

Seasonal asthma caused by airborne *Platanus* pollen

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Summary

This work describes three patients suffering from bronchial asthma after being naturally exposed to airborne plane-tree pollen. The three patients gave immediate response in skin tests and dual response in bronchial provocations using *Platanus hybrida* extract. There was specific seric IgE activity against this/these antigen(s) with the CAP system. The three patients also showed significant correlation ($P < 0.001$) between their rhinitis and asthma symptom-scores registered on their diary cards and plane-tree pollen counts, collected using the Burkard spore trap. Among 187 patients living in Madrid and who came to our centre with a history of rhinitis and/or seasonal asthma, we found a prevalence of positive skin-prick tests to *Platanus* of 56%, only surpassed by gramineous pollen (*Dactylis glomerata* and/or *Trisetum paniceum*) 92% and *Olea europaea* 63%. The aerobiological sampling of the pollen content of the air in Madrid, carried out between 1 January 1979 and 31 December 1993 revealed an airborne presence (per cent of total yearly pollen count, mean of 1979–1993) of 14.9% for the *Platanus*, 14.8% for grasses, 9.8% for *Olea* and 3.6% for *Plantago*. The *Platanus* is one of the most frequently found pollens in the atmosphere over Madrid. At present, in this geographical area, a high percentage of patients with pollinosis are sensitive to this pollen. At least in some of these patients *Platanus* pollen is capable of inducing rhinitis and bronchial asthma.

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Introduction

The *Platanus hybrida* or *Platanus acerifolia* (Maple leaf platanus) 'Plane tree' is one of the trees most frequently found in the majority of western European cities (Fig. 1) [1,2]. The specific name (*hybrida*) refers to the probable cross between an Euro-Asian species (*Platanus orientalis*) and a North-American species (*Platanus occidentalis*). Its flowering season in Europe is from March to June, depending on the climate and weather [2]. High concentrations of its pollen are trapped in air samplers located in Spain [3,4], Portugal [5], Great Britain [6], France [7], Switzerland [8], Belgium [9] and Italy [10]. There are, however, very few works on the allergenicity produced by the pollen and it is believed to be of minor and doubtful importance with regard to pollinosis [2].

Among patients suffering from hay fever, a low prevalence of sensitivity to this pollen has been described: 13% (Montpellier, France) [11], 5% (Naples, Italy) [12] and as yet we have not found any articles correlating the symptoms of patients and the count of these pollens, or on the capacity this pollen has of inducing specific bronchial response.

The purpose of the present study was to see the actual prevalence of sensitivity to *Platanus* pollen among the Madrid pollinosis patients. Also in this study we investigated the capacity of this pollen to induce asthma in three selected patients by correlating symptoms, pollen counts and specific bronchial challenges.

Materials and methods

Pollen counts

The pollen count was made according to a previously

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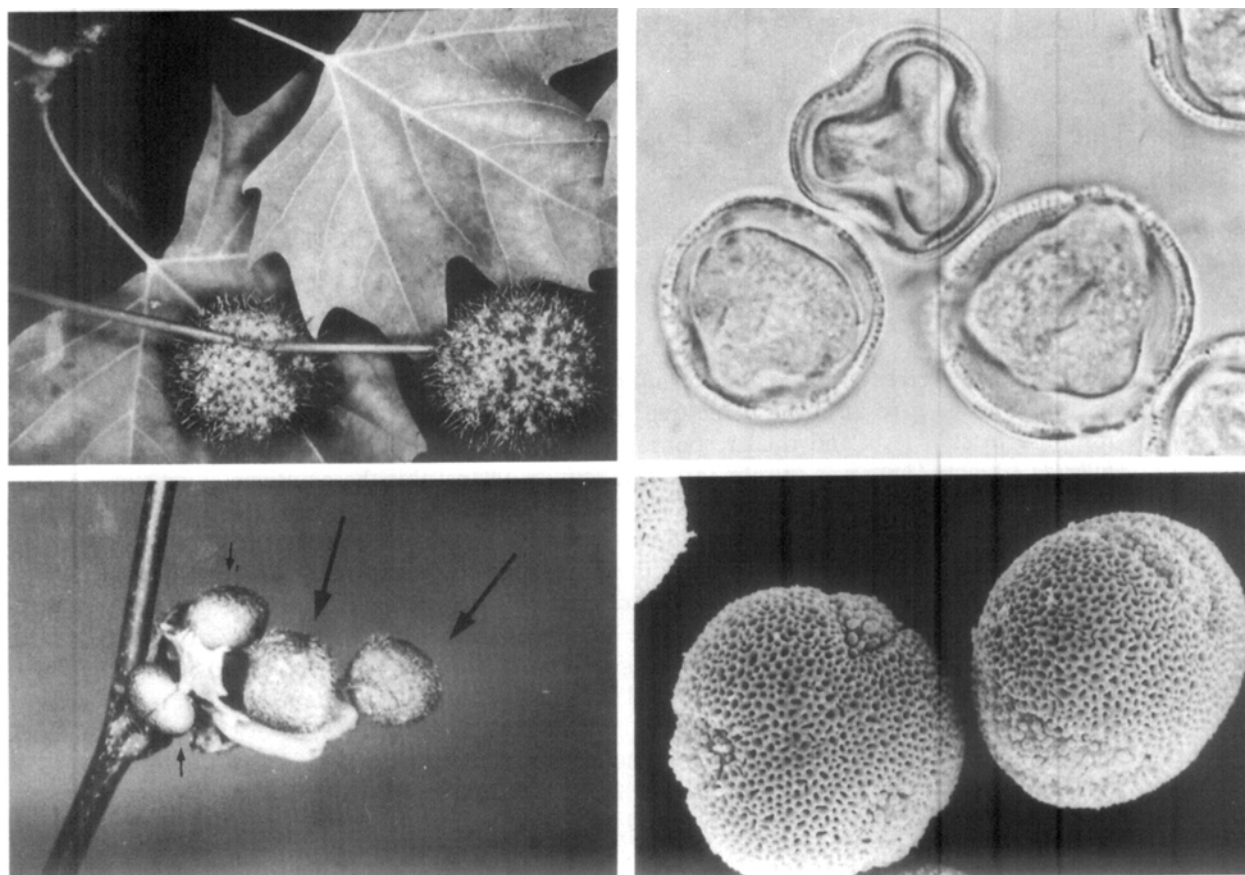


Fig. 1. *Platanus hybrida* branch showing globose heads of staminate flower (small arrows) and pistillate flowers (arrows). The fruit (above) is a globose head of top-shaped achenes. As can be seen in the microscopic photographs (optical section), the grains are trizonocolpate, suboblate or oblate spheroidal and small ($18 \times 25 \mu\text{m}$). By scanning electron microscope the grains show an exine microreticulate, with colpi and covered with membrane studded with small granulations.

described technique with a volumetric air sampler (Burkard spore trap) [13,14]. The trap was installed on the roof terrace of our centre which is 20 m high and is located in the north-east of Madrid urban area ($40^\circ 26' \text{N}$; $3^\circ 40' \text{E}$). The sampling period included the months of January–December 1979–1993.

Patients

Two men and one woman aged 41, 56 and 46 years respectively who came to our consulting rooms with a history of seasonal asthma and rhinoconjunctivitis of 6, 4 and 5 years' evolution were studied.

To discover the present prevalence of sensitivity to *Platanus* pollen, another 187 patients (84 women and 103 men), living in Madrid and aged between 6 and 7 (average age 27 years) who came to our centre throughout 1993 suffering from rhinitis and/or seasonal asthma

symptoms, were studied. The patients' evaluation was made by a physician's assessment that included, for each subject, a case history, a clinical examination and a skin-prick test.

Symptom scores

Ocular symptoms of redness, itching, lacrimation and nasal symptoms of sneezing, itching, congestion and rhinorrhoea were assessed by the three patients on a daily basis with the visual analogue scale where 0 = absence of symptoms, 1–3 = mild symptoms, 4–7 = moderate symptoms, 8–10 = severe symptoms. In the same way, bronchial symptoms of wheeziness, tightness, coughing and shortness of breath were assessed. Diary cards were kept daily from September 1992 until July 1993.

Collection of *Platanus hybrida* pollen

Morning selection and collection of mature male capitula was made. Collected inflorescences were immediately vacuum-dried at 35°C. Mechanical sieving was later undertaken with the dried product, 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, with the purified pollen, 24-h vacuum-drying at 35°C was carried out.

Preparation of *Platanus hybrida* extract

This was done using a previously described method [15]. Two grams of the *Platanus hybrida* extract were defatted with acetone, dried and extracted in 40 ml of 0.01 mol/l phosphate and 0.15 mol/l NaCl, pH 7.4 phosphate-buffered saline (PBS), and the mixture (1:20 w/v) was stirred for 12 h at 4°C. The suspension was filtered through a filter paper (Whatman Ltd, Maidstone, UK), dialysed against PBS and sterilized by 0.22 µm filtration (Millipore, Molsheim, France), having a final concentration of 395 µg/ml in protein, as determined by Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, California, USA).

Skin tests

All skin tests were performed by the prick technique, beginning with a dilution of 1:2 000 000 w/v of the *Platanus* extracts and were progressively increased 10-fold until a positive reaction was obtained.

A battery of commercially available common inhalant allergens (Aristegui, Bilbao, Spain) were also skin-prick tested. This battery included *Dermatophagoides pteronyssinus*, cat dander, *Alternaria tenuis*, *Olean europaea*, *Plantago lanceolata* and grass pollens. *Trisetum paniceum* and *Dactylis glomerata* were chosen, being considered the most representative grasses of our geographical area [16].

Five normal patients were tested with the *Platanus* extract as a control. Histamine phosphate (10 mg/ml) was used as a positive control, and 50% glycerol was used as a negative control. All skin-test sites were read after 15 min. A positive reaction was defined as a weal of at least 3 mm by 3 mm in the presence of a negative reaction to the 50% glycerol and a positive test with histamine phosphate.

Bronchial provocation tests

Bronchial challenges with methacholine and *Platanus* extract were performed according to the methods used

by Cockcroft *et al.* [17] and Juniper *et al.* [18], with some previously-described modifications [19]. These tests were performed during an asymptomatic period, out of season and results were expressed as PC20 FEV₁. Broncho-provocation testing with *Platanus* was performed in a double-blind protocol. A control test-day, with exposure to PBS inhaled for 2 min, was carried out to ensure that the patient's FEV₁ was stable over at least 8 h and that any possible physiological changes observed on challenge days were not attributable to normal circadian changes in pulmonary function or changes in effort [20]. The following inhalation tests were conducted on different days: *Platanus* extract in stepwise concentration of 1:2 000 000, 1:200 000, 1:20 000, 1:2000, 1:200 and 1:20 w/v. Two unexposed patients with asthma were also challenged but in a single-blind manner with a 1:20 w/v dilution after having obtained informed consent.

IgE measurement

Total IgE was determined with the Pharmacia CAP system IgE FEIA (fluoro-enzyme-immunoassay) and specific IgE for *Platanus hybrida* with Pharmacia CAP system. The CAP kits were purchased from Pharmacia Diagnostics, Uppsala, Sweden, and the assay was carried out according to the manufacturer's instructions [21]. Results were expressed in kU/l. Values >0.70 kU/l (class ≥ 2) were considered positive.

Statistical analysis

Linear regression was used to analyse the correlation between the *Platanus* pollen counts and the patients' symptom scores. The statistical significance of the correlation was evaluated with the Student *t*-test.

Results

Pollen counts and symptom scores

Platanus pollen counts mainly appeared in the months of March and April, with a predominance of the latter (Fig. 2). As can be seen from Fig. 3, there is an upward trend in the yearly *Platanus* pollen concentrations in the last half of the period under study, mainly due to the high counts obtained in the years 1988 and 1990–1993.

In the three patients a significant correlation was observed between the *Platanus* pollen counts and their rhinitis symptoms ($P < 0.001$) and bronchial asthma ($P < 0.001$) (Fig. 2). The patients reflected no symptoms on their diary cards outside the months of March and April. It was very typical, in these patients, to change in a

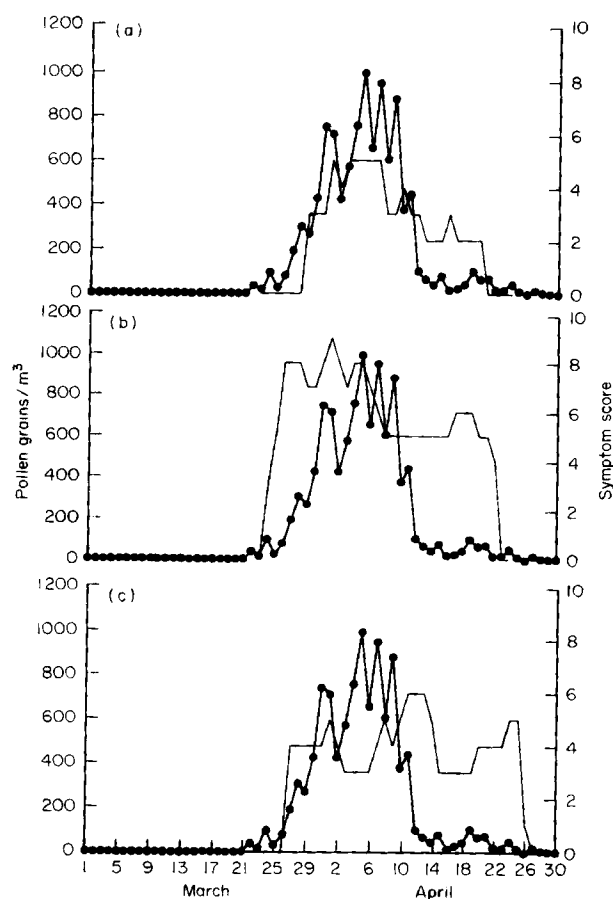


Fig. 2. Correlation between asthma symptoms observed by the patient on his/her diary card and *Platanus* pollen counts obtained during the same period using a Burkard spore trap. (a) Patient 1. (b) Patient 2. (c) Patient 3.

few days from complete good health to a very severe condition, as the plane-tree pollen count rose from zero to many hundred grains per cubic metre (Fig. 2). In contrast, at the end of the season, symptoms still

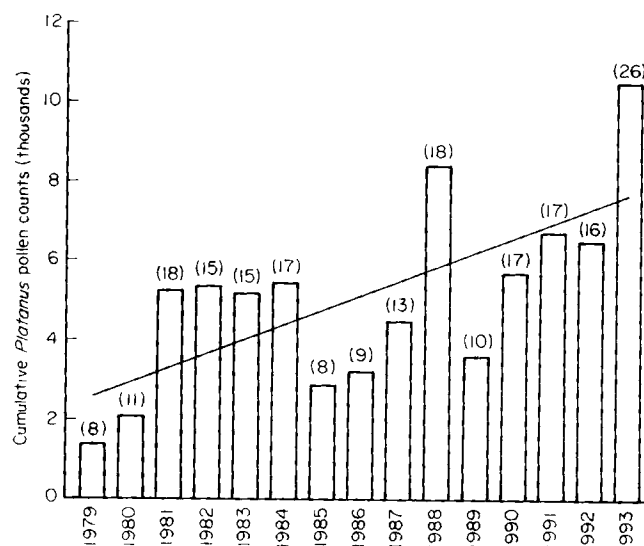


Fig. 3. Madrid's total annual atmospheric *Platanus* measured (the sum of daily averages over 1 year). In brackets the airborne presence of *Platanus* (per cent of total yearly pollen count).

persisted for a week when the pollen count had already fallen.

Skin tests and IgE measurement

As can be seen in Table 1, the three patients gave immediate weal-and-erythema response to the *Platanus hybrida* pollen extract, two of them even with dilutions as small as 1:2 000 000 w/v which shows a high sensitivity to this antigen(s). Significant IgE activity could be found against this pollen in all three patients. On the other hand, all these results were negative in the five non-atopical persons used as control.

Patient 3 also gave positive skin tests for cat dander and *D. pteronyssinus*, although in anamnesis the patient

Table 1. Patients' skin-prick test and IgE measurement data

Patients	Skin-prick tests Weal diameters (mm)					End-point titration with <i>Platanus</i> <i>hybrida</i> *(w/v)	Total IgE UI/ml	Specific IgE by CAP kU/l
	<i>Platanus</i> <i>hybrida</i>	<i>Trisetum</i> <i>paniceum</i>	<i>Olea</i> <i>europaea</i>	<i>Plantago</i> <i>lanceolata</i>	<i>D. pter.</i>			
1	6 × 5	4 × 4	6 × 4	—	—	1:200 000	20.9	1.55
2	8 × 6	5 × 4	4 × 4	4 × 3	—	1:2 000 000	117	13.7
3	12 × 9	6 × 5	8 × 6	7 × 5	5 × 4	1:2 000 000	596	59.8

* Extract dilution enough to elicit a small weal of 1–2 mm with flare and itching. *D. pter.* *Dermatophagoides pteronyssinus*.

Table 2. Presence of airborne pollen and skin-test positivity

	Airborne presence (%)	Skin-test positivity (%)
<i>Platanus</i>	14.9	56
<i>Poaceae</i>	14.8	92
<i>Olea</i>	9.8	63
<i>Plantago</i>	3.6	47

Airborne presence (per cent of total yearly pollen count, mean of 1979–1993) and skin-test positivity (per cent positive reactions in patient with pollinosis) in Madrid.

denied the appearance of symptoms in the presence of furry pets and/or household dust.

Sensitivity towards other pollens (grasses, *Olea* and *Plantago*, found in Madrid from May to July) [22], was also found in all three patients. In Table 2, one observes the prevalence of positive prick tests to pollen found in the 187 patients studied with rhinitis and/or seasonal

asthma. The greatest prevalence was towards grasses (*Dactylis* 87%, *Trisetum* 86%, *Trisetum* and/or *Dactylis* 92%). *Platanus* gave rise to 56% positivity which puts it in third place behind grasses and *Olea* (63% of positive skin reactions).

Bronchial provocation tests

Methacholine challenges showed slight bronchial hyper-responsiveness in the three patients (methacholine PC20 = 13 mg/ml in patient 1, 15 mg/ml in patient 2 and 1.2 in patient 3). On the control day of exposure to PBS, there was no significant change in spirometry throughout the day in any of the patients. In contrast, the three patients had an immediate and late asthmatic response after challenge with the 1:20 w/v (patient 1), 1:2000 w/v (patient 2) and 1:2 000 000 w/v (patient 3) dilution of the *Platanus hybrida* extract (Fig. 4).

In contrast, two non-atopic asthmatics that showed a methacholine PC20 of 0.9 and 4.5 mg/ml, did not react to the 1:20 w/v *Platanus hybrida* extract.

Discussion

It is a well known fact that in Madrid the most important cause of rhinitis and/or seasonal asthma are grass pollens, with the peak pollenization period between the months of May–June [4,16,22]. In this sense, the three patients showed an immediate cutaneous response towards grass pollen extracts. Apart from this, they all referred to the presence of asthma symptoms in the spring, which was suggestive of the fact that these pollens were in fact responsible for their symptoms, for which reason a specific immunotherapy was started for them. However, during the next check-up, 6 months later, when their diary cards of rhinitis and asthma symptoms could be contrasted with the atmospheric pollen counts measured in Madrid, the absence of correlation with grass pollen counts could be seen. There was, however, a significant correlation ($P < 0.001$) with *Platanus* pollen counts. These data were supported in all three patients by the obtention of a significant immediate and late bronchial response after inhaling an extract of this pollen. The positivity of the skin-prick tests and the presence of a high specific IgE activity against the antigen(s) of the *Platanus* pollen strongly suggest that a type I immunological mechanism could be involved as the underlying cause in these patients.

Certainly it may be suggested, that the asthma could have been induced by other tree pollens. In fact, during the patient's symptom period (from the last week of March to the last week of April) we observed, in addition to *Platanus* pollen, low concentrations of

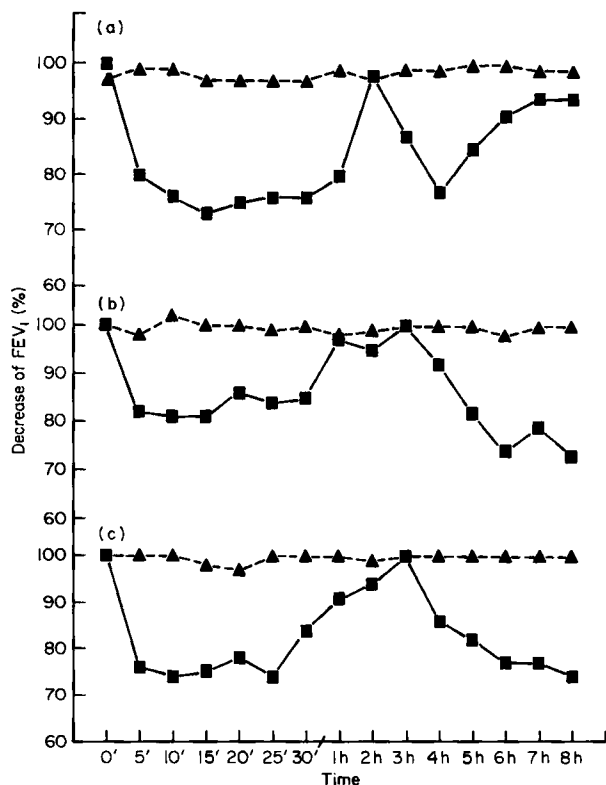


Fig. 4. Bronchial provocation test (■) with *Platanus hybrida* extract 1:20 w/v in (a) patient 1, (b) 1:2000 w/v in patient 2 and (c) 1:2 000 000 w/v in patient 3. The same test (▲) was performed with PBS on a control day.

Populus spp. pollen (4% of total yearly pollen counts) from 8 March to 7 April, and low concentrations of *Morus* spp. pollen (5% of total yearly pollen counts) from 1 to 22 April. However, it is unlikely that these two types of pollens are clinically relevant, due to the negative skin tests in the three patients with both *Morus alba* and *Populus alba* pollen extracts 1:20 w/v (not shown).

Although Anfoso *et al.* isolated a glycoprotein with a molecular weight of 22 000 Da as the major allergen from plane-tree pollen [25,26], in the revision of literature, plane-tree pollen is described as being a generally low allergenic, in spite of the high concentrations captured in the air samplers of a large part of Europe and the USA [2–6,22,23]. These data are in accordance with the low prevalence of positive skin reaction (only 2%) for plane-tree pollen obtained by us in a study undertaken in 1979, among patients with pollinosis in the Madrid area [4]. However, it strongly contrasts with the present high prevalence of skin sensitivity (56%) found among the 187 patients residing in Madrid and who came to our centre with a history of rhinitis and/or seasonal asthma.

The cause of this increase of positive prick tests to plane-tree pollen is unknown to us, although it could be due to an increase in the exposure of the inhabitants of Madrid to this pollen, as reflected in the pollen counts obtained in the period between 1979 and 1993. At present, together with grasses, *Platanus* it is the second most frequent taxon found in the atmosphere over Madrid (14% of total pollens), only surpassed by *Quercus rotundifolia* (17%), this last being, in contrast, much less allergenic [4]. The reason of this increase in the total yearly amount of this pollen could be due to:

- 1 The end of strong pruning of Madrid's plane trees which ended in 1987. Each winter almost all the branches of the *Platanus* trees were pruned to permit the growth of young branches, which reduced the total number of flowers per tree (and the pollen emitted) during the following spring.
- 2 The increase in the number of *Platanus* trees planted in the streets and parks of Madrid in the last few years. There being an estimated total of about 58 000 trees in 1979 in comparison to 73 000 today, which makes them the most common trees in Madrid.

These results appear to indicate that a change is occurring in the allergenicity of plane-tree pollen, considered years ago to be very slightly allergenic but perhaps whose present importance in contributing pollinosis symptoms is not to be underestimated, at least in the Madrid area.

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