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ORIGINAL ARTICLE

Detection of airborne allergen (Pla a 1) in relation to *Platanus* pollen in Córdoba, South Spain

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Abstract

Córdoba is one of the Spanish cities with the highest records of plane tree pollen grains in the air. Clinical studies have identified *Platanus* as a major cause of pollinosis. This fact provokes an important public health problem during early spring when these trees bloom. The objective of the study is to evaluate the correlation between airborne pollen counts and Pla a 1 aeroallergen concentrations in Córdoba, to elucidate if airborne pollen can be an accurate measure that helps to explain the prevalence of allergenic symptoms. Pollen sampling was performed during 2011–2012 using a Hirst-type sampler. Daily average concentration of pollen grains (pollen grains/m^3) was obtained following the methodology proposed by the Spanish Aerobiology Network. A multi-vial cyclone was used for the aeroallergen quantification. Allergenic particles were measured by ELISA using specific antibodies Pla a 1. The trend of *Platanus* pollen was characterized by a marked seasonality, reaching high concentrations in a short period of time. Airborne pollen and aeroallergen follow similar trends. The overlapping profile between both variables during both years shows that pollen and Pla a 1 are significantly correlated. The highest significant correlation coefficients were obtained during 2011 and for the post peak. Although some studies have found notable divergence between pollen and allergen concentrations in the air, in the case of *Platanus* in Córdoba, similar aerobiological dynamics between pollen and Pla a 1 have been found. Allergenic activity was found only during the plane tree pollen season, showing a close relationship with daily pollen concentrations. The obtained pollen potency was similar for both years of study. The results suggest that the allergenic response in sensitive patients to plane tree pollen coincide with the presence and magnitude of airborne pollen.

Key words

Platanus, aeroallergens, airborne pollen, Pla a 1, pollinosis

INTRODUCTION

Nowadays, *Platanus* pollen is being identified as a pollen type with an increasing incidence in cases of pollen allergy. Most patients sensitive to the plane tree live in an urban environment where this type of allergy is particularly problematic due to the high use of this ornamental tree [1]. At present, Córdoba is one of the Spanish cities with the highest records of plane tree pollen grains in the air [2]. The species used was *Platanus hispanica* Mill. Ex Muenchh (*P. hybrida* Brot.-*P. acerifolia* (Ait.) Willd.). This species is a hybrid between *Platanus occidentalis* L. (American origin) and *Platanus orientalis* L. (Oriental origin). These trees usually flower from March to April and fruit in late summer and autumn.

This fast growing tree tolerates a polluted atmosphere and offers an appreciated shadow in summer when high temperatures are recorded. On the other hand, several authors have demonstrated that *Platanus* plays an important role sequestering CO_2 in the cities [3]. The massive use of this ornamental tree in Córdoba provokes an important public health problem during early spring when these trees bloom [4]. Moreover, blooming and floral intensity is not conditioned by the frequent drought periods typical of the Mediterranean climate since these trees are irrigated artificially. However, rainfall during the pollen season produces, as usual, a washout, with a decrease in airborne pollen to the benefit of patients who suffer from pollinosis.

In Spain, *Platanus* pollen was described for the first time in Madrid in the 1990s as an important cause of pollinosis [5, 6, 7]. However, the characterization of plane tree allergens had been previously published by several European colleagues during the 1970s and 1980s [8, 9]. The most common symptoms associated with sensitivity to *Platanus* pollen are rhinoconjunctivitis and asthma, with a high incidence of asthma, compared with other pollen types [4]. Symptoms appear suddenly at the beginning of spring, usually during March, although some patients also show symptoms in September and October by falling leaves due to the pollen sticking to them [10]. The positive Skin Prick Testing (SPT), specific IgE, and also the proved occurrence of symptoms during the *Platanus* pollen season, stress the clinical importance of this pollen type in the city of Córdoba [4].

In the study of pollinosis, recent papers are being focused on aeroallergens rather than on airborne pollen counts, showing better results for studying the relationship with patient allergic response [10, 11, 12, 13, 14]. Air quality studies are necessary to improve the health and life conditions of patients suffering from allergy. It is known that particles larger than 20 μm are incapable of penetrating to the lower respiratory tract, but pollen grains release allergens when they come into contact with the mucosa, and could penetrate to the bronchi, thereby provoking asthma.

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In the case of *Platanus*, three allergens have been identified: a minor allergen (Pla a 3) and two major allergens (Pla a 1 and Pla a 2). Pla a 3 is an aeroallergen related to food allergy [15, 16], Pla a 2 is a glycoprotein (polygalacturonase) and Pla a 1 is a non-glycosilated protein of 18 kDa. During the early stages of the pollen hydration, these three proteins protect the membrane from osmotic changes and could break the emerging pollen tube [17, 18]. Pla a 1 represents 60% of IgE binding of *P. acerifolia* pollen extract, and has a prevalence of 84% among patients allergic to plane tree. This aeroallergen has also been detected in *P. occidentalis, P. orientalis* and *P. racemosa*.

Objective. The purpose of this study is to evaluate the correlation between airborne pollen and Pla a 1 aeroallergen in Córdoba. The main aim is to elucidate if airborne pollen can be accurate measure that helps to explain the prevalence of allergenic symptoms in patients, or if it would be necessary to incorporate new techniques to study allergen exposure.

MATERIALS AND METHOD

Study area. Córdoba (37°53'5.02"N, 4°46'44.95"W; 123 m.a.s.l.) is a medium- sized city located in southwest Spain, with 350,000 inhabitants and light industrial development. The cultivated land is mainly devoted to cereals and olive groves, while in natural areas the typical Mediterranean vegetation can be found. Ornamental flora is mainly based in *Citrus*, *Platanus*, *Celtis*, *Melia*, *Ulmus*, *Robinia*, *Cupressus*, *Populus*, *Acer* and *Ligustrum*. The climate is Mediterranean with a touch of continentality; the annual average temperature is 17.6°C and total annual rainfall – 536 mm (Spanish Meteorological Agency, AEMET, Spain).

Air sampling. Pollen sampling was performed continuously throughout 2011 and 2012, using a Hirst-type 7-day recording volumetric trap [19] with a suction flow rate of 10 L/min. Silicone fluid was used as adhesive for pollen capture. Samples were prepared and analysed counting four longitudinal transects along the slide, following the procedure recommended by the Spanish Aerobiology Network (REA) [20]. Data were expressed as daily average of pollen grains per cubic metre of air.

The start of the pollen season was defined as the first day on which a daily average of at least 1 pollen grains/m^3 was detected, followed by five subsequent days with 1 or more pollen grains/m^3. The end of the pollen season was defined as the last day on which a daily average of at least 1 pollen grains/m^3 was recorded when counts for the five following days were below this level. This protocol has been previously proved as an accurate method to define the *Platanus* pollen season [1]. The peak date was defined as the day on which maximum pollen counts were recorded and the pollen index (PI) was defined as the sum of daily values during the pollen season.

A continuous wind-oriented multi-vial cyclone sampler was used for the aeroallergen quantification. This is a low-volume sampler with a suction flow rate of 16.5 L/min (Burkard Manufacturing Co. Ltd., UK). The sampling efficiency of this apparatus has been previously described [21]. Daily airborne particles were collected dry, directly into a 1.5 mL Eppendorf vial, from February – May. These dates represent the *Platanus* pollen season, plus more-or-less two weeks before and after, trying to find possible aeroallergens during the blooming period. Daily airborne samples were stored at -20°C.

Both samplers were located on the same sampling station situated at 22m above ground level on the University Campus in the northeastern part of the city.

Immunochemical quantification of aeroallergens. Allergenic particles were measured by ELISA using specific antibodies Pla a 1.

Samples collected in Eppendorf tubes were analyzed following the protocol described by Takahashi et al. [22], with modifications proposed by Moreno-Grau et al. [14], and introducing certain new modifications that will be described below. After centrifugation at 11,000 g for 10 min, dry samples were extracted with 120 µl of phosphate buffer (50mM pH 7.0), to which 150mM of NaCl, 3mM of EDTA and 125mM of ammonium bicarbonate were added. Finally, 0.05% Tween 20 (Scharlau, Germany) was added, and the mixture stirred continuously at room temperature for 2 h. The extract was separated by centrifugation at 10,000 g for 10 min and stored in pellet form at -20°C.

The Pla a 1 content in aerosol samples was quantified using specific antibodies. ELISA plates (Greine, Frickenhausen, Germany) coated with 100 µl of the mouse anti-Pla a 1 polyclonal antibody (Bial Industrial Farmacéutica, Spain), 2 µg protein/ml, in phosphatebuffered saline (PBS), and incubated overnight at room temperature in a moist chamber. The wells were blocked (200 µl/well) with PBS-BSA-T (PBS with 1% bovine serum albumin (BSA) with 0.05% Tween 20) and incubated for 1 h at 37°C. Plates were then incubated for 1 h at 37°C with 100 µl/well of purified Pla a 1 (starting from a 12.5 ng/mL stock of Pla a 1, eight-fold serial dilutions were performed in PBS-BSA-T, containing 12.5–0.05 ng/mL, and controls prepared). 100 µl/well of extracted airborne samples were added. A standard curve was constructed from nine data points using a four-parameter logistic curve fit. After three washes with 200 µl PBS-T, plates were incubated for 1 h at 37°C with 100 µl/well of biotinylated polyclonal antibody Pla a 1 (Biotinylated rabbit antiserum anti-Pla a 1; Bial Industrial Farmacéutica) at 0.625 mg/mL. After three washes with 200 µl PBS-T, the plates were then incubated for 1 h at 37°C with 100 µl/well of streptavidin-conjugated peroxidase (0.25 mg/mL in PBSBSA- T; S5512 Sigma-Aldrich, St Louis, MO, USA). The enzyme activity of the monoclonal antibody conjugate was determined by adding 200 µl/well of ophenylenediamine (Sigma-Fast TM o-phenylenediamine dihidrochloride tablet sets Sigma p-9187) and incubated at room temperature in the dark. After 30 min, the reaction was stopped by adding 50 mL 3M H_2SO_4 and the absorbance was then measured at 492 nm. Results are expressed in picograms per millilitre, with reference to the protein content of the standard preparation, and subsequently transformed into picograms per cubic metre according to the volume sampled by the apparatus.

Natural protein was purified from *Platanus acerifolia* pollen extract using standard chromatographic methods and lyophilized in PBS containing 1% BSA and 0.05% Tween 20 [17].

Meteorological data. Meteorological data (temperature, relative humidity and rainfall) were obtained from the

Andalusian Regional Government Agroclimatic Information Network at the weather station on the outskirts of Córdoba city (UTM coordinates X: 341642.0; Y: 4192085.0).

Statistical analysis. Spearman correlation test was performed to evaluate statistical correlation between airborne pollen count and Pla a 1 aeroallergen. The strength of association between both variables with mean temperature (°C), rainfall (mm) and relative humidity (%) was assessed by a nonparametric Spearman correlation test. Finally, data were analyzed using simple linear regression analysis to investigate the relationship between pollen and aeroallergen levels, in an attempt to find a predictive formula for the aeroallergen load using the airborne pollen concentrations detected. Statistical analyses were carried out by using the SPSS version 17.0 software package for Windows, Microsoft ©. A p value <0.05 was considered statistically significant.

RESULTS

The trend of *Platanus* pollen type during 2011 and 2012 was characterized by a marked seasonality as this tree has a typical intense blooming, reaching high concentrations in a very short period of time.

In 2011 the pollen season started on 12 March and no allergens were detected before that day. In 2012, the pollen season also started on 12 March, but allergens were not detected until 14 March. The end of the pollen season occurred on 19 April in 2011 and on 29 April in 2012. The last day for allergens detection was on 5 April in 2011 and on 12 April in 2012.

The pollen peak was recorded on 20 March, with 459 pollen grains/m^3 in 2011, and on 23 March, with 130 pollen grains/m^3 in 2012 (Tab. 1). The maximum concentration of allergen occurred during the same pollen peak day in 2011, with 218 pg/m^3, while in 2012, it was 2 days delayed, occurring on 25 March with 79 pg/m^3. Normally, airborne pollen and aeroallergens follow similar trends.

Table 1 shows that the mean value for allergen content per pollen grains (pollen potency: average allergen release from pollen) shows a practically constant relationship in the two years of study, although slightly lower in 2012.

Table 1. Characteristics of the pollen season

	2011	2012
Pollen		
Peak value (pollen/m^3)	459	130
Peak Day	20 March	23 March
Pollen index (pollen/season)	3202	1322
Season length (days)	44	48
Pla a 1 allergen		
Peak Value (pg/m^3)	218	79
Peak Day	20 March	25 March
Allergen Index (pg/season)	1442	580
Pollen potency (pg/pollen)	0.450	0.439

Figure 1 shows a similar pollen and aeroallergen profile for both 2011 and 2012. However, it can be observed that during 2011 the value of the pollen index (PI) and the allergen index (AI) are approximately 2.5 times greater than in 2012 (Tab. 1; Fig. 2). The overlapping profile between both variables during both years suggests that pollen and Pla a 1 are significantly correlated (Tab. 2), overall for 2011, with higher significant correlation coefficients than for 2012. On the other hand, a higher significant correlation has been observed during post-peak than during pre-peak in the curve.

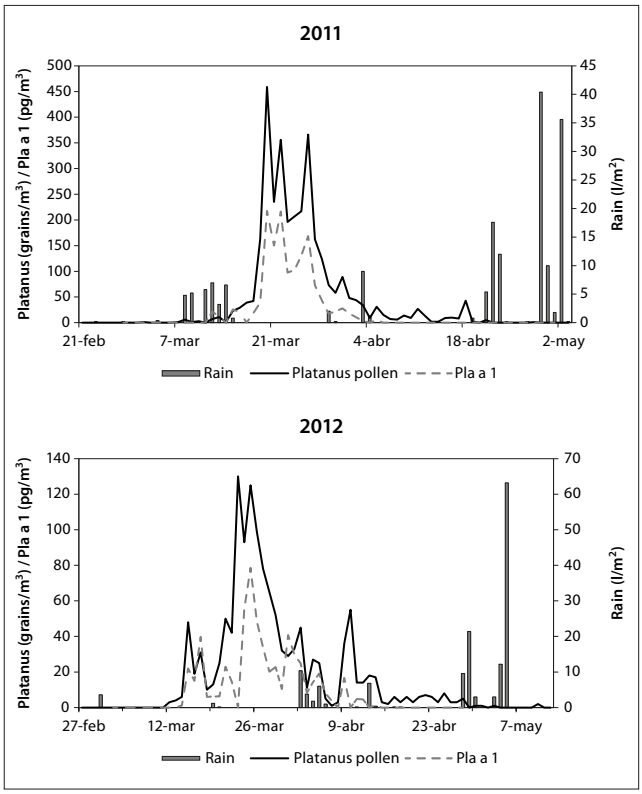

Figure 1. Daily values for *Platanus* pollen and allergen and precipitation recorded during the study period in Córdoba

Linear regression analysis showed a significant relationship between Pla a 1 and *Platanus* pollen in both years. However, higher R^2 coefficient value (0.946 and p<0.000) was found for 2011, comparing with 2012 (0.523 and p<0.000), and for the post-peak aspect to the pre-peak (Tab. 2; Fig. 3). It must be taken into account that for this species the pre-peak period is shorter and with lower pollen concentration than the post-peak period, as this pollen type appears abruptly in the air, reaching the peak pollen concentration in a short period of time.

Spearman correlation test was carried out to find a possible degree of association between daily average airborne pollen and aeroallergens with the main meteorological factors

Table 2. Spearman correlation test between *Platanus* pollen and allergen, plane pollen *vs* Pla a 1

		N	Spearman R	r^2	p	Slope
2011	Pollen Season	44	0.844**	0.946	0.000	0.518
	Pollen season Pre-peak	14	0.766**	0.945	0.000	0.447
	Pollen season Post-peak	30	0.926**	0.962	0.000	0.563
2012	Pollen Season	48	0.743**	0.523	0.000	0.390
	Pollen season Pre-peak	12	0.542	0.007	0.801	0.028
	Pollen season Post-peak	36	0.785**	0.803	0.000	0.537

** Correlation is significant at the 0.01 level (2-tailed)

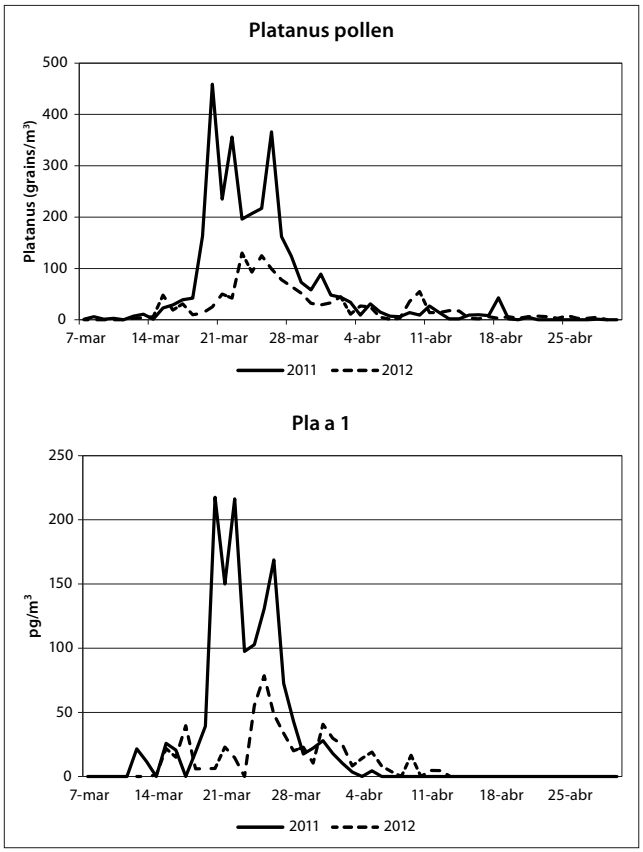

Figure 2. Comparison between data obtained in both years of study for *Platanus* pollen and allergen

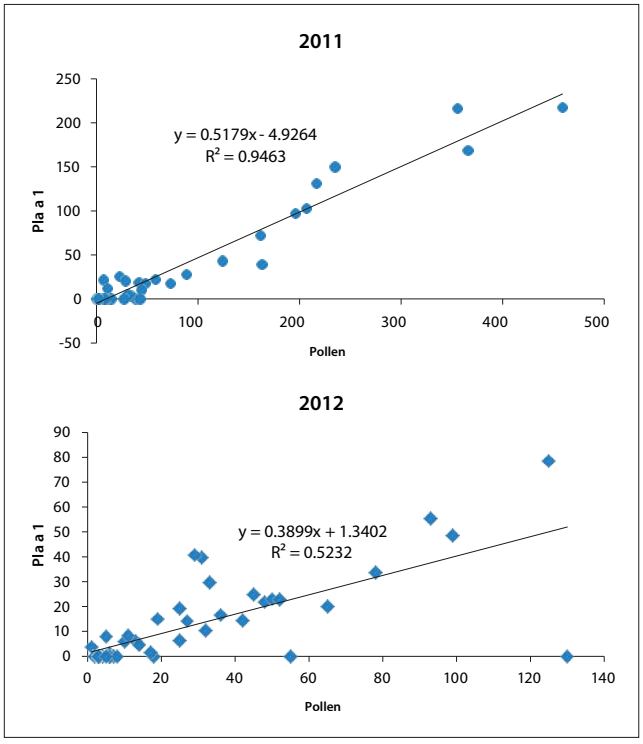

Figure 3. Linear regression analysis between Pla a 1 and Platanus pollen during the study years

(Tab. 3). In most cases, no significant correlation was observed between *Platanus* pollen and Pla a 1 with the main meteorological parameters. Both, pollen and Pla a

Table 3. Spearman correlation test between *Platanus* pollen and allergen and meteorological factors

	2011				2012			
	Temp	Hum	Rain	N	Temp	Hum	Rain	N
***Platanus* Pollen**								
Pollen season	-.455**	-.222	-.386**	44	.030	-.030	-.047	48
Pre-peak	-.590*	-.638*	-.611*	14	-.112	.552	-.075	12
Post-peak	-.592**	.266	-.100	30	.127	-.103	-.003	36
Pla a 1 allergen								
Pollen season	-.588**	.061	-.138	44	-.159	.048	-.003	48
Pre-peak	-.369	-.345	-.248	14	-.423	.592*	-.005	12
Post-peak	-.674**	.321	-.060	30	-.022	-.035	.016	36

** Correlation significant at the 0.01 level (2-tailed)
* Correlation significant at the 0.05 level (2-tailed)

1correlation with temperature was negative, but significant only during 2011, overall for the complete season and the post-peak with a significance of 99%, and in the case of *Platanus* pollen, also for the pre-peak with a significance of 95%. In the case of humidity, it was significant with a negative sign only during the pre-peak for pollen in 2011, and with a positive sign for Pla a1 during the pre-peak of 2012. Regarding rainfall, it was significant and negative for pollen records only in 2011 for complete season and pre-peak.

DISCUSSION

Some studies comparing airborne pollen and aeroallergens have found notable divergence during the periods preceding and succeeding the main pollen season. This was noticed for *Olea* [12] and also for *Platanus*, *Poaceae* and *Urticaceae* [10] in different areas of Spain. Different authors have concluded that during the periods out of the pollen season the allergenic activity could probably be due to the allergenic load of other parts of the plant other than pollen [23, 24, 25]. Nevertheless, a study on birch pollen season in five different European countries could only detect aeroallergens when pollen was present [11].

In the case of *Platanus*, the presented study shows similar aerobiological dynamics between airborne pollen and Pla a 1 in the air during the pollen season. The same results have also been reported by Rodríguez-Rajo et al. [26] in Ourense. However, Fernández-González et al. [10] reported that the presence of Pla a 1 allergen in the atmosphere of León is independent of the airborne *Platanus* pollen. These conclusions could probably be related with the low plane tree pollen levels recorded in León. In the case of Ourense and Córdoba, a higher pollen content in the air is usually detected, showing a clearer pollen curve [1, 10, 26]. This fact probably explains a closer relationship between pollen and Pla a 1 curves. It could also be the reason why better correlations were found in the presented study when pollen concentrations were higher, for instance, post-peak *vs.* pre-peak or 2011 *vs.* 2012. This could support the fact that lower pollen concentrations in the atmosphere provoke a lower relationship with the allergen content in the air. In the current study, allergenic activity was found only during the plane tree pollen season, showing a close relationship with daily airborne pollen concentrations, corroborating the very high and significant correlation coefficients obtained. This is an

interesting finding, taking into account the frequent presence of this tree in temperate areas, and its implication on pollen allergy.

Another interesting result is the similar pollen potency obtained for both years of study, showing that allergen released from *Platanus* pollen grains remains quite stable through the years, although it would be necessary to count them with more years of study to corroborate if this figure is similar over time. The pollen potency obtained for *Platanus* in Córdoba during the study years was lower than the pollen potency for *Betula* and *Olea*, according to the results obtained in different European countries [11, 13].

Some studies have reported that weather parameters show more influence on pollen dispersion than on allergen release [26]; however, this is not the case in the presented study. Although during 2011 a greater influence of meteorological parameters was found with pollen content in the air rather than with allergens, in 2012, only aeroallergens were significantly correlated, in this case, with the humidity.

In Córdoba, forcing units prior to the *Platanus* pollen season were reported as the most influencing parameter on pollen season start [1]. This fact has been also found for other tree species flowering in spring [27]. However, once the pollen season starts, meteorological parameters have a low influence on daily pollen concentrations, probably due to the fast blooming and more to an explosive process than to a response to other meteorological variables. Thus, the pollen season supposes a short period of time with low variations in weather parameters. Only during the year 2011 the plane tree pollen and allergen concentrations showed a significant negative correlation with temperature, probably due to the decrease in temperatures recorded in that year during the days with higher pollen records, as temperatures were higher at the beginning of March, decreasing through the month, and increasing again at the end of March. It is important to take into account that during March, at the beginning of spring, the weather is very changeable and temperatures do not show a clear tendency throughout the season, suffering a lot of oscillations in this period.

In respect to the effect of humidity, a significant and negative correlation was found for pollen emission, due to humidity making the pollen emission and transport difficult [28]. However, the positive correlation found with allergens means that this parameter favours allergen release from the pollen grains. This correlation was significant for the pre-peak when pollen grains are released from trees susceptible to the release of allergens, while during the post-peak, this relationship was not found, perhaps due to the importance of the contribution of pollen from resuspension with a lower implication in releasing allergens. Other authors have found that high atmospheric humidity may contribute to the release of allergenic proteins from pollen grains [29, 30].

The fact that the aeroallergen peak was delayed for two days in 2012 with respect to the pollen peak, could be related to the low humidity during the pollen peak day with humidity lower than 50%, which does not favour the allergen release.

In the case of rainfall, this parameter had only a significant influence during the pre-peak pollen season of 2011, this year, the rain recorded coincided with the beginning of the pollen season, and had a great influence in this period, while in 2012, rains occurred at the end of the pollen season and did not show a significant influence.

CONCLUSIONS

The results of the presented study suggest that plane tree allergen load in the atmosphere of Cordoba coincides with the presence and magnitude of airborne pollen, although it this a preliminary study that must be confirmed in the future. This study shows that in case of *Platanus* in Córdoba, the daily pollen concentrations can be accurately measured and helps to explain the allergen exposure in sensitive patients. In this case, pollen monitoring could be enough to offer accurate information to study the aeroallergen exposure in sensitive patients and to prevent symptoms, avoiding the need of using more expensive and time-consuming techniques to study the allergen content in the atmosphere. However, this conclusion cannot be extended to other pollen types or areas where, as many papers report, the aeroallergen content is not correlated with the pollen content of the air.

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