

REVIEW

Hypersensitivity to the allergens of the pollen from the olive tree (*Olea europaea*)

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Introduction

The olive tree (*Olea europaea*) is a long-lived, subtropical evergreen plant, with a characteristic knotty trunk, which has been cultivated and utilized by man for its fruit, oil and wood for at least 5000 years [1]. It is thought to have originated in Asia Minor and then to have spread throughout the Mediterranean Region, where it now grows in abundance in both cultivated (see Fig. 1) and wild states (*Oleaster*). Possible African origins from *Olea chrysophylla* or *Olea laperini* have also been suggested [2]. During the 16th century the tree was introduced into the Americas and then more recently to other areas with the preferred mediterranean-type climate, as found in parts of Australia and South Africa [1].

Intensive and wide spread growth of plant species, either as a result of natural phenomena, for example in the cases of birch (*Betula*) or of grasses (*Graminae*), or of man's activities, such as cultivation of rye-cereal (*Secale cereale*) or of the olive-tree, can often lead to respiratory hypersensitivity to the pollen in that significant proportion of the population which has a genetic predisposition. The probability that allergic problems may occur is high with anemophilous species which produce high levels of airborne pollen, and with those with long pollinating seasons that expose the individual for long periods of time. Readily extractable proteinaceous components from pollen may have particular molecular features associated with a propensity to induce IgE antibody, but this is still argued. Olive trees are insect-pollinated but high concentrations of wind-borne pollen can be measured locally [3] and can induce clinical hypersensitivity in a large number of atopic people.

Botanical considerations

The family *Oleaceae*, to which the olive tree belongs, contains some 400 species which include ash (*Fraxinus*), privet (*Liqustrum*), *Phillyrea*, *Forsythia* and jasmine (*Jasminum*) [1,4], though within the genus *Olea*, only the olive tree produces edible fruit [1].

A great number of olive tree varieties has been recognized, Pliny producing the first recorded classification of 15. Many authors have published subsequent classifications based on various anatomical features and habitats of the plant. Between 21 [6] and 80 [5] varieties have been recognized.

Recently a group of workers in Seville has studied differences in extractable components from the pollen of 16 varieties of olive tree [7-9]. The question of whether any observed differences in allergens from these varieties are qualitative or quantitative in nature has not yet been fully addressed, although preliminary studies by Spinozzi [10] have suggested that patients from the north and south of Italy, where different varieties of olives are grown, may respectively have different patterns of IgE specificity. Clearly, if major differences occur, then selection of a source of allergens for diagnostic and vaccine preparations for olive pollen allergy is not a simple matter.

Pollination

The olive tree produces small yellow-white flowers with four petals which appear between the end of January and the beginning of June, depending on latitude and climatic condition [3].

Both temperature and rainfall in March and April play a minor role in determining the amount of pollen produced. A more important factor is the 'Alternance of Production' observed with olive trees. This leads to alternating high and low levels of pollen from year to year [11].

Many pollen surveys have recorded the incidence of airborne olive pollen in particular in Italy, Spain and France, and surveys from Greece, Turkey and North Africa have also yielded positive counts. Airborne olive pollen has been recognized as an important cause of allergy in some regions of North America [12].

Olive pollen has featured highly in pollen surveys from most regions bordering on the Mediterranean Ocean. For example, in Naples the pollination period of *Olea* was reported by D'Amato [13,14] to last from the middle of April to the end of June, with olive pollen being the third most frequent airborne plant-allergen source after

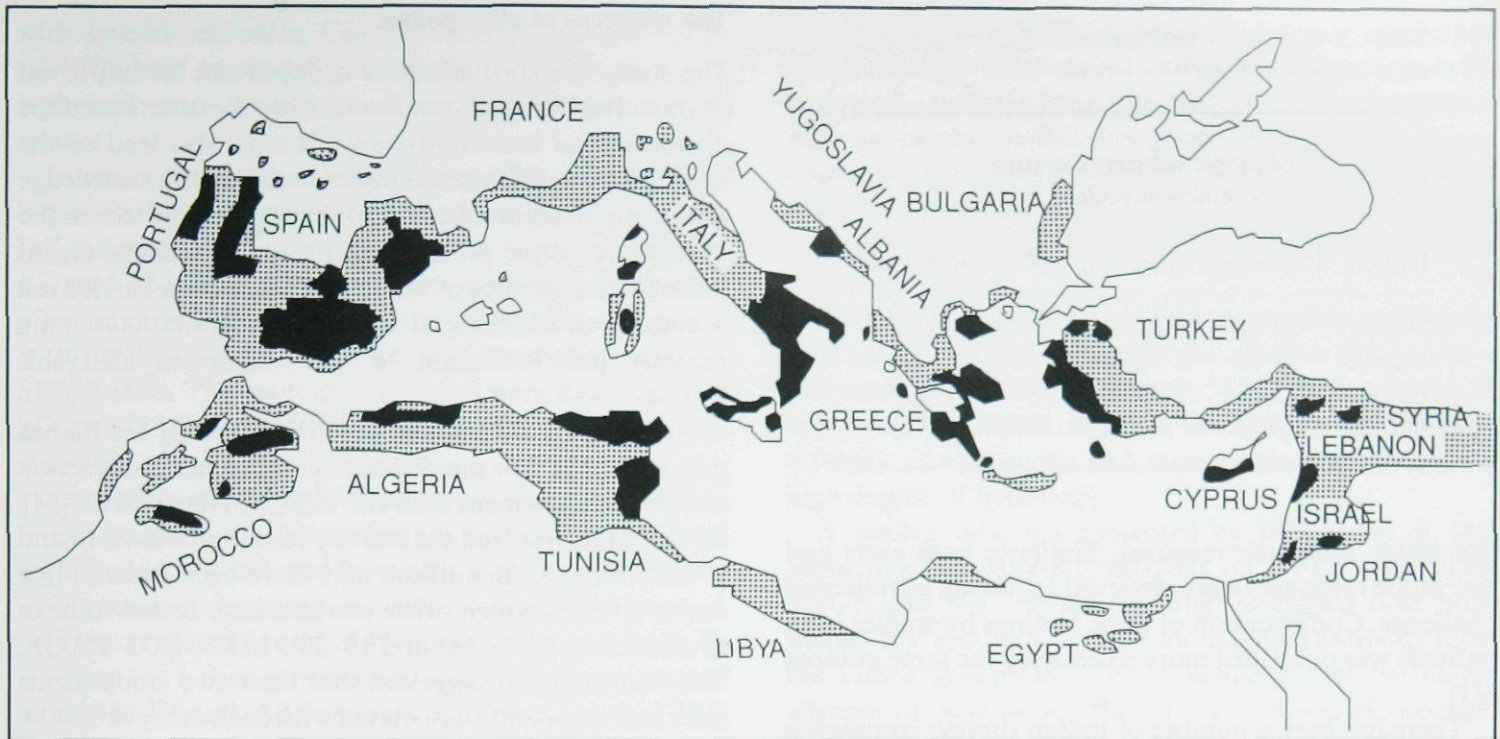


Figure 1. The principle olive growing areas of the Mediterranean region (reproduced by courtesy of the Institute of Biology, London). Light shading = low density. Dark shading = high density.

Table 1. Maximum values registered in 1983 for the pollen of olive in various Italian cities (Corriere Medico ad altre fonti) [16]

City	Date	grains/m ³
Ancona	12 June	42
Ascoli Piceno	1 June	250 (approx.)
Bari	22 May	1815
Cagliari	31 May	199
Catania	18 May	74
Firenze	15 June	37
Genova	8 June	251
Livorno	8 June	116
Napoli	3 June	783
Roma	1 June	100 (approx.)
San Remo	12 June	179

Urticaceae (*Parietaria*) and *Graminae*; olive pollen levels reached more than 100 grains/m³. In Montpellier, however, Oleaceae were only the sixth main source of pollens [15]. In Bari, Italy, where over 30% of the inland region is under cultivation with this tree, peaks of over 1800 grains/m³ have been recorded during May (Table 1) [16]. Data presented indicated that the large variations in olive pollen levels occurring in different regions of Italy are

dependent upon the extent of local olive tree cultivation as well as upon climatic conditions and other factors [12,16].

Clinical aspects

Respiratory allergy to the pollen of Oleaceae (ash, olive, privet and Phillyrea) has been recognized for many years [17,18] being reported mainly from countries in the Mediterranean region. Bousquet [15], working in Montpellier, France, which is on the northern fringe of the Mediterranean area, found that 15.2% of 326 atopics were skin test positive to Oleaceae, but he did not differentiate between the species. Confirmation of sensitivity was found by RAST assay and a good agreement was shown with levels of atmospheric pollen compared with those of other plant species. In a study carried out in central Spain [19], both asthma and rhinitis were claimed to be commonly caused by olive pollen. Confirmation of this diagnosis was again obtained by skin prick test and RAST studies.

Olive was considered by Tamir *et al.* [20] to be the most allergenic tree in Israel. They studied 28 patients whose nasal and bronchial symptoms were consistent with allergy to olive pollen, and who gave positive immediate skin-tests to olive pollen extract. Sixteen of these patients gave a positive immediate bronchial provocation response to olive pollen extract, half of them developing a

Table 2. Sensitivity to *Parietaria*, Graminae, and olive pollens in Italy

Region	% of atopic subjects sensitive to different pollens			Reference
	<i>Parietaria</i>	Graminae	Olive	
Napoli	82.02	38.12	23.11	[13]
Napoli	47.80	34.70	13.05	[3]
Roma	25.75	39.17	16.80	[22]
Bari	36.90	37.20	19.00	[16]

late phase asthmatic response. Similarly both early and late phase responses were observed following intradermal challenge. Confirmation of these findings by studies in 19 patients was published more recently by the same authors [21].

There has been a number of Italian surveys comparing skin tests with *in vitro* assays which have confirmed that the pollen of the olive tree can be the most common cause of respiratory allergy after *Gramineae* and *Parietaria* (see Table 2).

Other parts of the olive tree can produce allergic symptoms, both olive oil [23] and olive wood [24] have been reported to produce dermatitis consistent with type IV hypersensitivity.

Crossreactivity

Sensitization to the pollen of one species can sometimes lead to allergic reactions on challenge with pollen from a closely related species.

Bousquet [25] studied 20 patients presenting respiratory symptoms during the Oleaceae pollen season. Using skin test, RAST inhibition, tandem cross-immunoelectrophoresis, and iso-electric focusing, he demonstrated a high degree of crossreactivity, although no total identity, between olive, ash, *Phillyrea* and privet. Other authors have reported similar findings, in particular showing crossreactions between *Fraxinus* and *Olea europea* pollens [26,27].

Kernerman [28], working in Michigan, used inhibition of IgE ELISA and found crossreactions between olive, ash and privet, but in addition, between these and Russian Olive (*E. angustifolia*).

It seems likely therefore that individuals sensitized to olive pollen would react to inhalation of pollens from certain other members of the *Olea* family, and vice-versa. This could be of particular significance to allergic individuals travelling from one climatic region to another.

The allergens of olive pollen

The study of major allergens is important for improved characterization and standardization of extracts used for diagnosis and immunotherapy. It may also lead to the identification of future therapies based on the knowledge of epitope structure. Less work has been reported on the allergens of olive pollen than on many other allergens although two groups of workers in Spain have carried out a number of studies and other investigators have more recently paid attention to this important allergenic source.

Allergens of olive pollen were described by the Blanca group [29–33]. The major activity was found in a fraction at PI6.0 in agreement with the work of Hewitt *et al.* [34]. SDS-PAGE resolved the activity into fractions at 15 and 17 kD, though the authors may have been describing a major allergen which other workers later found to be in two bands at 17–18 kD and 19–20.5 kD by SDS-PAGE. The Blanca group suggested that these two components were non-covalently associated with each other at 32 kD, or even present as a higher molecular weight dimer, and that SDS resolved them into the two lower molecular weight species without the addition of reducing agents such as 2-mercaptoethanol or dithiothreitol. Similar views have been put forward by others as described below.

The most prolific authors in the area, the Lahoz group, working in Madrid, have been developing views over the last decade. An early study in 1982 [35] demonstrated the presence of two main allergenic fractions following Sephadex G100 separation of crude olive pollen extract, the main activity being found at approximately 65 kD as indicated by IgE reactivity *in vitro* and histamine release from the basophils of allergic individuals.

In a more detailed publication, the same groups [36] described at least 10 antigens separated by crossed immunoelectrophoresis and four major protein fractions separated by G100 Sephadex. An allergenic fraction was found at 160 kD, but most of the activity measured by inhibition of polyvinyl plate radioimmunoassay was seen at 60–65 kD.

Continuing work extended the knowledge of olive pollen allergens. Ion exchange separation on DEAE Sepharose produced a material that eluted at 500 mM NH_4HCO_3 and had the greatest IgE reactivity by RIA [37]. This material was further separated on Sepharose Con-A to produce a non-glycoprotein and a glycoprotein fraction (GLP500), the latter being highly reactive in RAST inhibition. This material was described as 'presenting itself in various molecular weight forms'. The authors suggested [38] that the main allergen of olive pollen was a glycoprotein of approximately 60 kD molecular weight

with several sub-units. Confirmation of the activity of GLP500 was presented [39] although activity could be separated into a variety of lower molecular weight species.

Further work by the same group published in 1988 [40] indicated that the proteins and antigen most reactive with IgE in sera from allergic patients were those at 17 kD, 19 kD and 42 kD. A monoclonal antibody (OL1), selected after immunization of mice with whole extract, reacted with the 17 kD and 19 kD protein bands separated by SDS-PAGE. The main glycoproteins were detected at 19 kD and 42 kD on SDS-PAGE by Con A and peroxidase staining.

The IgE in sera from 87% of patients sensitive to olive pollen (as indicated by case history and skin test) reacted with the 19 kD band, and from 68% with the 17 kD band. These results are consistent with those of Wheeler *et al.* [41] who reported figures of 88% and 65% respectively. In addition the Lahoz group found that 76% of sera reacted to the 42 kD band. All those sera that reacted to the 17 kD band also reacted to the 19 kD band, again in agreement with other published work [41]. The Lahoz group extended its studies [42] to produce two major allergenic fractions on DEAE Sepharose CL6B. The most important of these was separated at 0.3 M (NH_4HCO_3), as shown by RAST inhibition. This material was subjected to further fractionation with Sephadex and lentil lectin-B to produce two fractions. The glycoprotein fraction was mainly 19 kD with some 17 kD material, while the other which was more RAST active, was mainly 17 kD but contained some 19 kD material. It contained proteins that had a range of PI values (5.0, 5.3, 5.6). The authors named this material OL antigen I.

The fraction (OL antigen II) that eluted from DEAE Sepharose at 0.5 M (NH_4HCO_3) had a molecular weight of 8 kD on SDS-PAGE, but 10 kD under non-reducing conditions. It contained materials at PI 3.6, 3.8 and 4.0 and it did not react with the previously described monoclonal OL1.

Spinozzi [43] described major allergens at 19 kD and 21 kD, which could perhaps have been the allergens described by others at 17 kD and 19 kD, and an additional allergen at 30 kD. Rubio [44] also described allergens at 30 kD. Spinozzi observed that olive pollen allergic patients had IgG1 and IgG4 antibodies to non-allergen of other molecular weights.

More definitive studies by Villalba *et al.* [45] utilized G75 Sephadex to separate material at approximately 19 kD for further fractionation by reverse phase HPLC on an acetonitrile gradient. The authors named the peak containing the major allergen, *Ole e I*; it could be further resolved on SDS-PAGE into bands at 18 kD and 20.5 kD. The fraction at approximately 19 kD was separated again

on reverse phase HPLC to produce three isoallergen peaks. Complete amino acid analyses of material from the three peaks indicated that they had identical composition. This was partly confirmed by partial N terminal sequence homology between all three. The authors concluded that *Ole e I* is composed of 18 kD and 20.5 kD (glycosylated) materials with similar epitopes. A number of isoallergens of each exist.

Other authors [46], using other separation techniques, concluded that the major allergen of olive pollen was a multimeric unit at approximately 55 kD, with subunits at approximately 20 kD, and thus the allergen was basically a family of multimeric and monomeric proteins with a high degree of homology.

A similar view was presented by Wheeler *et al.* [41] using mild separation conditions on HPLC, and SDS-PAGE and IEF immunoblotting developed both with human IgE and with the OL1 monoclonal antibody from the Lahoz group [40]. They concluded that the major allergen of olive pollen *Ole e I* is composed of two sub-units with common epitopes at approximately 17 kD and 19 kD on SDS-PAGE and that they are derived from a loosely bound complex at approximately 60 kD. Other investigators have agreed with these findings (I. Taylor, personal communication). In addition, at least six isoallergens of *Ole e I* are present in the most active groups at PI 5.0 and PI 6.0, confirming other findings [42]. Data were also presented [41], showing the presence of other allergens which were apparently not related to *Ole e I* but to which some sera reacted strongly.

Conclusion

It is clear that olive pollen is a major cause of both rhinoconjunctivitis and asthma in those regions such as the Mediterranean area where large amounts of wind-borne olive pollen result from intensive cultivation. Crossreaction with other members of the *Oleaceae* could lead to allergic reactions occurring in individuals whose original sensitizing agent may have been pollen from a closely related species.

Despite the earlier rather confusing situation, a clearer picture of the major allergen of olive pollen is emerging. A protein or family of proteins with similar epitopes, to which the majority of olive pollen-allergies are sensitized, has now been subjected to physico-chemical characterization and some amino acid sequencing.

Most later studies have shown that the major allergen (*Ole e I*) is present at molecular weights of 17–18 kD and 19–20.5 kD, the latter material having possibly a higher degree of glycosylation. The allergen can be separated into at least six isoallergens with major bands at PI 5.0 and PI 6.0. There is evidence that the 17–18 kD and 19–20.5 kD species can be weakly and non-covalently bound

together to form higher molecular weight material at 55–65000 kD.

Other allergens, unrelated to *Ole e* I, are present in olive pollen, but characterization data are as yet inadequate.

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