



Pla a₁ aeroallergen immunodetection related to the airborne *Platanus* pollen content



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HIGHLIGHTS

- *Platanus* pollen a cause of respiratory allergy diseases in the cities.
- This paper informs about the Pla a₁ allergen concentrations in the atmosphere.
- Few researches intend to detect Pla a₁ allergens concentrations in the atmosphere.
- We apply Dot-Blot and Raman spectroscopy to corroborate aerobiological results.
- Allergenic risks could be forecasted some days in advance, with the model proposed.

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ABSTRACT

Platanus hispanica pollen is considered an important source of aeroallergens in many Southern European cities. This tree is frequently used in urban green spaces as ornamental specie. The flowering period is greatly influenced by the meteorological conditions, which directly affect its allergenic load in the atmosphere. The purpose of this study is to develop equations to predict the *Platanus* allergy risk periods as a function of the airborne pollen, the allergen concentration and the main meteorological parameters. The study was conducted by means two volumetric pollen samplers; a Lanzoni VPPS 2000 for the *Platanus* pollen sampling and a Burkard multivial Cyclone Sampler to collect the aeroallergen particles (Pla a₁). In addition the Dot-Blot and the Raman spectroscopy methods were used to corroborate the results.

The Pla a₁ protein is recorded in the atmosphere after the presence of the *Platanus* pollen, which extend the *Platanus* pollen allergy risk periods. The *Platanus* pollen and the Pla a₁ allergens concentration are associated with statistical significant variations of some meteorological variables: in a positive way with the mean and maximum temperature whereas the sign of the correlation coefficient is negative with the relative humidity. The lineal regression equation elaborated in order to forecast the *Platanus* pollen content in the air explain the 64.5% of variance of the pollen presence in the environment, whereas the lineal regression equation elaborated in order to forecast the aeroallergen a 54.1% of the Pla a₁ presence variance. The combination of pollen count and the allergen quantification must be assessed in the epidemiologic study of allergic respiratory diseases to prevent the allergy risk periods.

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1. Introduction

Platanus hispanica Miller ex Münchh, also known as London plane tree or sycamore, is frequently used as ornamental specie in parks, gardens and avenues of Southern European cities. It has been widely demonstrated that bioaerosols such as pollen grains give rise to human health problems as allergies and respiratory infections (Oteros et al., 2013). The special allergenic ability of the *Platanus* pollen has

been recognized by many researchers (Subiza et al., 1994; Varela et al., 1997; D'Amato, 2001; D'Amato 2011). So, the major allergen of *Platanus* pollen Pla a₁ was isolated and characterized (Asturias et al., 2002, 2003). This nonglycosylated 18-kDa protein (which belongs to the Invertase inhibitors proteins family) was recognized by up to 92% of monosensitized *Platanus* allergic patients and 83% of poly sensitized patients (Asturias et al., 2002). Even though, the real role of *Platanus* in respiratory allergies is difficult to assess because these trees bloom during the same period as other allergenic plants causing cross-reactivity episodes. Studies conducted by several authors noted cross-reactivity reactions between *Platanus* pollen and the pollen of Poaceae, Cupressus, Betula, Olea, Parietaria, Plantago, Artemisia and Chenopodiaceae (Varela et al., 1997; Asturias et al., 2002; Fernández-González et al., 2010) as

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well as some plant-derived foods (Miralles et al., 2002; García et al., 2010). Over recent years an increasing effect of aeroallergens on polysensitized patients has been observed that can be caused in part by the recent global warming (Damialis et al., 2007; Stach et al., 2007), which may imply a greater likelihood of the development of allergic respiratory problems in sensible subjects. Moreover, interactions of pollen with air pollution seem to modify the properties of both allergens and pollen, so that patients become more easily sensitized (D'Amato, 2001; D'Amato 2011).

In South and Central Europe the *Platanus* pollen is one of the main causes of pollinosis in urban areas contributing to the development of asthma and rhinoconjunctivitis processes (Fernández-González et al., 2010; Damialis et al., 2011; Alcazar et al., 2011; Nowak et al., 2012; Asero et al., 2012; Ozturk et al., 2013). In Spain the prevalence of positive skin prick test varies from values of the 8–9% sensitized people in the North Spain cities (Iglesias et al., 2007), around the 52–56% in Madrid (Varela et al., 1997) and sensitization rates of the 17% in the South-western cities (Alcázar et al., 2004).

The *Platanus* airborne pollen season takes place in the North Spain between March and April (Iglesias et al., 2007), occurring in the atmosphere during a short and abrupt period of time (Tedeschini et al., 2006; Alcázar et al., 2011). As consequence of the highly unstable climatic conditions during this period of the year, the weather parameters strongly influenced the release and the timing of the pollen. Several authors suggest that the temperature is the most influential parameter, affecting both the pollen season start and the daily pollen count (Sánchez-Reyes et al., 2009; Tedeschini et al., 2006). In urban areas the flowering period of this tree is also affected by cultural practices such as early pruning processes, when pruning is carried out at the start of flowering, the considerable movement of vegetation favors the release of a large number of pollen grains into the atmosphere, resulting in much higher concentrations (Iglesias et al., 2007). Therefore its flowering varies not only from one site to another, but also from one year to another (Iglesias et al., 2007).

The aim of the study was to quantify the *Platanus* pollen and the Pla a₁ allergen present in the air and to develop equations to predict the *Platanus* allergenic load in the atmosphere to prevent the allergy risk periods.

2. Material and methods

2.1. Aerobiological monitoring

The *Platanus* airborne pollen and the Pla a₁ allergen were sampled during the year 2012 by using two volumetric pollen samplers placed on the roof of the Science Faculty building in Ourense, near the town center (42°0'N, 7°5'W). A volumetric sampler (Lanzoni s.r.l. Bologna, Italy) Hirst type (Hirst, 1952) was used for the pollen sampling following the method of the Spanish Aerobiology Network (Galán et al., 2007). Pollen data were expressed as average of daily pollen grains per meter cubic of air when referring to daily mean values or pollen for total values. Pla a₁ aeroallergen was sampled by using a Multi-Vial Cyclone Sampler (Burkard Manufacturing Co Ltd. Hertfordshire, UK). The Pla a₁ proteins were collected directly into a 1.5 mL Eppendorf (dry condition) vial during the *Platanus* flowering period, and daily samples were stored at –20 °C.

Finally, in order to obtain fresh pollen samples, *Platanus* anthers were collected during the flowering season, dried at 27 °C and gently crushed. The pollen released from the anthers was passed through different grades of sieves and after stored at –20 °C (Sousa et al., 2012).

2.2. Dot-blot procedure

Platanus dry pollen extract and the Pla a₁ allergen samples were kept overnight in 1:7 (v/v) cold acetone for 12 h at –20 °C. Then precipitated protein sample was centrifuged at 13,200 rpm for 30 min at

4 °C. After acetone has evaporated, the recovered pellet was resuspended in 20 µL of phosphate buffer (500 mM pH 7.0; supplemented with 150 mM NaCl, 3 mM EDTA and 125 mM ammonium bicarbonate; 0.05% Tween 20 to 0.005%).

A series of protein extract sample drops (1 µL and 2 µL) were directly put onto a nitrocellulose membrane (Protran, Whatman® Schleicher & Schuell, Germany) and allowed to dry. The membrane was saturated during 1 h with 5% non-fat dry milk, 0.1% goat serum in TBS-T (20 mM Tris, 150 mM NaCl with 0.1% Tween 20) and incubated with Pla a₁ (1:2000) prepared in saturating buffer during 3 h at 4 °C on orbital shaking. After washing in TBS-T, dot blot was probed with goat anti-rabbit IgG-HRP (1:4000) in TBS-T during 1 h at 4 °C on orbital shaking. An ECL solution (Luminata Crescendo, Interface, Lda.) was used as a detection system. The chemiluminescent signal was exposed to AGFA medical X-ray film, developed by Fuji medical film processor model FPM 100A.

2.3. Raman spectroscopy procedure

Micro-Raman spectroscopic studies were carried out in samples of the fresh pollen and the Pla a₁ allergen sampled from the aerosol. A Jobin-Yvon Horiba LabRam system interfaced with an Olympus optical microscope was used at the Geology Centre of University of Porto and the spectra were obtained by means the 632.8 nm emission line of a He-Ne laser. An Olympus × 100 objective was used and 10 scans of 10 s each were performed on each analyzed particle. Spectra were obtained in the interval from 200 to 3100 cm^{–1}.

2.4. Enzyme-Linked ImmunoSorbent Assay (ELISA) procedure

The Pla a₁ samples collected into 1.5 mL Eppendorf vials were analyzed following the protocol proposed by Takahashi et al. (2001) with some modifications (Moreno-Grau et al., 2006). Dry samples were extracted with 120 µL of extraction buffer for 2 h. The extract was separated from the particulate matter by centrifugation at 4000 rpm for 10 min and stored in pellet form at –20 °C.

Aeroallergen contents 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 µg). Coated wells were blocked with 200 µL of phosphate-buffered saline (PBS) that contained 1% bovine serum albumin and 0.05% Tween 20 (PBS-B-T). Afterwards, they were sequentially incubated with 100 µL per well of purified allergens, or extracted airborne samples, with 625 ng/mL biotin-labeled allergen-specific rabbit antiserum, and 0.25 µg/mL streptavidin-peroxidase (Sigma) in PBS-B-T. All the incubations were performed at 37 °C for 1 h, with 3 washes of 200 µL per well of PBS-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 measured at 492 nm. Crude extracts were assayed as dilution series, 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 a monoclonal antibody Pla a₁, a natural antibody Pla a₁ and finally the biotinylated antibody Pla a₁ (Bial Industrial Farmacéutica, Spain).

2.5. Meteorological data

Weather data of maximum, minimum and average temperatures (°C), relative humidity (%), rainfall (mm) and wind speed (m/s) were supplied by the Ourense-Ciencias meteorological station of Meteogalicia, located next to the pollen volumetric trap and the allergens Cyclone sampler.

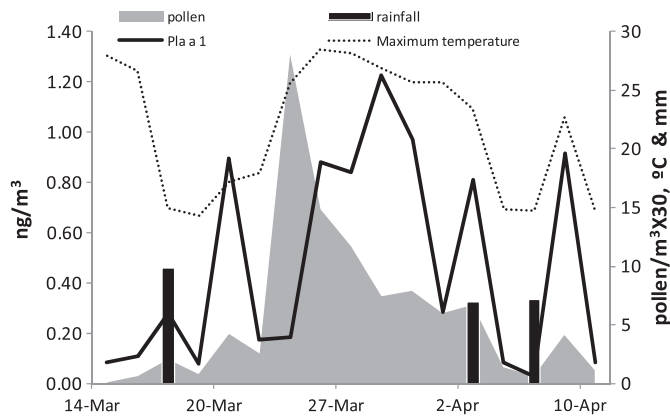


Fig. 1. *Platanus* pollen concentration (pollen/m³)/30 in gray area, Pla a₁ in black line, and principal weather variables, in black line maximum temperature and in black bars rainfall.

2.6. Statistical analysis

A Spearman test was performed in order to obtain the correlations between meteorological factors and daily concentration of airborne pollen and allergens. Considering the results obtained by the correlation analysis, stepwise backward regression equations were drawn up based in daily data in order to construct a model to predict the *Platanus* airborne pollen and aeroallergen presence in the atmosphere of Ourense. Moreover, a *t*-test for dependent samples was carried out in order to check whether significant statistical variations existed between the estimated and the real data. All statistical analyses were conducted by using the SPSS 19.0 statistical package.

3. Results

The presence of *Platanus* pollen and Pla a₁ allergen in the atmosphere was detected from the second half of March to the first decade of April (Fig. 1). This data was confirmed by the Dot-blot test conducted in which the IgG reactivity of both Pla a₁ samples (from the Burkard cyclone allergen and from the pollen crude extract) were compared (Fig. 2). The results of the Raman spectroscopic assay obtained, compared with the database of pollen spectra present in our laboratory, also confirmed the presence of *Platanus* pollen in the Burkard cyclone allergen samples during the *Platanus* flowering season (Fig. 3).

The airborne pollen registered showed a total amount of 7145 pollen grains during the pollen season with duration of 28 days (Fig. 1). The maximum daily pollen content in the air was registered in March 22th with 847 pollen grains/m³, moment in which an increase of the maximum temperature values and a decrease of the relative humidity were detected. Likewise, the results of the ELISA method showed a presence of Pla a₁ aeroallergen during the *Platanus* pollination period. A total allergen amount of 7.9 ng was registered during the pollen season whereas the maximum daily Pla a₁ allergen concentration of 1.2 ng/m³ was observed on March 28th (Fig. 1). In spite of the three days delay registered in the maximum values, there is a high degree of association between daily *Platanus* pollen count and Pla a₁ levels (Fig. 4).



Fig. 2. IgG reactivity of Pla a₁. A, Dot-blot test with *Platanus* pollen in the air and B, Dotted with *Platanus* pollen crude extract.

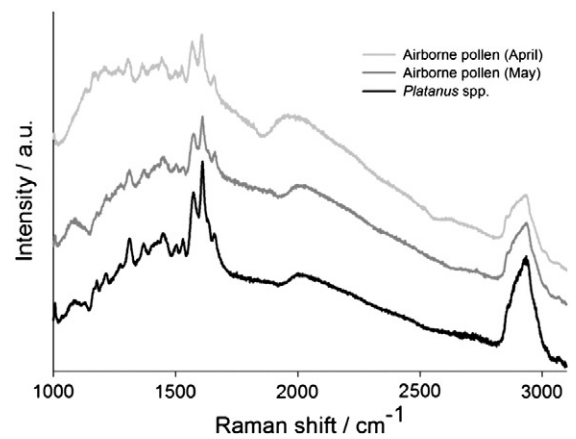


Fig. 3. Raman spectra obtained on the airborne pollen collected on April, on airborne pollen collected on May and, on *Platanus* spp. blank from our pollen database.

In order to determine the effects of the main meteorological parameters in the *Platanus* airborne pollen and Pla a₁ allergen concentrations, a Spearman correlation test was conducted (Table 1). A positive correlation with a 99% of significance ($p < 0.01$) between *Platanus* pollen content in the air and mean temperature was obtained. With a lower degree of association ($p < 0.05$) a positive correlation was obtained with maximum temperature and negative correlation with the relative humidity (Table 1). Similar correlations were obtained between Pla a₁ aeroallergen concentrations and the main meteorological parameters whereas with a $p < 0.05$ value (Table 1).

Taking into account the results obtained by the correlation analysis, regression equations were developed in order to forecast the *Platanus* allergy risk periods. The most accurate model for *Platanus* pollen includes as independent variables the Pla a₁ allergen concentration and the average temperature; the regression equation obtained accounted for the 0.645 of variance of the pollen counts. The most accurate model for Pla a₁ aeroallergen include as an independent variable the *Platanus* pollen content in the air; the regression equation obtained accounted for the 0.541 of the variance of the aeroallergen (Table 2). The accuracy of the equations was confirmed by means the fitted graphical comparison between predicted and observed data (Fig. 5) and the *t*-test no significant differences (Table 3).

4. Discussion

The plane trees have marked allergological interest in different areas of Central and Southern Europe (D'Amato et al., 2007). The flowering

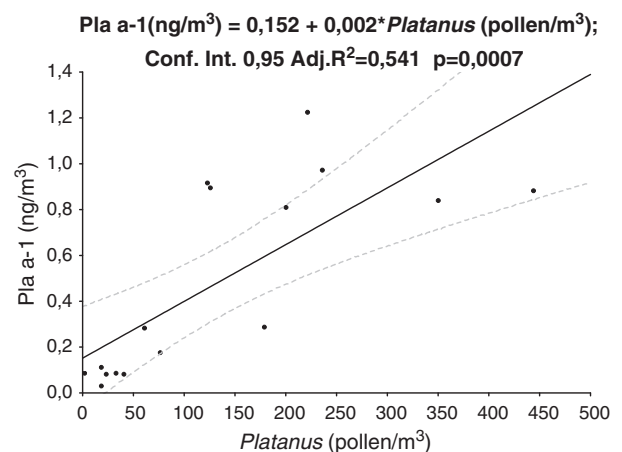


Fig. 4. *Platanus* pollen concentration (pollen/m³) versus Pla a₁ allergen values (ng/m³) during 2012 year in Ourense.

Table 1

Spearman correlation coefficients between *Platanus* pollen and Pla a_1 allergen and the main meteorological parameters during the main pollination period ($p < 0.01^{**}$, $p < 0.05^{*}$).

	<i>Platanus</i> pollen		Pla a_1 allergen	
	Spearman R	p-level	Spearman R	p-level
Maximum temperature	0.503*	0.039	0.568*	0.017
Minimum temperature	0.209	0.420	−0.145	0.579
Mean temperature	0.620**	0.008	0.522*	0.031
Relative humidity	−0.580*	0.015	−0.587*	0.013
Rainfall	−0.272	0.291	−0.324	0.205
Wind speed	−0.236	0.362	−0.227	0.381

occurs during March and early April characterized by a short and intense blooming period. As several authors noted (D'Amato, 2001; Marks et al., 2001; Pulimood et al., 2007; D'Amato et al., 2008; Rodríguez-Rajo et al., 2011) some discrepancies between the timing of pollen and aeroallergen appearance in the atmosphere were observed. The pollen allergens are primarily transported by pollen grains but can be also released to the microaerosol suspension smaller than the pollen grains (Knox et al., 1997; Solomon et al., 1983). Therefore, due to its smaller size, the presence of allergen peaks in the atmosphere could be previous than the pollen appearance in the air (Cabrera et al., 2002). Furthermore some studies showed that rainy episodes before the pollen peak concentration in the atmosphere produce an increase in the small aeroallergen fractions (Rantio-Lehtimäki et al., 1994; Moreno-Grau et al., 2006). After mechanical deposition of the pollen grains on various surfaces by the droplet action, the wet conditions allow the release of the proteins from the dry pollen which due to its small size could again become as aeroallergens. Under storms or rainfall conditions some cytoplasmic material, mainly starch granules and proteins, is abruptly discharged outside the pollen whereas the pollen grains were cleaned from the atmosphere. This could explain the appearance of protein peaks where rainfall occurs in the previous afternoon, due to short but heavy storms (Fernández-González et al., 2011). In the present study, the rain registered before the *Platanus* pollen peaks may explain the discordance between the *Platanus* pollen and the allergen Pla a_1 presence. The first allergen peak reached during 21st March (as well as the two last allergen peaks) took place after rainfall episodes occurred two days earlier. These weather conditions can be responsible by the increase of allergen in the air. Similar findings have been reported by Rodríguez-Rajo et al. (2011) in the Cartagena city, and by Fernández-González et al. (2010) in the Leon city. Buters et al. (2010) suggested that when the weather conditions are not favorable to pollen dispersion (i.e. presence of rain in the pollination period), the airborne pollen concentration decreases and the allergenic potency may be higher. Finally, the main allergen peak is also consequence of the intense release of pollen in the atmosphere. Recent studies pointed that

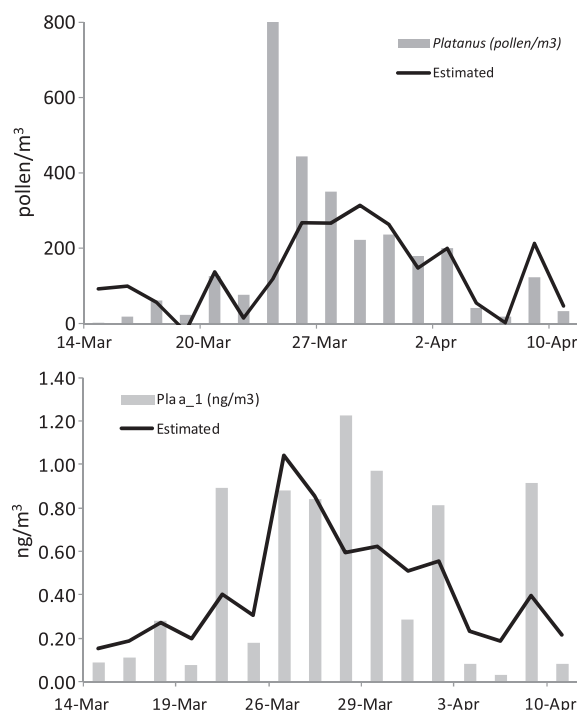


Fig. 5. *Platanus* pollen counted in gray bars and predicted values in black line.

the orbicules or Ubisch bodies, produced by tapetal cells and deposited in parallel sequences on the pollen wall layers (Suárez-Cervera et al., 1995; Vinckier et al., 2005) are likely involved in the pollen dispersion, increasing the aeroallergen level during flowering which is responsible for a higher risk of asthma (Rodríguez-Rajo et al., 2011).

Moreover, studies conducted by several authors revealed cross-reactivity reactions between the allergens of the *Platanus* pollen and the pollen allergens of different taxa such as Poaceae, Cupressus, Betula, Olea, Parietaria, Plantago, Artemisia and Chenopodiaceae (Varela et al., 1997; Asturias et al., 2002; Fernández-González et al., 2010) because the release of their pollen occurs during the same period. Therefore it is important to know if the allergen quantification corresponds with the real presence of Pla a_1 allergen in the atmosphere in order to avoid false positive cases. In this way, studies conducted by Rodríguez-Rajo et al. (2012) showed that the *Fraxinus* allergen Fra e_1 concentrations are clearly detected using Ole e_1 antibody (due to its high degree of homology with the ash major allergen Fra e_1) during the months of January and February, a period of the year without presence of olive pollen in the atmosphere. In the

Table 2

Parameters to forecast *Platanus* pollen (pollen/m³) using the concentration of Pla a_1 allergen (ng/m³) and mean temperature (Mean temp. in °C), and parameters to forecast Pla a_1 allergen (ng/m³) using *Platanus* pollen (pollen/m³). (The Beta is the standardized regression coefficient, S.E. Beta is its standard error, B is the unstandardized regression coefficient and S.E. B is its standard error).

	R	R ²	Adjusted R ²	F	D.F.	p-level	S.E.
	0.832	0.692	0.645	14.604	2.13	<0.000	77.218
<i>Platanus</i> pollen (pollen/m ³) = −157.192 + (171.127 * Pla a_1) + (15.971 * mean temp.)							
	Beta	S.E. Beta	B	S.E. B	t(13)	p-level	
Intercepted			−157.912	85.541	−1.846	0.088	
Pla a_1	0.561	0.177	171.127	53.976	3.170	0.007	
Mean temp.	0.398	0.177	15.971	7.095	2.251	0.042	
	0.756	0.571	0.541	18.707	1.14	<0.000	0.287
Pla a_1 (ng/m ³) = 0.152 + (0.002 * −Platanus pollen)							
	Beta	S.E. Beta	B	S.E. B	t(14)	p-level	
Intercepted			0.152	0.105	1.441	0.171	
<i>Platanus</i> pollen	0.756	0.175	0.002	0.001	4.325	0.001	

Table 3

t-test for the *Platanus* pollen counts and Pla a_1 allergen and Estimated values. Marked differences significant at $p < 0.05$, Diff: significant differences, s.d. Diff.: Standard deviations differences.

	Mean	s.d.	N	Diff.	s.d. Diff.	t	df	p-level
<i>Platanus</i> sp.	134.500	129.529						
Estimated	134.445	107.652	16	0.055	71.791	0.003	15	0.998
Pla a_1	0.485	0.424						
Estimated	0.421	0.258	16	0.064	0.0284	0.905	15	0.380

present study, the presence of *Platanus* pollen was confirmed by using Dot-blot test and Raman spectroscopy. The first test verified the presence of *Platanus* airborne pollen in the samples and in the crude extract. The Raman spectroscopy technique was used by the first time to identify airborne pollen by the spectrum obtained. Some authors observed different pollen-types spectra (Laucks et al., 2000; Ivleva et al., 2005; Schult et al., 2008). The conventional methods for pollen grains identification are based on the morphological analysis but with the Raman spectroscopy method we can identify pollen by spectra recognition having the advantage to be a rapid technique. In this way we used the Raman spectroscopy method to prove the presence of *Platanus* pollen in the study area during their flowering period (March to April).

The meteorological conditions determined the different phenological events in many plants, and in addition meteorological variables before flowering could control the level of pollen dispersion and the timing of pollen season. The correlation analysis showed high Spearman correlation coefficients between airborne pollen content or the concentration of allergen Pla a_1 and the meteorological parameters. The meteorological variables with a high degree of association were mean and maximum temperature and relative humidity. Similar findings have been mentioned in studies carried out by Iglesias et al. (2007) and Rodríguez-Rajo et al. (2011). Moreover, the presence of high *Platanus* pollen content in the air can be also related with the microenvironmental conditions and cultural practices. There are differences in areas with diverse degree of urbanization, in terms of quantity of pollen counted and therefore affecting the intensity of allergy. Previous studies conducted by Rodríguez-Rajo et al. (2011) showed more concentration of *Platanus* airborne pollen in Ourense than Cartagena and therefore a higher concentration of Pla a_1 allergens in Ourense. Other important factors for pollen dispersion were the weather conditions and the location of the study area. Several authors suggest that the duration of the pollen season and the intensity of the allergy season can also be modified by the climate change which it is different in each bioclimatic area (Tedeschini et al., 2006; Cecchi et al., 2010; Alcázar et al., 2011).

Finally, regression equations were developed in order to forecast the *Platanus* allergy risk periods. The Adjusted R^2 values ranged around 0.55–0.65. Similar findings were reported by Iglesias et al. (2007) for *Platanus* pollen in the same area with a R^2 coefficient value of 0.29–0.48. The graphical representation of the values predicted by the equations fitted with the observed data. Furthermore, the t-test for dependent samples shows that there is no significant difference at the 95% level between estimated and observed data.

5. Conclusions

The presence of *Platanus* allergens in the atmosphere during the March and April months was confirmed by using Dot-blot test and the Raman spectroscopy. A stronger influence of the meteorological parameters (mainly temperature) on the *Platanus* airborne pollen content and Pla a_1 aeroallergen was found. The equations developed to predict the *Platanus* pollen and allergen content in the atmosphere allow us to forecast the allergenic risks periods some days in advance in order to prevent sensitized people from pollinosis symptoms. The

combination of pollen count and the allergen quantification must be assessed in the epidemiologic study of allergic respiratory diseases.

Acknowledgments

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