Parietaria major allergens vs pollen in the air we breathe

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Key words
Allergens, Par j 1, Par j 2, Urticaceae pollen, PM10, PM2.5, airborne pollution.

ABSTRACT:

Background: *Parietaria* and *Urtica* are the genera from the Urticaceae family more frequent in Mediterranean and Atlantic areas. Moreover, both genera share pollination periods, and their pollen (of the main species) is so similar that there is no aerobiological evidence of the proportion of each of them in the airborne pollen identification, except in the case of *U. membranacea*. However, *Parietaria* is one of the most important causes of pollinosis and *Urtica* is not. Our aim is determine if airborne Urticaceae pollen concentrations show the aerodynamics of the two major allergens of *Parietaria* (Par j 1 and Par j 2) as well as the allergen distribution in the different-sized particles.

Methods: The air was sampled during the pollination period of *Urticaceae* using Hirst Volumetric Sampler and Andersen Cascade Impactor in two cities of Southern Spain (Córdoba and Granada). The samples were analysed by the methodology proposed by the Spanish Aerobiology Network (REA) and the minimum requirements of the European Aeroallergen Society (EAS) for pollen, and by ELISA immunoassay for allergens.

Results: The patterns of airborne pollen and Par j 1-Par j 2 were present in the air during the studied period, although with irregular oscillations. Urticaceae pollen and Par j 1-Par j 2 allergens located in PM2.5 showed positive and significant correlation during the period with maximum concentrations (March to April).

Conclusion: *Parietaria* aeroallergens show similar pattern of Urticaceae airborne pollen. Urticaceae pollen calendar is as a good tool for allergy prevention. On the other hand, important concentrations of Par j 1 and Par j 2 were located in the breathable fraction (PM2.5), which could explain the asthmatic symptoms in the allergic population to *Parietaria*.

Keywords: Par j 1-Par j 2 allergens, Urticaceae pollen, Biological air quality, ELISA analysis

1. Introduction

Allergic disorders constitute an important public health problem, which is increasing dramatically since the last decades. The pollen and spores are known to play an important role in respiratory allergies that appear especially during the flowering periods of plants. In Europe is estimated that the prevalence of pollen allergy affects up to 40% of the allergic population (D'Amato et al., 2007).

Urticaceae is a family of dicotyledonous plants with more than 1800 species. The better represented genera in the Mediterranean area are *Parietaria* L. and *Urtica* L. Both are wind-pollinated weed that are commonly found in the countryside and urban areas, growing on walls and soils with high nitrogen content. Moreover, the flowering of these genera is overlapped in time, beginning in winter and, in the case of *Parietaria*, being extended until autumn. Because pollen from different genera are similar under light microscopy (spheroidal, psilate and triporate), except for *Urtica membranacea* with smaller and periporate pollen, the pollen calendars are always displayed with the name of Urticaceae pollen type.

However, the clinical significance of these genera is different. *Parietaria* constitute the third most sensitizing allergen source after mites and grass pollens in South-East France (Charpin, 2000) and one of the main causes of asthma and rhinitis in Spain and Italy (D'Amato & Liccardi 1994; Alergológica 2005). On the contrary, *Urtica* pollen displays little allergenic activity. Bousquet et al. (1986), Vega-Maray et al. (2006a) and Tiotiu et al (2016) confirmed the absence of cross-reactive antigens between *Parietaria* and *Urtica* pollen grains and concluded the lowest allergy risk of this last genus to induce diseases.

The first proteomic map of *Parietaria* pollen shows that the 36% of the total proteins correspond to allergens (Barranca et al., 2010). The major allergens isolated and characterized are Par j 1 and Par j 2 with IgE of 95% and 82%, respectively. Both are two small non-specific lipid transfer protein (LTP) and present a conserved structure (Colombo et al., 1998). On the other hand, two minor allergens have been isolated and characterized: Par j 3 is a profilin protein (Asturias et al., 2004) and Par j 4 (Pj CBP) is characterized as Calcium-Binding Protein (Bonura et al., 2008).

In the last two decades, the Aerobiology focus the research on both, airborne pollen behaviour and aeroallergens (i.e. Moreno-Grau et al., 2006; De Linares et al., 2010; Jato et al., 2010; Galán et al., 2013; Buters et al. 2012, 2015; Alcázar et al. 2015; Plaza et al. 2016a, 2017). Knowledge on the dynamic of these particles is contributing to major information on airborne biological pollution. Several methods have been used for aeroallergen detection, such as Cyclone collector (i.e. Moreno-Grau et al., 2006; De Linares et al., 2014; Plaza et al 2016a; 2016b), Andersen cascade impactor (i.e. Schäppi et al., 1996; De Linares et al., 2007) or Chemvol® high-volume cascade Impactor (Buters et al. 2008; Albertini et al 2013; Galán et al 2013). Comparable results have been observed when comparing different samplers in the same place and years, i.e. Cyclone collector and Chemvol® high-volume cascade impactor in Córdoba (Plaza et al 2017). These studies have also shown similar dynamic between airborne pollen and aeroallergen but with discrepancies when exposition to different external events.

In the same way, there has been an increased interest in determining the size-fractions particles where these allergens are airborne (De Linares et al 2010; Esposito et al 2012; Buters et al 2012). Knowing that the Environmental Protection Agency (EPA) has determined that particles are classified in two size categories based on their penetration capacity into the lung as either: PM10 as particulate matter with an aerodynamic diameter of 10 μm and PM2.5 as fine particulate matter with an aerodynamic diameter of 2.5 μm (Esworthy, 2013), a comparison of allergen load of these two categories could reveal the different clinical symptoms that provoke these particles.

The main goal of this paper was to study the behaviour of Urticaceae pollen and the two major *Parietaria* allergens, Par j 1 and Par j 2, in Southern Spain (Córdoba and Granada). The specific goals have been to determine if the airborne Urticaceae pollen concentrations show the aeroallergens dynamics and study the allergen distribution in different-sized particles to establish whether this distribution could be related with the allergy symptoms.

2. Materials and methods

2.1. Area of study

The aerobiological study was carried out in two cities of Southern Spain (Córdoba and Granada). The aerobiological station of Córdoba (37º50'N, 04º45' W; 123 m.a.s.l.) is situated in the University of Córdoba in the north-eastern part of the city, while the station of Granada is localized in the University of Granada in the city centre (37º11' N, 03º35' W; 685 m.a.s.l.). Although the climate in both cities is Mediterranean (characterized by moderate annual temperature and summer drought), it presents important oscillations in temperature (summer-winter and day-night).

The genera of Urticaceae family present in Córdoba and Granada are Urtica and *Parietaria* (Castroviejo et al. 1993; Blanca et al. 2009). *U. dioica* L., *U. urens* L. and *P. judaica* L. are present in both cities while *U. membranacea* Poir. in Lam. only in Córdoba and *P. mauritanica* L. and *P. lusitanica* L. only in Granada. Although the flowering start of these species is variable, their flowering periods are usually overlapped. According to the Spanish handbooks of plants (Castroviejo et al. 1993; Blanca et al. 2009), the flowering start of *U. urens* occurs in January, *U. membranacea* and *P. lusitanica* in February, *P. judaica* and *P. mauritanica* in March, and *U. dioica* in April.

2.2. Sampling of Airborne Pollen and Allergens

For this study, airborne pollen behaviour was performed during the period 1993-2016. The monitoring was realized with a volumetric Hirst type Spore Trap (Hirst 1952). This collector was designed specifically for pollen, spores, and other particles suspended in the air, with an aspiration of 10L/min, comparable with the respiration of an average adult human.

Hirst samplers were placed at 22-23m above ground level. The counting method was that recommended by the Spanish Aerobiology Network, REA (Galán et al. 2007) and the minimum requirements of the European Aerobiology Society, EAS (Galán et al. 2017a). Terminology used in this paper follows the International Association for the Aerobiology (IAA) and EAS recommendations (Galán et al., 2017b). The daily pollen data are expressed as daily average of pollen per cubic metre of air (pollen/m^3). In this study, we analyse the data expressed in daily pollen and Annual Pollen Integral (APIn).

U. membranacea have a pollen type different from that of other Urticaceae species. The former has polipantoporate pollen with a smaller diameter of 9–12 µm while the others have triporate pollen with a diameter of 14-19 µm (Trigo et al 2008). In this study, only the dynamics of the Urticaceae pollen type was taken into account.

The aeroallergens were studied through a temporal study considering the years from 2006 to 2009 in the aerobiological station of Córdoba and a spatial study analysing the year 2006, in two cities (Córdoba and Granada). In both cases a cascade impact collector was used (Andersen 1958). The sampling took place during the middle hours of the day when pollen concentrations are highest (between 12 and 17h) (Díaz de la Guardia et al. 1998; Galán et al 2000). These collectors distribute the particles in different stages of size-fractions. The air flow through the impactor is controlled by a pump that draws in air at 30L/min (Lanzoni SPS 3001, Italy). The size discrimination of the particles is possible by the variation in the air velocity, which is led sequentially through a series of fibreglass Whatman® filters (Glass microfibre filters; type: GF/A) of descending pore size, this increasing the air velocity from the first stage to the last. The largest particles are deposited at the first stages while the smallest pass through the collector until being stopped by the correspondingly fine filter (Andersen 1958).

The samples were analysed by an indirect ELISA (De Linares et al. 2007). For each filter, 4 circular replicates (diameter 0.5 cm) were taken on a radial pattern. As a control, 4 replicates of 1 filter with no impact were used. The filters were submerged in 125 µL phosphate-buffered saline (PBS, pH 7.4) in microplate wells for 20 h at room temperature. The discs were removed and the wells cleaned with PBS-TW (0.3% Tween 20). After blocking during 1 h at 37 ° C with 200 µL/well of PBS containing 1% bovine serum albumin (Sigma, St. Louis, Mo., USA) and 0.3% Tween 20. After 3 washes with 200 µL PBS-TW (0.3% Tween 20), 125 µL horseradish peroxyidase (Polyclonal Swine Anti-Rabbit Immunoglobulins; Dako Cytomation, Glostrup, Denmark) diluted in PBS at a concentration of 1: 1,000, was added and incubated in the same conditions. Further washes were carried out by incubating at room temperature and in darkness with 125 µl O-phenylenediamine tablets (OPD; Sigma, St. Louis, Mo., USA) diluted in citrate buffer (1 tablet of OPD + 12.5 ml buffer citrate + 12.5 ml distilled water + 20 µl H 2 O 2). This reaction was stopped by adding 50 µL of HCl 3N. The results in all cases are expressed in nanograms of allergen per cubic metre. Par j 1-Par j 2 allergens were quantified using polyclonal antibody (Bial-Aristegui, Spain), which were isolated in the same fraction and identified by the fingerprinting of the peptide (Arilla et al., 2006). The standard curve was drawn from dilutions of Par j 1-Par j 2 allergens purified from *P. judaica* pollen extract by affinity chromatography (Bial-Aristegui, Spain; Arilla et al., 2006).

For a reliable comparison of the results for the two samples of two cities, these collectors functioned adjacently to Hirst samplers on the same timetable. The results in all cases are expressed in nanograms of allergen per cubic metre of air (ng/m^3).

2.3. Meteorological data

Daily series of Temperature (maximum, mean, and minimum), Precipitation and mean Related Humidity were used. Data were provided by the Andalusia Network of Agroclimatic Information (RIAA).

2.4. Statistical analysis

The reproducibility of ELISA technique was determined by mean the coefficient of variance percentage (%CV) being calculated as the standard deviation/mean × 100. 30 replicates in each city and year were used. In the case of Córdoba, the CV ranges from 8.33% to 6.53 and in Granada, 6.65%.

Spearman's correlation coefficients between daily data of Urticaceae pollen, Par j 1-Par j 2 allergen, allergen in Pm10 and PM2.5, and meteorological parameters were calculated during the allergen studied period. This analysis was carried out by using the SPSS version 19.0.

3. Results

3.1. Airborne pollen vs. aeroallergens

The meteorological parameters during the studied period were examined in each area (Table 1). In Córdoba, a warmer and rainier climate is observed (16.9ºC, 553.7 mm) than in Granada, with a colder and drier climate (15.2ºC, 267.4 mm).

Figure 1 shows average concentration of the Urticaceae airborne pollen during 24 years (1993-2016) and the annual patterns during the studied period. This pollen type is presented in the air throughout the year showing its maximum pollination in winter and spring in both cities. Córdoba registered a lower concentration than Granada, and showed an explosive increase in its concentrations at the beginning of spring. Instead in Granada, the higher concentrations were registered during end of spring.

Regarding the years with pollen and allergen detection, the four year aerobiological behavior in Córdoba followed similar dynamics to the average 1993-2016 for pollen (Figure 1). 2009 presented the longest pollen season (256 days) but the lowest Annual Pollen Integral (APIn) (1343 pollen/m^3), while 2006 presented the shortest season (134 days) and 2008 the highest APIn (3306 pollen/m^3). The peak day pollen concentration was higher in 2006 (400 pollen/m^3) than the others years (ranging from 151 to 53 pollen/m^3) (Table 1).

In Granada, the Urticaceae airborne pollen concentration recorded during 2006 followed similar patterns to the average 1993-2016, as in Córdoba, this year registered higher pollen concentrations than others years (Figure 1).

The comparative study of the two cities during the same year (2006) shows that the APIn in Córdoba was 2108 pollen/m^3, registering the peak day on 2st April (400 pollen/m^3). In Granada, this pollen type registered higher APIn (5957 pollen/m^3) and was presented in the air during more time (338 days) than on Córdoba (134 days), despite peak day was registered two days before with lower concentration (31th March, 194 pollen/m^3; Table 1).

The Spearman correlation test between Urticaceae pollen concentrations and meteorological variables during allergen study period (Table 2) showed significant and negative correlations with the temperature in both cities (except 2007 in Córdoba). On the other hand, relative humidity presented significant and positive correlation in 2006 and 2009 in Córdoba, and also during 2006 in Granada.

The aeroallergen study during the four years in Córdoba showed fluctuation in the analysed years (Table 1). The Allergen Season Integral (ASIn) and peak allergenic concentrations recorded differences; while in 2007 was detected 23016.9 ng/m^3 of Par j 1-Par j 2 in the 118 analysed days, in 2008 was detected 13037.8 ng/m^3 during the 170 analysed days. In the case of peak allergen days, while the highest concentration were detected in 2007 (February 27th), reaching the 856.1 ng/m^3; the lowest occurred in 2008 (June 12th) with 389.8 ng/m3. Only 2009 and 2008 registered moments where airborne allergens were not detected (18 and 7 days, respectively). Comparing Córdoba and Granada during 2006, the peak day in Córdoba occurred on March 29th with 450.3 ng/m^3, while in Granada occurred on May 9th, with 369.6 ng/m^3.

The aeroallergen dynamic of Par j 1-Par j 2 in both cities was characterized by its continued presence during the studied period, although with irregular oscillations (Fig. 1). In the case of Córdoba, when Urticaceae pollen registered the highest concentrations, aeroallergen behaviour was similar to pollen. However, before and after pollen season, allergen load was detected. On the other hand, Granada registered two allergen periods with different concentrations: 1st February to 30th April, with low levels but with similar dynamic with airborne pollen; and 1st May to 30th June, with high allergen concentrations and low pollen (Figure 1).

Results for the Spearman correlation test around the studied period are showed in Table 2. In Córdoba positive and significant correlation between Urticaceae pollen and Par j 1-Par j 2 during 2007 and 2008 were registered (0.225 and 0.212; $p<0.05$, respectively), but not in 2006 and 2009. In relation with the meteorological variables, aeroallergens showed significant correlation with mean and minimum temperature (2007, 2008, and 2009).

The spatial study shows that during 2006 non-significant correlation was observed between aeroallergens and pollen. However, if we analyse the period with maximum pollen concentration in both cities (1st March-30th April in Córdoba and 1st February-30th April in Granada), the correlation were positive and significant (0.440; $p<0.05$ and 0.283 $p<0.01$, respectively). In relation with the meteorological variables, allergens concentrations showed significant correlation with maximum temperature in Granada.

3.2. Airborne allergens in different-size particles

The distribution of Par j 1-Par j 2 allergen according to the particle sizes showed that the stage with larger particles (stage 1) registered the lower concentration of allergens (Table 3), lower than 10.2%. In Córdoba the highest allergens concentrations were localized in the different stages depending of the studied year. Comparing 2006 in both cities, in Córdoba the highest concentration (25.1%) were registered in stage 3 and in Granada in the stage 6 (35.6%) (<1.1µm).

According to EPA classification, the results obtained showed that the highest allergen load was localized in PM 2.5 in the both cities (ranging to 67.9% to 39.3% in Córdoba, and 72.1% in Granada during 2006), except in 2009 that the result was opposite, with higher concentrations in PM10.

Correlation analysis between allergen load (PM10 and PM2.5), airborne pollen concentration and meteorological variables are showed in Table 2. The results showed similar correlations when comparing aeroallergen with pollen, i.e, PM 2.5 showed significant positive correlations with Urticaceae pollen, while PM10 not. This analysis in relation with meteorological variables obtained non-significant results.

4. Discussion

4.1. Dynamics of Airborne particles related with Urticaceae pollen

One of the main goals when monitoring pollen and spores is to know the allergen exposition in the air to develop successful strategies for protecting human health and improve the quality of life of allergic patients. At the end of the 1990s, the studies of aeroallergens, based on immunological analysis, have been recognized as a good bio-indicator of the allergens presence and as a good tool for improving prevention mechanisms in allergic patients (Cecchi, 2013).

In this study, the daily average of Urticaceae pollen concentration (1993-2016) shows a constant presence throughout the year in both cities, although the highest levels are recorded between late winter and early spring. Both studied cities are characterized by showing variability in interannual behaviour. E.g. Córdoba registered average autumn concentration of 23 and 21 pollen/m3 during 1996 and 2000 respectively, while in the other years were ranging 6 to 1 pollen/m3. The same phenomenon was registered in Granada where the average autumn concentration was 11 pollen/m3 during 1997 and 2001, while in the other years were ranging 4 to 1 pollen/m3. These significant average concentrations have contributed to provoke that the mean pollen calendar show two peaks (spring and autumn) while it is not shown for the allergens during the studied years. In the other hand, 2006 has been characterized by an explosive flowering in few days and a peak day of 400 pollen/m^3 in Córdoba and 194 pollen/m^3 in Granada. This variable interannual and intraannual behavior has been observed in previous years in the same cities (Galán et al., 2000; Díaz de la Guardia et al., 1998) and in other regions of Mediterranean area (Belmonte and Roure, 1991; Trigo et al., 1996; Belmonte et al., 1999) due to the humidity is a determinant factor in the Urticaceae pollen concentration. In fact, the correlation between pollen and relative humidity in this study was positive and significant in both cities. On the other hand, the significant negative correlation with daily temperature in both cities could be due to drought stress, because the increased of temperature provokes withering of these plants.

Many studies have indicated that airborne pollen and allergens load have parallel dynamics with significant correlations during the period of maximum pollination (Spieksma et al. 1995, Schäppi et al. 1996, Spieksma and Nikkelss 1999, De Linares et al 2010; Buters et al., 2012). In the case of *Parietaria* allergens, significant correlations were obtained in Córdoba during 2007 and 2008 for all period studied while in 2006 (as occurred in Granada) the significant correlation was obtained during the period with maximum pollen concentration. These results coincide with another study on *Parietaria* allergens in Spain (Jato et al., 2010) with a low but positive correlation between Par j 1-Par j 2 and Urticaceae pollen in Cartagena (Southeaster Spain) and Ourense (Northwester Spain).

Although during March to April high values of pollen and allergens were reached, in May and June the allergen concentrations were higher than airborne pollen. With the botanical information obtained in the Spanish handbooks of plants (Castroviejo et al. 1993; Blanca et al. 2009), it could speculate that the high levels of Urticaceae pollen during March and April in both cities probably are due to overlap the blooming of Urtica and Parietaria plants. After these months, the flowers of Urtica wither while Parietaria continues to flower (especially P. judaica, which continues until the end of autumn) showing the real pollination of Parietaria (more low than Urtica) and high allergen load.

The temporal study realized in Cordoba during the four years showed that the years with maximum allergen concentrations, the pollen concentrations were lower and *vice versa*. 2009 was the year with higher allergens concentrations but lower pollen (Table 1). This year registered the more extreme meteorological conditions, with the highest temperatures, and the lowest precipitation and relative humidity of the period 2006-2009. As Chen et al. (2016) indicated, the pollen allergens could be associated with stress responses and metabolic events during pollen development. Although more studies are needed, perhaps the release of the *Parietaria* allergens is conditioned to stress, registering this significant increase levels in 2009.

4.2. Parietaria airborne allergens in different particles sizes

The allergen load in Andersen cascade Impactor showed differences in the distribution of these particles. Except in Córdoba during 2009, the maximum allergen concentrations were detected in PM 2.5. Several studies have speculated that the pollen grains can release allergens before germination, appearing smaller biological particles with equal or greater allergenicity (Suarez-Cervera et al., 2003; Vega-Maray et al., 2006b; De Linares et al. 2007; Prado et al., 2015). The present study has supported these results and has classified the particles according to size and to EPA categories. The major concentrations of aeroallergens registered have an aerodynamic size that can easily penetrate the lower respiratory zone and provoke asthmatic symptoms almost immediately. It could explain the high percentage of asthmatic symptoms in the patients sensitized to *Parietaria*. I.e. in Italy and Spain more than 50% of patients sensitized suffer asthma with severe bronchial hyper-responsiveness (D'Amato et al 2007 and Alergólogica 2005, respectively).

The analysis protocol carried out in this study has been focused to simulate the mucosal surface of the human tract respiratory, using phosphate-buffered saline (PBS, pH 7.4) as hydration method. Given that the allergens are located in the interior of the pollen grains (Casas et al., 1996; Vega-Maray et al., 2006b), if this pollen has not germinated during the hydration process and released proteins into the wells of the microplate, the primary antibody is incapable of detecting the existence of allergens, and therefore less activity is detected (De Linares et al. 2007). The use of saline buffer show that in natural conditions, the human respiratory tract is exposed to allergens located in different sizes particles. If the allergen concentration in PM10 particles is compared with PM 2.5, this study shows that there is an important allergen load located in particles low than 2.5 µm that can easily penetrate the lower respiratory zone and provoke asthmatic symptoms almost immediately.

Spearman correlation analysis have shown a positive and significant correlation between Urticaceae pollen vs Par j 1- Par j 2 and PM 2.5 in all studied period, except in 2009. This year, Córdoba registered the lowest precipitations and relative humidity of this period (2006-2009) and this situation could affect the allergen release per pollen.

In conclusion, the Urticaceae airborne pollen shows similar pattern of *Parietaria* allergens in the atmosphere. For this reason, the Urticaceae pollen calendar is a good tool for allergy prevention. On the other hand, important Par j 1 and Par j 2 concentrations are located in the breathable fraction, which could explain the asthmatic symptoms in the *Parietaria* allergic population.

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Table 1. Par j 1-Par j 2 allergens, Urticaceae pollen and meteorological parameters in Córdoba and Granada. Tmax (mean annual maximum temperature), Tmean (mean annual temperature), Tmin (mean annual minimum temperature), P (total annual rainfall), RH (mean annual Relative Humidity).

	Granada	Córdoba			
	2006	2006	2007	2008	2009
Par j 1-Par j 2 allergens					
Peak (ng/m^3)	369,6	450,3	856,1	389,8	3494,7
Peak day	9-May	29-Mar	27-Feb	12-Jun	11-Mar
Analyzed days	150	92	120	172	170
Days with allergen presence	150	92	118	170	152
Allergen Integral	21116,6	13459,6	23016,9	13037,8	18884,4
Urticaceae Pollen					
Peak (pollen/m^3)	194	400	53	151	56
Peak day	31-Mar	2-Apr	24-Mar	13-Mar	4-Apr
Analyzed days	365	351	364	358	349
Days with pollen presence	338	134	205	235	256
Pollen Integral during period allergen studied	4218	1764	1509	3037	1145
Annual Pollen Integral	5957	2108	1843	3306	1343
Meteorological data					
Tmax	23,4	24,6	24,3	24,0	26,0
Tmean	15,2	17,7	16,9	17	18,9
Tmin	8,0	11,5	10,4	10,4	11,9
P	267,4	553,7	521	660	436,4
RH	71,0	65,2	62,6	62,3	56,4

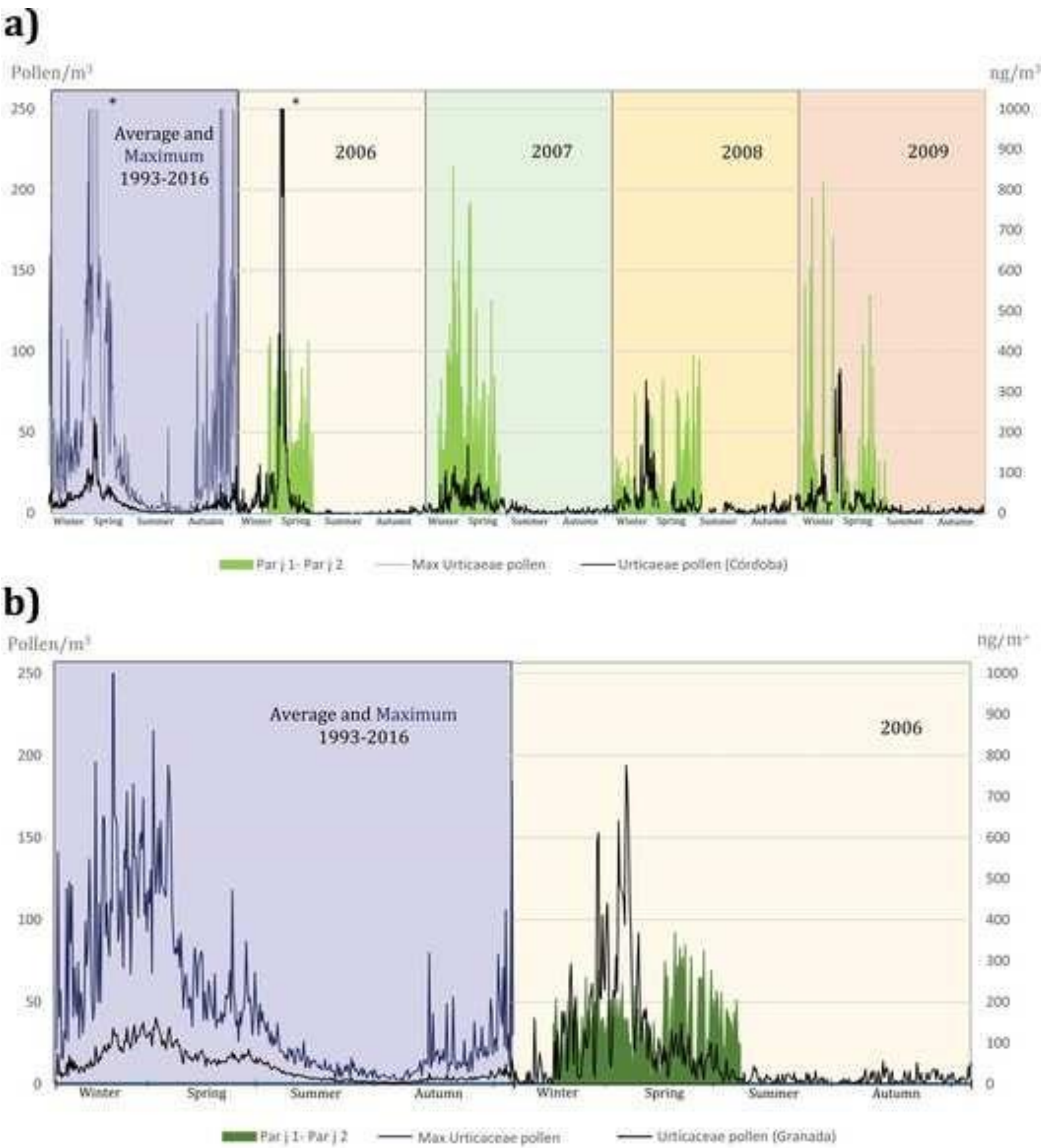
Table 2. Correlation coefficients for Urticaceae pollen, Par j 1-2 allergens, allergens in Pm 10 and PM 2.5, and meteorological factors over the total study period and during the Maximum Pollen Concentration of 2006 ** p<0.01; * p<0.05. Tmax (maximum temperature), Tmean (mean temperature), Tmin (minimum temperature), P (total rainfall), RH (mean Relative Humidity).

			Urticaceae Pollen	Par j 1-Par j 2	Tmax	Tmean	Tmin	P	RH
Córdoba	2009 (n= 170)	Urticaceae Pollen	1.000	0.089	-0.315**	-0.407**	-0.353**	-0.109	0.173*
		Par j 1-Par j 2	0.089	1.000	-0.133	-0.146	-0.173*	0.220	0.110
		PM 10	0.104	0.895**	-0.164**	-0.169**	-0.180*	0.021	0.155
		PM 2.5	0.041	0.844**	-0.129	-0.138	-0.173*	0.063	0.089
	2008 (n= 172)	Urticaceae Pollen	1.000	0.212*	-0.243**	-0.329**	-0.418**	-0.129	0.039
		Par j 1-Par j 2	0.212*	1.000	0.142	0.103*	0.209**	-0.027	0.088
		PM 10	0.137	0.893**	-0.150*	-0.179*	-0.191*	-0.031	-0.133
		PM 2.5	0.225*	0.953**	0.122	0.163*	0.163*	-0.011	-0.053
	2007 (n= 120)	Urticaceae Pollen	1.000	0.225*	0.024	-0.022	-0.113	-0.172	-0.037
		Par j 1-Par j 2	0.225*	1.000	-0.137	-0.212	-0.247**	0.052	0.033
		PM 10	0.134	0.800**	-0.057	-0.118	-0.200**	-0.066	-0.074
		PM 2.5	0.261*	0.870**	-0.192*	-0.264**	-0.264**	0.05	-0.129
	2006 (n= 91)	Urticaceae Pollen	1.000	0.145	-0.518**	-0.576**	-0.510**	0.184	0.431*
		Par j 1-Par j 2	0.415	1.000	-0.015	-0.013	-0.078	-0.046	-0.064
		PM 10	0.060	0.849**	0.104	0.121	0.083	0.041	-0.141
		PM 2.5	0.178	0.917**	-0.098	0.108	-0.173	-0.094	-0.005
	MPC 2006 (n= 27)	Urticaceae Pollen	1.000	0.440*	0.37	0.357	-0.071	-0.464**	0.562**
		Par j 1-Par j 2	0.440*	1.000	-0.236	-0.260	-0.137	0.044	0.195
		PM 10	0.350	0.446**	-0.081	-0.112	-0.209	-0.0255	-0.075
		PM 2.5	0.449*	0.995*	-0.249	-0.268	-0.119	0.068	0.205
Granada	2006 (n= 150)	Urticaceae Pollen	1.000	0.094	-0.250**	-0.369**	-0.409**	-0.017	0.182*
		Par j 1-Par j 2	0.094	1.000	0.166*	0.147	0.130	-0.156	-0.054
		PM 10	0.091	0.877**	-0.195*	-0.180*	-0.170*	-0.121	-0.091
		PM 2.5	0.089	0.972*	0.0133	0.112	0.094	-0.164	-0.035
	MPC 2006 (n= 89)	Urticaceae Pollen	1.000	0.283**	0.515**	0.375**	0.027	-0.388**	0.458**
		Par j 1-Par j 2	0.283**	1.000	-0.067	-0.091	-0.144	-0.087	0.055
		PM 10	0.127	0.495**	-0.196	-0.211**	-0.162	0.086	0.069
		PM 2.5	0.285**	0.985**	-0.058	-0.081	-0.139	-0.111	0.041

Table 3. Par j 1-Par j 2 concentrations in different particle-size fractions (expressed as total sum allergens and percentages) in Córdoba and Granada.

	Granada		Córdoba							
	2006 (1st Feb-30th June)		2006 (1st Feb-30th May)		2007 (1st Feb- 31st May)		2008 (9th Jan- 28th June)		2009 (13th Jan- 1st June)	
Stage (μm)	**ng/m^3**	**%**	**ng/m^3**	**%**	**ng/m^3**	**%**	**ng/m^3**	**%**	**ng/m^3**	**%**
1 (≥5.8)	2011.1	9.5	840.4	6.2	2337.4	10.2	961.5	7.4	1597.5	8.5
2 (<5.8-4.7)	2608.5	12.4	1800.9	13.4	3633.8	15.8	2336.9	17.9	6638.3	35.2
3 (<4.7-3.3)	1310.0	6.2	3380.5	25.1	4028.8	17.5	2494.8	19.1	3223.2	17.1
4 (<3.3-2.1)	3496.4	16.6	2544.1	18.9	3496.0	15.2	2729.4	20.9	2679.9	14.2
5 (<2.1-1.1)	4212.6	19.9	2474.7	18.4	3123.9	13.6	3276.6	25.1	2431.4	12.9
6 (<1.1)	7508.5	35.6	2419.0	18.0	6396.9	27.8	2840.3	21.8	2314.1	12.3
PM 10	5929.5	28.1	6021.9	44.7	10000.0	43.4	5793.2	44.4	11459.0	60.7
PM 2.5	15217.5	72.1	7437.8	55.3	13016.9	56.6	8846.4	67.9	7425.3	39.3
TOTAL	21116.6	100.0	13459.6	100.0	23016.9	100.0	13037.8	100.0	18884.4	100.0

Figure 1


a)
Pollen/m³
ng/m³
Average and Maximum 1993-2016
2006
2007
2008
2009
250
200
150
100
50
0
1000
900
800
700
600
500
400
300
200
100
0
Par j 1- Par j 2
Max Urticaeae pollen
Urticaeae pollen (Córdoba)
b)
Pollen/m³
ng/m³
Average and Maximum 1993-2016
2006
Winter
Spring
Summer
Autumn
Par j 1- Par j 2
Max Urticaceae pollen
Urticaceae pollen (Granada)

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Asunto: ER-19-483R1: Final Decision

Ms. No.: ER-19-483R1
Title: Parietaria major allergens vs pollen in the air we breathe Corresponding Author: Dr. Concepcion De Linares
Authors: Purificación Alcázar, PhD; Ana M. Valle; Consuelo Díaz de la Guardia, PhD; Carmen Galán, PhD

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