

Olea europaea pollen counts and aeroallergen levels predict clinical symptoms in patients allergic to olive pollen

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Background: Allergic symptoms are commonly related to atmospheric pollen counts in sensitized allergic individuals. However, concordance between symptoms, pollen counts, and aeroallergen concentrations is not always good.

Objectives: To determine the correlation between olive pollen counts, aeroallergen levels, and clinical symptoms in patients with allergic asthma or rhinitis in Ciudad Real (Spain).

Methods: Two types of samplers were used to determine pollen exposure: a Burkard spore trap to collect pollen grains and a high-volume air sampler to collect airborne particles. A total of 366 air filters were collected. After extraction, they were analyzed by specific immunoglobulin E enzyme-linked immunosorbent assay inhibition using a serum pool containing high titers of olive-specific immunoglobulin E. Twenty olive-pollen monosensitized patients were asked to record their daily symptoms before, during, and after the olive pollen season.

Results: Olive pollen was detected between April 21 and June 30, 2004. Symptoms showed positive and significant correlations with pollen counts ($r = 0.700$, $P < .001$) and aeroallergen levels ($r = 0.803$, $P < .001$). Using a Poisson regression model, relative changes in aeroallergen concentrations and pollen counts were found to be similar and significant. Threshold levels for the induction of symptoms were 162 olive pollen grains/m³ and 22.7 ng of olive pollen allergen/m³ (equivalent to 0.9 ng/m³ of Ole e 1).

Conclusions: Olive aeroallergen concentrations and pollen counts are positively associated with symptoms of rhinitis and asthma in olive-allergic patients. Both data may be used in the clinical follow-up of these patients.

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INTRODUCTION

Olea europaea belongs to the Oleaceae family and is one of the most common and economically important trees in Mediterranean countries, North and South America, Australia, and South Africa.^{1,2} In Spain, it is the second leading cause of hay fever, preceded by grass pollen and followed by members of the Chenopodiaceae and Urticaceae families. However, in regions of southern Spain, olive pollen is the main cause of allergic sensitization (84% of the sensitized individuals in Jaen and 87% in Ciudad Real).^{3–5}

Pollen counts have traditionally been used to monitor the intensity and length of the pollen season and the evolution of patients with hay fever.^{6,7} Although the mechanisms by which pollen grains induce seasonal rhinoconjunctivitis symptoms have been identified, the mechanisms in asthma are less clear. One of the main concerns is that pollen grains are too large to reach the lower airways.^{8–10} Pollen allergens derived from pollen grains, microsomal particles, or other parts of plants could be directly responsible for the allergic symptoms.^{11–14}

In recent years, the quantification of pollen allergens using immunochemical methods and the analysis of their relationship with pollen counts have received greater attention, demonstrating that the relationship is not always consistent.^{15–17} Meteorological factors, such as rain, humidity, and storms, may help to clear grass pollen grains from the atmosphere, but these same factors may produce an increase in allergen concentration. The rupture of pollen grains, or rising air currents, may return allergens from the ground into the atmosphere.^{18,19} Moreno-Grau et al²⁰ studied olive pollen counts and aeroallergen levels and found a close relationship between pollen counts and Ole e 1 concentrations. However, Fernández-Caldas et al.¹⁶ demonstrated a lack of correlation between red oak pollen counts and aeroallergen levels, showing the presence of oak aeroallergen outside the pollen season. Similar

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results were obtained by Argawal et al., analyzing the ragweed and *Alternaria* spp. seasons in Minnesota.^{21,22}

From a medical point of view, the most significant challenge remains to be able to correlate aerobiological data with respiratory symptoms in patients with allergies. Few clinical studies correlate pollen counts and aeroallergen levels with symptom scores, and the results are variable.^{23–25} In the case of grass²⁶ and *Parietaria judaica*²⁷ pollen, allergic symptoms showed similar correlations with pollen counts and aeroallergens; in contrast, grass aeroallergens are detected before and after the pollen season and show a closer relationship with symptoms of rhinitis or asthma than do pollen counts.²⁸

The objective of the study was to determine the correlation between olive pollen counts, allergen concentrations, and symptoms in individuals with allergies who were monosensitized to olive pollen and who resided in the province of Ciudad Real (situated 200 km south of Madrid).

METHODS

Patient Population

Patients suffering from rhinitis or asthma during the olive pollen season (symptoms mainly between April and July) and screened at the outpatient clinic of the Allergy Departments of Puertollano and Ciudad Real (Ciudad Real, Spain) were included in the study. The inclusion criteria were: (1) seasonal rhinitis or asthma of at least 2 years' duration based on the Allergic Rhinitis and Its Impact on Asthma²⁹ and Global Initiative for Asthma Guidelines³⁰ criteria; (2) monosensitization to olive pollen; (3) resident for at least 5 years in either city included in the study; and (4) aged between 14 and 49 years. The following exclusion criteria were applied: (1) perennial rhinitis or asthma, (2) nasal polyposis, and (3) sensitization to pollens other than olive. Written informed consent was obtained from participants and written parental consent for children. The study was approved by the Institutional Review Board.

Skin prick testing was performed with a battery of common aeroallergens (Laboratories ALK-Abelló, Madrid, Spain). The inclusion criterion was a positive skin prick test (>3 mm wheal diameter) to the olive pollen extract and a history of clinical symptoms during the olive pollen season.

Symptoms and Medication Diary Cards

All patients were given diary cards on which to record their daily consumption of antiallergic medication, and their conjunctival, nasal, and bronchial symptom scores according to the following scale²⁷:

- 0 = no symptoms.
- 1 = mild symptoms:
 - slight nasal obstruction, slightly runny nose, or occasional sneeze or itching of the eyes
- 2 = moderate symptoms:
 - moderate nasal obstruction, moderately runny nose, some sneezing and congestion, some ocular itching, or mild asthma
- 3 = severe symptoms:

- complete nasal obstruction, almost continuously runny nose, frequent sneezing or ocular symptoms, or asthma attacks

Graded symptoms were summarized with a weighted score for the drugs used:

- 0 = no drugs
- 1 = oral antihistamines or β -agonists
- 2 = nasal or bronchial corticosteroids
- 3 = systemic corticosteroids

From the 418 patients screened, 20 patients monosensitized to olive pollen were selected for enrollment in the 1-year study period.

Pollen Counts

Aerobiological sampling was carried out from January 1 to December 31, 2004, with a Burkard spore trap (Burkard Manufacturing Co., Rickmansworth, Herts, UK) placed at a height of 15 m in Ciudad Real. The adhesive tape (samples) was changed each Friday and divided into seven parts, one for each day of the week. Samples were prepared according to the usual technique, and they were examined under optic microscope with a 100 $\times$ objective lens. The pollen concentrations were expressed as pollen grains/m³ of air.

Air Sampling

A volumetric air sampler adapted for outdoor use (Air Sentinel, Quan-Tec-Air Inc., Rochester, Minnesota, USA)³¹ was used for aeroallergen collection and was run continuously during 2004. The Air-Sentinel was placed 15 m above street level in Ciudad Real. The air flow was 10 m³/h. Airborne particles were collected onto polytetrafluoroethylene filters (Quan-Tec-Air Inc.), composed of stretched Teflon with a polyester support backing and rated with a 99.9% efficiency at 0.3 μ m. The sampling time for each filter was 24 hours, which represents 240 m³ of air per sample. Filters were replaced at approximately the same time each day. After removal, filters were sealed in plastic bags and stored at 4°C until extraction.

Filter Extraction and Allergen Quantification

A total of 366 polytetrafluoroethylene filter membranes were individually placed in tubes containing 0.5 ml of 0.01M phosphate-buffered saline. Tubes were stirred until the filter was completely soaked and left for overnight extraction in a rotary mixer (Labinco B.V., Breda, The Netherlands) at 4°C. The samples were then centrifuged at 3,500 r.p.m. for 5 minutes, and the supernatant was removed and processed immediately to avoid loss of allergenic activity.

Total allergenic activity and allergen content were measured in eluted samples using validated enzyme-linked immunosorbent assay inhibition assays, employing a standard olive extract containing 260 μ g of Ole e 1 (determined using specific polyclonal antibodies anti-Ole e 1). Flat-bottomed microtiter plates (Immulon IV, Chantilly, VA) were coated with olive extract at a final concentration of 1 μ g of protein/well and incubated overnight at room temperature.

Various dilutions of the olive pollen extract were prepared to generate a standard curve. Fifty-milliliter aliquots of the filters were incubated for 2 hours at room temperature with pooled sera obtained from patients with allergies who were monosensitized to olive (dilution 1/10). Positive and negative controls were also included. The dilutions were then transferred to the olive-coated microplates and incubated overnight. After washing, 100 μ l of peroxidase-labeled antihuman immunoglobulin E (Ingenasa, Madrid, Spain) was added, and the preparation was allowed to stand for 30 minutes at room temperature. The plates were then washed again, developed for 30 minutes, and stopped with 1N H₂SO₄.

The percentage inhibition was calculated using the reference inhibition curve determined from the standard olive pollen. Allergen concentrations were extrapolated using the standard curve and were based on the inhibition capacity; the final results were expressed in micrograms of allergen per milliliter.

The threshold levels for olive pollen counts and olive allergen concentrations were defined as the levels required to cause symptoms in all subjects clinically sensitive to olive pollen (minimum symptom score = 1).³²

Meteorological Data

Detailed daily records of temperature, humidity, and wind speed and direction were obtained from the meteorological observatory in Ciudad Real (39°N, 41°E, 630 m above sea level).

Statistical Analysis

All analyses were carried out using SPSS version 17.0 for Windows. We analyzed the association between the number of symptoms and one or more independent variables. The variables considered were pollen count and aeroallergen concentrations, with the meteorological variables temperature and relative humidity being included as confounding variables. Because of the short time over which data were obtained, seasonal decomposition was not significant in this model. A systematic strategy was used to develop the model. This model was first analyzed by taking into account the effects of temperature and relative humidity on the daily number of symptoms. The relationships between the daily number of symptoms and temperature and relative humidity are not necessarily linear, and delayed effects can occur. To control those effects, the computation was performed with lags of up to 5 days for temperature, relative humidity, temperature squared, and relative humidity squared. A forward stepwise Poisson multiple regression analysis was then applied to the data. Variables were included according to the fit of the model to the data.

RESULTS

Patient Population

A total of 20 patients (11 women and 9 men) were included in the study. The mean age was 28.2 years (range, 10 to 51

Table 1. Characteristics of the 20 Monosensitized Patients

Patient no.	Age	Sex	Symptoms	Classification
1	21	F	R,C	SPR
2	48	F	R,C,A	PCA
3	33	M	R,C	MIR
4	13	M	R,C,A	CA
5	44	M	R,C,A	PCA
6	41	F	R,C,A	CA
7	17	M	R,C,A	CA
8	23	F	R,C	SPR
9	15	M	R,C,A	CA
10	42	F	R,C,A	CA
11	26	M	R,C,A	PCA
12	14	M	R,C,A	PCA
13	29	F	R,C,A	UA
14	20	F	R,C	SIR
15	18	F	R,C	MPR
16	32	M	R,C,A	PCA
17	19	F	R,C	MPR
18	41	F	R,C	SPR
19	37	M	R,C	MPR
20	32	F	R,C,A	PCA

Abbreviations: R,C, rhinitis, conjunctivitis; R,C,A, rhinitis, conjunctivitis, asthma; MIR, mild intermittent rhinitis; MPR, Mild persistent rhinitis; SIR, moderate-severe intermittent rhinitis; SPR, moderate-severe persistent rhinitis; CA, controlled asthma; PCA, partly controlled asthma; UA, uncontrolled asthma.

years). All patients were monosensitized to olive pollen and suffered from allergic respiratory tract symptoms; all had rhinoconjunctivitis (mild rhinitis only in 4 patients), and 12 (60%) suffered from asthma (uncontrolled in 1 patient, partially controlled in 6, and controlled in 5) (Table 1).

Pollen Counts

In 2004, the olive pollen season comprised the 21st of April to the 30th of June. The highest olive pollen peak was detected on the 5th of June with 443 grains/m³. The total quantity of olive pollen grains documented for the whole year 2004 was 3,493 grains. No olive pollen grains were detected during the rest of the year.

The filters were also analyzed by scanning electron microscopy at the Jardín Botánico in Madrid, Spain, identifying spherical components with a diameter of approximately 1 μ m located close to the pollen grains; these could correspond to the orbicules (Fig. 1).

The threshold level for olive pollen was 162 grains/m³ and for olive aeroallergens 22.7 ng/m³ (equivalent to 0.9 ng/m³ of Ole e 1).

Immunochemical Quantification of Airborne Allergens

Variable amounts of airborne olive allergens were detected throughout the year. The maximum values of aeroallergens coincided with the pollen season and the highest peak (35.2 ng of olive aeroallergen/m³) was detected on May 31.

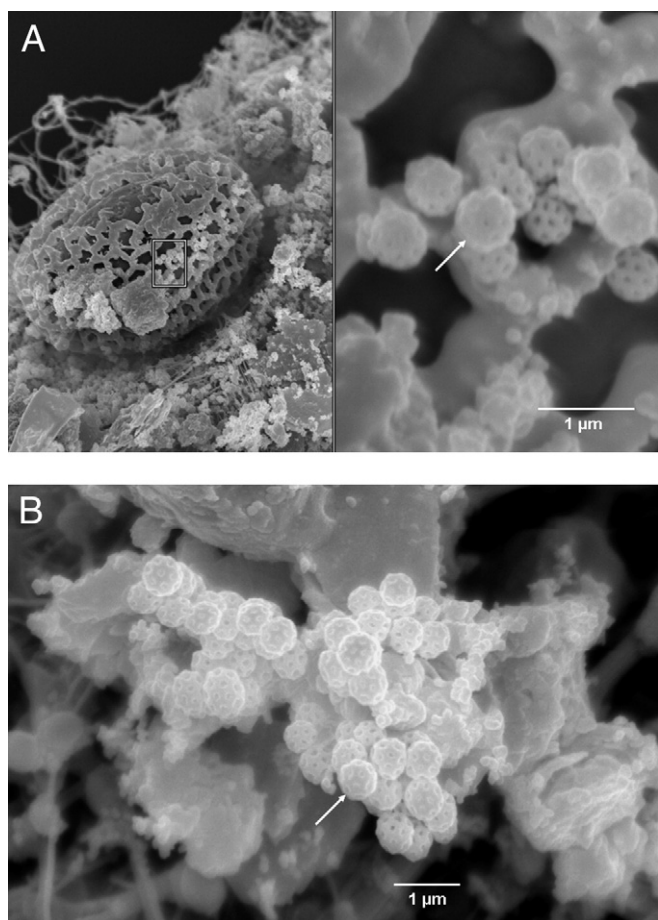


Figure 1. A, Electron microscopy picture of an olive pollen grain captured on an air filter. In Figure 1 on the right, small, spherical elements with a diameter of 0.5 to 1 μm can be observed between the trabeculae of the pollen (arrow). These may correspond to orbicules of *Olea europaea*. B, Clearer image of the spherical elements (arrow) compatible with orbicules of *Olea europaea*.

Correlation Among Symptoms, Pollen Counts, and Aeroallergen Levels

Table 2 shows the descriptive statistics for the daily number of symptoms, pollen counts, aeroallergen concentrations, and meteorological variables. A mean of 12.44 symptoms were registered during the pollen season. Figure 2A and B shows the time series for the number of symptoms–pollen counts

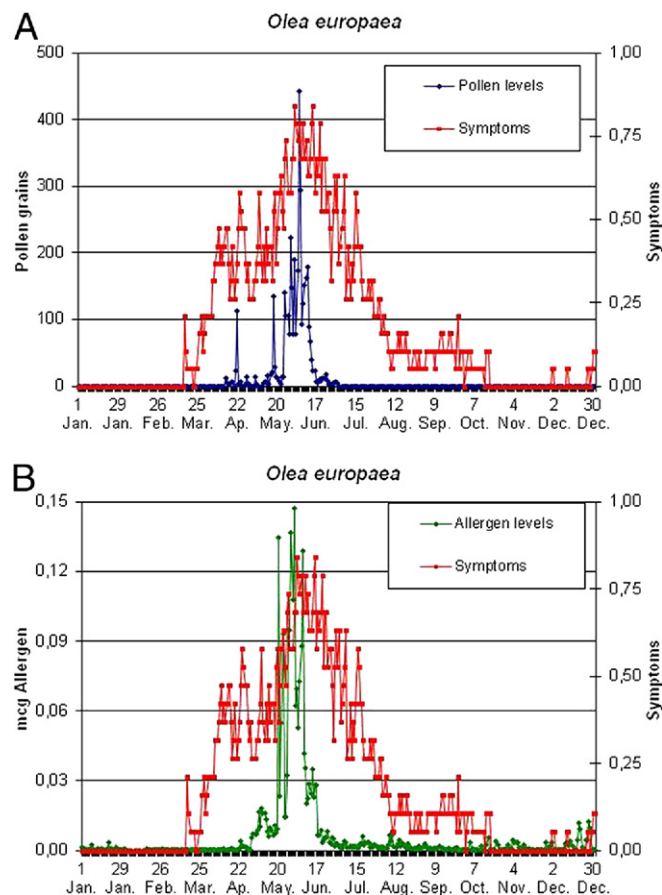


Figure 2. A, Correlation between pollen counts and symptoms in the study population ($r = 0.70$). The Y axis represents the values of pollen counts (left axis) and symptoms (right axis), respectively. The curves of each variable are referenced respect to its correspondent axis. B, Correlation between aeroallergen concentrations and symptoms in the study population ($r = 0.80$). The Y axis represent the values of aeroallergen concentration (left axis) and symptoms (right axis), respectively. The curves of each variable are referenced with respect to their correspondent axes.

and symptoms–aeroallergen levels, respectively. Figure 3 represents correlation between pollen grains and aeroallergen levels.

Poisson multiple regression test assumes a linear relationship between response and predictors. Correlations between the daily number of symptoms and the independent variables

Table 2. Descriptive Analysis of Daily Number of Symptoms, Pollen Grains, Aeroallergen Levels, and Meteorological Variables

	Mean	Median	SD	Min	Max	Range
No. of symptoms	12.44	12.00	6.05	1	24	23
Temperature	20.04	20.20	6.04	9.90	32.40	22.50
R.H.	57.66	55.00	14.45	31	89	58
Pollen	48.89	10.00	80.43	0	443	443
Allergens	6.89	2.34	9.23	0.001	35.262	35.261

Abbreviation: R.H., relative humidity.

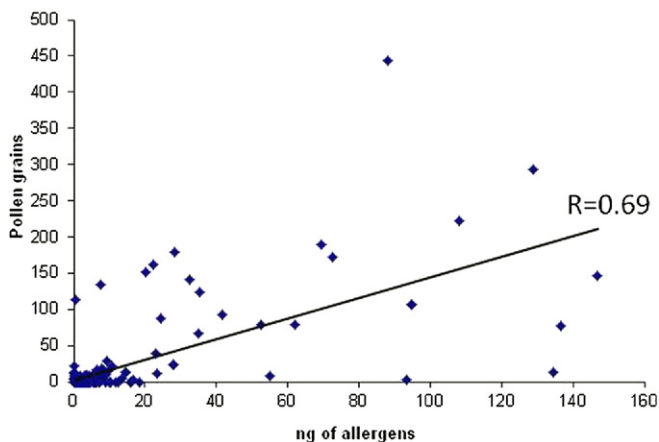


Figure 3. Correlation between aeroallergen concentrations and pollen grains ($r = 0.69$).

were significant. Positive correlations were obtained between symptoms and pollen counts ($r = 0.700$, $P < .001$), between symptoms and aeroallergen concentrations ($r = 0.803$, $P < .001$), and between the combined pollen counts and aeroallergen concentrations ($r = 0.650$, $P < .001$) were obtained.

The results of the Poisson regression model are shown in Table 3. Variables included were a 4-day lag for temperature, a 4-day lag for temperature squared, a 4-day lag for relative humidity, a 4-day lag for relative humidity squared, a 4-day lag for pollen count, and a 2-day lag for aeroallergen concentrations. Looking at the final column of Table 2 (incident rate ratios), one can see that relative changes in aeroallergen levels and in pollen counts are significant and similar.

DISCUSSION

The current study was conducted in Ciudad Real, Spain, where olive pollen counts are high during the pollen season (range between 400 and 1,200 grains/ m^3) because of the abundance of this tree in our region and the proximity of large olive groves in the neighboring provinces of Jaen and Córdoba.^{3,33} These high pollen counts are responsible for a large number of sensitizations. Other prevalent pollens can be identified in our area, particularly grasses and Chenopodiaceae, which are responsible for a substantial number of polysensitized individuals. In the first phase of the study, 418 individuals were considered eligible for inclusion in the

study, and symptom scores were collected in all of them. However, only the 20 olive-monosensitized individuals (5% of the initial population) were finally enrolled. The study was performed exclusively during the olive pollen season (April to June), because this avoided interference with other pollens of the Oleacea family (ash during the months of February and March and privet during the month of July), which have demonstrated cross-reactivity with olive.^{34,35} To establish the olive pollen season, we used the “98% method”: the season starts when 1% of the total count is achieved and ends when 99% is reached.³⁶

Previous studies have established a positive correlation between pollen counts and symptoms. However, some studies have shown that this correlation is not always uniform, particularly with respect to symptom intensity and duration, because some pollens may be too large to reach the small bronchioles and induce an allergic response.^{8,9} Furthermore, allergens are derived not only from pollen grains, but also from other parts of plants, such as leaves, stems, or small orbicules. These components release microparticles into the atmosphere with a similar capacity to induce allergic symptoms because of their ability to penetrate the respiratory tree; these reactions may be more severe than those caused by pollen grains.¹⁸ A number of studies conducted with other pollens (grasses,¹⁵ oak,¹⁶ and birch¹⁷) have demonstrated varying degrees of correlation between symptoms and pollen grain counts. The clinical significance of this discordance has received less attention, because most aerobiological studies have focused on an analysis of the relationship between pollen counts and allergen concentrations.

In the current study, we have demonstrated the presence of allergens in the air and analyzed the correlation between their concentration and the total pollen count. This analysis was performed during the olive pollen season to avoid possible interference with pollen grains from other trees related to olive, such as ash or privet. Our results demonstrate a positive correlation between symptoms (rhinitis and/or asthma) and pollen grain and allergen concentrations ($r = 0.70$ and $r = 0.80$, respectively). Although both correlations were significant, it was better with allergens levels. There was also a shorter lag difference between exposure and symptoms (2 days for allergens vs 4 days for pollen grains). The better correlation observed with allergens may be explained by the presence on the surface of the pollen grains of small particles

Table 3. Results from Poisson Regression Analysis

No. of Symptoms	Coefficient	Standard error	$P > z $	[95% Confidence interval]	RRI
Lag4(T)	0.341	0.071	<0.001	0.203, 0.479	1.406
Lag4(RH)	-0.076	0.030	0.013	-0.135, -0.016	0.927
Lag4(T ²)	-0.008	0.002	<0.001	-0.0112, -0.0042	0.992
Lag4(RH ²)	0.001	0.0002	0.005	0.0002, 0.0011	1.000
Lag4(pollen)	0.001	0.0004	0.002	0.0005, 0.0023	1.001
Lag2(allerg)	0.016	0.004	<0.001	0.0084, 0.0236	1.016

Abbreviation: RRI, rate ratio incidents.

(orbicules) (Fig. 1) that act as allergen carriers, or the release of allergens by plant parts, such as stems, leaves, and catkins. Mild symptoms observed in the previous allergen detection period could be related to the presence of allergens/pollen grains from ash as previously mentioned. Using scanning electron microscopy, Vinckier and Smets¹² studied the presence of orbicules in 15 allergenic European species, confirming the existence of these particles in all of them except Asteraceae and Oleaceae. In another article, the same author analyzed the presence of allergens in the orbicules of the same botanical species and detected only minor labeling for the major allergen Bet v 1 from birch.³⁷ However, Suárez-Cervera et al³⁸ confirmed the presence of Cry j 1, the major allergen of *Cryptomeria japonica*, on the orbicules of *C. japonica*, *Cupressus arizonica*, and *Cupressus sempervirens*. In the current study, we confirmed the presence of orbicules on the surface of olive pollen. We acknowledge that other plant parts, such as leaves, stems, and catkins, also may be responsible for these observations.

Pollen counts, or aeroallergen data, can be used in the management of patients allergic to olive pollen. The fact that olive allergens may be derived not only from pollen grains but also from other parts of the tree, such as orbicules and other tree parts, could shed light on this difference in favoring the detection of olive allergens for the follow-up of allergic individuals. Our results agree with those of Moreno-Grau et al.²⁰ In a similar study, D'Amato et al²⁷ analyzed the clinical course of patients allergic to *Parietaria judaica* pollen during the pollen season in Naples, detecting a significant association between pollen counts, allergen levels, and symptoms. Those findings differ considerably from the results of a recent study of grass pollen performed by our group,²⁸ in which rhinitis and asthma symptoms had a better correlation with allergen levels than traditional pollen counts.

Threshold pollen levels that induce symptoms have been suggested for Olea and grasses. In the case of *Olea europaea* pollen, Florido et al³³ established a limit of 400 grains/m³ to induce symptoms. In our study, a lower exposure (162 grains/m³) induced a significant allergic symptom reactivation in all of our patients. With regard to olive aeroallergens, the threshold level was 22.7 ng/m³, corresponding to 0.9 ng/m³ from Ole e 1.

Lower threshold levels have been established for grasses. Davies and Smith³² established a threshold level of 50 grains/m³ as the minimum value that will induce symptoms in grass-sensitized individuals. In other studies, only 30 to 35 grains/m³ were needed to stimulate 100% of patients.^{28,39}

The analysis of meteorological variables revealed a significant association between symptoms in olive-allergic patients and temperature and humidity. Temperature is one of the factors most closely related with olive phenology. Models have been created that determine the threshold temperature for olive tree flowering and the start of the pollen season.^{40,41} In our study, the direct effect of temperature on olive pollination is evident through its significant relationship with symptoms. Atmospheric humidity has also been related to an

increase in allergen concentration through osmotic rupture of the pollen grains, or because it acts as a vehicle to transport pollen allergens.^{18,42} In conclusion, we have demonstrated that olive allergen concentrations and pollen counts show a significant and positive correlation with clinical symptoms in olive pollen allergic monosensitized individuals. Aerobiological information systems based on pollen and spore counts should therefore be supplemented with information on aeroallergens. Further studies must be conducted to determine the threshold of sensitization or the capacity to stimulate an allergic response.

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