

## Sensitivity to *Cupressus*: Allergenic significance in Córdoba (Spain)

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### SUMMARY

The detection of high levels of Cupressaceae pollen concentration in the air from January to April for several years in our area prompted analysis of the incidence and allergenic significance of sensitivity to this pollen. Furthermore, this is the highest winter-blooming taxa in the city of Córdoba. Skin prick tests were carried out over a one-year period on 1532 patients suffering from respiratory disorders (asthma and/or rhinoconjunctivitis). A total of 42 variables were studied in Cupressus-positive and Cupressus-negative patients; the data obtained were analyzed using a statistical software package. Sensitivity to Cupressus was found in 13% of all outpatients attending the unit, 18% of patients with respiratory disorders and 35% of patients with pollinosis. No significant differences were found between Cupressus-positive (C+) and Cupressus-negative (C-) patients with regard to mean age, sex, patient environment (i.e., rural, semi-rural, urban), personal or family history of atopy, clinical symptoms or evolution after immunotherapy (which did not include this antigen). More C+ patients were found in the higher age brackets (over 25 years old;  $p < 0.05$ ); C+ patients showed greater duration ( $p < 0.05$ ) and slower development ( $p < 0.05$ ) of symptoms, and were also found to be more sensitive to other pollens ( $p < 0.001$ ). All the Cupressus-sensitive patients also reacted positively to Olea and Fraxinus, compared to 77% and 51% in the two Cupressus-negative groups.

**Key words:** Pollinosis - Cupressus pollen allergy - Cupressaceae aeropalynology

### INTRODUCTION

Cupressaceae is a family of conifers which includes three genera in our area: *Thuja*, *Cupressus* and *Juniperus*, with two wild species found in the province (*J. oxicedrus* and *J. communis*) and five ornamental species which grow in gardens (*T. orientalis*, *T. occidenta-*

*lis*, *C. macrocarpa*, *C. arizonica* and *C. sempervirens*); their pollen is found in the air of the city of Córdoba during the winter season, from January to April.

The pollen grains most frequently recorded in the atmosphere of Córdoba during the winter belong mainly to five taxa: *Ulmus minor*, *Fraxinus* sp., *Populus* sp., *Alnus glutinosa* and Cupressaceae. Cupressaceae pollen is by far the most commonly occurring during this period (30%). *Ulmus minor* pollen accounts for 1.2% of the total, *Fraxinus* pollen for 0.6%, *Populus* pollen for 2.8% and *Alnus glutinosa* pollen for 0.5% (1).

Given this prevalence, and in view of the lack of local data regarding sensitivity to Cupressaceae pollen, an analysis was made of its incidence and potential allergenic significance in order to establish its clinical profile.

### MATERIALS AND METHODS

In addition to the routine allergy tests, total IgE and a battery of skin prick tests for inhalant allergens were performed on all outpatients attending the Allergy Unit for respiratory disorders. These allergens included house-dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*, *Acarus sirus* and *A. destructor*), pollens from wild and cultivated grasses (with individual testing of *Lolium perenne* and *Cynodon dactylon*), and from *Olea europaea*, as well as other pollens (*Chenopodium*, *Artemisia*, *Plantago* and *Parietaria*), fungi (*Penicillium*, *Aspergillus*, *Alternaria* and *Cladosporium*) and epithelial allergens (dog, cat, cow and horse).

Skin prick tests were performed for *Cupressus* pollen using a 2% weight/volume extract (2 g raw material per 100 ml vehicle, commercially prepared by Laboratorio CBF-Leti, Spain) and following the protocol laid down by the European Academy for Allergy and Clinical Immunology (E.A.A.C.I.) (2). The extract was tested previously on a group of 50 subjects with no history of allergy; no false positives were found.

Those patients whose response to *Cupressus* took the form of wheals equal to or greater than those caused by the positive control (histamine phosphate at 10 mg/ml) were considered positive (2).

Patients were thus classified as *Cupressus*-positive (C+) or *Cupressus*-negative (C-) according to the results of the skin test.

No other diagnostic tests were performed: the *Cupressus*-specific IgE test is not commercially available in the country, and the provocation test was excluded on the grounds that this study was designed only to assess the incidence of sensitivity rather than hypersensitivity and clinical response.

### Variables analyzed

The  $\chi^2$  test was performed using the SIGMASDI statistical software package to analyze a total of 42 variables which included the following:

#### Age

Six age ranges were assessed (0-5, 5-10, 10-15, 15-20, 20-25 and >25 years old) in an attempt to ascertain the age at which *Cupressus* sensitivity is manifested in this population.

#### Sex

Results were analyzed for possible prevalence of sensitivity in males or females.

#### Patient environment

The province was divided into three areas with distinct biogeographical and demographic characteristics: north (dense shrub or forestal area with a low population density), center (no natural vegetation due to intensive agricultural activity, densely populated) and south (rocky and mountainous area, with extensive olive tree cultivation, scattered dense populations). Each one of these biogeographical areas is characterized by different bioclimatic factors, with special plant formations, which cause effects in a manner different from *Cupressus* phenology (the main one being the flowering). As the central zone is warmer, flowering occurs there two weeks earlier than in the other zones.

#### Clinical symptoms

Results were analyzed in order to establish whether patients reported only symptoms of rhinoconjunctivitis or whether these were associated with bronchial asthma.

#### Duration of symptoms

Symptom duration was classified as seasonal (lasting only the length of the standard pollen season in this area, i.e., April-June), longer seasonal (February-Sep-

tember) or perennial (where symptoms lasted all the year round, or occurred during some other season).

#### Sensitization

This variable was considered indicative of positive reaction to one of the antigens tested in the skin prick test, regardless of whether it had clinical significance in the subject concerned, and was thus used to determine monosensitization to *Cupressus* and most frequent associations with other antigens.

#### Response to immunotherapy (RI)

In the follow-up period, and in accordance with Spanish and European Committee norms, some patients received immunotherapy. *Cupressus* was excluded from the treatment, even where sensitization was recorded, in order to determine differences in the evolution of symptoms between C+ subjects and C- subjects after a one-year interval. Subjects were classified as showing favorable evolution, no change or a worsening of the respiratory symptoms.

Results for the three groups of patients (C+, C- and total number of patients with respiratory disorders) were compared for possible statistically significant differences.

The airborne pollen counts were performed using a Burkard spore-trap situated on the roof of the Faculty of Sciences at a height of about 15 m. Sampling was carried out from 1982 to 1991, using the sampling and counting technique described by Domínguez *et al.* (3). These data from over a ten-year period were used to calculate daily means, thus providing an average curve for the whole year.

## RESULTS

Sensitivity to *Cupressus* was recorded in 13% of the patients attending the Allergy Unit claiming respiratory symptoms, and in 18% of the patients complaining of respiratory disorders.

**Table 1**

Origin of patients according to geographical divisions of the province of Córdoba. Symptom duration.

	Total patients	C(+)	C(-)	Signif.
Origin				
North	9	8	10	ns
Center	60	62	58	ns
South	31	30	32	ns
Symptom duration (years)				
< 2	10	8	12	ns
2-4	23	20	26	ns
4-6	14	10	18	ns
6-8	17	16	18	ns
> 8	36	46	26	p < 0.05

N = 1500 (percentage).

**Table 2**  
Clinical profile. Sex, age and nature and length of symptoms.

	Total patients	C(+)	C(-)	Signif.
Sex				
Male	50	53	45	ns
Female	50	47	55	ns
Age (years)				
0-5	5	2	8	ns
0-10	12	10	14	ns
10-15	18	22	16	ns
15-20	16	18	12	ns
20-25	13	6	20	ns
> 25	36	42	30	$p < 0.05$
Clinical syndrome				
Rhinoconjunctivitis	49	52	42	ns
Rhinoconjunctivitis/asthma	51	48	58	ns
Others	0	0	0	ns
Duration of symptoms				
Seasonal	56	56	56	ns
Longer seasonal	12	19	6	$p < 0.05$
Perennial	32	25	38	ns

N = 1500 (percentage).

A positive reaction was observed in 35% of pollinosis patients. A comparison of sensitivity rates in adults and children under 14 revealed a higher incidence in adults (37% vs. 24%,  $p < 0.01$ ).

As Table 1 shows, no significant differences were recorded between any two of the three patient groups (C+, C- and total) with regard to patient environment. Moreover, *Cupressus*-sensitive patients had displayed symptoms for over 8 years ( $p < 0.05$  compared to other groups).

No statistically significant differences were observed with regard to personal or family history of atopy.

Table 2 records clinical information (nature of respiratory disorder, reason for attending the Unit and overall duration of symptoms, i.e., seasonal, longer than seasonal and perennial); symptom duration was longer for C+ than for C- patients ( $p < 0.05$ ). No significant differences were recorded with regard to sex, although the incidence of *Cupressus* sensitivity was found to be higher in patients over 25 years of age.

As Table 3 shows, 100% of *Cupressus*-sensitive patients were also sensitive to *Olea* and *Fraxinus*, compared to 77% and 51% for the other two groups. None of the patients in this study were sensitive solely to *Cupressus*, and no significant differences were recorded between groups regarding the evolution of patients after receiving immunotherapy in which this pollen was not included.

Cupressaceae constitute the largest proportion of winter blooming taxa in the city of Córdoba. Figure 1 shows annual pollen variation with the daily ten-year average. The pollen season started in December and finished at the end of March. Maximum concentrations were recorded in February and March, reaching the highest peaks in February, with a maximum daily aver-

**Table 3**  
Clinical profile. Sensitization and evolution after treatment with immunotherapy.

	Total patients	C(+)	C(-)	Signif.
Sensitization				
Monosensitive	16	0	22	ns
Polysensitive	84	100	78	$p < 0.05$
<i>Olea</i> sensitive	83	100	77	$p < 0.05$
Non- <i>Olea</i> sensitive	17	0	23	ns
<i>Fraxinus</i> sensitive	78	100	51	ns
Non- <i>Fraxinus</i> sensitive	22	0	49	ns
Evolution*				
Improvement	69	68	70	ns
Equal	24	26	22	ns
Worse	7	6	8	ns

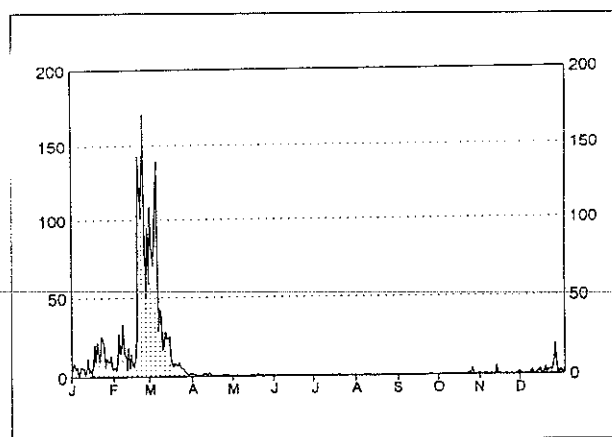
N = 1500 (percentage). \*Evolution on administration of immunotherapy (excluding *Cupressus*).

age over the ten-year period of 170 grains/m<sup>3</sup> in the air on 23 February. Maximum values for some years lay far from mean levels (i.e., 752 grains/m<sup>3</sup> on 22 February, 1983; 600 grains/m<sup>3</sup> on 19 February, 1988, and 993 grains/m<sup>3</sup> on 23 February, 1991). Very high values indeed to be taken into account if we compare them with the apparent allergenicity shown by this pollen.

## DISCUSSION

Although species of three genera are relatively common in the province of Córdoba, only those belonging to *Cupressus* and *Thuja* are sufficiently frequent, in terms of number of individuals, to produce either enough pollen to be detected during air sampling or enough allergen to cause sensitization in inhabitants of the area. However, *Thuja* is producing very little pollen in our area due to the bud cutting inherent to its cultivation as a hedge plant.

A number of authors, including Ariano, Aubert, Bousquet, Calistri, Caiaffa, Carbonnel and Panzini, have sought to establish the clinical and allergenic profile of conifer pollinoses in general, and of *Cupressus* pollino-

Fig. 1. Daily average pollen grains/m<sup>3</sup>/hour (1982-1991) of Cupressaceae.

sis in particular (4-9, 10-12). In their 1978 study, Michel *et al.* reported *Cupressus* to be one of the main agents responsible for winter pollinosis, due to the widespread presence of this pollen in the city of Montpellier (13).

Panzini, Zerboni and Ariano have concluded that sensitivity to Cupressaceae is a clearly defined clinical entity, whose allergenicity has yet to be clarified (12).

The study of *Cupressus* sensitivity in the province of Córdoba has led to similar conclusions, although analysis of results suggests that the following points should be highlighted:

Firstly, the absence of monosensitization (Table 3) – despite clearly reactogenic levels (4) – suggests that the resultant clinical syndrome is not sufficiently intense to prompt early medical consultation; this supposition is borne out by the greater incidence of *Cupressus* sensitivity in patients over 25 years of age, and by the prolonged duration (8 years) of symptoms prior to the study (Tables 1 and 2, respectively).

This sensitivity would appear to follow a subclinical course, leading patients to consult the doctor only when these subclinical effects are aggravated by sensitivity to other species – such as grasses and olive – with greater allergenic potential in this region.

The fact that 100% of C+ patients were also sensitive to *Olea* pollen (Table 3) may also support this hypothesis, rather than indicate a possible cross-reaction between Conifers and *Oleaceae*.

However, the degree of sensitivity to *Cupressus* pollen in this area is striking both for outpatients in general and for pollinosis patients: 37% of adult pollinosis patients were sensitive, as were 18% of all respiratory patients attending the Unit during the study period.

Symptoms in patients sensitive to *Cupressus* tended to be more persistent than those usually found in this area in patients sensitive to grasses and/or olive.

In conclusion, *Cupressus* sensitivity is common in Córdoba, and patients tend to delay medical consultation, generally suffer more prolonged symptoms, show clinical features similar to those provided by other pollinosis patients with respect to symptoms although not to intensity, and undergo progressive multisensitization.

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#### RESUMEN

Los granos de polen más frecuentes detectados en el aire de la ciudad de Córdoba durante el invierno pertenecen a la familia Cupressaceae. Los representantes de esta familia de coníferas pertenecen a tres géneros diferentes Thuja, Cupressus y Juniperus. El hecho de que hasta ahora no se disponga de datos clínicos respecto a la posible alergenicidad de su polen en Córdoba, unido a su alta concentración en el aire durante los meses de enero a abril, nos permite pensar en la posibilidad de que estas plantas sean responsables de las pollinosis invernales que ocurren en nuestra zona. Se realizaron pruebas cutáneas con extractos de polen de Cupressus a un total de 1532 pacientes con problemas respiratorios (asma y/o

rinoconjuntivitis), que representan un 73,77% del total de enfermos que acuden a la Unidad de Alergia. Se ha observado una sensibilidad al polen de Cupressus de un 13% respecto a todos los pacientes que se atienden en la Unidad de Alergia, un 18% de los pacientes con problemas respiratorios y un 35% de los pacientes diagnosticados de polinosis. No se han encontrado diferencias significativas entre pacientes Cupressus-positivo y Cupressus-negativo con respecto a edad, sexo, lugar de procedencia, datos históricos de atopia tanto personales como familiares, síntomas y evolución tras la inmunoterapia. Sólo se ha encontrado una diferencia significativa para una edad mayor de 25 años: los pacientes Cupressus-positivo presentan una duración mayor de los síntomas y un desarrollo lento en los mismos. La ausencia de monosensibilizaciones, a pesar de los altos niveles reactogénicos, sugiere que el síndrome clínico no es suficientemente intenso como para realizar consultas médicas en las fases tempranas. Esta suposición se basa en el hecho de que la mayor incidencia de sensibilidad a Cupressus se da en pacientes mayores de 25 años y en la prolongada duración de los síntomas. Esta sensibilidad parece seguir un curso subclínico, que lleva a que los pacientes sólo acudan a la consulta cuando la enfermedad se agrava por la sensibilidad al polen de otras especies (olivo y gramíneas). De hecho el 100% de los pacientes sensibles a Cupressus lo son también a Olea, lo que parece indicar una posible reactividad cruzada.

**Palabras clave:** Polinosis - Alergia al polen de *Cupressus* - Aeropalinología de Cupressaceae

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