

Variations in airborne pollen in central and south-western Spain in relation to the distribution of potential sources

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Abstract

The present study seeks to compare daily and hourly airborne pollen concentrations at eight different stations in Castilla-La Mancha (central Spain) and Extremadura (south-western Spain) and assess pollen distribution sources. Sampling stations were located 69–440 km apart in a straight line in Albacete, Toledo, Talavera de la Reina and Ciudad Real in Castilla-La Mancha, and in Badajoz, Plasencia, Santa Amalia and Zafra in Extremadura. Airborne pollen was collected using Hirst-type volumetric spore traps. *Quercus* was the most abundant pollen type at all stations except for Ciudad Real, where *Olea* pollen predominated. Comparisons of daily data between pairs of stations revealed statistically significant positive correlations in all cases for Poaceae and *Olea*. Comparisons of hourly data between stations indicated greater differences than daily data. Analysis of correlation coefficients and straight-line distances between stations revealed a strong negative correlation. Analysis of total airborne pollen data for the eight sampling stations suggests that airborne pollen concentrations decrease from west to east and from south to north, partly reflecting dominant wind patterns. A clear correlation was observed between airborne pollen concentrations and the surface area covered by olive crops in a 50 km radius around the sampling stations.

Keywords: *Extremadura, Castilla La-Mancha, airborne pollen monitoring, Quercus, Poaceae, Olea, Plantago*

Pollen grains are reproductive structures in vascular plants that produce and transport male gametes or the cells that produce them. In approximately one-fifth of all plants, pollen grains are transported by wind to the ovules in gymnosperms or the stigmata in angiosperms. One direct consequence of this form of pollen transportation is allergy in humans. The last few years have seen a progressive increase in both the prevalence and the severity of allergic respiratory diseases (D'Amato et al. 2007) impairing the quality of life of allergy sufferers. The World Allergy Organization

(Pawankar et al. 2011) has reported that allergies and asthma are becoming a major public health concern.

Volumetric spore traps can be used to determine airborne pollen seasonality; the data obtained may be useful as an indication of the threat to public health at a specific point in time. Since the airborne pollen spectrum for a given site reflects local pollen sources, there are differences between places. The assessment of the environmental factors giving rise to these differences is helpful for constructing predictive models (Singh & Mathur 2012).

Numerous aerobiological studies have compared two or more sampling stations in different areas, primarily with a view to assessing differences and similarities between stations, in order to justify their location and provide accurate values for ascertaining the influence of local environment on airborne pollen data for a given station. Comparative studies have also been used to evaluate possible trends or changes in the pollen season due to climate change (Recio et al. 2009; Jato et al. 2013).

Comparative studies using Hirst-type volumetric spore traps and data from sampling stations, which were more than 30 km apart, have been carried out in Europe. While most used two spore traps, some used three (Ekebom et al. 1997), four (Kupias et al. 1989; Spieksma et al. 1989; Ruffaldi & Greffier 1991; Jäger et al. 1996) or even nine (Emberlin et al. 1994). The average distance between stations was 500–800 km, ranging from 31 km (Strandhede & Wihl 1981) to 2334 km (Alcázar et al. 2009). The number of years analysed was typically between one and seven, but the following are noteworthy for their duration: an 11-year

study by Alcázar et al. (2009), a 13-year study by Jäger et al. (1996) and a 17-year study by Jato et al. (2013). The earliest published research used only graphical comparisons of results (Leuschner & Boehm 1981; Strandhede & Wihl 1981; Lejoly-Gabriel & Leuschner 1983; Caramiello et al. 1985; Rizzi-Longo et al. 1992). Correlation and regression analyses were only later included (Galán et al. 1995; Corden et al. 2002; Recio et al. 2009; Sabariego et al. 2011; Jato et al. 2013), and only one study (Alcázar et al. 2009) used the Wilcoxon signed-rank test. A useful review of the statistical methodology for this kind of work has been published by Trigo et al. (2000). In all these studies, the emphasis was on daily data, although in some cases, weekly, monthly or even whole-season data were included. Only one study (Corden et al. 2002) examined hourly data.

Although research has already been carried out using data from some of the sampling stations in the study area (Muñoz-Rodríguez et al. 2010; Pérez-Badia et al. 2010, 2011), the present study aspires to obtain and compare results between stations over a broad area of the central and south-

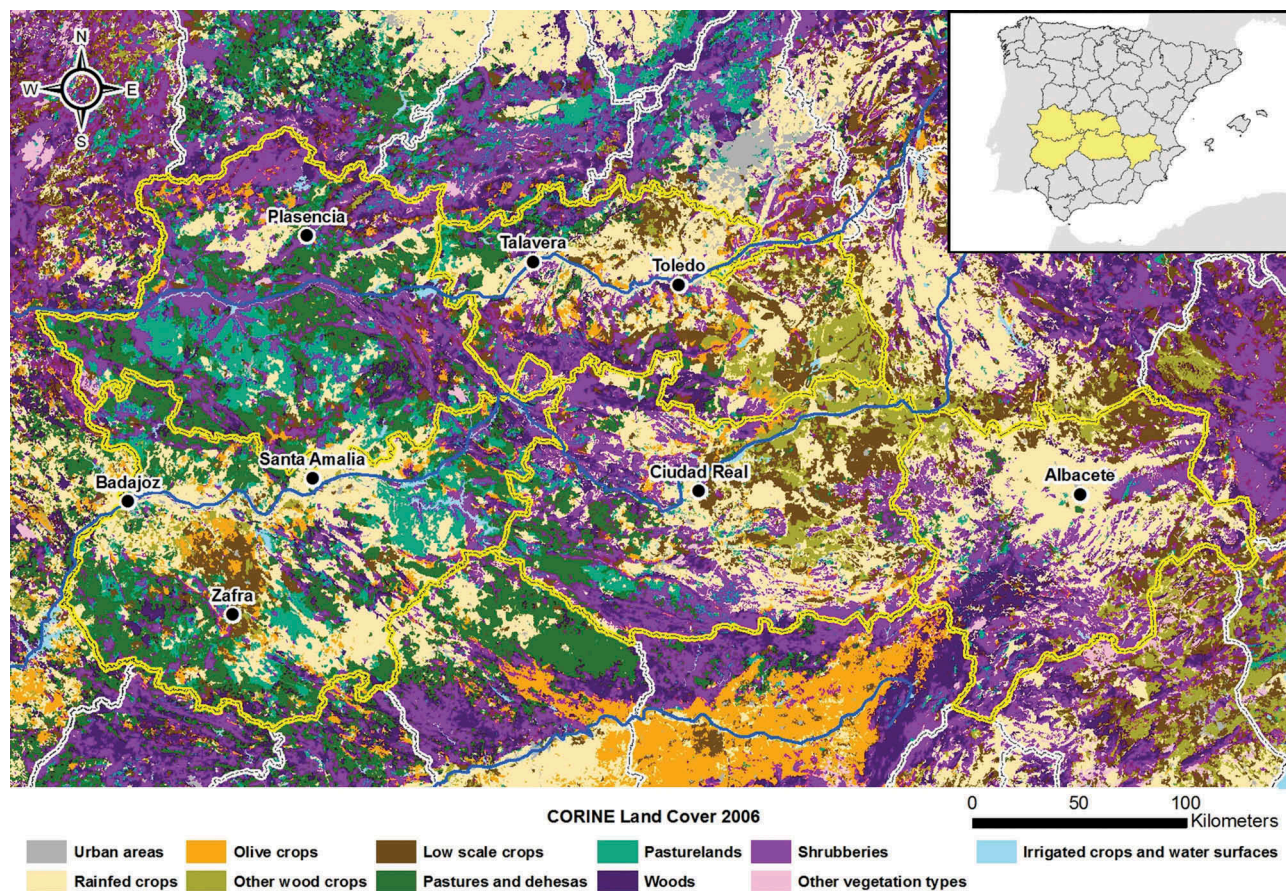


Figure 1. Locations where the aerobiological studies were performed. Main land cover for the study area, including pollen stations (CORINE map).

Table I. Pollen stations studied along with their geographical situations, metres above sea level (MASL), height above ground, annual average temperature and annual rainfall.

	Albacete	Badajoz	Ciudad Real	Plasencia	Santa Amalia	Talavera	Toledo	Zafra
Latitude	39.01	38.88	38.99	40.04	39.02	39.96	39.86	38.43
Longitude	1.86	6.97	3.93	6.08	6.01	4.83	4.02	6.62
MASL (m)	686	184	630	352	253	360	529	508
Level above ground (m)	22	16	18	17	6	14	17	6
Temperature (°C)	13.6	16.6	14.7	15.5	17.2	15.4	15.4	16
Rainfall (mm)	367	463	396	950	850	573	357	588
<i>Straight-line distances between pollen stations in kilometres</i>								
Badajoz	441.5							
Ciudad Real	179.2	263.8						
Plasencia	380.9	149.9	220					
Santa Amalia	351.6	84.9	181.6	112.9				
Talavera	275.7	219.5	133.9	107.6	145.8			
Toledo	209.3	275.1	96.7	176.5	194.1	69.9		
Zafra	400.5	69.9	225.2	180.8	74.5	217.5	260	
<i>Pollen index</i>								
<i>Quercus</i>	2796	15 854	6851	8498	4151	13975	14 864	14 947
<i>Olea</i>	1982	10 420	8595	2161	8805	6195	5486	8838
Poaceae	1317	9537	3378	3150	654	5670	3235	4740
<i>Plantago</i>	592	1372	586	847	967	774	352	918
<i>Main land use (ha)</i>								
Oaks forests	39 548	45 670	46 713	89 663	34 542	117 278	47 097	49 234
Irrigated crops	440	8166	8395	14 119	12 425	8684	5975	5415
Olive crops	2087	56 502	36 479	22 328	41 663	37 482	49 959	66 048
Rainfed crops	420 733	252 351	279 609	84 531	268 957	191 899	324 711	152 652
Grasslands	27 921	278 337	96 347	275 383	285 989	177 474	53 439	306 686

Note: straight-line distances between pollen stations in kilometres; Pollen Index for the types studied and the period analysed; main land uses around 50 km from each station (in hectares).

western Iberian Peninsula with a similar climate pattern. Daily and hourly airborne pollen concentrations for the most abundant pollen types (*Quercus*, Poaceae, *Olea* and *Plantago*) were collected in April and May; those pollen types represent at least 70% of the pollen captured, and the period analysed includes at least 55% of total-recorded pollen. Since all sampling stations are located within the same geographical area, comparative analysis took into account environmental factors as a probable source of potential variation. Correlations between land use and pollen data were also investigated. The aim of this work is, therefore, to explain variations in airborne pollen between monitoring sites taking into account the distribution of potential pollen sources around stations that have similar climate and are separated by several hundred kilometres.

Material and methods

Study area

Eight aerobiological stations were studied in April and May 2011 in central and south-western Spain

(Figure 1; Table I). Latitudes and longitudes covered ranges of 1.61 degrees of northern latitude (38.43°–40.04° N) and 5.11 degrees of western longitude (1.86°–6.97° W); the altitude of the sites ranged from 184 to 686 m above sea level. Straight-line distances between the stations ranged from 69.9 to 441.5 km; average values of temperature and rain for the stations studied are also considered (Table I).

Biogeographically, the area is located in the Mediterranean region (west Mediterranean subregion). The area belongs to two biogeographical provinces, namely the Mediterranean central Iberian Province (Castilian Subprovince) and the Mediterranean west Iberian Province (Lusitan-Extremadurean Subprovince) (Rivas-Martínez et al. 2002). Bioclimatically, the stations are situated in the Mesomediterranean Belt (Rivas-Martínez 2007).

The main land uses in the study area (Figure 1) include rain fed crops that dominate the plains, *Quercus* forests and shrubs that are abundant in mountainous areas, olive groves present in the most fertile areas, and irrigated crops located close to major rivers. Natural vegetation in the study area consists mainly of holm and cork oak

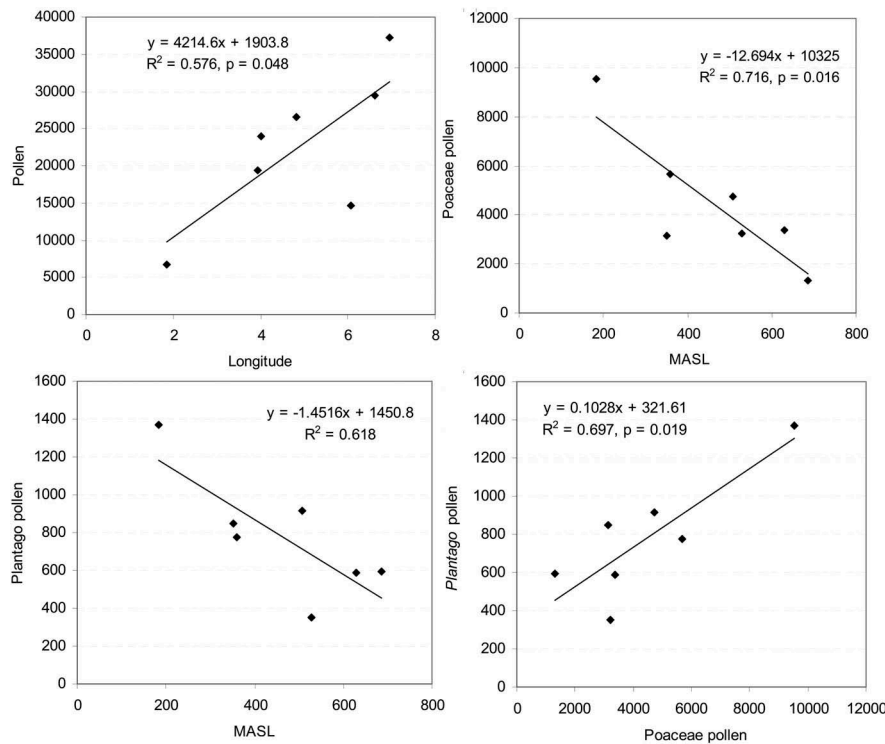


Figure 2. Relationships between summed pollen counts (Pollen Index) and longitude, between total Poaceae pollen counts and height above sea level (in metres above sea level [MASL]), between total *Plantago* pollen counts and height above sea level, and between *Plantago* and Poaceae pollen counts at the stations studied. Santa Amalia was not included due to lack of data.

forests (*Quercus ilex* L. subsp. *ballota* [Desf.] Samp., *Q. suber* L.), shrubs and pasturelands alternating with meadows. Junipers (*Juniperus* spp.), kern oaks (*Q. coccifera* L.), *Asparagus* spp., wild olives and other species (*Olea europaea* L. var. *sylvestris* Brot., *Phillyrea angustifolia* L.) are present in shrubs, bushes and undergrowth. Major Mediterranean scrub species include *Cistus* spp., *Cytisus* spp., *Retama sphaerocarpa* (L.) Boiss. and *Rosmarinus officinalis* L. The crop fields are mainly cereal and olive groves. Riparian vegetation is represented along the main rivers by ashes (*Fraxinus angustifolia* Vahl.), alders (*Alnus glutinosa* L.), willows (*Salix* spp.) and poplars (*Populus alba* L., *Populus nigra* L.). Grasses, nettles and goosefoot are among the most common herbaceous species. Ornamental species in city parks and gardens include cypresses (*Cupressus* spp.), elms (*Ulmus* spp.), plane trees (*Platanus* spp.) and glossy privet (*Ligustrum* spp.). See Table I for more specific information for each sample station.

All sampling stations have a Mediterranean climate with marked continental characteristics that include hot and dry summers with rain primarily during winter and spring. Annual average temperatures range from 13.6 to 17.2 °C, clearly varying as a function of altitude ($r = -0.886$, $p = 0.003$). Prevailing winds during the study period were south-easterly to westerly.

Aerobiological survey

Pollen was recorded using Hirst-type volumetric spore traps positioned 6–22 m above ground level (Table I). Sampling and analyses were carried out in accordance with the procedures recommended by the Spanish Aerobiology Network (Galán et al. 2007). The meteorological data were provided by the Spanish Meteorology Agency (AEMET, www.aemet.es/en/portada) from which average temperature and total rainfall were used.

Daily concentrations of the most abundant airborne pollen types (i.e. *Quercus*, Poaceae, *Olea* and *Plantago*) in April and May 2011 were analysed. The sums of the daily concentrations (Pollen Index, PI) were calculated for the study period (61 days, Table I). Due to technical problems, six days of data are missing from the Toledo station and only 21 days of data are available for the Santa Amalia station. Hourly data from ten continuous days were analysed along with the peak concentration values ($n = 240$). The selected days were 5–14 April for *Quercus* and 5–14 May for the other pollen types.

Statistical analysis

Two kinds of comparisons were performed: between pollen types and between stations; then, station

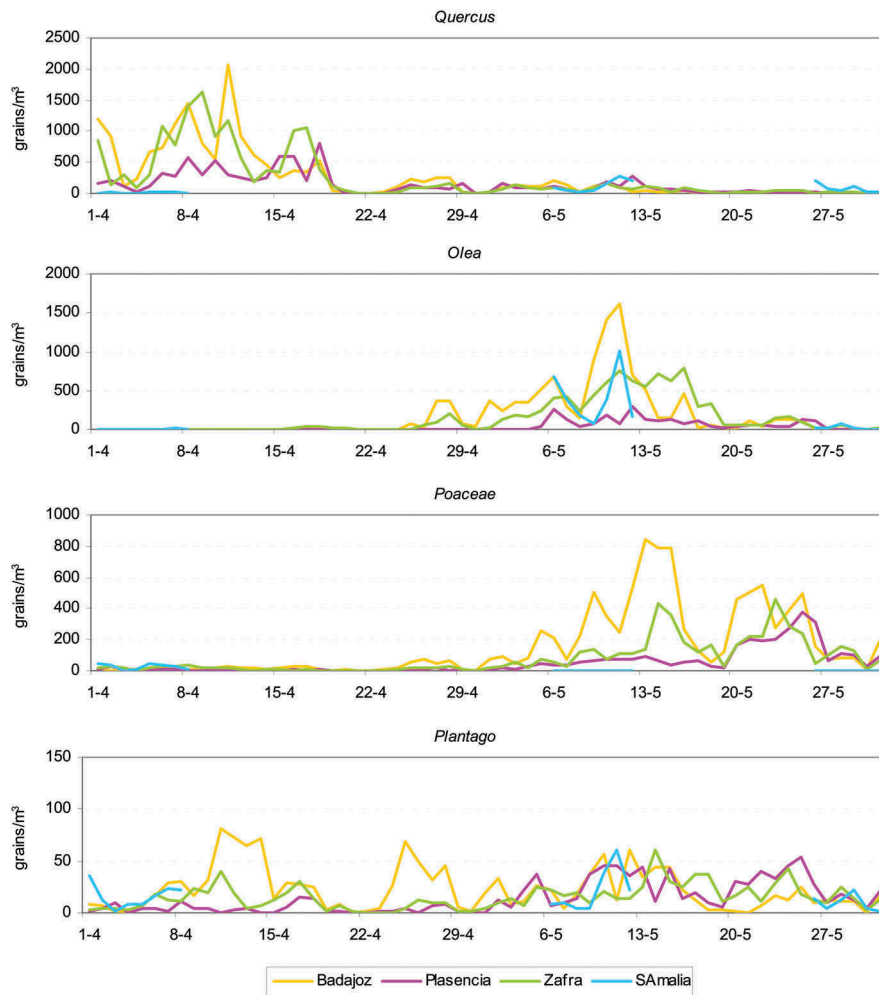


Figure 3. Daily pollen concentration (in grains/m³) over the study period for the sample stations at Badajoz, Plasencia, Zafra and Santa Amalia.

values were compared in relation to the distance between them. Correlation coefficients between stations were calculated using daily data from all four pollen types in aggregate. These correlation coefficients were then compared with the distances in kilometres between the respective pairs of stations using the Wilcoxon signed-rank test. Non-parametric statistics were used because the daily and hourly data did not fit a normal distribution. The Spearman correlation coefficient was used to compare the daily ($n = 61$) and hourly data ($n = 240$) of the different stations. For daily data, the whole period was considered and for hourly data the ten days, as outlined earlier, including the day with the pollen peak, were used. For the daily and hourly data, the Friedman test (χ^2) was calculated to compare the data for each pollen type, and the Wilcoxon signed-rank test (z) was performed on the pairs of stations. We have used this statistical test in the assumption that events at the same time should be considered paired data and they are not completely independent, for instance, phenology is a corollary of that.

We reckon that the assumption of independence of samples may be acceptable for this kind of data (Ziello et al. 2012), but we have preferred following the same methodology of previous similar works (Tormo-Molina et al. 1996, 2013) and the recommendation of Trigo et al. (2000). The hypothesis tested was that the closer the stations, the more similar are the airborne pollen records, depending on pollen type. Sums of daily concentrations (PI) were compared with longitude and metres above sea level using the Pearson correlation coefficient; uniformity of data was confirmed for these inputs through the Kolmogorov–Smirnov test. The SPSS 15.0® software package was used to perform the statistical analyses. For the Santa Amalia station, only hourly data were considered in the comparisons. To take the potential influence of height over ground of spore traps into account, Spearman correlation was calculated between PI and height of samplers.

The relationships between land use and pollen concentration were studied using Pearson correla-

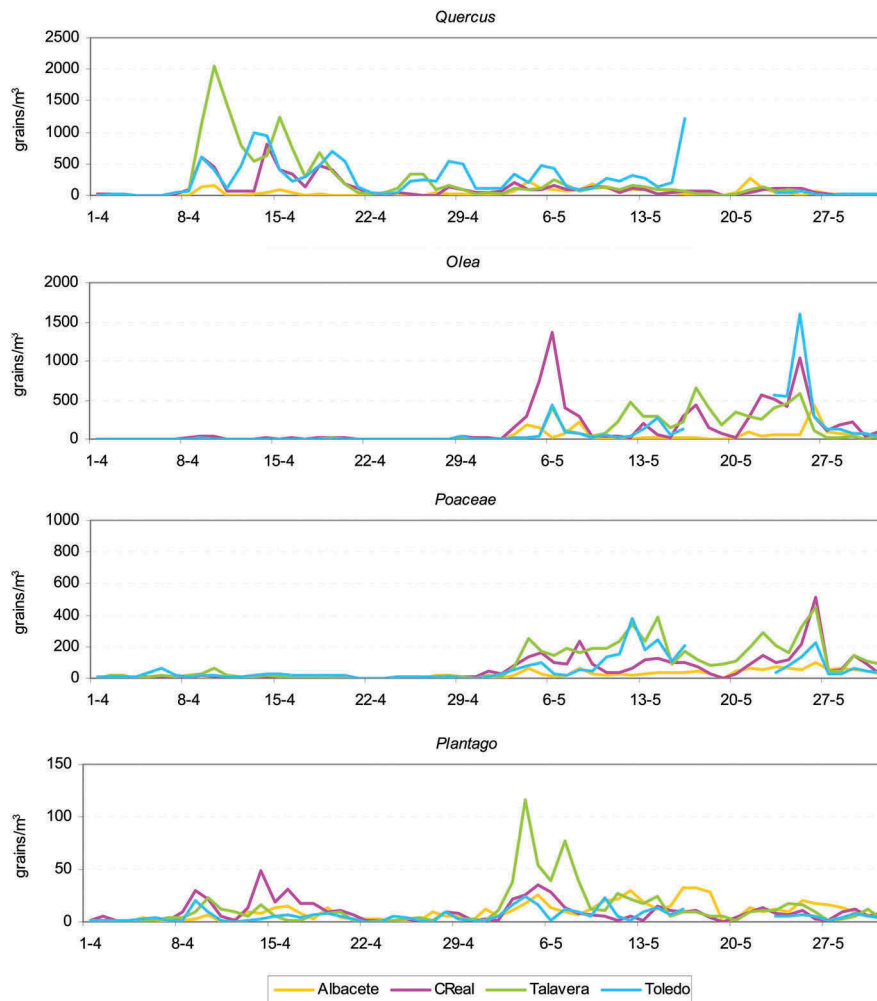


Figure 4. Daily pollen concentration (in grains/m³) over the study period for the sample stations at Albacete, Ciudad Real, Talavera and Toledo.

tion analyses between pollen concentration and the surface area (in km²) allocated for different land uses for 5, 10, 25 and 50 km areas around the pollen spore trap locations. CORINE Land Cover maps of the main land uses (1:100 000 scale) were elaborated with data from 2006 (IGN 2006).

Results

Quercus was the most abundant pollen type at all stations except Ciudad Real, where *Olea* was the most common. *Poaceae* was the third most abundant pollen type at all stations except Plasencia, where it was second. The least abundant pollen type was *Plantago*. The highest PI for all four types were recorded at the Badajoz station (37 182) and the lowest at Albacete (6687). Comparison of PI for each pollen type and total aggregate pollen for all four types, with latitude, longitude, height of spore traps above sea and ground level revealed statistically significant correlations between longitude and

total pollen from the sums of all four pollen types ($r^2 = 0.576$, $p = 0.048$) and between longitude and *Plantago* ($r^2 = 0.581$, $p = 0.046$). Moreover, height above sea level was negatively correlated with total pollen concentrations for *Poaceae* ($r^2 = -0.716$, $p = 0.016$) and *Plantago* ($r^2 = -0.618$, $p = 0.036$). The only statistically significant correlation between pollen types was recorded for *Plantago* and *Poaceae* ($r^2 = 0.697$, $p = 0.019$) (see Figure 2).

The highest daily pollen peaks were observed for *Quercus* in Badajoz (11 April, 2053 grains/m³) and in Talavera de la Reina (14 April, 2048 grains/m³). For *Olea*, daily peaks appeared at Badajoz (11 May, 1616 grains/m³) and Toledo (25 May, 1600 grains/m³). For *Poaceae*, a daily peak was reached at Badajoz (13 May, 847 grains/m³). For *Plantago*, there was a daily peak at Talavera (4 May, 116 grains/m³) (Figures 3, 4). The relation between samplers' height over ground and PI was not statistically significant except for *Quercus* pollen ($r = -0.793$, $p = 0.033$).

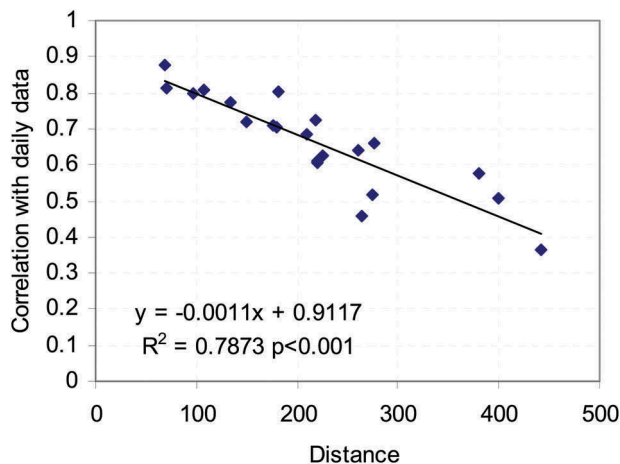


Figure 5. Relationships between pairs of pollen stations taking into account the distance between stations (in kilometres) and correlation coefficients based on the daily pollen concentrations.

The comparison of daily pollen data revealed different patterns for the different pollen types. For each type, data for 21 combinations of stations (pairs of stations) were calculated for correlation analysis, and only values with $p < 0.01$ were taken into account. The data from Santa Amalia were excluded from this analysis. For Poaceae and *Olea*, significant correlations were obtained for all combinations, and for *Quercus* and *Plantago*, significant correlations were obtained for 14 combinations. The Friedman test (Table II) showed that only daily average *Quercus* pollen concentrations were similar at all stations. For *Olea*, the Wilcoxon test indicated that daily average concentrations of airborne pollen were similar for some pairs of stations (Table III).

Comparisons between pairs of stations by correlation coefficients using all daily data revealed statistically significant correlations in all cases. A statistically significant negative correlation ($r = -0.887$, $p < 0.001$) was observed for distance between sampling stations and correlation coefficients for daily pollen data (Figure 5). Wilcoxon test results showed that the data were similar in only four cases and that the correlation with distance was not significant. The four cases with significant results in the Wilcoxon test were: Talavera de la Reina–Zafra ($z = -0.355$, $p = 0.723$), Ciudad Real–Toledo ($z = -0.632$, $p = 0.528$), Talavera de la Reina–Toledo ($z = -0.951$, $p = 0.341$) and Ciudad Real–Plasencia ($z = -1.693$, $p = 0.090$) (listed in order).

A lower degree of correlation was observed when hourly data from different stations were analysed. Of the 21 combinations, seven significant positive cor-

relations were found for *Quercus* and *Olea*; six for Poaceae and four for *Plantago*. The Friedman test indicated no similarity between stations attaining relatively high values (Table II). The analysis of hourly peaks revealed significant correlations in all cases, although peaks never occurred at the same time, even for pollen types with strongly correlated daily data; instead, hourly peaks occurred at different times of the day (Figures 6, 7), even for the stations closest to each other (Badajoz–Plasencia and Talavera de la Reina–Toledo).

Comparison of land use around sampling stations and airborne pollen concentrations revealed a significant positive correlation between the surface area given over olive crops within an area of 50 km in diameter and the sum of daily average pollen concentrations ($r^2 = 0.6876$, $p = 0.023$, Figure 8). No other clear relationships were found for other pollen types, distance between stations, and land uses.

Discussion

Two or more aerobiological sampling stations always attained different results. These differences may be attributable to the spore traps themselves, although this can only be assessed if all of the spore traps are used under the same conditions, as recently demonstrated (Tormo-Molina et al. 2013). When spore traps are used in different areas, environmental factors affecting recorded values include source distribution (Rizzi-Longo et al. 1992), geographical barriers (Leuschner & Boehm 1981; Kupias et al. 1989), spore trap height (Emberlin et al. 1994; Fernández-Rodríguez et al. 2014b), distance (Fernández-Rodríguez et al. 2014a) and climate (Kupias et al. 1989). It is generally accepted that greater distances between spore stations prompt greater differences due to environmental factors. Latitude is regarded as one of the main contributory factors (Kupias et al. 1989; Corden et al. 2002; Alcázar et al. 2009), largely due to resulting differences in the phenology of the sources (Spieksma et al. 1989). Nevertheless, in this study, this factor may be adduced because the latitude range is 1.6° . In this respect, one major concern is the extent to which pollen data from one aerobiological station may be extrapolated to another in terms of distance, since obtaining this kind of data can be time-consuming. Climate change intensity depends on location and data from different aerobiological sampling stations have been compared with a view to evaluating possible trends or changes in the pollen season due to climate change (Recio et al. 2009; Jato et al. 2013). Nevertheless, since the general climate in this study was similar for all sampling stations, this would seem

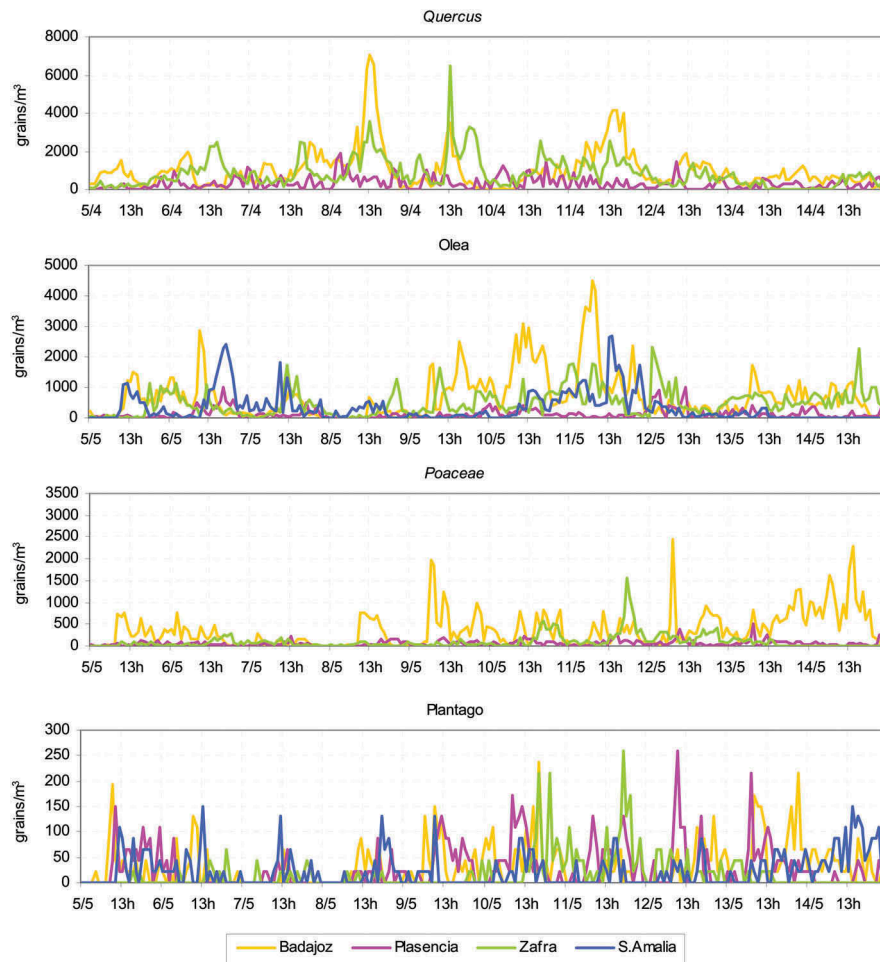


Figure 6. Hourly pollen concentration (in grains/m³) for two ten-day periods together with peak concentration values for the sample stations at Badajoz, Plasencia, Zafra and Santa Amalia.

to be at best a minor contributor to differences between data from different stations.

Altitude between spore traps affects mainly the amount of pollen recorded (Fernández-Rodríguez et al. 2014b) and this generally does not modify correlation values as used in this study. In any case, we cannot leave out this effect in order to explain some of the differences between locations.

Daily data showed that in an area, approximately 440 km × 180 km in size and with a similar climate and vegetation, the general pattern of airborne pollen was similar for the four main analysed pollen types. Nevertheless, differences were observed in the total amounts of pollen recorded. One trend suggested by the data is a decrease in airborne pollen concentrations from the southwest to the northeast, perhaps reflecting the dry, continental climate and the amount of vegetation (Albacete-Toledo versus Plasencia). The effect of sampling versus station altitude should also be considered since airborne pollen levels tend to be lower at higher altitudes (Leuschner & Boehm 1981).

As reported by Trigo et al. (2000), simple associations between pairs of stations, based on correlation coefficients, are not by themselves indicative of a similarity of the results: since data is time-dependent, only statistics for paired data can provide an accurate interpretation. In the present study, the results were only similar for certain pairs of stations, and in daily data, only for certain pollen types. The pairs of stations with similar results were not necessarily closest geographically, although the correlation coefficient revealed a clear trend in this regard. The widely raised question regarding the extent to which pollen data can be extrapolated from a given station to any other (Frenz et al. 1997) cannot be categorically answered. Wind may play an important role (Ekebom et al. 1997). In the present study, dominant winds from the west and south may be in correspondence with a general trend in the decrease of total pollen in the same main direction; nevertheless, this may not be adduced as a direct cause but an effect over rain distribution.

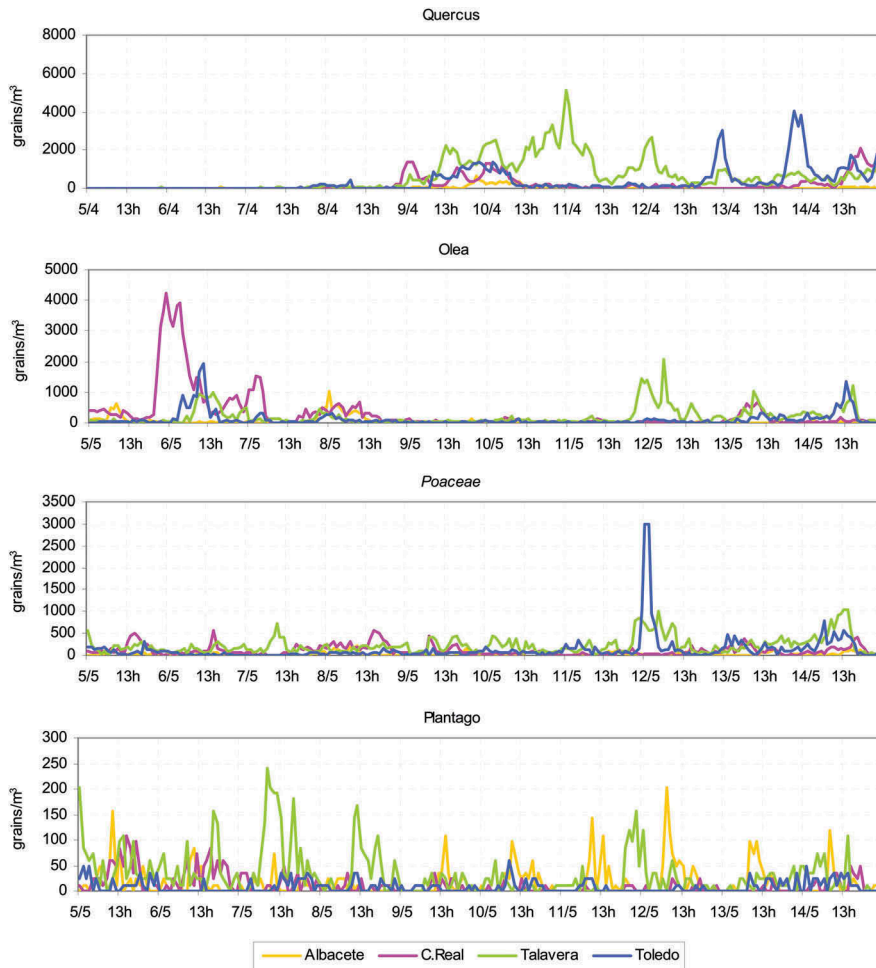


Figure 7. Hourly pollen concentration (in grains/m³) for two ten-day periods together with peak concentration values for the sample stations at Albacete, Ciudad Real, Talavera and Toledo.

There were no clear general patterns for peaks in hourly data. Local environmental factors affect the recorded results in different ways at different stations. Intradiurnal variations and hourly peaks reveal distribution patterns consistent with the varying distances of pollen-emitting sources from the trap (Vaquero et al. 2013). Such observations may reflect particular circumstances, including the arrival of previously released pollen at spore traps, which may occur on different days of the year, as shown in the present study when peaks are reached on different days. Pollen sources in the immediate vicinity of sampling stations are thus of great value in accounting for the airborne pollen concentrations recorded (Kupias et al. 1989; Emberlin et al. 1994). Attention has also been drawn to the influence of different responses of trees and herbaceous plants to previous rainfall (Cariñanos et al. 2004; García-Mozo et al. 2007; Stach et al. 2008), which must also be taken into account when interpreting data.

With regard to land use, the only significant correlation with airborne pollen concentrations was observed in the surface area of olive groves. For Poaceae and *Plantago* pollen types, comprising a wide range of species with diverse ecological preferences, it is more difficult to ascertain the possible correlation between land use and pollen levels. The regional presence of *Quercus* species, mainly in woods and 'dehesas', is so widespread that the influence of the most proximal stands may be very small (García-Mozo et al. 2012). This is consistent with the similarities found in the correlation of our daily results; nevertheless, we cannot discard the influence of height of the sampler. In contrast, the *Olea* pollen type corresponds to only one species with a clearly-defined land use (García-Mozo 2011); even so, ornamental olive trees may make a small contribution (Aira et al. 2011). This specificity, taken in conjunction with evidence relating phenology, crop levels and airborne pollen concentrations, enables close correlations to be

Table II. The results from the Friedman test between pollen types.

Friedman	Daily data		Hourly data	
	χ^2	<i>p</i>	χ^2	<i>p</i>
<i>Quercus</i>	6.952	0.434	583.4595	<0.001
<i>Olea</i>	19.349	0.007	465.7824	<0.001
Poaceae	38.739	<0.001	493.1752	<0.001
<i>Plantago</i>	29.601	<0.001	113.5711	<0.001

identified. Furthermore, since olive pollen is considered highly allergenic (Bonofiglio et al. 2013) and is a major cause of allergy in the Mediterranean area (Subiza et al. 2007), these results provide valuable information for allergy sufferers, enabling the identification of major sources of airborne pollen.

The importance of the relationships observed within the general context of this type of research resides in the size of the area studied (440 km in width) and its geographic features (the continentalism of the climate and vegetation); airborne pollen levels show local differences, but also point to a similar overall seasonal pollen pattern from the sampling stations studied. Although the conclusions are intended specifically for local usage, they may be valuable to any area with a Mediterranean climate with a relevant continental influence.

Conclusion

Comparison over a minor timescale revealed different results for pollen stations separated by a distance of 69 to 440 km, despite similarities in general climate, vegetation and land use. Similar results observed in any pair of stations appeared

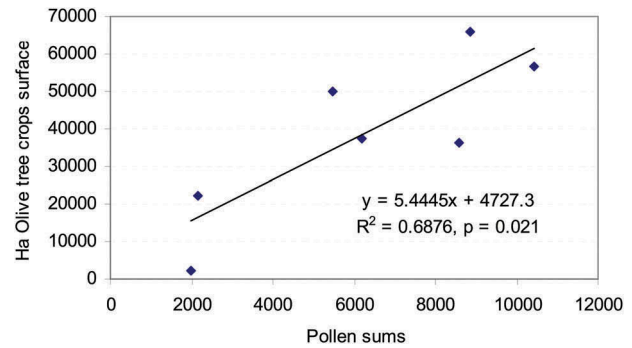


Figure 8. Comparison of total olive pollen counts and olive crop surface area in a 50 km area around pollen stations.

to be consistent with the distance between stations. From a public health viewpoint, this information may be useful for allergy specialists. Daily data masked relevant differences in hourly data. Hourly pollen levels did not follow any general pattern, and there were no similarities with regard to the timing of hourly peaks. In terms of land use, the surface area of olive tree crops in a 50 km area around the spore trap locations was found to exhibit a clear relationship with airborne pollen data.

This study highlights differences and similarities in patterns of airborne pollen concentrations between sampling stations and thus provides useful information regarding the potential siting of new spore traps. As a general rule, in the absence of geographical barriers and where land uses are similar, overall seasonal patterns in airborne pollen concentrations are likely to be similar. Nevertheless, hourly differences are evident, and may be responsible for allergy problems at a local level.

Table III. The results from the Wilcoxon test between pollen stations for the *Olea* pollen type. Statistical significant values (similarity) are given in bold.

	Badajoz	Ciudad Real	Plasencia	Talavera	Toledo	Zafra
Albacete	-3.130 0.002	-5.120 0.000	-0.350 0.726	-1.698 0.090	-1.969 0.049	-3.976 0.000
Badajoz		-0.804 0.422	-4.302 0.000	-0.679 0.497	-1.598 0.110	-0.319 0.750
Ciudad Real			-4.475 0.000	-2.192 0.028	-2.573 0.010	-3.976 0.000
Plasencia				-4.615 0.000	-3.033 0.002	-5.570 0.000
Talavera					-1.158 0.247	-2.178 0.029
Toledo						-2.382 0.017

Disclosure statement

No potential conflict of interest was reported by the authors.

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