

Aerobiological and allergenic analysis of Cupressaceae pollen in Granada (Southern Spain)

C. Díaz de la Guardia¹, F. Alba¹, C. de Linares¹, D. Nieto-Lugilde¹, J. López Caballero²

¹Departamento de Botánica. Facultad de Ciencias. Universidad de Granada. Granada, Spain

²Alergomedic. Clínicas de Alergia y Asma. Granada, Spain

Summary. Cupressaceae pollen has been cited in recent years as one of the major airborne allergens of the Mediterranean region, prompting us to conduct an exhaustive analysis on the aerobiological behaviour of this pollen in the Iberian Peninsula and the repercussion that it has had on the atopic population. The aerobiological study, performed from 1996 to 2003 in the city of Granada (S. Spain), used a volumetric Hirst collector. The results indicate that this pollen is present in the air most of the year, registering a high incidence during the winter months. This type of pollen behaved irregularly in the air, fluctuating yearly, seasonally, and within the same day. Temperature and humidity were the parameters that most directly influence the variability of this allergen, while rainfall prior to flowering increased pollen production. The predictive models used estimated a high percentage of the levels reached over the short term by this pollen in the atmosphere of Granada. The clinical study performed with atopic patients showed that some 30% of the population with pollinosis are sensitive to Cupressaceae pollen, affecting people of both genders equally. On the other hand, the most sensitive age group was 21-40 years of age, while children and the elderly registered almost negligible values. Most of the sensitive subjects resided within the city or in the metropolitan area, where environmental pollution reached high levels, while the pathology was found to be less frequent in rural zones. The most frequent symptoms were upper-respiratory ailments and an asthmatic profile.

Key words: Aerobiology, allergy, Cupressaceae pollen, predictive models, southern Spain.

Resumen. En los últimos años el polen de *Cupressaceae* se ha considerado uno de los principales alérgenos aéreos de la región Mediterránea, lo que motivó la realización de un análisis exhaustivo del comportamiento aerobiológico de este polen en la Península Ibérica y de la repercusión que tenía en la población atópica. En el estudio aerobiológico, llevado a cabo entre 1996 y 2003 en la ciudad de Granada (sur de España), se utilizó un captador volumétrico tipo Hirst. Los resultados indican que este polen se encuentra presente en el aire la mayor parte del año, pero registra una alta incidencia en los meses de invierno. Este tipo de polen mostró un comportamiento irregular en el aire, con fluctuaciones anuales, estacionales y en un mismo día. La temperatura y la humedad fueron los parámetros que influyeron más directamente en la variabilidad de este alérgeno, mientras que la lluvia antes de la floración incrementó la producción de polen. Los modelos predictivos utilizados estimaron un porcentaje elevado de los niveles alcanzados a corto plazo por este polen en la atmósfera de Granada. El estudio clínico realizado con pacientes atópicos mostró que un 30% de la población con polinosis es sensible al polen de *Cupressaceae*, afectando a ambos sexos por igual. Por otra parte, el grupo de edad más sensible fue el de 21 a 40 años, mientras que los niños y los ancianos registraron valores casi insignificantes. La mayoría de los sujetos sensibles residían en el núcleo urbano o en el área metropolitana, donde la contaminación ambiental alcanza niveles elevados, mientras que la patología fue menos frecuente en la zona rural. Los síntomas más frecuentes fueron afecciones de las vías respiratorias altas y un perfil asmático.

Palabras clave: Aerobiología, alergia, polen de *Cupressaceae*, modelos predictivos, sur de España.

Introduction

In southern Spain, the Cupressaceae family is represented both by autochthonous and by introduced species used for reforestation or, more frequently, for ornamental plants. Notable among these autochthonous plants are common juniper (*Juniperus communis* L., *J. oxycedrus* L.) and savin juniper (*J. phoenicea* L., *J. thurifera* L. and *J. sabina* L.), which are either trees or shrubs, forming part of the natural vegetation in woodlands and thickets throughout the Mediterranean region. Of the introduced species, the most frequent include Italian cypress (*Cupressus sempervirens* L.), Arizona cypress (*C. arizonica* Greene), Mexican cypress (*C. lusitanica* Miller), and Monterey cypress (*C. macrocarpa* Hartweg). Other Cupressaceae species are Lawson cypress (*Chamaecyparis lawsoniana* (A. Murray) Parl.), incense cedar (*Calocedrus decurrens* (Torr.) Florin), oriental arborvitae (*Platycladus orientalis* (L.) Franco), Western red cedar (*Thuja plicata* Lambert) and alerce (*Tetraclinis articulata* (Vahl) Masters), also used as ornamental plants.

Cupressaceae pollen is one of the most frequent of the annual pollen spectrum of this area, so that the pollen levels registered in certain periods of the year are extremely high, due both to the abundance of these species as well as their mass flowering. The high degree of pollen production of these species causes considerable biological pollution during the winter, which makes this type of pollen the most abundant in this period of the year, accounting for some 70% of the pollen spectrum. In addition, cypress pollen reaches significantly high concentrations in places of southern Spain, such as Jaén or Málaga [1-2] or the eastern part of the peninsula with a clear Mediterranean influence, such as Valencia or Barcelona [3-4], although annual quantities registered are lower than in Granada [5].

The allergenic importance of this family has been demonstrated by different authors in numerous works showing this pollen to be the cause of pollinosis, especially in Mediterranean countries such as Italy [6-8], France [9] [10], Israel [11], and Spain [12-14]. In addition, it has been shown in the last few decades that allergic reactions have significantly increased during the winter due to cypress pollen in the Mediterranean area [15-19] particularly due to the *Cupressus sempervirens* L. and *C. arizonica* Greene, which are considered a prime cause, and increasingly more common, of pollinosis.

Among the reasons for this significant increase in the allergy to Cupressaceae pollen is the greater exposure of the population to these plants [20], underestimation of its real prevalence by confusing it with winter colds [19], and the concomitant effect of environmental pollution on these allergenic particles [21]. Among specialists, the theory gaining acceptance and a general consensus is that respiratory illnesses appear with greater frequency in industrialized countries, especially among individuals living within urban areas with high pollution levels than among those living in rural areas. Also, the latest studies show that the rise in atopic respiratory ailments parallels the intensification of environmental pollution, particularly gases and particles [22, 23].

Similarly, current field studies in aerobiology corroborate a continual increase in airborne Cupressaceae pollens, a phenomenon that could considerably exacerbate allergies by intensifying the risk of exposure to these particles. This situation prompted the present preliminary study to determine the repercussion that Cupressaceae pollen has on the population in S. Spain.

Study area

The city of Granada is located in the SSE of the Iberian Peninsula (37° 11' N, 3° 35' W) in the Betic orological province of the Mediterranean region and in the Mesomediterranean bioclimatic level. Situated at the eastern end of the Genil Basin (altitude: 685 m), the city is surrounded by the mountains of the Betic and Sub-Betic ranges. The natural vegetation of this area includes holly oak (*Quercus ilex* L.) woodlands frequently replaced by degraded shrub-lands and pastures rich in grasses, accompanied by pine reforestation. The local climate is continental Mediterranean [24], with a marked continental character that accentuates the cold winters and warm summers, substantial swings in daily temperature, high insolation, low winds, and moderate precipitation with long dry periods. The topographical and climatic peculiarities characterizing the city of Granada and its environs favour the accumulation of particles in suspension, hampering the clearing of the atmosphere.

The population of the province of Granada is distributed in three large concentric rings. The first is a central one comprising the urban area, with medieval quarter and large avenues with modern buildings and dense traffic. The second ring is called the metropolitan area, which surrounds the urban area and contains the industrial zones and the new residential zones with ample garden areas. The third ring is the rural zone, with agricultural areas and country houses near the fields or natural vegetation (Figure 1).

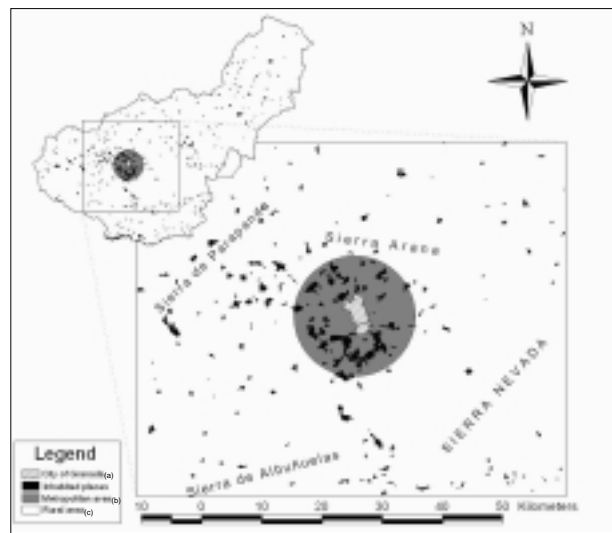


Figure 1. Province of Granada, distribution of the main population centres and indication of zoning: a) city of Granada, b) metropolitan area, c) rural zone.

Material and methods

This study was conducted in Granada (S. Spain) over 7 cypress-pollen seasons (1996/97-2002/03). A volumetric Hirst-type spore-trap [25] was located on the roof of the Faculty of Sciences (University of Granada), at 25 m above ground level, ensuring free air circulation. Daily pollen counts followed the methodology proposed by the Spanish Aerobiology Network, the REA, [26] and the data are expressed as number of pollen grains per cubic metre of air (grains/m^3). The main pollen season (MPS) was determined at 90%, taking the data compiled for September and August and following the methodology of Nilsson & Persson [27]. The most relevant data registered at the sampling stations during the MPS are shown. Graphs have also been plotted to show the average daily and diurnal counts for Cupressaceae, as well as the behaviour of the main meteorological parameters that influence the pollen content of the air. The degree of association between the daily observations of meteorological parameters (highest, mean and lowest temperatures; relative humidity; rainfall; and accumulated rainfall) and the pollen concentrations registered for the MPS of each period were submitted to a Spearman rank test.

The mathematical model selected to predict the Cupressaceae pollen concentrations was a stepwise multiple regression. The data for the pollen season 2002/03 were excluded from the model in order to use them to validate the predictive model established by comparing averages of the observed-predicted series by the Wilcoxon test. To improve the accuracy of the predictive equation, we included a new variable, dephased values from the pollen series, "Cupressaceae pollen concentrations recorded on the three previous days", which was closely correlated with the data series of pollen and helped plot the trends of the curves.

For the clinical study we carried out an observational transversal study of 610 patients who arrived consecutively at the Alergomedic Allergy Clinic (Alergomedic, Clínicas de Alergia y Asma de Granada), where they were attended for the first time between May 1999 and June 2003. In the present study, 347 individuals were selected for their seasonal symptoms and because they had come up positive in a pollen prick test, ruling out possible animal, food and drug allergies. The extracts

used in the prick test were grass pollen (*Lolium y Secale*), *Olea europaea*, *Cupressus arizonica*, *Artemisia vulgaris*, *Parietaria judaica*, *Plantago lanceolata*, *Chenopodium album*, *Platanus orientalis* and fungal spores (*Alternaria alternata*, *Cladosporium* and *Penicillium*). In recording the clinical data, we took into account five variables: age, sex, origin, antigen of the positive response and symptoms. This latter variable was in turn divided into 3 categories: 1) asthmatic profile (asthma, asthmatic rhinoconjunctivitis or rhinoasthma); 2) upper-respiratory ailments (rhinitis, conjunctivitis or rhinoconjunctivitis); and 3) dermatitis (urticaria). In addition, for the origin, three levels were established according to the classification proposed in Figure 1: a) patients residing in the urban centre of the city of Granada; b) inhabitants of residential zones surrounding the city or metropolitan area; and c) those living in rural zones of the province.

Results

The average daily rate of Cupressaceae pollen during the study period (1996-2003) is reflected in Figure 2. The pollen season develops over a long period from September to June-July, although pollen intensity peaks in February and March.

In all the periods studied, very high annual totals were recorded (Table 1), exceeding 14000 grains in most of the period (except 1998-99). Despite a general rising trend in the annual counts, these quantities fluctuate between years, varying from 9710 pollen grains in 1998-99 to 20412 in 2002-03. The cumulative method defining the Main Pollen Season (MPS) provides highly variable dates for the onset of the season, ranging from October to February, whereas the end remained quite stable (March and in exceptional cases April). This variability in defining the beginning date lengthened the MPS from 47 days (1999-00) to 147 days (1996-97). The peak day generally occurred in February and consistently with quantities higher than 1000 grains/ m^3 .

Figure 3 shows the evolution of the meteorological parameters (1996-03) which most directly influenced the airborne-pollen levels. Thus, temperature and insolation followed a downward curve towards the end of summer,

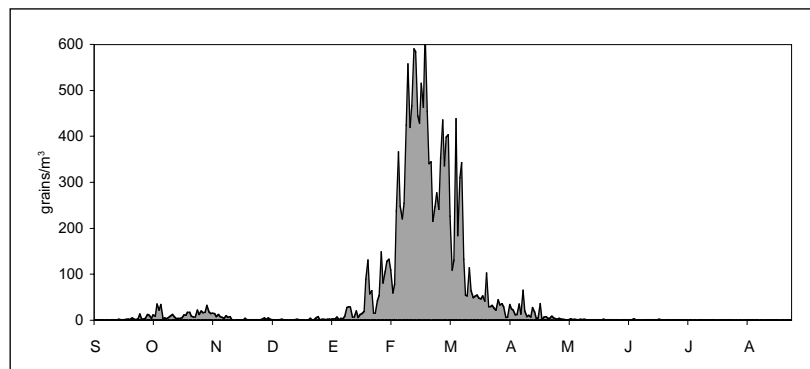


Figure 2. Daily average pollen concentration of Cupressaceae pollen during the study period (1996/97-2002/03).

Table 1. Total annual counts and main aspects of MPS of Cupressaceae pollen in the atmosphere over Granada.

Periods	Total grains grains/m ³	MPS			PEAK DAY	
		Starting date	Ending date	length n of days	Date	Value grains/m ³
1996/97	19091	29-10-96	24-03-97	147	16-02-97	1088
1997/98	17618	13-01-98	04-03-98	51	16-02-98	1837
1998/99	9710	28-01-99	20-03-99	52	09-03-99	2553
1999/00	14388	01-02-00	18-03-00	47	19-02-00	1240
2000/01	17028	22-01-01	22-03-01	60	07-02-01	1730
2001/02	16387	02-11-01	27-03-02	146	13-02-02	1211
2002/03	20412	29-01-03	12-04-03	74	05-03-03	2137

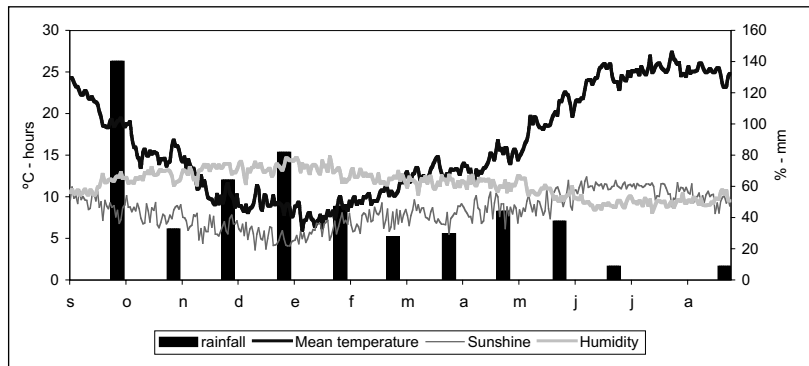


Figure 3. Average meteorological parameters for the study period (1996/97-2002/03).

Table 2. Spearman's correlation coefficients between daily Cupressaceae pollen during MPS and the principal meteorological parameters.

Variables	1996	1997	1998	1999	2000	2001	2002
Maximum temperature	0.671**	0.760**	0.719**	0.072	0.178	0.567**	0.432**
Minimum temperature	-0.318**	0.238	0.385**	-0.407**	-0.266**	-0.019	0.171
Mean temperature	0.358	0.742**	0.688**	-0.310*	-0.015	0.414**	0.427**
Sunshine	0.753**	0.419**	0.383**	0.318**	0.038	0.198*	0.100
Relative humidity	-0.622**	-0.479**	-0.152	-0.136	-0.192	-0.538**	-0.480**
Rainfall	-0.497**	-0.203	-0.175	-0.273	-0.201	-0.116	-0.141
Accumulated Rainfall	0.534**	0.652**	0.140	0.523**	0.212	0.197*	0.270*

* P ≤ 0.05, ** P ≤ 0.01.

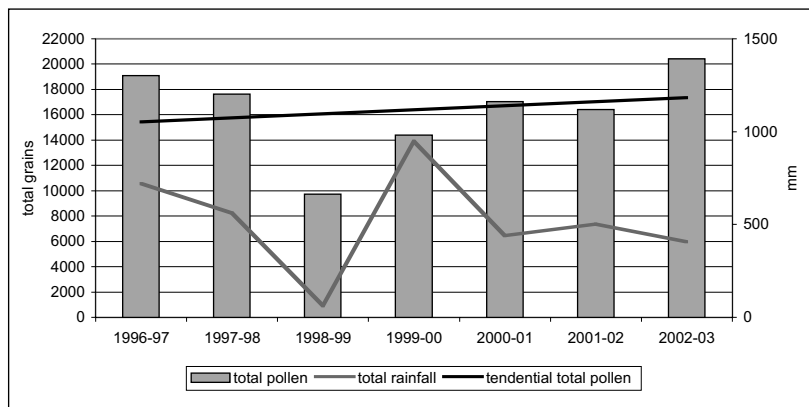


Figure 4. Relationship of total pollen and cumulative rainfall in the different pollen seasons analysed. Trend for total pollen production.

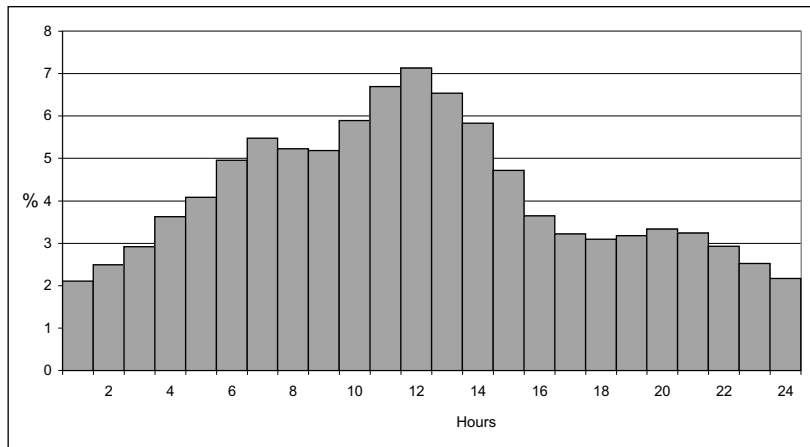


Figure 5. Average of the evolution of Cupressaceae pollen concentrations over the day for the study period 1996/97-2002/03.

Table 3. Predictive equation by stepwise multiple-regression analysis with indication of the equation derived at each step; r² regression coefficient.

Variables in the equation	Equation	r ²	F	Significance level
1 st Step a) 3-day before pollen	$y = 0.924 + 0.734a$	0.541	395.440	0.0000
2 nd Step a) 3-day before pollen b) Max temp	$y = 1.244 + 0.636a + 0.14b$	0.613	395.440	0.0000
3 rd Step a) 3-day before pollen	$y = 1.481 + 0.611a + 0.10b - 3.035 \cdot 02c$	0.619	272.728	0.0000

Table 4. Spearman's correlation coefficients and comparison of averages by the Wilcoxon test between daily Cupressaceae pollen during MPS of 2002-03 (observed) and data derived from linear regression (expected).

Spearman's correlation			Wilcoxon Test		Z	Significance level 2-Tailed P
r	Mean Rank		Sum of Ranks			
Observed-Expected	Observed negative	Expected positive	Observed negative	Expected positive		
0.316**	44.40	28.44	1865	910	-2.572	0.010

** P ≤ 0.01.

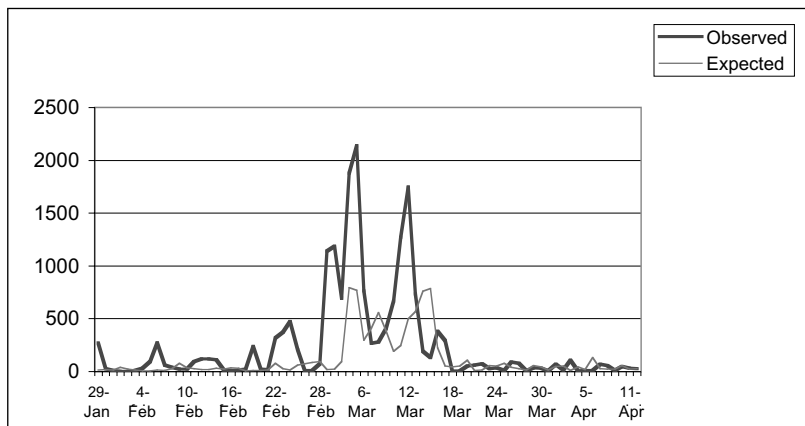


Figure 6. Graphic representation of the Cupressaceae pollen concentrations during the MPS of the period 2002-03 against the expected values, by predictive equations.

Table 5. Clinical percentage data grouped in different categories: total of the populational sample, proportion of poly- and monosensitive individuals, sex, age, origin, and symptoms.

	Total (n=104) (%)	Polysensitization (n=91) (%)	Monosensitization (n=13) (%)
Sex			
♀	50.0	50.5	46.2
♂	50.0	49.5	53.8
Age			
0-10	3.8	4.4	
11-20	18.3	20.9	
21-30	29.8	30.8	23.1
31-40	30.8	29.7	38.5
41-50	11.5	11.0	15.4
51-60	4.8	2.2	23.1
61-70	1.0	1.1	
Origin			
Urban centre	46.2	47.3	38.5
Metropolitan area	35.6	34.1	46.2
Rural zones	18.3	18.7	15.4
Symptomatology			
Asthma	45.2	50.5	7.7
RC	53.8	49.5	84.6
Dermatitis	1.0		

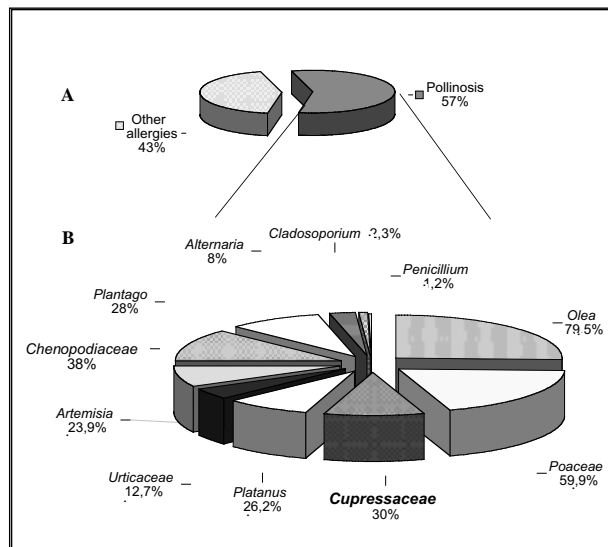


Figure 7. a) Total atopic population studied, percentage of pollinosis and other allergies (such as animals, foods or drugs). b) Percentage of pollen and spore sensitization.

reaching lows in January, followed by a new rise during February to a peak in July.

Humidity showed the opposite trend, so that the highest annual values occurred during the cold seasons, with lows in the dry season. Precipitation was heaviest in autumn and winter, followed by spring; during summer, the values were lowest.

The correlation test indicated the effect of the meteorological variables on the Cupressaceae pollen (Table 2). In general terms, this pollen correlated

positively with peak temperature, mean temperature, insolation and cumulative precipitation, but negatively with temperature lows, relative humidity and daily rainfall. The pollen/cumulative precipitation relationship was also evaluated by the graphic representation of the total Cupressaceae pollen production vs precipitation for September-August (Fig. 4). This modelling, together with the correlation analysis of the two variables, reflected a high degree of affinity between precipitation and the total pollen collected at the sampling station.

To estimate the behaviour of Cupressaceae pollen over an ideal day, the evolution of this pollen was averaged for the study years in percentages (Fig. 5). The pollen proved to be present in the air 24 h a day, the highest incidence occurring in the middle hours of the day and peaking at approximately 12.00 h. Table 3 presents the predictive equations established by multiple regression. This method provides a different equation at each step with the variables most closely related to Cupressaceae pollen. To simplify the mathematical model, we selected the equation of the second step, being more parsimonious and less complex, with an appropriate r^2 . This equation introduces two independent variables: pollen observations of previous three days; and peak temperature. The resulting r^2 was very satisfactory, predicting future concentrations at a very high confidence level (61%) and prognosticating the exposure risk to this pollen over a medium length of time.

The Wilcoxon test was used to compare the results from the regression equation and the pollen data found during the Cupressaceae pollen season of 2002-03 (Table 4), indicating that there were no significant differences between the two series compared. This was also corroborated by

the Spearman's correlation test, which indicated a high degree of association between the two variables, and by graphic representation (Fig. 6), showing the model to be capable of predicting the overall trend of this pollen in the air.

Among the population clinically diagnosed for pollinosis (347 records), some 30% (104 patients) presented sensitivity to Cupressaceae pollen (Fig. 7). The degree of this sensitivity is categorized in Table 5, which groups and cross-references the clinical data. Most of the patients were polysensitive (87.5%), while far fewer (12.5%) were monosensitive to Cupressaceae pollen. Moreover, males and females were equally affected, with a slightly higher number of monosensitive patients among males.

The symptoms in all these patients were mainly upper-respiratory or asthmatic, whereas dermatitis was uncommon. Polysensitive patients registered similar results, while among monosensitive subjects, 84.6% presented upper-respiratory symptoms (rhinitis, conjunctivitis, or rhinoconjunctivitis).

With respect to patient origin, the majority of the patients lived either in the city centre of Granada (46%) or in the metropolitan area (35%), and a minority (19%) lived in rural zones of the province. These proportions were similar for polysensitive subjects, but varied in the case of monosensitive, as the greatest amount of the latter lived in the metropolitan area of Granada (46%) and a somewhat lower percentage lived in the city centre.

The variable age was divided into decade categories, providing noteworthy data. More than half the patients, including polysensitive ones, were 21 to 40 years of age, while reactions to pollen were not common in children younger than 10 years of age or in the elderly. Among monosensitive subjects, this allergy affected primarily individuals between 21 and 60 years of age, with the highest frequency in the group of 31 and 40 years of age, with no case of monosensitivity found in subjects younger than 21 years.

Discussion

Aerobiological study

Seasonal behaviour

The continuous aerobiological sample provided data of general interest to the population of southern Spain, indicating the particle levels that inhabitants are exposed to throughout the year, and consequently the risk of an allergic reaction.

The analysis of seasonal behaviour of the Cupressaceae pollen revealed that the alternation of flowering of the different species keeps this pollen in the air almost the entire year. Nevertheless, pollen intensity is very high, between the second fortnight in January and the first in April, when primarily the species of the genus *Cupressus*, ubiquitous in urban vegetation, massively produces pollen [28].

This intense pollen production makes Cupressaceae pollen the most frequent particle found in aerobiological

sampling in Granada in winter, as reported in other Spanish cities with a Mediterranean climate, such as Málaga [29], Córdoba [30] or Lleida [4]. In agreement with these studies, we found high *Cupressus* pollen emission into the atmosphere in winter, making this particle a powerful allergen. In addition, the populations of places with a Mediterranean climate are more susceptible to developing allergies to cypress, given that, according to Belmonte et al. [14], this pollen appears more frequently in aerobiological samplings at sites where these ornamental species abound.

Data on the evolution of the total annual pollen not only reveal marked fluctuations between years (Table 1 and Figure 3), presumably due to the alternation of the production cycles of most of the tree species, but also reflect that pollen production has tripled in recent years [31]. As noted by numerous authors, the increased water reserves in the soil favour heavy production cycles among woody plants, producing the opposite effect when reserves are low. Figure 4 shows that when the annual precipitation surpasses the averages levels for Granada (402 mm), pollen production intensifies, being considerably lower when the rainfall does not exceed this value.

The duration and intensity of the MPS is closely linked to the length of the period registering allergic symptoms. In this way, we verified that the duration of the Cupressaceae pollen season presented pronounced yearly fluctuations (from 47 to 147 days), related directly to the meteorological conditions in the period prior to and during the season [32]. Thus, the precipitation during flowering causes excessive prolongation of the season and low pollen intensity (e.g. 1996/97 or 2001/02). On the other hand, excessively warm temperatures during pollen production shorten the MPS and prompt high concentrations (e.g. 1999/00).

The daily variation pattern enables us to establish the hours of highest risk of exposure during Cupressaceae pollen, revealing that the highest concentrations occur during the central hours of the day, coinciding with the highest temperatures and lowest humidity values. On the contrary, the pattern reverses in the evening, when rising humidity causes pollen grains to be deposited over any surface. The trend over the day found in Granada is similar to that of other points in Spain, such as, Córdoba [33] and Santiago de Compostela [34].

Correlation analysis

The correlation analyses (Table 2) showed that the evolution of Cupressaceae pollen in the air is strongly related to meteorological variables, so that peak temperature, mean temperature, and insolation generally augment the airborne-pollen content, encouraging both emission as well as dispersal. On the contrary, when the temperature lows descend in winter, emission is hampered and the higher humidity and precipitations provoke the aggregation and deposition of the pollen. These results coincide with those of numerous aerobiological works [34-36].

Regression analysis and model validation

The predictive models developed for the daily evolution of Cupressaceae pollen in Granada indicate that the equation can predict with 61% accuracy the seasonal dynamics of the pollen in the air. This model enables medium-term predictions, given that we can ascertain three days beforehand the independent variables that have been included in the equation ("pollen observations three days before" and "maximum temperature").

This information can be of utmost importance in providing warnings with some lead time for allergic people at risk of exposure to high pollen levels, or advising medical services for adopting preventive measures. In general, the literature reveals that temperature is the most influential variable in predictive models [35], although when we included the out-of-phase pollen series we considerably improved the predictive percentage.

The graphic comparison (Figure 6) of the predictive pollen series (expected) with the observed data over the MPS of 2002/03 (observed) reflect that the predictive data in general adjusted well to the evolution of the pollen season, delineating a seasonal dynamic very similar to the one actually observed. Although the model does not offer good results in predicting the highest values, we should emphasize that it allows predictions of both rising and falling trends with a high degree of effectiveness. In addition, the Spearman correlation test verifies the significant positive correlation indices between the numerical series (observed-expected), a fact that confirms that both of them present similar behavioural patterns. Finally, the Wilcoxon test corroborated that there were no significant differences between the series compared, confirming that the regressive model selected is valid to predict the evolution of airborne Cupressaceae pollen.

Clinical study

The increase in the prevalence of respiratory allergies ascribed to Cupressaceae pollen over recent years is related, according to numerous authors, to atmospheric pollution, although there is no consensus with respect to the particle that provokes this situation. Muranaka et al. [37] point to particles from diesel engines acting as an adjuvant. For others, such as Bousquet et al. [38] or Hrabina et al. [39] the cause is the considerable rise in the levels of airborne pollen produced by the overuse of these plants as ornamentals. The answer may simply be the better diagnostic quality of the tests used in recent years, replacing non-standardized extracts [40]. Finally, all the above factors may be involved.

In the present study, the use of specific standardized extracts with this allergen has revealed that some 30% of the patients with pollinosis present sensitivity to cypress pollen, a number close to that reported by Guerra et al. [41] in Córdoba (Spain), where 35% of the patients reportedly reacted positively in the Cupressaceae skin test. In places under the Mediterranean influence, the prevalence of reaction to this pollen is also high, being 35.1% in Rome [20], 32% in Tel-Aviv [11], 19.5% in Montpellier [38] and 13.4% in Morocco [42]. In other

places, the frequency of allergy sensitivity of patients with pollinosis is lower, probably due to a more restricted distribution of these taxa and consequently a lower concentration of this allergen in the air, such as Zaragoza, where the rate is only 3% [43].

The study of the symptoms reveals that in the Granada population the most frequent ailments are upper-respiratory and asthmatic, both appearing with the same frequency. These data are similar to those of numerous specialized publications, which also identify cypress pollen as the main cause of rhinoconjunctivitis and asthma in the Mediterranean area. The high percentage of monosensitive patients, 12% of the total Cupressaceae-sensitive cases studied in Granada, could be related to the fact that this pollen is practically the only one that we detect in the atmosphere of Granada during the winter period, as well as the overuse of cypresses in gardens and avenues. Similar results have been found in places with a similar airborne-pollen content and Mediterranean climate. Thus, in a study in Italy by the Italian Association of Aerobiology [44] it was reported that these represent 14.7% of the total, even reaching values of up to 19.3% in Latium [8].

It should be taken into consideration that the atmospheric, topographic, and urban characteristics of Granada discourage the dispersion of airborne particles, with the natural ventilation and atmospheric purification processes further accentuating this situation of exposure to airborne allergens for the population. In addition, studies on air-quality diagnosis [45] reveal that the limit values and the immission guideline established by the European regulations for air pollutants are amply exceeded (nitrogen dioxide, sulphur dioxide, carbon monoxide, particles in suspension, and ozone), evidencing higher levels of immission in winter. This high density of biological and non-biological particles during the winter could explain the high percentage of patients sensitized to this airborne allergen in Granada.

This hypothesis gains even more strength when we analyse the origin of the sensitive individuals. The great majority were from the urban centre of Granada and the metropolitan area (81%), where traffic and industry are dominant elements, whereas relatively few were from rural areas (19%) where atmospheric pollution is low. The added effect of environmental pollution on the *Cupressus* genus has recently been investigated by Midoro-Horiuti et al. [46], indicating that the prime allergen, *Cup a 3*, belongs to the PR-5 family of proteins, related to the pathogeny in plants, the expression of which is induced in situations of stress or by diesel and ozone particles [47, 48]. This bolsters the idea that the high index of dissolved pollutants in the atmosphere of Granada and the surrounding air could stimulate the synthesis of PR-5 proteins in cypress pollen and could also be responsible for the high frequency of allergic processes in the urban population.

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References

- Ruiz, L., Cano, E. & Díaz de la Guardia, C. Aerobiología en Andalucía: Estación de Jaén (1999). REA 2000, 6: 39-42.
- Recio, M., Trigo, M.M., Docampo, S. & Cabezudo, B. Aerobiología en Andalucía: Estación de Málaga. REA 2002, 7: 83-88.
- Totajada, B. & Mateu, I. Aerobiología en la Comunidad Valenciana: Estación de Burjassot (2000-2001). REA 2002, 7: 167-172.
- Belmonte, J. & Roure, J. Aerobiología en Catalunya: Estación de Lleida. REA 2002, 7: 155-160.
- Alba, F., Díaz de la Guardia, C. & Sabariego, S. Aerobiología en Andalucía: Estación de Granada. REA 2000, 6: 31-34.
- Caiaffa, M. F., Macchia, L., Strada, S., Bariletto, G., Scarpelli, F. & Tursi, A. Airborne *Cupressaceae* pollen in Southern Italy. Annals of Allergy 1993, 71: 45-50.
- Nardi, G., Canziani, A., Striani, P., Santini, N., Coccia, C., Seghetti, L. & Kranic, R. Cupressaceae pollen in the atmosphere of Ascoli Piceno (Central Italy) and sensitization of allergenic subjects. Aerobiologia 1996, 12: 269-271.
- Papa, G., Romano, A., Di Fonso, M., Viola, M., Artesani, M., Di Gioacchino, M. & Venuti, A. Prevalence of sensitization to *Cupressus sempervirens*: a 4-years retrospective study. Sci. Total Environ. 2001, 270 (1-3): 83-87.
- Charpin, D., Hughes, B., Mellea, M. Seasonal allergenic symptoms and their relation to pollen exposure in south-east France. Clin. Exp. Allergy 1993, 23: 435-439.
- Panzani, R. History of allergy to Cypress pollen. Allerg. Immunol. 2000, 32 (3): 142-144.
- Geller-Bernstein, C., Waisel, Y. & Lahoz, C. Environment and sensitization to cypress in Israel. Allerg. Immunol. 2000, 32: 92-93.
- González Romano, M. L. a. Estudio aeropolínico de Sevilla (1988-1990). An. Asoc. Palinol. Leng. Esp. 1993, 6: 39-50.
- Cabezudo, B. Trigo, M.M., Recio, M. & Toro, F.J. Contenido polínico de la atmósfera de Málaga: años 1992-1993. Acta Botánica Malacitana 1994, 19: 137-144.
- Belmonte, J., Canela, M., Guardia, R., Guardia, R.A., Sbai, L., Vendrell, M., Cariñanos, P., Díaz de la Guardia, C., Dopazo, A., Fernández, D., Gutiérrez, M. & Trigo, M.M. Aerobiological dynamics of the Cupressaceae pollen in Spain, 1992-98. Polen 1999, 10: 27-38.
- D'Amato, G., Spiekma, F.Th.M., Liccardi, G., Jäger, S., Russo, M., Kontou-Fili, K., Nikkels, H., Wüthrich, B. & Bonini, S. Pollen-related allergy in Europe. Allergy 1998, 53: 567-578.
- Michel, F. B., Dhivert, H., Marty, J.P., Alquie, M.C., Cour, P. & Guerin, B. Pollinoses hivernales. Rev. Franç. Allergol 1978, 18 (2): 83-88.
- Panzani, R., Zerboni, R. & Ariano, R. (1991). Allergenic significance of Cupressaceae pollen in some parts of the Mediterranean area. In G.D'AMATO, F.Th.M. SPIEKSMAS & S. BONIN (eds). Allergenic pollen and pollinosis in Europe, pp. 81-84. Blackwell Scientific publications, Oxford.
- Ariano, R., Panzani, R.C., Chiappella, M. & Augeri G. Pollinosis in a Mediterranean area (Riviera Ligure, Italy): ten years of pollen counts, correlation with clinical sensitization and meteorological data. J. Invest. Allergol Clin Immunol 1994, 4: 81-86.
- Mari, A., Di Felice, G., Barletta, B., Tinghino, R. & Pini, C. Cypress allergy: An underestimated pollinosis. Allergy 1997, 52: 355-356.
- Di Felice, G., Caiaffa, M.F., Bariletto, G., Allerni, C., Di Paola, R., Mari, A. Allergens of Arizona cypress (*Cupressus arizonica*) pollen: Characterization of the pollen extract and identification of the allergenic components. J. Allergy Clin. Immunol. 1994, 94: 547-555.
- Miyamoto, T. Epidemiology of pollution-induced airway in Japan. Allergy 1997, 52 (38): 30-36.
- D'Amato, G., P. & Liccardi, G. Outdoor environmental injury of airways and development of allergic respiratory diseases. Pulmon Pharmacol Therap 1998, 11: 369-374.
- D'Amato, G., Liccardi, P., Russo, G. & D'Amato, M. On the interrelationship between outdoor air pollution and respiratory allergy. Aerobiologia 2000, 16(1): 1-6
- Capel Molina, J. J. Los climas de España. Oikos-tau 1981, pp: 133-163.
- Hirst, J. M. An automatic volumetric spore-trap. Ann. Appl. Biol. 1952, 39: 257-265.
- Domínguez Vilches, E., Galán Soldevilla, C., Villamandos de la Torre, F. & Infante García-Pantaleón, F. Handling and evaluation of the data from the aerobiological sampling. Monografías REA/EAN 1991, 1: 1-8.
- Nilsson, S. & Persson, S. Tree pollen spectra in the Stockholm region (Sweden), 1973-1980. Grana 1981, 20: 179-182
- Tormo, R., Muñoz, A., Silva, J. & Gallardo, F. Pollen production in anemophilous trees. Grana 1996, 35: 38-46 (370).
- Recio, M., Trigo, M.M., Toro, J. & Cabezudo, B. Caracterización del contenido polínico invernal de la atmósfera de Málaga. Acta Botánica Malacitana 1996, 21: 65-70.
- Ruiz de Clavijo, E., Galán, C. & Infante, F. Variations of airborne winter pollen in southern Spain. Allergol. et Immunopathol 1988, 16 (3): 175-179.
- Díaz de la Guardia, C. & Alba, F. Aerobiología en Andalucía: Estación de Granada (1997). REA 1998, 4: 29-32.
- Keynan, N., Waisel, Y., Shomer-Ilan, A. & Tamir, R. Forecasting pollen pollution: correlation with floral development. Ann. Allergy 1989, 63: 417-420.
- Galán, C., Tormo, R., Cuevas, J., Infante, F., Domínguez, E. Theoretical daily variation patterns of airborne pollen in the South-West of Spain. Grana 1991, 30: 201-209.
- Aira, M. J., Dopazo, A. & Jato, M.V. Aerobiological monitoring of Cupressaceae pollen in Santiago de Compostela (MW Iberian Peninsula) over six years. Aerobiologia 2001, 17: 319-325.
- Galán, C., Fuillerat, M.J., Comtois, P. & Domínguez-Vilches, E. Bioclimatic factors affecting daily Cupressaceae flowering in southwest Spain. Int. J. Biometeorol 1998, 41: 95-100.
- Norris-Hill, J. & Emberlin, J. Diurnal variation of pollen concentration in the air of north-central London. Grana 1991, 30: 229-234
- Muranaka, M., Suzuki, S., Koizumi, K., Takafuji, S., Miyamoto, T., Ikemori, R. & Tokiwa, M. Adjuvant activity of diesel exhaust particles for the production of IgE antibody in mice. J. Allergy Clin. Immunol. 1986, 77: 616-623
- Bousquet, J., Knani, J., Hejjaoui, A., Fernando, R., Cour, P., Dhivert, H. Heterogeneity of atopy. I. Clinical and Immunologic characteristics of patients allergic cypress pollen. Allergy 1993, 48: 183-188.
- Hrabina, M., Dumur, J.P., Sicrad, H., Viatte, A. & Andre, C. Diagnosis of cypress pollen allergy: in vivo and in vitro

- standardization of a *Juniperus ashei* pollen extract. Allergy 2003, 58: 808-813.
40. Charpin, D. Epidemiology of cypress allergy. Allerg. Immunol. 2000, 32: 83-85.
 41. Guerra, F., Daza, J.C., Miguel, R., Moreno, C., Galán, C., Domínguez, E. & Sánchez-Gijo, P. Sensitivity to *Cupressus*. Allergenic significance in Córdoba (Spain). J. Invest. Allergol Clin Immunol 1996, IV: 117-120.
 42. Alaoui Yazidi, A. & Bartal, M. Sensibilisation cutanée au pollen de cyprès au Maroc: étude multicentrique. Allerg. Immunol. 2000, 32: 113-116.
 43. Pola Pola, J., Zapata Jiménez, C. & Sanz Turón, E. Polinosis en el área de Zaragoza. Rev. Esp. Alergol Immunol Clin. 1998, 13(2): 135-139.
 44. Aerobiology, Italian Association (2002). "An epidemiological survey of Cupressaceae pollenosis in Italy." J. Invest. Allergol Clin Immunol 12(4): 287-292.
 45. Ayuntamiento Granada. Diagnóstico Ambiental. In Ayuntamiento de Granada homepage [on line]. 2004. [Cited on 14th October 2004]. Available in World Wide Web: <http://www.granada.org/inet/wambiente.nsf/link/za1e>.
 46. Midoro-Horiuti, T., Goldblum, R.M., Kurosky, A., Wood, T.G., Schein, C.H. & Broods, E.G.. Variable expression of pathogenesis-related protein allergen in Mountain Cedar (*Juniperus ashei*) pollen. J. Immunology 2000, 164: 2188-2192.
 47. Van Loon, L. C., Gerritsen, Y. Protease activity and pathogenesis-related proteins in virus-infected Samsun NN tobacco leaves. Plant Sciences 1989, 63: 141.
 48. Linthorst, H. J. M. Pathogenesis-related proteins of plants. Crit. Rev. Plant Sci. 1981, 10: 123.

Dr. C. Díaz de la Guardia

Facultad de Ciencias
Universidad de Granada
Campus Fuente Nueva
E-18071 Granada, Spain
Phone: +34958243394
E-mail: cdiaz@ugr.es