

ORIGINAL ARTICLE

Platanus pollen allergen, Pla a 1: quantification in the atmosphere and influence on a sensitizing population

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Summary

Background The allergic response in susceptible patients does not always coincide with the presence and magnitude of airborne pollen counts. The prevalence of allergy to *Platanus* is currently moderate, although the percentage of monosensitized patients is low. This hinders accurate interpretation of the relationship between the amount of pollen inhaled and the patient's symptoms.

Objective This study aims to investigate the relationship between the atmospheric concentration pattern of Pla a 1 aeroallergen and the *Platanus* pollen.

Methods The pollen sampling was carried out using a Hirst-type volumetric trap (Burkard[®]) for pollen grains and a Burkard Cyclone sampler (Burkard[®]) for Pla a 1 allergen. Serum-specific IgE levels to *Acer* sp., *Artemisia vulgaris*, *Betula alba*, *Chenopodium album*, *Cupressus arizonica*, *Cynodon dactylon*, *Fraxinus excelsior*, *Lolium perenne*, *Pinus* sp., *Plantago lanceolata*, *Platanus acerifolia*, *Populus* sp., *Quercus ilex* and *Taraxacum officinale* allergens were determined using the EAST System (Hytec specific IgE EIA kit; Hycor Biomedical, Kassel, Germany).

Results The aerobiological dynamics of *Platanus* pollen grains and Pla a 1 differed considerably, particularly during the *Platanus* pollination period. Of the 118 subjects tested, sera from 34 contained specific IgE to *Platanus* pollen and all of them had specific IgE to other pollen types.

Conclusions The presence of the aeroallergen Pla a 1 in the atmosphere appears to be independent of *Platanus* pollen counts over the same period, which may be contributing to allergic symptoms and sensitization. The number of polysensitized patients displaying allergy to *Platanus* suggested that allergic symptoms were caused by co-sensitization or cross-reactivity involving a number of allergenic particles.

Keywords aeroallergens, Pla a 1, plane, pollen, pollinosis

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Introduction

Over the last 30 years, there has been considerable research into the relationship between inhaled allergen levels and patient-allergic response, with studies mostly focusing on pollen counts rather than on other airborne allergens [1–3]. The results suggest that the allergic response in susceptible patients does not always coincide with the presence and magnitude of airborne pollen counts. As a result, technologies and methods are being developed to enable aerosol sampling using low- and

high-volume samplers, and to measure aeroallergens using immunochemical and molecular tests [4–6].

The ability of *Platanus* pollen grains to trigger immediate hypersensitivity reactions has been documented since the 1970s; the reported prevalence of sensitization to this pollen in Mediterranean Europe ranges between 3% and 52% [7–10]. Major differences have been noted between airborne *Platanus* pollen counts, the degree of sensitization to this pollen and the clinical response. The prevalence of allergy to *Platanus* is currently moderate, although the percentage of monosensitized patients is

low. This hinders accurate interpretation of the relationship between the amount of pollen inhaled and the patient's symptoms. Moreover, the elevated number of polysensitized patients suggests that there may be some cross-reactivity between allergen fractions derived from different plant families, particularly involving Poaceae, *Betula*, *Olea europaea*, *Parietaria judaica*, *Plantago*, *Artemisia* and *Chenopodiaceae* [11].

Research has so far identified three *Platanus acerifolia* (Aiton) Willd pollen allergens, apart from profilins [12]: one 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 [13]. Pla a 2 is a glycoprotein (polygalacturonase) recognized by 83% of sera from monosensitized and polysensitized patients; it accounts for 52% of the total IgE-binding capacity of *Platanus acerifolia* [14]. Pla a 1 is a non-glycosylated 18 kDa protein and has sequence homology to invertase inhibitory proteins (PII) and pectin methylesterase inhibitor proteins (PMEI) [11, 15]. During the first minutes of pollen hydration, these proteins protect the membranes from osmotic changes, which could break the incipient pollen tube and abort the germinative process. At the same time, they promote the presence of simple sugars (glucose and fructose) necessary for the development of the pollen tube. Pla a 1 represents about 60% of the total IgE binding of *P. acerifolia* pollen extract, and has a prevalence of 84% among *Platanus*-allergic patients. This aeroallergen has also been detected in *P. occidentalis*, *P. orientalis* and *P. racemosa* [16].

Platanus acerifolia is a deciduous tree, planted in urban areas world-wide because of its resistance to diseases and to air pollution. Although it is very common in North American and European cities, its potential as an allergen is rated differently from country to country. Pollen emission – before or in some cases during leaf opening – is explosive, and takes place over slightly more than a month in spring, i.e. March/April or April/May depending on the weather conditions. Airborne pollen counts therefore vary during the flowering period [17, 18]. The values reported in the Iberian Peninsula range between 15% [19, 20] and over 46% of the total pollen count [21]. Airborne *Platanus* pollen has been detected as late as October or early November due to post-seasonal refloating, due to the presence of pollen grains attached to falling leaves. In recent years, airborne *Platanus* pollen in the Spanish city of León has accounted for just over 3% of the total airborne pollen count, due to late intense pruning.

A number of authors have reported the presence of the allergen Pla a 1 in the atmosphere, using analytical methods based on monoclonal and polyclonal antibodies. This protein remains in the air for some time both before and after peak pollen counts [22, 23].

In clinical terms, according to a multicentre study of pollen-allergy sufferers in Spain [24, 25], the percentage of subjects with rhinoconjunctivitis sensitized to *Platanus*

pollen ranges from over 22% in the Aragón and Madrid to 0% in Asturias. Among asthmatic subjects, the percentage increases to 36% and 17%, respectively. In the Castilla y León region, the percentage of sensitization is relatively low, at 2.4%.

This preliminary study aimed to measure airborne Pla a 1 allergen concentration, compared them with the airborne pollen concentration of *Platanus*, identify the environmental conditions likely to contribute to the release of this allergen in the air and evaluate the clinical applications of these data.

Materials and methods

Air sampling

Bioaerosol sampling was carried out in León, in the north-western Iberian Peninsula. Biogeographically, the city belongs to the Mediterranean region; the mean annual temperature is 11.1 °C and the total annual rainfall is 467.5 mm.

Pollen sampling was performed continuously throughout 2008 and 2009, using a Hirst-type [26] 7-day recording volumetric trap (Burkard Manufacturing Co. Ltd., Hertfordshire, UK) with a suction flow rate of 10 L/min. Samples were prepared and analysed following the procedure recommended by the Spanish Aerobiology Network [27], and the data were expressed as grains per cubic metre of air.

The atmospheric aerosol for the quantification of the allergenic fraction was sampled with a low-volume sampler, a continuous wind-oriented cyclone sampler with a suction flow rate of 16.5 L/min (Burkard Manufacturing Co. Ltd.). The sampling efficiency of this apparatus is described by Emberlin [28]. The atmospheric particles were collected dry directly into a 1.5 mL Eppendorf vial every 24 h of sampling, during the *Platanus* pollination period, from March to May. The samples collected each day were stored at –20 °C. Both samplers were located on the same sampling station situated at 15 m above the ground on a building in the University Campus.

Immunochemical quantification of aeroallergens

Samples collected in Eppendorf tubes were analysed following the recommended protocol [29], with certain modifications. After centrifugation at 11 000 g for 10 min, dry samples were extracted with 120 µL of phosphate buffer (50 mM pH 7.0), to which 150 mM of NaCl, 3 mM of EDTA and 125 mM of ammonium bicarbonate were added. Finally, 0.05% Tween 20 (Scharlau, Germany) was added, and the mixture was 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 (Greiner, Frickenhausen, Germany) were coated with 100 µL of the mouse anti-Pla a 1 monoclonal antibody 5C1 L-071 (Bial Industrial Farmacéutica, Spain), 5 µg protein/mL, in phosphate-buffered 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 20 µg/mL stock of Pla a 1, eightfold serial dilutions were performed in PBS-BSA-T, containing 50–0.4 ng/mL, and controls were prepared). At the same time, 100 µL/well of extracted airborne samples were added. The 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 L-071 Bial Industrial Farmacéutica, Spain) at 0.625 µg/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 µg/mL in PBS-BSA-T; S5512 Sigma-Aldrich, St Louis, MO, USA).

The enzyme activity of the monoclonal antibody conjugate was determined by adding 200 µL/well of *o*-phenylenediamine (Sigma-Fast TM *o*-phenylenediamine dihydrochloride tablet sets Sigma p-9187) and incubated for 30 min at room temperature in the absence of light. This reaction was stopped by adding 50 µL 3 M H₂SO₄ 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 were 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 [16].

Patients and sera

The most abundant local airborne pollen types, as identified in an aerobiological survey, were tested on 118 atopic student volunteers (36 males and 82 females) at the University of León (Spain), aged 19–32, after obtaining informed consent. All subjects displayed positive skin prick test (SPT) reactions to one or more of the following commercial pollen extracts: *Acer* sp., *Artemisia vulgaris*, *Betula alba*, *Chenopodium album*, *Cupressus arizonica*, *Cynodon dactylon*, *Fraxinus excelsior*, *Lolium perenne*, *Pinus* sp., *Plantago lanceolata*, *Platanus acerifolia*, *Populus* sp., *Quercus ilex* or *Taraxacum officinale* (Bial Indus-

trial Farmacéutica, Spain). Serum-specific IgE levels to these pollen allergens, as determined using the EAST System (Hytec specific IgE EIA kit; Hycor Biomedical, Kassel, Germany), were expressed in IU/reference values: < 0.35 IU/ml (class 0) to > 100 IU/mL (class 5).

Weather data

Meteorological data were supplied by a DAVIS weather station placed next to the Hirst-type 7-day recording volumetric trap and the Burkard Cyclone sampler. Temperatures (°C), rainfall (mm), relative humidity (%) and wind speed (km/h) were recorded continuously during a period of time 2 weeks before and 2 weeks after the aeroallergen and the pollen sampling period.

Statistical analysis

Data were compiled using Microsoft office Excel 07. Correlation analysis for non-parametric data used Spearman's Rank Correlation Coefficient. The level of significance was assessed between $P < 0.01$ and $P < 0.05$. SPSS statistical software, version 14.0 (SPSS Inc., Chicago, IL, USA), was used for all statistical analyses.

Results

Aerosol concentrations

Platanus pollen emission patterns in León in 2008 and 2009 differed considerably, although the flowering period – indicated by the presence of airborne pollen – was similar. In 2008, *Platanus* pollen was detected from 26 March to 29 May. In 2009, it was scarce or non-existent due to heavy pruning in January, which gave rise to minimal flowering. Nevertheless, airborne pollen was detected – albeit in negligible amounts – over an 8-week period from 16 March to 15 May. In 2008, the SPI (Seasonal Pollen Index) of *Platanus* pollen was 1514, accounting for 6.8% of the total annual pollen in León; in 2009, the SPI value was 40, accounting for no more than 0.5% of the total annual pollen. In 2008, the peak pollination period ran from 21 April to 12 May, with a maximum daily peak on 27 April of 273 grains/m³. In 2009, there was no real mean pollination period and the daily concentration did not exceed 4 grains/m³ (Figs 1 and 2).

Measurement of Pla a 1, major allergen of *Platanus*, showed that the airborne pollen and allergen concentration behaved differently. In 2008, relatively high pollen counts were recorded from 28 April to 8 May. During the main pollination period, curves for pollen and allergen concentration displayed similar behaviour; however, differences were apparent for the periods 3 weeks before and 3 weeks after the maximum pollen-emission period. The

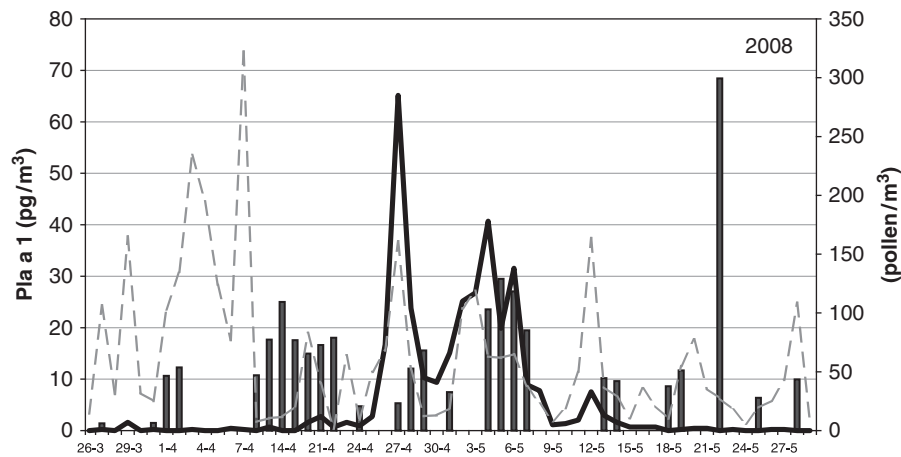


Fig. 1. *Platanus* pollen concentration (pollen/m³) (—), other pollen types (---) and Pla a 1 protein concentration (pg/m³) (■) in the city of León, Spain during 2008.

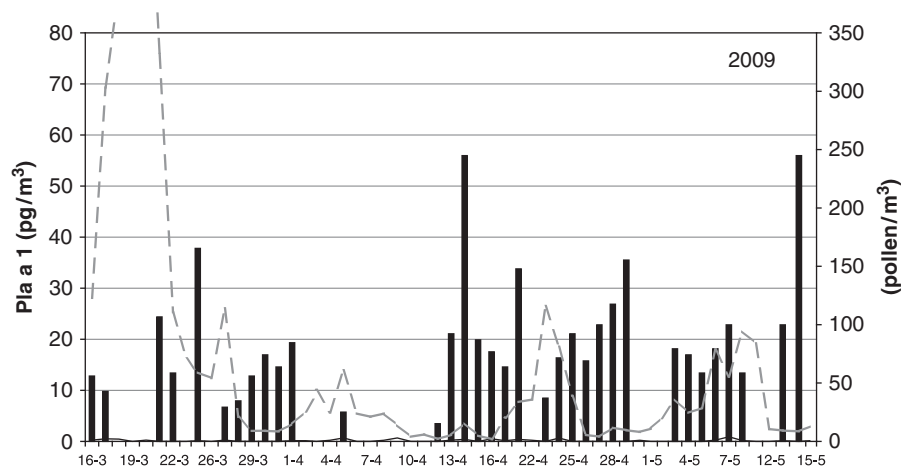


Fig. 2. *Platanus* pollen concentration (pollen/m³) (—), other pollen types (---) and Pla a 1 protein concentration (pg/m³) (■) in the city of León, Spain, during 2009. Please note that the peak value, off the graph, of other pollen types was 562 pollen/m³.

main peak for allergen activity in fact occurred after the maximum pollen-emission period (Fig. 1). In 2009, airborne Pla a 1 concentrations were similar to those recorded the previous year (1–60 pg/m³) from 16 March to 14 May (Fig. 2); the highest values did not correspond to the few pollen peaks recorded during that period. No significant correlations were observed between Pla a 1 allergen, *Platanus* pollen, most common pollen types during the same period and the main meteorological parameters (Table 1).

Clinical analysis

Of the 118 subjects tested, sera from 34 (28.8%) contained specific IgE to *Platanus* pollen, although the levels were relatively low: the mean value was 2.22 IU/mL, including six with a range of >3.5 to >17.5 IU/mL (class 3) and the rest with a range of >0.70 to >3.5 IU/mL (classes 1 and

2). All subjects had specific IgE to other pollen types, which always included grasses (Table 2). Statistical analysis of the correlation between *Platanus*-positive sera and sera positive to other pollen extracts revealed significant correlations ($P < 0.01$) with *Acer* sp., *Artemisia vulgaris*, *Betula alba*, *Chenopodium album*, *Fraxinus excelsior*, *Lolium perenne*, *Populus* sp., *Quercus ilex* and *Taraxacum officinale* (Table 3).

Discussion

This research was considered to examine the relationship between airborne *Platanus* pollen and Pla a 1 allergen concentrations. The aerobiological dynamics of both types of particle were found to differ considerably during the *Platanus* pollination and in particular during the period immediately before and after the main pollination

Table 1. Spearman's correlation coefficients between allergen Pla a 1 and *Platanus* pollen concentration, pollen concentration for most common types in the same seasonal period and the main meteorological parameters for 2008 and 2009

	Pla a 1 (2008)	Pla a 1 (2009)
<i>Platanus</i> pollen	0.177	0.084
<i>Betula</i> pollen	0.190	0.129
<i>Cupressus</i> pollen	-0.144	-0.306*
Oleaceae pollen	0.041	-0.496**
Poaceae pollen	-0.102	0.065
<i>Quercus</i> pollen	0.069	0.129
Salicaceae pollen	0.136	-0.270
Maximum temperature	-0.073	-0.182
Mean temperature	-0.023	-0.010
Minimum temperature	0.130	0.194
Rainfall	-0.002	0.220
Relative humidity	0.114	0.133
Wind speed	0.068	0.295

* $P < 0.05$.

** $P < 0.01$.

period. Similar findings have been reported for other genera of plants [2, 30, 31].

A number of authors have suggested that these variations may be due to the presence of allergenic particles emitted from other parts of the same plant; it is the case of Ubisch bodies in Poaceae, although later studies appear to show that most Ubisch bodies in fact remain within anthers even after the release of pollen grains [32]. Other authors have found that high atmospheric humidity may contribute to the release of allergenic proteins outside the pollen grain during the activation of the same [33, 34] or during the pollen tube growth under wet conditions (abortive germination). These processes may represent significant mechanisms of release of allergens in the air [35] but in the present study no significant correlations were observed between Pla a 1 and atmospheric humidity.

The re-suspension of pollen grains in the air [36] may bring allergenic particles into the atmosphere more than once; this could make it more difficult to interpret the variation of the Pla a 1 concentration, given that generally it is released during the first minutes of hydration of pollen grain [37]. However, the airborne Pla a 1 levels recorded in 2009 when the amounts of *Platanus* pollen were low suggest the involvement of other allergen sources, structurally or functionally similar to invertase and pectin methylesterase inhibitory proteins.

During the *Platanus* pollination period, in the atmosphere of the city of León, Spain, pollens types belonging to botanical families including Cupressaceae, Oleaceae, Betulaceae, Salicaceae, Poaceae and Fagaceae were found; however, only *Quercus*, *Betula* and the Salicaceae family were present at a significant concentration during the period when the allergen concentrations were high. Moreover, in the serum of subjects with specific IgE to the

extract of *Platanus acerifolia* pollen, there has been a high correlation with specific IgE to extracts of pollen of *Fraxinus* (Oleaceae), *Populus* (Salicaceae), *Quercus* (Fagaceae) and *Betula* (Betulaceae).

The large number of polysensitized patients displaying allergy to *Platanus* suggested that allergic symptoms were caused by co-sensitization or cross-reactivity due to different allergens. Co-sensitization to grass pollen, *Olea europaea*, *Parietaria judaica*, *Plantago*, *Artemisia* and Chenopodiaceae is quite common [38]. Cross-reactivity between pollen extracts from *Platanus acerifolia*, *Lolium perenne* and *Olea europaea* has also been reported in polysensitized subjects, but not in monosensitized individuals [11]. The major allergen, Que a 1 of *Quercus alba*, is a defence protein that quantitatively increases its presence when the plant has responded to a biotic stimulus [39]. This protein is very similar to Bet v 1 (17 kDa), both of which belong to Fagales allergen group 1, which also contains the Salicaceae, comprising mainly poplars (*Populus*) and willows (*Salix*), whose main allergens have not yet been characterized. The similarity in the molecular weight between Pla a 1, Que a 1 and Bet v 1, as well as their taxonomic proximity, suggests that they may share certain amino acid sequences; this might explain why Pla a 1 allergen was detected in the air when *Platanus* pollen was scarce or absent. Moreover, Pla a 1 contains a high percentage of amino acid residues identical to the invertase inhibitory proteins of various plants [15]. These proteins have several types of metabolic functions and are also involved in enzymatic activity related to carbohydrate metabolism [37] and in the plant development and defence reactions. Pla a 1 has a significant homology with specific proteins present in the pistil, involved in the mechanism of pollen-pistil interactions, as well as with certain stamen proteins not present in other plant tissues [15]. This may also account for cross-reactivity with other pollen types.

In a recent paper on Bet v 1, Buters et al. [40] suggest that when the weather conditions are not favourable to pollen dispersion, in other words at a low concentration, the allergenic potency may be higher. This hypothesis could explain the coexistence of both a low pollen concentration and a high allergen concentration. In fact, during the *Platanus* pollen season 2009, the meteorological conditions were: sunny days and clear nights, low humidity, no rain, no cloud cover and minimum temperatures considerably lower than those of the same period of 2008. In other words, the 2009 pollen season was colder and drier than that in 2008.

The results of this study show that the presence of Pla a 1 allergen in the atmosphere is independent of the amount of *Platanus* pollen that exists in the same period. Determine in the air at the same time the amount of allergen Pla a 1 in addition to plane pollen and other pollen types that can have common amino acid sequences with Pla a 1, is

Table 2. Serum-specific IgE levels (IU/ml) from 34 sensitized subjects to the pollen allergens of *Platanus acerifolia* (Pla), *Acer* sp. (Ace), *Artemisia vulgaris* (Art), *Betula alba* (Bet), *Chenopodium album* (Che), *Cynodon dactylon* (Cyn), *Fraxinus excelsior* (Fra), *Lolium perenne* (Lol), *Plantago lanceolata* (Plan), *Populus* sps. (Pop), *Quercus ilex* (Que) and *Taraxacum officinale* (Tar)

#	Age	Pla	Ace	Art	Bet	Che	Cyn	Fra	Lol	Plan	Pop	Que	Tar
1	23	1.7	2.2	2.4	2.0	1.3	16.9	3.1	100	2.9	2.9	—	7.3
2	20	2.3	0.6	3.1	4.3	1.8	39.4	7.6	> 100	13.3	—	—	8.8
3	21	9.0	4.1	6.3	5.2	10.4	64.4	11.9	> 100	24.6	6.3	4.5	13.2
4	20	1.2	0.9	0.7	1.4	0.8	8.0	2.1	81.9	1.1	0.7	—	4.6
5	19	< 0.3	—	—	0.8	2	38.2	5.5	> 100	0.8	< 0.3	< 0.3	10.5
6	18	1.3	—	4.9	0.4	0.9	—	1.3	34.5	2.4	—	1.2	6.6
7	21	1.0	—	4.6	< 0.3	0.4	5.1	—	13.8	0.9	—	—	2.8
8	18	0.4	—	1.6	—	—	—	—	2.9	< 0.3	—	—	0.6
9	32	1.4	0.7	0.7	0.8	1.1	4.0	2.3	11.1	2.2	0.6	1.5	1.4
10	17	1.3	0.6	1.2	1.5	0.8	1.3	1.5	5.3	30.6	—	0.6	—
11	23	0.6	< 0.3	—	< 0.3	< 0.3	1.4	1.3	13.6	—	< 0.3	—	2.4
12	19	0.6	< 0.3	0.6	< 0.3	< 0.3	5.3	1.2	56.4	4.8	0.6	—	3.2
13	23	0.6	0.6	4.3	< 0.3	0.4	8.3	1.5	48.5	36.1	0.8	< 0.3	5.3
14	22	1.4	—	1.7	1.9	1.5	—	4.2	100	1.6	2.3	< 0.3	8.7
15	21	3.0	—	—	5.9	—	3.5	—	52.2	—	—	—	3.6
16	26	0.6	< 0.3	0.4	1.1	0.7	2.8	—	10.1	0.4	0.7	—	2.4
17	23	6.5	2.3	5.6	6.3	10.5	16	12.5	56.4	9.3	9.2	4.9	7.1
18	18	3.7	—	3.9	4.2	3.1	14.6	4.7	52.2	4.6	4.6	1.2	—
19	19	2.3	0.7	1.7	1.0	1.4	6.7	—	60.3	1.2	2.9	0.6	3.1
20	20	0.8	< 0.3	0.8	0.4	0.5	11.3	—	49.9	< 0.3	0.5	—	1.9
21	19	5.2	1.1	2.0	4.2	4.9	14.4	4.9	> 100	9.0	5.1	2.2	7.5
22	23	1.4	< 0.3	3.0	0.7	1.4	11.6	3.1	91.9	0.5	0.6	1.4	5.1
23	22	2.0	2.8	2.2	5.0	0.4	—	2.8	0.4	3.6	3.9	1.5	0.7
24	28	4.7	—	—	—	10.9	32.9	9.9	> 100	—	—	—	—
25	22	11	—	9.6	7.4	9.8	90.7	10.8	> 100	—	9.2	—	14.5
26	25	< 0.3	< 0.3	—	—	—	—	—	—	< 0.3	—	—	—
27	21	1.1	—	—	—	3.6	> 100	3.8	> 100	39.8	1.4	—	9.8
28	20	< 0.3	—	—	—	< 0.3	< 0.3	—	—	—	—	0.5	—
29	20	0.4	< 0.3	—	—	—	9.2	2.8	100	—	—	0.4	—
30	19	0.9	0.6	1.0	—	0.7	7.0	6.7	85.5	1.8	—	0.5	6.2
31	22	2.2	< 0.3	0.6	2.3	2.4	5.6	3.3	85.5	0.7	—	1.6	3.6
32	22	0.5	0.6	—	—	4.9	0.5	—	< 0.3	3.0	0.5	—	0.6
33	22	1.9	1.7	5.3	—	1.5	15.5	—	50.3	—	4.4	—	7.4
34	27	0.5	—	< 0.3	0.4	0.4	3.1	1.2	13.3	< 0.3	< 0.3	0.8	1.0

Table 3. Spearman's correlation coefficients between the sera positive to *Platanus* (Pla) and to other pollen extracts analysed

	Ace	Art	Bet	Che	Cyn	Fra	Lol	Plan	Pop	Que	Tar
Pla	0.677**	0.543**	0.839**	0.696**	0.668**	0.790**	0.249	0.170	0.890**	0.786**	0.624**

** $P < 0.01$.

Acer sp. (Ace), *Artemisia vulgaris* (Art), *Betula alba* (Bet), *Chenopodium album* (Che), *Cynodon dactylon* (Cyn), *Fraxinus excelsior* (Fra), *Lolium perenne* (Lol), *Plantago lanceolata* (Plan), *Populus* sp. (Pop), *Quercus ilex* (Que) and *Taraxacum officinale* (Tar).

important information to interpret correctly the symptoms of patients with respiratory allergy.

The methods used for this type of studies certainly require further development, in order to ensure more reliable measurements [6]. Although it has been assessed that the Burkard cyclone sampler has the same sampling efficiency as the Burkard pollen trap [28], certainly there are other types of errors (systematic and random errors)

that may affect the entire method from the particles' sampling (e.g. sampler location) up to the analysis of the results (e.g. sample abundance) [41].

The measurement of allergens in the atmosphere carried out using the double-sandwich ELISA method does not allow the adoption of a single protocol for the immunochemical quantification of different plant allergens, and therefore, will require additional studies to

perform measurements of aeroallergens actually used in the routine.

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