

Short communication

Hypersensitivity to pollen of *Olea europaea* in Israel

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The incidence of skin-tested sensitivity to olive pollen allergens among subjects with suspected atopic respiratory allergy was investigated in various populations of Israelis. This incidence was correlated with the olive cultivars, with the abundance of trees in the patients' neighborhoods, and with the history of exposure of the studied populations to olive pollen. Positive skin reactions to olive pollen, among atopic patients of the Jewish population, is rather high where olive trees are abundant (66%), and lower (29%) where trees are scarce ($P < 0.003$). Sensitization was significantly lower ($P < 0.003$) among a population of Israeli atopic Arabs (16%), though these Arabs have lived in an olive-rich area for several generations.

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The olive is one of the major crops of the Mediterranean region, and the allergenicity of its pollen is well established and documented (1–11). In Israel, olive trees are abundant in Galilee, Samaria, Judaea, and the upper Jordan valley. Along the coastal plain, olive cultivation is less common, whereas in the Negev desert olive trees are rare. During the last decades, olive trees have been widely planted for ornamental purposes, an act that turned them into a new source of urban air pollution (11).

The earliest data on the role of olive pollen as a major factor in the etiology of allergy in Israel were established some 40 years ago (3, 12–14). Nevertheless, little attention was given to the divergence among the local populations of plants and people. Thus, the aim of the present study was to summarize and update the information about olive allergenicity, to obtain some quantitative information on the effects of the density of olive trees on the incidence of olive-induced allergy, and to clarify the effect of the intraspecific variability of the olive upon the incidence of sensitization to its pollen.

Material and methods

Preparation of pollen extracts

Pollen of *Olea europaea* was collected in the arboretum of the Volcani Center, Israel, from the fol-

lowing cultivars: Barnea, Manzanillo, Muhassan, Merhavia, Souri, Uovo di Piccione, Kalamata, Kadesh, Koronaiki, and hybrids of Kalamata and Barnea. Pollen of the cultivars Bosana, Nera di Gonnos, Palma, Pizz'e Carroga, and Tonda di Cagliari and that of *O. oleaster* was collected in Sardinia.

The flowering branches were shaken into paper bags, and the released pollen grains were collected and dried at 40 °C for 48 h. The pollen was then sifted through a silk cloth (Swiss Silk Bolting Cloth Co., Cat. No. T-100). The clean grains were kept dry, in sealed containers, at –20 °C until preparation of the extracts.

Skin tests were done with diagnostic extract of *O. europaea* (Center Laboratories, USA), and with autochthonous pollen extracts prepared by the following procedure. Clean pollen grains were sonicated in a sterile saline-glycerol buffer solution (pH 8.2, 2.8% NaHCO₃, 0.5% NaCl, 50% glycerol) (15). This is the standard solution in which commercial allergen extracts were supplied (cf. Center Laboratories). Sonication was done at 4 °C, for 4 min. The solution was then centrifuged for 30 min at 7600 g to allow precipitation of the solid remains. The supernatant was then filtered through a membrane with 45-µm pores. The protein content of the obtained extract was determined by the Lowry

procedure (16), with bovine serum albumin as a standard. After the protein content was determined, each of the autochthonous extracts was diluted to a standard concentration of 5 mg protein/ml solution. The extracts were stored at -20°C (17).

Patients

A total of 1573 patients suspected of allergy to pollen by their general practitioners were investigated. The patients came from urban and rural communities all over the country. Most of them were Jews, of various ethnic origins: Ashkenazi, Sephardic, Oriental, or mixed. Ninety patients were Arabs from Um-El-Fahem, a town in the Samaritan foothills, whose inhabitants have lived in this olive-rich area for at least a few generations. All patients were invited to the local clinic to answer a questionnaire on family and personal history and the present symptoms of allergy. Pollen allergy was confirmed on the basis of:

- 1) seasonal symptoms (rhinitis, cough, asthma, conjunctivitis, and skin symptoms)
- 2) family history of at least two affected first-degree relatives
- 3) positive skin reactions to at least two commercial pollen extracts of the common plants of Israel.

The patients were examined by allergologists and skin tested with a panel of 20 aeroallergens common in Israel. This included the commercial extract of *O. europaea* as well as 13 of the home-made extracts, prepared from well-defined olive cultivars (Table 2). A 1 mg/ml histamine solution was used as a positive, and buffered saline glycerol as a negative control (9). The degrees of reaction were defined as follows: 0 = none, 1 = wheal diameter of 2–4 mm diameter, 2 = wheal of 5–7 mm, 3 = wheal of 8–10 mm, and 4 = wheal diameter larger than 10 mm with pseudopodia (18). Venous blood samples were taken from the patients of Um-El-Fahem for genomic analysis: extraction was done by the salting-out procedure (19), and DNA typing was done by restriction fragment length polymorphism (20).

Statistical analysis

The epidemiologic data of prevalence of positive SPT to different olive cultivars were evaluated by ANOVA. The differences in the sample sizes were accounted for. The least significant difference (LSD) among the various populations was calculated.

Results

Of the 1573 patients suspected of respiratory allergy, 1144 were diagnosed as allergic by our criteria. The average percentage of patients with positive skin prick test (SPT) to the commercial olive extract and/or to one or more of the autochthonous extracts was 40% (Table 1). Two of the tested populations were beyond the average: that of Um-el-Fahem, which was significantly below the average ($P < 0.05$), and that of Jerusalem, which was significantly above it. The prevalence of sensitization to olive pollen among the Jewish populations was positively correlated with the density of olive trees in the neighborhood. Sensitivity to olive among the Arab patients was far below the prevalence in any of the tested Jewish populations.

Genomic DNA typing of this Arab population revealed that those who had positive SPT to the commercial extract had a higher phenotypic frequency of DR-7 and/or DQ2 ($P < 0.03$), as compared with those whose SPT to olive was negative (21). In our previous study of MHC class II antigens in the same Arab population, the DR-4 haplotype was not found in any of the 11 patients who were sensitized to olive pollen. On the other hand, DR-4 was found in 44% of the controls. Thus, DR-4 may be considered to protect against olive pollen sensitization.

No significant differences in monosensitization to olive were found among Jewish inhabitants of olive-rich settlements (2.2%), Jewish inhabitants of olive-poor areas (1.6%), and the Arabs of Um-El-Fahem (5.2%). No significant differences in sensitivity to olive pollen were found between allergic patients of urban (33–60%) and of rural communities (17–67%).

Table 1 Number of patients with proven allergy who had positive SPT responses to olive pollen, as compared with density of trees in their neighborhood

Region		Density of olive trees	No. of tested patients	No. and % of patients with positive SPT to olive*
Rural	Negev desert	Low	42	12 (29%)
	Coastal plain	Medium	624	246 (39%)
	Jordan valley	High	158	72 (46%)
	Um-El-Fahem	High	78	13 (16%)
Urban	Jerusalem	High	136	85 (66%)
	Tel Aviv	Medium**	106	50 (34%)
Grand average				40%

* LSD ($P < 0.05$) between means was 10.6. ** Mostly ornamental.

Large variations in SPT responses to the various cultivars were recorded. The SPT responses to common cultivars were significantly above, and those to rare cultivars were significantly below the average (Table 2). The electrophoretic analysis of the examined extracts showed a conspicuous difference in the content of the 18–20-kDa band, and, to a lesser extent, also in the proteins of the 42–44, 50, and 95-kDa bands. For details, see Waisel et al. (22).

Discussion

In Israel, olive pollen is an important inducer of clinical hay fever, as assessed by seasonal symptoms, SPT, and provocation tests (10, 23, 24). Israel is rich in numerous olive cultivars, some abundant and old, and others rare newcomers. Olive cultivars differ not only in their fruit quality but also in their pollen characteristics. This is expressed by differences in protein composition, and especially by the dissimilarity in the expression of *Ole e 1* (22). Thus, the intraspecific differences in allergenicity of olives should be given proper attention. *Ole e 1* was often regarded as the major allergen of olive pollen, though modern investigations have identified additional allergens (6, 25, 26). The presented results support those findings but add to them the fact that some cultivars can be twice as allergenic as others. Such differences imply that sensitization to olive may not be a general feature, but may depend upon the specific traits of the pollen sources.

The percentage of Jewish patients allergic to olive,

as correlated with the density of olive trees in their area, proves again that exposure of allergic patients to a large amount of allergenic pollen has a bad effect on their health, and that reduction in pollen pollution should be recommended.

The prevalence of sensitization to olive pollen among the Jewish population of Israel that immigrated to Israel this century differs from that of an Arab population that has lived for several generations in the midst of olive trees. The prevalence of olive sensitivity among Arabs was the lowest observed, i.e., 16% of the examined allergic patients. These sensitized subjects had a high frequency of DR-7 and/or DQ2, whereas unsensitized ones had a high frequency of DR-4. Apparently, exposure of a population to olive pollen for generations reduces the number of positive responders. This was recently observed in three additional places: in Sassari, Sardinia, where the number of positive skin test responders to olive was very low, as compared to other olive-rich areas in Italy (8, 27); in Australia, where olive sensitization was higher among new immigrants than among the indigenous population (28); and in Split, Croatia (29), where hypersensitivity was significantly higher among immigrants from olive-free areas than in the autochthonous population, which has been exposed to olive pollen for generations. This leaves us with a threefold problem: did the low prevalence of allergy in such populations result from the development of tolerance, is it spontaneous desensitization, or is it the long arm of natural selection? The solution of this problem requires a better knowledge of the genetic background of the olive cultivars, as well as investigation of additional human populations from areas that are rich in olives.

Table 2. Percentage of positive skin test responders to olive pollen allergens in subjects with suspected atopic respiratory allergy

Cultivars	Responses	
	% of allergic patients with positive SPT*	Mean score of reaction intensity (0–4 scale)
Commercial extract	39%	2.6
Muhassani (1)	31%	1.4
Souri (1)	27%	1.9
Manzamillo (1)	26%	1.7
Kalamata (hybrid) (2)	39%	1.6
Kadesh (2)	35%	2.1
Barnea (2)	29%	1.8
Merhavia (2)	25%	2.0
Kalamata (2)	25%	1.6
Koronaki (2)	23%	1.2
Uovo di Piccione (2)	22%	1.4
Barnea (hybrid) (2)	16%	2.0
Bosena (0)	26%	1.2
Palma (0)	18%	1.3

(1) Old and common cultivar.

(2) New cultivar; not common.

(0) Not found in Israel.

* LSD ($P < 0.05$) between means is 5.2.

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