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ORIGINAL PAPER

A contribution to the knowledge of Cupressaceae airborne pollen in the middle west of Spain

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Abstract This study investigates the pollen of Cupressaceae in the atmosphere of Salamanca during the years 2000–2007. Annual variations in the concentration of pollen in the atmosphere were analysed by the volumetric method using Hirst spore trap. From a quantitative point of view, Cupressaceae is the third most abundant airborne taxa in the city of Salamanca where it constitutes 11 % of the total pollen content as a mean value, displaying an increase in its atmospheric pollen index during the 8 years studied. This pollen type was mainly detected in the atmosphere during winter season, with an atmospheric pollen season registered between late January and June and maximum concentrations detected in late February and early March. The intradiurnal pattern reached a higher hourly concentration peak in the central hours of the day. Cupressaceae pollen concentrations exceeded known thresholds on more days in the last 4 years studied. The correlations obtained between daily pollen counts and different meteorological parameters showed that the airborne presence of this pollen type is positively associated with temperature during winter months. The results could be useful to improve the knowledge of this pollen type in the atmosphere and to prevent symptoms of allergic reactions in pollen-sensitive people.

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1 Introduction

The Cupressaceae family includes around 28 genera with more than 100 species of widely distributed evergreen trees and bushes in the northern and southern hemispheres (Farjon 2005). The Taxaceae family (9 genera, 16 species), which is similar to a pollen morphology point of view, has also been included in the Cupressaceae pollen type. This pollen type is stenopalynous, by reason of the light microscopy reveals no morphological differences at species or genus level, and it is defined as spheroidal, intectate and monoporate with a distinct annulus (Bortenschlanger 1990). However, some authors think that this type of pollen is inaperturate (Boucher et al. 2002).

The great amount of pollen grains produced by Cupressaceae, mainly in *Cupressus* genus (Hidalgo et al. 1999), leads to high airborne pollen levels in many cities of Africa (Berman 2007), America (Latorre and Caccavari 2009), Asia (Erkan et al. 2011), Europe (Boi and Llorens 2013) or Oceania (Green et al. 2004), especially in the Mediterranean area, due to the presence of native species and the use of non-native species for ornamental or forestry purposes. These atmospheric concentrations are related to the importance of some Cupressaceae pollen allergens in higher cases of respiratory allergic diseases, mainly in Southern Europe (Guerra et al. 1996; Papa et al. 2001) and West Asia (Geller-Bernstein et al. 2003; Sin et al. 2008), being responsible of winter pollen allergy (Caramiello et al. 1991) along with other pollen allergens such as *Alnus* (D'Amato and Spieksma 1990) and *Fraxinus* (Fernández-González et al. 2012). In fact, some allergic symptoms such as conjunctivitis, rhinitis and asthma caused by Cypress allergens (Mistrello et al. 2002) are reported in a large number of patients along the Mediterranean area (Caimmi et al. 2012), as a result of the high variability in the protein content and potency of the Cypress pollen extracts used for skin testing (Pico de Coaña et al. 2010). In the middle west of the Iberian Peninsula, 19.1 % of the allergic patients are sensitized to one of the main Cypress allergens, Cup s 1, according to specific IgE assay in serum (Rodríguez et al. 2011). The relationship between meteorological variables and Cupressaceae pollen levels is relevant for pollen-allergy prevention (Ocaña-Peinado et al. 2013), as these meteorological factors could increase pollen levels and influence the severity of allergy symptoms. That fact raises various works dealing with the influence of meteorological factors in Cupressaceae flowering period (Galán et al. 1998; Fuertes-Rodríguez et al. 2007).

In this study, we aimed to establish the aerobiological behaviour of this pollen type and to evaluate which meteorological factors are more influential on atmospheric pollen records. In addition, the knowledge of the daily and seasonal character is important for the diagnosis of pollinosis and subsequently to prevent the development of clinical symptoms in allergic patients, establishing the main season of their acute sensitivity.

2 Materials and methods

The study has been carried out in the city of Salamanca (40°58′N; 5°40′W) located in the middle west of the Iberian Peninsula at 800 m above sea level, and it has around 180,000 inhabitants. The climate is Mediterranean continental, characterized by a low annual rainfall level (382 mm) that determines a dry season in the summer period (Capel Molina 1981). Flora in the surrounding area includes species developed in natural formations as pastures with holm oaks (*Quercus ilex* L. subsp. *ballota* (Desf.) Samp.) or forests with oak trees (*Quercus pyrenaica* Willd.), both located at south and west of the city. Prickly juniper (*Juniperus oxycedrus* L.) is a common arboreal element in holm oak forests located in specific areas with a high level of insolation. According to data supplied by Salamanca City Council in 2003, the different Cupressaceae species cultivated in the city constitutes 6 % of total urban arboreal flora, mainly Mediterranean Cypress (*Cupressus sempervirens* L.) and Arizona Cypress (*Cupressus arizonica* Greene), but even with a few number of trees belonging to different species as California incense cedar (*Calocedrus decurrens* (Torr.) Florin), Lawson Cypress (*Chamaecyparis lawsoniana* (A. Murray) Parl.), Monterrey Cypress (*Cupressus macrocarpa* Hartw.) or Oriental thuja (*Platycladus orientalis* (L.) Franco). Other pollen-related families, such as Taxaceae, are scarcely represented in the city and the surroundings.

The aerobiological monitoring was performed by a Hirst type volumetric spore trap (Burkard 7-day recording volumetric spore trap—Burkard Manufacturing Co. Ltd.), placed on the roof of a central historical building at a height of 20 m above ground level (a. g. l.) from 1 January 2000 to 31 December 2007. The pollen trap has the air-flow rate of 10 L per min. The methods standardized according to the Spanish Aerobiology Network were used for sampling, slide preparation, pollen counting and data interpretation (Domínguez et al. 1991; Galán et al. 2007): four longitudinal traverses of contiguous fields along the length of the slide were performed, starting from the centre and separated by at least 1 mm to avoid oversampling or empty areas and using 400 magnifications in optical microscopy. This method allows us to examine 12.85 % of the surface area. Taxaceae species were included in the same type of pollen "Cupressaceae", because they are similar to a pollen morphology point of view (Trigo et al. 2008).

The annual pollen index (API) of each year was calculated by summing Cupressaceae daily pollen values. The atmospheric pollen season (APS) defined by Jato et al. (2006) was used in this work, and it was defined using the methodology proposed by Nilsson and Persson (1981), as the period from the time the sum of daily mean Cupressaceae pollen concentrations reaches 5 % of the total sum until the time when the sum reaches 95 %, i.e. the time with 90 % of the whole pollen amount. A 5-day running mean was calculated during the studied period and plotted to assess the seasonal trend.

In addition, the intradiurnal variations recorded during the APS for 2005–2007 period have been studied, taking into account three different models described by some authors (Galán et al. 1991; Sánchez Reyes et al. 2009). These models were developed from airborne pollen levels obtained hour by hour throughout a day (24 h). The first model calculated the value for each hour represented by the sum of the values corresponding to that hour, divided by the total number of days of the APS. However, in the second model, an ideal day was obtained, dividing this sum of values of each hour only by the number of days of the APS on which this pollen type was present. In the third model, it was important whether rainfall occurred or not. The daily average of the pollen type was calculated using the total number of days of the APS period as the denominator. But for the calculations of the hourly concentrations, we selected just the dry days with a daily value equal to or higher than the average previously calculated. In the graphical representation, columns represented the hourly percentage of pollen levels related to total daily pollen counts and we used a 3-h running mean to smooth the tendency, expressed as bi-hourly concentration.

Meteorological data were supplied by the Spanish State Meteorological Agency (AEMET), located 10 km east from the pollen sampling site. Nonparametric statistical correlation analyses (Spearman's test) were carried out between meteorological parameters (temperature, total hours of sunshine, rainfall, relative humidity, wind speed, frequency of calms and winds from the first, second, third and fourth quadrant) and pollen data (daily average of pollen grains/m^3 of air) during the whole period and each year analysed within the APS. Some authors revealed that correlations between meteorological factors and Cupressaceae pollen counts are more significant during winter season, from December 21 until March 20 (Díaz de la Guardia et al. 2006, Tortajada and Mateu 2008), developing also these correlations along this season in each year studied. The nonparametric Spearman's coefficient was chosen because daily pollen counts are not normally distributed. In addition, linear regression was used in order to evaluate possible trends in different parameters of APS as well as API during the studied period. Statistical analysis was carried out by SPSS v.12 software package.

3 Results

The study on Cupressaceae pollen indicated variation in annual pollen indexes (Fig. 1). The lowest pollen index was recorded in 2000 and the highest in 2007, with 1823 being the mean value. The percentages of these Cupressaceae pollen indexes in respect of total atmospheric levels from all pollen types ranged from 7.6 % in 2000 to 13.6 % in 2003, with 11 % as mean value. This interannual variation meant an increase in levels of this pollen type. Linear regression analysis displayed a positive slope (Fig. 1) with a high significance ($p < 0.05$), reflecting a trend to increased API values during the studied period.

Figure 2 shows the seasonal variations in the average of the daily pollen concentrations of Cupressaceae during the studied years as the 5-day running means of 8 years analysed from January 1 to December 31. This pollen type appeared in late November, increasing its daily levels from early February until the first week of March, when it reached the highest mean daily value. During the study period, the highest Cupressaceae pollen concentration was observed on 4 March 2007 and the lowest on 29 February 2000, with the earliest peak on 3 February 2002 and the latest on 12 March 2003 (Table 1).

The duration of the APS was 136 days on average, and it ranged between 84 and 162 days (Table 1). The earliest APS starting date was on 6 January 2004 and the latest on 11 February 2006, with January 29 as a mean value. The APS ended on average on June 11, ranging between 16 April 2007 and July 6 in the second year studied. These seasonal changes showed a decrease in the length and the ending date of APS. Linear regression analysis displayed a negative slope (Fig. 3) with a high significance ($p < 0.05$), reflecting a trend to a decrease in the length of APS and early end dates of the APS during the studied period.

Waisel et al. (2004) described the level of 50–60 Cupressaceae pollen/m^3 as being the threshold at which allergy symptoms were provoked in sensitized

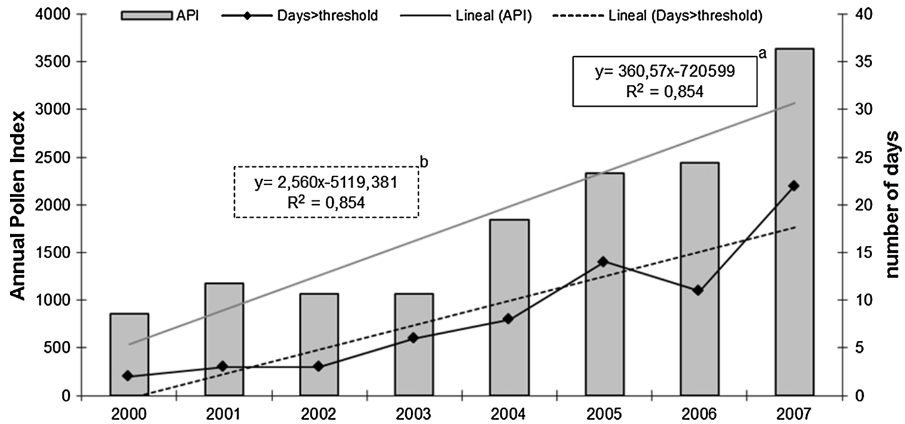

Fig. 1 Cupressaceae annual pollen indexes and number of days of airborne Cupressaceae pollen concentrations above known thresholds in the city of Salamanca from 2000 to 2007. In both cases, their trend was analysed by means of linear regression analysis. *a* Significance level of API slope ($p = 0.001$). *b* Significance level of days >threshold slope ($p = 0.001$)

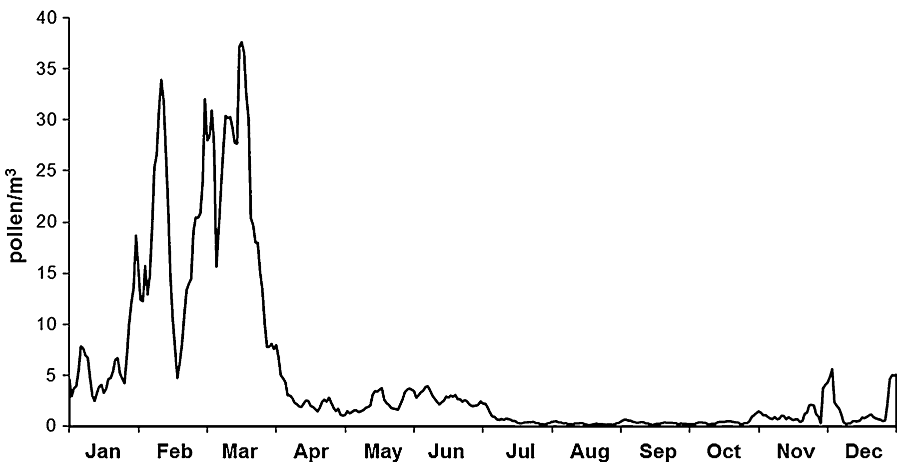

Fig. 2 Seasonal variation of Cupressaceae during 2000–2007 average (5-day running mean)

Table 1 Seasonal behaviour of airborne Cupressaceae pollen

	2000	2001	2002	2003	2004	2005	2006	2007	Mean
Annual pollen index	855	1180	1071	1071	1839	2327	2438	3635	1823
% total	7.6	0.8	13.6	3.1	0.8	1.1	1.8	0.7	10.9
Peak value	55	132	195	107	112	205	141	550	77
Peak day	29-Feb	24-Feb	3-Feb	12-Mar	7-Feb	13-Feb	11-Mar	4-Mar	4-Mar
Days >50–60 p/m^3	2	3	3	6	8	14	11	22	9
APS length	152	162	147	147	159	96	139	84	136
Start APS	31-Jan	27-Jan	29-Jan	3-Feb	6-Jan	8-Feb	11-Feb	24-Jan	29-Jan
End APS	30-Jun	6-Jul	23-Jun	28-Jun	7-Jun	13-May	28-Jun	16-Apr	11-Jun

APS atmospheric pollen season

patients. The number of days exceeding this threshold was low in the period 2000–2002 (Table 1), but increased in recent years. These days were distributed mainly in February and March, except scarce days in January 2002 and 2004, 1 day in November 2006 and in April 2007, and 2 days in December 2003.

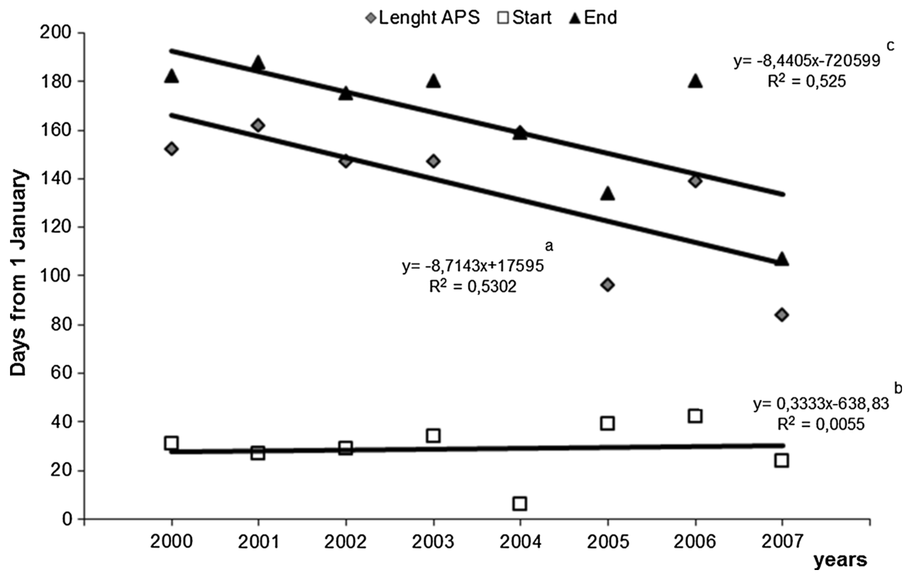

Fig. 3 Evaluation of APS length, starting and ending dates' (from 1 January) trends during the studied period by means of linear regression analysis. *a* Significance level of APS length slope ($p = 0.041$). *b* Significance level of APS start dates' slope ($p = 0.862$). *c* Significance level of APS end dates' slope ($p = 0.042$)

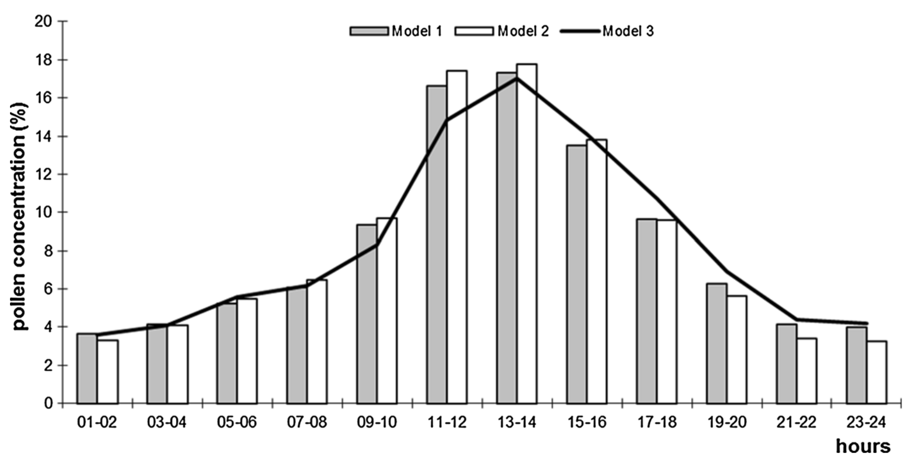

Fig. 4 Cupressaceae intradiurnal patterns during 2005–2007

The hourly distribution of Cupressaceae pollen in the atmosphere presented similar pattern in the three models: pollen levels increased from 9:00 to 16:00, when concentrations reached maximum percentages over the average total counts of the day, and after that, the values decreased until midnight (Fig. 4).

Correlations between daily pollen counts and the main meteorological parameters with a statistically significant positive correlation ($p < 0.01$) and medium values of Spearman's coefficients were found in two cases during the period 2000–2007: wind speed during year 2007 and also during its APS (Table 2). The same trend was observed in the case of negative correlation, showing four cases in APS of year 2000 with rainfall and relative humidity, as well as with frequency of calms during year 2007 and its APS. In addition, correlation analysis of temperature and rainfall with airborne pollen concentrations was performed during winter days (from December 21 to March 21), including the last days of the previous year (from 1999 to 2006). Table 3 displays a positive and significant correlation between pollen levels and temperature, mainly in the case of maximum temperature.

4 Discussion

The increase in airborne Cupressaceae concentrations in the atmosphere of Salamanca during the studied period, shown by regression analysis, was observed in

Table 2 Spearman correlation coefficients established for airborne Cupressaceae pollen concentrations and meteorological parameters along the atmospheric pollen season (APS)

Cupressaceae	T_{mean}	T_{max}	T_{min}	Sunshine	Rainfall	RH	Wspeed	CF	Wind NW	Wind NE	Wind SE	Wind SW
2000												
Annual	−0.15**	−0.042	−0.24**	0.043	−0.237**	−0.122**	−0.15**	0.111*	0.184*	0.164*	−0.185**	−0.059
APS	−0.158	0.068	−0.29**	0.092	−0.378**	−0.216**	−0.22**	0.33**	0.131	0.124	−0.130	−0.071
2001												
Annual	0.150**	0.112*	0.170**	0.095	0.076	−0.088	0.124*	−0.157**	−0.159**	−0.103	0.175**	0.073
APS	0.295**	0.225**	0.347**	0.152	−0.023	−0.116	−0.098	0.048	−0.041	0.073	0.030	−0.010
2002												
Annual	−0.28**	−0.20**	−0.34**	−0.054	−0.097	−0.087	−0.013	0.089	−0.035	0.035	0.049	−0.162**
APS	−0.27**	−0.18**	−0.33**	−0.19*	−0.154	0.102	−0.066	0.077	−0.038	0.255**	0.010	−0.086
2003												
Annual	−0.20**	−0.17**	−0.23**	−0.12*	−0.023	0.041	−0.007	0.009	−0.035	0.142**	0.046	−0.116**
APS	−0.131	−0.108	−0.100	−0.2**	0.122	0.089	−0.076	0.102	−0.154	0.310**	0.127	−0.130
2004												
Annual	−0.23**	−0.20**	−0.28**	−0.096	−0.028	0.102	0.020	−0.003	−0.058	0.003	0.097	−0.021
APS	−0.058	−0.018	−0.085	−0.126	−0.057	0.192*	0.056	0.204*	−0.120	0.188*	0.196*	−0.033
2005												
Annual	−0.044	−0.020	−0.083	0.011	0.029	−0.091	0.167**	−0.193**	−0.107*	0.067	0.141**	0.086
APS	0.122	0.166	0.067	−0.21*	−0.025	−0.141	−0.053	−0.003	−0.092	0.249*	0.044	−0.029
2006												
Annual	−0.12**	−0.083	−0.17**	0.3**	0.026	−0.090	−0.016	−0.062	−0.033	0.030	0.044	−0.009
APS	−0.188*	−0.153	−0.187*	0.248	0.032	0.082	−0.022	−0.151	0.086	0.062	−0.052	−0.329**
2007												
Annual	−0.17**	−0.21**	−0.16**	0.2**	0.141**	0.128*	0.365**	−0.453**	−0.185**	−0.023	0.286**	0.090
APS	0.468**	0.337**	0.277*	0.23*	−0.011	−0.232*	0.485**	−0.323**	−0.325**	−0.253*	0.302**	−0.013
Total period (2000–2007)	−0.13**	−0.10**	−0.15**	−0.031	−0.015	−0.018	0.014	−0.071**	−0.05**	0.034	0.074**	−0.015

T_{mean} = mean daily average temperature (°C). T_{max} = maximum daily average temperature (°C). T_{min} = minimum daily average temperature (°C). Sunshine = daily average sunshine (hours). Rainfall = total daily rainfall (mm). RH = daily average relative humidity (%). Wspeed = daily average wind speed (km/h). Wind NW = north-western winds (%). Wind NE = daily average frequency of north-eastern winds (%). Wind SE = south-eastern winds (%). Wind SW = south-western winds (%). CF = daily average frequency of calms (%)

Significance levels: * 0.95 %; ** 0.99 %

Table 3 Spearman correlation coefficients established for airborne Cupressaceae pollen concentrations and temperature and rainfall during winter season

Cupressaceae	T_{mean}	T_{max}	T_{min}	Rainfall
2000	0.437**	0.497**	0.343**	−0.145
2001	0.293**	0.385**	0.086*	−0.123
2002	0.502**	0.535**	0.321**	−0.168
2003	0.298**	0.381**	0.136	−0.080
2004	0.276**	0.323**	0.234**	0.009
2005	0.439**	0.479**	0.190	0.072
2006	0.525**	0.628**	0.295**	−0.068
2007	0.392**	0.371**	0.270**	0.118
Total winter season	0.446**	0.551**	0.259**	−0.059

T_{mean} = mean daily average temperature (°C). T_{max} = maximum daily average temperature (°C). T_{min} = minimum daily average temperature (°C). Rainfall = total daily rainfall (mm)

Significance levels: * 0.95 %; ** 0.99 %

other aerobiological stations during the first years of the twenty-first century (Tortajada and Mateu 2008; Ianovici 2009) and related with forestry and urban management, which could be the case in our study. A similar fact was described in the Iberian Peninsula for other abundant trees of *Quercus* genus (García-Mozo et al. 2006). Both cases could be not only related to the known effect of climate change on higher pollen levels and subsequently a great number of allergic processes (Osborne et al. 2000; Shea et al. 2008), but also coupled to other forestry and urban factors involved in tree management (Clot 2003). Nevertheless, the relationship between the increase in pollen concentrations and global change requires further detailed investigations.

The early end of APS in 2007 might be explained by urban management (pruning of trees) in some areas of the city of Salamanca, shortening the length of APS. This fact could suggest the removal of that year in regression analysis of trends in the length and ending dates of APS, but taking into account API and peak values registered, we thought all data should continue in order to clarify other observed trends.

Annual pollen index during 2000–2007 period registered in Salamanca was similar to other cities of the middle of Spain (Paulino et al. 2002; Valencia-Barrera et al. 2010), being lower than other points also located in the middle of the Iberian Peninsula (Gutiérrez Bustillo et al. 2002), probably linked to different town-planning conception. APS corresponded to that already described in other parts of the Mediterranean area (Tosunoğlu et al. 2013), with a long airborne presence from November to June, due to the phenology observed in different species of this family developed in the studied area, starting with *Cupressus* species and lasting with *Juniperus* species (Belmonte et al. 1999). In addition, the period of Cypress pollination may last some months, because of the gradual mechanism of microsporophyllous maturation and furthermore because pollination shows high variability, from year to year (Hidalgo et al. 2003). This case could explain the length of APS displayed in the city of Salamanca. However, a shorter APS linked to a decrease in its length was also displayed in Cupressaceae pollen at Badajoz, a city located in the south-west of Spain (Tormo-Molina et al. 2010). This fact could be related to a large number of pollen producing species in Cupressaceae and to their ornamental use in urban areas. The increase in the number of days exceeding medical thresholds during the studied period could be due to physiological adaptations to anomalous weather conditions combined with urban managements, but further studies with more years and more aerobiological stations are needed to understand this effect and its implications on allergic diseases.

The highest values recorded during the central hours of the day, specifically between 11:00 and 18:00, revealed that there were a similar pattern to that reported for Granada (Díaz de la Guardia et al. 2006) and Porto (Ribeiro et al. 2008), bringing into agreement with the observed occurrence of anthesis and pollen dispersion during daylight (Latalowa et al. 2005).

Taking into account weather conditions and their correlations with pollen levels, there was no relationship between meteorological parameters and the airborne pollen concentration, except some cases during 2000–2007 period. This could be connected with early winter phenology of Cypress. Analogous associations with temperature variables and daily Cupressaceae pollen counts have been established in different cities in the Mediterranean area (Kizilpinar et al. 2012; Sabariego et al. 2012). In the middle west of the Iberian Peninsula, other types of pollen produced by arboreal taxa revealed the same significant positive relationship with temperature (Rodríguez de la Cruz et al. 2008). Rainfall displayed no correlation with airborne concentrations of Cupressaceae pollen during the winter season, probably due to rainfall that occurred in the city of Salamanca from last December to March, as other authors stated in previous works (Iglesias et al. 1993). In this city and its surroundings, intense rainfall was registered in late spring and summer along the Mediterranean area (Romero et al. 1999), causing the known wash out effect of atmospheric particles (Smart et al. 1979), displaying a significant and negative correlation with pollen levels in the middle west of Spain (Rodríguez de la Cruz et al. 2012).

In our study, there was an increase in API with a positive trend obtained using regression analysis, as well as trends in shorter APS and their early ending dates. In addition, higher pollen levels were located in the central hours of the day, according to information provided by intradiurnal models, and there was a positive and significant correlation between airborne pollen concentrations and temperature during winter months. Pollen monitoring should be continued because this paper helps towards demonstrating that pollination of different species included in the Cupressaceae family, according to its abundance, should be taken into consideration as a vector of different aeroallergens, helping allergologists in establishing diagnosis, and it could also play an important role as a bioindicator of different effects in global change processes.

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