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POLLEN SPECTRUM AND RISK OF POLLEN ALLERGY IN CENTRAL SPAIN

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Abstract: The present work analyses the airborne pollen dynamic of the atmosphere of Toledo (central Spain), a World Heritage Site and an important tourist city receiving over 2 millions of visitors every year. The airborne pollen spectrum, the annual dynamics of the most important taxa, the influence of meteorological variables and the risk of suffering pollen allergy are analysed. Results of the present work are compared to those obtained by similar studies in nearby regions. The average annual Pollen Index is 44,632 grains, where 70–90% is recorded during February–May. The pollen calendar includes 29 pollen types, in order of importance; Cupressaceae (23.3% of the total amount of pollen grains), *Quercus* (21.2%), and Poaceae and *Olea* (11.5 and 11.2%, respectively), are the main pollen producer taxa. From an allergological viewpoint, Toledo is a high-risk locality for the residents and tourist who visit the area, with a great number of days exceeding the allergy thresholds proposed by the Spanish Aerobiological Network (REA). The types triggering most allergic processes in Toledo citizens and tourists are Cupressaceae, *Platanus*, *Olea*, Poaceae, Urticaceae and Chenopodiaceae-Amaranthaceae. Allergic risk increases in 3 main periods: winter (January–March), with the main presence of the Cupressaceae type; spring, characterized by Poaceae, *Olea*, *Platanus* and Urticaceae pollen types; and, finally, late summer (August–September), characterized by Chenopodiaceae-Amaranthaceae pollen type, which are the main cause of allergies during these months.

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INTRODUCTION

The high presence of airborne pollen in central Spain is due to the large number of anemophilous species in this area. Among them it is possible to remark the high concentrations due to natural species such as *Quercus*, *Populus*, Poaceae, Urticaceae, *Artemisia* and Chenopodiaceae-Amaranthaceae; to the ornamental species; Cupressaceae and *Platanus*; or even to crop species such as *Olea*. The diversity and composition of the pollen spectrum reflects the flora and the vegetation of an area. For this reason, in cities such as Madrid, Toledo and Ciudad Real, all of them located in central Spain, the pollen from Cupressaceae, *Populus*, *Quercus*, Poaceae and *Olea* are the most abundant in the atmosphere [18, 24, 34]

Together with their ecological and agronomic interest, aerobiological studies have a clear clinical interest, especially for allergic patients. Allergies have increased in developed countries during recent years constituting a health-care problem of growing importance. This increase affects both the number of affected patients and the severity of allergic reactions [7, 30]. Monitoring airborne pollen content enables identification of the pollen types causing pollinosis in a particular geographical area. This knowledge and the

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design of pollen calendars, are essential to reduce the exposure of allergic patients to pollen during the days of higher pollen concentration, and enable the development of preventive measures when planning outdoor activities. Pollen calendars offer information about pollen type diversity in the air, as well as both timing and intensity of the pollen season [8, 22, 38]. Moreover, continuous monitoring of airborne pollen grains offers us valuable aerobiological information, being one of the most accurate and valuable indicators on the impact of climate change [3, 14, 16, 17].

The scenario of the present work is the city of Toledo, at the centre of the Iberian Peninsula, north-west area of the Autonomous Community of Castilla-La Mancha. This work enhances previous results both analysing pollen and pollinosis in Toledo [18, 28]. The main objectives of the present work are to establish an actual pollen calendar, to study allergenic pollen types and to establish the influence of meteorological variables on daily pollen concentration, by applying statistical analysis in order to obtain a deeper analysis of the actual allergy pollen risk, with the aim of informing citizens and tourists on the state and situation of atmospheric pollen content in general and allergenic pollen content in particular.

MATERIAL AND METHODS

Area description. Toledo is the administrative capital of the Autonomous Community of Castilla-La Mancha, located at the centre of the Iberian Peninsula (39º 51´ N, 4º 01´ W), 529 m above sea level and on the banks of the River Tagus. It has around 83,000 inhabitants, was declared a World Heritage Site by UNESCO in 1986, and is an important tourist location receiving over 2 millions visitors every year.

Bioclimatically, Toledo lies in the Mesomediterranean belt, with a dry ombroclimate [39]. Winters are cold and summers are very dry, with high temperature differences between both. Average annual temperature is 15.4ºC; July is the warmest month (average temperature: 26ºC) and January is the coldest (average temperature: 6.4ºC). Regarding rainfall, average annual rainfall is 382 mm; October is the wettest month (43 mm) and July is the driest (8 mm) [26].

Biogeographically, Toledo is located in the Mediterranean Region (W Mediterranean Subregion) and, according to Rivas-Martínez *et al.* [39], it is located on the limit between two biogeographical provinces: the Mediterranean Central Iberian Province (Castilian Subprovince, Machean Sector) and the Mediterranean West Iberian Province (Lusitan-Extremadurean Subprovince, Toledan-Taganean Sector).

Natural vegetation is represented with holm oak forests (*Quercus rotundifolia* Lam.), shrubs and pasturelands alternating with meadows. Junipers (*Juniperus oxycedrus* L.) and wild olives (*Olea europaea* L. var. *sylvestris* Brot., *Phyllirea angustifolia* L.) are present in forest undergrowth, and in shrubs and bushes. The presence of the river Tagus enables the existence of riparian vegetation with shrubby willow vegetation (*Salix* spp.), ash trees of *Fraxinus angustifolia* Vahl, poplars (*Populus* alba L. and *Populus nigra* L.), and elms (*Ulmus minor* Mill.). Crop fields in the Toledo surroundings mainly occupy the Mancha territory and are cereal and olive crops. Ornamental flora, frequently highly allergenic, in the parks and gardens of Toledo, is represented by elms (*Ulmus pumila* L.), pines (*Pinus halepensis* Mill.), cypresses (*Cupressus sempervirens* L. and *Cupressus arizonica* Greene), olive trees (*Olea europea* L.), hybrid planes (*Platanus hispanica* Mill. ex Münchh), and glossy privets (*Ligustrum lucidum* Aiton). Other less-abundant species include mulberry trees, palm trees and cedars. Grasses, nettles, Urticaceae and Chenopodiaceae are the most characteristic herbaceous species in forest, ruderal and weed flora.

Airborne pollen and allergy risk. A Hirst Volumetric spore-trap (Lanzoni model, VPPS 2000) was used for aerobiological sampling. It was installed around 17 m above ground level, in the campus of the University of Castilla-La Mancha, located in the north-western area of Toledo. The methodological criteria followed for spore-trap placement, sampling and sample analysis follows the proposals of the Spanish Network of Aerobiology [15].

Calculation of the Main Pollen Season (MPS) comprises from the day in which 2.5% of total pollen is registered to the day in which 97.5% of total pollen is registered – i.e., it collects 95% of total registered pollen. MPS was carried out according to the methodology described by Andersen [2].

The pollen calendar of Toledo was created according to the model proposed by Spieksma [47]. Setting off from average daily concentration (grains/m^3), 10-day arithmetic means are calculated. These means approximately correspond to the third part of the month. Subsequently, data are grouped by following date-equivalence criteria and the 10-day arithmetic mean corresponding to the period under study (2003–2007) is calculated. Graphic representation is performed by matching each mean with one of the 11 types of exponential frequency [C: classes, V: values pollen grains (pg)/m^3; C1 V: 1–2; C2 V: 3–5; C3 V: 6–11; C4 V 12–23; C5 V: 24–43; C6 V: 50–99; C7 V: 100–199; C8 V: 200–399; C9 V: 400–799; C10 V: 800–1599; C11 V: 1600 or more.], according to Stix & Ferretti [48], and is represented in the shape of a histogram. Only pollen types with means greater than or equal to 1 grain/m^3 are represented. In the pollen calendar, the different pollen types are chronologically ordered according to the appearance of peaks. This structure enables a clear view of the pollen periods of each of the different taxa throughout the year.

The risk of pollen allergy was calculated by the number of days with different levels of risk of allergy diseases caused by the pollen of the main important taxa, according to the allergy thresholds proposed for the Spanish Aerobiology Network (REA) [15]. 'Moderately allergenic levels' were considered in case herbaceous taxa ranged from 15–30 pg/m^3 (Urticaceae) or 25 to 50 pg/m^3 (Chenopodiaceae-

Table 1. Mean Temperatures (ºC) and Rainfall (mm) for each year and seasons from 2003 to 2007.

			Tª (ºC)	Rainfall (mm)
2003	Season	Winter	8.2	76
		Spring	18.5	49
		Summer	26.6	6
		Autumn	12.9	172
	Year		**16.5**	**303**
2004	Season	Winter	8.3	82
		Spring	15.5	213
		Summer	25.7	27
		Autumn	13.0	83
	Year		**15.6**	**405**
2005	Season	Winter	6.0	30
		Spring	18.9	30
		Summer	25.9	5
		Autumn	12.3	91
	Year		**15.8**	**156**
2006	Season	Winter	7.2	58
		Spring	18.8	97
		Summer	26.8	17
		Autumn	14.2	205
	Year		**16.7**	**377**
2007	Season	Winter	8.9	37
		Spring	15.8	243
		Summer	24.9	25
		Autumn	11.9	76
	Year		**15.3**	**381**

Amaranthaceae, Poaceae). For arboreal taxa, from 25–50 pg/m^3 (Ulmaceae) and from 50–200 pg/m^3 for the remainder species (Cupressaceae, *Olea*, Pinus, *Platanus*, *Populus* and *Quercus*). The category 'highly allergenic levels' was given to cases above these values.

Meteorological data. The data of the different climatic variables analysed – maximum, minimum and average temperature (ºC); rainfall (mm); maximum, minimum and average pressure (mb); evapotranspiration (mm); wind speed (km/s); and sunshine hours – were provided by the Toledo-Buenavista meteorological station, located 2 km from the spore trap, through the database of the Spanish State Meteorological Agency (AEMET). Rather irregular inter-annual rainfall patterns were observed along this period (2003–2007); 2003 and 2005 were the driest years, showing very low rainfall values (303 and 156 mm, respectively), remaining far below the average value (382 mm). Regarding temperature, the values have been rather similar in summer and autumn (except a warmer autumn in 2006), and lower values were obtained for spring in 2004 and 2007 (15.5–15.8 ºC), and winter in 2005 and 2006 (Tab. 1).

Statistical analysis. To confirm statistically a possible relationship between daily pollen concentrations and the different meteorological parameters, such as rainfall, temperature (maximum, minimum and mean), and sunshine, a non-parametric Spearman's correlation test was performed. A non-parametric correlation analysis was used since the data are not normally distributed. This test was used in order to detect the possible significant influence of some meteorological variables on the pollen variation during the Pre-Peak period (PP). SPSS 15.0® software package was used.

RESULTS

Pollen spectrum. The average annual Pollen Index obtained in Toledo during the period under study (2003–2007) was 44,632 (Tab. 2). The highest value (56,000) was recorded in 2007 and the lowest (29,826) in 2004.

During these years, the highest pollen amounts were registered between February–May, when annual amounts range between 70–90% of the whole annual amount (Fig. 1). Average daily concentrations in this period are over 1,000 pg/m^3 on some days (Fig. 2), reaching their maximum amount on 1 May 2005 (2,337 pg/m^3). The month with the maximum annual pollen concentration varies according to the year (Tab. 2): it was March in 2003 (14,545 pg/m^3; 34% of total annual pollen) and April in 2004 (6,217 pg/m^3; 20.8% of total annual pollen), while it was May in the remainder years (18,103 pg/m^3, 33.6% of total annual pollen in 2005; 14,281 pg/m^3, 35.1% of total annual pollen in 2006; and 15,587 pg/m^3, 27.8% of total annual pollen in 2007). The period between September–December is that showing the lowest pollen concentration (levels remain below 1% of total annual pollen concentration).

The evolution of pollen concentration show the different peaks reached year by year (Fig. 2 and 3), mainly due to the pollen contribution of Cupressaceae in February–March, *Quercus* in April, and Poaceae and *Olea* between May–June. From mid- and late June, pollen concentrations undergo considerable reduction, as no significant peak is observed. However, small amounts of pollen are present in the atmosphere from the end of the summer from Chenopodiaceae-Amaranthaceae species, and the autumn flowering of *Artemisia* and some Cupressaceae species between October and December.

The most relevant pollen types during this period, in order of quantitative importance, are: Cupressaceae (23.3% of the total amount of pollen grains), *Quercus* (21.2%), and Poaceae and *Olea* (11.5% and 11.2%, respectively) (Tab. 3), showing wide inter-year variations in total amount, main pollen season, length of the pollen season, and maximum daily values (Tables 3 and 4). To a lesser extent, we have registered pollen from other species, such as *Populus* (8.3%), *Platanus* (4.8%), *Pinus* (2.7%) and *Ulmus* (1.9%). Regarding herbaceous species, apart from Poaceae, there stand out the pollen types Urticaceae (5.4%) and Chenopodiaceae-Amaranthaceae (1.7%). The maximum daily values

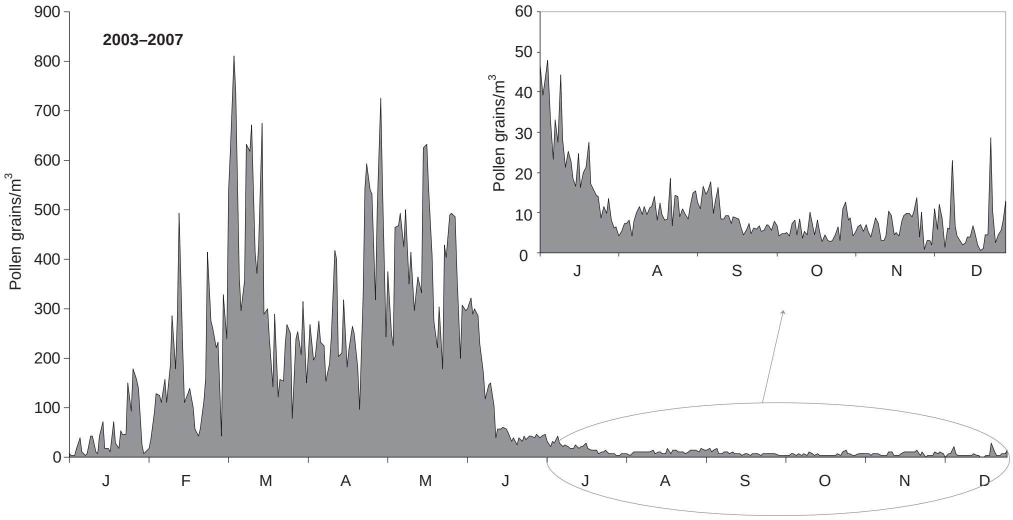

Figure 1. Seasonal fluctuations in daily average concentrations of total pollen. Mean values for period 2003–2007.

(Tab. 4) registered for the different tree pollen types correspond to *Quercus* (2,179 pg/m^3; reached on 1 May 2005), followed by Cupressaceae (1,834 pg/m^3; 16 March 2005), *Populus* (1,464 pg/m^3; 5 March 2003), *Olea* (1180 pg/m^3; 26 May 2005) and *Platanus* (905 pg/m^3; 27 March 2003). Poaceae (694 pg/m^3; 18 May 2003) and Urticaceae (474 pg/m^3; 13 April 2003) show the maximum values for pollen types of herbaceous species.

The Main Pollen Season (MPS) for each analysed type (Tab. 4) shows that pollen types Cupressaceae, *Pinus* and *Quercus* have a long pollen season, some of them exceeding 4 months. On the other hand, *Platanus*, *Populus* and *Ulmus* have the shortest pollen season (around 45 days). The pollen season of herbaceous types are generally longer, more than 5 months, since they include a wider variety of species. Thus, the presence of airborne pollen types Urticaceae, Poaceae and Chenopodiaceae-Amaranthaceae ranges between five–six months on average.

The pollen types included in the pollen calendar of the atmosphere of Toledo (Fig. 4) account for 29 types: 19 types from woody plants and 10 from herbaceous plants. The different taxa are ordered as a function of the pollination and the moment at which their maximum peaks appear. The scale is exponential, so that each step doubles, approximately, the previous one (Fig. 5). Regarding the diversity of the represented types, we shall point out the absence of certain taxa such as *Cannabis*, Cyperaceae, Juncaceae, *Mercurialis*, Palmae and *Sambucus*, which are present in the atmosphere of Toledo but do not reach the 1 grain/m^3 value on average; therefore, they are not included in this calendar.

During January and February, pollen types of tree species such as *Alnus*, Cupressaceae, *Fraxinus*, *Populus* and

Table 2. Monthly and annual values (total and percentages) of total pollen during the years studied.

	2003		2004		2005		2006		2007		2003–2007		SD	Max	Min
	Total	%	Total	%	Total	%	Total	%	Total	%	Average	%			
Jan	1,987	4.6	3,138	10.5	545	1.0	385	0.9	915	1.6	1,394	3.1	1,157.75	3,138	385
Feb	4,744	11.1	5,743	19.3	2,986	5.5	2,515	6.2	7,755	13.8	4,749	10.6	2,128.56	7,755	2,515
Mar	14,545	34.0	4,050	13.6	15,898	29.5	9,668	23.8	12,439	22.2	11,320	25.4	4,695.55	15,898	4,050
Apr	9,141	24.3	6,217	20.8	11,411	21.2	10,594	26.0	6,373	11.4	8,747	19.6	2,382.24	11,411	6,217
May	10,378	24.3	4,911	16.5	18,103	33.6	14,281	35.1	15,587	27.8	12,652	28.3	5,149.65	18,103	4,911
Jun	1,466	3.4	4,746	15.9	3,108	5.8	1,094	2.7	9,109	16.3	3,905	8.7	3,250.94	9,109	1,094
Jul	270	0.6	473	1.6	640	1.2	516	1.3	1,627	2.9	705	1.6	532.23	1,627	270
Aug	*	*	*	*	442	0.8	531	1.3	572	1.0	515	1.2	66.46	572	442
Sept	*	*	163	0.5	385	0.7	280	0.7	512	0.9	335	0.8	148.82	512	163
Oct	87	0.2	96	0.3	128	0.2	335	0.8	252	0.5	180	0.4	109.11	335	87
Nov	89	0.2	218	0.7	111	0.2	309	0.8	263	0.5	198	0.4	95.39	309	89
Dec	28	0.1	71	0.2	117	0.2	198	0.5	596	1.1	202	0.5	229.08	596	28
PI	42,756	100	29,826	100	53,874	100	40,706	100	56,000	100	44,632	100			

Pollen Index (PI), Standard deviation (SD), Maximum value (Max) and Minimum value (Min). * Incomplete data because there were problems with the collector sampler.

Table 3. Annual total counts of the main pollen types in the atmosphere of Toledo (2003–2007).

	Average 2003–2007	% Total pollen	Standard Deviation	Maximum value	Minimum value
Chenopodiacea- -Amaranthaceae	774	1.7	133.82	917	652
Cupressaceae	10,397	23.3	4,572.70	17,236	4,838
Olea	4,991	11.2	2,184.89	7,427	2,101
Pinus	1,184	2.7	408.55	1,687	805
Platanus	2,178	4.8	1,127.10	3,870	712
Poaceae	5,136	11.5	2,814.42	9,109	1,730
Populus	3,686	8.3	1,734.95	5,499	982
Quercus	9,469	21.2	4,622.70	17,305	5,361
Ulmus	858	1.9	372.78	1,418	483
Urticaceae	2,415	5.4	1,493.17	4,209	331

Ulmus reach high levels. However, herbaceous pollen types such as Urticaceae and Poaceae are also present but show lower levels. In February, pollen types *Fraxinus* and *Alnus* reach their maximum concentration in air, thus obtaining exponential classes 2 (3–5 grains/m^3) and 3 (6–11 grains/m^3), respectively.

March is characterized by high concentrations of Cupressaceae and *Populus* pollen, which reach their maximum concentration, corresponding to exponential class 8 (200–399 grains/m^3), in the first weeks of the month. Thus, Cupressaceae pollen, in spite of flowering in autumn, gathers between January–March. Moreover, the pollen type of *Populus* extends from February–late March (early April), their weekly average values being over 200 grains/m^3.

Ulmus show average values of exponential class 5 (25–49 grains/m^3) in mid-March and their flowering period extends from February–mid-April. March is also characterized by the presence of Poaceae and Urticaceae pollen, even reaching exponential classes 2 (3–5 grains/m^3) and 3 (6–11 grains/m^3) for the former and 4 (12–23 grains/m^3) and 5 (24–49 grains/m^3) for the latter. Besides, other pollen types such as Moraceae, *Pinus*, *Platanus*, *Quercus*, *Rumex* and *Salix* are also present.

Platanus and *Moraceae* show short flowering periods (less than 2 months). *Platanus* pollen remains in the air for only a few weeks (March–April) and reaches its maximum peak between late March–early April (Tab. 4): exponential class 6 (50–99 grains/m^3). Moraceae pollen is present from mid-March to late May: peaks in early April – exponential class 5 (24–49 grains/m^3).

A wider diversity of pollen types always appears in spring months (April–May). April shows the presence of 20 out of the 29 types studied. Besides, apart from *Moraceae*, Urticaceae also reaches its maximum concentration: exponential class 6 (50–99 grains/m^3). The pollen of Urticaceae is present throughout the year, with several peaks corresponding to its different species, although its flowering period mainly takes place during March and April.

May shows 19 out of the 29 types represented in the calendar and the highest concentrations of Poaceae, *Pinus*, *Quercus*, *Plantago* and *Olea*. Poaceae concentrations reach exponential class 7 (100–199 grains/m^3), although their presence in air remains steady throughout most of the year due to the great number of species in Castilla-La Mancha (more than 250 species). The *Pinus* pollen type reaches its maximum peaks (exponential class 4: 12–24 grains/m^3) in May, thus extending its pollen season from early spring (March) to early summer (July).

Quercus, together with Cupressaceae, is one of most important pollen types in Toledo. The existence of several species with different flowering periods means that the pollen of this group remains in the air for several months, although its maximum peaks are found in May (exponential class 8: 200–399 grains/m^3).

The pollen type *Olea* characterizes May and June. *Olea* is a species with biennial pollen production cycles – alternating years of high and low pollen production [12]. Maximum pollen concentrations reach exponential class 8 (200–399 grains/m^3). Herbaceous types *Plantago* and *Rumex* reach maximum concentrations of exponential class 3 (6–11 grains/m^3) in this period of the year.

June shows the appearance of *Ligustrum* pollen, whose presence is due to ornamental flora, and pollen of Myrtaceae and *Castanea*, although always in low concentrations. During June and July, the pollen concentration of most of the taxa commented so far begin to decrease, thus giving rise to the presence of pollen of herbaceous species whose flowering period corresponds to late summer, such as Chenopodiaceae-Amaranthaceae, present in the atmosphere of Toledo with maximum exponential values of class 3 (6–11 grains/m^3) between mid-August and mid-September.

Early autumn is characterized by the intermittent appearance of *Artemisia* pollen, reaching exponential class 2 (3–5 grains/m^3) in November. This period of the year is also characterized by the presence, although at lower concentrations, of a secondary peak of the Cupressaceae pollen type, which usually extends from October to December.

Pollen allergy risk. Allergenic pollens present in the atmosphere of Toledo correspond to the following types:

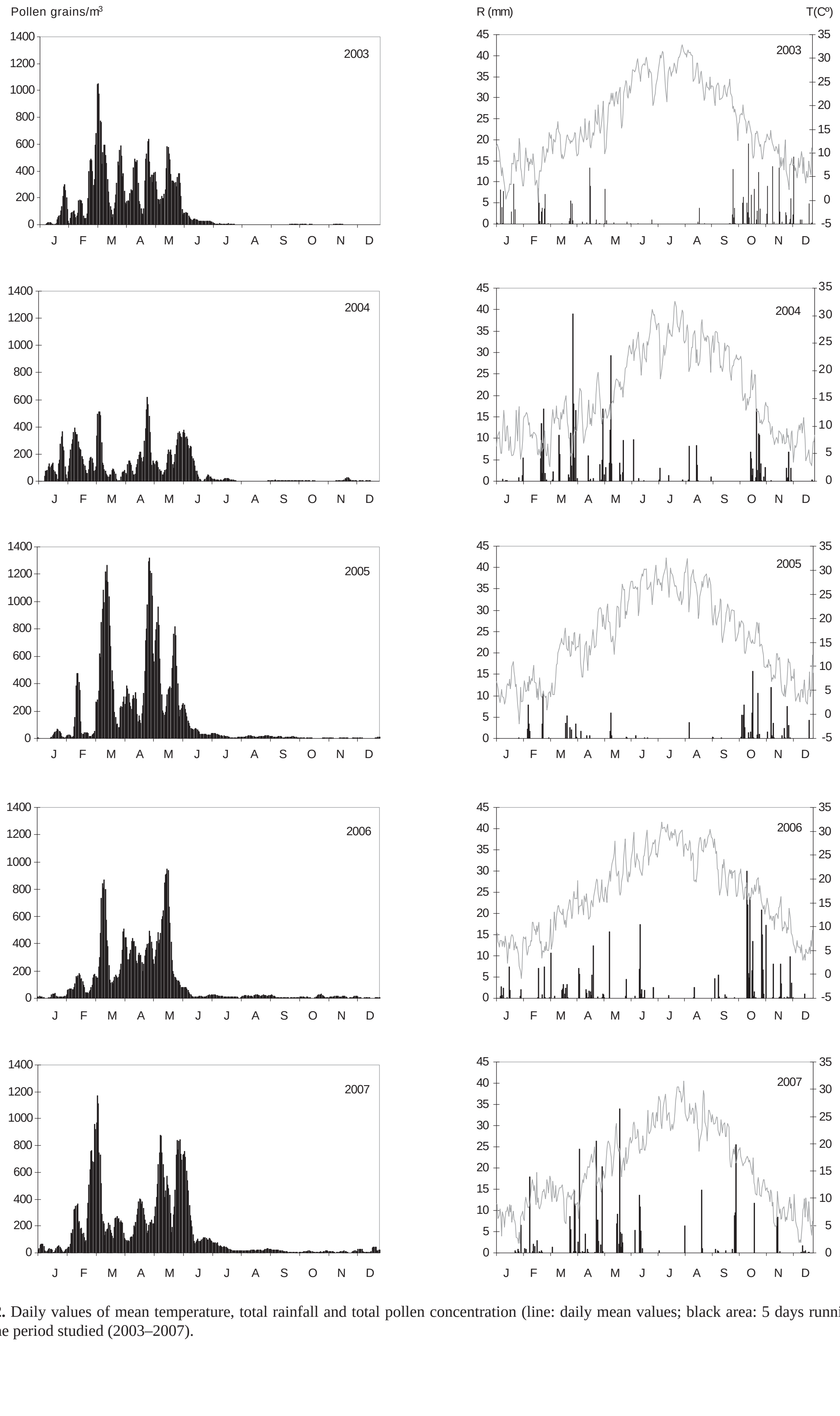

Figure 2. Daily values of mean temperature, total rainfall and total pollen concentration (line: daily mean values; black area: 5 days running mean) during the period studied (2003–2007).

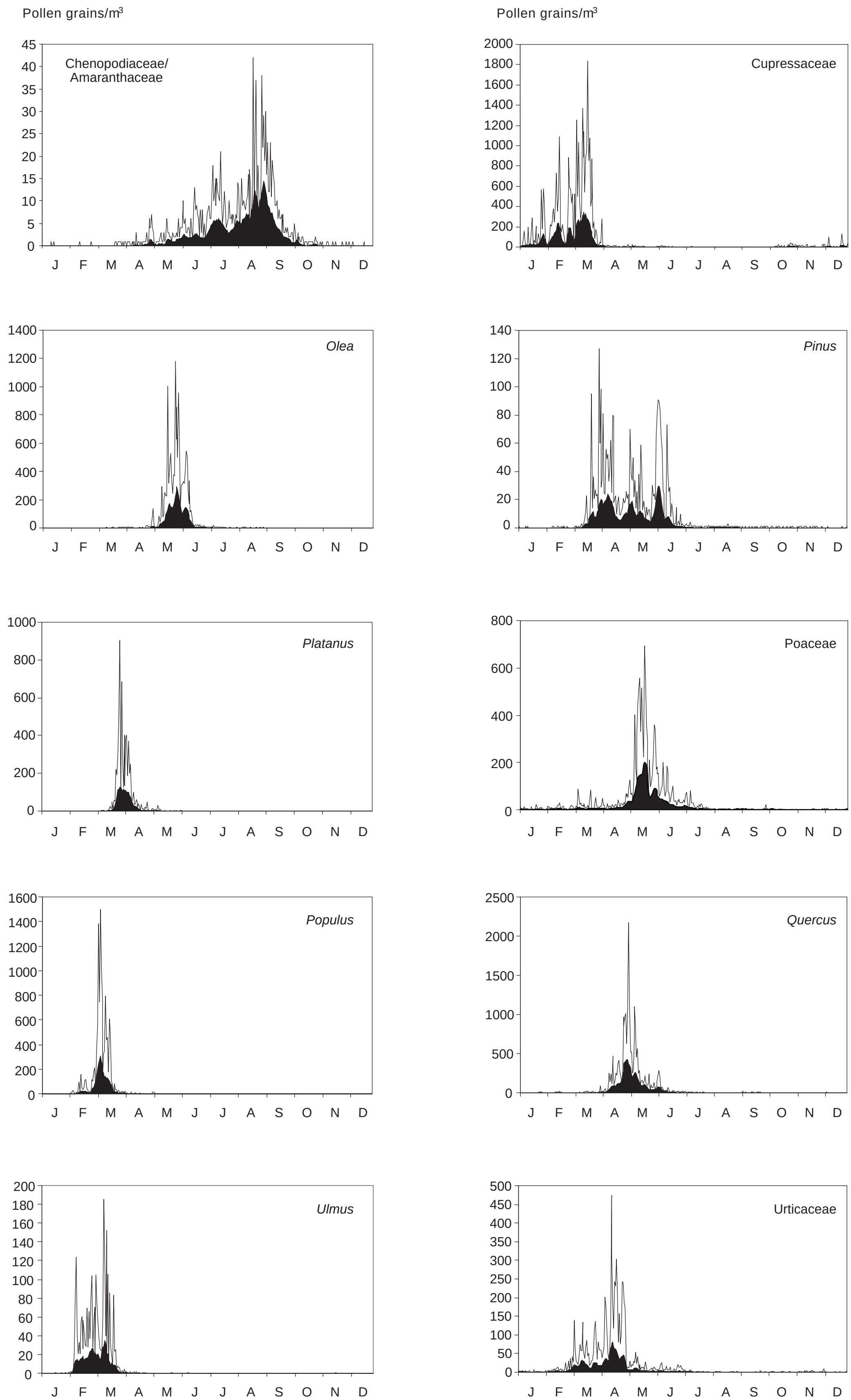

Figure 3. Five days running mean (black area) and maximum daily mean (line) values for pollen concentration, during the years studied (2003–2007) reached by the 10 most abundant pollen types. Note the different scales.

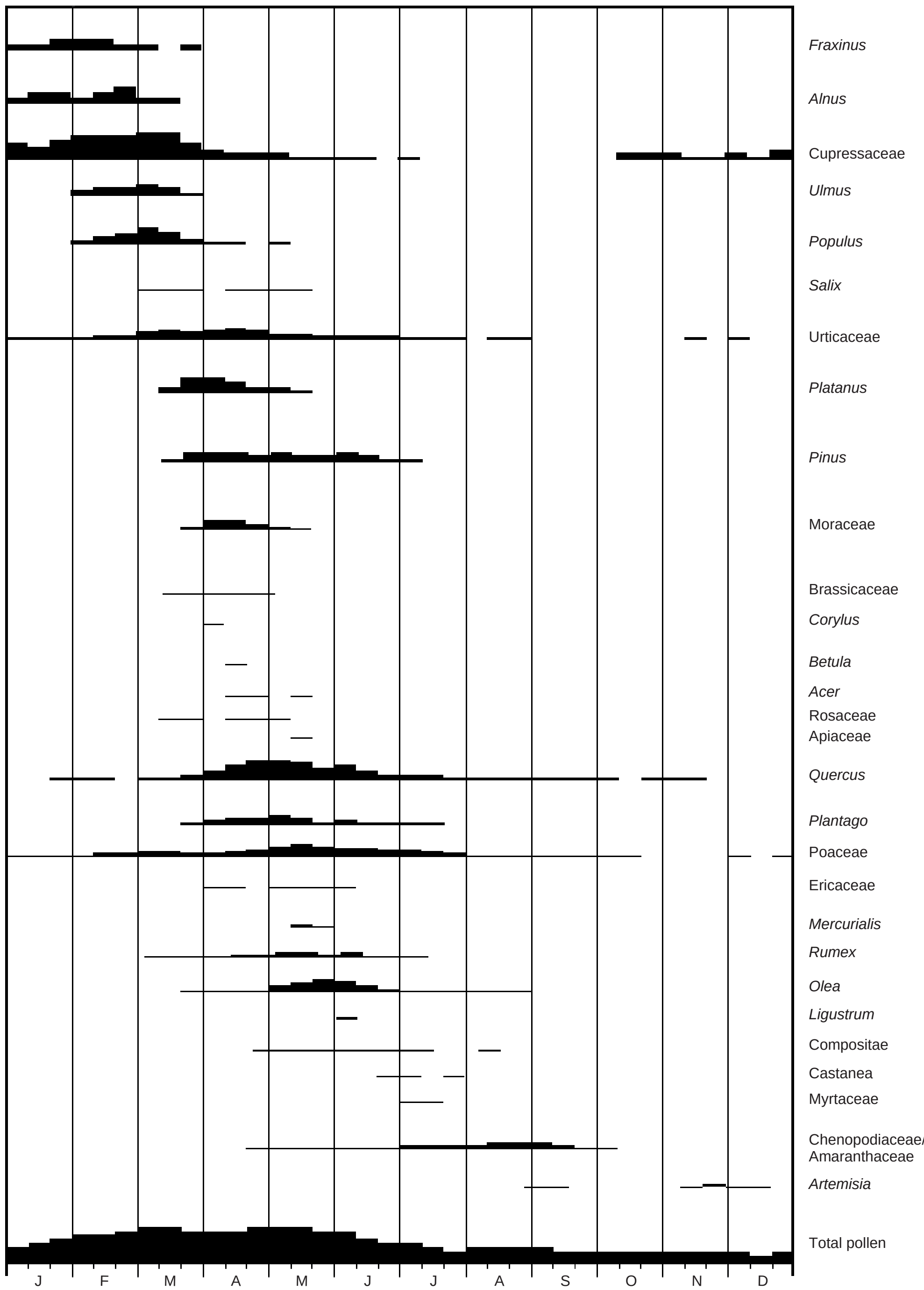

Figure 4. Pollen calendar of Toledo (2003–2007).

Fraxinus, *Alnus*, Cupressaceae, *Ulmus*, *Populus*, *Platanus*, Urticaceae, *Betula*, *Quercus*, *Plantago*, Poaceae, *Ligustrum*, *Castanea*, Chenopodiaceae-Amaranthaceae and *Artemisia*. They account for 51% of all pollen types in the pollen calendar. The average daily concentration values of these pollen types and their seasonal evolution determine the 3 main periods in which allergic processes take place (Fig. 1 and 4). The first allergic period corresponds to winter months (January–March), with airborne presence of allergenic pollen types *Fraxinus*, *Alnus*, Cupressaceae, *Ulmus* and *Populus*. The second allergic period corresponds to spring months, including the *Platanus* pollen season, followed by the seasons of Urticaceae, *Betula*, *Pinus*, *Quercus*, *Plantago*, *Olea*, *Ligustrum* and *Castanea*. Finally, the third allergic period takes place in late summer and autumn (August–November) with pollen types Chenopodiacee-Amaranthaceae and *Artemisia*.

The number of days in which atmospheric pollen concentration reaches high-enough risk levels to produce allergic reactions amongst Toledo population and visitors, vary according to the different pollen types. Thus,

Table 4. Characteristics of the Main Pollen Season (MPS) for the most important taxa in Toledo and number of days (Nº D) with daily pollen concentraion over an allergenically significant value (Galán *et al.*, 2007). DMV: Daily maximun value. MAL: Moderately Allergenic Levels, HAL: Highly Allergenic Levels. * Incomplete data because there were problems with the collector sampler.

		2003	2004	2005	2006	2007
Cupressaceae	Pollen season	23 Nov–16 Mar	09 Jan–09 Mar	03 Feb–01 Apr	01 Feb–16 Apr	14 Nov–07 Apr
	Season length	111	61	58	75	145
	DMV (Date)	886 (24 Feb)	878 (5 Mar)	1,834 (16 Mar)	690 (12 Mar)	1,258 (4 Mar)
	Nº D MAL/HAL	17/20 (37)	16/18 (34)	9/19 (28)	24/4 (28)	15/15 (30)
Chenopodiaceae-Amaranthaceae	Pollen season	*	*	29 Apr–28 Sep	29 Apr–08 Oct	17 May–27 Sep
	Season length	*	*	153	163	134
	DMV (Date)	*	*	18 (7 Jul)	42 (21 Aug)	37 (24 Aug)
	Nº D MAL/HAL	*	*	0/0	2/0 (2)	3/0 (3)
Olea	Pollen season	16 May–13 Jun	28 May–9 Jun	09 May–26 Jun	01 May–08 Jun	01 May–26 Jun
	Season length	29	23	49	39	57
	DMV (Date)	490 (29 May)	338 (10 Jun)	1,180 (26 May)	1,003(18 May)	955 (30 May)
	Nº D MAL/HAL	9/6 (15)	8/3 (11)	14/8 (22)	13/9 (22)	11/13 (24)
Pinus	Pollen season	16 Mar–12 Jun	20 Mar–28 Jun	04 Apr–14 Jul	01 Apr–10 Jun	16 Mar–06 Jul
	Season length	89	101	102	71	113
	DMV (Date)	43 (22 Mar)	98 (31 Mar)	89 (05 Jun)	81 (04 Apr)	127 (30 Mar)
	Nº D MAL/HAL	0/0 (0)	3/0 (3)	8/0 (8)	3/0 (3)	8/0 (8)
Platanus	Pollen season	22 Mar–08 Apr	31 Mar–18 Apr	31 Mar–08 May	27 Mar–26 Apr	16 Mar–03 May
	Season length	18	19	39	31	49
	DMV (Date)	905 (27 Mar)	247 (07 Apr)	402 (01 Apr)	400 (04 Apr)	292 (25 Mar)
	Nº D MAL/HAL	9/6 (15)	3/1 (4)	4/4 (8)	9/2 (11)	9/2 (11)
Poaceae	Pollen season	10 Apr–04 Jul	22 Apr– 15 Jul	18 Feb–26 Nov	12 Feb–02 Sep	18 Feb–21 Jul
	Season length	86	85	282	203	154
	DMV (Date)	694 (18 May)	360 (28 May)	138 (08 May)	516 (15 May)	557 (13 May)
	Nº D MAL/HAL	14/17 (31)	17/20 (37)	8/6 (14)	16/24 (40)	26/43 (69)
Populus	Pollen season	19 Feb–13 Mar	08 Feb–19 Mar	12 Mar–04 May	07 Mar–20 Mar	25 Feb–24 Mar
	Season length	23	41	54	14	28
	DMV (Date)	1,464 (05 Mar)	704 (06 Mar)	138 (16 Mar)	791 (11 Mar)	991 (06 Mar)
	Nº D MAL/HAL	13/4 (17)	12/2 (14)	7/0 (7)	3/7 (10)	6/7 (13)
Quercus	Pollen season	08 Apr–08 Jun	07 Apr–14 Jun	17 Apr–26 Jun	08 Apr–04 Jul	08 Mar–11 Jul
	Season length	62	69	71	88	126
	DMV (Date)	965 (25 Apr)	495 (27 Apr)	2,179 (01 May)	466 (2 May)	366 (10 May)
	Nº D MAL/HAL	26/15 (41)	21/6 (27)	20/20 (40)	30/9 (39)	10/16 (26)
Ulmus	Pollen season	06 Feb–26 Mar	06 Feb–21 Mar	13 Feb–21 Mar	10 Feb–24 Mar	14 Feb–24 Mar
	Season length	49	45	37	43	39
	DMV (Date)	55 (13 Feb)	124 (08 Feb)	185 (09 Mar)	105 (01 Mar)	69 (20 Feb)
	Nº D MAL/HAL	5/1 (6)	2/7 (9)	6/7 (13)	3/6 (9)	3/3 (6)
Urticaceae	Pollen season	28 Feb–02 Jun	13 Feb–25 Jul	02 Mar–04 Dec	07 Feb–14 Nov	14 Feb–09 Jul
	Season length	95	164	278	281	146
	DMV (Date)	474 (13 Apr)	243 (24 Apr)	27 (21 May)	136 (26 Mar)	303 (19 Apr)
	Nº D MAL/HAL	16/21 (37)	5/8 (13)	0/0	14/29 (43)	32/31 (63)

3 groups: Poaceae, *Quercus*, and Cupressaceae, exceed amounts considered as 'moderately allergenic levels'; annual average is around 30 days, out of which around 15 are qualified as 'highly allergenic'.

These amounts mean that 1) Poaceae exceeds 'moderately allergenic levels' between a minimum of 2 and a maximum of 10 weeks throughout the year; 2) *Quercus* and Cupressaceae between 4 and 6 weeks; 3) Urticaceae up to 9 weeks; 4) *Olea*: 15 days on average (between 2 and 4 weeks a year). The prevalence of these levels for *Populus*, *Platanus*, *Ulmus* and *Pinus* is between one and two weeks.

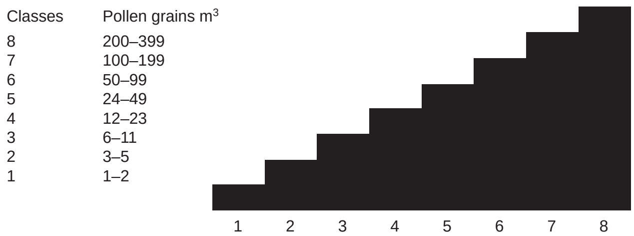

Figure 5. Classes and pollen concentration values represented in the calendar graph.

Meteorological influence. The result of relating data of pollen concentration in the Pre-Peak period to those from meteorological variables (temperature, rainfall, hours of sun, wind speed, atmospheric pressure and evapotranspiration) (Tab. 5) is that, in general, correlations are positive for temperatures. In the case of average temperature, these correlations are positive and significant for all taxa, except for *Ulmus*. For rainfall, correlations were negative in 9 out of the 10 taxa studied. Correlations were significant only in the cases of Chenopodiaceae-Amaranthaceae and *Populus*, with not very high correlation coefficients (r = -0.193 and r = -0.304, respectively). Correlations between hours of sunlight and pollen in the pre-peak period only turned out to be significant in the case of the pollen group of Chenopodiaceae-Amaranthaceae, Poaceae and *Ulmus*. The results obtained for wind speed were positive for some taxa and negative for others. A significant and positive correlation was obtained in the case of *Olea*. Finally, evapotranspiration – closely related to temperature – mainly shows positive and significant correlations in most taxa under study. High pressures – related to atmospheric stability – are in general positively correlated to the taxa under study (Tab. 5).

DISCUSSION

The results obtained in the present work provide similar pollen amounts to those reported year by year in nearby areas such as Ciudad Real, located in the southern area of the same Region of Castilla-La Mancha [10, 34, 45] and Madrid, located 70 km north [23, 24, 43]. Nevertheless, these pollen concentrations are lower than those registered in other areas bio-climatically similar, such as Jaén [42] and Cáceres [51], due to high *Olea* pollen concentrations from the abundant olive crops in the former and the high pollen levels of Poaceae, holm oaks and cork oaks produced in the pasturelands of the latter.

Regarding the preliminary results obtained in Toledo [18], a high number of similarities is observed regarding the peak amounts reported, as well as the beginning of the presence of airborne pollen in most of the taxa studied. Nevertheless, there is an important difference here, the inclusion of 19 new taxa. A high incidence of Cupressaceae, *Populus*, *Quercus*, Poaceae and *Olea* pollen grains was also noted. These 5 pollen types are also predominant in the neighbouring provinces of Madrid [24] and Ciudad Real [20, 34], both of which are located on the same Mesomediterranean bioclimatic belt showing similar vegetational characteristics. Nevertheless, we must highlight that *Olea* registers lower concentrations in Madrid than in Toledo and that *Populus* concentrations are lower in Ciudad Real. There are also noticeable differences regarding the concentrations obtained for the Urticaceae type: much higher values are obtained in Toledo probably due to the presence of a higher number of Urticaceae species favoured by the hygro-nitrophyllous environments near the River Tagus. On the other hand, the incidence of *Platanus* pollen in Toledo is much lower than in Madrid and Ciudad Real, since this species is less used in the ornamentation of streets, parks and gardens of Toledo.

Regarding the pollen calendars of towns located in more distant areas of Spain, greater differences are observed: taxa such as *Populus*, *Ulmus* and *Platanus* show rather lower registers in the province of Málaga [11, 12, 36, 37]. However, pollen types such as *Pinus*, or other more thermophile species such as *Casuarina* or Palmae, are more abundant in southern and eastern regions [4]. Northern regions are characterized by the fact that Cupressaceae and

Table 5. Coefficients of correlation between daily pollen pre-peak concentration and the main meteorological parameters based on the Spearman correlation test (significance levels **99%, *95%).

	Cupressaceae	Chenopodiaceae-Amaranthaceae	*Olea*	*Pinus*	*Platanus*	Poaceae	*Populus*	*Quercus*	*Ulmus*	Urticaceae
T mean (ºC)	0.333**	0.580**	0.534**	0.229*	0.577**	0.579**	0.563**	0.742**	0.033	0.232**
T max (ºC)	0.556**	0.577**	0.467**	0.268**	0.448**	0.584**	0.543**	0.666**	0.124	0.254**
T min (ºC)	0.265**	0.526**	0.530**	0.124	0.619**	0.496**	0.435**	0.638**	-0.075	0.121*
Rainfall (mm)	-0.108	-0.193**	-0.087	-0.161	0.224	-0.121*	-0.304*	-0.112	0.043	-0.076
Sun hours	0.060	0.237**	0.107	0.145	-0.212	0.395**	0.231	0.203	0.303*	0.125
Wind velocity (km/h)	0.076	-0.014	0.385**	-0.142	0.026	-0.043	0.132	-0.023	-0.040	-0.013
Evapotranspiration (mm)	0.362**	0.442**	0.675**	0.168	0.191	0.460**	0.484**	0.573**	-0.165	0.167**
Atmospheric pressure (millibars)	0.232**	0.089	-0.077	0.038	-0.395	0.166**	0.450**	-0.067	0.312*	0.008

Olea have rather less importance; on the contrary, pollen types such as Urticaceae, *Castanea* or *Betula* have greater importance [5, 40, 41].

As many authors have already pointed out, flowering, pollination and dispersion of pollen grains are closely related to meteorological parameters, especially to temperature and rainfall [1, 19, 41, 44]. Within the studied period, it was observed that the driest years (2003 and 2005) were followed by the years with the lowest pollen production (2004 and 2006) (Tab. 1 and 2): drought is likely to condition the pollen production of the following year. The statistical results obtained correlating climatic variables and daily pollen concentrations were similar to those obtained by García-Mozo *et al.* [18]. The positive and significant correlation obtained between wind speed and olive pollen concentrations may explain the origin of the pollen of this species in areas far away from the spore-trap, as observed by other authors [44], being ascertained by the existence of slight peaks prior to their maximum peak in its main pollen season. In the case of Urticaceae, obtained correlations are of the same sign as those obtained by Rodríguez *et al.* [41] for the same meteorological variable in Lugo (NW Spain).

Regarding allergies, during the winter period, the presence of airborne allergenic pollen types such us *Fraxinus*, *Alnus* and Cupressaceae is noticeable. The first 2 of them have low incidence among the Toledan population, due to their low amounts. On the contrary, Cupressaceae, the most abundant pollen type, can be considered as the main cause of winter pollinosis [13, 27, 28]. Daily concentrations producing moderately allergenic levels are detected in an average of 32 days during 5 weeks. The species belonging to this pollen type are part of the city, but also of the environment of the town of Toledo (*Juniperus oxycedrus* L.), being particularly present in parks and gardens. In them, *Cupressus sempervirens* L. and *Cupressus arizonica* Greene are predominant but we can also find *Cupressus macrocarpa* Hartw, *Cupressocyparis leylandii* Dallim. & A.B. Jacks., *Chamaecyparis lawsoniana* (A. Murray) Parl. and *Platycladus orientalis* (L.) Franco. The incidence of pollinosis has been increasing in Toledo [50]. According to Moral [26] and Suárez-Cervera *et al.* [49], this incidence could be due to the currently growing use of these plants in parks and gardens, and to the currently growing pollution and the interaction of this pollen type with diesel particle pollution. The prevalence of positive skin tests for Cupressaceae represents 30% [6, 50].

The second allergic period begins with the *Platanus* pollen season (late March and April). Although this species has a short pollen season, several days exceed the "high risk" threshold. Moral *et al.* [28] and Subiza *et al.* [50] indicate a prevalence of *Platanus* pollen sensitivization around 52%, being responsible for the appearance of allergic symptoms in 28% of pollen allergic patients during this time of the year in the population of the central area of the Iberian Peninsula [52]. As occurs with Cupressaceae, allergies have increased in the last years [50] due to the increasing use of *Platanus* (*Platanus hispanica* Mill. ex Münchh = *Platanus acerifolia* (Aiton) Willd.) as an ornamental species in streets and new parks and gardens [46].

The continuous flowering of Urticaceae species provoke the presence of pollen in the atmosphere of Toledo for a high number of months a year, starting in early spring. One of the genera producing a high number of allergies in this group, *Parietaria*, presents a moderate activation threshold and its pollinosis affects to 10–30% of allergic patients in Spain [25]. The other genus at the central area, *Urtica*, has a prevalence of 49% in positive skin tests among pollinosis patients [28, 50]. During the period of this study, a high number of days exceeded moderate allergenic level, being most of them days with a high allergenic risk.

Betula pollen, which causes important allergic diseases in northern Europe [7], has scarce importance in Toledo. It is found in April, but in low amounts. *Pinus* pollen is also present in April. Although this genus has particular allergenic interest in areas with large natural, planted or reforested pine forests [21], in Toledo the days reaching allergy risk concentrations represents less than a week.

The *Quercus* pollen reaches high amounts, being the second in importance after Cupressaceae. From the viewpoint of allergies, the low allergenic capacity of the species that can be found in Toledo would explain the absence of pollinosis related to *Quercus* in this region [33].

Poaceae pollen is the main cause of pollinosis almost all over the world, and also in Europe, due to the high allergenic capacity of pollen and the high number of species present in large extensions of most European ecosystems. Together with *Olea*, it is responsible for most spring allergies in central Spain and also in Castilla-La Mancha [50]. The prevalence of positive skin tests among pollinosis patients is 75% for Toledo [50]. Davies *et* Smith [9], and Ong *et al.* [31] consider that 25 pg/m^3 average daily concentrations trigger moderate allergic processes, while over 50 pg/m^3 concentrations may give rise to clinical symptoms in sensitive patients [29]. In Toledo, along the pollen season of Poaceae, average daily concentrations over 25 pg/m^3 are obtained 38 days a year on average (moderately allergenic levels), and concentrations over 50 pg/m^3 are obtained around 22 days a year on average (highly allergenic levels).

Olea europaea L. pollen has an outstanding importance in pollinosis in southern and central Spain [50]. Castilla-La Mancha is, behind Andalucia, the second olive-oil producer Spanish region [19]. The highest concentrations of *Olea* pollen are obtained in May, when concentrations over 50 pg/m^3 have been registered during the last 5 years. The prevalence of positive skin-test from olive tree pollen is 58% and represents the second cause of pollinosis in Toledo [50].

Plantago pollen mainly appears from April–June, but in low amounts. Allergic patients to *Plantago* (it sensitizes 25–75% of patients) are usually also allergic to Poaceae pollen [50]. Besides, both pollen seasons coincide. *Ligustrum* and *Castanea* allergenic pollens have low concentrations in June, so they scarcely induce any pollinosis.

Finally, as already mentioned, the third allergic period takes place in late summer and autumn, mainly from August–September and is caused by the Chenopodiacee-Amaranthaceae pollen type. Despite the small amounts registered, they are very aggressive pollens, being the third cause of pollinosis in Toledo [32]. Allergy to these species is closely related to the presence of these plants in gardens, irrigated crops, pools and salt marshes. In November, *Artemisia*'s is the only pollen type which may produce some kind of allergy among sensitive population (38.31%, according to Moral *et al.* [28]).

To conclude, release and dispersion of allergenic pollens of wild plants cannot be controlled, but the knowledge of the main risk allergy periods, in conjunction with the control of the species planted in parks and gardens, may help to prevent pollinosis symptoms in patients and their subsequent public health costs.

CONCLUSIONS

The pollen spectrum of Toledo is typically Mediterranean, with a wide variety of taxa, similar to those of nearby localities. The highest pollen production in the atmosphere of Toledo are detected from February–May (70–90%), being Cupressaceae, *Quercus*, Poaceae and *Olea* the most important pollen types, in abundance order. The evolution of pollen concentration shows different peaks year by year, mainly due to the pollen contribution of Cupressaceae in February–March, *Quercus* in April–May, and Poaceae and *Olea* between May–June. From mid-late June onwards, pollen concentration decreases drastically. The results of correlation analyses between meteorological variables and daily pollen concentrations during the pre-peak period were significant in a high number of cases. The values of daily, monthly and annual concentrations of pollen types producing allergies identify a great number of days a year in Toledo as highly allergenic. The types triggering most allergic processes in Toledo citizens and tourists are Cupressaceae, *Platanus*, *Olea*, Poaceae, Urticaceae and Chenopodiaceae-Amaranthaceae. Allergic processes take place in 3 main periods – namely, winter (January–March), with the main presence of the Cupressaceae type; spring, characterized mainly by Poaceae, *Olea*, *Platanus* and Urticaceae pollen types; and, finally, late summer (August–September), characterized mainly by Chenopodiaceae-Amaranthaceae pollen types.

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