
Correlation between *Olea europaea* and *Parietaria judaica* pollen counts and quantification of their major allergens Ole e 1 and Par j 1-Par j 2

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Background: In patients with pollinosis, allergic symptoms are often correlated with the number of airborne pollen grains, although this correlation is not always close. The direct measurement of the concentration of aeroallergens has only recently been introduced and is an important advance in public health information systems.

Objective: To compare specific quantification of aeroallergens Ole e 1 and Par j 1-Par j 2 *Olea* and *Urticaceae* pollen counts.

Methods: The Hirst method sampler and the Burkard Cyclone sampler were used for pollen count and allergen quantification, respectively. The aerosol was extracted and quantified for Ole e 1 and Par j 1-Par j 2 content using enzyme-linked immunosorbent assay procedures.

Results: Day-to-day variations were observed in both the pollen count and the amount of allergens. Pollen counts and aeroallergen quantification were closely correlated with 99% significance (*Olea*/Ole e 1: $R = 0.892$, $P < .001$; *Urticaceae*/Par j 1-Par j 2: $R = 0.734$, $P < .001$).

Conclusion: The technique for the sampling and quantification of aeroallergens presented in this article, based on enzyme-linked immunosorbent assay and applied to the protein extracts directly obtained from the bioaerosol, represents an important advance in the epidemiologic study of allergic respiratory diseases.

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INTRODUCTION

Type I allergic disorders, such as rhinoconjunctivitis, atopic eczema, and bronchial asthma, are global problems that afflict up to 25% of the population in industrialized countries.^{1,2} Allergens from dry pollen grains are cytoplasm proteins,^{3–5} but in gymnosperms they are also situated in orbicules and walls.^{6,7} During hydration in the germination medium or isotonic media, these allergens elute within minutes.^{3–5,7,8} The same or similar biochemical processes can occur in the mucosa of sensitized patients and may induce allergic reactions.^{3–5,9,10} Although this mechanism might be responsible for symptoms of rhinoconjunctivitis, it cannot explain the occurrence of allergic asthma during or after the pollination period.^{11–17} Various submicronic particles may be responsible for allergic asthma, because intact pollen grains are unlikely to intrude into the deeper airways because of their size.^{12,18–25}

Methods for measuring allergen exposure are based on counting pollen grains in air samples^{26–29} or using antibody-

based allergen reaction systems,^{15,19,22,30,31} as well as polymerase chain reaction analysis^{32,33} or surface plasmon resonance.³⁴ The most essential information for pollinosis patients is the amount of airborne allergens and their temporal distribution.²⁷ Future epidemiologic studies should measure allergens rather than pollen grain counts.

In this study, the airborne content of the pollen types *Olea* and *Urticaceae* was quantified using conventional count and microscopy identification techniques, whereas the allergenic content of the atmospheric bioaerosol was quantified using an enzyme-linked immunosorbent assay (ELISA) double sandwich technique for the allergens Ole e 1 and Par j 1-Par j 2. These allergens are present in pollen from olive trees (*Olea europaea*) and weeds (*Parietaria judaica*), which constitute the most important allergen sources in Mediterranean countries.^{35–38}

METHODS

Bioaerosol Sampling

For traditional pollen counts, the well-known Hirst³⁹ method sampler VPPS 2000 (Bologna, Italy) developed by Lanzoni was used. It is an exclusive programmed sampler for particles between 2 and 200 μm in diameter, with a driving speed of 2 mm/h and a flow of 10 L/min. A 345-mm Melinex tape impregnated with a silicone mixture was exposed for 7 days.

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After sampling, it was cut into 48-mm intervals and each piece was put on a 76 × 26-mm microscope slide and covered with glycerol gelatin (Sigma, St Louis, MO) that contained fuchsin as a staining agent.

For aeroallergen quantification, we used a Burkard Cyclone sampler (Burkard Manufacturing Co Ltd, Hertfordshire, England), which consisted of a continuous volumetric sampler with wind orientation, using a single reverse-flow miniature cyclone with an air throughput of 16.5 L/min. The instrument offers 100% efficiency sampling down to a 1.06- μ m range, decreasing to 93.82% for the size range 0.82 to 0.75 μ m. The efficiency of particle capture in the pollen size ranges was evaluated at the Pollen Research Unit.²⁰ Particles were collected directly into a 1.5-mL Eppendorf vial. This device was working from March 17 through July 1, 2003. The samples correspond to daily periods on weekdays and to aggregates during weekends and holidays. Airborne samples were stored at -20°C. Both samplers were located in the same sampling station.

Pollen Counts

Pollen identification was conducted by experienced personnel and was based on comparison with reference slides and pollen. An Olympus BH-2 microscope with D-Plan optics (Olympus Technicas, Barcelona, Spain) was used for counting at ×500 magnification. Four longitudinal transects were analyzed. The number of pollen grains was calculated as previously described.⁴⁰ To compare pollen count with aeroallergen concentrations, total pollen concentrations were calculated in the samples taken in the same periods used to collect the aeroallergens.

Meteorological Data

Rain data were obtained from the Spanish National Institute of Meteorology and aggregated in the same periods used to sample and quantify the aeroallergens.

Extraction of Allergens From Airborne Samples

Airborne samples collected in 1.5-mL Eppendorf vials were extracted using a modification of the method described by Takahashi et al.³⁴ Dry samples were extracted with 110 μ L of extraction buffer (50-mmol/L phosphate buffer, pH 7.4, that contained 150-mmol/L sodium chloride, 125-mmol/L ammonium bicarbonate, 3-mmol/L EDTA, and 0.005% Tween 20) for 2 hours at room temperature. The extract was separated from the particulate matter by centrifugation at 5,000g for 10 minutes and stored at -20°C.

Quantification of Ole e 1 and Par j 1-Par j 2 by ELISA

Ole e 1 and Par j 1-Par j 2 contents in aerosol samples were quantified using specific 2-site antibody ELISAs as previously described.^{36,41} Briefly, microtiter plates (Maxisorp, Nunc, Denmark) were coated overnight at 4°C with specific monoclonal antibody (5A3 at 0.25 μ g per well for Ole e 1 and 5D4 at 0.5 μ g per well for Par j 1-Par j 2). Coated wells were blocked with 200 μ L of phosphate-buffered saline that contained 1% bovine serum albumin and 0.05% Tween 20 (PBS-

B-T). Afterward, they were sequentially incubated with 100 μ L per well of purified Ole e 1, Par j 1-Par j 2, or extracted airborne samples, biotin-labeled allergen-specific rabbit antiserum (2.5 μ g/mL for Ole e 1 and 625 ng/mL for Par j 1-Par j 2), and streptavidin-peroxidase (0.25 μ g/mL) (Sigma) in PBS-B-T. All the incubations were performed at 37°C for 1 hour, with 3 washes of 200 μ L per well of PBS-B-T between successive steps. Finally, wells were incubated at room temperature in the dark with a solution of *o*-phenylenediamine (Sigma). The reaction was stopped after 30 minutes by adding 3M H₂SO₄, and the absorbance was then measured at 492 nm. Crude extracts were assayed as dilution series in triplicate, and the concentrations were interpolated from the linear portion of the standard curve.

Ole e 1, used as reference material, was purified from olive pollen extract by gel filtration and reverse-phase chromatography, as previously described.⁴² Par j 1-Par j 2 allergens, used as reference material, were purified from *P. judaica* pollen extract by affinity chromatography using a specific polyclonal rabbit antibody as previously described.⁴¹ Dose response curves obtained for purified Ole e 1 and Par j 1-Par j 2 were shown (Fig 1).

Correlation of ELISA Results and Pollen Grain Counts

Pollen concentration was compared with allergen concentration for the same intervals. Data were analyzed using the Pearson correlation coefficient and linear regression to investigate the correlation between pollen and aeroallergen levels. SPSS statistical software, version 11.0 (SPSS Inc, Chicago, IL) was used for all statistical analyses.

RESULTS

Pollen Counts

The pollen type *Olea* appears in the Cartagena aerosol in the spring, the first appearance usually occurring during the second half of April. Pollen counts indicate an intense bloom lasting for approximately 8 weeks, with maximal daily concentrations in May. In 2003, the main pollination period was from May 2 to June 5, reaching a maximum count on May 16 with 453 grains/m³. The total count for this period was 1,950 grains/m³. Figure 2A shows the *Olea* total grains measured in each of the samples taken to identify the aeroallergens.

The pollen type Urticaceae comprises pollens of the *Urtica* and *Parietaria* genera that cannot be distinguished morphologically.¹³ Urticaceae is present in the Cartagena bioaerosol throughout the year, with maximum values in the spring (in April and May). In 2003 we counted a total of 2,168 grains/m³ of this pollen type: 2,021 grains/m³ from February 27 through July 1. The highest counts were 78 grains/m³ on April 27 and 77 grains/m³ on May 25. Figure 2B shows the total grain counts of Urticaceae in the period corresponding to each one of the samples taken to quantify aeroallergens.

Aeroallergen Quantification

Extracts obtained from samples collected from May 2 through June 5, 2003, were used for Ole e 1 quantification,

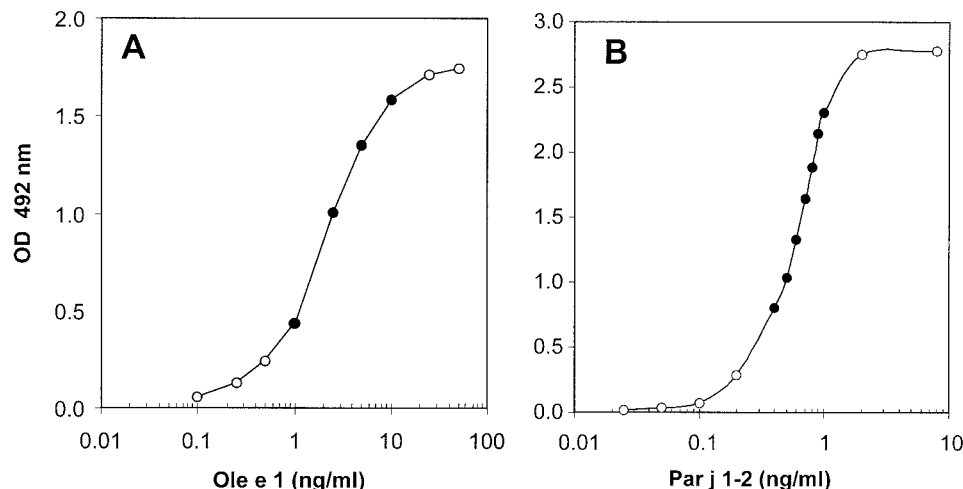


Figure 1. Enzyme-linked immunosorbent assay dose response curve for Ole e 1 purified from olive pollen extract (A) and Par j 1-Par j 2 purified from *Parietaria judaica* pollen extract (B). OD 492 nm indicates absorbance at 492 nm vs protein concentration. Closed circles indicate the practical working range used for detection.

and samples from March 17 through July 1, 2003, were used for Par j 1-Par j 2. Figure 2 shows the concentrations of the aeroallergens Ole e 1 and Par j 1-Par j 2 measured in the atmospheric bioaerosol of Cartagena. Approximately 60 grains of *Olea* are necessary per sample to detect Ole e 1 in the air. To detect the allergenic content of Par j 1-Par j 2 in the bioaerosol sample extracts, approximately 30 grains of Urticaceae (*Parietaria*) are needed per sample.

Correlation of ELISA Results and Pollen Grain Counts

Figure 3A shows the scatter diagram of the concentrations of Ole e 1 vs the concentration of *Olea* grains present in the samples during the same periods. The Pearson correlation coefficient for Ole e 1 and *Olea* is 0.892 ($P < .001$), the determination coefficient R^2 is 0.796, the slope of the regression line is 0.012, and the y-intercept is 4.35. Figure 3B shows the scatterplot of the concentrations of Par j 1-Par j 2 vs the counts of Urticaceae. The Pearson correlation coefficient for Par j 1-Par j 2 and Urticaceae is 0.734 ($P < .001$), the determination coefficient R^2 is 0.538, the slope of the regression line is 0.799, and the y-intercept is 187.36.

DISCUSSION

The onset of allergic symptoms in patients with pollinosis is often correlated with the number of airborne pollen grains.^{18,26,43} However, this correlation is not always close,²⁷ and pollen counts do not reflect the allergenic activity of airborne particles,¹⁵ because allergens may also be carried in a microaerosol suspension smaller than pollen grains.^{11,12} Furthermore, meteorologic conditions may favor the elution of allergenic proteins in humid environments^{3-5,7} or cause abortive pollen germination in which the pollen tubes break and release respirable micronic and submicronic particles that contain the major allergens.¹⁷ Thus, the aeroallergens present as submicronic particles in the atmospheric aerosol and those

coming from pollen grains inhaled by sensitive people are the triggers for allergic symptoms. Therefore, a good approach in aerobiology is to quantify the total allergenic load (pollen grains and allergenic proteins) of the bioaerosol for each of the main allergens in the known pollen types of a given geographic area.

The Burkard Cyclone sampler combined with immunochemical assays used in this study was a simple method for sampling and quantifying aeroallergens. This sampling method was highly efficient in terms of capturing all sizes of pollen grains and micrometric and submicrometric particles,²⁰ which made it ideally suited for the purposes of our investigation. The sampling conducted through the direct collection of aerosol particles in an Eppendorf tube facilitated the elution of aeroallergens and the final collection in a single sample. This process simplified the analytical determination by eliminating the manipulation of multiple fractions, which makes the entire procedure more cumbersome and expensive. Therefore, we believe that this sampling method could be easily and quickly implemented in aerobiological laboratories that routinely perform pollen counts. The ELISA sandwich method used for allergen quantification shows a high degree of reproducibility and specificity.³⁶

Day-to-day variations were observed in both the pollen count and the amount of allergens. Airborne allergenic activity for Ole e 1 shows a first peak between May 15 and 22 and a second peak between May 22 and 26. The quantification of aeroallergens using the immunologic method was significantly correlated with pollen counts during the period studied. In the case of *Olea*, the quantification of aeroallergens closely follows the pollen counts (Fig 3A), with a Pearson correlation coefficient of 0.892 ($P < .001$; $R^2 = 0.796$) between the counts of *Olea* and the concentrations of Ole e 1; this indicates the presence of allergens in the samples coin-

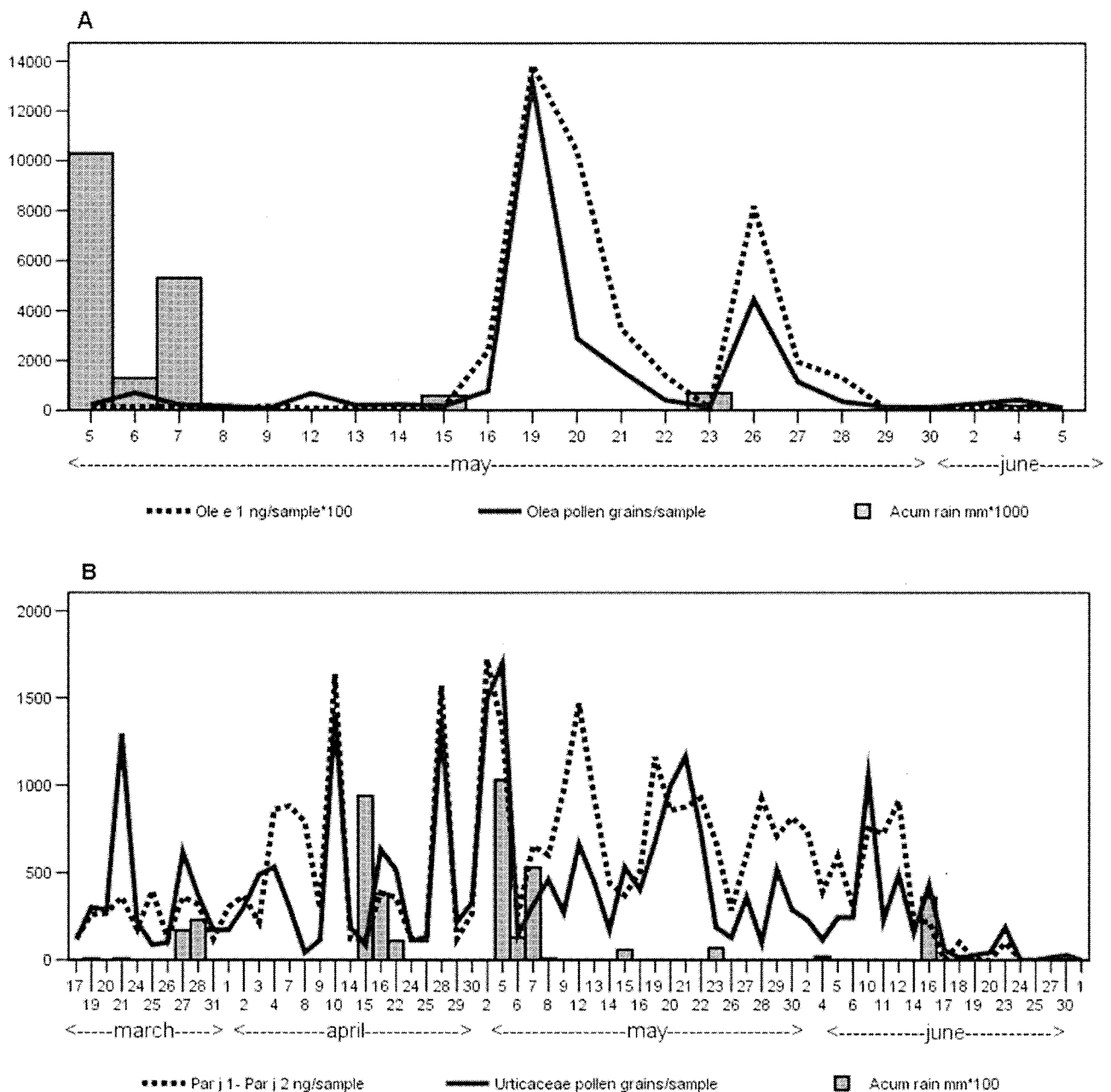


Figure 2. Concentrations of *Olea* (A) and Urticaceae (B) pollen obtained with the Hirst method, reported as accumulated values for aeroallergen sampling period (solid line). The Ole e 1 (A) and Par j 1-Par j 2 (B) allergen concentrations were determined by the enzyme-linked immunosorbent assay technique (dashed line). Accumulated precipitation for aeroallergen sampling period is also shown (bars).

ciding with the presence of the pollen type in the air. Proteins similar to the Ole e 1 allergen were detected in different species of the Oleaceae family (*Syringa vulgaris*, *Fraxinus excelsior*, and *Ligustrum vulgaris*).⁴⁴ Nevertheless, *S vulgaris* and *F excelsior* were not present in the area of our study, and the flowering of *L vulgaris* began after the pollination of *Olea*. For this reason, the Ole e 1 allergen detected in the

bioaerosol corresponds to the *Olea* pollination and corroborates the correlation between the *Olea* pollen counts and the allergen quantified.

Par j 1-Par j 2 allergen content also displayed fluctuations similar to those shown by the pollen counts. Only at the beginning of the period under study were the Urticaceae pollen counts higher than the aeroallergen concentrations.

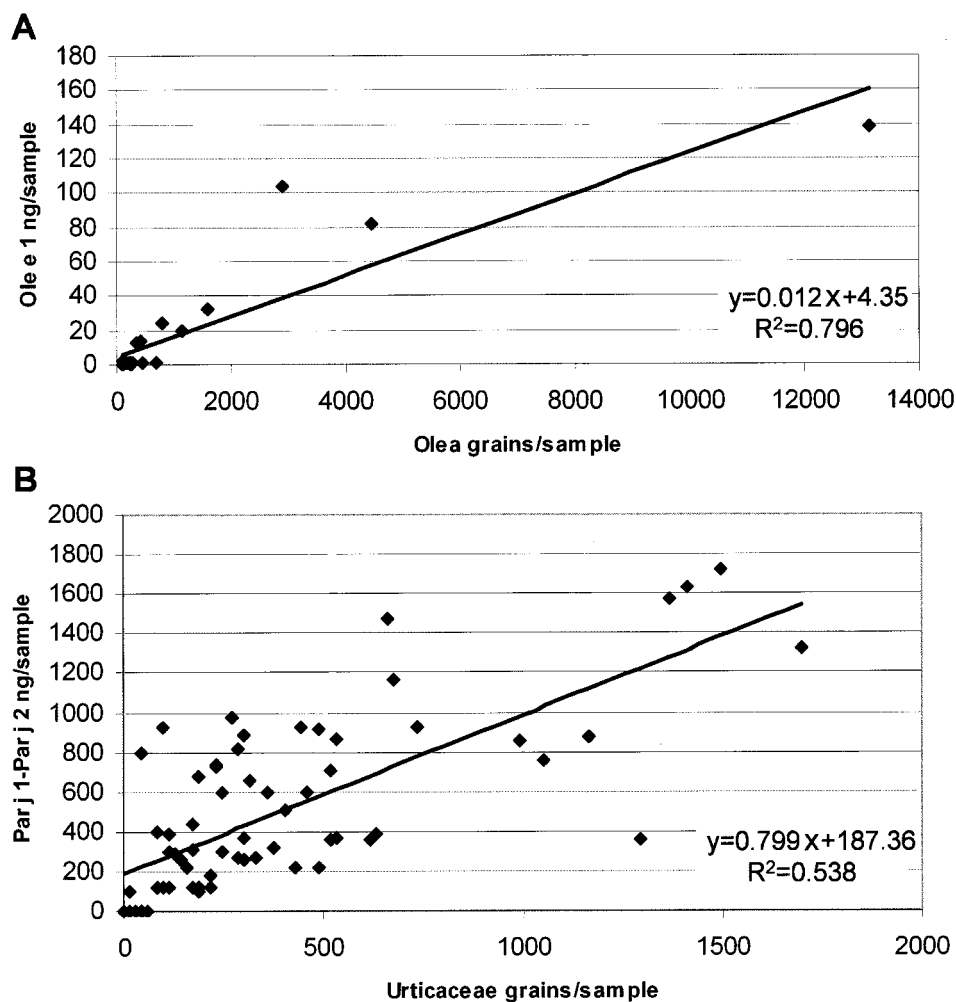


Figure 3. Correlation between pollen counts and aeroallergen quantitation for *Olea* vs Ole e 1 (A) and Urticaceae vs Par j 1-Par j 2 (B).

After April 4, the aeroallergen concentration values were equal to or higher than the pollen counts. We found 3 periods in which the aeroallergen concentration exceeded the values of the pollen counts in samples from April 6 to April 9, May 7 to May 14, and May 27 to June 12. The second period was preceded by an accumulated precipitation value of 16.9 mm. An increase in aeroallergen concentrations after rain episodes has been reported,^{17,43} and the present results appear to confirm the hypothesis that pollen aeroallergens are released during rain episodes and either become resuspended on the surface of particles or become airborne independently.^{11,12,19} Some authors argue that rain episodes result in an increase of small airborne antigenic fractions because the pollen deposited on various surfaces releases its proteins when wetted and, after drying, this material may again become airborne.¹⁹

Lower correlation (Pearson correlation coefficient = 0.734; $P < .001$; $R^2 = 0.538$) was found between Urticaceae counts and Par j 1-Par j 2 content (Fig 3B). This weaker

association could be due to the presence of pollen grains from *Urtica* and *Parietaria*, both present in the study area, which are undistinguishable with an optical microscope.¹³ *Parietaria* is one of the most important causes of pollinosis in Mediterranean countries,³⁵ whereas *Urtica* is a nonallergenic plant and does not cross-react with *Parietaria*.^{13,35} Therefore, the results obtained could be valuable for epidemiologic studies that compare allergic symptoms with the real concentrations of *Parietaria* aeroallergens.

In summary, these results emphasize the importance of measuring allergen exposure through the detection of the allergen load in the aerosol, rather than relying solely on counting pollen grains. This method is highly specific, which enables the identification of the specific allergens of pollen grains that are undistinguishable morphologically. The application of this specific and quantitative antigen-antibody technique to control the content of allergens in the air represents an important advance in the epidemiologic study of allergic respiratory diseases.

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