



Assessment between *Platanus* pollen and Pla a 1 allergen in two cities of North-western Iberian Peninsula

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Abstract *Platanus* pollen is considered as a major cause of pollinosis in many southern European cities. This tree is commonly used in parks, avenues and gardens as ornamental species. The flowering period is short and abrupt between March and April, since this tree is characterized by having fast and explosive blooming process. This fact induces an important public health problem when *Platanus* bloom. The objective of the study is to evaluate the pollen and allergen concentration in two cities in the north-west of the Iberian Peninsula, Porto (Portugal) and Ourense (Spain), and analyse how weather parameters and environmental pollutants can affect the patterns of pollen and allergen airborne distribution. The pollen sampling was conducted, during 2018, using a Hirst volumetric sampler, and Pla a 1 aeroallergen was collected using a Burkard multivial cyclone sampler. The pollen counting was obtained following the Spanish Aerobiology Network methodology. Aeroallergens were quantified by ELISA method using a Pla a 1-specific antibody. *Platanus* pollen

concentrations are seven times lower in the Porto atmosphere than in Ourense, but the Pla a 1 content is only 2 times higher in Ourense city. These differences can be explained by the influence of air pollutants in plants and meteorological factors. *Platanus* airborne pollen and Pla a 1 aeroallergen were correlated with some meteorological parameters, namely temperature, rainfall and relative humidity, and with atmospheric pollutants such as NO₂ and O₃. The results suggest the existence of different allergenic activities in the atmosphere of both cities.

Keywords Plane tree · Elisa · Aeroallergen · Pollutants · Weather variables

1 Introduction

Over recent decades, airborne pollen is considered as the main cause of asthma, rhinitis and related allergic diseases (D'Amato et al. 2007) with a tendency to an incidence increase (Bousquet et al. 2011). In Europe, allergic disorders are an important public health problem since 20% of the total people suffers from pollen-induced allergies (World Allergy Organization 2013). In addition, in industrialized countries, between 5 and 30% of the population is affected by pollen-related allergic diseases (Cecchi 2012).

Currently, authorities are keeping in mind that variations in urban planting can have an important role

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in determining which pollen types are most abundant in the air of the cities, and therefore the potential allergenic airborne pollen risks in the urban environments (Li et al. 2018). In the last years, the pollen spectrum in the cities has changed due to the use of wind species that produce large amounts of pollen in green spaces (Alcázar et al. 2004; Charpin et al. 2005). Among the common trees planted in urbanized area are the plane trees. These trees have an important allergological interest in central and south European cities as a consequence of their abundance and the release of pollen that can induce allergenic reaction in the sensitized people (D'Amato et al. 2007). *Platanus* phenology is characterized by having fast and explosive blooming process, thus, the pollen season supposes a short and abrupt period of time (Alcázar et al. 2015). The flowering period usually lasts from March to April, whereas fruit set occurs in late summer and autumn.

Meteorology plays a determinant role in the pollen production by the plant and the timing of the pollen season. Temperature is usually the weather variable with most influence on the timing of the main pollen season start (Majeed et al. 2018). Relative humidity can make difficult pollen emission and transport (García-Mozo et al. 2007); however, high atmospheric humidity may contribute to the release of allergenic proteins from pollen grains (Suárez-Cervera and Seoane-Camba 2005; Suárez-Cervera et al. 2008a).

Another parameter that can contribute to the increase in allergenic diseases and asthma in urban areas is the environmental pollution (Azizi 2011; North et al. 2014). Air pollutants such as particulate matter, NO₂ or O₃ can exacerbate the allergenic reactions by their interaction with pollen grains or the plants (Ghiani et al. 2012). The relationship between airborne pollen and the atmospheric concentration of fine/ultrafine particulates should be taken into account for human health studies (Feng et al. 2011; Lu et al. 2011).

Finally, topography, the slopes of the cities or differences in the height of the buildings and in the width of the streets can create microclimate conditions producing accumulation of airborne particles as well as induce an advance/delay on the pollen season, thus affecting the airborne pollen and allergen patterns (Maya-Manzano et al. 2017; Mimet et al. 2009).

All the aforementioned parameters can cause modifications in plant phenology, which at the same

time induce changes in the time of pollen allergens dispersion. The main allergenic protein of the plane tree is Pla a 1, a non-glycosylated protein with a molecular weight of 18 kDa (Asturias et al. 2002, 2003; Fernandez-Gonzalez et al. 2010). This protein represents around 60% of the total IgE binding to *Platanus* pollen extract and can be used for specific diagnosis of *Platanus* pollen allergy (Asturias et al. 2003; Pazouki et al. 2009).

The objective of the present study was to quantify airborne *Platanus* pollen and Pla a 1 content in Porto (Portugal) and Ourense (Spain) cities, both located in the north-western of the Iberian Peninsula and to analyse how weather parameters and environmental pollutants can affect the patterns of pollen and allergen distribution. This study allowed to quantify, for first time, the Pla a 1 allergen content in the air of the Porto city.

2 Materials and methods

The study was carried out during 2018 in two cities located in the north-western of the Iberian Peninsula: Porto (41°9'7.20"N, 8°38'13.20"W) and Ourense (42°20'41.05"N, 7°51'20.46"W). Porto is the second largest Portuguese city, bordered by the Atlantic Ocean and the Douro River; the annual mean temperature is around 15 °C, the humidity ranges between 75 and 80%, and the total annual rainfall varies between 1000 and 1200 mm (Miranda et al. 2001). Ourense is located in north-western Spain at the bioclimatic Mediterranean region; this area has an annual average temperature of 14 °C and 772 mm of annual total precipitation (Martínez-Cortizas and Pérez-Alberti 1999).

The data in both locations were obtained following exactly the same protocols and methods. Aerobiological monitoring of *Platanus* pollen and Pla a 1 allergen were conducted by means of two volumetric traps placed on the roof of the Science Faculty buildings in each study area. A Hirst-type sampler (Hirst 1952) was used for the pollen sampling following the method of the Spanish Aerobiology Network (Galán et al. 2007). The same sampler model, Lanzoni VPPS 2000, was used in both locations in order to avoid errors in the flow calibration (Oteros et al. 2017).

Pollen data were expressed as pollen grains for total pollen values or pollen per meter cubic of air when

referring to daily mean values. Pla a 1 aeroallergen was sampled by using a multivial cyclone sampler (Burkard Manufacturing Co Ltd. Hertfordshire, UK). The 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 and quantified by means an enzyme-linked immunosorbent assay (ELISA) following the protocol proposed by Takahashi et al. (2001) and Moreno-Grau et al. (2006). The samples were extracted with 120 μL buffer for 2 h, and then, the particulate matter was separated by centrifugation at 4000 rpm during 10 min and stored at -20°C . Pla a 1 proteins were quantified using specific 2-site antibody ELISA as previously described (Arilla et al. 2002, 2006). The microtiter plates were coated over night at 4°C with a Pla a 1 monoclonal antibody. Wells were blocked with PBS-BSA-T that contained 1% bovine serum albumin and 0.05% Tween 20. After the plates were incubated with natural antibody allergens or airborne samples were extracted, then, we add a secondary Pla a 1 biotinylated antibody and 0.25 $\mu\text{g}/\text{mL}$ streptavidin-peroxidase (Sigma). All incubations were performed at 37°C for 1 h, and we make 3 washes with buffer (PBS-T) between successive steps. Finally, the plates were incubated at room temperature in the dark with o-phenylenediamine (OPD) (Sigma), the reaction at 30 min with H_2SO_4 (3 M) was stopped, and the absorbance was measured at 492 nm. The extracts were assayed as dilution series, and the daily concentrations were interpolated from the linear portion of the standard curve in ng/m^3 of air.

The period covered by the main pollen season (MPS) for each study year and sampling station was determined by means of criteria proposed by Andersen (1991). This method considered the first day of the MPS when the accumulated sum of pollen reached the 2.5% of the annual pollen integral and the final date when the 97.5% of the pollen grains were registered.

Meteorological data (maximum, minimum, average temperatures, rainfall and relative humidity) and pollutants data (NO_2 , O_3 , PM_{10} and $\text{PM}_{2.5}$) were considered in the study, and their average values can be seen in Table 1. In Porto, the meteorological data were continuously monitored by the meteorological station of the Faculty of Sciences of the University of Porto located 7 m apart from the pollen and allergen samplers at the same altitude. The pollutants concentrations were provided from the “Sobreiras”

monitoring station (placed 2 km apart from the sampler) which is integrated in the Air Quality Monitoring Network of Porto-MA, managed by the Regional Commission of Coordination and Development of Northern Portugal, under the responsibility of the Ministry of Environment (<http://qualar.apambiente.pt/>). In Ourense, the data were provided by the “Ourense-Ciencias” meteorological station of Meteogalicia. The meteorological station was placed at 400 m from the sampler, and the pollutant station was located at 2 km from the pollen and aeroallergen traps.

Statistical analysis was performed by means of a nonparametric Spearman’s correlation test in order to analyse the relationship between daily pollen and allergens concentrations and meteorological and pollutant parameters. Principal component analysis (PCA), a multidimensional method, was performed with the objective to detect any relationships between the variables as well as to categorize the independent variables according to their influence on the variation of the dependent one-pollen (Tipping and Bishop 1999). The package STATISTICA 5.1 was used for the statistical analysis.

3 Results

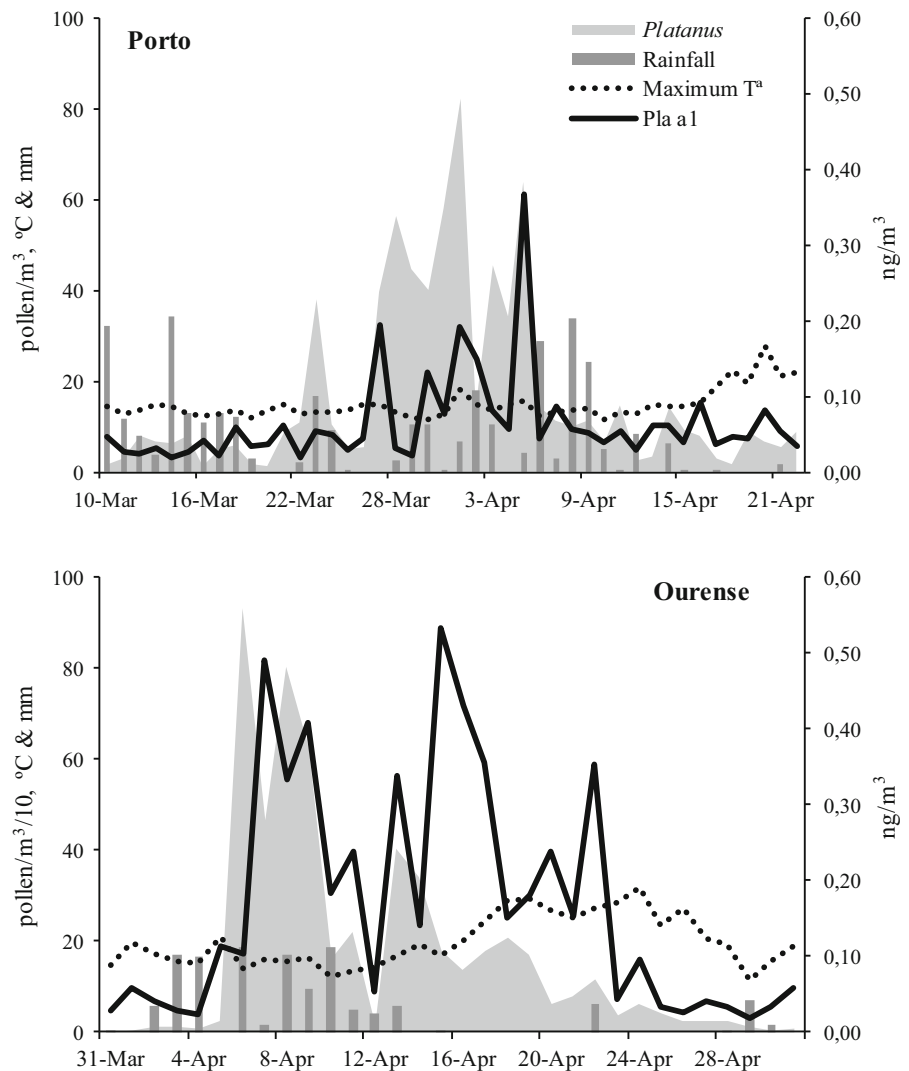
The presence of *Platanus* pollen and allergen (Pla a 1) in the Porto atmosphere was detected from the second 10 days of March to the second 10 days of April, whereas in Ourense from the last day of March to the first day of May (Fig. 1). The airborne annual pollen integral registered showed a total amount of 750 pollen grains/ m^3 in Porto during the pollen season with a length of 44 days (the main pollen season lasted from March 10 to April 22). The maximum daily pollen content in the air was reached in April 1 with 82 pollen grains/ m^3 ; the peak coincides with an increase in maximum temperature and a decrease in relative humidity (Table 1, Fig. 1). Likewise, the results of ELISA test showed the presence of Pla a 1 aeroallergen during the *Platanus* pollination period. The annual allergen integral amount was 2.9 ng/m^3 , and the Pla a 1 daily peak was reached in April 5 with a 0.37 ng/m^3 (Table 1). In Ourense, the *Platanus* pollen season length was 32 days (the main pollen season started on March 31 and finished on May 1), with an annual pollen integral of 5383 pollen grains (Table 1). The

Table 1 Average pollen (pollen/m³), pollen peak (pollen/m³), pollen peak date, *Platanus* pollen integral (pollen), start and end of main pollen season (MPS) and length of main pollen season (MPS) (days)

	<i>Platanus</i>	Pla a l	Max temp.	Min temp.	Avg temp.	Rain	RH	Wind speed	NO ₂	O ₃	PM ₁₀	PM _{2.5}
Porto												
Mean	17	0.06	14.88	9.87	12.43	7.93	80.61	2.79	45.74	67.74	22.77	5.9
Peak	82	0.37	27.72	17.02	21.26	34.43	92.54	6.79	107.5	95.75	57	12
Peak date	1-Apr	5-Apr	20-Apr	20-Apr	20-Apr	14-Mar	2-Apr	10-Mar	12-Apr	29-Mar	21-Apr	22-Mar and 17-Apr
TOT.	750	2.9				348.8						
Start MPS	10-Mar											
End MPS	22-Apr											
Length MPS	44											
Ourense												
Mean	168	0.17	19.89	6.8	12.71	4.23	71.09	1.35	24.95	43.01	14.62	9.51
Peak	932	0.53	31.9	12.3	19.2	18.8	88	2.1	55.07	65.13	33.3	17.81
Peak date	6-Apr	15-Apr	24-Apr	23-Apr	24-Apr	6-Apr	11-Apr	3-Apr	6-Apr	28-Apr	21-Apr	21-Apr
TOT.	5383	5.4				135.2						
Start MPS	31-Mar											
End MPS	1-May											
Length MPS	32											

Average allergen (ng/m³), allergen peak (ng/m³), allergen peak date and allergen integral (ng). Mean, peak and peak date of maximum, minimum and average temperature (°C), rainfall (mm), relative humidity (%), wind speed (m/s), NO₂ (µg/m³), O₃ (µg/m³), PM10 (µg/m³) and PM2.5 (µg/m³)

Fig. 1 Pollen grains (grey area), allergen concentration (black line) maximum temperature (black points) and rainfall (grey bars) during the study period in Porto and Ourense areas



maximum daily pollen content was 932 pollen grains/m³ on April 6 (Table 1, Fig. 1) influenced by the hourly rainfall pattern detected (Fig. 2). The annual Pla a 1 integral amount in Ourense was 5.4 ng, and the maximum daily peak was observed on April 15 with 0.53 ng/m³ (Table 1).

In order to individually determine the effects of the main meteorological parameters, the pollutant gases and the particulate matter in the *Platanus* pollen and Pla a 1 allergen airborne content, a spearman correlation test was conducted (Table 2). In Porto, the test showed a positive correlation with 99% of significance ($p < 0.01$) between *Platanus* pollen and Pla a 1 allergen, whereas with a lower degree of association ($p < 0.1$) with the Pla a 1 concentration 1 day before,

NO₂ 3 days before and O₃ values during the same day. The Pla a 1 allergen showed a high level of correlation ($p < 0.01$) with the pollen concentrations of 2 and 3 days before as well as with the allergen concentration of 3 days. With a lower degree of significance, positive association with temperatures in the same day ($p < 0.05$) and with the NO₂ values of 2 days before ($p < 0.1$), whereas a negative correlation with PM_{2.5} content in the previous day ($p < 0.05$) was observed (Table 2). In Ourense, we found a positive correlation between *Platanus* pollen and Pla a 1 in the same day and 1 and 2 days before, rainfall 3 days before, relative humidity 2 and 3 days before ($p < 0.01$) and NO₂ values 2 days before ($p < 0.05$). With 99% of significance level but negative, a correlation with

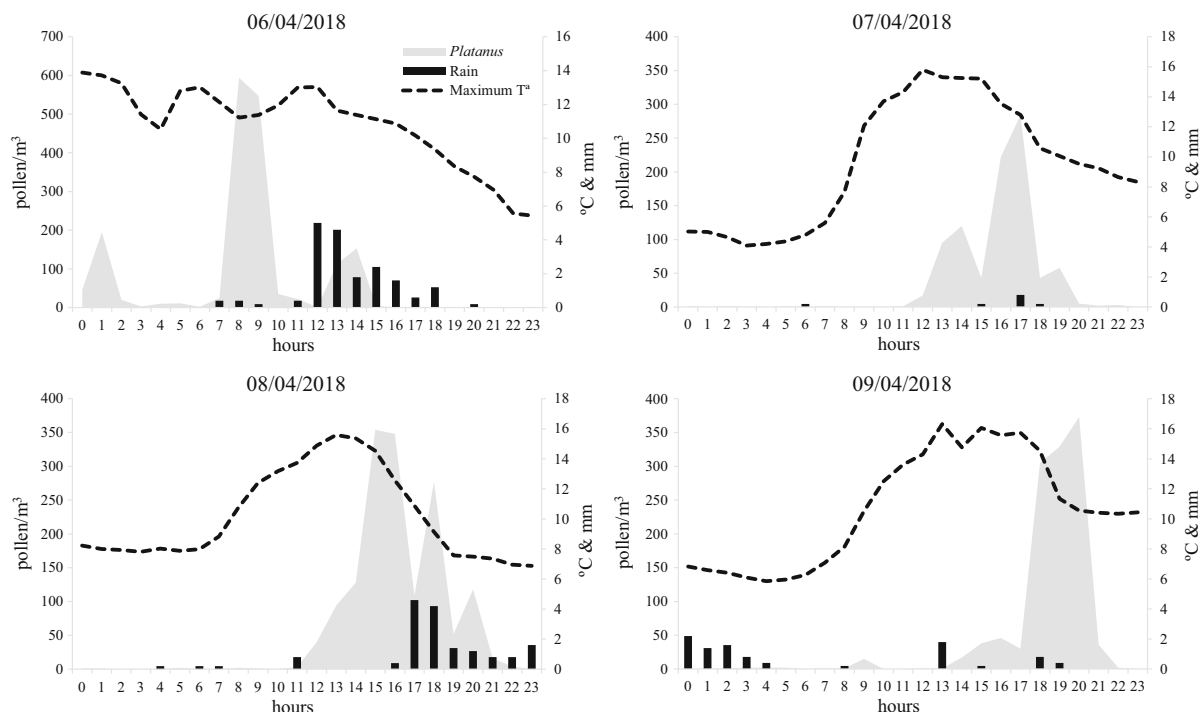


Fig. 2 Hourly distribution of *Platanus* pollen, maximum temperature and rainfall in Ourense during the maximum pollen peaks

maximum temperature 3 days before and O_3 2 and 3 days before was observed. Similar correlations were observed between Pla a 1 allergen and meteorological variables or pollutant gasses (Table 2).

In Porto, the PCA analysis extracted 3 principal components, since they had eigenvalues greater than or equal to 1.0 (Table 3). These components account for around the 67% of the variability in the original data. In general term, the three PCAs were correlated as follows: component 1—maximum, minimum and average temperatures and PM_{10} ; component 2—relative humidity, rainfall, wind speed, O_3 and NO_2 ; and finally component 3—*Platanus* pollen, Pla a 1 allergen and $PM_{2.5}$ (Table 3). In Ourense were extracted 4 principal components which together account for around 85% of the variability in the original data. This fact suggests that the four hypothetical variables explained the majority of total variance with a 15% loss of information. In general, the four PCs were correlated as follows: component 1—maximum, minimum and average temperature, relative humidity and PM_{10} ; component 2—wind speed, O_3 , NO_2 and $PM_{2.5}$; Component 3—rainfall; and component 4—*Platanus* pollen and Pla a 1 allergen (Table 3). The results of

PCs analysis were plotted on two-dimensional figures. These plots showed that in Porto the *Platanus* pollen and Pa a 1 allergen were mainly related to temperature and wind speed (Fig. 3) and in Ourense, pollen and allergen concentrations are influenced by the relative humidity and rainfall (Fig. 4). In addition, the pollen and allergen were related variables in two studied areas (Figs. 3, 4).

4 Discussion

Platanus spp. is a common species used as ornamental tree in the parks and gardens along the European cities. Several authors refer that the pollination period of *Platanus* takes place from March to April characterized by a short and intense booming period (D'Amato et al. 2007; Fernández-González et al. 2013; Maya-Manzano et al. 2017). Accordingly, in the present study the main pollen season (MPS) was short lasting from 32 to 44 days; in Porto, it took place from the second 10 days on March to April 22 occurring later in Ourense, from the last day of March to the first day of May. Compared with a previous study conducted in

Table 2 Spearman correlation test between *Platanus* pollen or Pla a 1 allergen and the main meteorological and pollutants variables during the same day and 1, 2 and 3 previous days ($p < 0.01$ is marked in bold)

	Porto		Ourense	
	<i>Platanus</i>	Pla a 1	<i>Platanus</i>	Pla a 1
<i>Platanus</i>		0.443***		0.768***
<i>Platanus</i> _1	0.622***	0.261*	0.723***	0.640***
<i>Platanus</i> _2	0.433***	0.401***	0.562***	0.668***
<i>Platanus</i> _3	0.363**	0.433***	0.354*	0.407**
Pla a 1	0.443***		0.768***	
Pla a 1_1	0.269*	0.188	0.655***	0.612***
Pla a 1_2	0.189	0.227	0.522***	0.628***
Pla a 1_3	0.207	0.419***	0.258	0.285
Rainfall	0.083	− 0.158	0.137	− 0.007
Rainfall_1	− 0.171	− 0.159	0.011	0.050
Rainfall_2	− 0.240	− 0.056	0.184	0.155
Rainfall_3	− 0.230	− 0.182	0.475***	0.371**
Maximum T	− 0.027	0.374**	0.065	0.131
Maximum T_1	0.059	0.000	0.051	− 0.117
Maximum T_2	0.043	− 0.075	− 0.184	− 0.212
Maximum T_3	− 0.019	0.084	− 0.473***	− 0.440**
Minimum T	0.124	0.325**	− 0.130	− 0.152
Minimum T_1	0.079	0.130	− 0.134	− 0.235
Minimum T_2	0.040	0.036	− 0.267	− 0.439**
Minimum T_3	− 0.162	− 0.048	− 0.390**	− 0.382**
Average T	0.088	0.371**	0.009	0.031
Average T_1	0.041	0.073	− 0.019	− 0.143
Average T_2	0.059	0.031	− 0.229	− 0.292
Average T_3	− 0.087	0.017	− 0.434**	− 0.427**
Relative humidity	0.121	− 0.102	0.311*	0.190
RH_1	0.031	− 0.058	0.241	0.310*
RH_2	− 0.126	− 0.065	0.482***	0.486***
RH_3	− 0.226	− 0.033	0.678***	0.562***
Wind speed	0.146	0.042	− 0.216	− 0.159
Wsp_1	− 0.051	− 0.155	− 0.167	− 0.250
Wsp_2	0.033	− 0.029	− 0.222	− 0.163
Wsp_3	0.014	− 0.103	− 0.072	− 0.142
NO ₂	0.004	0.102	0.196	0.207
NO ₂ _1	0.125	0.284*	0.250	0.256
NO ₂ _2	0.213	0.185	0.439**	0.269
NO ₂ _3	0.265*	0.241	0.325*	0.447**
O ₃	0.267*	0.009	− 0.425**	− 0.447**
O ₃ _1	0.012	− 0.015	− 0.484***	− 0.518***
O ₃ _2	− 0.010	0.129	− 0.588***	− 0.513***
O ₃ _3	0.009	− 0.118	− 0.394**	− 0.492***
PM ₁₀	0.040	0.156	0.072	0.097
PM ₁₀ _1	0.130	− 0.028	0.040	− 0.033
PM ₁₀ _2	0.127	− 0.125	− 0.109	− 0.184
PM ₁₀ _3	0.130	0.025	− 0.361*	− 0.343*

Table 2 continued

	Porto		Ourense	
	<i>Platanus</i>	Pla a 1	<i>Platanus</i>	Pla a 1
PM _{2.5}	– 0.225	– 0.166	0.150	0.264
PM _{2.5–1}	– 0.248	– 0.386**	0.120	0.105
PM _{2.5–2}	– 0.193	– 0.231	0.076	0.043
PM _{2.5–3}	– 0.249	– 0.021	– 0.106	– 0.115

*** $p < 0.01$, ** $p < 0.05$,* $p < 0.1$ **Table 3** Eigenvalues, per cent of variance and dimension weights of principal component analyses performed with *Platanus* pollen, Pla a 1 allergen, weather parameters and atmospheric pollutants in Porto and Ourense (values < -0.5 and > 0.5 marked in bold)

Table of components	Porto			Ourense			
	1	2	3	1	2	3	4
Eigenvalue	3.40	2.78	1.81	4.69	3.21	1.20	1.06
Per cent of variance	28.37	23.17	15.10	39.10	26.73	10.03	8.83
Cumulative percentage	28.37	51.54	66.64	39.10	65.83	75.86	84.69
<i>Platanus</i>	0.073	0.017	0.860	– 0.108	0.111	0.558	0.686
Pla a 1	0.221	– 0.067	0.816	– 0.066	0.120	– 0.079	0.935
Maximum T	0.845	– 0.320	0.036	0.853	0.111	– 0.394	0.032
Minimum T	0.887	0.223	0.127	0.837	– 0.076	0.131	– 0.132
Average T	0.945	– 0.115	0.116	0.950	0.059	– 0.248	– 0.040
Relative humidity	0.007	0.553	– 0.308	– 0.590	0.268	0.549	0.266
Rainfall	– 0.123	0.786	– 0.107	– 0.293	– 0.053	0.885	0.022
Wind speed	0.062	0.833	0.078	0.071	– 0.902	– 0.008	– 0.065
O ₃	– 0.110	0.802	0.194	– 0.100	– 0.869	– 0.109	– 0.243
NO ₂	– 0.064	– 0.658	– 0.065	0.048	0.633	0.586	– 0.062
PM ₁₀	0.724	0.118	– 0.008	0.805	0.482	– 0.180	– 0.023
PM _{2.5}	0.460	– 0.180	– 0.527	0.597	0.702	– 0.133	0.010

Porto, the MPS duration was longer, but the starting date was close to the average date for *Platanus* flowering (Ribeiro and Abreu 2014). In Ourense, Fernández-González et al. (2013) noted a lower duration of 28 days for the *Platanus* MPS, but the pollen season started early in the second half of March. The delay observed in the MPS can be related to the influence of the meteorological factors since different studies showed an advance in the *Platanus* pollen dispersion with increase in winter/spring temperatures (Norris-Hill 1998; Sabariego Ruiz et al. 2008). Furthermore, on the days immediately before the beginning of flowering, high temperature and the absence of rain favour the fast pollen release. The meteorological conditions registered in our study, with a period of rain before the start of the MPS, induced

the delay of the pollen release into the atmosphere due to the slow anthers opening.

The annual pollen integral in our study was much lower in Porto (750) compared with Ourense (5382). In Porto, the value was similar to that pointed out during a data set period of 2003–2012 with 844 *Platanus* pollen (Ribeiro and Abreu 2014), whereas lower than that noted for Ourense with 7148 pollen (Fernández-González et al. 2013). This decrease in the annual pollen integral was induced by rainfall episodes during the period of high pollen presence in the atmosphere. The pollen peak coincides with an increase in the relative humidity, mainly caused by strong rainfall (18.8 mm). But this rain occurred mainly during the hours of the day with theoretically maximum pollen presence in the atmosphere, between

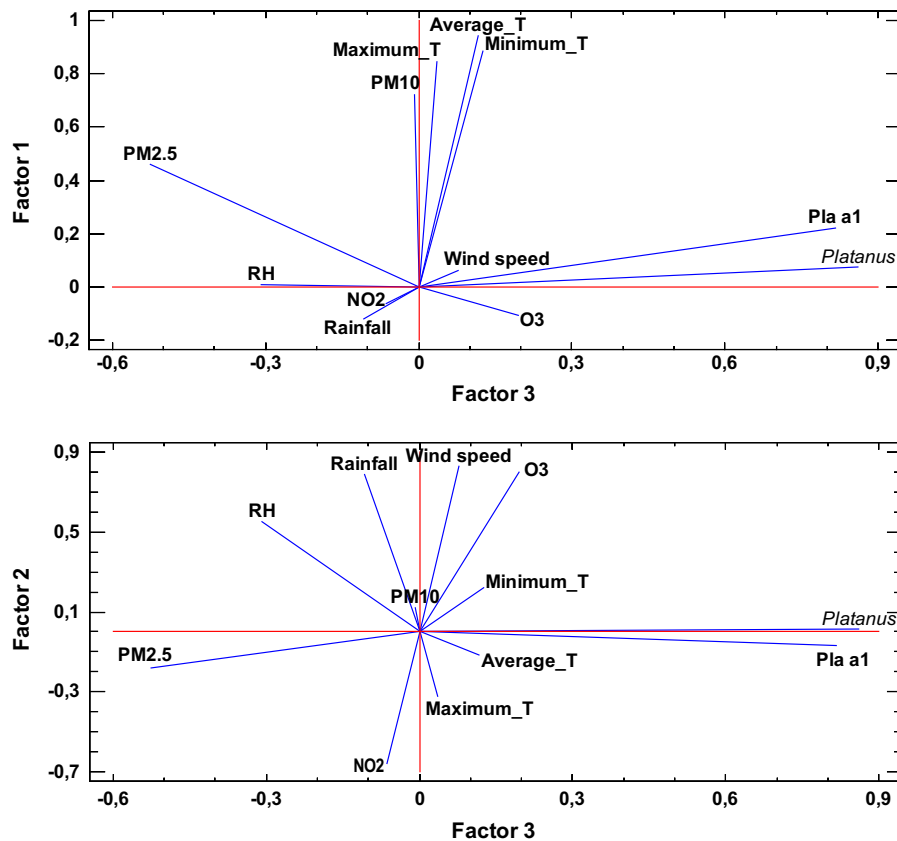


Fig. 3 Plot of principal components analysis (PCA) in Porto. Factor loadings: factor 3 versus factor 1 and factor 3 versus Factor 2. Rotation varimax. Extraction principal components

12:00 and 18:00 h (Aira et al. 2011). This fact could induce an important reduction in the pollen peak concentration which should be twice that registered during the year 2018.

Although the daily *Platanus* pollen and allergen counts showed a high association degree, the allergen peak was slightly delayed 4 days in Porto and more than the double amount of days in Ourense (9 days). Furthermore, we want to highlight that the results obtained in this study noted a different allergenic potency in the atmosphere of both locations. While the *Platanus* pollen concentrations are seven times lower in the Porto atmosphere, the Pla a 1 content is only 2 times higher in the Spanish city. This behaviour can be explained by several factors such as differences in the allergen content of the pollen, influence of air pollutants in plants, meteorological factors or soil nutrient deficit. Fernández-Caldas et al. (2007) pointed out that in some plants there is an annual oscillation in the allergenic potency (individual

histamine equivalent prick units) and in the allergenic proteins in the pollen grains, which was attributed to environmental aspects such as temperature and rainfall. Galan et al. (2013) noted, in some plant species, differences in the allergenic load of pollens between locations. Even high-potency pollen could be transported over 400 km to areas with low-potency pollen, resulting in > 40% of the yearly exposure (Galan et al. 2013).

Different studies point out the traffic as a source of pollution, since the motor vehicle exhaust emissions are a major source of air pollutant in industrialized and developed countries (Azizi 2011). In these urban areas, the plants develop defence strategies in response to environmental stressors such as atmospheric pollutants. One of these strategies is the regulation of defensive proteins or pathogenesis-related (PR) proteins (Armentia et al. 2002; Cortegano et al. 2004; Suárez-Cervera et al. 2008b). Therefore, air pollution can act as a stress factor and stimulate the expression

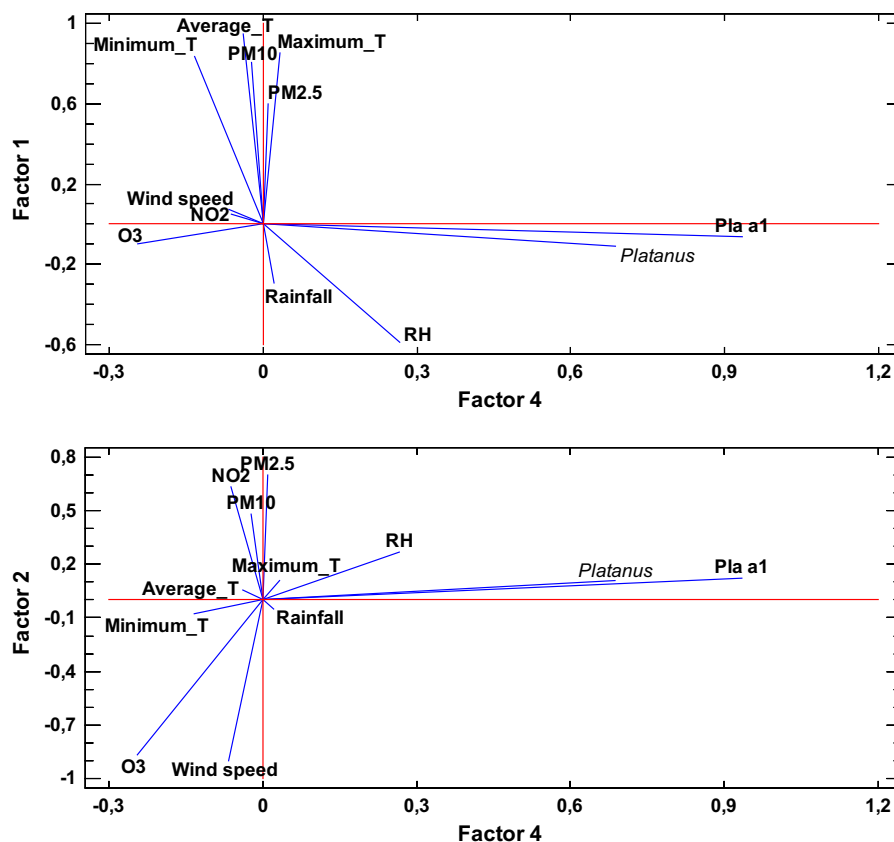


Fig. 4 Plot of principal components analysis (PCA) in Ourense. Factor loadings: factor 4 versus factor 1 and factor 4 versus factor 2. Rotation varimax. Extraction principal components

of these defensive proteins (Aina et al. 2010). Different studies pointed out that Pla a 1 proteins are involved in plant development and defence mechanisms (Sturm and Tang 1999; Fernandez-Gonzalez et al. 2010; Hong et al. 2010). Also, it was observed that *Platanus* airborne pollen-specific allergens can suffer modifications after being exposed to atmospheric gaseous pollutants (Ribeiro et al. 2017). This can justify that in Porto the allergenic protein load in the atmosphere was higher as in Ourense traffic road and pollution levels are lower than in Porto and as a consequence, the NO₂, O₃ or PM₁₀ concentrations are almost two times lower in the atmosphere. In this way, statistical results detected a high degree of association between pollen or allergen concentrations and NO₂ levels in the previous days. These results are according to some studies that identified air pollution as a stress factor, increasing the expression of Pla a 1 allergen involved in plant defence mechanism (Sedghy et al. 2017). Otherwise correlation analysis showed

decreases in the airborne pollen and allergen content under high atmospheric O₃ content during the previous days in the case of Ourense. These low levels of O₃ registered in the city do not reach the threshold necessary to trigger ecological responses in the plane trees. Nonetheless, it was observed that when plane pollen was in vitro exposed to concentrations of O₃ equal to the limit value for the human health protection in Europe (120 µg/m³), an increase in Pla a 1 Abs content was observed (Ribeiro et al. 2017).

Other factors that can affect the pollen and protein content were the meteorological parameters, such as temperature, relative humidity and rainfall. These weather variables can directly influence the allergen content differences between years (Buters et al. 2010). In the present study, the airborne pollen peak occurred before the aeroallergen peak in both cities. This fact may be due to the occurrence of precipitation, since under strong rainfall conditions some cytoplasmic material, mainly proteins and starch granules, can be

abruptly discharged from the pollen grain (Fernández-González et al. 2011, 2013). In our study, the precipitation registered before the pollen peak can explain the discordance between pollen and allergen content in the air. In Porto, the *Platanus* and Pla a 1 contents were positively correlated with temperature as it is noted by other authors for this taxa (Fernández-González et al. 2013). By the contrary in Ourense, the correlation was positive with rainfall and with relative humidity but negative with maximum temperature. When we analysed hourly daily data for this city, we can observe that during the days with the higher pollen counts, the peaks were detected from 7:00 to 11:00 am, moment in which rainfall started and was non-stop until 20:00 pm. This fact induced an increase in average daily relative humidity and a decrease in average daily temperature and therefore an unusual positive correlation of airborne pollen concentration with rainfall and relative humidity and a negative correlation with temperature. Other authors also noted negative correlations with temperature for *Platanus* in the south of Spain (Alcázar et al. 2015). When the PCFA was evaluated, the same behaviour was detected. In the Porto, *Platanus* pollen and Pla a 1 aeroallergen matches in the same quadrant of temperatures, and in the contrary quadrant than rainfall and relative humidity. In Ourense, the *Platanus* and Pla a 1 concentrations are close to rainfall and relative humidity variables due to the unusual hourly precipitation regime detected during the peak days.

5 Conclusions

In our study, the *Platanus* spp. main pollen season was shorter and earlier in Porto than in Ourense that lasted, respectively, 32 and 44 days, occurring in the beginning of March in Porto and in the last days of March in Ourense.

Daily *Platanus* airborne pollen and allergen counts showed a high statistically significant association degree, but the allergen peak was slightly delayed in the two cities.

This study allowed to quantify, for first time, the Pla a 1 allergen content in the air of the Porto city. Our findings showed different allergenic potency in the atmosphere of both locations. While the *Platanus* annual pollen integral is seven times lower in the Porto

atmosphere, the Pla a 1 content is only 2 times lower than in the Spanish city.

Also, airborne pollen and Pla a 1 aeroallergen were correlated with some meteorological parameters, namely temperature, rainfall and relative humidity, and with atmospheric pollutants such as NO₂ and O₃.

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