

Correlation between airborne *Olea europaea* pollen concentrations and levels of the major allergen Ole e 1 in Córdoba, Spain, 2012–2014

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Abstract *Olea europaea* L. pollen is the second-largest cause of pollinosis in the southern Iberian Peninsula. Airborne-pollen monitoring networks provide essential data on pollen dynamics over a given study area. Recent research, however, has shown that airborne pollen levels alone do not always provide a clear indicator of actual exposure to aeroallergens. This study sought to evaluate correlations between airborne concentrations of olive pollen and Ole e 1 allergen levels in Córdoba (southern Spain), in order to determine whether atmospheric pollen concentrations alone are sufficient to chart changes in hay fever symptoms. The influence of major weather-related variables on local airborne pollen and allergen levels was also examined. Monitoring was carried out from 2012 to 2014. Pollen sampling was performed using a Hirst-type sampler, following the protocol recommended by the Spanish Aerobiology Network. A multi-vial cyclone sampler was used to collect aeroallergens, and allergenic particles were quantified by ELISA assay. Significant positive correlations were found between daily airborne allergen levels and atmospheric pollen concentrations, although there were occasions when allergen was detected before and after the pollen season and in the absence of airborne pollen. The correlation between the two was irregular, and pollen potency displayed year-on-year variations and did not necessarily match pollen-season-intensity.

Keywords Olive · Aeroallergens · ELISA · Airborne pollen · Ole e 1 · Pollinosis · Aerobiology · Pollen · Pollen index · Meteorological parameters

Introduction

Hay fever caused by pollen allergens is among the most common chronic diseases in Europe, and its incidence has risen over recent decades (Bousquet et al. 2007; Cecchi et al. 2010). Airborne pollen concentrations have traditionally been used as a means of estimating exposure to aeroallergens and as the basis for information provided to local allergy-sufferers. Today, national and regional monitoring networks provide essential data on the spatial and temporal distribution of airborne pollen.

However, recent research has shown that airborne pollen concentrations alone do not always provide a clear indication of actual allergen exposure; nor do they always coincide with symptom intensity in patients sensitive to a given pollen type (D'Amato et al. 2007; Brito et al. 2011). Variations in aeroallergen concentrations have been observed under certain environmental conditions (De Linares et al. 2007; Fernández-González et al. 2010; Buters et al. 2012). Differences in pollen potency reported for different sites in Europe may well be attributable to specific local conditions, including long-range transport and other factors (Buters et al. 2012; Galán et al. 2013).

Olive pollen (*Olea europaea* L.) is the second largest cause of pollinosis in the southern Iberian Peninsula (Sánchez-Mesa et al. 2005), and it is the leading cause of hay fever in some areas of Andalusia, where up to 80 % of patients suffering from allergy are sensitized to this pollen type (Sánchez-Mesa et al. 2005; Barber et al. 2008). Ole e 1 is the most representative allergen, accounting for over 10 % of the total

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pollen protein content in Oleaceae (Obispo et al. 1993); it is found only in pollen, and not in other parts of the plant (Villalba et al. 1994).

Olive is extensively cultivated in the southern Iberian Peninsula. Spain is the world's largest olive producer in terms of surface area, and 60 % of Spain's olive groves are located in Andalusia, mostly in the provinces of Córdoba and Jaén. The greater incidence of olive-pollen allergy may be due to an increase in the surface area planted, as well as to changes in farming practices, with the introduction of intensive methods and of improved irrigation systems prompting an increase in flowering (Barranco et al. 2005).

In a molecular epidemiological study, Barber et al. (2008) showed that olive-pollen sensitization measured by Ole e 1 reactivity appeared to reach a ceiling in terms of prevalence and average IgE values in the population exposed to intermediate concentrations of olive pollen, noting that it would therefore be interesting to monitor actual Ole e 1 levels in addition to pollen concentrations, with a view to documenting possible correlations more accurately.

This study sought to examine the correlation between airborne olive-pollen and Ole e 1 concentrations in Cordoba, and to determine the influence of weather variables on pollen concentrations and allergen release.

Materials and methods

Study area

The city of Cordoba is located in southwestern Spain (37° 53' N, 4° 45' W), at a height of 123 m.a.s.l. It has a Mediterranean climate with some continental features. The annual mean temperature is 18.1 °C and annual average rainfall is 591 mm (30-year averages, 1982–2011, provided by the Spanish Meteorology Agency-AEMET). Cordoba is Spain's second largest olive-producing province, with an area of 343,812 ha given over to this crop.

Pollen and airborne allergen monitoring

The study was carried out from 2012 to 2014 during the olive-pollen season (March to June), with an additional margin of 2 weeks both before and after the season to enable possible allergen detection outside the flowering period. Sampling was performed using two samplers located close to each other, at 22 m above ground level in two buildings at the University Campus of Rabanales, located in the northeast of the city.

Airborne pollen sampling was performed using a Hirst-type volumetric spore trap (Hirst 1952), following the procedures laid down in the Spanish Aerobiology Network (REA) Management and Quality Manual (Galán et al. 2007) and in compliance with the minimum requirements set out by the

European Aerobiology Society (EAS) (Galán et al. 2014). Concentrations were expressed as the daily average number of pollen grains per cubic meter of air. For the purposes of this study, the pollen-season start-date was taken as the first day on which 50 or more pollen grains/m³ were recorded with the five consecutive days recording 50 or more grains/m³, and the end-date as the first day with values of below 50 pollen/m³ followed by five following days with equal or lower values.

Ole e 1 was detected using a low-volume cyclone sampler (Emberlin 1995). Particles were collected in a 1.5-ml Eppendorf vials located in a carousel, with an air throughput of 16.5 L/min (Burkard Manufacturing Co., Ltd.). The carousel is programmed to change the vial every day at 12:00 and an operator introduces new vials once a week. The instrument is sensitive to wind direction, so the sampling orifice always faces the prevailing wind. The airborne allergens were collected for 24 consecutive hours from 12:00 to 12:00 next day. This technique is highly efficient over a wide range of captured particles, with 90 % efficiency for the size range 0.82–0.75 µm. Daily samples were stored at –40 °C until extraction.

Extraction and quantification of airborne allergens

Extraction of samples from the cyclone sampler was performed following Takahashi et al. (2001) as modified by Moreno-Grau et al. (2006). Samples collected in 1.5-ml Eppendorf vials were centrifuged for 3 min at 6000 rpm, so that particles were deposited on the bottom. Samples were then resuspended in 120 µl of extraction buffer (50 mmol/L phosphate buffer at pH 7.4 containing 150 mmol/L sodium chloride, 125 mmol/L ammonium bicarbonate, 3 mmol/L EDTA, and 0.005 % Tween 20) for 2 h at room temperature with constant stirring. They were then centrifuged at 4000 rpm for 10 min and the supernatant was collected taking care not to take the pellet. Four 30-µl aliquots were made in order to avoid having to thaw the sample several times if performing more than one analysis; aliquots were finally stored at –40 °C until analysis.

Airborne allergen concentration in aerosol samples was quantified by Double Sandwich ELISA, in which antigen extract was trapped between two antibodies, following Rodríguez Rajo et al. (2011), Moreno-Grau et al. (2006), and Arilla et al. (2002, 2005, 2006). Ninety-six-well microplates (Greinerbio-one, Germany) were coated with 100 µl of the corresponding monoclonal antibody 5A3 (Bial Industrial Pharmaceutical, Spain), 5 mg/ml diluted in PBS and incubated overnight at 4 °C in a humidity chamber. The next day, the plate was blocked with 200 µl/well of BSA-PBS-T (PBS + 1 % BSA and 0.05 % Tween 20) and incubated 1 h at 37 °C. Later, 100 µl/well of the calibration curve and sample extracts, diluted with BSA-PBS-T according to daily pollen amounts, were added and incubated for 1 h at 37 °C. After three washes with 200 µl PBS-T buffer (PBS + Tween 20 0.05 %), plates

were incubated for 1 h at 37 °C with 100 µl/well of polyclonal antibody (Biotinylated rabbit anti Ole e 1, Bial Industrial Farmaceutica, Spain at 0.5 mg/ml). Plates were then washed with 200 µl PBS-T and incubated for 1 h at 37 °C with peroxidase-conjugated streptavidin (0.25 mg/ml in PBS-BT). Finally, wells were incubated for 10 min at room temperature with 100 µl TMB in the dark. The reaction was stopped by adding 100 µl of 3 M H₂SO₄ and absorbance was measured at 450 nm.

The standard curve was generated with a series of dilutions (0.10 to 50 ng/ml) of a purified preparation of Ole e 1, performed in duplicate.

The final curve was based on the average of the two absorbance readings, in order to minimize potential error. Samples were analyzed in duplicate, and daily concentrations were interpolated from the standard curve and expressed in picograms per cubic meter of air. Pollen concentrations were compared with allergen concentrations for the same time intervals.

Weather data

Weather data used were rainfall (mm), maximum, minimum and average temperature (°C), relative humidity RH (%), and wind speed (m/s), as supplied by the Andalusia Regional Government Agroclimatic Information Network at a weather station located on the outskirts of Córdoba city (UTM coordinates 37° 51' 25" N, 04° 48' 10" W).

Statistical analysis

Spearman's rank correlation test was performed to evaluate correlations between pollen and airborne allergen concentrations and also to examine their correlation with major weather variables. Statistical analysis was performed using the SPSS version 14 software package for Windows.

Results

For the 3-year study period, the rainiest pollen season was 2012. The highest humidity was recorded during 2013 pollen season, with an average of 63 %, though rainfall and average temperature were lower than in other years. The warmest year was 2014. For the period prior to the pollen season, 2012 recorded the driest and coldest winter. 2013 had the wettest and the most humid winter, and 2014 was the warmest winter (Table 1).

The olive pollen season started from mid-April to the beginning of May. Peak day occurred from the beginning of May to mid-May and the season ended from early to mid-June (Table 2). Year-to-year differences were observed in the timing and intensity of the pollen season, with a general advance of both the start date and the peak pollen date over the study period; the advance was particularly marked in 2014.

The rainiest pollen season, recorded in 2012, displayed a shorter and smoother pollen-season curve, i.e., a lower pollen peak than in the following years, but with an annual pollen index (PI) similar to that of 2013. A higher PI was detected in 2014 (Table 2).

Higher airborne allergen concentration was detected in 2012 than in the other 2 years (Table 2). A striking feature for year 2014 was the detection of airborne allergens at the start and the end of the pollen season, when airborne pollen concentrations were very low (Fig. 1).

Regarding the results for pollen potency (pollen vs. allergens), the highest value was recorded in 2012 and the lowest in 2014 (8.74 and 1.56 pg Ole e 1/pollen grains, respectively).

Spearman correlation tests showed a significant positive and high correlation ($r = 0.740$ in 2012; $r = 0.726$ in 2013; $r = 0.560$ in 2014) between daily allergen and airborne pollen concentrations for all study years, significant at the 0.01 level in the three cases. The highest correlation was obtained in 2012 (Table 3).

A positive correlation was recorded between maximum and minimum temperature and both olive-pollen and Ole e 1 allergen concentrations; the correlations were especially marked for pollen concentrations. Both parameters correlated negatively with average relative humidity and precipitation, although the correlation was only significant in 2013, while in 2014 it was significant only for pollen. A significant negative correlation was observed between pollen concentrations and wind speed in all three study years, and between allergen concentrations and wind speed only in 2012 (Table 3).

Discussion

Different papers have reported possible relationships for different pollen types and airborne allergens at various sites, e.g., for *Parietaria* vs. Par j 1 and *Olea* vs. Ole e 1 (Moreno-Grau et al. 2006), for *Betula* vs. Bet v 1 (Buters et al. 2012) and for *Platanus* vs. Pla a 1 (Alcázar et al. 2015). In the latter two studies, allergens were detected only when pollen was present. Some authors, however, report disparities between allergen and pollen curves, e.g., for *Plantago* (González-Parrado et al. 2014) and *Platanus* (Fernández-González et al. 2010).

In general, earlier research has highlighted a divergence between airborne allergen and pollen concentrations in the periods preceding and following the main pollen season, e.g., for grasses (Spiekma et al. 1995; Rodríguez Rajo et al. 2011), for *Olea* (De Linares et al. 2007), for *Platanus* (Fernández-González et al. 2010; Rodríguez Rajo et al. 2011), and for *Urticaceae* (Rodríguez Rajo et al. 2011). In *Platanus* (Fernández-González et al. 2010), airborne pollen and Pla a 1 concentrations did not follow exactly the same trend during the pollen season, and especially during the period immediately before and after the main pollen season; however, these

Table 1 Main meteorological conditions in Córdoba, Spain, in 2012–2014

		2012	2013	2014
Before pollen season (from 1-1 to 31-3)	Average temperature (°C)	9.1	9.9	10.0
	Precipitation (mm)	19.5	415.6	242.4
	Relative humidity (%)	92.8	99.8	98.8
During pollen season	Average temperature (°C)	20.4	18.9	20.6
	Precipitation (mm)	117.0	26.4	55.2
	Relative humidity (%)	56.4	63.0	58.6

findings may be due to the generally low pollen concentrations recorded in the study area (Alcázar et al. 2015).

According to Brito et al. (2011) and Moreno-Grau et al. (2006), this difference between airborne pollen and allergen concentrations during the period before and after pollen season has been attributed to possible free allergens from pollen grains or other parts of the plants in response to certain atmospheric conditions and subsequently suspended in the air for a variable period of time. Our results support these observations; however, Ole e 1 release occurs only from pollen (Villalba et al. 1994).

A number of authors have noted that high atmospheric humidity may favor release of the protein from airborne pollen grains or even from the pollen tube (Fernández-González et al. 2010; Buters et al. 2012, 2015). Here, humidity appeared to exert no clear influence, probably due to specific features of the local climate, characterized by dry and warm weather with continental features and stormy episodes in spring. In general, research suggests a stronger correlation of weather parameters with pollen dispersal than with allergen release (Rodríguez Rajo et al. 2011), although some studies report a strong correlation between temperature and allergen concentrations, indicating that allergens may be more sensitive than pollen grains to temperature (Brito et al. 2011; Galán et al. 2013).

Table 2 Characteristics of 2012–2014 pollen seasons

	2012	2013	2014
Pollen			
Pollen season start	6–5	27–4	16–4
Pollen season end	13–6	14–6	1–6
Season length (days)	39	49	47
Peak value (pollen/m ³)	3654	4886	4280
Peak day	16–5	13–5	5–5
Pollen index PI (pollen grains)	20,625	21,977	40,060
Ole e 1 allergen			
Peak value (pg/m ³)	36,052	6267	3838
Peak day	17–5	10–5	3–5
Allergen index (pg)	180,209	79,161	62,366
Pollen potency (pg/pollen grains)	8.74	3.60	1.56
Pollen potency pre-peak	10.64	2.12	1.64
Pollen potency post-peak	9.56	5.75	1.68

However, it is important to note that airborne pollen concentrations primarily reflect flowering phenology; it is thus important to examine correlations with weather-related parameters more closely related to plant phenology and plant pollen emission. By contrast, release of aeroallergens from pollen is prompted by other parameters linked less to plant phenology than to pollen exposure. Here, strong correlations were recorded with airborne pollen concentrations, a variable linked to olive flowering phenology.

The airborne allergen recorded—albeit at low concentrations—both before and after the pollen season, especially in 2012 and 2014, probably came from outside the study area. A number of authors have attributed the local presence of airborne allergens outside the main pollen season to medium- and long-range transport. For example, airborne allergens detected in Évora (Portugal) have been traced to southern Spain (Galán et al. 2013). This might account for the recording of airborne allergens at the start of the pollen season, when pollen concentrations were still very low, particularly in 2012. Aeroallergens bind to smaller particles than pollen grains and can thus more readily be transported from distant areas.

Temperature plays a key role in the timing of the pollen season (Aguilera et al. 2014). The warm weather in 2014 probably encouraged early flowering of olive trees and thus an early start to the pollen season. Airborne allergens were detected at an earlier stage (April) than in the previous 2 years (May). By contrast, the drier, colder winter of 2012 gave rise to a delayed start to the pollen season. During the rainiest year (2012), pollen potency was higher than during the other 2 years, possibly due to higher release of allergens from pollen grains, but lower pollen concentrations were detected. This indicates that although during the pollen season the correlation between daily allergen concentration and humidity is not significant, specific humidity increases recorded during stormy episodes, usually during spring in Córdoba, may have caused a one-off increased release of allergens and they can be present in the air the rest of the season.

Differences between the curves for pollen grains and airborne allergens may also be explained in part by the presence of other species (*Fraxinus* sp., *Syringa vulgaris*, *Ligustrum* sp.) which contain Ole e 1 and its pollen grains may be released before and after the olive pollen season (Brito et al. 2011; Obispo et al. 1993). In our study area, however, the

Fig 1 Airborne olive pollen and airborne allergen Ole e 1 concentrations in Córdoba (Spain) over the study period

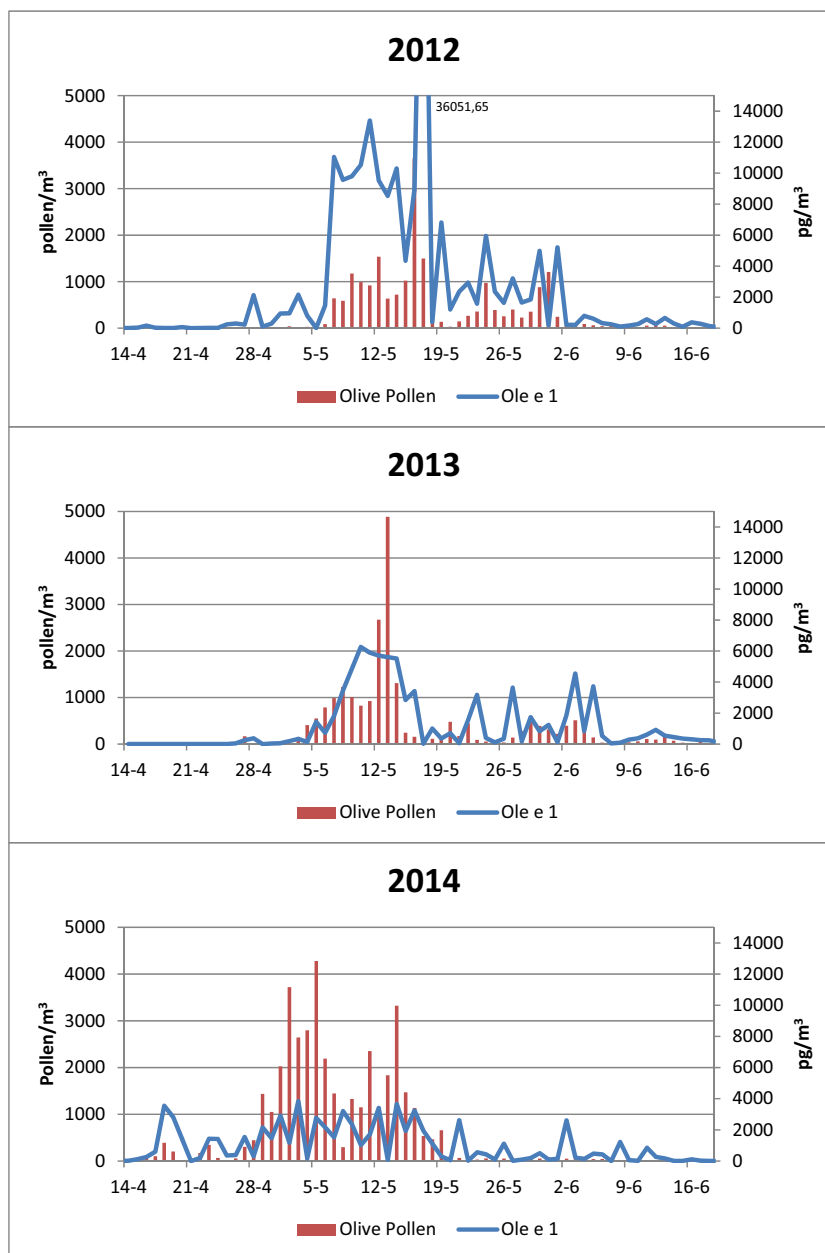


Table 3 Spearman correlations between olive pollen and Ole e 1 concentrations, and weather variables

		pg/m ³	Maximum temperature (°C)	Minimum temperature (°C)	Average temperature (°C)	Precipitation (mm)	Relative humidity (%)	Wind velocity (m/s)
2012	<i>N</i> = 39	pg/m ³	.260	-.278	.090	.182	-.050	-.477 ^b
		Pollen/m ³	.740 ^b	-.009	.293	-.117	-.290	-.533 ^b
2013	<i>N</i> = 49	pg/m ³	.551 ^b	.327 ^a	.562 ^b	-.387 ^b	-.519 ^b	-.145
		Pollen/m ³	.726 ^b	.239	.502 ^b	-.440 ^b	-.542 ^b	-.351 ^a
2014	<i>N</i> = 47	pg/m ³	.328 ^a	.309 ^a	.365 ^a	-.215	-.207	-.162
		Pollen/m ³	.560 ^b	.422 ^b	.690 ^b	-.463 ^b	-.584 ^b	-.417 ^b
2012–14	<i>N</i> = 135	pg/m ³	.339 ^b	.123	.273 ^b	-.059	-.212 ^a	-.342 ^b
		Pollen/m ³	.674 ^b	.420 ^b	.339 ^b	-.239 ^a	-.329 ^b	-.472 ^b

^a Correlation significant at the .05 level (2-tailed)

^b Correlation significant at the .01 level (2-tailed)

pollen curves did not match, *Fraxinus* blooming several months before olive, and *Ligustrum* blooming nearly 1 month after the end of the olive flowering period (Cariñanos et al. 2002; Martínez et al. 2015). Concentrations of *Fraxinus* and *Ligustrum* pollen types of sufficient magnitude to modify airborne allergen concentrations were not observed during the olive-pollen season.

It is important to remark that a clear positive correlation was noted between airborne olive pollen concentrations and levels of the major airborne allergen Ole e 1 ($p < 0.01$) for all study years (Table 3), indicating that pollen grains are the main source of allergens in the air. Therefore, pollen grains can be a good indicator for the amount of allergens in the air and an important fact for allergy prevention. However, like recent studies show (Buters et al. 2015), the ratio between the two varied and pollen potency was not constant over the study period, since the amount of pollen and allergens is not a constant value every year.

Conclusions

The results of this study have shown that although the correlation between pollen grains and allergens is very high and significant, the relationship between pollen and allergen concentrations is irregular and not always an increase in pollen grains results in an increase in allergen concentration. Sometimes a gap between the peaks for both variables is observed. Moreover, at the end of the season, there are allergens even when pollen concentrations are very low or zero. This may be due to the small size for allergen particles that could remain easily in the air or they may come from other remote areas where flowering occurs later, being transported from long distance. Furthermore, pollen potency does not always match pollen-season severity; potency displayed marked year-on-year variations, since pollen concentrations do not reflect the concentrations of allergens. Correlations among airborne allergens, airborne pollen, and different weather-related parameters do not account for variations in pollen potency; it may depend on other factors. Thus, although airborne pollen certainly provides useful information, additional information on airborne allergens is required in order to ensure that data supplied to allergy-sufferers is as accurate as possible.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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