

## Original article

# Allergenicity and cross-reactivity of Russian olive pollen (*Eleagnus angustifolia*)

**Background:** The purposes of this study were: to determine the prevalence of sensitization and immunochemical characterization of *Eleagnus angustifolia* pollen (Russian olive) that belongs to the family Eleagnaceae.

**Methods:** A total of 134 patients with rhinoconjunctivitis and/or asthma were studied. Its allergenicity, cross-reactivity with olive pollen and the presence of *Ole e 1* and *Ole e 4*-like molecules were evaluated.

**Results:** *Eleagnus angustifolia* pollen was detected from May to June. Seventy-three of 134 (30.5%) had positive skin test to *E. angustifolia*, all of them were positive to olive. There was a good correlation between specific immunoglobulin (Ig)E levels to *E. angustifolia* and *Olea europaea* ( $r = 0.77$ ,  $P = 0.002$ ). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) immunoblots revealed major IgE-binding bands in the *E. angustifolia* extract of 43 and 63.7 kDa. The *E. angustifolia* extract was not able to inhibit olive, whereas *O. europaea* inhibited *E. angustifolia* up to 41%. The presence of *Ole e 1*- and *Ole e 4*-like allergens in *E. angustifolia* extract was confirmed by enzyme-linked immunosorbent (ELISA) inhibition assays. Nasal challenge with *E. angustifolia* was positive in three of six patients with positive skin test to both pollens and negative in five patients with positive skin test only to *O. europaea*.

**Conclusions:** This study confirms that *E. angustifolia* is capable of sensitizing individuals in Madrid. A minimal-to-moderate cross-reactivity with olive pollen was established, suggesting some cross-reactivity but not excluding co-sensitization.

**J. Sastre<sup>1</sup>, M. Lluch-Bernal<sup>1</sup>,  
A. M. G. Bustillo<sup>2</sup>, J. Carnés<sup>3</sup>,  
F. Marañón<sup>3</sup>, M. Casanovas<sup>3</sup>,  
E. Fernández-Caldas<sup>3</sup>**

<sup>1</sup>Fundación Jiménez Díaz, Servicio de Alergia, Universidad Autónoma de Madrid, Madrid;

<sup>2</sup>Department of Botany, Faculty of Pharmacy, Universidad Complutense de Madrid, Madrid;

<sup>3</sup>C.B.F. LETI S.A., Tres Cantos, Madrid, Spain

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Joaquín Sastre MD, PhD  
Fundación Jiménez Díaz  
Servicio de Alergia  
Avda. Reyes Católicos 2  
28040 Madrid  
Spain

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*Eleagnus angustifolia* (Russian olive, oleaster or silverberry in English, or árbol del paraíso, matapolilla, panjé, or olivo de Bohemia in Spanish) belongs to the family Eleagnaceae. It has invaded zones along watercourses in many arid and semiarid regions of the world, and is also used as an ornamental tree in many European cities. The colours of the leaves vary from silvery grey-green to dark green (Fig. 1) and the trees pollinate during the spring. Although mainly pollinated by insects, Russian olive pollen can be identified in air samples. The pollen is 40–56 microns in diameter (Fig. 1).

Its allergenicity was suggested in 1992 by Kernerman et al. (1) in the United States of America. The investigators performed inhibition studies to determine if pollen extracts of *Olea europaea*, ash (*Fraxinus americana*), privet (*Ligustrum vulgare*), and Russian olive (*Eleagnus angustifolia*) could inhibit immunoglobulin (Ig)E antibody binding to olive pollen extracts. The inhibition studies demonstrated that the tree pollen extracts were capable of inhibiting specific IgE-binding to the olive extract in a dose–response manner. The IgE-immunoblots demonstrated several proteins

common to olive, ash, and privet. The authors concluded that there is a high degree of cross-reactivity between native Michigan trees and olive trees. This cross-reactivity is the most likely cause for skin test reactivity to olive pollen extracts in allergic patients residing in Michigan. To the best of our knowledge, no other reports have been published on the potential allergenicity of Russian olive pollen.

In the Madrid area, there are approximately 2000 *E. angustifolia* trees (personal communication, Madrid, City Hall) and its pollen is monitored in the ambient air of the city since 1997.

The objectives of this study were: (i) to determine the aerobiology of this pollen in the area of Madrid, (ii) to determine the prevalence of positive skin test among patients with rhinoconjunctivitis and/or asthma, (iii) to determine the clinical relevance of sensitization to *E. angustifolia* by nasal challenges, (iv) to study its allergenicity by immunoblots and its allergenic cross-reactivity with olive pollen, (v) to evaluate the presence of *Ole e 1* and *Ole e 4*-like molecules in the extract of *E. angustifolia*.



Figure 1. (A) *Eleagnus angustifolia* tree, (B) flowers and (C) pollen of *E. angustifolia* ( $\times 40$  magnification).

## Materials and methods

### Pollen counts

*Elaeagnus angustifolia* pollen counts were determined daily since 1997 in the station of the Faculty of Pharmacy at the Universidad Complutense of Madrid, and in other Madrid stations, which belong to the Spanish aerobiological monitoring network. Pollen was collected using a Burkard volumetric Spore-Trap located at the roof of the building. The sampling period included from January to December, 1998–2002.

### Pollen extracts

*Elaeagnus angustifolia* and *O. europaea* pollens (Biopol Laboratory, Inc., Spokane, WA, USA) were extracted overnight, 1 : 20 w/v in phosphate-buffered saline (PBS) 0.01 M, dialyzed in 3.5 kDa

dialysis membranes and freeze-dried. The freeze-dried extract was reconstituted in 50% glycerine solution at a concentration of 2 mg/ml and filtered through a 0.2 micron filter for skin testing.

### Patient population

A total of 134 consecutive patients, who complained from seasonal rhino-conjunctivitis and/or asthma symptoms, were evaluated in an outpatient clinic. Skin prick tests were performed following recommendations of the European Academy of Allergy and Clinical Immunology.

### Skin test reagents

Standardized allergen extracts of mites, pollens, animal danders, moulds and cockroaches were obtained from C.B.F. LETI S.A.

(Madrid, Spain). A 50% glycerine/saline solution and HCl histamine solution at 10 mg/ml were used as negative and positive controls, respectively. A skin test was considered positive if the wheal was at least 3 mm greater than the negative control.

#### Specific IgE by direct ELISA and specific IgE inhibitions

Specific IgE-binding to *E. angustifolia* and *O. europaea* extracts and inhibition experiments were conducted using methods previously described (2, 3). A result was considered positive when a serum bound four times more than a negative control.

Specific IgE inhibition experiments were conducted in a similar manner; 125 µl of serial dilutions of pollen extracts (at equal protein concentrations), respectively, were mixed with 125 µl of a serum pool, diluted 1 : 10, containing high titres of specific IgE to *E. angustifolia* or *O. europaea* allergens. These mixtures were shaken for 2 h at room temperature. After the inhibition period, 100 µl of these mixtures were added to the coated wells (as previously described) for an overnight incubation. Afterwards, the assay was completed as for the specific IgE determinations. *Ole e 1* and *Ole e 4* were purified following methods previously described (2, 3) and used as inhibitors in the same assay.

#### SDS-PAGE and immunoblots

Separation by electrophoresis was accomplished in acrylamide gels according to the method of Laemmli (4). Pollen extracts were used under reduced conditions; 15% separating and 4% packing gels were used. Immunoblots with individual sera and serum pools were carried out with both extracts. Separated proteins were transferred to polyvinylidene fluoride (PVDF) membranes, Immobilon-P (Millipore, Bedford, MA, USA) in a Trans Blot (BioRad, Hercules, MA, USA) tank in a basic buffer. After the transfer, the membranes were allowed to dry and incubated overnight in a humid chamber at room temperature with the individual sera (diluted 1 : 3), or serum pools of patients (diluted 1 : 40) in a PBS 0.01 M Tween 20 solution. The strips were then washed with 0.1% PBS Tween and incubated for 2 h at room temperature with peroxidase-labelled monoclonal human anti-IgE (Ingenasa, Madrid, Spain) at a concentration of 1 µg/ml and 3% human serum albumin (HSA). After several washes, the enzymatic reaction took place in a solution composed of a 0.1 M citrate buffer (pH 5.0) containing 10 mM ethylenediaminetetraacetic acid (EDTA) and 1% dextran sulphate (1 ml), tetramethyl benzidine (TMB) in dimethyl sulphoxide (DMSO) (20 µl) and 1 M H<sub>2</sub>O<sub>2</sub> (2 µl).

#### Nasal challenges with *E. angustifolia*

Nasal challenges were performed in six patients with positive skin tests to *O. europaea* and *E. angustifolia* and in five patients positive to *O. europaea* but negative to *E. angustifolia*. Nasal corticosteroids and antihistamines were withdrawn 3 weeks and 3 days before the start of the study, respectively. Before starting nasal challenges, patients waited for 30 min in order to give the nasal mucosa time to acclimatize to local conditions. Nasal challenges were performed with two increasing doses of *E. angustifolia* extract (0.1 and 1 mg/ml) at 15 min intervals after a sham-challenge with PBS. Extracts were sprayed into each nostril by means of a nasal pump spray delivering a fixed dose of 0.125 ml solution. The nasal response was measured 15 min after each challenge. Nasal responsiveness was monitored by the number of sneezes, and acoustic rhinometry (Rhinometrics SRE 2100, Assens, Denmark). Minimal cross-sectional area (MCA) measured at the head of the inferior

turbinate was used as end-point. Three measurements in each nasal cavity were performed during quiet oral respiration within 1 s at each allergen concentration and automatically integrated by computer. Averages of the pre- and post-triplicate measures were used for analysis. The presence of a decrease in MCA of 30% of baseline and five or more sneezes over a period of 15 min was considered as positive test (5). The study was performed in the month of October–November to minimize exposure to pollens. All patients signed written informed consent forms.

## Results

#### Pollen counts

*Eleagnus angustifolia* pollen appeared from mid-May to June. Counts varied from 0 to 5 grains/m<sup>3</sup> and total annual count from 0 to 32. Total annual counts varied from 0 to 54 grains in the different stations in Madrid. During these 2 months, the most frequent pollen types collected were *O. europaea* and grass pollens.

#### Skin test results

Ninety-four of the 134 subjects (70.1%) had a positive skin test to pollens (grasses, weeds and trees); 73 (54.4%) had a positive reaction to *O. europaea* (olive) and 41 (30.5%) were positive to *E. angustifolia*. All patients with a positive skin test to *E. angustifolia* were also positive to olive and other pollens. Seventy percentage of patients were also sensitized to perennial allergens, mainly pet danders and mites.

#### Nasal challenge with *E. angustifolia*

Three of six patients skin test positive to *O. europaea* and *E. angustifolia* had a positive nasal challenge with *E. angustifolia*. All five patients with positive skin tests to *O. europaea* but negative to *E. angustifolia* had negative nasal challenges.

#### Specific IgE by direct ELISA

A total of 26 sera were evaluated for specific IgE to both extracts. Seventeen sera were positive to both extracts and a significant correlation between specific IgE levels to *E. angustifolia* and olive ( $r = 0.77$ ,  $P = 0.002$ ) was obtained in this group. Nine sera were positive to olive and negative to *E. angustifolia*. When all the sera were analysed, the correlation coefficient was  $r = 0.89$ ;  $P < 0.0001$ .

#### ELISA inhibition

A serum pool containing the 17 positive sera was prepared and used in enzyme-linked immunosorbent (ELISA) inhibition assays. The ELISA inhibition experiments demonstrated minimal-to-moderate cross-reactivity with

*O. europaea*. The *E. angustifolia* extract was not able to inhibit olive at the concentrations tested, whereas *O. europaea* inhibited *E. angustifolia* up to 41%.

Presence of *Ole e 1*- and *Ole e 4*-like allergens

The presence of *Ole e 1*- and *Ole e 4*-like allergens in *E. angustifolia* extract was confirmed by ELISA inhibition.

*Ole e 1* needed 1.03 µg to get 50% inhibition of the *O. europaea* extract and 0.54 µg of the *E. angustifolia* extract. *Ole e 4* needed 0.39 µg to get 50% inhibition of the *O. europaea* extract and 2.51 µg to get the same inhibition of *E. angustifolia* extract.

SDS-PAGE and immunoblots

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of both pollens is shown in Fig. 2. Different band pattern was observed. The SDS-PAGE immunoblots using highly reactive serum revealed major IgE-binding bands in the *E. angustifolia* extract with molecular weights of 37, 43, 63.7 and 77.4 kDa in 40%, 65%, 50% and 45% of patients, respectively (Fig. 3). Figure 4A, B show the results of the immunoblot inhibition experiments. After incubation of the *O. europaea*- and *E. angustifolia*-positive serum pool with olive extract, no bands were visualized in the *E. angustifolia* extract. When the same serum pool was incubated with *E. angustifolia* extract, no bands were detected in the *E. angustifolia* extract, suggesting a high degree of cross-reactivity.

Discussion

In this study, we describe the allergenicity of *E. angustifolia*, a tree which is used in Madrid for ornamental and wind-cutting purposes. This tree is frequently planted in other European cities as ornamental trees and, therefore, sensitization to its pollen cannot be discarded in other

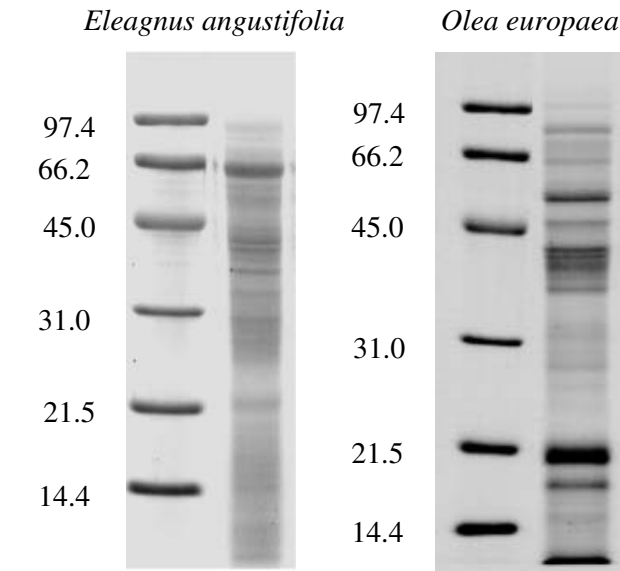


Figure 2. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of *Olea europaea* and *Eleagnus angustifolia* pollen extracts.

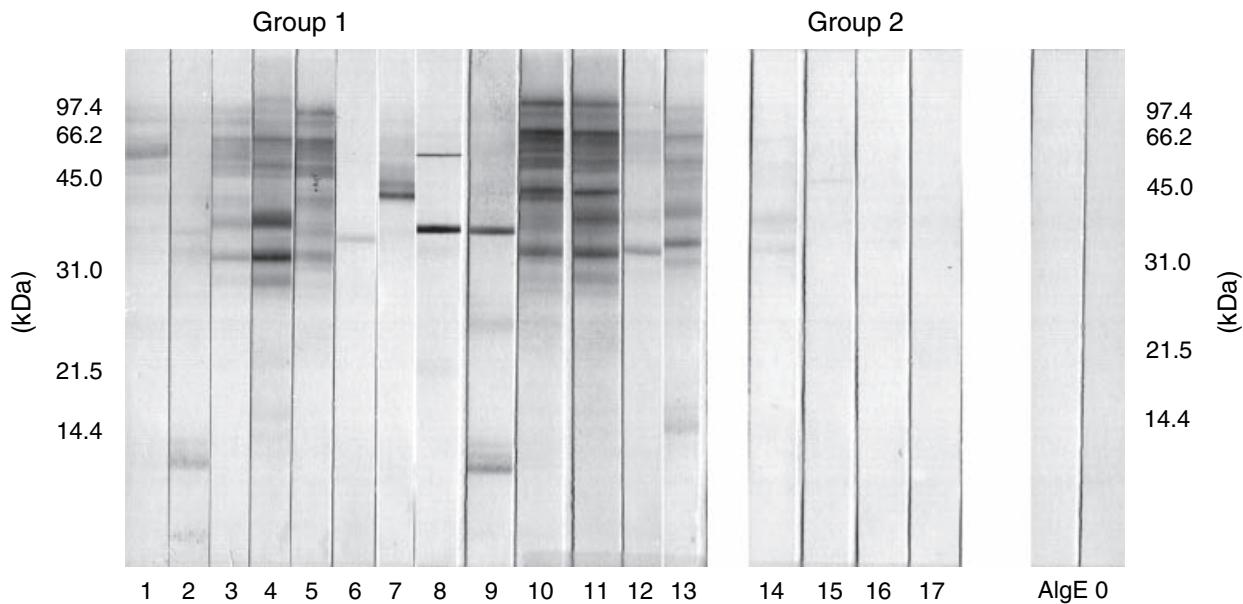


Figure 3. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) immunoglobulin (Ig)E immunoblots of *Eleagnus angustifolia* pollen extract. Group 1 sera of patients allergic to *E. angustifolia* and *Olea europaea*; group 2, sera from patients allergic only to *O. europaea*.

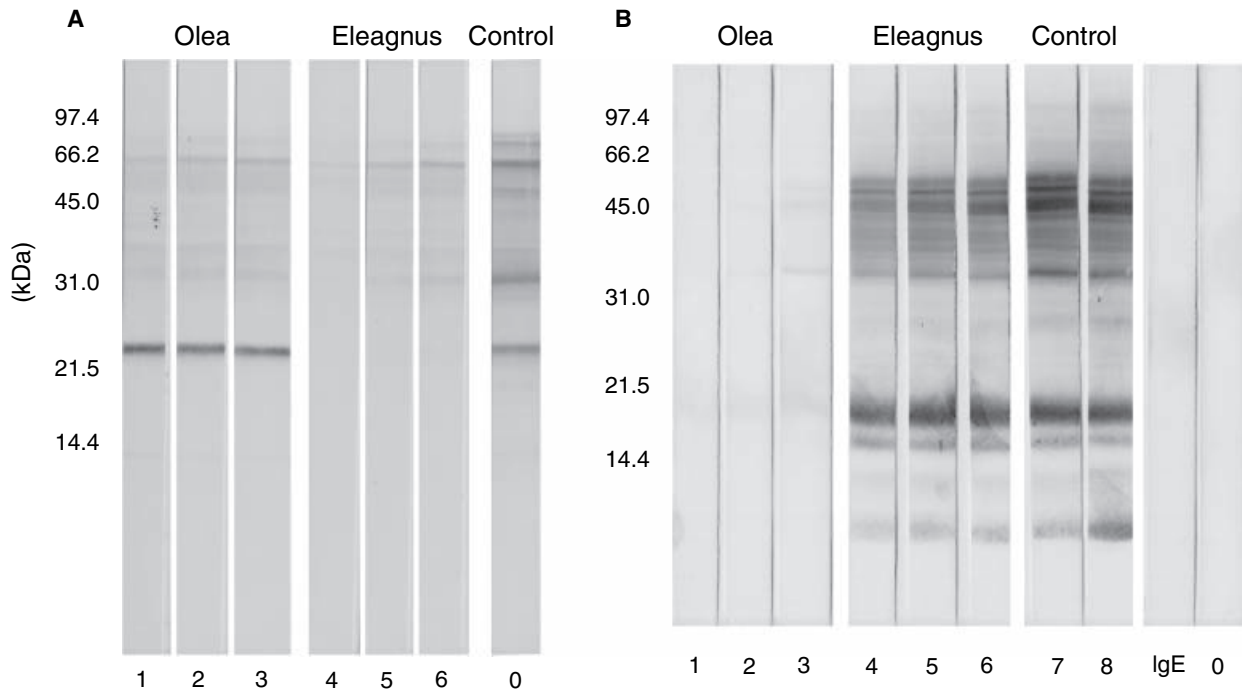


Figure 4. (A) Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) immunoblots inhibition assays. Solid-phase *Eleagnus angustifolia* with a pool of sera of *E. angustifolia*-sensitized patients. Inhibitors: 1, *Olea europaea* 2 mg/ml; 2, *O. europaea* 1 mg/ml; 3, *O. europaea* 0.5 mg/ml; 4, *E. angustifolia* 2 mg/ml; 5, *E. angustifolia* 1 mg/ml; 6, *E. angustifolia* 0.5 mg/ml; 0, control. (B) SDS-PAGE immunoblots inhibition assays. Solid-phase *O. europaea* with a pool of sera of *E. angustifolia*-sensitized patients. Inhibitors: 1, *O. europaea* 2 mg/ml; 2, *O. europaea* 1 mg/ml; 3, *O. europaea* 0.5 mg/ml; 4, *E. angustifolia* 2 mg/ml; 5, *E. angustifolia* 1 mg/ml; 6, *E. angustifolia* 0.5 mg/ml; 7 and 8, controls; IgE, IgE background; 0, blank.

countries. Local exposure in backyards, alleys or boulevards where this tree is planted must be considered, as, in these areas, pollen counts may exceed those registered in pollen counting stations (6, 7).

The pollination of *E. angustifolia* takes place in Madrid from May to June, coinciding with the pollination period of olive trees and grasses. The pollen counts of *E. angustifolia* registered at local pollen stations can be considered as low, but must be considered in the context of the 2000 trees planted in and around the city. A cause of low *E. angustifolia* pollen count obtained in a roof level station could be the relative large size (40–56  $\mu$ ) of the pollen in comparison with other allergenic pollens such as grasses or olive.

Pollen counts which produce symptoms in pollen allergic individuals are variable (8) and, based on the data presented in this study; we cannot exclude the possibility that *E. angustifolia* pollen induces sensitization and symptoms. Therefore, authorities and allergists should be aware of the sensitization potential of *E. angustifolia* and it seems warranted to closely follow a potential increase in sensitization to this pollen. Increases in sensitization have occurred in cities where plane trees, Cypress and olive trees have been widely planted (9–11).

It cannot be excluded that a positive skin test to olive pollen in areas where olive trees are not present may be

due to a sensitization to *E. angustifolia*, or other cross-reactive pollens as has been suggested by Kernerman et al. (1). In this study, conducted in Michigan, USA, where olive trees are nonexistent, nine of the 12 olive skin test-positive patients (75%) confirmed that they were exposed to 1, or more of the studied trees in their yards. Five patients had also traveled to areas where olive trees were grown. Gonzalez et al. (12) have studied immunological cross-reactivity of olive pollen with other pollens but *E. angustifolia* was not included in the study.

Sensitization to *E. angustifolia* pollen by prick test in Madrid was detected in 30.5% of patients with rhinitis and/or asthma and in 44% of pollen-sensitive patients. All patients with a positive skin test to *E. angustifolia* were positive to olive. However, none of the patients was monosensitized to *E. angustifolia* pollen. Because of the coincident pollination with olive and grasses, it was not possible to determine a relationship between exposure and clinical symptoms. We were able to establish a good correlation between specific IgE levels to *E. angustifolia* and olive pollen in a group of patients sensitive to both allergens ( $r = 0.77$ ,  $P = 0.002$ ). However, the correlation coefficient was even better when all the sera were included in the analysis ( $r = 0.89$ ,  $P < 0.0001$ ).

The results of the ELISA inhibition experiments demonstrated moderate cross-reactivity with an olive

pollen extract. The *E. angustifolia* extract was not able to inhibit olive, whereas the olive extract inhibited *E. angustifolia* up to 41%. Ole e 1 and Ole e 4, major allergens in olive pollen (13,14), seem to be present in the *E. angustifolia* pollen extract, as suggested by the ELISA inhibition assays. These findings deserve further studies, since Ole e 1-like molecules have been described in pollen extract of other plant species (15) and may be implicated cross-reactivity reactions.

The allergenic profile of *E. angustifolia* indicates the presence of several allergenic bands in the range 37 to 77 kDa. An allergen of 43 kDa was recognized by 65% of patients with positive SPT to *E. angustifolia* and another band of 63.7 kDa by 50% of sensitized patients, which could represent major allergens.

The clinical relevance of sensitization to *E. angustifolia* was confirmed by nasal challenge tests in a group of selected patients. Three out of six patients sensitized to *E. angustifolia* had a clinical response after being challenged with an extract of *E. angustifolia*. However, none of the 5 patients negative to *E. angustifolia*, but positive to *Olea europaea* did have a positive response. This fact implies that a nasal challenge test with

*E. angustifolia* could be a better and more sensitive test to discriminate between sensitized individuals with or without rhinitis.

In summary, this study confirms the presence of *E. angustifolia* pollen in the atmosphere of Madrid and the sensitizing capacity of this pollen. We have also confirmed that the inhalation of *E. angustifolia* allergens may induce nasal symptoms in sensitized patients. A minimal-to-moderate cross-reactivity was established with *O. europaea* pollen allergens, suggesting cross-reactivity but not excluding co-sensitization. *Eleagnus angustifolia* pollen extract contains approximately 20 IgE-binding bands. Ole e 1- and Ole e 4-like molecules are present in extracts of *E. angustifolia* pollen. To the best of our knowledge, this is the first prospective study to analyse the prevalence of sensitization to this pollen in Europe.

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### References

1. KERNERMAN SM, MCCULLOUGH J, GREEN J, OWNBY DR. Evidence of cross-reactivity between olive, ash, privet, and Russian olive tree pollen allergens. *Ann Allergy* 1992;**69**:493–496.
2. BOLUDA L, ALONSO C, FERNÁNDEZ-CALDAS E. Purification, characterization and partial sequencing of 2 new allergens of *Olea europaea*. *J Allergy Clin Immunol* 1998;**101**:210–216.
3. BOLUDA L, SASTRE J, CASANOVAS M, FERNÁNDEZ-CALDAS E. Determination of Ole e 1 by enzyme immunoassay and scanning densitometry. Validation by skin-prick testing. *J Immunol Methods* 1999;**223**:17–26.
4. LAEMMLI UK. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* 1970;**227**:680–685.
5. GANSLMAYER M, SPERTINI F, TERRIEN M, LEIMGRUBER A, RAHM F, MOSIMANN B. Evaluation of acoustic rhinometry in a nasal provocation test with allergen. *Allergy* 1999;**54**:974–976.
6. STEWART GA. Pollens and allergic disease: do not overlook your own backyard. *Clin Exp Allergy* 1993;**23**:537–541.
7. GINER MM. Unexpected pollinosis or insufficient pollen records? Importance of regional monitoring. *Int J Environ Health Res* 2002;**12**:187–192.
8. FRENZ DA. Interpreting atmospheric pollen counts for use in clinical allergy: allergic symptomology. *Ann Allergy Asthma Immunol* 2001;**86**:150–157.
9. VARELA S, SUBIZA J, SUBIZA JL, RODRIGUEZ R, GARCIA B, JEREZ M et al. *Platanus* pollen as an important cause of pollinosis. *J Allergy Clin Immunol* 1997;**100**:748–754.
10. PANZANI R, CENTANNI G, BRUNEL M. Increase of respiratory allergy to the pollens of cypresses in the south of France. *Ann Allergy* 1986;**56**:460–463.
11. D'AMATO G, SPIEKMA FT, LICCARDI G, JAGER S, RUSSO M, KONTOU-FILI K et al. Pollen-related allergy in Europe. *Allergy* 1998;**53**:567–578.
12. GONZALEZ EM, VILLALBA M, RODRIGUEZ R. Allergenic cross-reactivity of olive pollen. *Allergy* 2000;**55**:658–663.
13. CARNÉS SÁNCHEZ J, IRAOLA VM, SASTRE J, FLORIDO F, BOLUDA L, FERNÁNDEZ-CALDAS E. Allergenicity and immunochemical characterization of six varieties of *Olea europaea*. *Allergy* 2002;**57**:313–318.
14. QUIRALTE J, FLORIDO F, ARIAS DE SAAVEDRA JM, GOMEZ A, SAENZ DE SAN PEDRO B, GONZALEZ E et al. Olive allergen-specific IgE responses in patients with *Olea europaea* pollinosis. *Allergy* 2002;**57**(Suppl. 71):47–52.
15. OBISPO TM, MELERO JA, CARPIZO JA, CARREIRA J, LOMBARDERO M. The main allergen of *Olea europaea* (Ole e I) is also present in other species of the Oleaceae family. *Clin Exp Allergy* 1993;**23**:311–316.