

Platanus pollen as an important cause of pollinosis

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Objective: The existence of *Platanus* pollinosis is not generally accepted despite the production of very large quantities of airborne *Platanus* pollen in many cities of the United States and Europe. The aim of this study was to investigate if *Platanus* pollen really contributes to the symptoms of the patients with pollinosis in the Madrid area.

Methods: We carried out systematic skin prick testing with *Platanus* pollen extract on 47 patients seen in our allergy center with spring-summer pollinosis symptoms. Each patient maintained symptom score diaries before, during, and after the *Platanus* pollination season. The average symptom scores were calculated and compared with the *Platanus* pollen counts. Measurements of specific IgE by ELISA and immunoblotting also were performed in each patient.

Results: The *Platanus* skin tests were positive in 33 of the 39 patients first seen with seasonal symptoms during *Platanus* pollen season and only in three of the eight patients without symptoms during *Platanus* exposure (Fisher's exact test; $p < 0.05$). Twenty-two of the 33 *Platanus*-positive skin test patients also had a positive ELISA result. Furthermore, the average 24-hour rhinitis symptom scores of the 39 patients first seen with seasonal symptoms during March through April showed significant correlation with *Platanus* pollen counts ($r_s = 0.57$, $p < 0.05$). The immunoblot results suggest that a 17 kd pollen protein could be a major allergen in patients with *Platanus* pollinosis.

Conclusions: *Platanus* pollen is an important cause of pollinosis in Madrid. A protein with a molecular weight of 17 kd appeared to be its major allergen. (J Allergy Clin Immunol 1997;100:748-54.)

Key words: *Platanus* pollen, London plane tree, airborne pollen, allergenic pollen, pollinosis

The plane trees, or sycamores, are widely grown as street and shade trees in the United States, the most common being *Platanus occidentalis* (American sycamore) and *Platanus acerifolia* or *hybrida*, a hybrid between *Platanus occidentalis* and *Platanus orientalis*, called London plane tree. Likewise, the London plane tree is one of the trees most frequently found in the majority of western European cities.¹ However, despite

Abbreviations used

SPT:	Skin prick test
PBS:	Phosphate-buffered saline
PAGE:	Polyacrylamide gel electrophoresis

all plane-tree species being wind-pollinated and shedding large amounts of buoyant pollens, there are very few works on the allergenicity produced by this pollen, and it is believed to have a minor and doubtful importance with regard to pollinosis.²⁻⁷

Recently we have reported that *Platanus hybrida* pollen was able to cause rhinitis and asthma symptoms in three patients living in Madrid, Spain.⁸ Furthermore, we also observed that at the present in Madrid there is a very high prevalence (56%) of patients with pollinosis with positive SPT results to *Platanus* pollen. However, in nearly all cases a positive reaction to grasses was also observed. For this reason it was difficult to estimate the true clinical relevance of *Platanus* pollen in the Madrid area.⁹

The aim of this work was to investigate whether *Platanus* pollen actually contributes to the pollinosis symptoms of patients with seasonal rhinoconjunctivitis, seasonal asthma, or both having positive SPT results to both *Platanus* and grass pollen extracts in the Madrid area.

The presence of a cross-reactivity between *Platanus* and grasses was also investigated, as well as the protein pattern of reactivity.

METHODS

Patients

We have evaluated the percentage of positive SPT responses to common aeroallergens in one group of 70 patients of either sex who had come spontaneously to our clinic consecutively in 1994. Patients were selected for a history of seasonal or perennial rhinitis, asthma, or both. Each patient's evaluation was made by physician assessment that included a case history, clinical examination, and SPT. From this group we have selected the patients with positive SPT responses to pollens and with seasonal clinical symptoms during spring, summer, or both. There were 47 patients of either sex, with ages ranging from 8 to 57 years (median, 29 years), born and still living in or around Madrid.

A second larger group of 262 patients of either sex with seasonal allergy was chosen at random and studied in our center

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TABLE I. Presence of airborne pollen and positive SPT responses

Family or genus	%	Positive SPT responses		
		Species	Common name	(%)
<i>Platanus</i> spp	59	<i>Platanus hybrida</i>	London plane tree	74
<i>Quercus</i> spp	15	<i>Quercus rotundifolia</i>	Live oak	17
<i>Populus</i> spp	7	<i>Populus alba</i>	White poplar	24
<i>Morus</i> spp	7	<i>Morus alba</i>	White mulberry	39
<i>Pinus</i> spp	4	<i>Pinus sylvestris</i>	Scotch pine	6
Cupressaceae	2	<i>Cupressus arizonica</i>	Arizona cypress	18
Poaceae	1	<i>Dactylis glomerata</i>	Orchard grass	100
Others	5			

Airborne presence in March through April (percent of the total March through April pollen counts from 1995) and positive SPT responses (percent positive reactions in 47 patients with pollinosis) in Madrid.

from 1994 to 1995. Their ages ranged from 5 to 62 years (median, 26 years). Seasonal allergy was confirmed by history and positive SPT results to pollen extracts (wheal diameter of 3 mm or greater).

Pollen counts

The pollen counts were made according to a previously described, standardized technique with a Burkard spore trap for 7 days.⁹ The trap was installed on the roof of our center, which is 20 m high, in the east of the Madrid urban area. The sampling period included the months of January to December 1995.

Symptom scores

Ocular symptoms of redness, itching, and lacrimation and nasal symptoms of sneezing, itching, congestion, and rhinorrhea were assessed by all 47 patients (group I) on a daily basis with the visual analogue scale as follows: 0 = absence of symptoms, 1 to 3 = mild symptoms, 4 to 7 = moderate symptoms, and 8 to 10 = severe symptoms. In the same way bronchial symptoms of wheezing, tightness, coughing, and shortness of breath were assessed. Diary cards were kept daily from February 1995 until June 1995.

Collection of pollen samples

Selection and collection of mature male capitula was done in the morning. Collected inflorescences were immediately vacuum-dried at 35°C. Mechanical sieving was later done with the dried product by using 3 mm, 0.5 mm, 160 µm, 80 µm, and 40 µm mesh sieves. Microscopic control was then undertaken to ensure that the purity of the pollen was at least 98%. Finally, 24-hour vacuum drying of the purified pollen at 35°C was carried out.

Preparation of pollen extracts

Preparation of pollen extracts was accomplished with a previously described method.¹⁰ Two grams of *Platanus* pollen were defatted with acetone, dried, and extracted in 40 ml of 0.01 mol/L phosphate and 0.15 mol/L NaCl phosphate-buffered saline (PBS; pH 7.4), and the mixture (1:20 wt/vol) was stirred for 12 hours at 4°C. The suspension was filtered through a filter paper (Whatman Ltd., Maidstone, U.K.), dialyzed against PBS, and sterilized by 0.22 µm filtration (Millipore, Molsheim, France).

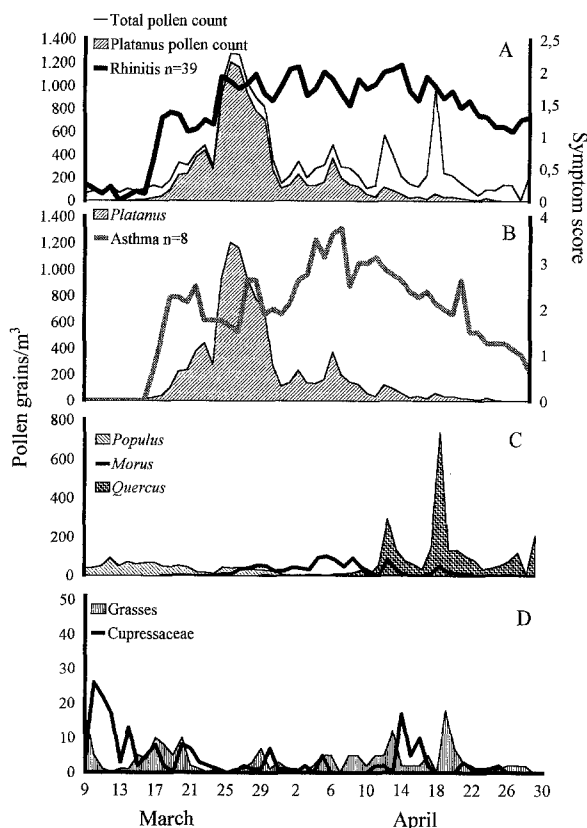


FIG. 1. A, Correlation between mean rhinitis symptom scores recorded by 39 pollinosis patients on their diary cards and *Platanus* pollen counts obtained with use of a Burkard trap (Spearman rank correlation, $r_s = 0.57$, $n = 53$, $p < 0.001$, two-tailed significance). B, Correlation between mean asthma symptom scores recorded by eight pollinosis patients on their diary cards and *Platanus* pollen counts (Spearman rank correlation, $r_s = 0.58$, $n = 53$, $p < 0.001$, two-tailed significance). C and D, Daily mean values of the *Populus* spp, *Morus* spp, *Quercus* spp, grasses, and *Cupressaceae* pollen counts in Madrid in 1995.

Poa annua, *Dactylis glomerata*, *Cupressus arizonica*, *Populus alba*, *Morus alba*, and *Quercus rotundifolia* extracts also were performed in the same way.

Protein concentration was determined by the use of Bio Rad protein assay (Bio Rad Laboratories, Richmond, Va.), with a final concentration of 850 µg/ml in protein for *Platanus* extract and 1250 µg/ml for *Dactylis glomerata* extract.

Skin tests

In all 47 patients and 10 atopic patients without pollinosis used as control subjects, SPTs were performed with *Cupressus*, *Populus*, *Morus*, *Platanus*, *Dactylis*, and *Quercus* extracts (Immunotek Lab, Madrid, Spain). A battery of other common inhalant allergens were also used for skin prick testing. This battery included *D. pteronyssinus*, cat dander, *Alternaria*, *Cladosporium*, and *Aspergillus* extracts (Leti Laboratory, Madrid, Spain).

Histamine chlorhydrate (10 mg/ml) was used as a positive control and 50% glycerol in PBS was used as a negative control. All SPTs were read after 15 minutes. A positive reaction was defined as a wheal of at least 3 mm by 3 mm in the presence of a negative reaction to the 50% glycerol and a positive test result with histamine chlorhydrate.

TABLE II. Characteristics of the 47 patients

Group	Sex		Age (median)	Clinical manifestations (%)†					Total IgE (IU/ml)	Specific IgE‡		Positive SPT result (%)	
	M	F		SC	KJ	A	(A)	NS		Platanus	Dactylis	Platanus	Dactylis
I	5	6	33	54	36	0	18	46	385 ± 79	0.23 ± 0.02	1.33 ± 0.24	0	100
II	28	8	27	91	78	22	33	9	410 ± 52	0.82 ± 0.10	1.28 ± 0.13	97	100
Control*	3	7	23						104 ± 34	0.15 ± 0.04	0.09 ± 0.07	0	0

The 47 pollinosis patients were classified into two groups according to the absence (group I, $n = 11$) or presence (group II, $n = 36$) of positive SPT and/or ELISA responses to *Platanus* pollen.

SC, Spasmodic coryza; KJ, conjunctivitis; A, asthma; (A) patients with seasonal asthma in spring and/or summer but without asthma symptoms in March through April of 1995; NS, no pollinosis symptoms in March and/or April.

*Ten atopic subjects without pollinosis used as control subjects.

†Patients with symptoms in March and/or April.

‡Results are expressed in optical densities at 492 nm as mean ± SEM.

TABLE III. Comparative SPTs among patients with pollinosis with or without sensitization to *Platanus* pollen

SPTs	Patients allergic to <i>Platanus</i> pollen ($n = 155$)	Patients not allergic to <i>Platanus</i> pollen ($n = 107$)	Wilcoxon rank	
			Z-score	p
+Control (histamine)	4.55	4.30	-1.79	0.07
-Control (glycerolsaline)	0.12	0.03	-1.00	0.31
<i>D. pteronyssinus</i>	0.49	0.06	-2.36	0.01
Cat dander	1.96	1.21	-1.50	0.13
Dog hair	1.28	0.76	-0.83	0.40
Horse hair	0.91	0.49	-0.88	0.37
<i>Blatta orientalis</i>	0.67	0.19	-1.47	0.14
Feathers	0.22	0.19	-0.71	0.47
<i>Cupressus</i> pollen	3.12	1.30	-6.79	<0.01
<i>Dactylis</i> pollen	7.25	5.62	-7.61	<0.01
<i>Olea</i> pollen	5.92	2.75	-7.98	<0.01
<i>Alternaria</i>	1.21	0.37	-4.50	<0.01
<i>Cladosporium</i>	0.80	0.27	-3.90	<0.01
<i>Aspergillus fumigatus</i>	0.57	0.08	-3.55	<0.01
Atopy score	24.39	13.29	-8.66	<0.01

Results are expressed in mean wheal diameters (mm) subtracted from the negative control.

In vitro tests

Total IgE and specific IgE measurement. Total IgE FAST (3M Diagnostic System Inc., Santa Clara, Calif.) was performed according to the manufacturer's instructions. Specific IgE to *Platanus* and *Dactylis* extracts was determined by ELISA, as described.¹⁰ Briefly, protein extracts (5 µg/ml) were bound to 96-well polystyrene microplates (Costar, Cambridge, Mass.) by overnight incubation at 4° C. After washing with PBS-0.1% Tween, plates were neutralized with 1% bovine serum albumin (1 hour at room temperature), washed, and incubated with undiluted serum samples (4 hours at room temperature). Plates were washed and incubated with 1:1000 dilution of mouse monoclonal antibody to human IgE (Sigma Chemical Co., St. Louis, Mo.). After the incubation period and a washing step, wells were filled with peroxidase-labeled goat antibodies to mouse IgG (Sigma). After a final washing, a substrate solution was added and the color was allowed to develop. The enzymatic reaction was stopped with HCl, and the absorbance values were measured at 492 nm. Results were considered positive when

above the mean +3 SD of a control group (10 atopic patients without pollinosis).

ELISA was also used to carry out inhibition assays, as previously described in detail.¹⁰

Extracts from *Platanus* and *Dactylis* adjusted in protein were used as inhibitors. *Aspergillus fumigatus* was used as an unrelated antigen for control of specificity.

Polyacrylamide gel electrophoresis (PAGE) and immunoblotting. *Platanus* and *Dactylis* extracts were diluted in 0.1 mol/L Tris HCl, 2% sodium dodecyl sulfate, 10% glycerol, and 0.01% bromophenol blue. Electrophoresis and immunoblotting were carried out as described.¹¹ Proteins were stained with 1% Coomassie blue stain, and their molecular weight was estimated with commercial protein standards. Once the protein bands were transferred to nitrocellulose sheets, they were neutralized and incubated with the sera to be tested (overnight at 4° C). After washing, the sheets were incubated with a dilution of mouse monoclonal anti-human IgE (Sigma), then with a second antibody (anti-mouse IgG-biotin conjugated), and finally with

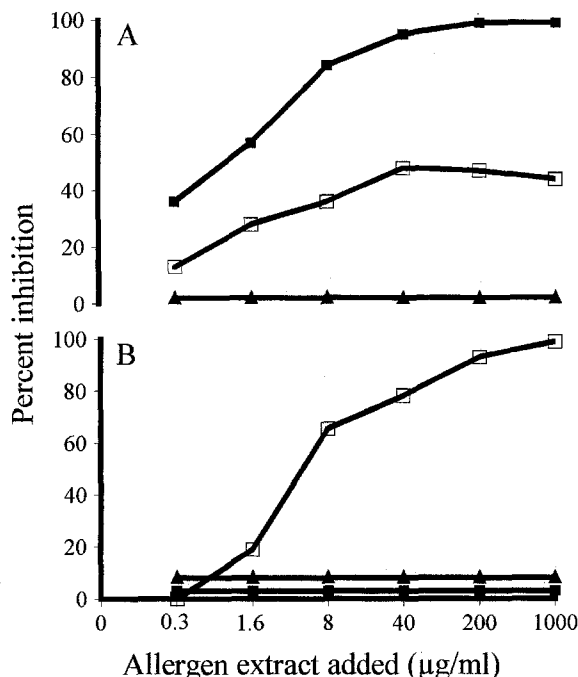


FIG. 2. ELISA inhibition assays to *Platanus* and *Dactylis* pollens with use of pool of 10 patient serums with specific IgE to both *Platanus* and *Dactylis* pollen extracts. **A**, *Platanus hyrida*; **B**, *Dactylis glomerata* with *Platanus* pollen inhibitor (filled squares), *Dactylis* pollen inhibitor (open squares), and *Aspergillus fumigatus* inhibitor (filled triangles).

streptavidin conjugated with alkaline phosphatase. After a final washing, the membranes were soaked in a substrate solution, and the enzymatic reaction was stopped by rinsing with distilled water.

Statistical analysis

Spearman rank correlation was used to analyze the correlation between the pollen counts and patient symptom scores. The Wilcoxon test was used to analyze the statistical association between specific IgE and SPT results and to assess differences between the mean wheal diameters to allergens tested in patients with pollinosis who had positive and negative SPT responses to *Platanus* pollen extract. Comparison of March to April pollinosis symptoms with positive SPT results to *Platanus* pollen was done with the two by two contingency table (Fisher's exact test).

RESULTS

SPT and pollen counts

The frequency of positive SPT results for all studied pollen allergens in the group of 47 patients with pollinosis of either sex is summarized in Table I. The percent values have been calculated on the total number of patients with positive SPT results, considering also the polysensitizations.

All patients had a positive reaction to grass pollens, and 74% of them showed, in addition, SPT results positive to *Platanus* pollen, 39% to *Morus* extract, and 24% to *Populus* extract. None of the control subjects had positive SPT responses to the allergen battery. As can be

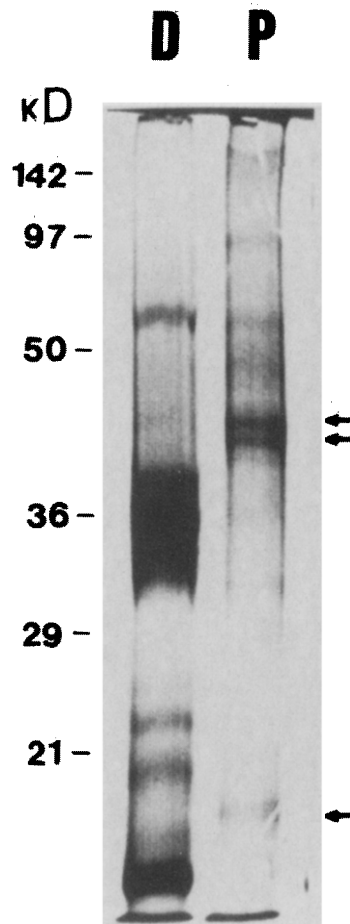


FIG. 3. PAGE of *Dactylis* (D) and *Platanus* (P) pollen extracts (Coomassie blue staining). Arrows indicate *Platanus* major protein bands (45, 43, and 17 kD).

seen in Fig. 1, *Platanus* pollen counts mainly appeared from mid-March to mid-April. During these 2 months, the most frequent pollen types collected in our spore trap were *Platanus* (59% of the total pollen sampled March to April), *Quercus* (15%), *Populus* (7%), *Morus* (7%), *Pinus* (4%), *Cupressaceae* (2%), and grasses (1%) (Table I and Fig. 1).

Symptom scores

To investigate the contribution of *Platanus* pollen in the March through April pollinosis symptoms, our 47 patients were classified into two groups according to the absence (group I, $n = 11$) or presence (group II, $n = 36$) of positive SPT, ELISA, or both types of results to *Platanus* (Table II).

As shown, while 54% of group I patients had symptoms in March through April, these symptoms were present in almost all patients of group II (91%). In other words, only 9% of this latter group were free of symptoms versus 46% of group I (Fisher's exact test; $p < 0.05$).

In most patients, symptoms were associated with oculorhinitis, although asthma was present in eight of them. Among patients with rhinitis symptoms in

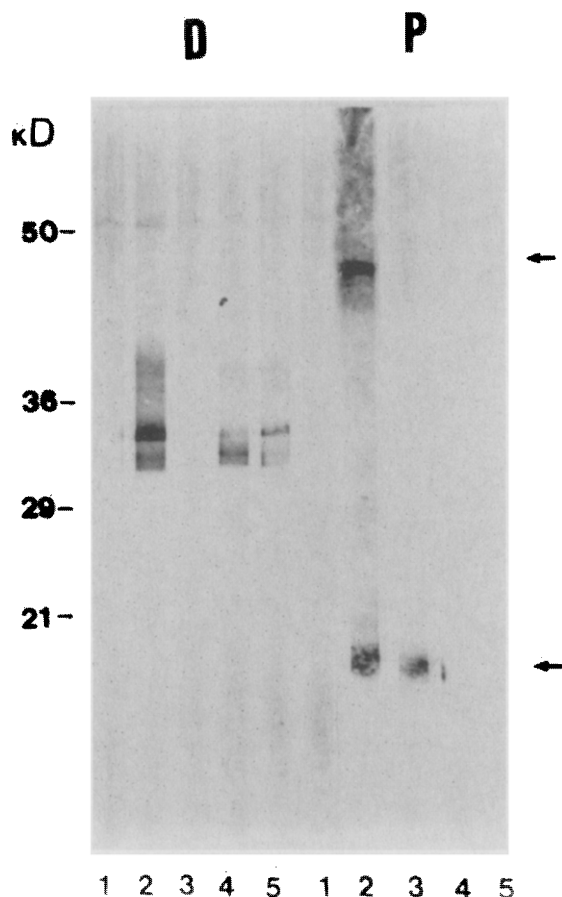


FIG. 4. Immunoblotting on *Dactylis* (D) and *Platanus* (P) pollen extracts. Line 1, D-P- pooled sera; line 2, D+P+ pooled sera; line 3, D-P+ pooled sera; line 4, D+P-serum; and line 5, D+P- serum. Arrows indicate 45 and 17 kD *Platanus* protein bands.

March through April, it was possible to observe a close dose-response relationship between *Platanus* pollen counts and mean rhinitis symptom scores ($r_s = 0.57$, $p < 0.05$) (Fig. 1). Furthermore, it was also possible to observe a close association between the onset and duration of the mean asthma symptom scores with the onset and duration of *Platanus* pollen counts in those eight patients with asthma ($r_s = 0.58$, $p < 0.05$) (Fig. 1).

Clinical characteristics of *Platanus* pollen-sensitized patients

As can be seen in Table II, 52% of group II patients had spring, summer, or both types of asthma, although only eight of them had asthma symptoms in March through April of 1995. By contrast, the prevalence of asthma in group I patients (i.e., with negative SPT results to *Platanus*) was only 27%.

Given the low number of cases studied, we performed a retrospective study by revising 262 medical records chosen at random of patients with pollinosis diagnosed in our center during 1994 to 1995. In this second group

of patients we were able to observe that seasonal asthma was more frequent among pollinosis patients sensitized to grasses and *Platanus* pollen (96 of 155) than among patients sensitized only to grasses or other pollens than *Platanus* (50 of 107) (chi-square test; $p < 0.05$).

Because prevalence of asthma in a population is linked to the number of positive skin test responses, we performed a study to determine the degree of atopy in patients allergic to grass with or without plane tree allergy. As shown in Table III, the cumulative mean wheal diameters for 12 common allergens was determined and expressed as an atopy score. Statistically significant differences were seen in the cases of seven allergens: *D. pteronyssinus*, *Cupressus*, *Dactylis*, *Olea*, *Alternaria*, *Cladosporium*, and *Aspergillus* (Table III). Furthermore, the atopic score was significantly greater in patients with plane tree allergy (24.39 vs. 13.29; Wilcoxon $p < 0.001$), which suggests, in fact, a greater degree of atopy between patients allergic to plane tree compared with patients not allergic to plane tree.

In vitro studies by ELISA

All 47 patients were tested for specific IgE to *Platanus* and *Dactylis* pollen extracts by ELISA (Table II). As shown, IgE reactivity to *Platanus* was detectable in 69% of the group II patients but in none of the group I patients. Thus a significant correlation between the specific IgE and SPT results existed (Wilcoxon; $p < 0.05$). Because most of the *Platanus*-positive sera were reactive with *Dactylis* pollen as well (72%), ELISA inhibition studies were carried out to test cross-reactivities between both pollen extracts (Fig. 2). As shown, reactivity with *Platanus* was partially inhibited with *Dactylis* extract (Fig. 2, A), whereas the converse was not the case (Fig. 2, B). A rather similar inhibitory effect was also seen with other pollen extracts (e.g., *Olea* spp., *Fraxinus* spp.; data not shown) but not with an unrelated antigen (Fig. 2). Of note, those few sera with detectable IgE to *Platanus* but not to *Dactylis* pollen could not be inhibited by the latter (not shown).

In vitro studies by PAGE and immunoblotting

The foregoing results prompted us to assess IgE reactivity to either *Platanus* and *Dactylis* by immunoblotting. Fig. 3 shows the protein pattern of *Dactylis* and *Platanus* pollen extracts as detected by PAGE. As can be seen, a rather different pattern was apparent without sharing major protein bands. These were of 17, 43, and 45 kD in *Platanus* pollen extract. As shown in Fig. 4, IgE from pooled sera reacting with both *Dactylis* and *Platanus* recognized different bands. The pattern of reactivity of these sera (D+P+) with *Dactylis* pollen (line 2) was similar to that found with those (D+P-) reacting with *Dactylis* alone (lines 4 and 5). All sera with reactivity with *Platanus* pollen (lines 2 and 3) recognized the 17 kD band. Pooled sera reacting with both *Dactylis* and *Platanus* pollen (line 2) also recognized the 45 kD band (Fig. 4). Nevertheless, by analyzing every serum in this pool, we noted that reactivity with the 45 kD band was a

particular feature of some sera. All sera, however, reacted with the 17 kd band (not shown).

DISCUSSION

Aerobiologic studies

Platanus pollen appeared to be impacted in our trap in March and April (59% of the total pollen sampled in these two months). It was the pollen most frequently found in the atmosphere of Madrid in 1995, contributing 19% of the total pollen sampled in this year and presenting a highest 10-day running mean of 704 grains/m³ of air. These high *Platanus* pollen counts were similar to previous years in this area⁸ and are similar to those described in other European cities.^{12, 13} On the contrary, the grass pollen counts in these two months (March and April) were rather low (highest 10-day running mean of 6 grains/m³) compared with the grass pollen counts sampled in May and June (highest 10-day running mean of 47 grains/m³). Likewise, the contribution of the other pollens (*Cupressus*, *Populus*, *Morus*, and *Quercus*) in the March and April sample total load were very scarce compared with the *Platanus* counts (Table I and Fig. 1).

Clinical studies

The prevalence of positive SPT results to pollens that we obtained in the 47-patient group of this study (Table I) did not differ significantly from the results obtained in a previous study in which we examined a larger population of 411 pollinosis patients,⁹ which suggests that these 47 patients included in this study are representative of the pollinosis patients visiting the outpatient clinic.

Of these 47 patients, 39 (83%) had symptoms of rhinitis, asthma, or both during the period from March to April. All of them had positive SPT responses to grasses, and in 32 it was possible to observe a positive SPT response to *Platanus* pollen extract as well.

The question was whether the pollinosis symptoms were caused by the *Platanus* pollen or to the small concentrations of airborne grass pollens that also appeared in March and April (Fig. 2). First, 32 of 39 patients with symptoms in March and April were sensitized to *Platanus* pollen, whereas only three of the eight patients without symptoms were sensitized (Fisher's exact test; $p < 0.05$). This points to *Platanus* pollen as an important contributor in pollinosis symptoms from March to April because all patients had positive SPT responses to grasses irrespective of whether they had symptoms. Second, it was possible to obtain a significant correlation between the average 24-hour rhinitis symptom scores and the *Platanus* pollen counts (Spearman rank correlation $r_s = 0.57$, $p < 0.05$). In eight of our 47 patients it was also possible to observe, in their diary cards, symptoms of asthma, showing a clear relationship between the onset and duration of the mean asthma symptom scores and the beginning and duration of *Platanus* pollen counts ($r_s = 0.58$, $p < 0.05$). However, the most severe asthma symptoms appeared between 10

and 15 days after the *Platanus* peak day (daily mean of 1201 grains/m³ of air), when the pollen count had already dropped to about 200 *Platanus* grains/m³ (Fig. 1). We do not know the reason for this, but perhaps it could be due to the presence of *Platanus* pollen allergenic aerosol fraction ($< 5 \mu\text{m}$), as described for ragweed and other pollens.¹⁴⁻¹⁶ The peak of such allergenic particles has been described as occurring somewhat later than the peak of the pollen count and correlates more closely with the peak of clinically recognized ragweed-induced asthma.¹⁴

One fact that attracted our attention was that seasonal asthma, whether in spring or summer, was more frequent among pollinosis patients sensitized to both *Platanus* and grass pollen (96 of 155) compared with pollinosis patients sensitized only to grasses or grasses and other pollens different from *Platanus* (50 of 107) (χ^2 test; $p < 0.05$). This might be related to a greater degree of atopy seen in the patients allergic to plane tree (Table III) because the prevalence of asthma in a population has been linked to the number of positive SPT results.¹⁷⁻²¹ Thus *Platanus* pollen sensitivity might be considered as a marker for atopic degree, being a risk factor for seasonal asthma in pollinosis patients at least in the Madrid area.

In vitro studies

IgE reactivity to *Platanus* pollen extract could be demonstrated in most (66%) but not all patients with positive SPT results to this pollen. Most of these patients also had specific IgE to *Dactylis*. Moreover, with the use of these sera, a significant cross-reactivity was found between *Dactylis* and *Platanus* pollen extracts by ELISA inhibition studies. It is interesting to note that *Dactylis* inhibition was not shown by *Platanus* in its turn. This might be due to qualitative and/or quantitative differences of the shared epitopes in each pollen extract. Differences in substances competing to be bound to the ELISA support also can be considered.¹¹ The fact that other tree pollens (e.g., *Fraxinus* and *Olea*) also inhibited *Platanus* pollen suggests the presence of epitopes in *Platanus* pollen that are shared by pollens of different plants. Although this is not surprising, because cross-reactivities among tree and grass pollens have been described,²²⁻²⁴ tree pollen or *Dactylis* pollen extracts were unable to inhibit *Platanus* reactivity of those sera with detectable IgE reactivity just to the latter, although positive SPT results to grasses were also found. The basis of such a cross-reactivity between *Platanus* and *Dactylis* pollen is not clarified by this study. The protein pattern detected by use of either Coomassie blue or immunoblotting was rather different. All *Platanus*-positive sera recognized a 17 kd band, whereas a low number of them also recognized a 45 kd band and other proteins of higher molecular weight. Although these latter sera were included within those having reactivity with both pollens, we could not detect shared bands clearly. Nevertheless, slight but detectable minor bands in the range of 30 to 35 kd were seen in *Platanus* pollen extract that

might be antigenically related to group 1 and group 5 major allergens of grass pollen. Other possibilities, such as cross-reacting conformational epitopes or a cross-reacting allergen being a carbohydrate in nature and therefore not detectable by immunoblotting, can also be considered.²⁵ The fact that IgE reactivity to *Platanus* pollen was in most cases directed to 17 kd protein is consistent with the early results of Anfosso et al.^{26, 27} which described a 22 kd protein allergen in this pollen as detected by gel filtration.

REFERENCES

- Lewis WH, Vinay P, Zenger VE. Airborne and allergenic pollens of North America. Baltimore, Md.: The Johns Hopkins University Press; 1983. p. 77-80.
- Frankland AW. Pollen allergens in Great Britain. In: Charpin J, Surinych R, editors. Atlas of European allergenic pollens. Paris: Sandoz; 1974. p. 131-41.
- Bousquet J, Cour P, Guerin B, Michel FB. Allergy in the Mediterranean area. I. pollen counts and pollinosis of Montpellier. Clin Allergy 1984;14:249-58.
- Solomon WR, Mathews KP. Aerobiology and inhalant allergens. In: Middleton E, Reed CH, Ellis EF, Adkinson NF, Yunginger JW, editors. Allergy: principles and practice, Vol 1. 3rd ed. St. Louis, Mo.: CV Mosby; 1988. p. 312-42.
- D'Amato G, Lobefalo G. Allergenic pollens in the southern Mediterranean area. J Allergy Clin Immunol 1989;83:116-22.
- Spieksma FTM. Regional European Pollen Calendar In: D'Amato G, Spieksma FTM, Bonini S, editors. Allergenic pollen and pollinosis in Europe. London: Blackwell Scientific Publications; 1991. p. 49-65.
- Spieksma FTM, Noland N, Frenguelli G, Van Moerbeque D. Polen atmosférico de Europa. Bruselas: UCB S.A.; 1993. p. 54-5.
- Subiza J, Cabrera M, Valdivieso R, Subiza JL, Jerez M, Jimenez JA, et al. Seasonal asthma caused by airborne *Platanus* pollen. Clin Exp Allergy 1994;24:1123-9.
- Subiza J, Jerez M, Jiménez JA, Narganes MJ, Cabrera M, Varela S, et al. Allergenic pollen and pollinosis in Madrid. J Allergy Clin Immunol 1995;96:15-23.
- Subiza J, Subiza JL, Hinojosa M, Garcia R, Jerez M, Valdivieso R, et al. Anaphylactic reaction following the ingestion of chamomile tea: a study of cross-reactivity with other composite pollens. J Allergy Clin Immunol 1989;84:353-8.
- Subiza JL, Caturla A, Pereira LF, Camargo MC, Bustos A, Boimorto R, et al. Evidence that a putative anti-idiotypic monoclonal antibody may actually be recognizing circulating immune complexes. J Autoimmun 1992;5:363-77.
- Belmonte J. Concentración polínica en Barcelona. Orsis 1988;3:67-75.
- Subiza E, Subiza J, Jerez M. Arboles, hierbas y plantas de interés alergológico en España. In: Basomba A, Conde J, Cortada JM, editors. Tratado de Alergología e Inmunología Clínica. Vol IV. Madrid: Luzán; 1986. p. 257-366.
- Agarwal MK, Swanson MC, Reed CE, Yunginger JW. Immunochemical quantitation of airborne short ragweed, *Alternaria*, antigen E, and Alt-I allergens: a two-year prospective study. J Allergy Clin Immunol 1983;72:40-5.
- D'Amato G, Gentili M, Russo M. Detection of *Parietaria judaica* airborne allergenic activity: comparison between immunochemical and morphological methods including clinical evaluation. Clin Exp Allergy 1994;24:566-74.
- Spieksma FTM, Nikkels BH, Dijkman JH. Seasonal appearance of grass pollen allergen in natural, pauci-micronic aerosol of various size fractions: relationship with airborne grass pollen concentration. Clin Exp Allergy 1995;25:234-9.
- Asher MI, Pattemore PK, Harrison AC, Mitchell EA, Rea HH, Stewart AW, et al. International comparison of the prevalence of asthma symptoms and bronchial hyperresponsiveness. Am Rev Respir Dis 1988;138:524-9.
- Woolcock AJ, Peat JK, Salome CM, Yan K, Anderson SD, Schoeffel RE, et al. Prevalence of bronchial hyperresponsiveness and asthma in rural adult population. Thorax 1987;42:361-8.
- Britton WJ, Woolcock AJ, Peat JK, Sedgwick CJ, Lloyd DM, Leeder SR. Prevalence of bronchial hyperresponsiveness in children: the relationship between asthma and skin reactivity to allergens in two communities. Int J Epidemiol 1986;15:202-9.
- Peat JK, Salome CM, Woolcock AJ. Factors associated with bronchial hyperresponsiveness in Australian adults and children. Eur Respir J 1992;5:921-9.
- Miles J, Cayton R, Ayres J. Atopic status in patients with brittle and non-brittle asthma: a case-control study. Clin Exp Allergy 1995;25:1074-82.
- Howlett BJ, Hill DJ, Knox RB. Cross-reactivity between Acacia (wattle) and rye grass pollen allergens: detection of allergens in Acacia (wattle) pollen. Clin Allergy 1982;12:259-68.
- Swoboda I, Scheiner O, Kraft D, Breitenbach M, Heberle-Bors E, Vicente O. A birch gene family encoding pollen allergens and pathogenesis-related proteins. Biochim Biophys Acta 1994;18:457-64.
- Ebner C, Hirschwehr R, Bauer L, Breiteneder H, Valenta R, Ebner H, et al. Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). J Allergy Clin Immunol 1995;95:962-9.
- Aalberse RC, Koshte V, Clemens JG. Cross-reactions between vegetable foods, pollen and bee venom due to IgE antibodies to a ubiquitous carbohydrate determinant. Int Arch Allergy Appl Immunol 1981;66:259-60.
- Anfosso F, Soler M, Mallea M, Charpin J. Isolation and characterization in vitro of an allergen from Plane-tree (*Platanus acerifolia*) pollen. Int Arch Allergy Appl Immunol 1977;54:481-6.
- Anfosso F, Lerys R, Charpin J. The allergen of Plane-tree pollen. Allergy 1980;35:196-8.

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