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ORIGINAL ARTICLE

Molecular aerobiology – *Plantago* allergen Pla l 1 in the atmosphere

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Abstract

Introduction. Exposure to airborne pollen from certain plants can cause allergic disease, but allergens can also be found in non-pollen-bearing fractions of ambient air. This may explain why the allergic response in susceptible patients does not always coincide with the presence and magnitude of airborne pollen counts. *Plantago* pollen is an important cause of pollinosis in northern Mediterranean countries, but it is difficult to determine its incidence in allergies because *Plantago* pollen appears in the atmosphere at the same time as grass pollen.

Objective. The study aimed to investigate the relationship between the atmospheric concentration of Pla l 1 aeroallergen and *Plantago* pollen, and its incidence in a population group.

Materials and method. Pollen was sampled using a Hirst-type volumetric trap (Burkard™) and Burkard Cyclone sampler (Burkard™) for Pla l 1 allergen. Allergen was determined with a Pla l 1-specific ELISA. Serum-specific IgE levels to several plant allergens were measured with the EAST system.

Results. The aerobiological dynamics of *Plantago* pollen grains and Pla l 1 did not follow the same trend, whereas the sum of *Plantago* with some other pollen types showed a more similar behaviour. Of the 118 subjects tested, sera from 52 contained IgE to *Plantago* pollen, but only 5 were monosensitized.

Conclusions. The presence of Pla l 1 in the atmosphere depends not only on *Plantago* pollen but also on the pollen of other species from the Oleaceae family. Knowledge of the behaviour of allergen Pla l 1 in the atmosphere can help understand better asthma exacerbations associated with aeroallergens.

Key words

plantain, aeroallergens, Pla l 1, pollen, pollinosis

INTRODUCTION

The urban atmospheric environment contains many different airborne microorganisms, and our bodies need to be exposed to large amounts of biological particulate matter to develop a tolerance to foreign proteins. A hypo-responsive immune system would not have learnt to differentiate between self and non-self compatibility, and it has long been known that pollens and mould spores can induce symptoms of allergic respiratory diseases. These illnesses are progressively increasing in western countries due to environmental, anthropogenic and climatic factors. Changes in the growth pattern of plants, seasons of growth and length of the phenological period can change the geographic distribution of certain types of plants. Due to climate change, some pollen seasons are expected to start earlier and last longer. On the other hand, the natural or artificial introduction of non-autochthonous vegetation in urban areas has caused major changes in the immunological response of many patients to new respiratory allergens, and increased pollen-food reactions. This vegetation includes herbaceous plants native to certain areas and ecosystems, which readily expand and colonize other spaces to find suitable soil and climate conditions. Metabolic changes in these plants cause different responses, such as loss of leaves, double blooms or important variations in the duration of the floral phenological periods. At the same time, metabolic changes can modify the morphology of visible plant features, e.g., the size and shape of leaves and flowers, pollen grain size and aperture number. Modifications of this type have been observed in some species of the genus *Plantago*, specifically *Plantago lanceolata* L., a biannual herbaceous plant with ubiquitous distribution, especially in temperate zones. This plant grows well in the despoiled or removed soils often found around cities. Depending on environmental conditions, the morphology of *Plantago* pollen can vary widely. Many studies suggest that particles smaller than 20 microns enter the respiratory tract [1, 2] and when in contact with the mucous membranes, pollen grains release allergens that can penetrate the bronchial tubes causing asthma symptoms [3]. In addition, the air contains allergenic particles of plant origin smaller than pollen particulate (0.1–1 micron). These particles can pass through the nasal passages and enter the bronchi, quickly provoking an allergic response [1, 4, 5]. Research into these particles could yield more information on their allergenic activity in the atmosphere, and several

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studies have analysed the airborne concentration of allergens such as *Artemisia* (Art v 1) [6]; *Betula* [7, 8, 9, 10]; *Lolium* (Lol p 1) [11]; *Olea europaea* [12, 13], *Parietaria judaica* (Par j 1–2) [13, 14] and *Platanus* (Pla a 1) [15, 16]. *Plantago lanceolata* (plantain or English plantain) pollen is a major cause of pollinosis in temperate zones [17, 18, 19], but its allergenicity varies widely depending on the bioclimatic region. The incidence of *P. lanceolata* allergy has been reported in Australia [20] and in the Mediterranean area of Europe [21]. Approximately 30% of individuals are sensitized to this pollen, but most of them are also sensitized to pollen from other herbaceous plants, mostly grasses (most being polysensitized and therefore also allergic to the pollen of the herbaceous plants, mainly Gramineae). This makes it very difficult to evaluate the specific symptoms of plantain due to the concomitant presence of grass pollen and *Plantago* in the atmosphere. A high prevalence of *Plantago* pollen has been described in Spain [22, 23] with percentages reaching 45%, whereas cases of monosensitized individuals have only been described in the regions of Murcia and Castilla y León in Spain [24].

To date, one allergen of *Plantago* – Pla l 1 – has been analyzed and purified. This allergen is a polymorphic and hydrophilic protein with two bands in electrophoresis, one of approximately 17 kDa and the other of 20 kDa, corresponding respectively to non-glycosylated and glycosylated forms of the allergen. *Plantago* Pla l 1 allergen reacts with 93% of patients' sera [25] and is located outside the membranes in the extracellular spaces of the plant.

Clinical examination of individuals monosensitized to *Plantago* in the city of León, Spain, disclosed that when these individuals begin to show respiratory symptoms, few or no *Plantago* pollen grains are present in the atmosphere with airborne levels remaining <5 grains/m^3 for a long time. For this reason, the present study analysed the relationship between *Plantago* pollen concentration and the presence of the Pla l 1 allergen in the atmosphere of the city. The influence of climatic parameters on atmospheric *Plantago* release and variations in the concentration of this allergen were also studied.

MATERIALS AND METHOD

Ambient air sampling. The aerobiological samples were collected continuously during 2008, 2009 and 2010 at the University Campus of León in north-west Spain using a volumetric sampler, the Burkard Spore Trap [26], with an air flow of 10L/min. The counting method was that recommended by the Spanish Aerobiological Network (REA) [27]. The daily pollen data are expressed in grains per cubic meter (grains/m^3). 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 Company Ltd., Rickmansworth, UK) [28]. The atmospheric particles were collected dry, directly into a standard 1.5 mL Eppendorf vial every 24 h of sampling. The aeroallergens were sampled during the *Plantago* pollination period, from 25 April – 28 July 2008 and 2010, and from 25 April – 31 July 2009. The samples were stored at -20 °C. Both samplers were installed on a terrace 15 m above ground level.

Extraction and analysis of Pla l 1. Allergens were extracted using a modification of the method described by Takahashi et al. [29]. Dry samples (after centrifugation at 14,000 r.p.m., 1 min) were extracted with 120 µL of extraction buffer (50 mM phosphate buffer, pH 7.4, containing 150 mM sodium chloride, 125 Mm ammonium bicarbonate, 3 Mm EDTA and 0.005% Tween 20) for 2 hours at room temperature. The extract was separated by centrifugation at 4,000 r.p.m. for 10 min and stored at -20 °C.

Pla l 1 contents in aerosol samples were quantified using a double antibody sandwich ELISA method. The ELISA plates previously coated with 5 µg/well of a Pla l 1-specific monoclonal antibody (Abelló SA, Madrid, Spain) and then blocked (200 µL/well) with PBS-BSA-T (PBS with 1% bovine serum albumin with 0.005% Tween 20). The plates were then incubated for 1 h at 37 °C with 100 µL/well of the diluted test samples, and with purified Pla l 1 (Abelló SA), starting from a stock of Pla l 1 of 16.92 ng/mL, followed by seven serial dilutions of 1/3 in PBS-BSA-T, as reference curve and controls. After three washes with 200 µL PBS-T, plates were incubated for 1 h at 37 °C with 100 µL/well of a rabbit Pla l 1-specific polyclonal serum (Abelló SA) and incubated for 1 h at 37 °C with 100 µL/well of GAR/H+L/PO Goat Anti-Rabbit-H+L-Peroxidase, Calbiochem, Nottingham, UK) after three washes with 200 µL PBS-T. Finally, the enzyme activity of the antibody was determined by adding 200 µL/well of *o*-phenylenediamine (Sigma-Fast™ *o*-phenylenediamine dihydrochloride tablet sets Sigma) and incubated for 30 min at room temperature in the absence of light. This reaction was stopped by adding 50 µL 3M H_2SO_4 and the absorbance was then measured at 492 nm. The results of absorbance in the presented test samples were interpolated from the linear portion of the standard curve to establish the concentration of Pla l 1 allergen. Results were expressed in picograms per millilitre, with reference to the protein content of the standard preparation, and were subsequently converted into picograms per cubic metre according to the volume sampled by the apparatus. Natural protein was purified from *Plantago lanceolata* pollen extract.

Meteorological data. Meteorological data were supplied by the Department of Applied Physics of León University. Temperature, rainfall, relative humidity and wind speed were recorded continuously in the same periods of aeroallergen sampling.

Patients and sera. In the presented study, 118 students (36 males and 82 females; aged between 20–32 years) from the University of León in north-west Spain were included after obtaining their informed consent. Criteria for the final selection were a clinical history compatible with pollen allergy (rhinitis, rhinoconjunctivitis, or asthma), no previous immunotherapy and residence in León province for at least the previous four years. Patients were evaluated out of the pollen season (September – December, 2009–2010). All subjects displayed positive skin-prick test (SPT) reactions to one or more of the commercial pollen extracts (Bial Industrial Farmacéutica, Bilbao, Spain) for the most abundant pollens present in the area, according to the results of the León outdoor environment aerobiological survey. Histamine and saline solutions were used as positive and negative controls, respectively. Wheals equal or greater than 3 mm in average diameter were considered positive. Serum-specific IgE levels

to these pollen allergens were determined with the EAST System, Hytec specific IgE EIA kit (Hycor Biomedical, Kassel, Germany). Specific IgE levels were expressed in IU/ reference values: < 0.35 IU/mL (class 0) to > 100 IU/mL (class 5).

Previously, the authors had received a favourable report from the Ethics Committee of the University of León to perform the study.

IgE immunoblotting. *P. lanceolata* pollen proteins were separated by SDS-PAGE at 12.5% polyacrylamide under reducing conditions [30] and electrophoretically transferred onto polyvinyldifluoride membranes – PDVF, Hybon-P (GE-Healthcare, Uppsala, Sweden) [31]. After blocking, membranes were incubated at 4 °C overnight with diluted human sera. After washing, bound antibodies were detected with horseradish peroxidase-conjugated anti-human IgE (Dako, Copenhagen, Denmark). The blot was washed and developed by the ECL+ Western blotting detection system (GE-Healthcare, London, UK).

Statistical analysis. Correlation analysis was performed for non-parametric data using Spearman's rank correlation coefficient. The level of significance was assessed between P < 0.01 – P < 0.05. SPSS statistical software, version 14.0 (SPSS Inc, Chicago, IL, USA) was used for all statistical analyses.

RESULTS

Plantain pollen and Pla l 1 in the atmosphere. In general, the *Plantago* pollen season in the atmosphere of León is long, usually lasting from March – September, due to the flowering of two taxa in the urban area: *P. lanceolata* and *P. coronopus*. *Plantago* pollen was present in the city from March – September in 2008, and from March – October in 2009. However, the pollen was detected in the air nearly a month later in 2010, between mid-April – mid-October. During 2008, the seasonal pollen index-SPI (sum of daily pollen concentration during the analysed period) of *Plantago* pollen was 855, accounting for 4.8% of the total annual pollen in León. In 2009, the SPI value was 383, accounting for no more than 3.9%, and during 2010, the SPI value was 579, accounting for 3.9% of the total pollen count. In 2008 and 2009, the most intense pollination period occurred from May – June with a maximum peak of 51 grains/m^3 on 12 May 2008 and 16 grains/m^3 on 1 June 2009. In 2010, this period was irregular with the highest peaks (21 grains/m^3) observed on 29 April and 6 June (Fig. 1).

The atmospheric concentration of pollen and the Pla l 1 allergen did not follow exactly the same trend. The highest values of Pla l 1 were obtained on 4 May (82 pg/m^3) and 31 May (166 pg/m^3) in 2008 and 2009, respectively, whereas in 2010 high values were intermittent and the highest peak (123 pg/m^3) was obtained on 6 July. These dates did not coincide with the maximum values of *Plantago* pollen concentration. In 2008, the peak pollen concentration occurred eight days after the maximum protein value and four days before in 2009. In 2010, the highest allergen concentration was observed fifteen days after the peak pollen concentration. The sum seasonal protein concentration was lower in 2008 than in the other two years (426), although the airborne concentration of allergen was more continuous (Fig. 1).

Statistical analysis. With Spearman's correlation coefficient, the atmospheric concentration of Pla l 1 allergen, *Plantago* pollen concentration, Oleaceae pollen concentration and the sum of Oleaceae and *Plantago* pollen concentration was used. Data was also used from other major pollinic types in the atmosphere during the time of *Plantago* pollination, as well as meteorological parameters: temperature, relative humidity, precipitation, wind speed and solar radiation (Tab. 1). In 2008, low significant correlations were obtained with the *Plantago* pollen concentration (0.414), but the sum of Oleaceae and *Plantago*, *Quercus, Salix, Rumex* pollen concentration showed a significant and positive correlation (0.569). Taking into account the meteorological parameters for 2008, a significant and positive low correlation was observed with relative humidity and precipitation. In 2009, the correlation values were lower than the year before. A major level of association was obtained with the sum of *Plantago*, Oleaceae, *Quercus* and *Rumex* pollen concentration (0.463), followed by the total annual pollen. In terms of climatic parameters, Pla l 1 was correlated with the minimum temperature and the relative humidity during 2009, but the value was very low and negative. In 2010, there was no significant correlation with almost any parameter, except for *Salix* pollen concentration, maximum, minimum and mean temperature and solar radiation, but always with very low values.

Table 1. Spearman´s correlation coefficients between allergen Pla l 1 and *Plantago* pollen concentration, Oleaceae, Poaceae, *Quercus*, *Salix* and *Rumex* pollen concentration, the sum of *Plantago* and Oleaceae pollen concentration, the sum of *Plantago*, Oleaceae, *Quercus* and *Rumex* pollen concentration in same seasonal period and main meteorological parameters for 2008 and 2009. * P< 0.005, ** P<0.01

Year	2008	2009	2010
Allergen	Pla l 1	Pla l 1	Pla l 1
Plantago	0.414**	0.299**	0.224
Oleaceae	0.216	0.374**	-0.176
Poaceae	-0.229	0.263*	0.166
Quercus	0.608**	0.409**	-0.012
Salix	0.383**	0.153	-0.324**
Rumex	0.437**	0.452**	0.218
The sum *of Plantago* and Oleaceae	0.415**	0.389**	0.216
The sum of *Plantago*, Oleaceae, *Quercus* and *Rumex*	0.569**	0.463**	0.140
Seasonal Pollen Index (SPI)	0.278*	0.417**	0.180
Maximum temperature	-0.276*	-0.018	0.366**
Minimum temperature.	0.026	-0.235*	0.265*
Mean Temperature	-0.192	-0.132	0.348**
Precipitation	0.468**	-0.021	-0.065
Relative Humidity	0.483**	-0.243*	-0.149
Wind Speed	0.076	-0.129	-0.002
Solar radiation	-0.292*	0.080	0.314**

Patient sensitization. All patients tested had a positive-SPT reaction to some commercial extracts from *Acer* sp., *Artemisia vulgaris, Betula alba, Chenopodium album, Cupressus arizonica, Cynodon dactylon, Fraxinus excelsior, Lolium perenne, Olea europaea, Pinus* sp., *P. lanceolata, Platanus acerifolia, Populus* sp., *Quercus ilex* and *Taraxacum officinale*. Specific IgE to *P. lanceolata* pollen extract was detected in 52 out of 118 sera analyzed (44.1%) (Tab. 2).

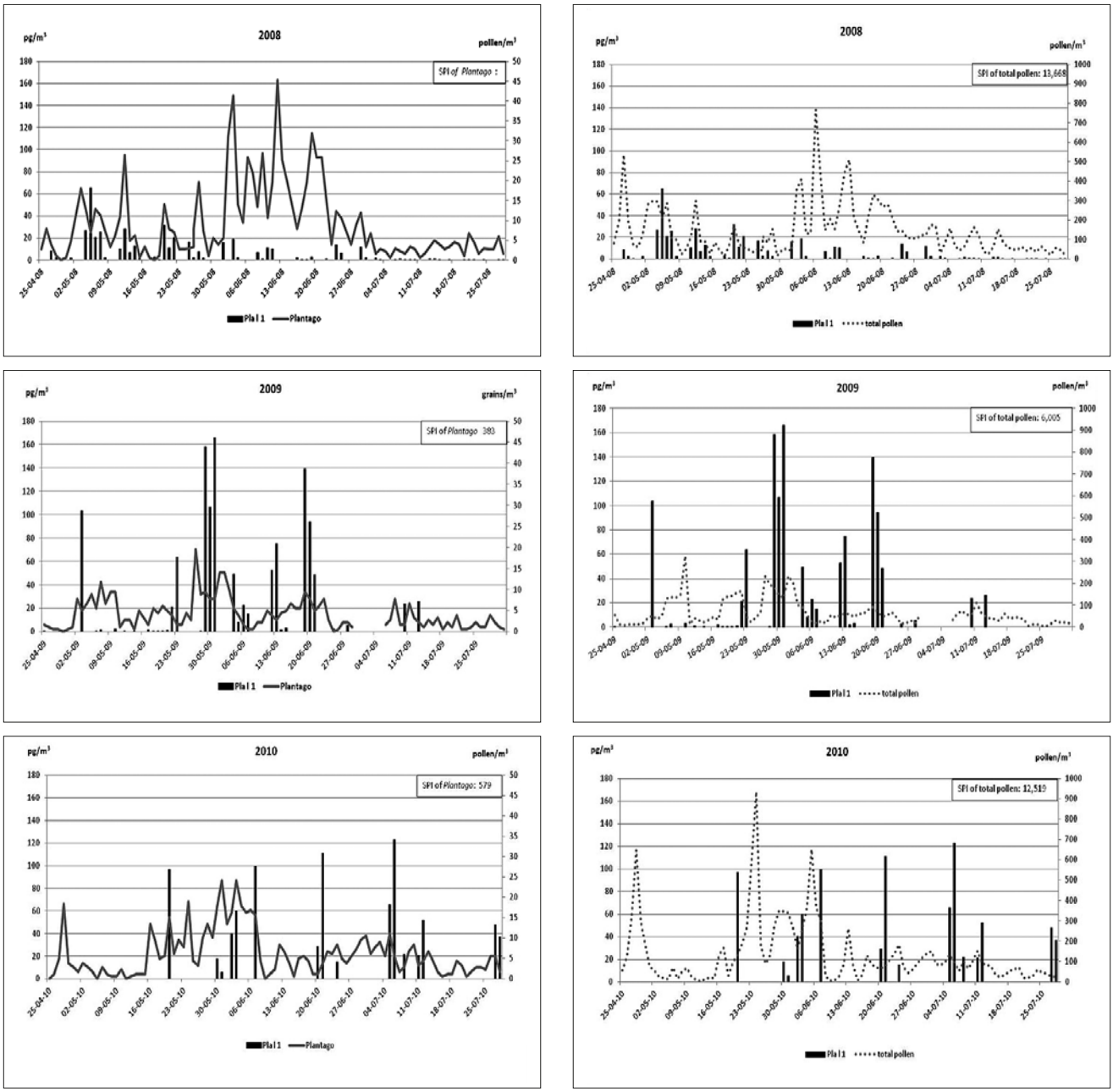

Figure 1. *Plantago* pollen concentration (pollen/m^3) (——); total pollen types (• • • •) and Pla l 1 protein concentration (pg/m^3) (■■■). SPI – Sum of daily pollen concentration during the analysed period

The average specific IgE level to *Plantago* pollen extract was 8.9 IU/mL including one with a range > 100 IU/mL (class 5), six in the range 17.5–99.9 IU/mL (class 4), 18 in the range 3.50–17.49 IU/mL (class 3), 20 in the range 0.70–3.49 IU/mL (class 2), and seven in the range 0.35–0.69 IU/ml (class 1). Only five patients were monosensitized to *P. lanceolata* pollen extract (one class 1, three class 2, and one class 3), while the remainder presented specific IgE to other pollen types. Statistical analysis of the correlation between *Plantago*-positive sera and sera positive to other pollen extracts revealed low but significant correlations ($P < 0.01$) with *Acer, Artemisia, Chenopodium, Platanus* and *Populus* (Fig. 2).

Immunodetection with P. lanceolata pollen extract. Allergenic profiles of serum samples using *P. lanceolata* extract were obtained from five patients IgE monosensitized to *P. lanceolata* pollen extract, and eight polysensitized patients (Fig. 3) according to skin prick test using *Plantago lanceolata* extract. Bands at 16.6 and 18.5 kDa, corresponding to the two isoforms of Pla l 1 (non-glycosylated and glycosylated, respectively) were detected in the majority of the sera. Sera from monosensitized patients (lanes 9–13) with a low specific IgE value reacted only with the two bands. In addition to these two bands, sera from polysensitized patients (lanes 1–8) also reacted with bands at 32.0, 36.3 and 45.0 kDa (Fig. 3).

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

Age	*Plan.*	*Ace.*	*Art.*	*Bet.*	*Che.*	*Cy.*	*Fra.*	*Lol.*	*Plat.*	*Pop.*	*Que.*	*Tar.*
21	3.8					4		46.3				4
23	2.9	2.2	2.4	2	1.3	16.9	3.1	100	1.7	2.9		7.3
20	13.3	0.6	3.1	4.3	1.8	39.4	7.6	>100	2.3			8.8
20	0.4	<0.35	<0.35	<0.35	<0.35		<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
21	24.6	4.1	6.3	5.2	10.4	64.4	11.9	>100	9	6.3	4.5	13.2
22	15.9	<0.35		<0.35	<0.35	<0.35	6.4	1.1				
20	1.1	0.9	0.7	1.4	0.8	8	2.1	81.9	1.2	0.7		4.6
20	3.8											1.6
19	0.8			0.8	2	38.2	5.5	>100	<0.35	<0.35	<0.35	10.5
18	2.4		4.9	0.4	0.9		1.3	34.5	1.3		1.2	6.6
21	0.9		4.6	<0.35	0.4	5.1		13.8	1			2.8
26	2				0.9		1.6	0.8		2.2		0.8
22	1.9				2.4	8.4		16.5				3
23	0.8				0.6	0.6		2.5			0.7	
32	2.2	0.7	0.7	0.8	1.1	4	2.3	11.1	1.4	0.6	1.5	1.4
17	30.6	0.6	1.2	1.5	0.8	1.3	1.5	5.3	1.3		0.6	
24	13.8				0.6	17.1	3.2	100				7.5
19	10.2		2.1		1.7	41.8	3.6	>100				12
19	4.8	<0.35	0.6	<0.35	<0.35	5.3	1.2	56.4	0.6	0.6		3.2
21	>100				4.4	66.4		88.6				
23	36.1	0.6	4.3	<0.35	0.4	8.3	1.5	48.5	0.6	0.8	<0.35	5.3
22	1.6		1.7	1.9	1.5		4.2	100	1.4	2.3	<0.35	8.7
26	0.4	<0.35	0.4	1.1	0.7	2.8		10.1	0.6	0.7		2.4
23	9.3	2.3	5.6	6.3	10.5	16	12.5	56.4	6.5	9.2	4.9	7.1
25	3.3											
23	1.2				1.3	6.3	79.7	38.3				2.5
18	4.6		3.9	4.2	3.1	14.6	4.7	52.2	3.7	4.6	1.2	
18	57.3				1.2	4.5	1.8	30.8				3.1
19	1.,2	0.7	1.7	1	1.4	6.7		60.3	2.3	2.9	0.6	3.1
32	1.2											
22	1.8				<0.35	5.7	1.5	43.6				1.3
19	9	1.1	2	4.2	4.9	14.4	4.9	>100	5.2	5.1	2.2	7.5
22	2.4		1.2			15.4		>100				9.2
23	0.5	<0.35	3	0.7	1.4	11.6	3.1	91.9	1.4	0.6	1.4	5.1
24	8.8				0.5			3.9				<0.35
21	1.2											
22	3.6	2.8	2.2	5	0.4		2.8	0.4	2	3.9	1.5	0.7
21	76.7							4.4				
20	2.5				<0.35	10.5	1.5	90.7				5.3
21	39.8				3.6	>100	3.8	>100	1.1	1.4		9.8
22	10.6				1.1	0.8		2.3				
19	4.3				1.2		4.4			0.9		2.2
19	0.6											
19	1.8	0.6	1		0.7	7	6.7	85.5	0.9		0.5	6.2
22	0.7	<0.35	0.6	2.3	2.4	5.6	3.3	85.5	2.2		1.6	3.6
20	13.5							3.4				0.8
22	3	0.6			4.9	0.5		<0.35	0.5	0.5		0.6
31	7.5											
20	0.6			2.4	1.3			6.1			0.4	1.2
24	8.3				<0.35	19.4	1.1	50.3				3.3
23	4							0.5				
22	0.4				0.5							

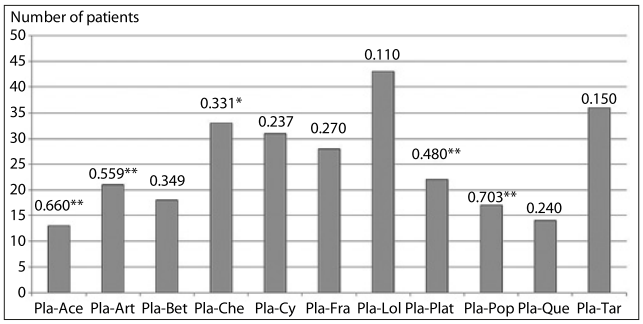

Figure 2. Spearman´s correlation coefficients (**P < 0.01, *P < 0.05) and number of patients with sera positive to *Plantago* (Pla) and to other pollen extracts analyzed: *Ace.* (*Acer* sp.), *Art.* (*Artemisia vulgaris*), *Bet.* (*Betula alba*), *Che.* (*Chenopodium album*), *Cyn.* (*Cynodon dactylon*), *Fra.* (*Fraxinus excelsior*), *Lol.* (*Lolium perenne*), *Plat.* (*Platanus hispanica*), *Pop.* (*Populus* sp.), *Que.* (*Quercus ilex*) and *Tar.* (*Taraxacum officinale*)

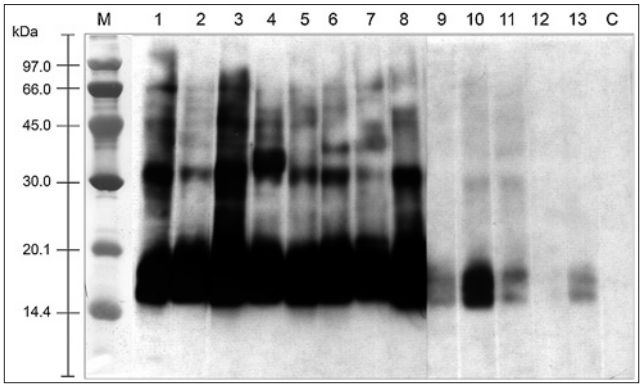

Figure 3. Reactivity of serum from patients sensitized to *P. lanceolta* pollen extract. Lane M – molecular weight markers; lanes 1–8 – sera from polysensitized patients; lanes 9-1 – sera from monosensitized patients; lane C – control sera pool (western blot)

DISCUSSION

In the presented study, the aerobiological dynamics of *Plantago* pollen and Pla l 1 concentrations did not follow the same trend during the period of plantain flowering. Other reports found protein concentration peaks before and after the pollination period, and a major similarity during the pollination period for other types of plants [10, 11, 12, 16, 32]. Some authors emphasize that these variations may be due to the presence of allergenic particles released from other parts of the same plant or from Ubisch bodies. A study on Gramineae [33] proved that most of the Ubisch bodies are not emitted but remain within the anthers, even after the release of allergenic pollen grains. In *Plantago lanceolata*, Ubisch bodies remain in the anther after the pollen has been dispersed (personal observation). In the presented study, correlations were found between Pla l 1 and pollen concentrations of the other pollen types such as Oleaceae, *Quercus* and Salicaceae. Pla l 1 is an extracellular glycoprotein whose function is not defined and these proteins can be found in other plants whose pollen proteins share the isoforms and variants of a given protein. For example, Pla l 1 and members of the Ole e 1 family [34, 35] have common epitopes and very similar amino acid sequences. Plants of the Oleaceae family (*Fraxinus, Syringa, Forsythia, Ligustrum*) were flowering in the city of Leon during the period of the study and could have produced cross-reactions. This could be one explanation why the total amount of allergen (1194) was much higher in 2009 that in the other two periods (426 in the 2008 and 841 in the 2010), although the *Plantago* seasonal pollen index-SPI was the lowest of the three years analyzed, whereas the Oleaceae SPI (289) was double the pollen concentrations in 2008 and 2010. In addition, a great amount of grass pollen was present and also showed some similarity to the minor grass allergen Phl p 11 [36]. Weber reported a cross-reactivity between plant allergens of groups of the Fagales order (*Salix, Populus, Quercus*) and an Ole e 1-like protein family [36]. These taxa were plentiful in the atmosphere of the city during the first phase of the *Plantago* pollination period. Hence, the similarity of the Pla l 1 and Ole e 1 amino acid sequences could also explain the discrepancy between the pollen and allergen curves.

Most Pla l 1 allergen was observed during the phenological phases 1 and 2 of plantain, when between 25% – 75% of the *P. lanceolata* flowers were open, emphasizing their cross-reactivity with other groups of plants. However, during phenological phase 3 (75% – 100% of flowers open) *P. coronopus* was also in flower. This species is small in size and pollinated in summer, but well represented in the study area; this would also explain the allergen peaks detected in the atmosphere during July and August. Some authors emphasize that pollen's capacity to release allergens may derive from the type of flower structure and weather conditions at the time of pollen emission [10]. However, in the presented study it was found that the pollen concentration was inversely proportional to the airborne allergen concentration, meaning that the years with higher *Plantago* pollen counts had lower amounts of allergen and *vice versa*. This may be related with the plant's degree of 'allergen synthesis', which will ensure better reproductive success during periods of low pollen production due to environmental stress, as demonstrated for other plants [37].

According to the correlation results, the meteorological parameters having most impact on atmospheric Pla l 1 concentration in 2008 were relative humidity and rainfall. The very low correlation of these parameters in 2009 and 2010 was due to the 10% lower humidity and 50% less rainfall compared to 2008. Some authors have suggested that high atmospheric humidity has an optimum effect on the release of allergenic proteins from pollen grains during their metabolic activation [38] and pollen tube growth in air known as 'abortive germination' [39]. In general, these aeroallergens are highly stable proteins even under extreme environmental conditions, but some of them remain attached to the pollen surface after their emission, or are dispersed by other pollen vectors and particles present in the air at the same time. In addition, the agglomeration of these molecules, under certain humidity conditions [40], often allows aeroallergens to float in the air for periods of time depending on meteorological conditions, such as convective air movements, wind gust, calm periods or rainfall.

Recent experimental studies [41] describe a direct relationship between temperature increase and CO_2 in the atmosphere and further development of the biomass of certain herbaceous plants such as pollen production and, consequently, allergenicity of plants. The explanation is based on the fact that an increase of these parameters increases the photosynthetic capacity of the plants, and some plants enriched in CO_2 produce a high percentage of unsaturated oils that make them more antigenic. However, it is difficult to prove these facts in nature, especially in areas with low industrialization as in the city of Leon where in the last ten

years there have been no substantial changes in CO_2 levels and the temperature has not shown large annual variations. In the presented study, there were no significant changes in temperature over the three years, and in any case, it was a period of time too short to assume the effects of climate change on the production of aeroallergens. In addition, because of the climatic conditions of the city of Leon, in spring there are frequent strong winds that easily disperse atmospheric particles of any origin, and in early summer storms that may partly explain the increased presence of airborne allergens at certain times. Several authors noted that pollen aeroallergens are released during rain episodes [42, 43, 44].

Many allergic patients were found polysensitized to plantain, as only five patients (about 10%) were monosensitized in the presented study. Similar results were reported in Japan by Nakamura et al. [45] who failed to find any monosensitized individuals. The high percentage of individuals polysensitized to *Plantago* pollen may be due to co-sensitization or cross-reactions between different allergenic particles. Weber suggested that in most cases sensitization to grass pollen, *Betula*, *Olea europaea*, *Parietaria judaica*, *Plantago*, *Artemisia* and Chenopodiaceae coexist [36]. This was clinically confirmed in the presented study by the correlation between IgE from patients sensitive to *Plantago* and IgE for some of these groups of plants. The main IgE-reacting proteins among sera from mono- and polysensitized patients were detected at 16.6 and 18.5 kDa, corresponding to the 17 and 20 kDa of non-glycosylated and glycosylated Pla l 1 described by Calabozo et al. [18].

CONCLUSIONS

This is the first study on the Pla l 1 allergen in the atmosphere. In general, the atmospheric concentration of the Pla l 1 aeroallergen depends not only on the concentration of *Plantago* pollen, but also on factors, such as other pollen types and meteorological conditions, i.e., rainfall and relative humidity. This fact and the correlations obtained with the serum patients suggest a possible cross-reactivity between *Plantago* (Pla l 1) and Oleaceae (Ole e 1) – Fagales pollen allergens.

The impact of climate change on vegetation, and in particular on the reproducing of plants, is totally dependent on the type of geographical area and its climate; therefore, it is necessary to have historical pollen data collected fir at least 30 years in order to analyze trends and tendencies correctly. The aerobiological monitoring of allergens is a strategic tool for monitoring the presence of pollen in the atmosphere currently considered allergens and the appearance of pollens that normally do not belong to the pollen atmospheric spectrum of a certain geographical area. The molecular aerobiology, which is the measurement of allergen content of the pollen and/or airborne particles, could be important because there are sufficient scientific evidences on possible cross-reactivity between other proteins of biological origins which could amplify the allergic respiratory response.

Knowledge of the behaviour of allergen Pla l 1 and similar molecules in the atmosphere, can help better understanding of asthma exacerbations associated with aeroallergens.

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