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Urtica membranacea and the importance of its separation from the rest of the Urticaceae in aeropalynological studies carried out in the Mediterranean region

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Abstract

The aerobiological behaviour of the *Urtica urens* and *Urtica membranacea* pollen types was analysed in Badajoz and Mérida (SW Spain) in order to determine the importance of separating them in studies carried out within the area of distribution of *Urtica membranacea*. In the two study sites, Urticaceae pollen concentrations in the atmosphere ranged between 1055.7 and 2866.2. This puts them in an intermediate position among the locations that have been studied in the Iberian Peninsula. Annually, more than 25% of these amounts corresponded to *Urtica membranacea* pollen. The *Urtica urens* pollen type was present in the atmosphere almost all year round, although its maximum concentrations occurred in February. Its daily concentrations correlated negatively with temperature, wind speed, and westerly winds, and positively with relative humidity, calm periods, and easterly winds. The annual index seemed to be negatively influenced by winter rainfall, and positively by winter maximum temperatures. The hourly pattern of pollination showed low concentrations throughout the night, and a single peak between noon and early afternoon. *Urtica membranacea* pollen was present in the atmosphere from February to May, and its maximum concentrations occurred between March and April. While its daily variations did not seem to be consistently influenced by any of the meteorological factors analysed, the annual indices positively correlated with the minimum temperatures, which also affected the beginning and the duration of the principal pollination period. The hourly pattern of pollination showed that its presence was not restricted to the early afternoon, but that, on occasions, its maximum concentration occurred at the end of the day (up to midnight).

Key words: *Aerobiology, meteorological factors, allergy, Urticaceae,* Urtica membranacea *type,* Urtica urens *type, Spain*

Introduction

The Urticaceae family includes five genera in Europe (Heywood, 1964), but only two have species with a wide distribution, *Urtica* and *Parietaria*. *Urtica* has four species with a wide distribution in the Mediterranean region: *U. dioica* L., *U. urens* L., *U. membranacea* Poriet (=*U. dubia* Forssk.) and *U. pilulifera* L. *Parietaria* also has four species with a wide distribution in the Mediterranean region: *P. officinalis* L., *P. judaica* (=*P. diffusa* Mert. & W.D.J. Koch in Röhl), *P. mauritanica* Durieu in Duch. and *P. lusitanica* L.

The pollen of the Urticaceae has been the subject of many studies, and its aerobiological behaviour has been analysed from different standpoints. Some studies, including those of Belmonte et al. (1999), González-Minero et al. (1998) and Mañas et al. (1990), conducted in the Iberian Peninsula, have centred on comparing its presence between different sampling sites. Others have analysed the behaviour at specific locations. These include the work of Díaz de la Guardia et al. (1998) in Granada, Galán et al. (2000) in Córdoba, Trigo et al. (1996) in Málaga, and Vega et al. (2003) in León, and, outside the Iberian Peninsula, of Arobba et al. (1992) in Genova

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(Italy), Corden & Millington (1991) in Derby (UK), Emberling & Norris-Hill (1991) in London (UK), Fornaciari et al. (1992) in Perugia (Italy), and Rizzi-Longo et al. (2004) in Trieste (Italy).

The family has also been the subject of other types of studies: the analysis of pollen production by different populations (Guàrdia & Belmonte, 2004), and comparisons between different placements of the trap at a given site (Arobba et al., 2000), of different heights of the trap (Alcázar et al., 1998; Fiornina et al., 1999; Hart et al., 1994), of different trapping methods (Belmonte et al., 2000), of different sample analysis techniques (Cariñanos et al., 2000), and of different forms of processing the data (Belmonte & Canela, 2002). Lastly, one must cite the work of Spieksma et al. (2003) who analysed the upward trend of concentrations of this family's pollen at five sites in NW Europe.

The aerobiological interest of this family is fundamentally due to the allergenicity of the pollen of *Parietaria* spp. This genus is regarded as particularly important in countries bordering the Mediterranean since its pollen provokes symptoms in patients throughout its long flowering period (D'Amato et al., 1991). In Spain, the frequency of sensitization to *Parietaria* pollen with respect to monosensitized (only to *Parietaria*) and polysensitized (grasses and/or olive, as well as *Parietaria*) patients varies between 13% and 43% on the Mediterranean coast (Alergológica, 1995). The importance of this becomes clear when considering that Urticaceae pollen appears in the atmospheric records of all of Spain's aerobiological stations (Belmonte et al., 1999).

The threshold concentration of *Parietaria* pollen at which sensitized patients begin to be symptomatic is 30 grains/m^3 according to D'Amato et al. (1991), and 10 – 15 grains/m^3 according to Negrini et al. (1992). On the contrary, the pollen of the species of *Urtica* is allergenically irrelevant. While Corbi et al. (1985) found only weak cross-reactivity of the pollen of this genus with that of *Parietaria*, Bousquet et al. (1986), using RAST inhibition, isoelectric focusing, and skin tests, demonstrated the total absence of cross-reactivity.

The family is palynologically narrow, with all of its representatives except one having trizonoporate pollen grains of the *Urtica urens* type. The exception is *Urtica membranacea*, which has smaller, polypantoporate grains (Díez, 1987). Aeropalynologically, the pollen grains of the *Parietaria* species are not distinguishable from those of the rest of the wild species. The result is that, in epidemiological studies, the data concerning this highly allergenic species are contaminated by the presence of allergenically innocuous pollens. Nonetheless, nearly all the studies published on the group, including those mentioned above, consider a single pollen type that includes all the species of the Urticaceae, even though the pollen of at least *Urtica membranacea* could be separated. The only exception is the work of Galán et al. (2000) who used the data of a 2-year study to determine the influence of meteorological parameters on the pollination of the Urticaceae, separating out *Urtica membranacea* and dividing the rest into groups of spring or autumn phenology.

Urtica membranacea is a species with a Mediterranean distribution, and its presence affects a great number of aerobiological stations in that area. The present work was, therefore, aimed at determining the potential influence on subsequent aerobiological studies of separating the *U. membranacea* from the *U. urens* type, in particular on the interpretation of the results with respect to allergies. Our research team has been discriminating the two types since 1993, and publishing the corresponding data independently since 1997 (Gonzalo et al., 1997) and has gathered a continuous series of daily data over more than 10 years, the period covered by the present study.

The principal objective was to analyse and compare the behaviour of the pollen types in the atmosphere of Badajoz over 10 years, with particular reference to the 3 years of data recorded at the Mérida aerobiological station, which is some 60 km from that of Badajoz. By means of this comparison, we can check whether the variability of behaviour described in the family as a whole can be explained on the basis of the different phenology and distribution of the various species involved, as proposed by several authors (e.g., Belmonte et al., 1999).

In Spain, the Urticaceae are represented by four genera comprising a total of ten species, of which *Soleirolia* Gaudich. and *Forsskaolea* L. are restricted to the Balearic Islands and to the Province of Almería. In the rest of the Peninsula, the species belong to the genera *Urtica* L. (five species) and *Parietaria* L. (three species) (Paiva, 1993). In Badajoz and Mérida, the representatives and their dates of flowering are shown in Table I.

Material and methods

The study was conducted in Badajoz and Mérida, in SW Spain. The two sites are 60 km apart, and both belong to the same mesomediterranean bioclimatic zone and to the same biogeographical province, the Luso-Extremadurense (Ladero, 1987). They are located in the flood plains of the River Guadiana, an area strongly influenced by farming activities; indeed, the percentage of land area devoted to crops is 66.4% in Badajoz and 70.3% in Mérida, with 31.3% and 11.9%, respectively, under irrigation (MAP, 1985a, b).

The study was performed using Burkard volumetric traps, which provided the counts from which

③ Table I. Representative species and their flowering dates.

	Paiva (1993)	Ruiz (1995)
Urtica dioica L.	April – September	May – August
Urtica urens L.	(January) March – October (December)	March – April
Urtica membranaceae Poir.	February – September	March – April
Parietaria judaica L.	March – October	February – October
Parietaria mauritanica Durieu	March – May	March – May
Parietaria lusitanica L.	March – May	March – May

the continuous hourly and daily pollen concentrations in the air were calculated (Hirst, 1952). In Badajoz, the data were collected from 1994 to 2003, and in Mérida from 1996 to 1998. Because of problems in the infrastructure of the two aerobiological stations, there were interruptions in the data series: in Badajoz, from 28 April to 1 May 1995, and in Mérida, from 28 May to 6 June 1996.

The data used in the study correspond to the two pollen types into which the Mediterranean Urticaceae are divided: the *Urtica urens* and the *Urtica membranacea*. For each type, the daily concentrations were determined for each year at each location. The annual indices were calculated by summing the daily concentrations for each year.

For each year (from January to December), the principal pollination period was determined following the method of Nilsson & Persson (1981): the beginning and end of the period are taken to be the days on which the accumulated concentration reaches 5% and 95%, respectively, of the annual index. Also, for purposes of comparison with data from other aerobiological stations at which the two Urticaceae pollen types have not been separated, the sum of the two annual indices and the percentage represented by each were calculated.

For each type and each year, the daily concentrations corresponding to the common principal pollination period were compared between the two sites, using Pearson's correlation coefficient (r), the probability that $r = 0$ considered significant if $p < 0.05$.

In order to establish a pollination model for these pollen types at the two locations, their mean daily pollination levels were calculated, taking, for a given date, the arithmetic mean of the concentrations determined in each year. The resulting curves were fitted with a polynomial of order 8, which is the most widely used parametric model for pollen data (Comtois & Sherknies, 1991; Belmonte & Canela, 2002).

Meteorological data were acquired from the Territorial Meteorological Centre of Extremadura for the Badajoz and Mérida meteorological stations, and consisted of the mean, maximum and minimum temperatures (°C); relative humidity (%); rainfall (mm); wind speed (hm); and the periods of calm and of winds (hours) from the NE (quadrant 1), SE (quadrant 2), SW (quadrant 3) and NW (quadrant 4) (hours). No data corresponding to relative humidity or wind speed were available for 2002 or 2003 in Badajoz, nor for 1997 in Mérida when neither of the other wind parameters were available.

Pearson's correlation test was used to study correlations between the daily concentrations of the two pollen types in Badajoz and Mérida and the daily values of the meteorological parameters. Only data corresponding to the principal pollination period were considered. The significance level was taken to be $p < 0.05$.

Daily meteorological data were used to calculate the accumulated rainfall and the mean values of the mean, maximum, and minimum temperatures, and of the relative humidity, for autumn, winter, and spring. Pearson correlation coefficients were calculated to relate the annual index, the start and end dates (taken as the day number in the calendar year) of the principal pollination period and its duration, with those seasonal meteorological data, using for each period the values for the autumn of the preceding year and those of the winter and spring of the year under consideration. In the case of the *Urtica urens* type, since no significant correlations were found, the analysis was repeated using the annual indices reached in two periods: until the end of August, and from the beginning of September. These periods are similar to those used in the work of Galán et al. (2002). ①

From the hourly concentrations, the mean and 95% confidence interval for each hour of the day were calculated using the data for each day on which the two types were present during the ten years of the study in the case of Badajoz, and the 3 years in the case of Mérida. All the hourly data are solar hours, not GMT.

Results

Table II summarizes the observed annual characteristics of the pollination of the *U. urens* and *U. membranacea* types during the 10 years of the study. With respect to the sum of the concentrations of the two types (for subsequent comparison with the data from other Mediterranean sites), the values for Badajoz range from 1055.7 to 2866.2 with a mean of 1972.4, and those for Mérida (3 years of study) range from 1233.0 to 2234.5 with a mean of 1741.0.

Table II. Summary of the data corresponding to the *Urtica urens* and *Urtica membranacea* pollen types in Badajoz and Mérida. The value 'Annual index' represents the sums of the daily pollen concentrations (grains/m^3). The percentages are calculated with respect to the total of Urticaceae pollen. The principal pollination period data express the day number starting from January 1.

	Urtica urens type					*Urtica membranacea* type				
			Main pollen season					Main pollen season		
	Annual index	%	Onset	End	Span	Annual index	%	Onset	End	Span
Badajoz										
1994	1327.2	65.9	13	345	333	686.2	34.1	42	170	129
1995	1323.6	75.0	20	201	182	441.9	25.0	32	148	117
1996	414.6	39.3	48	333	286	641.1	60.7	81	141	61
1997	1587.9	78.0	12	329	317	447.9	22.0	43	169	127
1998	1436.1	62.5	10	323	314	862.3	37.5	37	142	106
1999	992.2	70.9	15	360	346	407.4	29.1	53	144	92
2000	1818.5	73.6	17	343	236	653.8	26.4	45	150	106
2001	1174.4	45.9	17	173	157	1386.5	54.1	49	141	93
2002	738.2	58.8	67	349	283	517.7	41.2	68	149	82
2003	1770.1	61.8	26	253	328	1096.1	38.2	58	133	76
Mérida										
1996	721.9	58.5	25	363	339	511.1	41.5	57	163	107
1997	1300.0	74.0	12	346	335	455.7	26.0	39	149	111
1998	1332.8	59.6	11	330	320	901.7	40.4	35	141	107

With respect to the two types independently, the mean annual index of *U. urens* pollen in Badajoz was 1258.3 and in Mérida 1118.2. In the case of *U. membranacea*, the mean annual index in Badajoz was 714.1 and in Mérida 622.8. In terms of the percentage of *U. membranacea* type pollen in the annual index of Urticaceae pollen, these data represent, in the atmosphere of Badajoz, between 25.0% in 1995 and 60.7% in 1996, and in Mérida between 26.0% in 1997 and 41.5% in 1996. The annual concentrations in Badajoz of the two types of Urticaceae pollen were not significantly correlated ($r = 0.211$; $p = 0.5576$).

The principal pollination period of the *U. urens* type was highly variable. In Badajoz, it usually began in January (6 of the 10 years studied) and ended in December (5 of the 10 years) or November (3 of the 10 years), the mean duration was 278.2 days. In Mérida the period began in January in all 3 years of the study, and ended in December in 1996 and 1997, and in November in 1998 (mean duration 331.3 days).

In the case of *U. membranacea*, in Badajoz the principal pollination period began in February and ended in May in 8 of the years studied. Its mean duration was of 98.9 days. In Mérida, it began in February in all 3 years, and ended in May in 1997 and 1998, and in June in 1996 (mean duration 108.3 days).

In both cases it could be said that there are two pollination periods, but for easier data managing it is better to consider only one. Figure 1 shows the mean daily concentrations for the two pollen types studied, and for the annual index of the Urticaceae, together with the best-fit order-8 polynomial curve. Results reveal the similarity in behaviour between Badajoz and Mérida. This was confirmed by the correlation study for the 3 years that the two sites have in common: the daily concentrations in Badajoz and Mérida during the common principal pollination period were significantly correlated for both pollen types ($p < 0.0001$ in all cases except for the case of *U. membranacea* pollen in 1996 which had a value of $p = 0.0095$). In addition to showing the principal pollination period of *U. membranacea*, Figure 1 also shows the principal pollination period of the *U. urens* type, which can be divided into two stages, the first centred in March and the second occurring in the last months of the year.

Table III lists the correlations found in Badajoz between the daily concentrations of the two pollen types and the different meteorological parameters considered.

In the case of the *U. urens* type, in 9 of the 10 years there was a significant negative correlation with the three temperature parameters, in 6 of the 8 years of available data, a significant positive correlation with relative humidity, but in no year was there any significant correlation with rainfall. With respect to wind speed (for which there were 8 years of data available), in 7 years there was a significant negative correlation, and in 4 of those years there was a corresponding positive correlation with the calm periods. The significant correlations with wind directions were positive in 6 years with winds from quadrant 1, and in 8 years with quadrant 2; they were

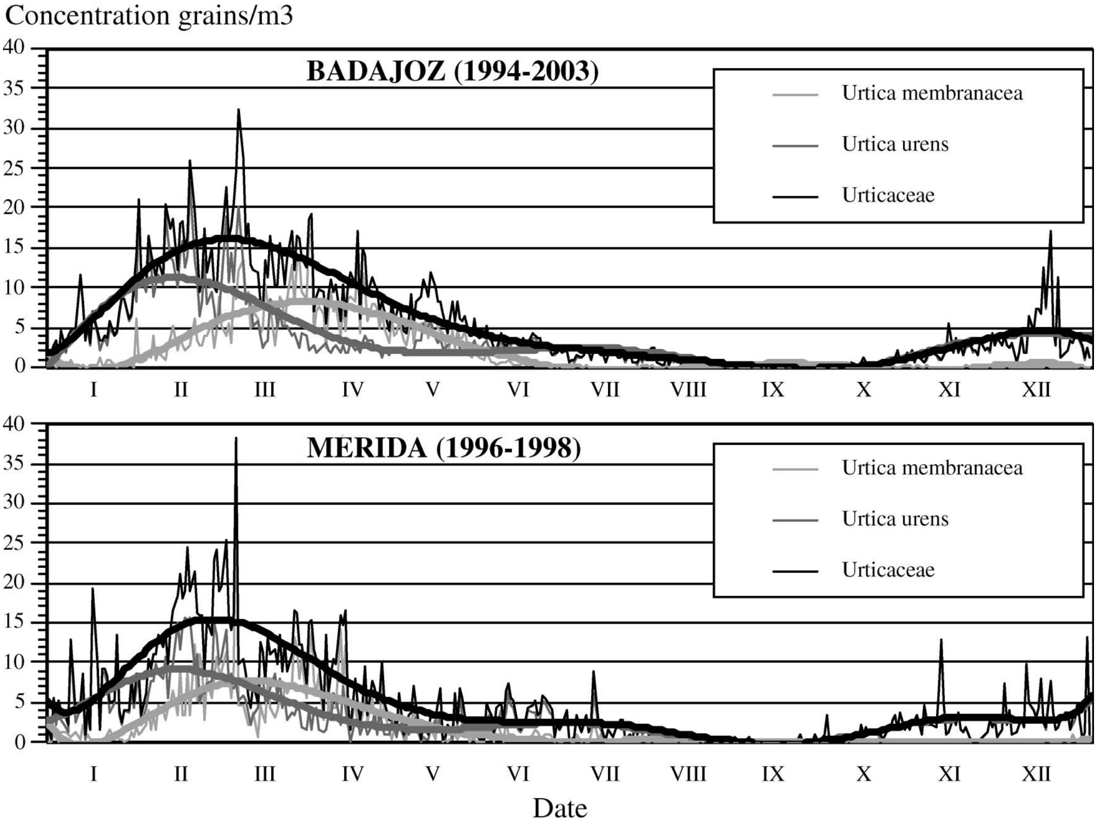

Figure 1. Mean concentrations of the *Urtica urens* and *Urtica membranacea* pollen types, and of the sum of the two, in Badajoz and Mérida during the years of study. In each case, the curve is the order-8 polynomial fit.

Table III. Significant ($p < 0.05$) coefficients of correlation between the daily values of different meteorological parameters and the daily concentrations of pollen of the *Urtica urens* (A) and *Urtica membranacea* (B) types in Badajoz. No relative humidity or wind speed data were available for 2002 and 2003.

	Mean temp	Max temp	Min temp	Relative humidity	Rain	Wind speed	Calm periods	NE wind	NW wind	SW wind	NW wind
(A) *Urtica urens*											
1994	−0.256**	−0.202**	−0.302**	0.191**		−0.227**	0.253**	0.186**	0.191**	−0.147*	−0.159*
1995	−0.379**	−0.379**	−0.332**	0.438**		−0.194*			0.189		−0.289**
1996	−0.211**	−0.177*	−0.236**	0.138					0.147		−0.127
1997	−0.346**	−0.258**	−0.368**	0.263**		−0.157*		0.219**	0.290**	−0.242**	−0.170*
1998	−0.229**	−0.203**	−0.238**	0.272**		−0.128		0.198**	0.391**	−0.284**	−0.218**
1999						−0.129	0.121				
2000	−0.230**	−0.140	−0.327**			−0.214**	0.115	0.213**	0.213**	−0.238**	−0.134
2001	−0.219*	−0.164	−0.280**	0.227*		−0.216*	0.223*	0.212*	0.569**	−0.280**	−0.254*
2002	−0.214**	−0.157*	−0.262**	–		–	0.180*	0.232**		−0.206**	
2003	−0.280**	−0.245**	−0.306**	–		–	0.322**		0.217**	−0.219**	−0.160*
(B) *Urtica membranacea*											
1994							0.204				
1995				0.286*							
1996											
1997	−0.238*		−0.250*		−0.179	−0.242*					
1998							0.267*	−0.331**			
1999	0.276*	0.299*		−0.340*					−0.234		0.264
2000	0.426**	0.326**	−0.376**						−0.256*		0.204
2001								−0.295*	−0.219		0.380*
2002				–	−0.226	–					
2003	0.254		0.299*	–		–		−0.352*	−0.257	0.387**	

*$p < 0.01$; **$p < 0.001$.

negative in 7 years with quadrant 3, and in 8 years with quadrant 4.

In the case of *U. membranacea* (Table III), there were not only fewer significant correlations than for the previous type, but also there was a lack of consistency between years in the sign of the correlations that were found. Perhaps the most noteworthy were the 2 years of significant negative correlations with rainfall, and the existence of some negative correlations with periods of winds from quadrants 1 and 2, and some positive correlations with those from quadrant 4.

Table IV lists the significant correlations for the 3 years of study in Mérida. The correlations found for the *U. urens* concentrations were: negative with the temperatures in all 3 years; positive with the relative humidity in 1 year; positive with the calm periods, and negative with wind speed in one of the 2 years of available data; for the wind direction, positive with quadrants 1 and 2, and negative with quadrants 3 and 4. In the case of *U. membranacea*, no significant correlation was found in more than one of the years of analysis.

For the 10 years of study in Badajoz, a correlation study was carried out between some of the characteristics of the annual pollination given in Table II for both pollen types (annual index, and beginning, end, and duration of the principal pollination period) and some seasonal meteorological parameters (rainfall, relative humidity, and mean, maximum, and minimum temperatures, for autumn, winter and spring). In the case of the *U. urens* type, no significant correlations were found. In the case of *U. membranacea*, however, the annual indices were positively correlated with the winter minimum temperatures ($r = 0.636$; $p = 0.0482$), the beginning of the principal pollination period was positively correlated with winter rainfall ($r = 0.706$; $p = 0.0225$) and negatively with the maximum temperatures of the same season ($r = 0.673$; $p = 0.0330$), and this last factor was positively correlated with the duration of the principal pollination period ($r = 0.727$; $p = 0.0173$).

Given that the principal pollination period of *U. urens* presented two peaks (Figure 1), the correlation study with the seasonal meteorological parameters was repeated with the annual indices of this type divided into two: one until August and the other starting from September. In the first case, two significant correlations were observed: a negative one with winter rainfall ($r = -0.633$; $p = 0.0496$), and a positive one with winter maximum temperatures ($r = 0.710$; $p = 0.0215$). In the second case, there were no significant correlations.

Figure 2 shows the hourly patterns of the concentrations of the two Urticaceae pollen types in Badajoz and Mérida. The *U. urens* type in Badajoz tended to be present at low concentrations during the night and through early morning – in particular, from 01:00 to 10:00. The concentrations then started to rise, reaching a maximum between 12:00 and 15:00, and then declining during the afternoon and evening to the nocturnal minimum.

The *U. membranacea* type in Badajoz had minimum concentrations between 07:00 and 10:00. There was then a rise until 13:00, followed by a slight decline between 16:00 and 18:00 and a second rise to the maximum between 20:00 and 24:00, after which there began a steady decline until the morning minimum. In Mérida, the pattern differed strongly in that, having reached the maximum concentration at 13:00, the concentrations did not rise again during the late afternoon and evening.

Discussion

The annual index of Urticaceae pollen places Badajoz and Mérida in an intermediate position among the 15 Spanish aerobiological stations studied by Belmonte et al. (1999), below northern coastal sites and some locations in the south, and above the more inland zones.

Table IV. Significant ($p < 0.05$) coefficients of correlation between the daily values of different meteorological parameters and the daily concentrations of pollen of the *Urtica urens* and *Urtica membranacea* types in Mérida. No relative humidity or wind data were available for 1997.

	Mean temp	Max temp	Min temp	Relative humidity	Rain	Wind speed	Calm periods	NE wind	NW wind	SW wind	NW wind
Urtica urens											
1996	−0.203*	−0.161	−0.252**				0.174*			−0.162	
1997	−0.388**	−0.283**	−0.476**	–		–	–	–	–	–	–
1998	−0.245**	−0.208**	−0.272**	0.235**		−0.209**	0.276**	0.206**	0.300**	−0.336**	−0.220**
Urtica membranacea											
1996				0.194				−0.233		0.222	0.201
1997	−0.225		−0.288*	–		–	–	–	–	–	–
1998							0.198				

*$p < 0.01$; **$p < 0.001$.

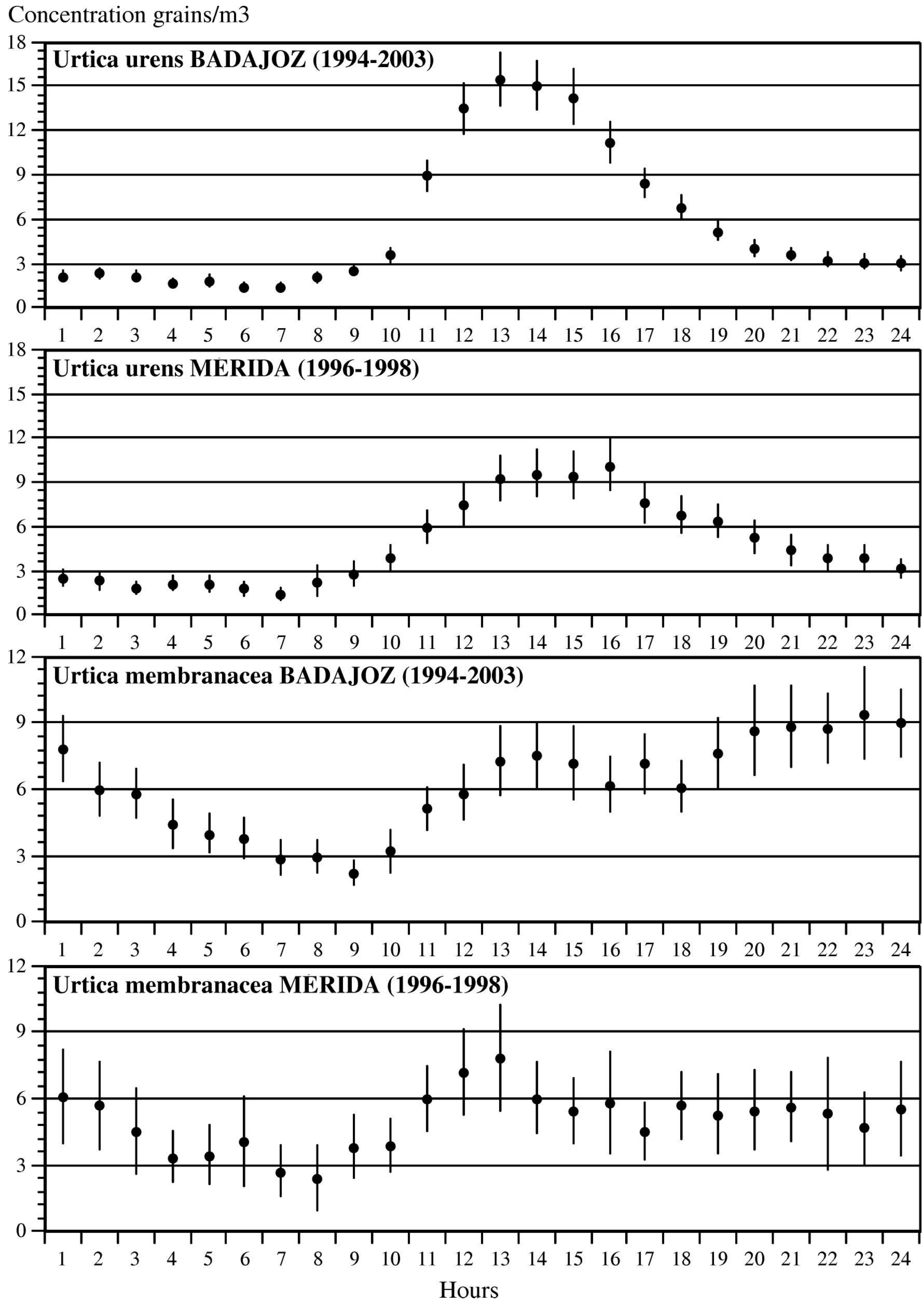

Figure 2. Mean hourly concentrations of the *Urtica urens* and *Urtica membranacea* pollen types in Badajoz and Mérida, representing in each case the arithmetic mean of all the days on which the type appeared in the years of the study, as well as the 95% confidence interval.

The annual index of the *U. urens* type were greater than those of *U. membranacea* in 8 of the 10 years studied in Badajoz, and in all 3 years in Mérida. For both types, there were interannual variations in the concentrations, but, given the lack of correlation between the two in Badajoz, it seems that the factors determining these concentrations are independent. The percentage represented by the *U. membranacea* pollen was always greater than 25% at both sites, indicating the importance of the pollination of this species. This percentage of non-allergenic pollen – which is included with the rest of the Urticaceae type in other studies carried out in the Mediterranean area – could lead to annual and local variations in epidemiology, in that the greater the presence of *U. membranacea* pollen in the atmosphere, the higher would be the real threshold concentration of Urticaceae pollen needed to provoke symptoms. This is especially so given that this threshold is in many cases determined by relating the local concentrations of Urticaceae pollen to its effect on allergic patients (D'Amato et al., 1991; Negrini et al., 1992).

The data relating to the beginning, end, and duration of the principal pollination period of the *U. urens* type in Badajoz and Mérida are similar to those from some sites reported in the work of Belmonte et al. (1999). The interannual differences are also similar. This pollen is present in the atmosphere almost all year round, having a mean principal pollination period of some nine months in Badajoz. On the contrary, for *U. membranacea* the period was far shorter – some 3 months – and with less interannual variability at the two study sites. This is logical, as various authors have noted (Belmonte et al., 1999; Galán et al., 2000), since the aerobiological behaviour of the pollen of the Urticaceae is due to the integration of several species, so that the pollination will be spread out over a broader period than that due to a single species. This remains true even if that single species is *U. membranacea* which, according to the data of Paiva (1993), has a long flowering period (Ruíz 1995, however, gives a narrower period of flowering for the species).

For both types, the behaviour at the two sites was very similar for the 3 common years of study. This was confirmed by the significant correlations between their daily concentrations, and by the polynomials that fitted their means. Such a similarity was to be expected given the similar environments and climate of the two cities. Indeed, these were factors that, according to Belmonte & Canela (2002), determined the appearance of high coefficients of correlation between different Catalonian sites. According to Belmonte et al. (1999) and Spieksma et al. (2003), human activity also has an important effect on the recorded concentrations of Urticaceae, but again, in the present study, the two cities have environments that are subject to similar anthropogenic influences.

At both locations, the dynamics of the *U. urens* type presented two maximum pollination periods, one centred in March and the other, smaller in magnitude, in November and December. During the rest of the year, the concentrations remained low, and practically disappeared around September. The two stages of pollination that were observed coincide with those defined by Galán et al. (2000), who suggest that, at least in Córdoba, the second stage belongs to the pollination of *Parietaria judaica*. This possibility would require, however, direct confirmation from studies on the flowering of the species of the family, since, according to Paiva (1993), the flowering of other species included in the type, such as *Urtica urens*, reaches these months. The two stages are also similar to those found by Belmonte et al. (1999) for Andalusian sites, by Mañas et al. (1988) for sites in the Balearic Islands, and by Munuera et al. (2002) in Murcia.

The daily concentrations of this pollen type seem to be negatively correlated with the temperature parameters. This could be a reflection of the long principal pollination period and the absence of high concentrations during part of the summer when in Spain the maximum temperatures are reached. This same relationship has been found in other studies on pollen of the Urticaceae family (Trigo et al., 1996). Nevertheless, it is worth mentioning that Rizzi-Longo et al. (2004) in Trieste found positive correlations in most of the 9 years of their study between the mean, minimum, and maximum temperatures and the daily concentrations of Urticaceae pollen. These correlations were present both when they considered the complete principal pollination period, and when they split it up into its ascending, maximum, and descending phases, although when they used accumulated instead of daily temperatures the correlations were negative except for the ascending phase. This coincides with the findings of Díaz de la Guardia et al. (1998) and Vega et al. (2003). Also Galán et al. (2000) found a positive influence of temperature during the spring and autumn pollination of the *U. urens* type in 1 of the 2 years of their study, although in the other year the spring correlation was of the opposite sign. In spite of these results, the authors affirm that temperature constitutes the most important factor acting on the spring and autumn concentrations of the *U. urens* type, since they appeared in the predictive formulas that were determined for the 2 years.

Relative humidity was positively correlated with the concentrations of the *U. urens* type. This contrary effect to that of temperature had already been observed in work on the pollen of the Urticaceae family as a whole (Trigo et al., 1996). As was to be expected from their results commented on in the paragraph above, Rizzi-Longo et al. (2004) found no significant correlations between the daily concentrations and this parameter, although there were significant negative correlations when they considered all 9 years of their study period together, for the complete principal pollination period and for its ascending and descending phases considered separately. In this, they again coincided with the observations in Granada by Díaz de la Guardia et al. (1998) and Vega et al. (2003). These last workers found positive correlations with the absolute humidity and with the wet-bulb temperature, which were of greater significance than the correlations with relative humidity. They, therefore, recommended the use of the former two parameters in these studies.

In the present work, there were no significant correlations with rainfall in either of the two locations, despite the summer period of minimum concentrations also being the driest period in a Mediterranean climate. This lack of effect has also been reported in other studies, such as those of Galán et al. (2000), Corden & Millington (1991), and Fornaciari et al. (1992). Díaz de la Guardia et al. (1998) and Rizzi-Longo et al. (2004), however, found this parameter to have a significant negative correlation with the daily concentrations.

Wind seems to diminish the concentrations of the *U. urens* type, as revealed by their significant negative correlations with wind speed —already noted in the study of Rizzi-Longo et al. (2004) – and positive correlations with the calm periods. While this is, in principle, paradoxical for the pollination of an anemophilous species, it could be related to the active pollen release mechanism in these species, with the moment of release being determined by environmental conditions (Galán et al., 2000). It could also be conditioned by the proximity of the sources to the trap, which would result in the wind acting to dilute the pollen in the environment, thereby reducing its concentrations. This might explain the negative influence of wind for some other pollen types studied in the same two locations, in particular, that of the Amaranthaceae and Chenopodiaceae (Muñoz et al., 2000), whose sources are located in the surroundings of both cities. It is not valid in the case of the Urticaceae, however, whose distribution is more disperse. Rather, one might consider that wind acts directly by restricting pollen release in these species, as previously proposed for the pollination of Plantago in Badajoz and Mérida (Tormo et al., 2001).

With respect to the positive correlations found between the concentrations of the *U. urens* type and the easterly winds (quadrants 1 and 2), and the negative correlations with the westerly winds (quadrants 3 and 4), these can not be interpreted on the basis of the location of the pollen sources relative to the traps as is the case with other types (Silva et al., 2000), since the distribution of the species is widely dispersed around both traps. Instead, the explanation could be that, at least at the Badajoz site, the temperatures are negatively correlated with winds from quadrants 1 and 2, and positively with winds from quadrants 3 and 4.

None of the seasonal meteorological parameters could explain the interannual variations in the pollination of the *U. urens* type in Badajoz, neither the differences in the annual indices, nor those in the beginning, end, and duration of the principal pollination period. Nevertheless, rainfall and winter maximum temperatures seemed to affect negatively and positively, respectively, the concentrations of this type collected in the first stage, i.e. until August. The concentrations collected from September onwards remained independent of these parameters.

Interestingly, dry warm winters lead to high annual concentrations of the *U. urens* type, while, with respect to daily concentrations, rainfall seems to have no influence at all, and temperatures are consistently negatively related. An explanation could be that the two factors act physiologically on the individuals and the populations, and that they act in different directions for the release, transport, and/or daily capture of the pollen grains. There is at least partial support for this hypothesis in the findings of Belmonte et al. (1999) for the Iberian Peninsula showing that the pollination of the Urticaceae family is more abundant in temperate zones than in the cold regions of the interior.

The variability of the correlations with the parameters at the two sites could be due to differing species compositions, or to other factors such as the height of the trap. Indeed, according to Hart et al. (1994), this latter factor can cause variations in the influence of the different meteorological parameters.

The daily pattern of the *U. urens* type pollination followed a diurnal model, with a single peak of maximum concentrations between noon and the first hours of the afternoon. This pattern is similar to that described by Galán et al. (1991) in Córdoba, Díaz de la Guardia et al. (1998) in Granada, La Sera & Domínguez (1996) in La Laguna (Canary Islands), and Trigo et al. (1996, 1997) in Málaga. The latter authors explain this model on the basis of these being the warmest hours of the day, and thus favourable to the release of pollen. The pattern is also similar to the models described by Fornaciari et al. (1992) in Perugia, Corden & Millington (1991) in Derby, Emberling & Norris-Hill (1991) in London, and Kasprzyk et al. (2001) at five Polish locations (most of the pollen of the last two works corresponded to *U. dioica* and *U. urens*). There are variations between these studies in the hour at which the maximum concentrations are recorded. Some authors suggest that this is due to the temperatures at each location. Hart et al. (1994) in Leicester, however, in describing a similar model, found a considerable delay as the height of the trap increased. Lastly, it has to be mentioned that Nitiu (2004) in Argentina founds a pattern of concentrations that is practically constant throughout the day.

As noted above, the pollination period of *U. membranacea* was shorter, with a single maximum between the months of March and April. Its daily variations seemed to be more independent of the meteorological parameters considered, and the correlations with temperature and humidity had changing signs, in contrast with the uniformity of the relationships of these factors with the pollination of the *U. urens* type. In some years, rainfall had a negative effect on the concentrations, which could be due to it cleansing the atmosphere of pollen. Galán et al. (2000) in Córdoba found significant positive correlations between the concentrations of *U. membranacea* and the temperatures in one of the years of their study; in the other, however, the only significant correlation was with the minimum temperatures, and it was negative; this contrasted with the appearance of a correlation of the same sign with relative humidity. Neither did these workers find any significant correlations with rainfall for this pollen type.

In the few years in which the correlations with wind speed and calm periods were significant, these had the same sign as for the case of *U. urens*. The wind direction correlations, however, seemed to have the opposite sign, i.e. in some years easterly winds reduced the concentrations and westerly winds increased them, both in Badajoz and in Mérida. There are therefore notable differences between the two pollen types in the response of their daily concentrations to meteorological variations.

The annual indices of this pollen type were positively correlated with the winter minimum temperatures. Cold winters, therefore, reduce the pollination rate. This point seems to create a certain analogy between the two pollen types studied. At a general level, winter temperatures can be said to positively affect the Urticaceae group, although in the case of *U. membranacea* the correlation was with the winter minimum temperatures, while in the case of the *U. urens* it was with the winter maximum temperatures. Nevertheless, the winter maximum temperatures did affect the principal pollination period of *U. membranacea*, by advancing the beginning and lengthening the duration.

Similarly, winter rainfall, which for the *U. urens* type reduced the annual index until August, seemed to delay the beginning of the principal pollination period in the case of *U. membranacea*, although it had no effect on the annual indices.

The hourly pattern of concentrations for *U. membranacea* was also different from that of the *U. urens* type. In the former case, the concentrations did not decline during the afternoon, but, at least in the case of Badajoz, continued to rise until the end of the day (midnight).

Conclusions

Urticaceae pollen in the atmosphere of Badajoz and Mérida reaches an intemediate concentration compared with the rest of Spanish stations. Pollen of *U. membranacea* can be separately studied in aerobiological works from the rest of the species of the family (*U. urens* type); it can represent more than 25% of total Urticaceae pollen, and since it is non allergenic, the habitual threshold used in epidemiology (total Urticaceae pollen) must take this fact into account. The *U. urens* pollen type is present in the atmosphere almost all year round, but *U. membranacea* pollen has a far shorter period.

U. urens shows two maximum pollination periods (March and November – December). The daily concentrations are negatively correlated with temperature and positively correlated with relative humidity; wind seems to diminish the concentrations and perhaps affects the active pollen release mechanism present in these species. Wind direction correlations can be better explained by a relation between temperature and wind directions than by a pattern of source distribution. The hourly daily pattern shows a single peak of maximum concentrations between noon and the first hours of the afternoon.

U. membranacea pollen has shorter pollination period with a single maximum (March – April); its daily variations do not show clear relations with meteorological parameters. The hourly pattern of concentrations is different from that of the *U. urens* type, as the concentrations did not decline during the afternoon.

There are hence major differences in the behaviour of the pollen of *U. membranacea* from that of the rest of the Urticaceae included within the *U. urens* type. These differences affect both the calendar of appearance of the pollen and its hourly pattern. Second, the two types respond differently to daily variations in meteorological conditions, and third, the two types present independent interannual variations of annual indices. By including the pollen of *U. membranacea* within a single pollen type together with the rest of the Urticaceae, a further factor of variation to the daily and annual differences in concentrations is added. This affects the study of the potential influence of different environmental parameters on those differences. Finally, considering its lack of allergenicity, it is recommended that, in Mediterranean aerobiological stations within the distribution area of *U. membranacea*, its pollen should be distinguished as an independent type.

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