

Ole e 4 and Ole e 5, important allergens of *Olea europaea*

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The pollen of *Olea europaea* is one of the most important aero-allergens in Mediterranean countries and elsewhere, including the USA, Argentina and Japan (1–4). The inhalation of the olive pollen is one of the most common causes of sensitization and allergic respiratory symptoms in genetically susceptible individuals in areas where olive cultivars are abundant.

Every year the high levels of olive pollen occur from May to June and the quantities vary every spring season, depending on the current climatic conditions and the weather of the first months of the year. As an example, in the Madrid area, the levels of olive pollen fluctuated between 150 grains/m³ in 1995 and 2000 grains/m³ in 1999 (Fig. 1, adapted from www.seaic.es).

In the last years there has been an increasing interest in the identification, purification, characterization and quantification of allergens in olive pollen allergen extracts (5,6) and some allergens of olive tree have been extensively studied. At the present time, nine allergens of *O. europaea* have been described, and several more await full characterization and sequencing.

In 1998 Boluda et al. (7), described the allergenic composition of different batches of olive pollen collected in Spain and the United States, and established qualitative and quantitative differences in the concentration of some allergens, in particular Ole e 1 and Ole e 4.

O. europaea is a tree belonging to the family Oleaceae. More than 30 genera and 600 species have been described in this family. The worldwide distribution of the different members of this family depends mainly of the specific biological requirements of the genera. It is therefore, a cosmopolitan

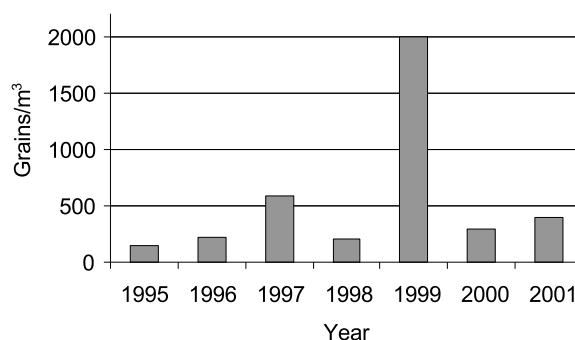


Figure 1. Number of pollen grains per m³ of *Olea europaea* during the last 6 years in Madrid, Spain.

botanical family. Most genera and species belonging to this botanical family are wind-pollinated trees or bushes.

An important and interesting aspect of the study of olive pollen allergy is the high degree of cross-reactivity that exists among trees and plants belonging to the same botanical family. Although, *O. europaea* is rare in northern Europe, other plants of the same family are very common in Nordic regions. Olive tree pollen contains the majority of the antigens triggering the clinical response in patients whose primary sensitization is to *O. europaea*. Other species, such as *Fraxinus excelsior* (ash tree), *Ligustrum vulgare* (privet) and *Syringa vulgaris* (lilac) contain similar allergens (8,9). More recently we have also described allergenic cross-reactivity between *O. europaea* and *Eleagnus angustifolia* (Russian olive) (10), an ornamental tree belonging to the family Eleagnaceae and closely related to Olive trees. These species seem to share not only Ole e 1 but also Ole e 4 homologs. *E. angustifolia* is widely planted in

Spain and other areas of the world, including central and northern Europe. It is also frequent in the margins of rivers and other humid areas and pollinates in the spring. Its allergenicity has been previously suggested in the USA by Kernerman et al. (11). In our study conducted in Madrid, Spain, all patients with a positive skin test to Russian olive were also positive to olive; but not all olive-positive patients were positive to Russian olive. There was a good correlation between specific IgE levels to Russian olive and olive ($r = 0.8$) in a group of patients sensitive to both allergens. By ELISA inhibition, the Russian olive extract was not able to inhibit olive, whereas an olive extract inhibited Russian olive up to 41%. This study confirms the sensitizing capability of Russian olive pollen in Spain. A minimal to moderate cross-reactivity was established, suggesting some cross-reactivity but not excluding cosensitization.

Antigenic and allergenic profile of *O. europaea* pollen extract

The antigenic profiles of several batches of *O. europaea* pollen show a wide range of proteins from less than 10 to more than 100 kDa. Nine of these proteins have been described as allergenic and have a variable degree of specific IgE recognition. Based on the amount of allergens present in olive, we expect more allergens to be described in the future. The isoelectric focusing pattern of the extracts shows bands in the pI range of 4 and 9.5; the majority of the allergens have an acidic isoelectric point. The majority of the described allergens have several isoforms.

The allergenic profile of the olive pollen extracts, as recognized by the specific IgE of olive pollen sensitized patients shows several IgE binding proteins/peptides. One of the most recognized proteins corresponds to Ole e 1, a dimer protein with a molecular weight of approximately 19 kDa. A large proportion of allergens can be detected between 30 and 60 kDa, and also a protein with low a molecular weight of approximately 10 kDa, described as Ole e 6. Allergen recognition varies depending on the geographic location of the patients and some discrepancies have been described between patients exposed to large quantities of olive pollen and patients residing in the Madrid area (12).

An important aspect of the study of *Olea* pollen extracts is the allergenic variability of the extracts, especially between those prepared with pollen collected in Spain, and in the USA. Recent experiments conducted in our Laboratory to compare

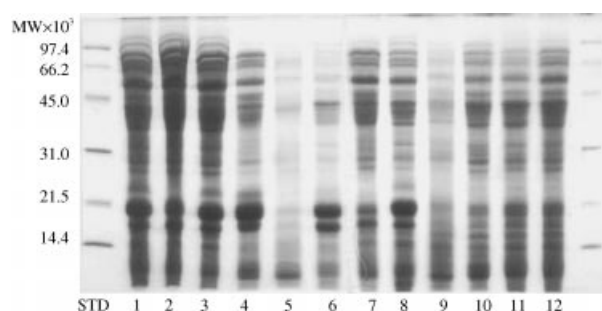


Figure 2. SDS-PAGE of 12 batches of *Olea europaea*. From 1 to 6 correspond to pollen collected in Spain and from 7 to 12 correspond to pollen collected in the USA.

batches of Olive pollen extracts made with pollen collected in California and Spain showed significantly different allergenic and antigenic patterns. While Spanish extracts showed a high concentration of Ole e 1, the most predominant allergen in American extracts revealed Ole e 4 as one of the main allergens (Fig. 2). These intraspecies differences among trees of the same species were described some years ago by different authors (13,14). Antigenic and allergenic differences in the composition of olive pollen extracts have also been suggested for different cultivars (15). These variations may be due to the existence of more than 260 hundred varieties of Olive trees (16). We have recently conducted a study on the allergenic variability of six different varieties during 4 years and have identified important variations in the allergenic composition of the six varieties and in the same varieties during the 4 years of the study.

Allergens

O. europaea pollen is the source of important allergens. In areas where this plant is abundant, the rate of sensitization in the total population is high and comparable to the rate of sensitization to grasses. Olive pollen is among the group of pollens whose allergenicity is better characterized. The degree of characterization of the allergenicity of olive pollen is comparable to that of other well studied allergen sources such as *Betula verrucosa*, some grasses and *Dermatophagoides pteronyssinus*.

One of the main allergens of *O. europaea* is Ole e 1, which was first described by Villalba et al. (17,18). This allergen has two main variants. One of them is nonglycosylated form and has a molecular weight of 18.5 kDa. The second variant is a glycosylated polypeptide with a molecular mass of 20 kDa. Both forms are widely recognized by the sera of sensitized individuals. Ole e 2 is an olive

pollen profilin with a molecular weight approximately 15 kDa, described by Asturias et al. in 1997 (19) and Ledesma et al. in 1998 (20). Ole e 3 was described by Batanero et al. (21) in 1996. It is a protein with a molecular weight of 9.2 kDa and with capacity to bind calcium. In 1998, Boluda et al. (22) purified and partial sequenced Ole e 4 and Ole e 5 simultaneously. Ole e 5 shows a high degree of homology with superoxide dismutase of plants.

A cysteine enriched allergen, designed as Ole e 6, was described in 1997 by Batanero et al. (23). The allergen has a molecular weight of approximately 10 kDa and exhibits a high capacity to bind IgE. Ole e 7 exhibits a high degree of polymorphism. Its molecular weight varies between 9.8 and 10.3 kDa and has no homology with any known protein. Ole e 7 was described in 1999 by Tejera et al. (24). Ole e 8 is a protein around 21 kDa with capacity to bind calcium. It was described by Ledesma et al. (25) in 2000. Ole e 9 is the most recently described allergen of *O. europaea* pollen. It is a β 1,3 glucanase and it has been described by Huecas et al. (26). The allergen has a molecular weight of approximately 46.4 kDa and is involved in the allergic response of 65% of patients with olive tree pollinosis.

Ole e 4 and Ole e 5

Boluda et al. described the allergenic composition of several batches of olive pollen collected in the United States using Spanish sera (7). The IgE binding pattern of these pollen batches differed from previous reports, especially when Spanish pollen was used (27,28). A great variability in the allergenic composition of *O. europaea* extracts has been described and it has been demonstrated that olive tree from different cultivars produce pollen with different allergenic characteristics. It has been suggested that the allergenicity of the olive tree pollen could depend from the variety of the olive tree (29). This fact may have important consequences from an allergological point of view. Since different varieties may produce pollen with a variable allergen load, olive pollen sensitive patients may experience a stronger or weaker allergic response after the inhalation of olive pollen, depending on the variety of the olive pollen inhaled. It may also have implications when discussing pollen counts in different regions, since some varieties could produce more allergenic pollen than others, thus, needing smaller amounts of pollen to induce sensitization and symptoms.

Therefore, exposure to pollen may not be synonymous of antigen exposure.

Boluda et al. demonstrated, using American olive pollen, that the frequency of IgE binding to Ole e 1 was low (40%) (7), in contrast with previous studies that reported an IgE binding frequency higher than 80% (27). These differences in the allergenic composition can be attributed to the source of the pollen used, the variety of the olive tree and to the content of specific allergens. The Ole e 1 content of some of these extracts was 15 μ g of Ole e 1/mg of protein. This quantity can be considered as low, since extracts from other olive tree pollens may contain more at least three times more allergen per mg of freeze dried extract. Extracts prepared using pollen collected in Spain regularly contain higher concentrations of Ole e 1. The clinical implications of these finding remain to be established.

We concentrated our efforts towards the purification of Ole e 4, because this allergen shows a high capacity of IgE binding, and Ole e 5, a new described allergen with a molecular weight of 16 kDa. Ole e 4 has, at least two isoforms in the pI range of 4.6–5.1. Ole e 5 has, at least five isoforms with isoelectric points ranging from 5.1 to 6.5, similar to Ole e 1 (30). Thus, Ole e 5 shows a higher degree of polymorphism than Ole e 4.

IgE binding to Ole e 4 and Ole e 5 was studied by immunoblotting, direct IgE binding and IgE inhibition. The allergenicity of Ole e 4 and Ole e 5 was not altered during the chromatographic steps, because they retained their ability to bind specific IgE. Ole e 4 fixed higher amounts of IgE than Ole e 5 by immunoblotting. However, these allergens captured similar amounts of specific IgE in solid phase assays. In IgE inhibition experiments, Ole e 4 exhibited a relatively high inhibition capacity; the inhibition capacity of Ole e 5 was lower. The two proteins were sequenced to obtain the primary structure of the antigens. Ole e 4 was fragmented with trypsin and the sequences of two internal fragments of Ole e 4 did not show any homology with other known proteins, at the time of publication, based on the Protein Sequence Data Bank (SWISS-PROT). However, the N-terminal sequence of Ole e 5 showed a high degree of homology with superoxide dismutases (SOD) of several plant species and less homology with SODs from distant phylogenetic species (Tables 1

Table 1. Amino acid sequence of two internal regions of Ole e 4

Ole e 4 Fragment 1	... AFANTGVEIV ...
Ole e 4 Fragment 2	... SIDTYLFSLYDEDK ...

Table 2. N-terminal sequence alignments of Ole e 5 with SOD sequences of different species

	10	20	30	%
<i>Ole e 5 (Olea europaea)</i>	VKAVTVLNSSEGPHGIVYFAQEGDGPTTV ...			
<i>Spinacia oleracea</i> (spinach)	M±KAV± VL±SSEG ±± G±VYFAQEGDGPTTV ...			79.3
<i>Ipomoea batatas</i> (sweet potato)	VKAV± VL± SSEG ±± G±± ±F± QEGDGPTTV ...			72.4
<i>Zea mays</i> (maize)	VKAV± VL± SSEG ±± G±± ±F± QEGDGPTTV ...			72.4
<i>Oryza sativa</i> (rice)	VKAV± VL± SSEG ±± G±± ±F± QEGDGPT±V ...			68.9
<i>Lycopersicum esculentum</i> (to mato)	VKAV±± ± LNSSEG ±± G±± ±F± Q± G±± PTTV ...			65.5
<i>S. cerevisiae</i>	VKAV± VL±±±±±±±±±±±±±±±±±±±±TV ...			27.5
	200	210	220	
<i>Escherichia coli</i>	VKAV± VL±±±±±±±±±±±±±±±±±±±± G ±±±±±±±± ...			24.1
		320	330	340
<i>Mus musculus</i> (mouse)	TVK±± TVL±±±±±±± P ±±±± V ±±±±±±±±±±±±±±±± ...			24.1
	280	290	300	
<i>Homo sapiens</i>	FVK± VTVL ±±±±± G ±±±±±±±±±±±±±±±±±±±±±±± ...			24.1
	14	20	30	40
<i>Bos taurus</i> (bovine)	V± A±± VL± S ±±±±±±±±±±±±±±±±±±±± G ±±±±±±±± ...			20.7

and 2). It is well established that SOD and other elements implicated in oxidative processes are implicated in allergic diseases, specially asthma (31,32). In the last few years, SOD, in particular MnSOD, has been identified as an allergen in different organisms, such as *Hevea brasiliensis* (Hev b 10) (33) or *Aspergillus fumigatus* (34). Ole e 5, Hev b 10 and the allergen of *A. fumigatus* are the only allergens described so far with SOD homology.

The existence of epitopes in the glycan moiety as a cause of cross-reactivity has also been described in olive pollen. Cross-reactivity due to an epitope in the carbohydrate moiety of Ole e 1 was demonstrated between Ole e 1 and glycoproteins-like ascorbate oxidase, peroxidase and bromelain (35). Recent experiments conducted in our laboratory, suggest the possibility that Ole e 1 and Ole e 4 share antigenic and allergenic epitopes. The contribution of both allergens to the total allergenicity of an olive pollen extract is also being investigated. The allergenic contribution of Ole e 1 to the total allergenicity of the extract is greater than that of Ole e 4. Ole e 1 is 7.6 times more potent than Ole e 4 when referred to the whole extract. Ole e 1 and Ole e 4 are 2.3 and 17.6 times less potent than the whole extract, respectively. The parallelism

obtained in the IgE inhibition curves of the whole extract, Ole e 1 and Ole e 4 suggest that the purification process did not alter the structure of these allergens and that both allergens contain most of the allergenic epitopes of the extract. However, the inhibition capacity of Ole e 1 is greater than that of Ole e 4, suggesting that the number of common epitopes is greater in Ole e 1 than in Ole e 4 or that they are better exposed in the Ole e 1 molecule.

More experiments with olive tree pollen are necessary to completely understand the complex allergenic composition of this pollen. The study of its allergenicity and cross-reactivity with other pollens is crucial to fully understand the role that Olive pollen plays in allergic diseases such as allergic rhinitis and asthma. The differences encountered in the concentrations of important allergens, such as Ole e 1 and Ole e 4 (36), in different pollen batches should be analysed in further detail. The potential existence of less allergenic, or hypoallergenic varieties of olive tree could have a great importance from a commercial, prevention and environmental control point of view, since olive trees are currently being planted as ornamental trees in many countries. The introduction of less allergenic trees in gardens and parks seems therefore warranted.

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