# **ORIGINAL ARTICLES**

## AEROBIOLOGICAL STUDY IN EAST-CENTRAL IBERIAN PENINSULA: POLLEN DIVERSITY AND DYNAMICS FOR MAJOR TAXA

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Abstract: A study was made of airborne pollen counts in Cuenca (east-central Iberian Peninsula, Spain), using data obtained over a 3-year period (2008–2010). This is the first such study carried out in the World Heritage city of Cuenca, situated in the large region of Castilla-La Mancha. Air monitoring was performed using the sampling and analysis procedures recommended by the Spanish Aerobiology Network. Sampling commenced in mid-2007, and provided the first recorded pollen-spectrum for the area. The greatest pollen-type diversity was recorded in spring, whilst the highest pollen counts (over 80% of the annual total) were observed between February and June. The lowest counts were found in September, November and December. The 10 leading taxa, in order of abundance, were: Cupressaceae, Quercus, Urticaceae, Pinus, Olea, Poaceae, Populus, Platanus, Chenopodiaceae-Amaranthaceae and Plantago. The pollen calendar was thus typically Mediterrean, and comprised the 27 pollen types reaching 10-day mean counts of over 1 grain/m3 of air. Maximum concentration values during the day were recorded between 12:00-20:00, coinciding with the highest temperatures and lowest humidity levels. The pollen types responsible for most allergies in the city of Cuenca, ordered by the number of days on which risk levels were reached, were: Poaceae, Urticaceae, Cupressaceae, Olea, Platanus and Chenopodiaceae-Amaranthaceae.

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#### **INTRODUCTION**

Airborne pollen counts can be used to chart the successive flowering of different wind-pollinated species [13] and to monitor the effects of climate change on plant species [5, 14, 17]. They are also a useful tool for the study of respiratory allergies [9, 29], the prevalence and severity of which have risen sharply over recent years, prompting a marked decline in the quality of life for allergy-sufferers. Allergic rhinitis affects up to 25% of the world population, and asthma around 18% [7]; pollen is the most common cause [39]. Recent studies have highlighted the effect of environmental pollution on the allergic potential of pollen [38], noting that high levels of airborne inorganic pollutants,

Received: 14 February 2011 Accepted: 2 May 2011 together with a constant increase in pollen particles, are jointly responsible for the increasing incidence of allergies in urban populations. In Spain, pollen accounts for 40% of cases of rhinoconjunctivitis and 27% of cases of asthma [3]. Pollen-count monitoring has enabled identification of the local pollen types triggering respiratory allergies in a number of geographical areas, and pollen calendars have been used to demonstrate the diversity of airborne pollen types as well as the duration and intensity of local pollen seasons [10, 24, 49]. This information is essential for the study and treatment of allergies; at the same time it helps to reduce the exposure of pollen-allergy sufferers, enabling preventive measures to be taken and open-air activities to be carefully planned. This study reports the airborne pollen counts in Cuenca, a city located in the region of Castilla-La Mancha, in eastcentral Spain. Airborne pollen monitoring started here in mid-2007, and this is the first paper to present the data obtained to date. The aims of the study were as follows: to establish the airborne pollen spectrum (mean annual counts and highest and lowest counts recorded for major taxa); to draw up a pollen calendar (mean weekly pollen-count dynamics in the course of an ideal year); to chart the distribution of pollen counts in the course of the day; and to identify allergenic pollen types.

#### MATERIAL AND METHODS

**Study area.** Cuenca is located at 956 m above sea level, in east-central Spain (40° 04' 34.54" N, 2° 07' 49.20" W). It is a World Heritage city, and a major tourist venue, with a population of around 56,000 inhabitants. Situated between the rivers Júcar and Huécar, in bioclimatic terms, Cuenca lies within the supramediterranean belt [50]. Mean annual temperature is 11.7°C. January is the coldest month (mean temperature 3.1°C) and July the warmest (mean temperature 21.9°C). Mean annual rainfall is 571 mm. May is the wettest month (72 mm) and July the driest (19 mm). In biogeographical terms, Cuenca is situated in the Mediterranean Region (W Mediterranean Subregion), in the Mediterranean an Central Iberian Province (Castilian Subprovince) [50].

The city is surrounded by cropland - mainly cereals and forest vegetation. Mediterranean forest vegetation predominates in the hills of the Serranía de Cuenca, to the north of the city, comprising pines (Pinus halepensis Miller, Pinus pinaster Aiton and Pinus nigra Arnold ssp. salzmanii (Dunal) Franco), junipers (Juniperus spp.) and oaks (Quercus ilex L. spp. ballota (Desf.) Samp., Quercus faginea Lam.). Major Mediterranean scrub species include Cistus spp., Thymus spp. and Rosmarinus officinalis L., among others. Riparian vegetation along the rivers Júcar and Huécar comprises mainly willow (Salix spp.) and poplar (Populus alba L. and Populus nigra L.). Grasses, nettles and goosefoot are amongst the most common herbaceous species. Ornamental species in the city's parks and gardens include abundant cypresses (Cupressus sempervirens L.), Judas trees (Cercis siliquastrum L.), elms (Ulmus pumila L.), glossy privet (Ligustrum lucidum Aiton) and others.

Airborne pollen and allergy risk. Airborne pollen was monitored over a 3-year period, from 2008–2010. A Hirst volumetric spore-trap [27], installed on the roof of the Museum of Sciences of Castilla-La Mancha, in the city's Old Town, was used for aerobiological sampling. Samplings and analysis procedures were those recommended by the Spanish Aerobiology Network [15].

The pollen calendar for the city of Cuenca was constructed using the model suggested by Spieksma [56]. Daily mean counts (grains/m<sup>3</sup>) were used to calculate 10-day arithmetic means during the study period, and results were expressed in the form of a histogram in which each 10-day mean corresponded to an exponential frequency class, following Stix and Ferretti [57]. The histogram included all pollen types displaying a 10-day mean equal to or greater than 1 grain/m<sup>3</sup>. In the pollen calendar, pollen types were ordered chronologically, by maximum 10-day peaks. This representation enabled the pollen seasons of the various taxa over one year to be visualized at a glance.

The Main Pollen Season, i.e. that portion of the pollen season during which most pollen is recorded, was calculated following Andersen [4]. This method includes 95% of the seasonal total pollen count, starting on the day on which 2.5% of total pollen was recorded and ending on the day on which 97.5% of total pollen was registered. Intradiurnal variations in pollen counts were analysed for 8 of the most abundant local taxa (Cupressaceae, *Quercus, Pinus, Olea,* Poaceae, *Populus,* Chenopodiaceae-Amaranthaceae and *Plantago*), following Galán *et al.* [18]. Results were represented graphically, showing bi-hourly percentage values, using official Spanish time (UTC + 1 hour during autumn and winter and UTC + 2 hours during spring and summer).

The risk of pollen allergy was calculated in terms of the number of days on which pollen counts exceeded the risk threshold established by the Spanish Aerobiology Network [15]; thresholds for Chenopodiaceae-Amaranthaceae pollen were modified to reflect the view of certain authors [44, 58] who argue that just a few grains of this pollen type are sufficient to trigger symptoms in patients allergic to it. Thus, the term 'moderately allergenic levels' was thus used to denote counts for herbaceous taxa ranging from 15–30 grains/m<sup>3</sup> (Urticaceae, Chenopodiaceae-Amaranthaceae) or from 25–50 grains/m<sup>3</sup> (*Plantago*, Poaceae) and for arboreal taxa from 50–200 pg/m<sup>3</sup> (Cupressaceae, *Olea, Pinus, Platanus, Populus* and *Quercus*). The term 'highly allergenic levels' was used to denote levels above these values.

**Meteorological data and statistical analysis.** Meteorological data were provided by the weather station located in the city of Cuenca, near the pollen trap, belonging to the Spanish Meteorology Agency (AEMET). The following meteorological variables were subjected to statistical analysis: daily mean, maximum and minimum temperature (°C), rainfall (mm), hours of sunlight (sun hours), wind speed (m/s), and atmospheric pressure (mb). The SPSS 15.0<sup>®</sup> software package was used for all statistical analyses. The correlation between meteorological variables and pollen counts during the pre-peak period (PP), i.e. the period from the start of the season up to the peak day (the day on which the maximum daily pollen count was recorded), was analysed using a non-parametric Spearman's correlation test, since data distribution was not normal.

#### RESULTS

**Pollen spectrum.** Over the 3-year period (2008–2010), a total of 115,609 pollen grains was recorded (Tab. 1). The

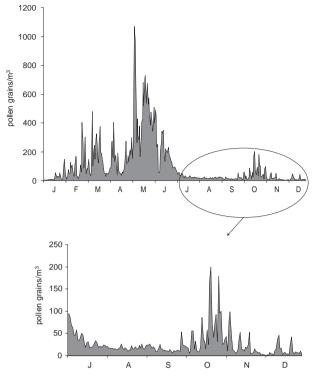


Figure 1. Seasonal fluctuations in daily average total pollen counts. Mean values for the period 2008–2010.

highest total annual count (44,848) was observed in 2009 and the lowest in 2010 (33,461). Over the study period, between 80%–90% of the annual pollen count was recorded from February to June (Tab. 1, Fig. 1). The month with the highest pollen counts varied: June in 2008 (10,668 grains/m<sup>3</sup>, 28.6% of total annual pollen), and May in both 2009 and 2010 (20,220 grains/m<sup>3</sup>, 45% and 14,202 grains/m<sup>3</sup>, 42.4%, respectively, of total annual pollen). Average daily counts over this period exceeded 1,000 pollen grains/m<sup>3</sup> on some days (Fig. 2), the maximum occurring on 7 May 2009 (1,524 grains/m<sup>3</sup>). Peaks were recorded at various points throughout the year (Fig. 1, Fig. 3), due largely to the contribution of Cupressaceae pollen in February–March, *Quercus* in April, Urticaceae in May and both Poaceae and *Olea* between May and June.

From late June onwards, pollen counts declined sharply until October, when an increase was recorded (Fig. 1). In late summer (August and September), pollen came largely from Chenopodiaceae-Amaranthaceae species flowering at this time. Some autumn-flowering Cupressaceae species were responsible for the increase in counts in October (mean 1,634 grains/m<sup>3</sup>, i.e. 4.2% of the total pollen). The lowest counts were recorded in September, November and December (mean 1.2, 0.8 and 1.8%, respectively).

The most abundant pollen type throughout the study period was Cupressaceae, accounting for 23.6% of total annual pollen (Tab. 2). Other major types, in order of quantitative importance, were: Quercus (19.8%), Urticaceae (17.1%), Pinus (10%), Olea (7.3%) and Poaceae (6.4%). Marked year-on-year variations were observed in total pollen counts, main pollen season, pollen-season duration, and maximum daily counts (Tab. 2, Tab. 3). Smaller amounts of pollen from other tree species were recorded, including *Platanus* (2.3%) and *Populus* (4.8%). Other herbaceous species with moderately high counts, in addition to Urticaceae and Poaceae, were Chenopodiaceae-Amaranthaceae (1.5%) and *Plantago* (1.2%). The highest maximum daily counts (Tab. 3) for tree pollen were recorded for Cupressaceae (1,399 grains/m<sup>3</sup> on 9 March 2009), followed by Olea (800 grains/m<sup>3</sup>; 9 May 2009), Quercus

Table 1. Monthly and annual total pollen counts (total and percentages); monthly mean temperatures (°C) and rainfall (mm) over the study period.

ths	2008		2009		2	2010 2008		08-2010 SD		Max	Min	2008			2009		2010	
Months	Total	%	Total	%	Total	%	Mean	%				T (°C)	R (mm)	T (°C)	R (mm)	T (°C)	R (mm)	
Ι	1,760	4.7	377	0.8	162	0.5	766	2	867.2	1,760	162	7.8	42.3	3.9	70.9	4.2	65	
II	2,798	7.5	4,231	9.4	1,298	3.9	2,776	7.2	1,466.6	4,231	1,298	7.9	31.2	6.3	48.3	5.3	85.2	
III	3,345	9.0	8,908	19.9	1,398	4.2	4,550	11.8	3,897.4	8,908	1,398	9.1	17.3	10.3	48.1	7.7	39.8	
IV	4,574	12.3	4,256	9.5	2,811	8.4	3,880	10.1	939.6	4,574	2,811	11.8	114	10.3	52.9	12.5	89.9	
V	9,699	26.0	20,220	45.0	14,202	42.4	14,707	38.2	5,278.6	20,220	9,699	14.1	105	17.8	1.5	14.1	66.5	
VI	10,668	28.6	3,503	7.8	7,077	21.1	7,083	18.4	3,582.5	10,668	3,503	19.4	63.7	22.6	10.7	19.1	80.5	
VII	1,631	4.4	748	1.6	1,031	3.1	1,137	2.9	450.9	1,631	748	23.2	4.9	25.5	0	25.8	0.9	
VIII	723	1.9	389	0.9	478	1.4	530	1.4	173	723	389	24.3	9.2	25.7	1.2	24.0	85.6	
IX	411	1.1	240	0.5	784	2.3	478	1.2	278.2	784	240	18.5	55.1	19.4	58.2	19.1	34.4	
Х	685	1.8	1,313	2.9	2,903	8.7	1,634	4.2	1,143.2	2,903	685	13.7	122.1	16.0	46.9	12.9	60.1	
XI	675	1.8	558	1.2	821	2.5	685	1.8	131.8	821	558	6.7	27.5	10.4	9.6	7.1	43.3	
XII	331	0.9	105	0.2	496	1.5	311	0.8	196.3	496	105	4.8	40.4	5.4	182.1	5.4	83.3	
PI	37,300	100	44,848	100	33,461	100	38,537	100	5,793									

Pollen Index (PI), Standard deviation (SD), Maximum value (Max) and Minimum value (Min).

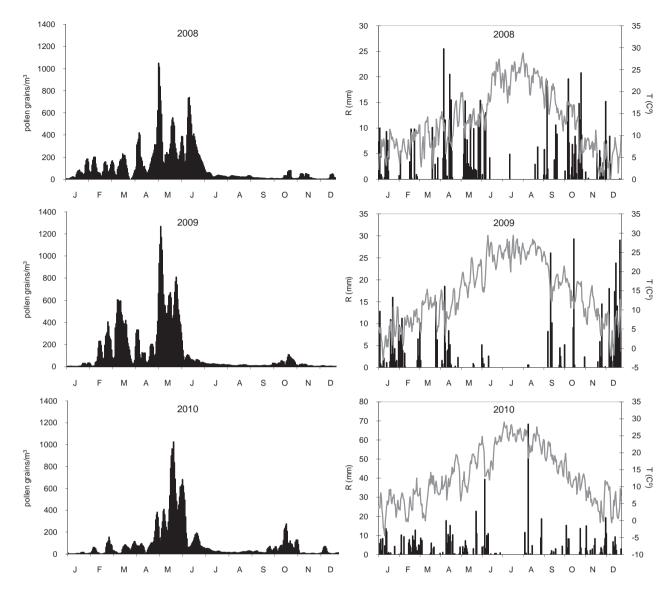


Figure 2. Daily values for mean temperature, total rainfall and total pollen counts (line: daily mean values; black area: 5-day running mean) during the study period (2008–2010).

**Table 2.** Annual total airborne pollen counts for the major pollen types inCuenca (2008–2010).

Pollen types	Average 2008–2010	% Total pollen	Standard Deviation	Maximum value	Minimum value
Chenopodiacea- Amaranthaceae	575	1.5	75.8	633	602
Cupressaceae	9,106	23.6	3,108.9	12,694	7,211
Olea	2,812	7.3	1,580.6	4,458	1,306
Pinus	3,870	10	805.3	4,719	3,117
Plantago	453	1.2	93.2	558	381
Platanus	897	2.3	414.6	1,282	458
Poaceae	2,447	6.4	410.5	2,820	2,007
Populus	1,864	4.8	498.0	2,276	1,309
Quercus	7,631	19.8	1,359.1	9,061	6,356
Urticaceae	6,575	17.1	231.6	6,840	6,411

(786 grains/m<sup>3</sup>; 7 April 2008), *Populus* (502 grains/m<sup>3</sup>; 20 March 2009), *Pinus* (680 grains/m<sup>3</sup>; 27 May 2009) and *Platanus* (313 grains/m<sup>3</sup>; 10 April 2008). Finally, Urticace-ae (1,129 grains/m<sup>3</sup>, 6 May 2008) and Poaceae (185 grains/m<sup>3</sup>; 25 May 2010) displayed the highest counts among herbaceous species.

The Main Pollen Season data for each pollen type analysed (Tab. 3) showed that Cupressaceae, Chenopodiaceae-Amaranthaceae, Urticaceae and Poaceae have long pollen seasons, lasting over 4 months, since they include a wide variety of species which flower consecutively over the whole pollen season. *Platanus* and *Populus* have the shortest pollen season (around 50 days).

A total of 27 pollen types were included in the pollen calendar for the city of Cuenca (Fig. 4, Fig. 5); of these, 16 were for tree species and the other 11 for herbaceous species. In January and February, small amounts of *Fraxinus*, *Alnus* and *Cedrus* pollen were recorded, represented

Table 3. Characteristics of the Main Pollen Season for the major taxa in Cuenca; number of days on which daily pollen counts exceeded allergy-risk thresholds.

		2008	2009	2010
Cupressaceae	Pollen season	09 Oct–25 May	26 Oct–07 May	10 Oct-08 Jun
	Season length	230	194	238
	DMV (Date)	398 (29 Jan)	1399 (09 Mar)	245 (23 Feb)
	Days >50 to 200 grains/m <sup>3</sup>	39	32	28
	Days >200 grains/m <sup>3</sup>	8	16	2
Chenopodiaceae- Amaranthaceae	Pollen season Season length DMV (Date) Days >15 to 30 grains/m <sup>3</sup> Days >30 grains/m <sup>3</sup>	16 Apr–18 Sep 156 22 (02 Sep) 6 0	02 May–17 Nov 200 16 (02 May) 1 0	29 Apr-06 Oct 161 31 (01 Sep) 4 2
Olea	Pollen season	27 Apr-11 Aug	07 May–23 Jun	09 May–02 Jul
	Season length	107	49	55
	DMV (Date)	109 (23 May)	800 (09 May)	564 (25 May)
	Days >50 to 200 grains/m <sup>3</sup>	4	17	8
	Days >200 grains/m <sup>3</sup>	0	5	3
Pinus	Pollen season	05 Apr–13 Jul	05 Apr-15 Jun	20 Apr–05 Jul
	Season length	100	72	77
	DMV (Date)	225 (12 Jun)	680 (27 May)	181 (03 Jun)
	Days >50 to 200 grains/m <sup>3</sup>	20	14	18
	Days >200 grains/m <sup>3</sup>	3	5	0
Plantago	Pollen season Season length DMV (Date) Days >25 to 50 grains/m <sup>3</sup> Days >50 grains/m <sup>3</sup>	07 Apr–22 Jul 107 24 (21 May) –	04 Apr–19 Jul 107 17 (14 May) –	26 Apr-01 Aug 98 23 (25 May) - -
Platanus	Pollen season	22 Mar–24 Apr	01 Apr-05 Jun	04 Apr–24 May
	Season length	34	66	51
	DMV (Date)	313 (10 Apr)	302 (05 Apr)	65 (24 Apr)
	Days >50 to 200 grains/m <sup>3</sup>	6	1	1
	Days >200 grains/m <sup>3</sup>	1	2	0
Poaceae	Pollen season	01 Apr-31 Aug	22 Mar–21 Aug	02 May–07 Sep
	Season length	153	153	129
	DMV (Date)	150 (23 May)	99 (19 May)	185 (25 May)
	Days >25 to 50 grains/m <sup>3</sup>	18	13	8
	Days >50 grains/m <sup>3</sup>	15	12	17
Populus	Pollen season	09 Feb–09 Apr	14 Feb–07 Apr	18 Mar–23 Apr
	Season length	61	53	38
	DMV (Date)	238 (19 Mar)	502 (20 Mar)	125 (30 Mar)
	Days >50 to 200 grains/m <sup>3</sup>	9	6	11
	Days >200 grains/m <sup>3</sup>	2	2	0
Quercus	Pollen season	06 Apr–14 Jul	05 Apr–25 Jun	27 Apr-15 Jun
	Season length	100	82	50
	DMV (Date)	786 (07 Apr)	369 (25 Apr)	472 (31 May)
	Days >50 to 200 grains/m <sup>3</sup>	34	33	22
	Days >200 grains/m <sup>3</sup>	10	12	11
Urticaceae	Pollen season	14 Apr–01 Set	19 Apr–24 Set	30 Apr-19 Aug
	Season length	141	159	112
	DMV (Date)	1129 (06 May)	1076 (06 May)	1084 (21 May)
	Days >15 to 30 grains/m <sup>3</sup>	10	15	23
	Days >30 grains/m <sup>3</sup>	45	27	26

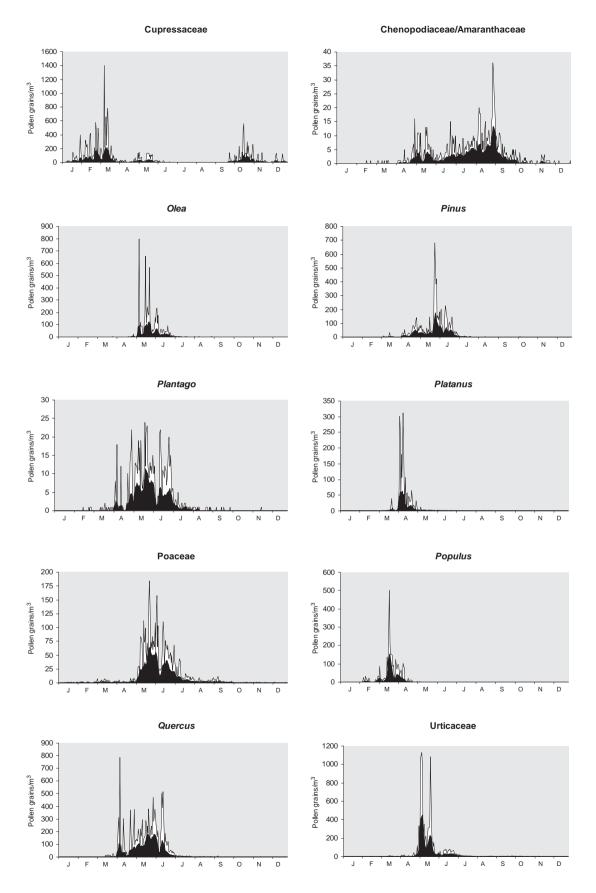


Figure 3. Five-day running mean (black area) and maximum daily mean (line) values during the study period (2008–2010) for the 10 most abundant pollen types. Note different scales.

by exponential class 1 (1-2 grains/m<sup>3</sup>); pollen from Ulmus, Populus and Cupressaceae were placed respectively in exponential classes 4 (12-23 grains/m<sup>3</sup>), 2 (3-5 grains/ m<sup>3</sup>) and 7 (100-199 grains/m<sup>3</sup>). During the same period, minimal pollen counts were recorded for Urticaceae and Poaceae. High pollen counts for Cupressaceae continued into March (exponential class 7; 100-199 grains/m<sup>3</sup>), while at mid-month Ulmus recorded 10-day averages corresponding to class 5 (25–49 grains/m<sup>3</sup>). During the last fortnight in March, Populus attained its highest counts (exponential class 6; 50–99 grains/m<sup>3</sup>), which persisted into April. By March, other pollen types started to appear, including Pinus, Platanus, Quercus, Rumex, Acer, and Salix, whilst Poaceae and Urticaceae pollen persisted. The greatest diversity of pollen types was detected in April and May (17 and 16 types, respectively). In April, Platanus pollen recorded its highest counts (exponential class 5; 24–49 grains/m<sup>3</sup>), while in May the highest airborne pollen counts for *Quercus* and *Pinus* were observed (class 7; 100-199 grains/m<sup>3</sup>), and also for Poaceae and Olea (class 6; 50-99 grains/m<sup>3</sup>). Plantago pollen counts also peaked in May (class 3; 6-11 grains/m<sup>3</sup>), as did Rumex (class 2; 3-5 grains/m<sup>3</sup>). Urticaceae pollen counts, observed throughout most of the year, peaked during the first week in May (class 8; 200–399 grains/m<sup>3</sup>), while the first low counts were recorded for Ericaceae, Compositae, Rosaceae and Apiaceae. Ligustrum pollen was first recorded in June (class 3; 6-11 grains/m<sup>3</sup>), when low counts were also detected for Cyperaceae and *Castanea* pollens. By the end of the month, pollen counts for these taxa had started to decline and remained low during July. In August and September, overall pollen counts remained low, major contributors including Artemisia, whose flowering period starts in late August and continues until November, and Chenopodiaceae-Amaranthaceae species, whose airborne concentrations peaked during these months (class 3; 6–11 grains/m<sup>3</sup>). October saw high counts for Cupressaceae pollen, when some species flowered; peaks were recorded late in the month (class 6;

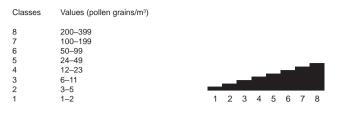


Figure 4. Classes and pollen concentration values represented in the calendar graph.

50–99 grains/m<sup>3</sup>), and considerable amounts were detected both in November and December.

Analysis of intradiurnal variations in pollen counts (Fig. 6) showed that most pollen types were present in small amounts in the early part of the day. From 10:00 onwards, counts started to increase, generally peaking at around noon or in the afternoon/evening (12:00-20:00). Cupressaceae pollen counts increased sharply from 12:00, peaking at 14:00. Plantago and Pinus displayed similar behavior, peaking at 12:00 and 16:00, respectively. Chenopodiaceae-Amarantaceae and Populus pollen counts peaked at 18:00, the latter recording a marked increase from 14:00 to 18:00. By contrast, Olea counts remained fairly steady throughout the day, with slight increases at 12:00, 14:00 and 22:00 (Fig. 6). Daily Poaceae pollen counts increased from 10:00 onwards, with slight peaks at 12:00 and 22:00. Finally, *Quercus* pollen attained its highest counts at 12:00, 14:00, 22:00 and 24:00.

**Meteorological influence.** Pollen counts during the study period, together with temperature and rainfall data, are shown in Figure 2. The March mean temperature was considerably higher in 2009 (10.29°C) than in 2008 and 2010, and pollen counts were also high for that month (8,908 grains/m<sup>3</sup>) (Tab. 1). A relation was observed between pollen counts in May and June, and rainfall both in the count-month and the preceding month. In 2008, the cleansing effect of heavy rains in May (105 mm) prompted

 Table 4. Coefficients of correlation between daily pollen pre-peak (PP) concentration and major meteorological variables, using the Spearman correlation test (significance levels: \*\*99%, \*95%).

Meteorological variables	Cupressaceae	Chenopodiaceae- Amaranthaceae	Olea	Pinus	Plantago	Platanus	Poaceae	Populus	Quercus	Urticaceae
T mean (°C)	0.540**	0.612**	0.751**	0.444**	0.467**	0.465**	0.651**	0.358**	0.576**	0.652**
T max (°C)	0.608**	0.590**	0.573**	0.519**	0.333**	0.207	0.510**	0.235*	0.448**	0.606**
T min (°C)	0.252**	0.600**	0.783**	0.148	0.531**	0.446**	0.639**	0.283**	0.677**	0.537**
Rainfall (mm)	- 0.349**	-0.377**	-0.318*	-0.455**	-0.059	0.020	-0.132	-0.050	-0.012	-0.237
Sun hours	0.509**	0.314**	0.067	0.470**	0.022	-0.116	0.279**	0.097	0.046	0.451**
Wind velocity (m/s)	- 0.113**	0.061	0.335*	-0.048	-0.025	0.021	0.116	0.032	0.093	-0.399**
Atmospheric pressure (millibars)	0.364**	0.200 **	0.284	0.459**	-0.054	-0.295	0.163	-0.366**	0.056	0.274*

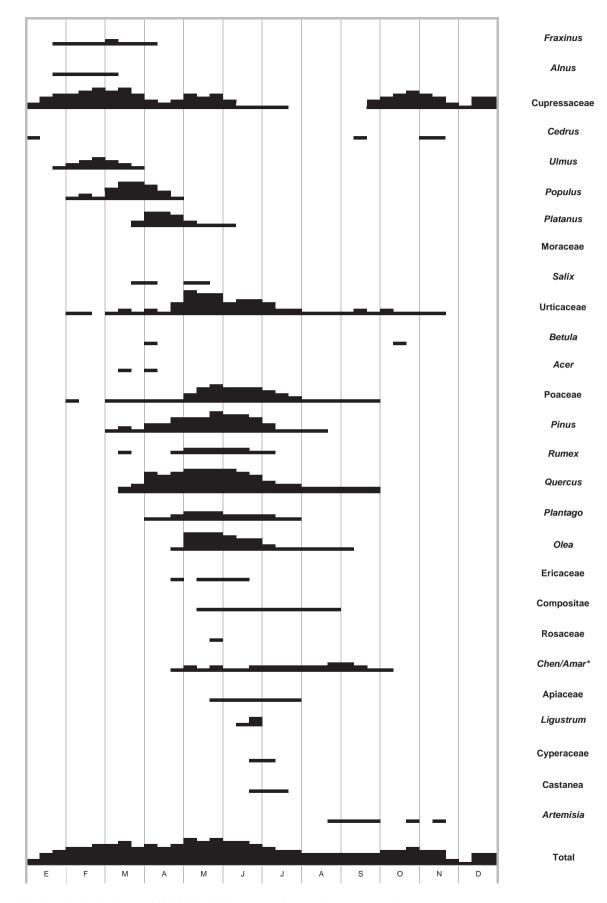
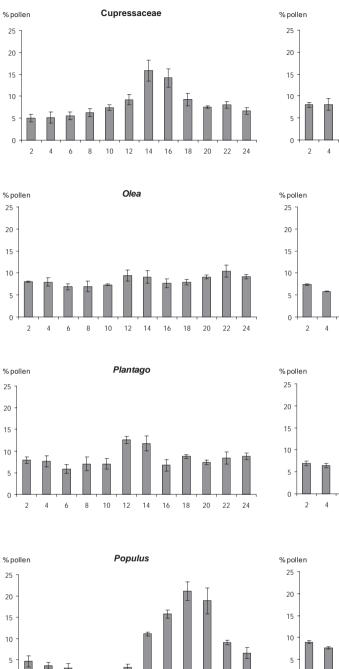
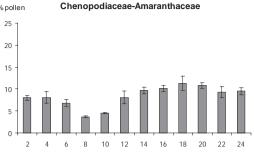
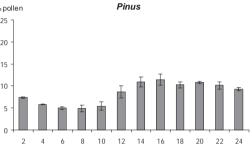
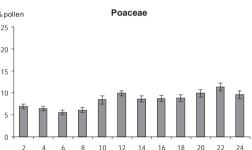


Figure 5. Pollen calendar for Cuenca (2008–2010). (\**Chen/Amar = Chenopodiaceae-Amaranthaceae*).









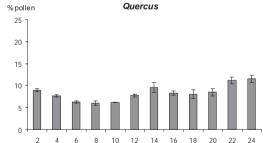


Figure 6. Mean (± standard error) bi-hourly distribution of pollen counts over 24 hours, expressed as percentages, for the pollen types: Cupressaceae, Chenopodiaceae-Amaranthaceae, *Olea, Pinus, Plantago*, Poaceae, *Populus* and *Quercus*.

a decline in pollen counts (9,699 grains/m<sup>3</sup>) compared to May 2009 and May 2010 (20,220 and 14,202 grains, respectively); however, the same effect prompted an increase in pollen counts in June 2008 (10,668 grains/m<sup>3</sup>). In 2009, the reverse occurred: high pollen counts and low rainfall (1.5 mm) in May led to low counts in June (3,503 grains/ m<sup>3</sup>) (Tab. 1).

10 12

14 16

2

6 8

18 20 22 24

Analysis of the correlation between pollen counts during the pre-peak period and meteorological variables (temperature, rainfall, hours of sunlight, wind speed and atmospheric pressure, disclosed a significant positive correlation between pollen counts and temperature for all taxa (Tab. 4). A negative correlation with rainfall was observed for 9 of the 10 taxa studied, although it attained statistical significance only for Cupressaceae, Chenopodiaceae-Amaranthaceae, *Olea* and *Pinus*. Correlations between pre-peak pollen counts and hours of sunlight were positive, but significant only for Cupressaceae, Chenopodiaceae-Amaranthaceae, *Pinus*, Poaceae and Urticaceae. Correlations with

wind speed were positive for some species, including *Olea*, and negative for Cupressaceae and Urticaceae. High pressure, linked to atmospheric stability, generally displayed a positive correlation with pollen counts for the taxa studied; only *Populus* exhibited a significant negative correlation.

Pollen allergy risk. The average of daily counts of allergenic pollen types and their seasonal evolution (Fig. 1, Fig. 5) determined that the incidence of these pollen types in Cuenca can be studied in 3 periods. From October to March, the major allergenic pollens were Cupressaceae, Fraxinus and Alnus; from April to June, pollen was recorded from Platanus, Pinus, Urticaceae, Olea, Poaceae, Plantago, Ligustrum and Castanea. Finally, in August and September, the major allergenic pollens were Chenopodiaceae-Amaranthaceae and Artemisia. The number of days on which pollen counts reached allergy-risk levels varied as a function of pollen type. Poaceae, Cupressaceae and Urticaceae attained moderately allergenic levels on an average of 30 days per year; High-risk levels were recorded for Poaceae pollen on around 15 days, Urticaceae on over 15 days, and Cupressaceae on 9 days. At least moderate allergenic risk levels were attained by Pinus over 3 weeks of the year, by Olea over 2 weeks, and by Chenopodiaceae-Amaranthaceae and *Platanus* on only a few days.

### DISCUSSION

A mean annual pollen count of 38,537 grains was recorded during the study period. This figure is lower than that reported for other cities in the Castilla-La Mancha region, including Toledo [20, 42] and Ciudad Real [45], but higher than the counts recorded elsewhere in central Spain, including Madrid, [54] and in other inland areas such as León [61], belonging to the same bioclimatic belt (supramediterranean) as Cuenca.

The diversity attested to in the pollen calendar for Cuenca is similar to that reported for other Spanish cities, in which a relatively small number of pollen types account for most of the total pollen count (Tab. 2), whilst a larger number contribute few grains [6, 8, 12, 26, 47, 48]. In Cuenca, as in Ciudad Real and Toledo [22, 42], the major pollen types in order of frequency are Cupressaceae (23.6%) and Quercus (19.8%), whose airborne pollen originates from ornamentals in the city's trees and parks (Cupressus spp.) and from local natural Mediterranean forest vegetation, dominated by junipers (Juniperus spp.) and oaks (Quercus ilex L. spp. ballota (Desf.) Samp., Quercus faginea Lam.). Airborne Urticaceae pollen counts (17.1%) were higher in Cuenca than elsewhere in Castilla-La Mancha and central Spain [22, 26, 42], due partly to abundant herbaceous communities dominated by Urticaceae species in riparian habitats along the rivers which flow on either side of the city, providing optimum soil moisture and organic matter supply, and partly to the widespread presence of Parietaria judaica in the walls of buildings in the Old

Town. Other abundant pollen types included Pinus (10%) and Olea (7.3%). Pinus pollen comes from pine woods on the outskirts of the city and in the Serranía de Cuenca; counts were considerably higher than those recorded in Madrid and Toledo [25, 42], but comparable to those reported for some cities in north-eastern Spain [52]. Olivepollen counts were quite high, given that Cuenca is not an olive-growing area; the pollen originates mostly in other parts of the province, such as La Alcarria, La Mancha and La Manchuela. Other major pollen sources include Populus (4.8%) in riparian vegetation, Poaceae (6.4%), Plantago (1.2%) and Chenopodiaceae-Amaranthaceae (1.5%) in forest herbaceous understorey, ruderal and weed flora, and Platanus (2.3%) in ornamental parks and gardens. Platanus pollen counts in Cuenca were lower than those recorded in other cities in the region (e.g. Ciudad Real and Toledo) and elsewhere in central Spain, including Aranjuez and Madrid [42, 45, 54]. A number of species, although present in the atmosphere, were excluded from the pollen calendar because counts were below the minimum 10-day average of 1 grain/m3 established for inclusion. They included: Brassicaceae, Cannabis, Casuarina, Corylus, Juncaceae, Mercurialis, Myrtaceae, Palmae and Sambucus.

A strong correlation was observed between airborne pollen counts and meteorological variables, especially temperature and rainfall. Findings matched those reported by other authors [2, 17, 21, 48, 51, 55]. Statistical analysis showed that temperature and hours of sunlight exerted a strong positive influence on pollen counts during the pre-peak period, the correlation with temperature being significant for all taxa (Tab. 4). By contrast, rainfall interrupted pollen emission, prompting lower daily counts, but prolonged flowering, thus increasing total airborne pollen counts [35]. Similar findings have been reported both in nearby cities such as Toledo [20, 42], and elsewhere in the Iberian Peninsula [1, 11, 12, 16, 28]. The positive influence of wind speed is evident in the presence of pollen from plants growing some distance away from the trap, and even beyond the city; a significant positive correlation was thus found between wind speed and Olea pollen counts. This phenomenon, also reported by other authors [42, 55], was apparent in the small peaks preceding the maxium peak for the main pollen season (Fig. 3). The significant negative correlation observed between wind speed and both Cupressaceae and Urticaceae pollen counts suggests that this pollen originates mostly in areas close to the trap. Similar results are reported for Urticaceae pollen in Lugo and Toledo [42, 52].

Analysis of intradiurnal patterns showed that peak counts for most pollen types tended to be recorded at around midday or in the afternoon; the difference served to indicate the distance of pollen sources from the trap, i.e. the timelag between pollen release and trapping [18, 43, 52]. Cupressaceae, *Plantago* and *Pinus* recorded peak counts at 14:00, 12:00 and 16:00; similar findings are reported for other Spanish cities including Lugo, Córdoba and Toledo

[18, 43, 52]. Peaks coincided with the highest temperatures and lowest relative humidity levels. Chenopodiaceae-Amaranthaceae pollen counts increased after 10:00, peaking in the evening (18:00), whereas in other Spanish cities, including Málaga and Córdoba, peaks occur at around noon [18, 59]. Populus counts peaked at 18:00, i.e. 2 hours later than in Toledo [43]. Peak Chenopodiaceae-Amaranthaceae and Populus counts were recorded at 18:00, suggesting that the sources were outside the city. Of the other 3 pollen types studied, *Quercus* counts displayed peaks at 3 different times (14:00, 22:00 and 24:00); the earliest peak probably corresponded to pollen from sources near the trap, whilst the 2 later peaks were prompted by sources from more distant woodland and from the Serranía de Cuenca; similar findings are reported for Toledo [43]. Hourly pollen counts for both Olea and Poaceae displayed little variation throughout the day; the lowest levels were recorded early in the morning (6:00 and 8:00), and small increases were noted at 12:00 and 22:00, probably caused in the case of Olea by pollen from sources further away from the trap; the increased observed for Poaceae reflects the fact that pollenrelease patterns vary widely among the species belonging to this family. Similar results have been reported for the cities of Málaga and Porto, as well as in the Hornachuelos Natural park in Córdoba [19, 49, 59]. Nevertheless, studies in other areas [32, 33, 34, 36, 40, 41], show that the daily behaviour of Poaceae pollen is irregular which could be due to the large number of species comprising the family, all of which have different flowering periods, other opening times and thus pollen-release times; pollen counts also differed as a function of differing weather conditions in the various areas studied.

Analysis of seasonal variations in mean daily allergenic pollen counts in Cuenca showed that, from October–March, Cupressaceae was the main cause of pollen allergy; in central Spain, the prevalence of sensitization to this pollen type among pollen-allergy sufferes is high (58% in Madrid, 30% in Toledo [58]). It is the single most abundant type in the city, accounting for 23.6% of total pollen, and counts are elevated by October (Fig. 1, Fig. 3). From January to March, other airborne allergenic pollen types detected included *Fraxinus* and *Alnus*, but these presented little allergenic threat since counts were low.

The second major allergy period started in early spring, i.e. late March and April, with *Platanus* pollination. The *Platanus* pollen season was short, airborne pollen being recorded on an average of 50 days of the year. However, while in other Spanish cities including Aranjuez, Madrid or Barcelona [54, 60], high *Platanus* counts are recorded, in Cuenca this genus accounted for only 2.3% of total pollen, and only exceeded the threshold of 50 grains/m<sup>3</sup> on a few days in the year (Table 3). *Pinus* pollen was also recorded in March, and counts peaked in May. Although allergy to *Pinus* pollen is considered uncommon and clinically insignificant [23], the abundant pine woods around the city and in the province of Cuenca prompt high local

pollen counts, which may affect local allergy-sufferers, a finding also reported in some areas in the northern Iberian Peninsula [23, 31].

Urticaceae pollen was present in the air during much of the year, although flowering mainly took place in May and June. One of the most allergenic genera is *Parietaria*, abundant on the walls of the Old Town. In Spain, between 10%–30% of allergy-sufferers are sensitive to *Parietaria* pollen [37]. During the study period, Urticaceae accounted for 17.1% of total pollen, and on many days counts exceeded the allergy-risk threshold of 15 grains/m<sup>3</sup> (Tab. 3).

In Europe and most temperate areas, Poaceae pollen is a major cause of allergies and rhinoconjunctivitis [30, 46]. In central Spain and in the Castilla-La Mancha region, Poaceae and Olea are the main cause of spring allergies [3]. The prevalence of Poaceae-positive skin prick tests among pollen-allergy sufferers exceeds 75% [58]. In Cuenca, Poaceae accounted for 6.4% of total pollen; the highest airborne counts were recorded in May and June every year, and on peak days counts exceeded 100 grains/m<sup>3</sup>. Moreover, allergy-risk thresholds were reached on a large number of days: mean daily counts exceeded 25 grains/m<sup>3</sup> on an average 27 days of the year, and counts of over 50 grains/ m<sup>3</sup> were recorded on an average of 15 days. Similar findings have been reported for other central Spanish cities, including Madrid and Aranjuez, while the number of days when counts reach risk levels is higher in Toledo [42, 53].

*Olea* pollen is a major cause of allergies in central and southern Spain. In Castilla-La Mancha, 55.6% of rhinoconjunctivitis-sufferers are sensitive to *Olea* [39]. The highest concentrations were recorded in May, and in the course of pollen seasons counts exceeded the high-risk threshold of 50 grains/m<sup>3</sup> on an average of 12 days per year; higher figures have been reported for other cities in the region, including Toledo [42].

*Plantago* pollen appeared mainly from April–June, but counts were always low. Patients allergic to *Plantago* also tend to be sensitive to graminae, whose flowering season overlaps with that of *Plantago*. Allergenic *Ligustrum* and *Castanea* pollen was also recorded in June, but in low amounts with little effect on allergy-sufferers.

The third pollen-allergy period was the summer, mainly August and September, due to Chenopodiacee-Amaranthaceae pollen. Although pollen counts in Cuenca were not particularly high, rarely exceeding 15 grains/m<sup>3</sup>, this is an agressive pollen type, and the third most important cause of pollen allergies in Spain, after Poaceae and *Olea* [58].

#### CONCLUSIONS

Airborne pollen counts in Cuenca displayed considerable seasonal variability, peak counts being observed in May and June, when over half the total pollen was recorded. The wide range of pollen types reflects the diversity of vegetation, comprising ornamental species (Cupressaceae, 23.6%; *Platanus*, 2.3%), tree species growing in riparian habitats (Populus, 4.8%), herbaceous plants (Urticaceae, 17.1%; Poaceae, 6.4%; Plantago, 1.2% and Chenopodiaceae-Amaranthaceae, 1.5%) and forest vegetation, with particularly high pollen counts for Quercus (19.8%) and *Pinus* (10%). The meteorological variable displaying the strongest positive correlation with airborne pollen counts was temperature, whilst rainfall exhibited the strongest negative correlation. A study of the intradiurnal variation of pollen disclosed 2 behaviour patterns. The first was followed by the majority of pollen types studied and showed a single concentration peak throughout the day reached between 12:00-22:00; by contrast, Olea, Poaceae and Quercus had 2 or 3 small concentration peaks as pollen reached the trap from sources at varying distances. The pollen types responsible for most allergies in Cuenca were Cupressaceae, Platanus, Olea, Poaceae, Urticaceae and Chenopodiaceae-Amaranthaceae. In some cases, pollen counts and the number of allergy-risk days were similiar to those reported for other cities in central Spain, including Madrid, but counts were generally lower than those recorded in other cities in the Castilla-La Mancha region, such as Toledo.

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