

Positive skin tests to pollen extracts of four species of *Pistacia* in Israel

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Summary

Fifty-eight atopic and nine non-atopic volunteers, all residents of the Tel Aviv metropolitan area, Israel, were skin tested with extracts made from filtered pollen of *Pistacia atlantica*, *P. lentiscus*, *P. palaestina* and *P. vera*. Twenty-four out of the fifty-eight patients examined (41.4%) had positive skin reactions to at least one of the four examined species of *Pistacia*: seven of the patients reacted to all the examined extracts, whereas the other seventeen reacted to only some of them. Fifteen patients reacted to extracts of *P. atlantica*, sixteen patients reacted to extracts of *P. lentiscus*, ten patients reacted to extracts of *P. palaestina* and seven patients reacted to extracts of pollen of *P. vera*. None of the healthy, non-atopic volunteers reacted to any of the extracts. To the best of our knowledge, this is the first report of immunological reactions to pollen extracts of the genus *Pistacia*.

Introduction

It is generally accepted that wind pollinated plants are potent allergens, in part because of the abundant dispersal of their pollen grains. Most investigations in this field refer to wild plants, which have a dominant impact on the pollen content of the air [1,2].

The genus *Pistacia* comprises ten species of trees in the whole of the temperate regions of the world. According to Zohary [3,4] the centre of origin of the genus is Central Asia, though the genus has a discontinuous pattern of distribution. The various species of *Pistacia* are spread mainly around the Mediterranean, and over the Irano-Turanian regions but appear also in the mountainous regions of East Africa as well as in the Sino-Japanese and Mexican regions (Fig. 1). One species (*P. vera* L.) produces edible nuts and is, therefore, grown commercially in several countries in the Middle East, including Israel.

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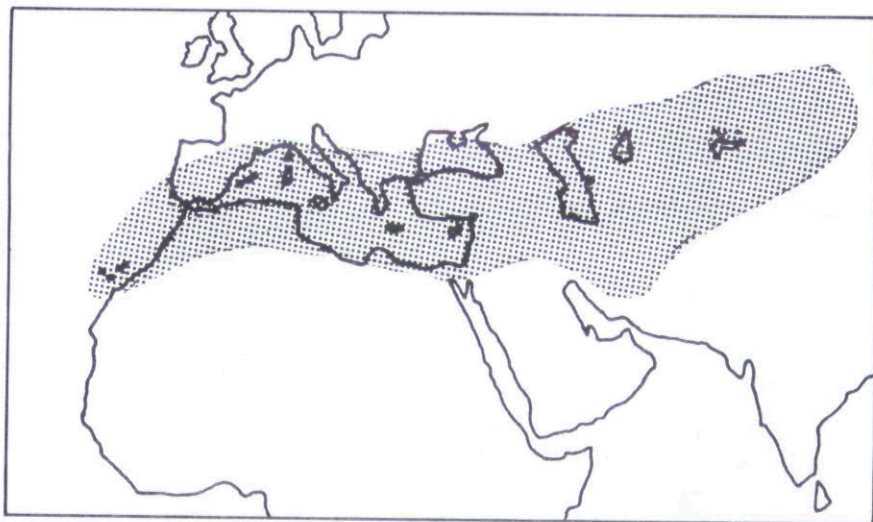
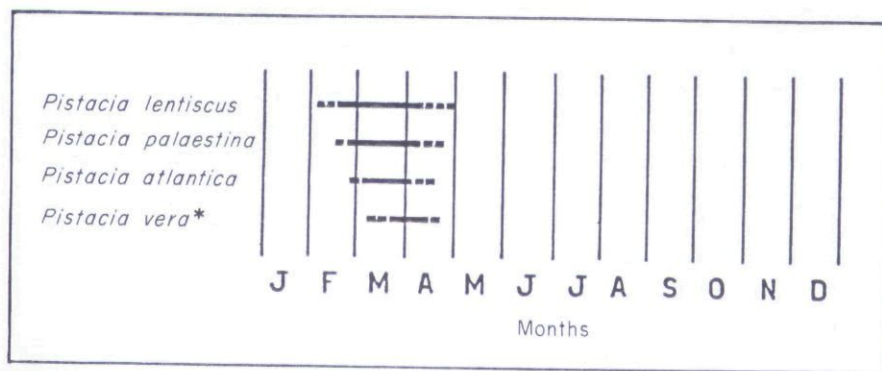


Fig. 1. General outlines of the distribution area of the Mediterranean species of the genus *Pistacia*.



*1986 flowering season in Avdat, central Negev.

Fig. 2. Flowering season of the four *Pistacia* species in Israel. (—) Main flowering period, (---) scattered flowering (1980–1986).

Some four species of *Pistacia* (*P. atlantica* Desf., *P. lentiscus* L., *P. palaestina* Boiss., and *P. vera* L. are common in the Mediterranean region [5].

Pistacia atlantica is a steppe plant of the Middle East and North Africa. It is typical to the transition zones between the Mediterranean mountains and the Deserts.

Pistacia lentiscus appears in large numbers in the natural plant communities of practically all the Mediterranean countries. Furthermore, *P. lentiscus* is planted widely as a cover plant or in hedges, and its concentrations around human habitations in those regions can be extremely high.

Pistacia palaestina is an East Mediterranean species. It is less common in the coastal plain of the countries of this region but is very common in their lower mountain ranges.

Trees of *P. vera* are planted in Israel on a small scale and their distribution is largely confined to the eastern Lower Galilee and to a few stands in the Negev mountains. However, plantations are very common in Turkey, Iran and Iraq.

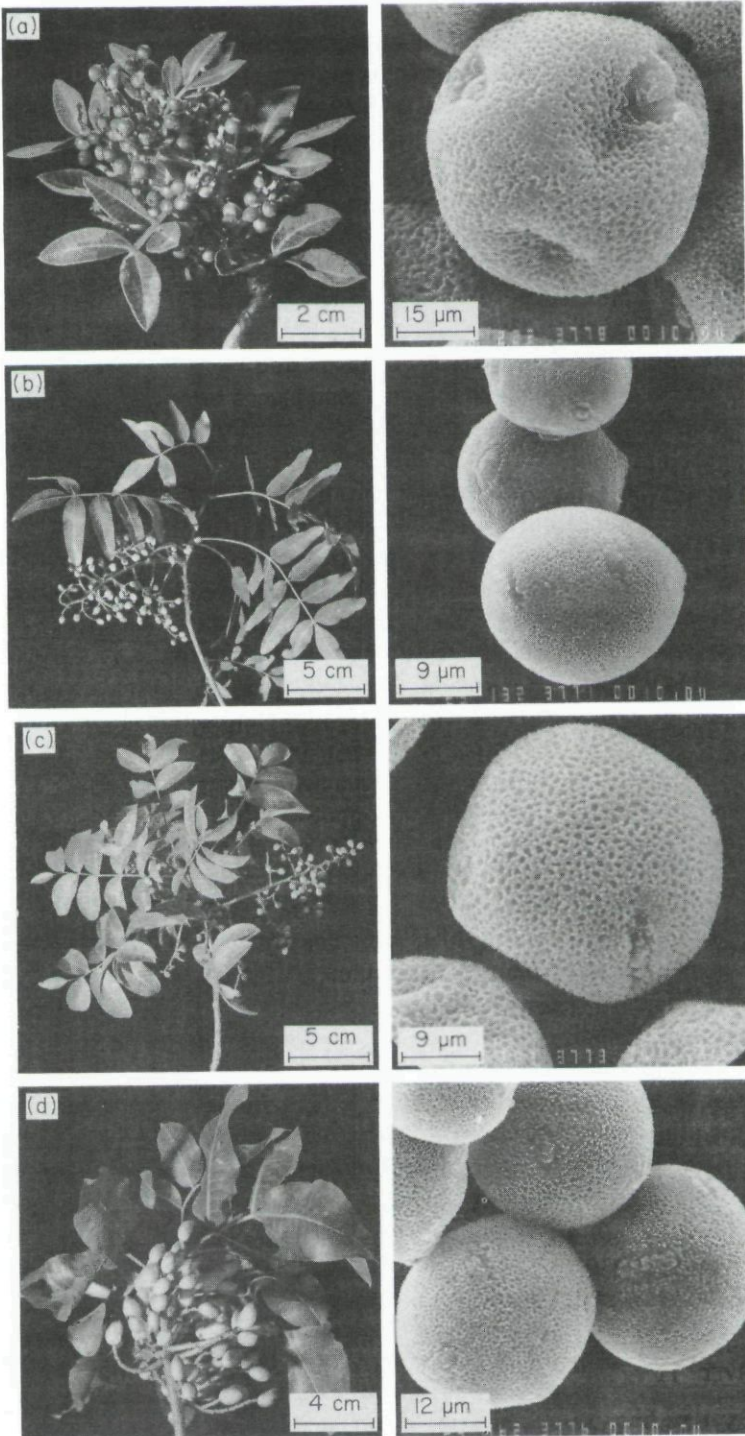


Fig. 3. Fruiting branches (left) and SEM images of pollen grains (right) of: (a) *Pistacia lentiscus*, (b) *P. atlantica*, (c) *P. palaestina* and (d) *P. vera*.

The flowering season of the deciduous species of *Pistacia* in Israel is rather short and is limited to a period of 3–5 weeks during the spring. Flowering of the evergreen species may spread over a longer period, but is mostly limited to 4–6 weeks between February and April (Fig. 2). Such periods of flowering are typical for many Mediterranean species.

The various species of the genus *Pistacia* are dioecious. The plants are wind pollinated and the male specimens produce large masses of pollen grains. The pollen grains are spheroidal, porate with typical long and narrow colpi and with reticulate exine (Fig. 3). Their size is 22–35 μm .

Subjects and methods

Two groups of volunteers with a total of sixty-seven persons, were selected for the study: one group of nine was comprised of students and university staff without previous records of hay fever. The second group was of fifty-eight atopic patients previously known to us for 2 years or more as suffering in the spring from hay fever, rhinoconjunctivitis and asthma. They had been previously tested and showed at least one positive immediate skin reaction to a battery of twelve of the common pollen extracts used in Israel. All patients live in the coastal plain of Israel in the Tel Aviv metropolitan area, where *P. lentiscus* is common and where some plants of *P. palaestina* and of *P. atlantica* also occur. Most of the patients have lived in this area for longer than 5 years. The patients are exposed annually to airborne pollen of *Pistacia*, although the concentration of such pollen in the air and the proportions of pollen of each species are unknown.

Pollen for extraction was collected from identified specimens of *P. lentiscus*, *P. palaestina*, and *P. atlantica* in the Botanic Gardens of Tel Aviv University, Tel Aviv, Israel. Pollen of *P. vera* was collected from the Avdat area in the central Negev.

Flowers with closed anthers were cut off the trees, put in paper envelopes and dried at 40°C. The released pollen grains were filtered through Swiss silk with a mesh opening of 59 μm (Bolting Cloth Manufacturing Co. Ltd, Zurich). Purity of the pollen was examined microscopically. Clean pollen was sealed in dry glass vials and preserved desiccated at –20°C until extraction.

Extractions were made by sonicating of the pollen grains in a sterile water solution of NaHCO_3 (2.75 g/l) and NaCl (5 g/l) at pH 8.2, for 1 hr at 4°C (Ultrasonic Cleaner, 50/60 Hz, 125 Watts, Branson Conn., U.S.A.). Shorter sonication periods were found to be ineffective in breaking the pollen. The extracts were then diluted in glycerol 2:1, centrifuged at 2500 $\times g$, and passed through a membrane filter (0.45 μm). Protein content was determined according to Lowry *et al.* [6]. The protein content of most extracts in the tested batches was 1.62–2.40 mg/ml. Nevertheless, each person was tested with extracts of almost the same protein contents. Reference protein was BSA dissolved in 33% glycerol.

Sonication was used as a standard procedure for preparation of pollen extracts. This was done to shorten the extraction time, to avoid contamination and proteolysis and to improve extraction of small pollen samples.

All sixty-seven volunteers were tested by the modified skin-prick test method [7] with extracts of four pollen species and a negative control solution (NaCl 0.9%). In all the cases examined the reaction to the control solution was negative.

The tests were made according to Pepys [7]. They were conducted during the

Table 1. Results of positive skin tests of twenty-four patients to pollen extracts of four species of *Pistacia*

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total positive reactions
<i>P. lentiscus</i>	0	4	36	0	10	14	0	10	4	4	0	13	0	10	0	0	30	150	14	0	25	12	20	16	16
<i>P. atlantica</i>	0	4	0	4	0	0	4	0	25	4	4	20	10	0	0	0	33	166	8	4	17	0	52	11	15
<i>P. palaestina</i>	95	0	0	0	0	0	0	0	10	4	0	15	0	0	17	0	32	120	7	0	0	7	33	0	10
<i>P. vera</i>	—*	—	—	—	—	—	—	—	—	—	0	18	0	0	20	21	54	25	32	0	0	0	30	0	7

* — not tested.

Table 2. Classification of the tested patients according to their responses to the various species or species combinations of *Pistacia*

	<i>P. atlantica</i>	<i>P. lentiscus</i>	<i>P. palaestina</i>	<i>P. vera</i>
Number of responders	15	16	10	7
% of responders out of the patients	28.3	27.6	18.5	28.0
Number of responders to one species only	5	5	1	1
% of responders to one species only out of all responders	33.3 (5 out of 15)	31.3 (5 out of 16)	10.0 (1 out of 10)	14.3 (1 out of 7)
Number of responders to two species only	3	4	2	1
% of responders to 2 species out of all responders	20.0 (3 out of 15)	25.0 (4 out of 16)	20.0 (2 out of 10)	14.3 (1 out of 7)
Number of responders to all examined species (either 3 or 4)	7	7	7	5
% of responders to all examined species out of all responders	46.7 (7 out of 15)	43.8 (7 out of 16)	70.0 (7 out of 10)	71.4 (5 out of 7)

summers of 1985–1986, i.e. not during the flowering season of *Pistacia*. None of the examined individuals was either ill or on any medication at the time of testing. A reaction was considered positive if the weal was larger than 4 mm².

Results

Results presented in Tables 1 and 2 show that twenty-four (41.4%) out of the fifty-eight atopic patients had positive skin reactions to at least one of the pollen extracts of *Pistacia*. Fifteen (28.3%) out of fifty-three atopic patients had positive skin reactions to *P. atlantica*, sixteen (27.6%) out of fifty-eight atopic patients had positive skin reactions to *P. lentiscus*, ten (18.5%) out of fifty-four atopic patients had positive skin reactions to *P. palaestina* and seven (28.0%) out of twenty-five atopic patients had positive skin reactions to *P. vera* (Table 2). Twelve of the patients reacted to one species only, whereas seven patients reacted to all the examined species. Two of these were not tested to *P. vera*. Five patients reacted to two of the examined species. An additional group of twenty-seven atopic patients from the Upper Galilee was also tested to the four species of *Pistacia* pollen extracts. Thirteen (48.2%) of those patients responded to at least one species. Thus, % sensitivity in that population to *Pistacia* pollen is similar to that of the allergic patients in the Coastal plain. None of the healthy, non-atopic volunteers reacted to any of the extracts.

Discussion

All the persons examined live in the same general area and are periodically exposed to *Pistacia* pollen. None of the healthy control persons reacted to *Pistacia* pollen extracts,

which indicates that the reactions were not due to a non-specific irritation. Some 41% of the atopic patients examined showed positive skin tests to at least one species of *Pistacia*. This means that *Pistacia* pollen extracts can elicit skin sensitizing antibodies in atopic persons. In Israel, hay fever is mostly due to grass-pollen sensitivity. The peak in grass flowering overlaps the flowering season of *Pistacia*. Therefore, it would be very difficult to determine clinically whether the hay fever symptoms resulted from exposure to *Pistacia* pollen alone. Furthermore, it seems reasonable to assume that trees of the genus *Pistacia* should be regarded as plants with a significant potential of allergenicity. It is interesting to note that no previous reports could be found in the literature regarding the allergenicity of *Pistacia* pollen [8,9]. Neither are extracts of *Pistacia* pollen found in commercial catalogues of firms producing pollen extracts for immunological tests and treatments.

Most of the examined patients showed sensitivity only to one or to two of the species, whereas seven patients (29.2% of responders) were sensitive to all the investigated species of *Pistacia*. The relatively large number of patients that responded to one or two species only strongly indicates that, although a certain antigen may be common to all species, the various species still have different and specific antigens. An alternative explanation might be a previous exposure of the patients to pollen of all the four species. The question of what part is played by the common allergens and what part is played by the specific ones, is still open.

This seems to be the first report of immunological sensitivity to pollen of trees of *Pistacia*. Probably, this results from the fact that the species of this genus are rather rare in North America and in Central and Northern Europe and, therefore, have been overlooked until now. Nevertheless, allergy to *Pistacia* might be of prime importance to the people of the Mediterranean basin and the Middle East.

Acknowledgments

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