

Models for forecasting airborne Cupressaceae pollen levels in central Spain

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Abstract The influence of meteorological variables on airborne Cupressaceae pollen levels in central Spain was analyzed, and prediction models based on polynomial and multiple regressions were used to predict pollen counts throughout the pollen season. The Cupressaceae pollen type was selected in view of both its abundance in the atmosphere of the central Iberian Peninsula (particularly from January to March) and its allergenic importance. Sampling was performed uninterruptedly over a 5-year period, using a Hirst volumetric sampler and the sampling method established by the Spanish Aerobiology Network. Temperature displayed the strongest (positive) correlation with Cupressaceae pollen counts. Polynomial and multiple regression analysis showed that maximum temperature was the most influential variable included in prediction models. The prediction equations obtained for the study period were reasonably satisfactory, accounting for 48% and 59% of the variation in airborne pollen levels.

Keywords *Cupressus* spp. · *Juniperus* spp. · Allergenic pollen · Meteorological variables · Polynomial and multiple regression analysis · Central Spain

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Introduction

The Cupressaceae family includes 21 genera and around 130 species of anemophilous trees and shrubs in the northern and southern hemispheres. In the Mediterranean region, this family is widely represented both by native species, typically in woodland, and by non-native species used for ornamental purposes and in some reforestation programs. In Spain, junipers (*Juniperus communis* L., *Juniperus oxycedrus* L., *Juniperus thurifera* L., *Juniperus phoenicea* L. and *Juniperus sabina* L.), and the Cartagena cypress (*Tetraclinis articulata* (Vahl) Mast.) are native species, whilst introduced species include members of the genera *Cupressus*, *Platycladus* and *Chamaecyparis* (Amaral 1986). The species *Cupressus arizonica* Greene, *Cupressus macrocarpa* Hartweg and, particularly, *Cupressus sempervirens* L. are a common feature of parks and gardens in towns and cities.

Cupressaceae pollen grains are characterized as spheroidal, intectate and monoporate with a distinct annulus (Bortenschlager 1990), and are stenopalynous, i.e., light microscopy reveals no morphological differences at species or genus level. Cupressaceae, and particularly cypresses, produce abundant pollen (Hidalgo et al. 1999) and contribute heavily to total airborne pollen counts in much of the Mediterranean area. In Italy and Albania, Cupressaceae pollen accounts for between 20% and 40% of total pollen recorded (Mandrioli et al. 2000; Priftanji et al. 2000). In the Spanish cities of Madrid and Toledo, located in the center of the Iberian Peninsula, Cupressaceae are reported to account for 22.5% and 23.3%, respectively, of total pollen counts (Gutiérrez et al. 2001; Pérez-Badia et al. 2010), and their pollen persists in the atmosphere over more than half the year, reflecting the relatively long flowering period of this family, i.e., from October to April.

This pollen type is of both aerobiological and clinical interest, since it is responsible for winter pollen allergies (Caramiello et al. 1991; D'Amato et al. 1992) in most Mediterranean countries, including France, Italy and Spain (Charpin 2000; Papa et al. 2001; Guerra et al. 1996; Subiza et al. 2007). In Spain, the highest prevalence of Cupressaceae-positive skin-tests is found in the central area of the Iberian Peninsula, with rates of 58% in Madrid and 30% in Toledo (Subiza et al. 2007). Research into the relationship between meteorological variables and Cupressaceae pollen production is of great interest for pollen-allergy prevention, since it enables the construction of prediction models aimed at forecasting pollen season start- and end-dates, duration, intensity, daily counts, etc. (Galán et al. 1998; Teranishi et al. 2000; Díaz de la Guardia et al. 2006; Fuertes-Rodríguez et al. 2007).

The present study sought to chart the influence of meteorological variables on Cupressaceae pollen counts in the central Iberian Peninsula, and to construct prediction models based on polynomial and multiple regressions in order to forecast Cupressaceae pollen counts. Cupressaceae is one of the most abundant pollen types in the atmosphere of central Spanish cities, and is thus of considerable allergenic importance. It is hoped that this research will improve the quality of life of pollen-allergy sufferers, including both local residents and the thousands of tourists visiting the area every year.

Materials and methods

Area of study

This study was carried out in the city of Toledo (39°51'N, 4°01'W) over a 5-year period (2005–2009). Toledo is located in the center of the Iberian Peninsula, on the banks of the river Tagus, 529 m a.s.l. It has been declared a World Heritage City and is visited by more than 2 million tourists every year. Its climate is Mediterranean with continental influence. Bioclimatically, according to the classification drawn up by Rivas-Martínez et al. (2002), Toledo lies in the Mesomediterranean belt, with a rainfall pattern characteristic of dry Mediterranean areas. Marked temperature oscillations are reported throughout the year: temperatures are low in winter (January: mean 6.4°C) and high in summer (July: mean 26°C). Annual mean temperature is 15.4°C. Rainfall is scarce (mean 382 mm) and is concentrated mainly in spring and late fall, with a summer drought (Ministerio de Medio Ambiente 2001).

Biogeographically, Toledo is part of the Mediterranean Region (W Mediterranean Subregion) and, according to Rivas-Martínez et al. (2002), is located on the border between two biogeographical provinces: the Mediterranean Central

Iberian Province (Castilian Subprovince, Manchegan Sector) and the Mediterranean West Iberian Province (Lusitan-Extremadurean Subprovince, Toledan-Taganean Sector). The local landscape comprises mostly cropland—mainly cereals and olive groves—whilst natural vegetation is composed largely of Mediterranean forest dominated by the holm oak [*Quercus ilex* L. subsp. *ballota* (Desf.) Samp.] and Mediterranean scrub dominated by *Cistus* spp., *Erica* spp., *Rosmarinus officinalis* L., *Thymus* spp., *Lavandula stoechas* L. subsp. *pedunculata* (Miller) Samp. ex Rozeira, among others.

Airborne pollen counts, meteorological data and statistical analyses

Airborne Cupressaceae pollen was collected with a Hirst-type volumetric spore trap (Hirst 1952), located on the roof of a building belonging to the University of Castilla-La Mancha in Toledo, approximately 17 m above ground level. The sampler sucks in air at a rate of 10 l/min, and pollen grains are trapped on a adhesive-coated Melinex® strip. Sampling and slide analysis were carried out in accordance with the protocol developed by the Spanish Aerobiology Network (Galán et al. 2007). Slides were analyzed daily under a light microscope (400x magnification), with four longitudinal sweeps per slide. Results were expressed as daily average number of pollen grains per cubic meter of air (grains/m³).

The main pollen season (MPS) is interpreted as the portion of the pollen season during which most pollen is recorded, and was determined, according Nilsson and Persson (1981) because with it, we achieved the best predictive models. It is also the methodology followed by some authors that worked in Cupressaceae pollen, such as Galán et al. (1998), Aira et al. (2001) and Tortajada and Mateu (2008). This method includes 90% of the seasonal total pollen count, starting on the day by which 5% of total pollen has been recorded and ending on the day by which 95% of total pollen has been registered. The Cupressaceae pollen season is long, and does not correspond to natural years. The annual cycle was taken as starting in October and ending in the following September.

Meteorological data were provided by the Toledo-Buenavista weather station, located 2 km from the spore trap, using the Spanish Meteorological Agency (AEMET) database (<http://www.aemet.es/en/portada>). The following meteorological variables were analyzed: maximum temperature (Tmax); mean temperature (Tmean); minimum temperature (Tmin); hours of sunlight (HS); relative humidity (H); rainfall (R); and wind speed (WS). Daily values for each variable were recorded, together with means for each day and for the 2 preceding days (Tmax_r, Tmean_r, Tmin_r, HS_r, H_r, R_r and WS_r).

Table 1 Characteristics of the main Cupressaceae pollen season. MPS Main pollen season

	Year	2005	2006	2007	2008	2009	Mean (2005–09)
MPS	Start day	3 February 2005	31 January 2006	14 November 2006	25 December 2007	7 January 2009	11 January
	End day	31 March 2005	15 April 2006	6 April 2007	20 March 2008	20 April 2009	21 March
	Season duration (days)	57	76	144	86	104	93
	Total pollen	16,141	4,206	10,820	16,572	9,480	11,444
	Peak day (date)	16 March 2005	12 March 2006	4 March 2007	16 January 2008	25 February 2009	10 March
	Peak day (concentration)	1,834	690	1,258	1,473	707	377
Total grains		17,223	4,422	11,389	17,447	9,981	12,092

Statistical analysis included correlation and regression analysis. The SPSS 15.0® software package was employed for all statistical analyses (<http://www.spss.com>). Correlation analysis was used to study the relationship between daily mean Cupressaceae pollen counts over the MPS and each of the meteorological variables indicated before. A non-parametric statistic (Spearman's correlation test) was used, since the data were not normally distributed.

Prediction models based on regression analysis were used to predict pollen counts. To apply regression analysis (parametric statistic) a logarithmic transformation ($\log [x + 1]$, $x = \text{daily mean Cupressaceae pollen concentration} - \text{grains} / \text{m}^3$) was carried out. After checking that the data were adjusted to a normal distribution, regression analysis based on both polynomial and multiple regressions was used to predict Cupressaceae pollen counts in central Spain. The results

obtained using the two methods were compared. For polynomial regression analysis, only one variable is included in the predictive equation, i.e., the variable best accounting for the variation in Cupressaceae pollen counts. Multiple regression allows examination of the simultaneous influence of the independent variables (meteorological variables) on the dependent variable (daily pollen concentration), and stepwise analysis was used, since it takes into account collinearity between variables and yields a different equation at each step using the variables correlating most strongly with pollen counts. The most parsimonious and least complex equation—that with the best R^2 value—was selected. In order to improve the predictive ability of the model, pollen counts for preceding days were also included (Cup_1: pollen count for the previous day; Cup_2: pollen counts for the 2 preceding days; Cup_3: counts for the 3 preceding days).

Table 2 Mean values of temperature (Tmean), rainfall and Cupressaceae pollen registered during winter, MPS and rainfall collection over the 2 months prior (2 MP) to the main pollen season

		Tmean (°C)	Rainfall (mm)	Pollen Cupressaceae (grains)
2005	Winter	6.2	33.3	15,564
	MPS	8.9	43.0	16,141
	2 MP to MPS		3.8	
2006	Winter	7.3	60.6	4,010
	MPS	10.7	57.4	4,206
	2 MP to MPS		33.6	
2007	Winter	8.4	38.5	10,530
	MPS	8.7	140.8	10,820
	2 MP to MPS		176.4	
2008	Winter	9.4	32.3	16,657
	MPS	9.0	35.0	16,572
	2 MP to MPS		28.8	
2009	Winter	7.3	125.0	9,309
	MPS	9.4	191.3	9,480
	2 MP to MPS		62.2	

Table 3 Spearman's correlation coefficients between daily Cupressaceae pollen concentration during the main pollen season and meteorological variables. Mean values of each day and the 2 previous days are also considered (_r)

	Cupressaceae 2005-08
Maximum temperature (°C)	0.634**
Tmax_r (°C)	0.674**
Mean temperature (°C)	0.594**
Tmean_r (°C)	0.607**
Minimum temperature (°C)	0.371**
Tmin_r (°C)	0.396**
Hours of sunlight (HS)	0.229**
HS_r	0.314**
Rainfall (mm)	-0.069
R_r (mm)	-0.152*
Relative humidity (%)	-0.271**
H_r (%)	-0.217**
Wind speed (m/s)	0.075
WS_r (m/s)	0.031

** $P \leq 0.01$, * $P \leq 0.05$

Table 4 Polynomial and multiple regression equations obtained to forecast the daily Cupressaceae pollen. Tmax: maximum temperature; Tmax_r: mean value of maximum temperature for each day and for the two preceding days; Cup_1: pollen count for the previous day

	Predictive equation	R^2
Polynomial regression	$y = -2.579 + 0.444 \text{ Tmax_r} - 0.010 \text{ Tmax_r}^2$	0.48
Multiple regression	$y = 0.502 \text{ Cup_1} + 0.074 \text{ Tmax} - 0.275$	0.59

The prediction models were constructed using data for 2005–2008. Pollen data for 2009 were used for the external validation of the resulting models. To verify the validity of the models obtained, the correlation between data observed in 2009 and data predicted using the model based on the 2005–2008 period was determined by means of Spearman's correlation test. The distributions of the values of observed and predicted data were also compared using the Wilcoxon signed-rank test.

Results

Maximum pollen counts were recorded between January and March (Table 1). MPS length ranged from 57 days in 2005 to 144 days in 2007. The highest total counts during MPS were recorded in 2005 and 2008 (16,141 and 16,572 grains, respectively). The peak daily count was reached between late February and mid-March, except in 2008 when it was reported in mid-January. The maximum daily value was 1,834 grains/m³, registered in 2005 (Table 1).

Table 2 shows meteorological data (mean temperature, rainfall) for the winter season, and for the MPS of each year, together with Cupressaceae pollen counts recorded in these periods. The rainiest winter was in 2009 (125 mm), while the driest were in 2005 and 2008 (less than 34 mm). The rainiest main pollen seasons were observed in 2007 and 2009 (140.8 and 191.3 mm, respectively), while rainfall remained below

58 mm in the other years. Rainfall over the 2 months prior to the pollen season was significant in 2007 and 2009, although rather less in 2009 (176.4 mm and 62.2 mm respectively). The highest mean temperatures during the MPS were noted in 2006 (10.7°C), while the lowest were recorded in 2007 (8.7°C). Finally, the coldest winter was in 2005 (6.2°C), and the warmest in 2008 (9.4°C).

Correlations between daily average Cupressaceae pollen counts during the MPS and the main meteorological variables were significant and positive for temperature (Tmax, Tmax_r, Tmean, Tmean_r, Tmin, Tmin_r) and hours of sunlight (HS, HS_r), and significant and negative for rainfall (R_r) and relative humidity (H, H_r) (Table 3). Polynomial regression showed that Tmax_r was the variable best accounting for airborne Cupressaceae pollen behavior, with $R^2=0.48$ (Table 4). In multiple regression, the equation containing the variable Cup_1 (Cupressaceae pollen count for the previous day) and Tmax (maximum temperature) displayed the strongest correlation with Cupressaceae pollen counts, accounting for 59% of variation in pollen levels (Table 4).

The Wilcoxon signed-rank test (Table 5) was used to compare the distribution of observed and predicted data. The results of polynomial and multiple regressions revealed no significant differences between these two data series ($P>0.05$). Spearman's correlation test indicated a high degree of association between the two data series when multiple regression was used ($r=0.607^{**}$), and a weaker association when polynomial regression was used ($r=0.340^{**}$).

Discussion

Cupressaceae pollen persists in the atmosphere of Toledo over a long period (October–April) due to the consecutive flowering of different Cupressaceae species (Pérez-Badía et al. 2010). However, the pollen season occurs mainly in January, February and March, coinciding with the flowering of the *Cupressus* species most widely grown in the area, which are responsible for producing large

Table 5 Wilcoxon signed-rank test to compare the distribution of concentrations of Cupressaceae pollen data from 2009 (observed) and derivatives of the polynomial and multiple regressions for 2005–2008 (predicted). Spearman's correlation coefficients between observed and predicted data

				Significance level
Polynomial regression				
Wilcoxon signed-rank (observed–predicted)	Negative ranks $N^- = 31$ Positive ranks $N^+ = 29$	Average = 28.65 Average = 32.48		$P = 0.842$
Spearman correlation (observed–predicted)	$r = 0.340^{**}$			$P = 0.008$
Multiple regression				
Wilcoxon signed-rank (observed–predicted)	Negative ranks $N^- = 33$ Positive ranks $N^+ = 26$	Average = 30.76 Average = 29.04		$P = 0.326$
Spearman correlation (observed–predicted)	$r = 0.607^{**}$			$P \leq 0.001$

amounts of airborne pollen. Similar behavior has been reported in other Spanish cities (Belmonte et al. 1999).

Significant year-on-year variations were observed with regard to the start, end and duration of the MPS, and also with regard to the total annual pollen index recorded. These variations are attributable to differences in weather conditions. Some winters were colder and drier, while others were warmer or wetter; the high rainfall recorded over the pollen season in 2007 and 2009 gave rise to a longer MPS duration (104 and 144 days, respectively) and reduced pollen counts compared to 2005 and 2008.

Similar findings have been reported by other authors, who also note a positive correlation between rainfall during the pollen season and pollen-season duration (Díaz de la Guardia et al. 2006; González-Minero and Candau 1996). Rainfall interrupts pollen emission, thereby avoiding rapid flowering exhaustion and thus extending the presence of airborne pollen (Keynan et al. 1989). Our results showed that high values of rainfall during the MPS do not mean an increase in the pollen recorded during this period but tends to lengthen the duration of the pollen season.

No relationship was observed between rainfall over the 2 months prior to the main pollen season and Cupressaceae pollen production: the highest rainfall values were recorded prior to the 2007 and 2009 pollen seasons, but total pollen counts were not very high in these years (Table 2). However, other studies carried out in various areas of Spain have reported a positive correlation between rainfall prior to the MPS and pollen production (Díaz de la Guardia et al. 2006).

The results of correlation analysis between daily mean Cupressaceae pollen counts and meteorological data were comparable to those noted by other authors (Galán et al. 1998; Aira et al. 2001; Díaz de la Guardia et al. 2006, Tortajada and Mateu 2008), who highlight the positive effect of temperature and hours of sunlight, and the negative influence of rainfall and relative humidity, on airborne pollen levels.

Regarding prediction models, when we compare the results obtained from the two models, multiple regression get better results (accounting for 59% of variation in pollen levels) than polynomial regression (accounting for 48% of variation in pollen levels). We included meteorological variables and values of pollen counts for the preceding days, as independent variables in the multiple regression, in order to improve the prediction of the variation in levels of pollen in the air. This procedure has been used by other authors such as Rodríguez-Rajo et al. (2004), Sabariego et al. (2008) and Díaz de la Guardia et al. (2006).

The two prediction models confirm that mean daily Cupressaceae pollen counts could be predicted on the basis of maximum temperature (polynomial and multiple regression) and pollen counts for the previous day (multiple regression). Since weather forecasts provide highly accurate temperature data, these models can be readily used to forecast

Cupressaceae airborne pollen counts in the short- and medium-term.

In this study, we present only results related to statistical analyses. We agree with Galán et al. (1998) that, with more field data, particularly phenological data, it should be possible to produce biological models, i.e., models that could reproduce the whole pollination cycle of Cupressaceae.

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