

Cupressaceae pollinosis in the Madrid area

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Summary

Background There is no previous report of Cupressaceae pollinosis in the Madrid area.

Objective To ascertain the presence of Cupressaceae pollinosis in the Madrid area.

Methods Thirty-six patients were studied who had winter pollinosis symptoms and a positive skin test to *C. arizonica*. They were skin tested with *C. sempervirens* (Abelló, Madrid), *C. arizonica* (in-house extract) and other common inhalant allergens (Abelló). Total IgE was measured and specific IgE to *C. arizonica*, *C. sempervirens* and *J. sabinoides* was determined.

Results Six of the patients were only sensitized to Cupressaceae pollens, whereas 30 patients had also sensitivity to other pollens (oleaceae 28, plantain 27, grasses 25, chenopodium 25, compositae 23, *Parietaria* 9) and 18 patients were also sensitized to other inhalant allergens (animals 17, mites 4, moulds 2).

Conclusion These results confirm the existence of allergy to Cupressaceae pollens in Madrid (Spain).

Keywords: Cypress pollen, pollinosis, allergy, IgE

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Introduction

Cupressaceae pollens have been reported as a cause of respiratory allergy in several countries, such as southern France [1,2], Italy [3], South Africa [4], the United States [5] and Australia [6].

The Cupressaceae family comprises a number of species that grow naturally or are cultivated as ornamental plants. The taxonomy of the Cupressaceae family is shown in Table 1 [7].

Cupressaceae pollinosis occurs during the winter months and usually produces rhinitis and conjunctivitis, while bronchial asthma has been recorded only in patients with sensitization to other aeroallergens [2].

In some Mediterranean regions where Cupressaceae are present, sensitization to pollen allergens of these trees may have been underestimated. In Spain, Cupressaceae pollen allergy has not previously been recorded, even

though there are high counts of Cupressaceae pollens in some areas [8,9].

In 1993, one female patient complained of allergic rhinoconjunctivitis during the winter season and she attributed this to *Cupressus arizonica* pollen exposure. All skin tests with usual inhalants were negative. She was then skin tested with an in-house *C. arizonica* pollen extract, which came out positive. All patients with winter pollinosis symptoms were skin tested with Cupressaceae pollens. This study deals with 36 patients diagnosed as having Cupressaceae pollinosis.

Materials and methods

Patients

Thirty-six patients (21 females, 15 males), aged from 12 to 62 years (mean age 31.4 ± 12.1) were studied at our outpatient clinic between February and August 1994. All patients had symptoms during the winter (December–March) and a positive skin-prick test to *C. arizonica* pollen extract.

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Table 1. Taxonomy of Cupressaceae

Sub-family	Genera	Species
Cupressoideae	Cupressus	<i>C. sempervirens</i> <i>C. arizonica</i>
	Chamaecyparis	<i>C. lawsoniana</i> Parl
Thujoideae	Thuja	<i>T. orientalis</i> <i>T. occidentalis</i>
	Juniperus	<i>J. sabinoideis</i> <i>J. communis</i> <i>J. oxycedrus</i>

Pollen counts

Madrid city pollen counts were obtained from the General Pardiñas Centre of Allergy and Clinical Immunology (Dr J. Subiza). They were obtained with a volumetric spore trap (Burkard spore trap) using the method previously described [10]. The counts were available between 1989 and 1994. Pollens counts are shown in terms of annual totals, the highest daily counts and 10 day mean values. They are expressed as the average number of grains of pollen per m³ of air.

Pollen

C. arizonica pollen was collected in January 1994 in Madrid. Pollen purity (>99%) was assessed by microscopic analysis.

Preparation of *C. arizonica* extract

The pollen (14 g) was extracted in 0.05 mol/L NH₄HCO₃ pH 8, containing 0.01 M thiourea, at 4°C for 5 h by magnetic stirring. After extraction, the pollen was separated from the supernatant by centrifugation at 16 000 g at 4°C for 20 min. The supernatant was thoroughly dialysed against 10 mM glycine, at 4°C for 24 h with a molecular weight cut-off of 3500 Da. The dialysed extract was centrifuged at 30 000 g at 4°C for 20 min. The supernatant was passed through a 0.45 µm pore size filter (Millipore Corp, Bradford, Mass, USA), freeze-dried and lyophilized. Protein concentration was assessed according to the method of Lowry *et al.* [11] and came out as 3% (3 mg of protein content in 100 mg of dry weight material).

Skin-prick tests (SPTs)

SPTs were performed on the volar side of the forearm with a prick standardized lancet (DHS, Leverkusen,

Germany) according to the Subcommittee on skin tests of the European Academy of Allergology and Clinical Immunology [12]. Histamine clorhydrate (10 mg/mL) and normal saline solution were used as positive and negative controls, respectively. The maximum weal diameter and its orthogonal perpendicular were measured 15 min after the prick and the mean of both diameters was taken as the test size. A mean weal diameter at least 3 mm larger than that produced by the negative control was considered as positive [12].

Available commercial skin-prick test extracts (Abello, Madrid, Spain) from mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*), danders (cat, dog), moulds (*Alternaria tenuis*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Penicillium notatum*) and pollens (*Olea europaea*, *Fraxinus excelsior*, *Lolium perenne*, *Cynodon dactylon*, *Artemisia vulgaris*, *Chrysanthemum leucanthemum*, *Chenopodium album*, *Parietaria judaica*, *Plantago lanceolata*) were tested on all patients.

A commercial non-standardized extract from *Cupressus sempervirens* pollen 1:20 w/v (Abello, Madrid, Spain) and a non-standardized in-house extract from *Cupressus arizonica* (500 µg protein content/mL) were tested in all patients.

Total serum IgE

Total serum IgE was measured by MEIA IMx system (Abbot Diagnostics, Chicago, Illinois, USA) following the manufacturer's instructions.

Serum specific IgE

Specific IgE to *C. sempervirens* and *Juniperus sabinoideis* was measured by CAP FEIA (Pharmacia Diagnostics, Uppsala, Sweden) following the manufacturer's instructions. Specific IgE to *C. arizonica* was measured by Phadezym RAST (Pharmacia Diagnostics, Uppsala, Sweden) following the manufacturer's instructions.

C. arizonica pollen extract was coupled to cyanogen-bromide activated paper disks according to the procedure of Ceska *et al.* [13].

A CAP level equal or greater than class 1 (0.35 kU/L) and a RAST level equal or greater than class 1 (0.35 kU/L) were both taken as positive.

Statistical analysis

IgE values were transformed into their decimal logarithmic values for statistical analysis. The geometric means of total IgE and specific IgE to Cupressaceae pollens were calculated.

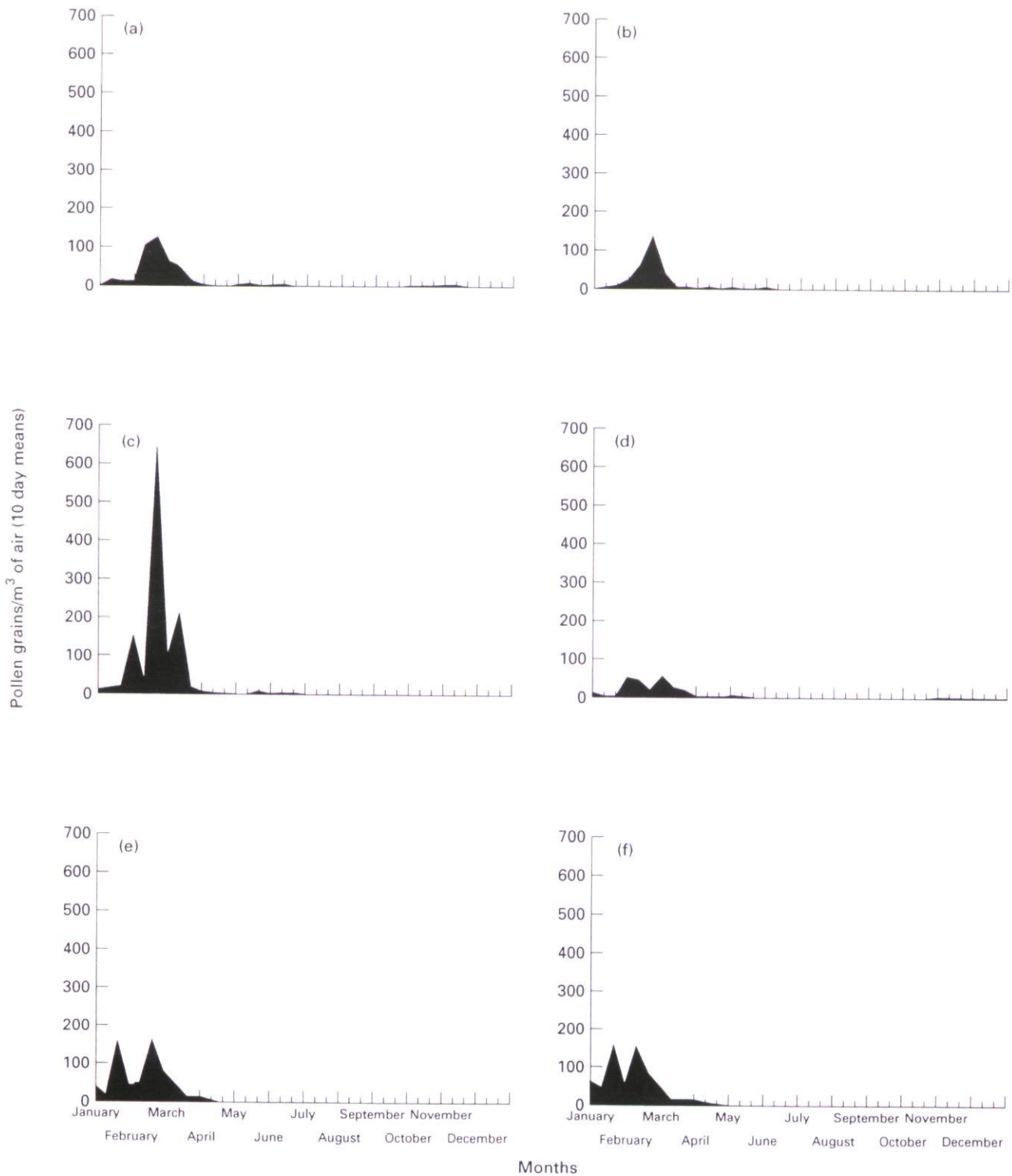


Fig. 1. Cupressaceae pollen counts. Daily counts of airborne Cupressaceae pollens in 6 successive years (1989 to 1994) in Madrid. The curves are based on 10 day running means of pollen counts (per cubic meter in 24 hours). Charts are based on data supplied by Dr. J. Subiza. (a) 1989. (b) 1990. (c) 1991. (d) 1992. (e) 1993. (f) 1994.

Results

Pollen counts

Figure 1 shows the variations in pollen counts from 1989 to 1994, expressed as 10-day mean values. According to these data the Cupressaceae pollen season in Madrid would begin in January and end in March.

The annual total of these pollen grains, as sum of daily mean values, ranges from 2466 grains/m³ in 1992 to 11 432 grains/m³ in 1991. The highest concentration of these pollens occurred in February in every year, as can be seen in Fig. 1. During these 6 years the highest pollen concentration was on 21 February 1991 (3306 grains/m³) (Table 2).

Patient characteristics

Age, sex, skin tests and total and specific IgE to Cupressaceae pollens of the 36 patients are shown in Table 3. All patients had symptoms during the winter. The type of symptoms reported by patients can be seen in Table 3.

Skin tests

SPTs were positive to *C. sempervirens* in 35 patients (97%) and to *C. arizonica* in 36 patients (100%).

Six patients were sensitized only to Cupressaceae pollens and the other 30 had also sensitivity to other pollens (oleaceae 28, plantain 27, grasses 25, chenopodium 25, compositae 23, *Parietaria* 9). Some patients had also sensitivity to other allergens (danders 17, mites 4, moulds 2).

Specific IgE

Specific serum IgE to *C. sempervirens* pollen was

Table 2. Airborne Cupressaceae pollen counts in Madrid. Highest values of daily mean counts from 1989 to 1994 (pollen grains per m³ of air)

Year	Day	Pollen counts
1989	14 February	642
1990	22 February	414
1991	21 February	3306
1992	5 February	228
1993	22 February	660
1994	15 December	450

(Data supplied by Dr J. Subiza).

Table 3. Age, sex, skin tests and total and specific IgE to Cupressaceae pollens

Age (mean $\pm$ SD years)	31.4 $\pm$ 12.1
Sex (M/F ratio)	15/21
Skin tests (% positive)	
<i>C. sempervirens</i>	35/36 (97%)
<i>C. arizonica</i>	36/36 (100%)
Total IgE (kU/L) (SD)	248.5 (76.5–806.8)
Specific IgE (% positive)	
IgE- <i>C. sempervirens</i>	35/36 (97%)
IgE- <i>J. sabinoides</i>	36/36 (100%)
IgE- <i>C. arizonica</i>	24/36 (67%)
Specific IgE (SD)*	
<i>C. sempervirens</i> (kU/L)	4.37 (1.91–10.00) (n = 35)
<i>J. sabinoides</i> (kU/L)	15.47 (5.73–41.75) (n = 36)
<i>C. arizonica</i> (PRU/mL)	0.63 (0.38–1.06) (n = 24)
Symptoms	
Rhinitis (%)	36/36 (100%)
Conjunctivitis (%)	32/36 (89%)
Asthma (%)	29/36 (81%)

* Patients with positive CAP or RAST.

detectable in 97% of patients, *C. arizonica* in 61% of patients and *J. sabinoides* in 100%.

Positive serum specific IgE levels to *C. arizonica* pollen (Phadezym RAST) ranged from 0.35 to 2.04 PRU/mL. A RAST class 2 was found in 10 sera, whereas 14 sera had a RAST class 1.

Positive serum specific IgE levels to *C. sempervirens* pollen (CAP System) ranged from 0.5 to 15.9 kU/L. A CAP class 3 was found in 20 sera, 13 sera had a CAP class 2, and only two sera CAP class 1.

Serum specific IgE levels to *J. sabinoides* pollen (CAP System) ranged from 0.50 to 67.3 kU/L. A CAP class ≥ 4 was found in 16 sera, 18 sera had a CAP class 3, one serum CAP class 2 and one serum CAP class 1.

The mean of specific IgE to *C. sempervirens*, *C. arizonica*, and *J. sabinoides* in patients with positive CAP or RAST is shown in Table 3.

Discussion

An awareness of airborne pollens that are present in a particular area is important for identifying the respiratory allergies that may occur there. Our data show the great importance of airborne Cupressaceae pollen grains in the Madrid area.

The predominant Cupressaceae species in the Madrid

area are *C. sempervirens*, *C. arizonica* and *J. oxycedrus* (J. A. Jiménez García, pers. comm.).

We have studied 36 subjects sensitized to Cupressaceae pollens, whose diagnosis was made on the basis of clinical symptoms, skin tests and specific IgE.

As in previous studies, rhinitis and conjunctivitis were the most frequent symptoms [2,14]. We have found asthma in 81% of the patients, a higher percentage than that reported by other authors (7–19%) [14].

Serum *C. sempervirens* specific IgE was detected in 97% of the patients, but *C. arizonica* in only 61%. One possible explanation for this could be that the RAST test is less sensitive than the CAP test [15].

We have also found serum specific IgE to *Juniperus sabinoides* in 100% of the patients. This pollen is another member of the Cupressaceae family that is not present in Spain. This positiveness may be due to crossreactivity between *Cupressus* and *Juniperus*, as previous studies suggest [16] or to the presence in Spain of other plants of the same genus, such as *J. oxycedrus* or *J. communis* (very frequent in some parts of our area) (J. A. Jiménez García, pers. comm.).

The higher IgE to *J. sabinoides* than to *C. sempervirens* may indicate that the genus *Juniperus* is more allergenic than *Cupressus*.

The results of our study confirm the presence of Cupressaceae pollen allergy in Madrid (Spain), a non-Mediterranean climate area. The sensitivity to these pollens allergens may have been underestimated in the past, and resultant symptoms could have been misinterpreted in some cases as viral infections or intrinsic diseases.

The increasing use of Cupressaceae trees in the plantations and gardens (pers. obs.) could be one reason for an increase in Cupressaceae pollen in the atmosphere and, hence the appearance or increase of Cupressaceae pollinosis. Another reason for the increase of Cupressaceae pollen allergy could be an interaction of these pollens with air pollution, thereby acting as an allergen adjuvant, as suggested by some authors with related pollens [17].

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