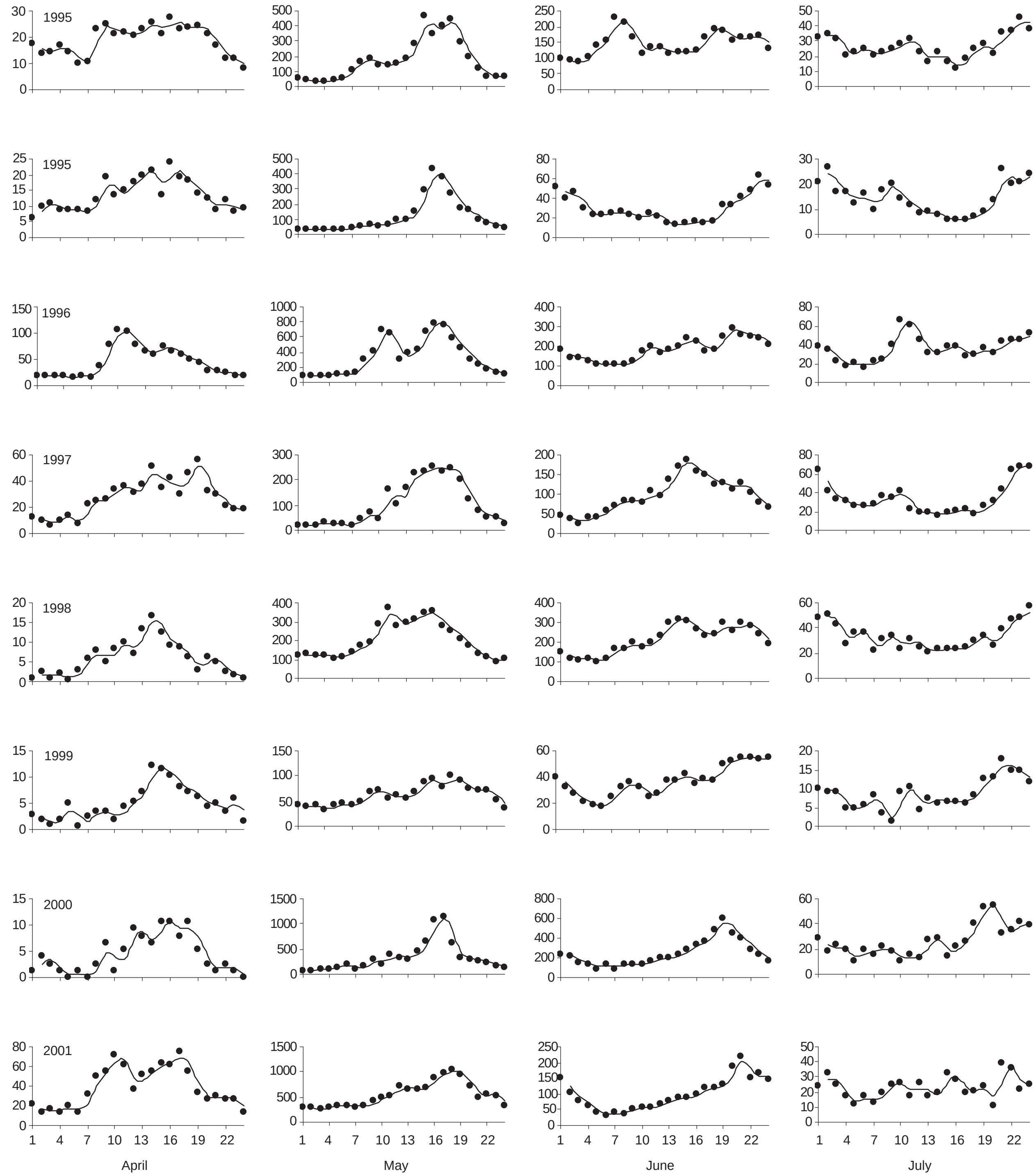
years. In each monthly pattern, one observes clear peaks of high concentrations. One observes that there are 3 hour intervals in which high concentrations are reached in all 4 months: one centred around 10:00 h, denominated the A or morning peak; another around 15:00 h, denominated the B or afternoon peak; and another at the end of the day, denominated the C peak. There is also sometimes a peak during the early morning, with the exact hour depending on the month. Often, successive peaks are not distinguished, as there is no intervening decrease in the concentration. April 1997 is an example (Fig. 2).

In April, the pattern is mainly determined by the A and B peaks, with the B peak being common to all the years of the study, and attaining the maximum concentrations in all years except 1996 and 2007 in which the A peak is greater. Only in 1996 and in 2001 was the 50 grains/m^3 threshold surpassed by both peaks and in 2006 is surpassed by B peak.

In May, the pattern is fairly uniform for all the years, consisting of the A and B peaks with varying relative importance. In most of the year, the B peak predominates over the rest, but in 1996 and 1998 the curves are bimodal, and in 2008 the A peak predominates. The 50 grains m^3 threshold is surpassed in every year, at least between 09:00 h–22:00 h.

In June, the patterns are very varied, although the one that occurs most often is that formed by the A, B, and C peaks, generally in increasing order of importance. There appear high concentrations in the early morning, which attain maximum values in 1994. The 50 grains/m^3 mean concentration threshold is surpassed in a very varied manner, depending mainly on the intensity of the pollination of that year and on the dominant peaks. It was surpassed by the C peak during the night in almost every year.

In July, the same peaks appear as in the preceding month, but now the maximum is attained during the night in every



year except 1996, when it occurs in the morning during the A-interval. The concentrations only surpass the 50 grains/m^3 threshold at night in 5 of the 15 study years, and in the morning in the aforementioned case of 1996.

Having observed the independence of the different peaks in the annual and monthly patterns, we then carried out a correlation analysis between the mean concentrations at the different times of day in the period from April to July of each year, and the monthly and seasonal values of rainfall, temperature, and relative air humidity, to determine whether the Poaceae pollen concentrations at the different times of day are influenced in the same way by these meteorological parameters. Table 2 lists the cases in which there was some significant correlation ($p<0.05$) with the concentrations of at least one time of day.

All the significant correlations with rainfall, mean temperatures, and relative air humidity were positive in sign.

With respect to rainfall, we consider of interest their effect on December over all the pollen concentrations, as well as the effect of autumn and winter rainfall over the

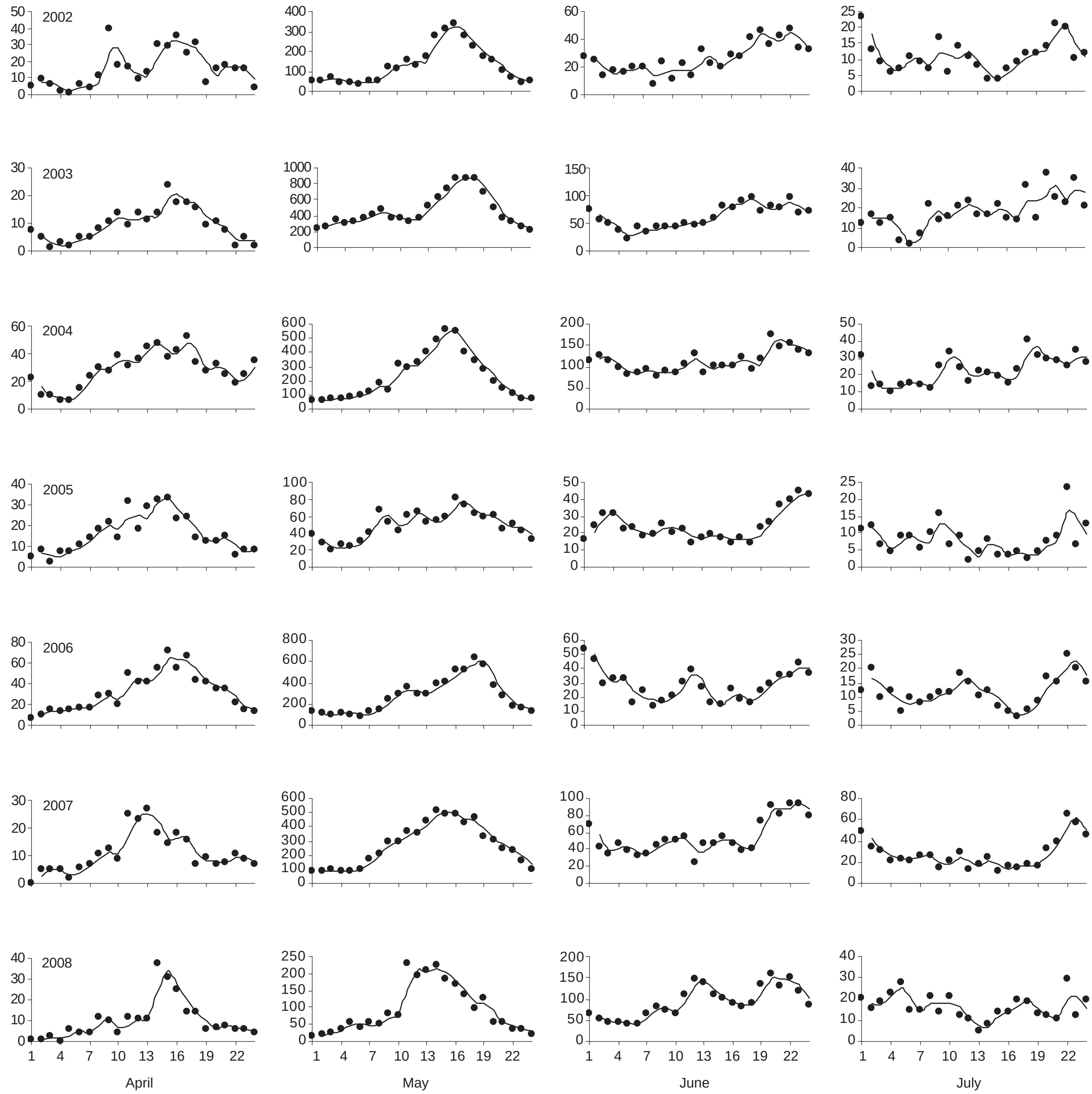


Figure 2. Mean Poaceae pollen concentrations at different hours of the day for the months of April to June, in the different years studied (solar hour x-axis). Plotted are mean concentrations (grains/m^3 y-axis) (dots), and the curve obtained by taking the mean of each value with the preceding and following values (black line).

pollen concentration between 09:00 h–14:00 h. With respect to mean temperature, those of autumn affected the concentrations between 09:00 h–11:00 h and between 13:00 h–15:00 h; and December temperatures showed significant correlations with the concentrations recorded at all the hours of the day. Lastly, with respect to the mean values of relative air humidity, that of February affected the concentration of 07:00 h and between 13:00 h–15:00 h; that of March affected nearly all the hours; that of April affected the concentrations to 06:00 and between 15:00 h–17:00 h; and that of winter affected to 02:00–09:00 h; from 12:00 h–15:00 h and to 24:00 h.

Table 3 gives the results of the correlation analysis between the daily meteorological parameters and the concentrations recorded at each hour of the day from April–July. The temperature parameters – daily minimum, mean, and maximum – were positively correlated with the concentrations between 19:00 h–08:00 h, and there was negative correlations between 14:00 h–16:00 h. Observing the differing influence of the temperature parameters in the

Table 3. Correlations between daily values of the different meteorological parameters and the hourly concentrations from April–July (n=1821). Significant correlations are indicated as * p<0.05, ** p<0.01, and *** p<0.001, together with their sign (Min Tª: minimum temperature, Max Tª: maximum temperature, RH: relative air humidity, W Vel: wind velocity, 1 (NE), 2 (SE), 3 (SW), and 4 (NW): winds out of quadrants).

Hours	Min Tª	Mean Tª	Max Tª	RH	Rain	W Vel	Calms	1(NE)	2(SE)	3(SW)	4(NW)
1	***+	***+	***+	***–	**–	*–		***+	*+	***–	
2	***+	***+	***+	***–	**–	**–		***+	**+	***–	*–
3	***+	***+	***+	***–	*–	**–		***+	*+	***–	
4	***+	***+	***+	***–	**–	*–		***+	*+	***–	
5	***+	***+	***+	***–	*–	*–		***+		***–	
6	***+	***+	***+	**–	**–			***+		***–	
7	***+	***+	***+	***–	**–	*–		***+		***–	
8		**–	***+	**–		***–		***+		***–	
9			*–			***–		***+	*+	***–	
10						**–		*+	**+	**–	
11										*–	*+
12									*–		**+
13									*–		***+
14	*–	*–		*+					**–		***+
15	*–	**–	*–	**+				*+	**–		***+
16	*–			*+	*–			*+	**–		***+
17					**–				***–		***+
18					**–	*–			**–		***+
19		*+	**+		**–	***–			*–	**–	***+
20	*+	***+	***+	***–	***–	***–		*+	*–	***–	**+
21	***+	***+	***+	***–	***–	***–		**+	*–	***–	***+
22	***+	***+	***+	***–	***–	***–		***+		***–	**+
23	***+	***+	***+	***–	***–	***–		***+		***–	*+
24	***+	***+	***+	***–	***–	***–		***+		***–	

different months independently (Tab. 4), one sees that in April and May the mean and maximum temperatures showed a positively correlated influence at all hours of the day, while the minimum temperatures were correlated less consistently. In June, there was a positive influence of these 3 parameters on the concentrations from 23:00 h–04:00 h, with the interval being shorter in the case of the minimum temperatures, and a negative influence from 11:00 h–22:00 h. Lastly, in July there were few significant correlations, noteworthy being those negatives between the minimum temperatures and the concentrations at 23:00 h and 24:00 h, and those positives between maximum temperatures and the interval 06:00–07:00 h.

The relative air humidity acted in the opposite sense to the temperature. The correlations were positive between 14:00 h–16:00 h, and negative between 20:00 h–08:00 h. The influence of the rainfall was similar, having a negatively correlated effect in the interval 16:00–08:00 h.

With respect to the wind parameters, wind speeds were negatively correlated with the nocturnal and early morning pollen concentrations, while the calm periods had no influence on the concentrations. The effects of wind direction were as follows: winds from quadrant 1 (NE) had a positive influence from 20:00 h–10:00 h and between 15:00 h–16:00 h; those from quadrant 2 (SE) had a positive influence from 01:00 h–04:00 h and for 09:00 h–10:00 h, and a negative influence between 12:00 h–21:00 h; those from quadrant 3 (SW) had a negative influence from 19:00 h–11:00 h, and no positive influence at any hour; and those from quadrant 4 (NW) had a negative influence at 02:00 h, and positive between 11:00 h–23:00 h. Table 5 gives the results of the independent analyses carried out for the influence of the wind direction in April, May, June, and July. Quadrant 1 winds in April, May, and June generally had a positive effect in the interval 20:00–10:00 h, whereas in July the significant correlations were sporadic and mostly negative in sign. Quadrant 2 winds in May had a negative effect between 12:00 h–21:00 h, and in June a positive effect between 01:00 h–10:00 h, and negative between 14:00 h–18:00 h. Quadrant 3 winds always acted negatively, noteworthy being the influence in May between 19:00 h–10:00 h, and in June at every hour. Lastly, quadrant 4 winds in April, May, June and July had a positive influence on the concentrations meanly between 11:00 h–24:00 h.

In view of the different patterns of the hourly variation in Poaceae pollen concentrations observed in the 15

Table 4. Correlations between the daily values of the mean, maximum, and minimum temperatures and hourly concentrations in April. Significant correlations are indicated as * p<0.05, ** p<0.01, and *** p<0.001, together with their sign.

Hours	Min T^a				Mean T^a				Max T^a			
	April	May	June	July	April	May	June	July	April	May	June	July
1	***+	***+	**+		***+	***+	**+		***+	***+	**+	
2	**+	***+	**+		***+	***+	**+		***+	***+	**+	
3	***+	***+			***+	***+	*+		***+	***+	*+	
4	***+	***+			***+	***+	*+		***+	***+	**+	
5	***+	***+			***+	***+			***+	***+		
6	***+	***+			***+	***+			***+	***+		*+
7	*+	***+			***+	***+			***+	***+		*+
8	*+	***+			***+	***+			***+	***+		
9	**+	***+			***+	***+			***+	***+		
10		*+			**+	***+			**+	***+		
11	*+	*+	***–		*+	***+	***–		*+	**+	**–	
12		***+	***–		***+	***+	***–		***+	***+	**–	
13	***+	***+	***–		***+	***+	***–		***+	***+	***–	
14	***+	*+	***–		***+	***+	***–		***+	***+	***–	
15	**+		***–		***+	***+	***–		***+	**+	***–	
16	*+		***–		***+	***+	***–		***+	***+	***–	
17	**+	*+	***–		***+	***+	***–		***+	***+	***–	
18	*+	***+	***–		***+	***+	***–		***+	***+	***–	
19	***+	***+	**–		***+	***+	*–		***+	***+		
20	**+	***+	*–		***+	***+			***+	***+		
21	***+	***+	**–		***+	***+		*–	***+	***+		*–
22	**+	***+	*–		***+	***+			***+	***+		
23		***+		*–	***+	***+			***+	***+		
24	*+	***+		*–	***+	***+		*–	***+	***+	*+	

years of study in Badajoz, it is clear that they correspond to the sum of a series of peaks whose relative importance varies from year to year. There particularly stand out, because of their frequency and greater importance in almost all the years, the afternoon rise in concentrations, between 14:00 h–18:00 h. There occur various peaks before noon, between 08:00 h–12:00 h. Lastly, subsequent to the aforementioned afternoon peak, others were recorded occurring between 18:00 h–19:00 h, and even some later peaks were observed.

The monthly patterns also seem to be the result of the additive effects of one or more peaks, and the variability in these patterns seems to depend on the relative importance of the different peaks in each year and in each month. The times of day at which the peaks appear show them to be the same as were described in the annual patterns, so that the months in which the highest concentrations occurred, especially May, were usually responsible for the shape of the annual graphs. It is nonetheless interesting to observe the patterns of the other months, since curiously the grass pollen thresholds necessary to trigger allergic processes are generally surpassed during the morning and afternoon in April and May, but during the night in July, with the situation in June being intermediate, and variable from year to year.

The peaks do not present the same phenology. Thus, the 2 with the greatest importance in determining the total annual pollination level, i.e., the A (morning) and B (afternoon) peaks, have a variable importance in April and May, although it seems that in the latter month the B peak is always higher, except for 2008. The A peak is longer-lasting, however, being observable even in July, when the hours that corresponded to the B peak often present a minimum in the pollen concentrations. On the contrary, the C (nocturnal) peak only becomes important from June onwards.

DISCUSSION

The independence with which the 3 periods of high concentrations has been defined varied annually and monthly, and the changes observed in their relative abundance, could mean that they each correspond to the different rhythms of pollination of the various species and genera in the family, which in conjunction give rise to variable annual and monthly patterns. This idea was put forward by Norris-Hill [44] who interprets the presence of different peaks as

Table 5. Correlations between daily values of periods of winds out of quadrants 1 (NE), 2 (SE), 3 (SW), and 4 (NW) and hourly concentrations in April, May, June, and July. Significant correlations are indicated as * p<0.05, ** p<0.01, and *** p<0.001, together with their sign.

Hours	1 (NE)				2 (SE)				3 (SW)				4 (NW)			
	April	May	June	July	April	May	June	July	April	May	June	July	April	May	June	July
1		***+	**+				**+			**–	**–	**–				
2	*+	**+	**+			*+	*+	*+		**–	***–	**–				
3	**+	**+	***+	*+			**+			***–	***–	**–				
4		**+	***+				*+			***–	***–					
5		**+	***+							**–	***–	*–				
6		*+	***+				*+			**–	***–	*–				
7		*+	***+					*+		**–	***–	*–				
8		***+	***+							***–	***–	**–				*+
9		*+	***+				*+			**–	***–	**–				*+
10		*+	**+				*+			*–	***–	*–				**+
11											***–	*–	**+	*+	***+	***+
12					*–	*–					***–		*+	**+	***+	**+
13					*–	*–					***–	*–	*+	**+	***+	**+
14				*–	*–	**–	*–				**–	*–	***+	***+	***+	***+
15						**–	*–				**–		**+	***+	***+	*+
16				*–		**–	*–				**–		**+	***+	***+	***+
17						***–	*–				**–			***+	***+	*+
18						**–	*–				**–	*–		***+	**+	***+
19						**–				**–	*–			***+	**+	***+
20	*+	***+				*–			*–	***–	**–	*–		***+	**+	*+
21		***+		*–		*–		*–	*–	***–	***–	*–		**+	***+	***+
22	**+	***+							**–	***–	***–	**–		*+	***+	***+
23	**+	***+	***+						*–	***–	***–	***–			*+	***+
24	**+	***+	***+						*–	***–	***–	***–				***+

reflecting the flowering of different grasses, but relates the monthly variations in hourly patterns to the amount of pollen that has accumulated in the atmosphere throughout the pollination period. This explanation is in accordance with the results reported by Liem [35] that each Poaceae species has its own pattern of daily periodicity in the release of pollen. There are even species such as Cynodon dactylon with several moments of anthesis in the day [62]. Also, Kasprzyk *et al.* [33] observe a fixed hourly pattern in *Secale cereale* that is independent of the year and the location, while for the rest of the grasses the pattern presents temporal and spatial variations. The explanation could also be sought in the dependence of the hourly concentrations on the meteorological conditions [2, 43, 46, 61], or even in the height at which the traps are placed, since Norris-Hill [44] found different hourly patterns in nearby areas on the basis of this variable.

Apart from these explanations, and taking into account that the average length of pollen in anemophilous species is lesser than in entomophilous ones [7, 13] with values ranging by 21.5 ± 27.2 h, and that this is environmentally influenced mainly with temperature and relative air humidity [6], we could expect that an hourly pattern of theses species would be adapted to the conditions in which they grow [36, 37, 66, 69].

These maxima not only explain the monthly and annual patterns found in the present study, but could also explain the existence of different patterns found at other locations in the Iberian Peninsula. Thus, the maxima between 06:00 h–17:00 h in Cáceres [65], Córdoba [22], Granada [2], and Málaga [10] could reflect the action of the A and B peaks, and would therefore correspond to the mean pattern given for May in the present work. In Córdoba, Cariñanos *et al.* [11] show other nocturnal peaks that could correspond to the present work's C peak, while in Granada Alba *et al.* [2] also describe a peak between 02:00 h–06:00 h which is similar to that described here in some years during the month of April. In Orense, the peak found by Iglesias *et al.* [32] between 18:00 h–23:00 h would correspond to the C peak, so that it resembles more the patterns given in the present study for June and July.

Outside of the Iberian Peninsula, the pattern reported by Käpylä [34] in Finland, with maxima between 07:00 h–10:00 h and between 14:00 h–18:00 h, would be determined by the A and B peaks. Bricchi *et al.* [8] in Italy find maxima between 16:00 h–20:00 h, which could be a combination of B and C peaks. Norris-Hill [43] in London finds high concentrations between 16:00 h–24:00 h, similar to the case cited above for Orense, which would be similar to the C peak. In Argentina, Pérez *et al.* [46] found in Buenos Aires a maximum at 08:00 h in 2 of the years of the study, and 2 peaks in the other year, one from 14:00 h–16:00 h and the other at 22:00 h, so that the 3 peaks observed in the present work could be present in that zone. In La Plata, however, Nitiu [42] found only the peak centred on 14:00 h in the 3 years of the study. Hence, the spectrum of species in a zone, and the combination of their daily rhythms of pollination, give rise to different local patterns of the hourly Poaceae pollen concentrations.

Other researchers have shown that the meteorological conditions prior to the flowering of grasses affect different aspects of their phenology, such as the onset or the duration of their pollination period [5, 12, 17, 19, 23, 27, 43, 45, 50, 59, 60]. The aim of the present work, however, has been, to determine the influence of these factors on the concentrations at different hours of the day, so that only in some cases can comparisons be made with the results of those studies.

The pollen levels at the different times of day respond differently to the influence of the monthly and seasonal values of rainfall, temperature, and relative air humidity. This perhaps lends support to the theory that the peaks in hourly concentrations correspond to different sources within the grasses, which, being a heterogeneous group of species, respond differently to the influence of the environment. The exception is the influence of the December temperatures. These effect positively the mean concentrations at most hours of the day, which is translated into a net effect of this factor on the pollination of the grasses, as has been shown in the work of Goldberg *et al.* [25] and Frei [18].

Rainfall prior to flowering has a positive effect on the annual pollination level of grasses [4, 20, 53, 64]. In the analysis carried out in the present study on the different hours of the day, December rains affect almost all the hours, but the autumn and winter rains, act positively on the concentrations recorded between 09:00–14:00 h. This matches fully the A or morning peak which occurs between 08:00–12:00 h in the months of the analysis. One observes that in the years of greatest autumn rainfall (1996 and 1998) this peak reaches magnitudes in May similar to those of the B peak. The autumn temperatures also affect this peak positively. With respect to relative air humidity, probably the most remarkable is the positive influence of April values on peak B. An explanation of this phenomenon might be that the group of species responsible for this pollination peak consists fundamentally of annual grasses, in which rainfall during the germination phase would affect the size of their populations and therefore the magnitude of their flowering. This idea was posited by Andersen [4] for different herbaceous plants, and by Subiza [63] for the particular case of steppe grasses.

With respect to the influence of the daily values of the meteorological parameters on the daily pollen concentrations at the different hours of the day (Tab. 3), one must first consider the case of the temperature parameters. In principle, an overall analysis of the April-July period shows a parallel behaviour of the 3 temperature parameters, with there being negative correlations during the period between 14:00 h–16:00 h in which the B pollination peak occur. Thus, although Spieksma *et al.* [61], Moseholm *et al.* [40], and Bricchi *et al.* [8] indicate that the maximum hourly concentrations occur when the air is warm and dry, the results of the present study are that, on the contrary, it is during the hours of the middle of the day when the observed relationship between temperature and pollen levels is nil or negative. This discrepancy is resolved when one examines the results of the independent analyses for each month. One finds that what happens at the hours between 11:00 h–22:00 h is that the correlation changes sign, passing from positive in April and May to negative in June, and thereby resulting in the loss of significance in the overall analysis. The reason for the change in sign is that the April-July period is one of rising temperatures, so that the analysis is reflecting the fact that grasses reach their pollination levels between certain limits of temperature. In the relatively cooler months of April and May, the net effect of warmer temperatures is positive. In the hot months of June and July, this positive relationship can only be effective during the cool night hours. During the hottest hours of the middle of the day, at least in June, the relationship is inverted since the hypothetical upper temperature limit for pollination would be surpassed, maybe because pollen viability depends on these temperatures. This idea would explain the change from patterns of pollination centred on the hours of daylight (April and May) to patterns of night pollination (July).

At a general level, these results are consistent with the behaviour outlined by Martin *et al.* [37] who find that temperature has a direct (positive) effect in the periods January–June and October–November, and an inverse (negative) effect in the periods July–September and December–January. In this same line, and also in a general sense, Fernández *et al.* [16] find a positive correlation between concentration and temperature for the months in which the temperatures reach 20–25°C, while in July and August, with higher temperatures, the concentration does not increase in the same way, and Galán *et al.* [21] find in Córdoba that the correlation between temperature and daily grass pollen concentrations is positive in the period prior to the pollination maximum, and negative afterwards.

Most authors recognize the negative influence of the relative air humidity and rainfall on the observed daily concentrations, because of the effect of washing pollen particles out of the atmosphere and of inhibiting the opening of the anthers [20, 31, 53]. Nonetheless, Emberlin & Norris-Hill [31] found a significant positive correlation with rainfall in London. In the particular case of grass pollens, Pérez *et al.* [46] find that relative air humidity below 48% tends to increase pollen levels, but they determined this influence for daylight hours, and it then has to be recalled that in Badajoz the influence of the relative air humidity was positive at times around 13:00–16:00 h.

With respect to the influence of the wind, the negative correlations found with wind speed periods coincide with those found at the daily level by Emberlin & Norris-Hill [15] in London, but are contrary to the findings of Smart *et al.* [58] and Bringfelt *et al.* [9] of positive correlations between grass pollen levels and wind speed. These differences are explained by Valencia *et al.* [68] as due to the wind acting to dilute pollen concentrations in some locations, but to facilitate the release of pollen from the anthers in others.

The way in which the wind direction acts on the daily concentrations has to be judged in terms of the environment of the trap, in particular the location of the sources of pollen [57]. The positive correlations that are found in the analysis of the results are of particular interest since they indicate the presence of such sources. As an antecedent of this relationship, Alba *et al.* [1] describe the importance of winds coming from the SW and SE of the province of Granada in determining the appearance of peaks of high pollen concentrations between 02:00 h–06:00 h.

Firstly, the explanation of the lack of any positive correlation with quadrant 3 is that this quadrant is occupied fundamentally by the urban area of Badajoz. It therefore contains no major source of grass pollen.

Quadrant 1, corresponding mostly to irrigation crops, contains many habitats suitable for the species of this family, including the group of neophytes introduced into habitats where the humidity is maintained during a great part of the year. Winds from this quadrant act positively on the concentrations recorded between 20:00–10:00 in April, May, and June, and hence on the high concentrations of A and C peaks observed in these months. These results allow one to understand the persistence of the A peak in the hourly patterns of April until July, since the humidity of the environment in this quadrant would permit the maintenance of the flowering of the species responsible for that peak.

Quadrant 4, which is occupied by pasture and dryland crops, seems to be the source of the pollen grains that cause the B peak, since it is positively correlated with the concentrations recorded between 11:00 h–23:00 h. The species involved are therefore pterophytes and spring flowering perennials, which end their flowering when the humidity conditions cause them to wither. This is confirmed by the significant positive correlations between the relative air humidity and the concentrations found from 14:00 h–16:00 h. The hypothesis is consistent with the findings of Kasprzyk *et al.* [33] concerning the hourly rhythm of *Secale cereale* L. – a species that fits the above description – which presented its maximum pollination between 10:00 h–16:00 h in the 2 years and in the 2 locations in Poland analyzed in that study. Its pollen season is from the end of May to the beginning of June. It should be mentioned, however, that in this same species, Sen *et al.* [54] find 2 peaks, one between 08:00 h–12:00 h, and another between 14:00 h–16:00 h, so that this species could contribute to both the A and B peaks.

Nevertheless, the difference in the persistence of these peaks throughout the pollination period of the grasses is consistent with the hypothesis put forward by Galán *et al.* [58] that short cycle grass species flower from May to the first fortnight of June, and the long cycle species flower later, thereby separating the pollination period into 2 groups of different flowering dates.

CONCLUSIONS

Our conclusion is that in the grasses there exist different hourly patterns of pollen release, that over the course of the prolonged pollination period the relative abundance of some species over others determines the general pattern corresponding to the family. Also, we think that the meteorological conditions prior to pollination influence this abundance of some species over others and therefore affect the general hourly pattern of the family, and that one must bear in mind factors of wind direction and location of the sources that could alter these patterns. In spring, the predominant species have a basically evening pattern of pollen release. They are replaced as summer approaches by species with a nocturnal pattern of pollen release. In some cases, morning patterns of release have been found both in spring and in summer. The temperature of the December prior to flowering is very important for pollination. Warmer temperatures lead to higher levels of pollen in the atmosphere at all hours of the day.

It is apparently the annual grasses that present a morning pattern of pollination. These are the most strongly affected by the meteorology of the preceding months, with autumn rainfall and autumn temperatures playing a major role in their growth and subsequent contribution to pollen in the atmosphere. The grasses with an evening pattern of pollen release are affected by the April temperatures. The hourly variations of pollen levels are more affected by the daily rainfall and temperature during the night hours, while the daily wind direction is the most decisive factor during the daytime hours. The location of the pollen sources is therefore of great importance in establishing the hourly distribution patterns of pollen in the atmosphere and this need further work to have accurately conclusion. The importance of these meteorological parameters, however, is not the same over the entire pollen season. Whereas in spring the daily temperatures generally act in a positive sense on the pollen levels, towards summer their influence becomes negative. The case is somewhat similar with respect to the influence of the wind direction, although the location of the sources then conditions the results.

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