

The combination of airborne pollen and allergen quantification to reliably assess the real pollinosis risk in different bioclimatic areas

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Abstract Exposure to allergens represents a key factor among the environmental determinants of asthma. The most common information available for pollinosis patients is the concentration of pollen grains in the bioaerosol and their temporal distribution. However, in recent years, discordance between pollen concentrations and allergic symptoms has been detected. The purpose of this research is to evaluate the relationship between pollen counts and the atmospheric aeroallergen concentrations in different Spanish bioclimatic areas. For the monitoring of allergen content in the air, a quantitative antigen–antibody

technique combined with the Cyclone sampling methodology was used. The study was conducted during 2007 by considering some of the most common allergens that induce pollinosis in each area: *Platanus* and *Urticaceae* in Ourense and Cartagena, and *Poaceae* in Ourense and León. In Ourense, pollen counts and aeroallergen concentrations coincided for the three pollen types studied, and the pollen and allergen data associated with the meteorological factors were highly significant for the pollen counts. In Cartagena (for *Platanus* and *Urticaceae*) and León (for *Poaceae*), the low correlations between pollen counts and allergen concentrations obtained could be due to the specific bioclimatic conditions. In contrast, the higher allergen concentrations found in the atmosphere in Cartagena and León compared to Ourense could be related to the existing pollutant levels there, inducing a higher expression of plant pathogenesis-related proteins in the plants of polluted cities. The combination of pollen counts and allergen quantification must be assessed to reliably estimate exposure of allergic people to allergens in different bioclimatic areas.

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1 Introduction

Nowadays, Type I pollen allergy disorders caused by airborne pollen proteins are on the rise and have a

remarkable clinical impact all over Europe. Exposure to allergens represents a key factor among the environmental determinants of asthma. Respiratory problems have now reached an estimated 20% of the population in industrialized countries (De Weed et al. 2002). The most common information available for pollinosis patients is the amount of pollen grains in the bioaerosol and their temporal distribution (D'Amato 2001). However, in recent years, discordance between pollen concentrations and allergic symptoms has been detected (D'Amato 2007). Different processes that occur in the anther and pollen grains can explain the presence of other allergenic particles in the atmosphere. During germination processes, the pollen hydration facilitates the elution of allergenic proteins in just a few minutes (Casas et al. 1996; Castells et al. 2002; Suárez-Cervera et al. 2003; Suárez-Cervera and Seoane-Camba 2005; Vega-Maray et al. 2004). Allergens can be extruded from pollen grains in wet conditions similar to the *in vitro* and *in vivo* germination processes (Suárez-Cervera et al. 2003; Suárez-Cervera and Seoane-Camba 2005). These allergens could also be released as micro-droplets and deposited on different parts of the plants such as leaves and stems and later dispersed again into the atmosphere (Taylor et al. 2004). Another possibility is that eluted allergens might be transferred from the hydrated pollen and bound to other particulate matter, such as diesel exhaust carbon particles in polluted air (Knox et al. 1997). Meteorological conditions, especially abrupt changes in the weather, might cause the fragmentation of pollen, generating aerosols that contain allergens, which provoke an increase in allergenic particles (Pehkonen and Rantio-Lehtimäki 1994; Taylor et al. 2004). Finally, microparticles from orbicules or Ubish bodies emitted from the anthers during dehiscence are also considered as possible carriers of pollen-derived allergens (Christensen et al. 1972; Seoane-Camba and Suárez-Cervera 1986; El-Ghazaly and Jensen 1986; Suárez-Cervera et al. 1995, 2008a; Rodríguez-Rajo et al. 2010).

Moreover, due to their size, intact pollen grains have difficulty in entering into the deeper airways (Solomon et al. 1983; Suphioglu et al. 1992; Ayuso et al. 1995). Consequently, many cases of the allergic asthma detected in pollen-sensitive patients could be provoked by much smaller particles. Among them, submicronic ($<1 \mu\text{m}$) and paucimicronic ($<10 \mu\text{m}$)

particles containing aeroallergens can penetrate through the nasal passages to the bronchia, quickly provoking allergic symptoms (Pehkonen and Rantio-Lehtimäki 1994; Spieksma et al. 1995; D'Amato et al. 1998; Moreno-Grau et al. 2006; De Linares et al. 2007). Therefore, these particles should be considered in epidemiological studies, and the allergenic proteins from pollen should also be measured.

Allergy and asthma episodes caused by airborne proteins produce a decline in the quality of life of allergy-sufferers and an increase in socioeconomic costs all over Europe. The purpose of this research is to evaluate the relationship between pollen counts and the atmospheric aeroallergen concentrations in different Spanish bioclimatic areas by considering some of the most common allergens that induce pollinosis in each area. Such information could make it possible to establish correlations with clinical symptoms and to prove if differences between geographical areas are found.

2 Materials and methods

2.1 Areas of study

The study was conducted in 2007 in three Spanish cities, which correspond to different biogeographical regions and climates (Fig. 1).

Ourense ($42^{\circ}20'N$ $7^{\circ}52'W$), located in the Northwest, has a temperate oceanic sub-Mediterranean



Fig. 1 Bioclimatic location of the studied sites

bioclimate and it is included in the Mediterranean region, in the transition between both the Mediterranean and Eurosiberian regions. León (42°34'N 5°35'W) is also located in the Northwest of Spain in the Mediterranean region, with a Mediterranean Pluviseasonal-Oceanic bioclimate. Finally, Cartagena (37°36'N 0°59'W) is located in the Southeast of the Iberian Peninsula, belonging to the Mediterranean Region with a Mediterranean Xeric Oceanic bioclimate.

2.2 Pollen and aeroallergen monitoring

Pollen and aeroallergens in the atmosphere were analyzed from the most important sources of pollinosis in each bioclimatic area. Therefore, *Platanus* pollen, Pla a 1 proteins, Urticaceae pollen and Par j 1–2 proteins were studied in Ourense and Cartagena, and Poaceae and Lol p 1 proteins in Ourense and León (as *Lolium* is one of the most representative genus of the Poaceae family in the studied areas).

Pollen sampling was performed using Hirst type (LANZONI VPPS 2000) volumetric traps located on tops of buildings at similar heights (between 10 and 20 meters above ground level). Melinex tape coated with a 2% silicone solution was used as the pollen-trapping surface. The exposed tape was cut into seven pieces, which were mounted on separate glass slides. Pollen grains were counted following the model proposed by the Spanish Aerobiological Network (REA), based on four longitudinal transects along the slides (Galan et al. 2007). Daily mean pollen counts were expressed as pollen/m³ of air.

Three Burkard Cyclone samplers (Burkard Manufacturing Co Ltd.) were used for aeroallergen quantification. This is a continuous volumetric sampler with wind orientation that uses a single reverse-flow miniature cyclone with an air throughput of 16.5 L/min. The instrument offers 100% efficiency sampling down to a 1.06- μ m range, decreasing to 93.82% for the size range 0.82–0.75 μ m. The efficiency of particle capture within the pollen size ranges was evaluated at the Pollen Research Unit (Emberlin 1995). Particles were collected directly into a 1.5 mL Eppendorf vial. The samples correspond to daily periods on weekdays and to aggregates during weekends and holidays. Airborne samples were stored at –20°C.

Pollen and aeroallergen samplers were located at the same sampling station.

2.3 Extraction of allergens from airborne samples

Airborne samples collected in 1.5 mL Eppendorf vials were extracted using a modification of the method (Moreno-Grau et al. 2006) described by Takahashi et al. (2001). Dry samples were extracted with 110 μ L of extraction buffer (50-mmol/L phosphate buffer, pH 7.4, that contained 150-mmol/L sodium chloride, 125-mmol/L ammonium bicarbonate, 3-mmol/L EDTA, and 0.005% Tween 20) for 2 h at room temperature. The extract was separated from the particulate matter by centrifugation at 5,000g for 10 min and stored at –20°C.

2.4 Quantification of allergens by ELISA

Aeroallergen contents in aerosol samples were quantified using specific 2-site antibody ELISAs as previously described (Arilla et al. 2002, 2006). Microtiter plates (Maxisorp, Nunc, Denmark) were coated overnight at 4°C with a specific monoclonal antibody (5D4 at 0.5 μ g). Coated wells were blocked with 200 μ L of phosphate-buffered saline that contained 1% bovine serum albumin and 0.05% Tween 20 (PBSB-T). Afterwards, they were sequentially incubated with 100 μ L per well of purified allergens, or extracted airborne samples, with 625 ng/mL biotin-labeled allergen-specific rabbit antiserum, and 0.25 μ g/mL streptavidin-peroxidase (Sigma) in PBSB-T. All the incubations were performed at 37°C for 1 h, with 3 washes of 200 μ L per well of PBSB-T between successive steps. Finally, wells were incubated at room temperature in the dark with a solution of o-phenylenediamine (Sigma). The reaction was stopped after 30 min by adding 3 M H₂SO₄, and the absorbance was then measured at 492 nm. Crude extracts were assayed as dilution series in triplicate, and the daily concentrations were interpolated from the linear portion of the standard curve expressed as ng/m³ of air. Allergens used as reference material were shown in Table 1.

2.5 Meteorological data

Meteorological data were supplied by the Spanish Meteorological Agency (AEMET) and aggregated in the same periods used to sample and quantify the aeroallergens.

Table 1 Allergens used as reference material

Pollen type	Antibodies		
	Monoclonal (coat)	Purified	Biotined
<i>Platanus</i>	Mouse anti-Pla a 1	Natural Pla a 1	Biotinylated rabbit anti-Pla a 1 polyclonal antibodies
Poaceae	Mouse anti-Lol p 1	Natural Lol p 1	Biotinylated mouse anti-Lol p 1 monoclonal antibody (9A4)
<i>Parietaria</i>	Mouse anti-Par j 1/Par j2	Natural Par j 1/Par j 2	Biotinylated rabbit anti-Par j1-Par j 2 polyclonal antibodies

2.6 Statistical analysis

The Spearman's test was used to test for correlations between pollen and allergen concentrations and the main weather-related factors: rainfall (mm), relative humidity (%), maximum, minimum and mean temperatures (°C), and wind speed (m/s). Finally, data were analyzed using linear regression analysis to investigate the correlation between pollen and aeroallergen levels. SPSS statistical software, version 11.0 (SPSS Inc, Chicago, IL), was used for all statistical analyses.

3 Results

3.1 *Platanus*

The *Platanus* pollen grains and Pla a 1 allergen were detected in the atmospheric aerosol during the early spring, throughout the second half March and the first of April (Fig. 2). Pollen counts indicate an intense bloom lasting for approximately 2–3 weeks, with maximal daily concentrations the March 24th with 460 pollen grains/m³ in Ourense and March 20th in Cartagena with 79 pollen grains/m³ (Table 2). Maximum allergen concentration was delayed 2 days in Ourense and advanced 3 days in Cartagena in relation to the pollen peak date (Fig. 2). The annual total *Platanus* pollen collected in Ourense was 2.399 pollen grains, and the Pla a 1 sum during the sampled period was 0.1201 ng while 701 pollen grains and 0.0218 ng of Pla a 1 were registered in Cartagena.

3.2 Poaceae

Airborne Poaceae pollen grains and Lol p 1 allergens were detected all over the months of June and July at both localities of the Northwest of Spain, Ourense and León. The maximum values were detected during

the first fortnight of July in both cities (Fig. 2). The total allergenic load in Ourense was 4,750 pollen grains and 22.239 ng of Lol p 1 proteins while 1,517 pollen grains and 13.563 ng of Lol p 1 in León (Table 2). Of the three aeroallergens studied, Poaceae is the most important from a quantitative point of view.

3.3 Urticaceae

Finally, Urticaceae pollen grains and Par j 1–2 allergens were detected in Ourense and Cartagena during the most part of the year being June and July in Ourense and the spring months, March, April and May, in Cartagena when it achieves the higher pollen concentration (Fig. 2). Slight differences were observed between the two cities as in spite of the Urticaceae pollen concentration was lower in Cartagena (459 as annual total pollen) than Ourense (1,417 as annual total pollen), lower Par j 1–2 total levels were registered in Ourense than Cartagena (a total of 0.217 and 0.572 ng, respectively). There is some discordance between daily pollen and Par j 1–2 concentrations, especially during July in Ourense and May in Cartagena, when the Urticaceae pollen concentration maintained or even increased simultaneous with the decrease of Par j 1–2 concentration (Fig. 3).

3.4 Linear regression analyses

The curves obtained (Fig. 2) showed that the aeroallergen daily distribution curve follow very closely the variations of the daily mean pollen concentrations in the most of the taxa and period studied. However, there are important differences in some cases, especially in Cartagena, Southeast xeric region of Spain (Fig. 2).

Linear regression analysis showed important relationship between Pla a 1 allergen quantification and

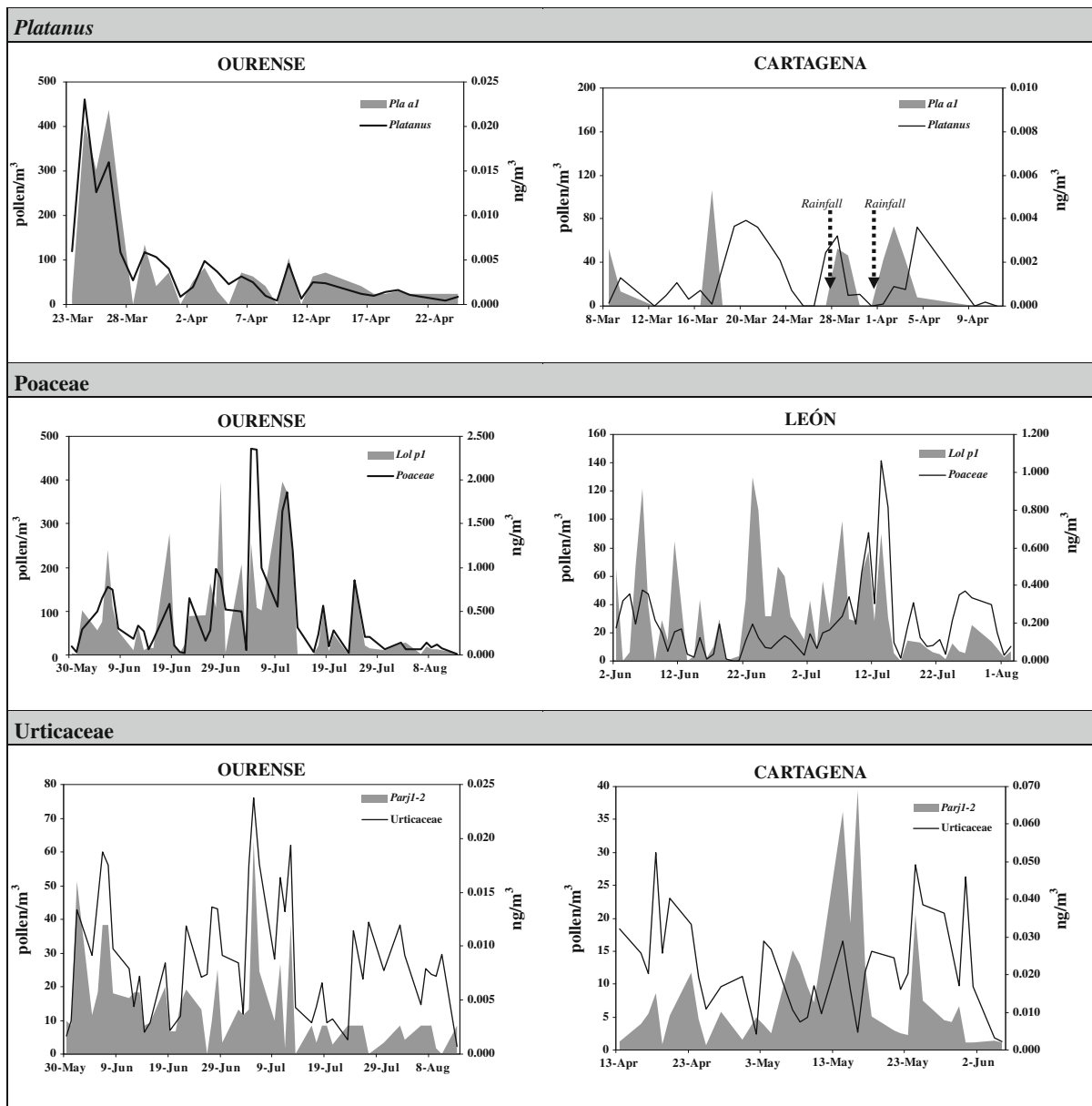


Fig. 2 Allergen values (ng/m^3) versus pollen concentration (pollen/m^3) in Ourense, Cartagena and León during 2007

Platanus pollen counts in the Northwest suboceanic area of Ourense, with high R^2 coefficient value of 0.851 and $p < 0.000$ (Fig. 3). Lower correlation was obtained between Pla a 1 allergens and *Platanus* pollen counts in the Southeast xeric region of Cartagena, with R^2 coefficient value of 0.021 and $p < 0.401$ (Fig. 3).

Lol p 1 allergen quantification and Poaceae pollen counts present a R^2 coefficient value of 0.467 and

$p < 0.000$ in the Northwest suboceanic area of Ourense (Fig. 3). In the Northwest continental area of León, lower relationship was observed between Lol p 1 allergen quantification and Poaceae pollen counts with a R^2 coefficient value of 0.123 and $p < 0.004$ (Fig. 3).

Finally, low correlation was observed between Urticaceae daily pollen and Par j 1–2 concentrations (Fig. 3) mainly during July in Ourense and May in

Table 2 Pollen and allergen concentrations during 2007 in each bioclimatical region

		<i>Platanus</i>		Poaceae		Urticaceae	
		Pla a1 (ng/m ³)	Pollen (pollen/m ³)	Lol p1 (ng/m ³)	Pollen (pollen/m ³)	Par j1–2 (ng/m ³)	Pollen (pollen/m ³)
Ourense	Total	0.1201	2,399	22.2392	4,750	0.2166	1,417
	Average	0.0414	83	0.4361	93	0.0043	28
	Maximum value	0.0218	460	1.9807	471	0.0198	76
	Date Maximum value	26-Mar	24-Mar	28-Jun	4-Jul	5-Jul	5-Jul
Cartagena	Total	0.0218	701			0.5720	459
	Average	0.0008	24			0.0141	13
	Maximum value	0.0530	79			0.0691	30
	Date Maximum value	17-Mar	20-Mar			16-May	18-Apr
León	Total			13.5634	1,517		
	Average			0.2339	26		
	Maximum value			0.9719	142		
	Date Maximum value			23-Jun	13-Jul		

Total, total annual sum; average, total annual average; maximum value, maximum value registered; date maximum value, date of the maximum value

Cartagena (Fig. 2). In general, the Par j 1–2 quantification and Urticaceae pollen counts represent a R^2 coefficient value of 0.434 and $p < 0.000$ in Ourense. However, lowest not significant relationship was registered between pollen counts and allergen concentration in the Mediterranean Xeric area of Cartagena (R^2 coefficient value 0.052 and $p < 0.674$).

Spearman's correlation test was carried out to find a possible degree of association between daily mean airborne pollen and allergens concentrations and the main meteorological factors (Table 3). Allergen correlation was significant in a numerous cases, and the signs for Spearman's coefficient were generally constant. Results showed that the parameters exerting the greatest positive influence, with 99.9% significance, were maximum, and mean temperatures and correlation sign was negative with rainfall and relative humidity parameters. Spearman correlations coefficients were higher in the case of pollen grains and in the Northwest zone of Spain.

4 Discussion

4.1 Pollen counts versus protein concentrations

In Ourense, in the Atlantic Northwest Spanish region, pollen counts and allergen protein atmospheric

concentrations coincided for the three pollen types studied, and therefore R^2 coefficients were very high. Moreover, the pollen and allergen protein data associated with the meteorological factors was highly significant in the case of pollen counts. This indicates that weather factors, such as mild temperatures, constant high humidity, and abundant rainfall (Rodríguez-Rajo et al. 2004) have more influence on pollen dispersion than on allergen release. Nevertheless, in the last years, the period of pollen exposure and the time when symptoms exist often do not coincide (Masullo et al. 1996; D'Amato 2007). In addition, discordance between pollen and spore concentrations and allergic symptoms has been detected in different regions (D'Amato 2001; D'Amato et al. 2008; Marks et al. 2001; Pulimood et al. 2007). Although pollen allergens are primarily transported by pollen grains (D'Amato 2001), they may also be carried in a microaerosol suspension smaller than pollen grains (Knox et al. 1997; Solomon et al. 1983). This is the case in Cartagena (for *Platanus* and *Parietaria*) and León (for Poaceae) where the day-to-day variations between pollen counts and allergen concentrations showed much lower Spearman correlations or R^2 coefficients.

Different mechanisms would have to be considered to understand the discordance between pollen and allergen content and the lack of a relationship between them. In general, allergen peaks are previous

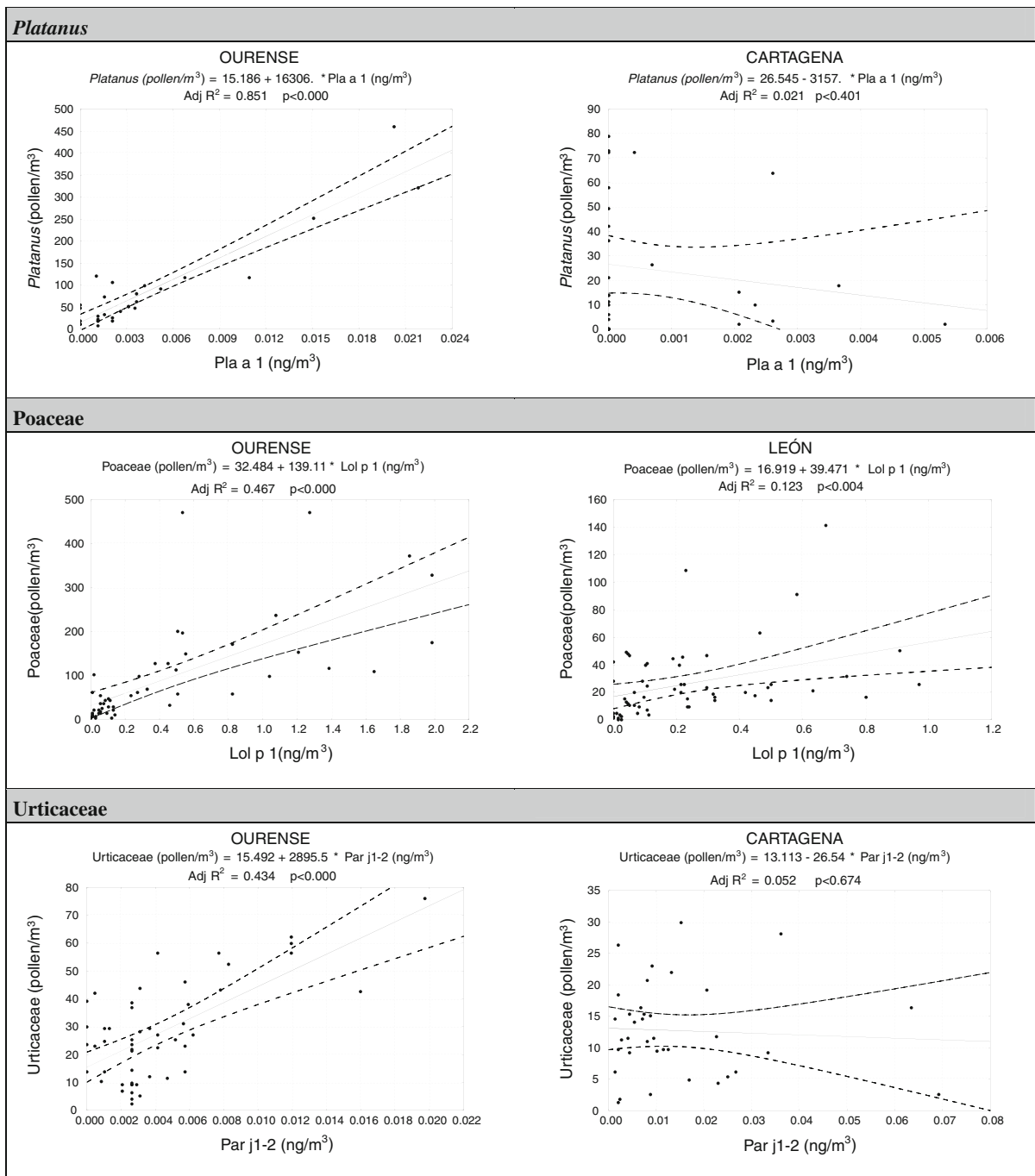


Fig. 3 Regression equations pollen versus allergen protein content

to the presence of pollen in the atmosphere (Cabrera et al. 2002). Some authors argue that rain episodes some days before this, result in an increase in small airborne antigenic fractions because the pollen deposited on various surfaces releases its proteins

when wetted and, after drying, this material may become airborne again (Rantio-Lehtimäki et al. 1994; Moreno-Grau et al. 2006). It is believed that allergens are dispersed faster than pollen grains in the atmosphere due to their small size. Studies conducted by

Table 3 Spearman correlation coefficients between pollen grains and allergen daily mean concentrations and the most important meteorological factors

	Pla a 1		Lol p1		Par j 1-2	
	Ourense	Cartagena	Ourense	León	Ourense	Cartagena
Allergen Spearman correlations						
Maximum temperature	-0.090	0.024	0.324***	0.278**	0.182	-0.155
Mean temperature	-0.134	0.062	0.279**	0.176	0.179	-0.097
Minimum temperature	-0.241	-0.042	0.036	-0.032	0.110	-0.132
Humidity	-0.268	-	-0.480***	-0.184	-0.210	-
Rainfall	-0.374**	0.043	-0.429***	-0.381***	0.056	0.074
Wind speed	-0.014	-	-0.225	-0.170	-0.144	-
	Platanus pollen		Poaceae pollen		Urticaceae pollen	
	Ourense	Cartagena	Ourense	León	Ourense	León
Pollen Spearman correlations						
Maximum temperature	-0.508***	-0.038	0.417***	0.710***	0.481***	0.020
Mean temperature	-0.542***	-0.276	0.369***	0.574***	0.452***	-0.139
Minimum temperature	-0.648***	-0.379**	0.073	0.155	0.157	-0.260
Humidity	-0.029	-	-0.358***	-0.401***	-0.423***	-
Rainfall	-0.292	-0.054	-0.312**	-0.353***	-0.535***	0.335
Wind speed	-0.216	-	-0.255*	-0.166	-0.292**	-

* $p < 0.1$; ** $p < 0.05$; *** $p < 0.01$

Suárez-Cervera and Seoane-Camba (2005) showed a rapid and abundant elution of Pla a 1 protein from *Platanus* pollen grains after hydration during the germination process. In Ourense, in the Atlantic Northwest Spanish region, the uniform and continuous wet conditions may produce the liberation of Pla a 1 allergens at the same time as pollen dispersion in the bioaerosol. On the contrary, in Cartagena, in the Mediterranean Xeric region, the habitual lack of rain combined with the irregular rainfall periods (Moreno-Grau et al. 2000) may explain the discordance observed between *Platanus* pollen counts and Pla a 1 allergen concentrations. The rain cleans pollen grains from the air, provoking the liberation and dispersion of Pla a 1. The two most important peaks of Pla a 1 allergens were obtained in Cartagena during March and April, coinciding with the high rainfall registers before these peaks. On the other hand, an increase in aeroallergen concentrations after rain episodes has been reported (Grote et al. 2003; Moreno-Grau et al. 2006). The present results could confirm the hypothesis that pollen aeroallergens are released during rain episodes and after that, they become resuspended either on the surface of particles

or become airborne independently (Busse et al. 1972; Solomon et al. 1983; Rantio-Lehtimäki et al. 1994).

Another possibility is that allergens could also be dispersed through the orbicules or Ubisch bodies from the anther wall, as well as through non-specific plant debris (Davis 1967; Pacini and Franchi 1993; D'Amato et al. 2002; Vinckier and Smets 2001; Suárez-Cervera et al. 2003, 2008a). The presence of labeled allergenic proteins in the orbicules was reported for Poaceae (El-Ghazaly et al. 1999; Márquez et al. 1997; Suárez-Cervera and Seoane-Camba 2005), Cupressaceae (Suárez-Cervera et al. 2003, 2008a), *Fraxinus* (Rodríguez-Rajo et al. 2010) and *Parietaria*. These orbicules are most likely involved in the dispersion of pollen grains, and they increase the allergen level in the air resulting in a higher risk of asthma. The orbicule dispersion could also be another mechanism of the emission of pollen allergen paucimicronic particles during dehiscence.

Finally, the correlation analysis conducted showed high Spearman correlation coefficients between airborne pollen content and meteorological factors. However, weak or not significant correlations were recorded between meteorological factors and the

allergenic protein load. Therefore, weather factors have a clear influence on the presence of pollen in the atmosphere while the increase in aeroallergen concentrations could be associated with special meteorological conditions, such as thunderstorm episodes (Schäppi et al. 1999; D'Amato 2001). In these cases, the pollen grains suffer a rupture due to the osmotic shock, and they release part of their content, including starch granules carrying allergens into the atmosphere (Schäppi et al. 1999; D'Amato et al. 2007). Epidemic asthma has been associated with various thunderstorm episodes in Italy, United Kingdom and Australia (D'Amato et al. 2007, 2008; Pulimood et al. 2007). Thunderstorm episodes are two to three times more frequent in the Mediterranean Pluviseasonal-Oceanic region of León than in Ourense during the Poaceae pollination period (Aemet 2009), and they could be a cause of the increase in aeroallergen concentrations. The abundant starch granules characteristic of the Poaceae cytoplasm (Taylor et al. 1994; Márquez et al. 1997) could be the source of new allergenic particles. Moreover, in Poaceae, numerous orbicules associated with allergens cover the surface of the anther (Márquez et al. 1997; Suárez-Cervera and Seoane-Camba 2005) and may be dispersed into the atmosphere due to thunderstorm episodes.

4.2 Air pollution factors

Quantitative variations were also recorded among the cities studied. The relationship between Urticaceae pollen and Par j 1–2 concentration was seven times higher in Cartagena than in Ourense while the ratio between Poaceae pollen and Lol p1 concentration was two times higher in León than in Ourense. In spite of the lower *Parietaria* and Poaceae pollen recorded in Cartagena and León, higher allergen protein concentrations were registered. This difference could be explained not only by the different representation of the species in the pollen types identified in each city (Jato et al. 2009) but also by the environmental conditions. The allergenic potential of the same pollen type could vary between years and geographical areas, and meteorological and pollution factors have been considered as the possible cause of these variations (Buters et al. 2008; Schäppi et al. 1999; Suárez-Cervera et al. 2008b). When the emission levels of the most important contaminants in the atmosphere in Ourense, León and Cartagena were evaluated

(Tecnoambiente 2009), it was found that the average pollution levels in Cartagena and León were almost two times higher than those of Ourense (in Cartagena 70.07 $\mu\text{g}/\text{m}^3$ of O_3 , and 7.78 $\mu\text{g}/\text{m}^3$ of SO_2 , in León 59.31 $\mu\text{g}/\text{m}^3$ of O_3 and 11.35 $\mu\text{g}/\text{m}^3$ of SO_2 , and in Ourense 40.98 $\mu\text{g}/\text{m}^3$ of O_3 and 6.68 $\mu\text{g}/\text{m}^3$ of SO_2).

Since plants lack an immune system, they have to protect themselves against external attacks through different strategies. Among these, the pathogenesis-related (PR) proteins form part of the plant defense system and can be found in some pollen grains (Hoffmann-Sommergruber 2000). The major allergen of *P. judaica* Par j 1–2 is a PR-14 protein called lipid transfer protein (LTP), which is located in the pollen cytoplasm (Vega-Maray et al. 2004). This protein could be widely expressed under stressful conditions, participating in the defense mechanism of plants (Zuidmeer and van Ree 2007). Recently, the PR-5 protein Cup a 3 was revealed in the pollen of cypresses from polluted air areas (Cortegano et al. 2004; Suárez-Cervera et al. 2008b). The same findings were found for the Beta-expansin Lol p 1, the major allergen of Poaceae, which is involved in pollen tube growth (Knox et al. 1997; Grobe et al. 1999; Armentia 2003). Moreover, the incorporation of pollutants on the pollen grain has been found by different authors who consider that they could alter the pollen surface as well as the release of allergens (Alaimo et al. 2000; Lombardo et al. 2001), increasing the risk of allergies. *Parietaria* is considered an accumulator of pollutants (Wolterbeek 2002), and a high concentration of trace elements have been detected in pollen and leaf samples (Alaimo et al. 2005). The high allergen concentration found in the atmosphere in Cartagena and León could be related to the existing pollutant levels there, which may induce a higher expression of plant PR proteins in these cities compared to Ourense.

5 Conclusions

The application of this specific and quantitative antigen–antibody technique combined with the Cyclone sampling methodology for the monitoring of allergen content in the air represents an important advance in the epidemiological studies of allergic respiratory diseases.

Climate and contamination levels produce a high variability of the allergenic load in the atmosphere in different bioclimatic areas.

Consequently, our results show that the combination of pollen counts and allergen quantification must be assessed to reliably estimate the exposure of allergic people to allergens.

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References

- Aemet. (2009). *Numero medio mensual de días de tormenta en España (1971–2000)*. <http://www.aemet.es/es/elclima/datosclimatologicos/valoresclimatologicos?l=7031&k=mur>. Accessed January 30, 2010.
- Alaimo, M. G., Lipani, B., Lombardo, M., Orecchio, S., Turano, M., & Melati, M. R. (2000). The mapping of stress by lead dosage in the predominant plants of Palermo, Italy. *Aerobiologia*, *16*, 47–54.
- Alaimo, M. G., Vizzi, D., & Melati, M. R. (2005). Histochemical properties and trace element concentrations in *Parietaria* L. from urban sites (Palermo, Italy). *Aerobiologia*, *21*, 21–31.
- Arilla, M. C., Eraso, E., Ibarrola, I., Algorta, J., Martínez, A., & Asturias, J. A. (2002). Monoclonal antibody based method for measuring olive pollen major allergen Ole e 1. *Annals of Allergy, Asthma & Immunology*, *89*, 83–89.
- Arilla, M. C., González-Rioja, R., Ibarrola, I., Mirw, A., Monteseirín, J., Condez, J., et al. (2006). A sensitive monoclonal antibody-based enzyme-linked immunosorbent assay to quantify *Parietaria judaica* major allergens, Par j 1 and Par j 2. *Clinical & Experimental Allergy*, *36*, 87–93.
- Armentia, A. (2003). Contenido alergénico de las gramíneas y polución. *Alergología e Inmunología Clínica*, *18*, 97–101.
- Ayuso, R., Carreira, J., & Polo, F. (1995). Quantization of the major allergen of several *Parietaria* pollens by an anti-Par j 1 monoclonal antibody-based ELISA: analysis of cross reactivity among purified Par j 1, Par o 1 and Par m 1 allergens. *Clinical & Experimental Allergy*, *25*, 993–999.
- Busse, W. W., Redd, C. E., & Hoehme, J. H. (1972). Where is the allergic reaction in ragweed asthma? II: Demonstration of ragweed antigen in airborne particles smaller than pollen. *Journal of Allergy and Clinical Immunology*, *50*, 289–293.
- Buters, J. T., Kasche, A., Weichenmeier, I., Schober, W., Klaus, S., & Traidl-Hoffmann, C. (2008). Year-to-year variation in release of Bet v 1 allergen from birch pollen: Evidence for geographical differences between west and south Germany. *International Archives of Allergy and Immunology*, *145*, 120–130.
- Cabrera, M., Martínez-Cócera, S., Fernández-Caldas, E., Carnés, J., Boluda, L., Tejada, J., et al. (2002). *Trisetum paniceum* (wid oats) pollen counts and aeroallergens in the ambient of Madrid, Spain. *International Archives of Allergy and Immunology*, *128*, 123–129.
- Casas, C., Márquez, J., Suárez-Cervera, M., & Seoane-Camba, J. A. (1996). Immunocytochemical localization of allergenic proteins in *Parietaria judaica* L. (Urticaceae). *European Journal of Cell Biology*, *70*, 179–188.
- Castells, T., Arcalis, E., Moreno-Grau, S., Bayo, J., Elvira-Rendueles, B., Belchi, J., et al. (2002). Immunocytochemical localization of allergenic proteins from mature to activated *Zygophyllum fabago* L. (Zygophyllaceae) pollen grains. *European Journal of Cell Biology*, *81*, 107–115.
- Christensen, J. E., Horner, H. T., & Lersten, N. R. (1972). Pollen wall and tapetal orbicular wall development in *Sorghum bicolor* (Gramineae). *American Journal of Botany*, *59*, 43–58.
- Cortegano, I., Civantos, E., Aceituno, E., del Moral, A., López, E., Lombardero, M., et al. (2004). Cloning and expression of a major allergen from *Cupressus arizonica* pollen, Cup a 3, a PR-5 protein expressed under polluted environment. *Allergy*, *59*, 485–490.
- D'Amato, G. (2001). Airborne paucimicronic allergen-carrying particles and seasonal respiratory allergy. *Allergy*, *56*, 1109–1111.
- D'Amato, G. (2007). Allergenic pollen and pollen allergy in Europe. *Allergy*, *62*, 976–990.
- D'Amato, G., Cecchi, L., & Liccardi, G. (2008). Thunderstorm-related asthma: Not only grass pollen and spores. *Journal of Allergy & Clinical Immunology*, *121*, 537–538.
- D'Amato, G., Liccardi, G., D'Amato, M., & Cazzola, M. (2002). Outdoor air pollution, climatic changes and allergic bronchial asthma. *European Respiratory Journal*, *20*, 763–776.
- D'Amato, G., Liccardi, G., & Frenguelli, G. (2007). Thunderstorm-asthma and pollen allergy. *Allergy*, *62*, 11–16.
- D'Amato, G., Spieksma, F., Liccardi, G., Jager, S., Russo, M., Kontou-Fili, K., et al. (1998). Pollen related allergy in Europe. *Allergy*, *53*, 567–578.
- Davis, G. L. (1967). The anther tapetum, Ubish granules and hay fever. *Australian Journal of Science*, *30*, 235–236.
- De Linares, C., Nieto-Lugilde, D., Alba, F., Díaz de la Guardia, C., Galán, C., & Trigo, M. M. (2007). Detection of airborne allergen (Ole e 1) in relation to *Olea europaea* pollen in Spain. *Clinical & Experimental Allergy*, *37*, 125–132.
- De Weed, N. A., Bhalla, P. L., & Singh, M. B. (2002). Aeroallergens and pollinosis: molecular characteristics of cloned pollen allergens. *Aerobiologia*, *18*, 87–106.
- El-Ghazaly, G., & Jensen, W. A. (1986). Studies of the development of wheat (*Triticum aestivum*) pollen. *Grana*, *25*, 1–19.
- El-Ghazaly, G., Moate, R., Cresti, M., Wallis, B., Takahashi, Y., Ferreira, F., et al. (1999). Localization and release of allergens from tapetum and pollen grains of *Betula pendula*. *Protoplasma*, *208*, 37–46.
- Emberlin, J. (1995). Analysis of allergens on airborne particles. In *Progress and problems. Europäisches Pollenflug-symposium* (pp. 48–61), ISSN 0933-1160.
- Galan, C., Cariñanos, P., Alcazar, P., & Dominguez, E. (2007). *Spanish aerobiology network: Management and quality manual*. University of Córdoba.

- Grobe, K., Becker, W. M., Schlaak, M., & Petersen, A. (1999). Grass group I allergens (beta-expansins) are novel, papain-related proteinases. *European Journal of Biochemistry*, *263*, 33–40.
- Grote, M., Valenta, R., & Reichelt, R. (2003). Abortive pollen germination: a mechanism of allergen release in birch, alder and hazel revealed by immunogold electron microscopy. *Journal of Allergy and Clinical Immunology*, *111*, 1017–1023.
- Hoffmann-Sommergruber, K. (2000). Plant allergens and pathogenesis-related proteins: What do they have in common? *International Archives of Allergy and Immunology*, *122*, 155–166.
- Jato, V., Rodriguez-Rajo, F. J., Seijo, M. C., & Aira, M. J. (2009). Poaceae pollen in Galicia (NW Spain): Characterisation and recent trends in atmospheric pollen season. *International Journal of Biometeorology*, *53*, 333–344.
- Knox, R. B., Suphioglu, C., Taylor, P., Desai, R., Watson, H. C., Peng, J. L., et al. (1997). Major grass pollen allergen Lol p 1 binds to diesel exhaust particles: implications for asthma and air pollution. *Clinical & Experimental Allergy*, *27*, 246–251.
- Lombardo, M., Melati, M. R., & Orecchio, S. (2001). Assessment of the quality of the air in the city of Palermo through chemical and cell analyses on *Pinus* needles. *Atmospheric Environment International*, *35*, 6435–6445.
- Marks, G. B., Colquhoun, J. R., Girgis, S. T., Koski, M. H., Treloar, A. B., Hansen, P., et al. (2001). Thunderstorm outflows preceding epidemics of asthma during spring and summer. *Thorax*, *56*, 468–471.
- Márquez, J., Seoane-Camba, J. A., & Suárez-Cervera, M. (1997). The role of the intine and cytoplasm in the activation and germination processes of Poaceae pollen grains. *Grana*, *36*, 328–342.
- Masullo, M., Mariotta, S., Torrelli, L., Grazinani, E., Anticoli, S., & Mannino, F. (1996). Respiratory allergy to *Parietaria* in 348 subjects. *Allergology Immunopathology*, *24*, 3–6.
- Moreno-Grau, S., Angosto, J. M., Elvira-Rendueles, B., Bayo, J., Moreno, J., & Moreno-Clavel, J. (2000). Effects of Meteorological parameters and plant distribution on Chenopodiaceae-Amaranthaceae, *Quercus* and *Olea* airborne concentrations in the atmosphere of Cartagena (Spain). *Aerobiologia*, *16*, 17–20.
- Moreno-Grau, S., Elvira-Rendueles, B., Moreno, J., García-Sánchez, A., Vergara, N., Asturias, J. A., et al. (2006). Correlation between *Olea europaea* and *Parietaria judaica* pollen counts and quantification of their major allergens Ole e 1 and Par j 1–Par j 2. *Annals of Allergy, Asthma & Immunology*, *96*, 858–864.
- Pacini, E., & Franchi, G. G. (1993). Role of the tapetum in pollen and spore dispersal. *Plant System Evolution*, *7*, 1–11.
- Pehkonen, E., & Rantio-Lehtimäki, A. (1994). Variations in airborne pollen antigenic particles caused by meteorological factors. *Allergy*, *49*, 472–477.
- Pulimood, T. B., Corden, J. M., Bryden, C., Sharples, L., & Nasser, S. M. (2007). Epidemic asthma and the role of the fungal mold *Alternaria alternata*. *Journal of Allergy & Clinical Immunology*, *120*, 610–617.
- Rantio-Lehtimäki, A., Viander, M., & Koivikko, A. (1994). Airborne birch pollen antigens in different particles sizes. *Clinical & Experimental Allergy*, *24*, 23–28.
- Rodriguez-Rajo, F. J., Iglesias, I., & Jato, V. (2004). Allergenic airborne pollen monitoring of Vigo (NW Spain) in 1995–2001. *Grana*, *43*, 164–173.
- Rodriguez-Rajo, F. J., Vega-Maray, A. M., Asturias, J. A., Jato, V., Seoane-Camba, J. A., & Suarez-Cervera, M. (2010). The relationship between tapetum cells and microspores based on protein localization in *Fraxinus angustifolia* (oleaceae) pollen grains. *International Journal of Plant Sciences*, *171*, 34–52.
- Schäppi, G. F., Taylor, P. E., Staff, I. A., Rolland, J. M., & Suphioglu, C. (1999). Immunologic significance of respirable atmospheric starch granules containing major birch allergen Bet v 1. *Allergy*, *55*, 478–483.
- Seoane-Camba, J. A., & Suárez-Cervera, M. (1986). On the ontogeny of the oncus in the pollen grain of *Parietaria officinalis* ssp. *judaica* (Urticaceae). *Canadian Journal of Botany*, *64*, 3155–3167.
- Solomon, W. R., Burge, H. A., & Muilenberg, M. L. (1983). Allergen carriage by atmospheric aerosol, I: Ragweed pollen determinants in smaller micronic fractions. *Journal of Allergy and Clinical Immunology*, *72*, 443–447.
- Spieksma, F. T. M., Nikkels, A. H., & Dijkman, J. H. (1995). Seasonal appearance of grass pollen allergen in natural, pauci-micronic aerosol of various size fractions; relationship with airborne grass pollen concentration. *Clinical & Experimental Allergy*, *25*, 234–239.
- Suárez-Cervera, M., Castells, T., Vega-Maray, A., Civantos, E., Pozo, V., Fernández-González, D., et al. (2008a). Effects of air pollution on Cup a 3 allergen in *Cupressus arizonica* pollen grains. *Annals of Allergy, Asthma & Immunology*, *101*, 57–66.
- Suárez-Cervera, M., Marquez, J., & Seoane-Camba, J. A. (1995). Pollen grains and Ubisch bodies development in *Platanus acerifolia*. *Review of Paleobotany and Palynology*, *85*, 63–84.
- Suárez-Cervera, M., & Seoane-Camba, J. A. (2005). In A. L. Valero Santiago & A. M. R. A. Cadahía García (Eds.), *Biología celular del polen: Origen y función de los alérgenos polínicos. Polinosis II. Polen y Alergia*. SL and Laboratorios Menarini SA.
- Suárez-Cervera, M., Takahashi, Y., Vega-Maray, A. M., & Seoane-Camba, J. A. (2003). Immunocytochemical localization of Cry j 1, the major allergen of *Cryptomeria japonica* (Taxodiaceae) in *Cupressus arizonica* and *Cupressus sempervirens* (Cupressaceae) pollen grains. *Sexual Plant Reproduction*, *16*, 9–15.
- Suárez-Cervera, M., Vega-Maray, A., Castells, T., Rodríguez-Rajo, F. J., Asturias, J. A., Le Thomas, A., et al. (2008b). An approach to the knowledge of pollen and allergen diversity through lipid transfer protein localization in taxonomically distant pollen grains. *Grana*, *47*, 272–284.
- Suphioglu, C., Singh, M. B., Taylor, P., Bellomo, R., Holmes, P., Puy, R., et al. (1992). Mechanism of grass pollen induced asthma. *Lancet*, *339*, 569–572.
- Takahashi, Y., Ohashi, T., & Nagoya, T. (2001). Possibility of real-time measurement of an airborne *Cryptomeria japonica* pollen allergen based on the principle of surface plasmon resonance. *Aerobiologia*, *17*, 313–318.

- Taylor, P. E., Flagan, R. C., Miguel, A. G., Valenta, R., & Glovsky, M. M. (2004). Birch pollen rupture and the release of aerosols of respirable allergens. *Clinical & Experimental Allergy*, *34*, 1591–1596.
- Taylor, P. E., Staff, I. A., Singh, M. B., & Knox, R. B. (1994). Localization of the two major allergens in rye-grass pollen using specific monoclonal antibodies and quantitative analysis of immunogold labeling. *Histochemical Journal*, *26*, 392–401.
- Tecnoambiente. (2009). *Troposfera, org-Portal de Calidad del Aire y Contaminación Atmosférica, España*. <http://www.troposfera.org/modules.php?name=datos>. Accessed January, 28, 2010.
- Vega-Maray, A., Fernández-González, D., Valencia-Barrera, R., Polo, F., Seoane-Camba, J. A., & Suárez-Cervera, M. (2004). Lipid transfer proteins in *Parietaria judaica* L. Pollen grains: immunocytochemical localization and function. *European Journal of Cell Biology*, *83*, 493–497.
- Vinckier, S., & Smets, E. (2001). The potential role of orbicules as vector of allergens. *Allergy*, *56*, 1129–1136.
- Wolterbeek, B. (2002). Biomonitoring of trace element air pollution: principles, possibilities and perspectives. *Environmental Pollution*, *120*, 11–21.
- Zuidmeer, L., & van Ree, R. (2007). Lipid transfer protein allergy: Primary food allergy or pollen/food syndrome in some cases. *Current Opinion in Allergy Clinical Immunology*, *7*, 269–273.