

REVIEW

Pollen-related allergy in the European Mediterranean area

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There is a body of evidence which suggests that the frequency of atopic diseases—particularly those induced by pollen allergens (rhinoconjunctivitis and/or bronchial asthma)—is increasing because of interaction with air pollution; in fact, atopic diseases have become more common during the past two decades [1–3].

Irritant gases and airborne matter have both adjuvant activity for specific IgE production against common allergens in experimental animals and enhancing effects on allergic symptoms in sensitized subjects [4–6].

This trend is also evident in the Mediterraneans, where the climate, characterized by mild winters and dry summers, facilitates the growing of a characteristic vegetation with the production of various types of allergenic pollen, some of which are different from those of central and northern Europe [7]. However, in the context of the Mediterranean area, several concurrent climatic and geographical factors promote the growth of various vegetations with many wind-pollinating plant families and species. As a result, the frequency of allergic sensitization to pollen is different, for example, between northern Italy on the one hand, with a continental and rather middle European climate, where allergy to grass pollen is more important, and southern Italy on the other hand, with a Mediterranean sub-tropical climate, where *Parietaria* is the major cause of pollinosis and Olive is responsible for many sensitizations [8].

The pollinosis situation in the northern Mediterranean area is halfway between the typical Mediterranean situations and that of central Europe.

In the latter area, and particularly in the southern part of France, mean annual pollen counts show that grass pollen and Cupressaceae pollen are the most frequent causes of pollinosis.

Some Mediterranean pollen types, such as Oleaceae, *Platanus* or *Parietaria*, are also of importance. Plantain is also represented in relatively large amounts [9].

Pollen calendars

Data on the presence and prevalence of allergenic airborne pollens, obtained from both aerobiologic studies

and allergologic investigations, make it possible to design a pollen calendar with the approximate flowering period of the plants in the area in which the sampling is carried out. In this way, even though allergenic plants experience considerable fluctuations in pollen production and dispersal from year to year reflecting patterns in pre-seasons weather and the conditions prevailing at the time of anthesis, it is usually possible to compare the chances of high atmospheric allergenic pollen concentrations in different areas, which can provide useful information for the allergic patient when his/her trips are being planned or in preventing the possible appearance of severe hay fever symptoms by appropriate medication or other measures [7].

In the Mediterranean area we find a typical vegetation with the presence of some allergenic plants, such as *Parietaria*, *Olea europaea* and Cupressaceae, besides various species of Gramineae and mugwort [7–10].

The aerobiologic sampling of the pollen content of the atmosphere, carried out in various Mediterranean cities, led us to identify three pollen seasons: (i) a low winter season (from December to end of March), marked by the presence of the pollens of such trees as Cupressaceae (*Cupressus* and *Juniperus*), Corilaceae (Hazel), Acaciae (Mimosa) and some Betulaceae. (ii) a high spring–summer pollen season (from April to July) of marked allergological interest, dominated by the pollination of Gramineae, *Parietaria* and *Olea*. In this period, from March to May, we also find the *Platanus* pollination, which has some allergenic importance in areas such as southern France. (iii) a summer–autumn season (from August to October), in which we find the second, lower peak of *Parietaria* and sometimes of Gramineae and the pollens of herbaceous plants, such as mugwort and Chenopodiaceae.

Considering allergenic pollen as a whole in the Mediterranean area, Gramineae are the most important cause of pollinosis [7–11]. Until the beginning of April no considerable atmospheric concentrations of grass pollen are reported in the Mediterranean area [10–12].

From the second half of April the pollination period with emergency of clinical symptoms starts in central-southern Italy, Greece and southern Spain, followed by northern Spain, southern France, northern Italy and former Yugoslavia [10].

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The seasonal pollen concentration and grass pollinosis symptomatology increase progressively in May and June in about all European Mediterranean areas, decreasing progressively in July.

Even though Gramineae are the most important cause of pollinosis in the Mediterranean area, other typical pollen grains play an important role. In this paper we are dealing with these typical allergenic pollens, in particular *Parietaria*, Olive and Cypress.

Parietaria

Parietaria officinalis and *P. judaica* are the most common species within the genus *Parietaria*. Others are *P. lusitana*, *P. mauritanica* and *P. cretica* [13].

These highly productive, anemophilous species are characterized by numerous, grouped, individually small and drab, scentless florets. *P. judaica* is thermophilic and heliophilic; consequently, it grows in coastal Mediterranean countries, such as Spain, southern France, Italy, the former Yugoslavia, Albania, and Greece [14–24].

The habitat of *P. officinalis*'s is hilly or mountainous (under 1000 m altitude) in Spain, France, northern Italy, Austria, Bulgaria, Czech and Slovak Republics, Romania, and in the south-western regions of the former USSR [14–16,18,21,23–25]. *Parietaria* pollen grains are small (mean diameter 12–16 μm), light, and spheroidal (Fig. 1).

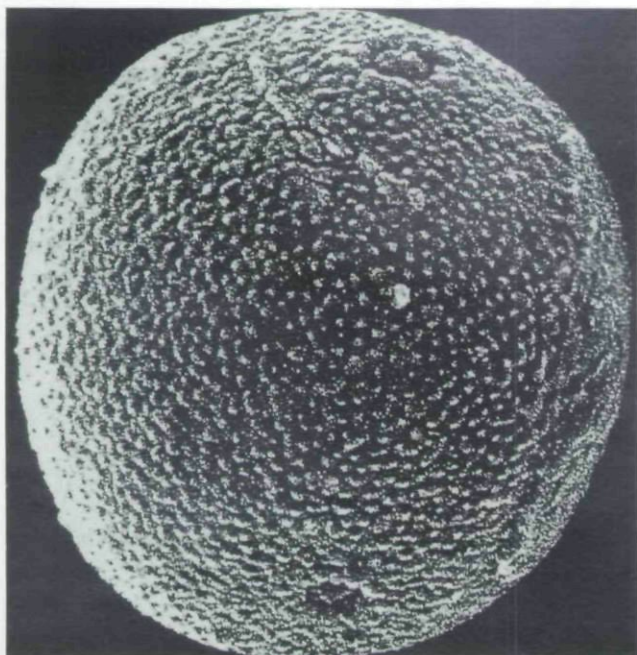


Fig. 1. Pollen grain of *Parietaria judaica* shown by scanning microscopy (original magnification $\times 4000$).

These characteristics favour wind pollination and penetration into airways. A large number of triporate pollen grains can be spread by propulsion (spring release) from the anther's flexible filaments.

Factors influencing the pollen output include temperature, humidity, and light intensity. The anthers of *Parietaria* open during favourable weather, particularly when it is warm and dry. They tend to open during daylight hours, from early morning to late afternoon.

The pollination periods of *Parietaria* and *Urtica dioica*, other non-allergenic species of Urticaceae family, overlap. In addition, it is not possible to distinguish the pollen grains by means of microscopic analysis, despite slight differences in size. Therefore, it is preferable to quote as 'Urticaceae' (the family including *Parietaria* as well as the genus *Urtica* [nettles]) the counts of atmospheric pollen without attempting to discriminate between genera or species [14,18,21].

The duration of *Parietaria*'s pollen season varies with the climate. In southern Italy it can persist from February to December [14,18,20,21], resulting in almost perennial symptoms, whereas in northern Italy and southern France and on the Mediterranean coast of Spain, pollination occurs on average between April and September [14–16,19,23,26,27].

The main *Parietaria* cycle of flowering in southern Italy, particularly in the Naples area, begins in March and continues until July [18,28]. Peak pollination occurs in May and June. In September and October a lower peak is detectable.

A prodigious output of pollen ensures the reproductive success of the plant. The mean pollen counts from the end of April to the end of June vary between 100 and 500 pollen grains/ m^3 of air, sometimes reaching peaks of 700–800/ m^3 of air. This depends on climate and on yearly variations in pollen production.

The pollen extracts of *P. officinalis* and *P. judaica* contain allergens recognized by IgE and IgG serum antibodies in patients with *Parietaria* allergy. Such allergens are, to a large extent, immunologically cross-reactive [26,29–31].

Pollen extracts of either species have been fractionated by several different methods [23,26,30,32–41]: the reported numbers of IgE-binding components are nine (*P. judaica*) and eight (*P. officinalis*) respectively. Individual differences in the array of IgE antibody specificities for allergens fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) have been assessed by comparing the immunoblot patterns of individual sera [38]. *P. officinalis* and *P. judaica* pollen extracts contained a 10–15 kDa band on electrophoresis. This corresponded to a major allergen, since it was present in each individual pattern; from one to eight

additional bands (18–90 kDa) appeared sporadically (in approximately 50% of the patterns).

The major allergens of *P. judaica* and *P. officinalis* have been purified from pollen extracts [36,42–47]. *Par o* I [45] and *Par j* I are glycoproteins: the first has an apparent molecular weight of 14.5 kDa. Reports on the molecular weight of the second are contradictory: there is general agreement on a molecular weight of 10–15 kDa by SDS-PAGE in reducing conditions [42,43,48,49], while using size-exclusion high-performance liquid chromatography (HPLC) analysis (non-reducing conditions) the reported molecular weight varies from 13 to 26 kDa [42,43,48].

The sequence of 12 NH₂-terminal amino-acid residues of *Par o* I has been determined [45]. It is often impossible to purify allergens from pollen extracts as single molecular species: it has been suggested that this micro-heterogeneity, which is a serious limit to studies on the structure/function relationship, may reflect events at the genetic level (allelic, isotypic, species variability) and/or the epigenetic (post-translational modifications of proteins, such as addition of carbohydrate chains) [44]. Purified *Par j* I and *Par o* I preparations contain closely related molecular species different for charge and electrophoretic mobility [45,49]. Polo *et al.* [49] report that charge heterogeneity of *Par j* I is decreased by deglycosylation. High-performance ion-exchange chromatography and immunoaffinity chromatography suggest that *Par j* I is a very unstable molecule which tends to fragment in solution. Several fragments obtained from *Par j* I have shown positivity toward an anti-*Par j* I monoclonal antibody, suggesting that they retain the structure of the allergenic determinant [50].

Recently, cDNA technology has been used to define the physicochemical and immunochemical structure of pollen allergens [29,51–56]. Preliminary reports suggest that the recombinant allergens are individual members of families of proteins that are represented in the mixture of isoforms composing the natural allergen [53,54]. Preliminary experiments to clone the cDNA genes specifying the major *P. judaica* allergen have been performed by d'Abusco *et al.* [57]. A cDNA expression library constructed in the vector lambda gt 11 [57,58] has been immunoscreened with rabbit *Par j* I antisera and with sera of allergic patients to identify clones with inserts encoding epitopes of the major allergen. A short cDNA insert expressing a fusion protein with *Par o* I/*Par j* I cross-reactive epitope(s) for human and rabbit antibodies has been characterized.

The relationship between the physicochemical structure of *Par j* I and its IgE-binding ability has been studied by Polo *et al.* [49]: their findings strongly suggest that the epitopes for the human IgE antibody are located mostly in the protein moiety and are conformational, as the IgE-

binding activity was maintained after deglycosylation, but abolished by reduction and alkylation of the native allergen.

IgE-binding proteins from partially purified fractionation of the pollen extracts include three *P. judaica* polypeptides (20, 30, and 40 kDa by SDS-PAGE) cross-reactive with *Par j* I. These were isolated by affinity chromatography with a mouse monoclonal antibody. Low molecular weight IgE-binding components were separated by gel filtration of *P. judaica* and *P. officinalis* pollen extracts [59,60]. Leaves and stems of *P. judaica* have also been found to have allergenic quality [61].

Di Felice *et al.* [62] have reported the ability of *P. judaica* pollen extract to induce antigen-specific proliferation of peripheral blood mononuclear cells (BPMC) in *Parietaria* allergic and non-allergic subjects.

Clinical data

At the clinical level, sensitizations to *P. judaica* and *P. officinalis* cannot be distinguished because of the high allergenic crossreactivity between these two major species of *Parietaria*.

Sensitization to *Parietaria* pollen greatly varies according to the geographic area. Pollinosis caused by *Parietaria* is less frequent before the age of 10 years [63]. A greater frequency of reactivity to *Parietaria* in women than in men has been reported in Europe [20,64] and in the US [65].

Sensitization to *Parietaria* is more common among people living in coastal towns than among country dwellers and inland urban inhabitants. The prevalence of sensitization to *Parietaria*, considering mono- and polysensitization, among pollinosis patients ranges from 40 to 80% in southern Italy and the Ligurian coast [18], from 10 to 60% in Mediterranean Spain [24], and it is approximately 45% in southern Greece [66] and 25% in Mediterranean France [26] (Table 1).

Table 1. Per cent of skin-test positivity to *Parietaria* pollen allergens in subjects of the Mediterraneans with suspected atopic respiratory allergy

Country	%	Reference
Southern France	15.2	[100]
Spain	36.3	
Greece	37.2	[101]
Northern Italy	4–12	[67]
Central Italy	9–18	
Southern Italy	12–30	

The extraordinarily long persistence in the atmosphere of *Parietaria* pollen in the Mediterranean area is responsible for a multi-seasonal symptomatology. This can be almost perennial in some areas for some subjects. Even in areas where the pollination period is shorter, late asthmatic reaction (LAR) may challenge patients both in summer and in autumn [68].

The clinical and immunological reactivity of patients allergic to multiple-pollen spp. such as *Parietaria* has not been sufficiently investigated up to now [14,69]. Bronchial asthma or its equivalents, almost always associated with rhinoconjunctivitis, is present in 52% of the monosensitized *Parietaria* patients in central and southern Italy, reaching a peak of 60% in Naples and Rome, whereas it affects 40% of patients suffering from pollinosis caused by grasses [18]. *Parietaria* hay fever is often a frustrating experience for both patients and clinicians, possibly because of the long period and the high peaks of pollination [70,71].

Oleaceae

The Olive (*Olea europaea*) is a long-lived tree which has been grown by man for more than 5000 years for its fruit, oil and wood [72]. It probably originated in Asia Minor and then spread across the whole Mediterranean basin [73–75]. A wild form of Olive is referred to as 'Oleaster', but there are other arboreal forms that vary according to the country in which they grow; for instance, in Spain there are now about 10 varieties [76] and many more are to be found in Italy [77]. Still, it is not clear whether all these varieties of Olive trees are able to produce different allergens. In Italy, the different varieties of *Olea*, found in the northern and southern parts of the country, can induce different IgE-mediated reactions [78]. *Olea europaea* pollen is small (mean diameter 17–21 μm), spheroidal, isoporal and tricolporate; the equatorial contour is subtriangular while the meridian one is circular or lightly elliptic (Fig. 2).

The pollination of the *Olea* is entomophilous but also anemophilous when pollen production is abundant [79]. The pollination of the *Olea* lasts from January to early April according to latitude and local climatic conditions which, moreover, are variable from year to year [80].

The pollination season in Italy varies from one region to another. In Campania and Puglia (southern Italy) it lasts from early April to late June with peaks from late May to the first 2 weeks of June [10,18,70,81]. In Umbria (central Italy) and Liguria (northern Italy) it lasts until July [82,85,86]. However, the pollination period of the *Olea* is not very long, lasting only about 1 month.

As far as the correlation between the course of temperature during the months preceding the flowering of

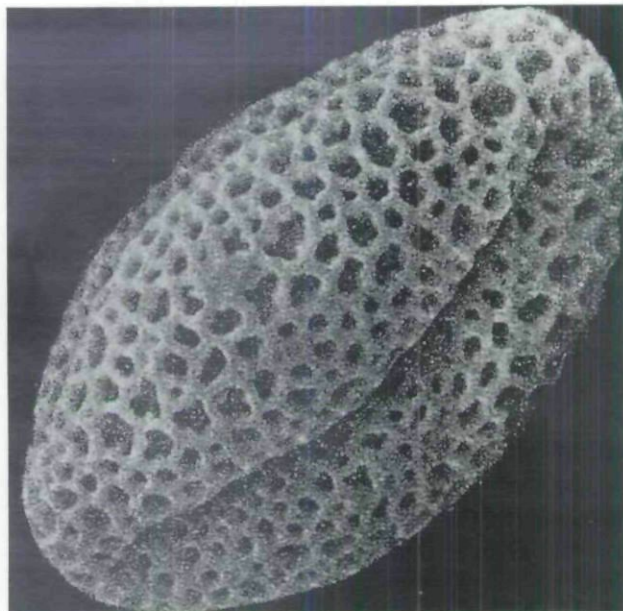


Fig. 2. Pollen grain of *Olea europaea* shown by scanning microscopy (original magnification $\times 4000$).

the tree and the beginning of pollination is concerned, a lower mean temperature delays the start of pollination while a higher temperature hastens it [83–85].

A precocious start of the pollination season is frequently characterized by a high pollen productivity. This trend, coupled to the records of mean temperatures of months preceding the flowering season, could represent, if confirmed, a means to forecast the start and severity of the pollen season of the *Olea* [83].

An agronomic phenomenon typical of the *Olea*, as well as of a number of other trees, is the rotation of productivity with the succession of seasons with higher and others with lower pollen production [80,81,86]. The highest atmospheric pollen concentration varies between 100–200 grains/ m^3 of air, which is the most frequent level in the Mediterranean area [18] and 2000–3000 grains/ m^3 in some areas such as Puglia, where the Olive is intensely cultivated [81].

Studies on the allergenic content of the *Olea europaea* pollen are currently in progress. Early studies on the allergen characterization of *Olea* pollen are those by Blanca *et al.* [87], who found 20 fractions with allergenic activity using IEF, RAST and RAST-inhibition. The major allergenic protein that was found at PI 6 through the use of SDS-PAGE was constituted by two sub-units of 15 and 17 kDa with a molecular weight of about 32 000 kDa.

Similar results were obtained by Hewitt *et al.* [87], who found various acid proteic fractions. Using gel chroma-

tography, Vela *et al.* [89] identified and described 10 different fractions and a major allergenic content of about 65 000 kDa, probably made up of aggregated basic and acid proteins. Likewise, studies by Lauzurica *et al.* showed a major allergenic glycoprotein of about 60 000 kDa consisting of different subunits of minor molecular weight in Olive pollen raw extract [90].

Several other authors [91–94] subsequently identified the major allergens of *Olea* pollen as proteins of 17, 19 and 42 kDa separated by SDS-PAGE, which could bind specific IgE in high percentage putted with serum of atopic subjects with *Olea* sensitization. A monoclonal antibody (OL-1) obtained from the immunization of mice with whole *Olea* extract was able to react with proteic fractions of 17 and 19 kDa separated by SDS-PAGE.

Other authors described an additional allergen of 30 kDa besides allergenic components superimposable to those already shown [95].

Studies carried out by Villalba *et al.* [96] made it possible to know the results of fractionization of allergenic proteins of 19 kDa until aminoacid analysis showing that *Ole e I* is composed of glycosilated proteins of 18 and 20.5 kDa with similar epitopes and different isoallergenic shapes. Barber *et al.* [97] showed that allergenic proteins of *Olea europaea* are highly variable, probably as a result of physical factors affecting the plant's maturation. This author identified the major allergenic activity in one multimeric unit of 55 000 kDa with a sub-unit of 20 000 kDa. Also free proteins were found of molecular weight 20 000 kDa with arcs of RAST inhibition like those of multimeric unit. On the basis of such results we can surmise that the allergenic activity of *Olea* pollen is probably associated with a protein family of polymers with a high degree of homology.

Lombardero *et al.* [98] showed that monoclonal antibodies OL-2 and OL-7 are able to identify two different epitopes of the *Ole e I*. These results confirm those obtained by Villalba *et al.* [96] who identified two fractions of about 18 and 20 kDa by SDS-PAGE.

Monoclonal antibodies were also used to study effects of major allergen depletion on the biological capacity of an extract. It was shown that *Ole e I* constitutes almost the totality of the biological capacity of a whole extract and the nearly total disappearance of the biological activity of an allergenic extract is due to its removal. Only 10% of atopic subjects with allergenic sensitization to Olive are in fact not sensitized to *Ole e I* [98].

Studies by Baldo *et al.* confirmed that in *Olea europaea*'s allergenic extract the proteic bands that more frequently or with intensity bind specific IgE belong to the following regions of molecular weight: approximately 14, approximately 19–20, approximately 40 and approximately 70 kDa [99].

Table 2. Per cent of skin-test positivity to *Olea europaea* pollen allergens in subjects of the Mediterraneans with suspected atopic respiratory allergy

Country	%	Reference
France	25	[26]
Spain	10–60	[24]
Greece	22	[66]
Northern Italy	9–44	[67]
Central Italy	20–33	
Southern Italy	15–70	

Olea europaea's pollinosis is characterized by a sometimes severe nasal and/or bronchial symptomatology; the prevalence among allergic people varies greatly according to geographical areas and the strength of tree cultivation [100–102] (Table 2).

It is possible to observe a great prevalence of nasal and conjunctival symptomatology in respect of asthmatic symptoms and the prevalence of the polysensitization (especially with other pollens) in respect of monosensitization. We can frequently demonstrate the development of perennial symptoms without correlation with the *Olea* pollination season in our monosensitized patients [103]. In the Mediterraneans allergic sensitization to other Oleaceae such as *Fraxinus* and *Ligustrum* is infrequent. While *Fraxinus* pollen may reach atmospheric concentrations of risk to atopic patients [104,105], *Ligustrum* pollen is present in very low concentrations. Only in conditions of local exposure (e.g. when cutting the *Ligustrum* hedge) can pollinosis symptoms develop [79]. However, we need to consider that a pollinosis patient allergic to *Olea* pollen and living in the Mediterraneans may, when travelling to northern Europe, presents allergic symptoms due to inhalation of pollen from other Oleaceae, such as *Fraxinus*. In fact there is a high degree of crossreactivity among Oleaceae pollens [106,107]. Recently, Obispo *et al.* demonstrated that the main allergen of *Olea europaea* (*Ole e I*) is also present in other species of the Oleaceae family [108].

Cypress

In the Mediterraneans, particularly Italy, France and Israel, allergy to Cupressaceae pollen, although apparently not widespread, has been increasing in the last two decades, probably because of interaction with air pollution, as occurs in other forms of pollinosis [4].

In some Mediterranean regions, where Cupressaceae are present, sensitization to pollen allergens of these trees is probably underestimated [109,110]. *Cupressus semper-*

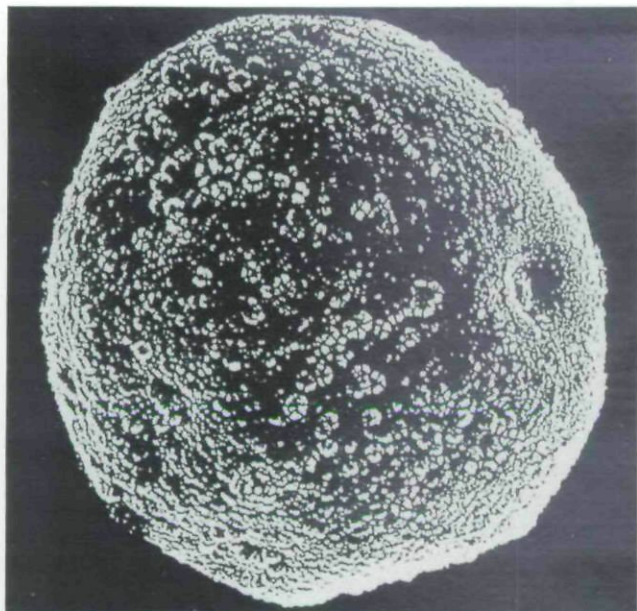


Fig. 3. Pollen grain of *Cupressus sempervirens* shown by scanning microscopy (original magnification $\times 4000$).

virens and *Cupressus arizonica* are the most common species within the genre *Cupressus*, *Juniperus* and *Thuja* are other genera of the Cupressaceae family. *Cupressus* pollen grain is spheroidal, isopolar and inaperturate (sometimes a pseudopore is present) with a diameter between 22 and 28 μm (Fig. 3). The *Juniperus* grains are identical with those of *Cupressus*, although often smaller.

Atmospheric concentration of *Cupressus* pollen are frequently very high, with peaks higher than 1000 grains/ m^3 of air [109]. Cour *et al.* demonstrated that Cypress pollination is characterized by a wide variability and in the southern part of France it can represent up to one-third of the total pollen grains [100]. The pollination season of *Cupressus* usually starts in January, continuing until the end of March or the first 2 weeks of April, with peaks between mid-February and the end of March [10, 109, 110].

Analysis of *C. sempervirens* pollen allergens proved difficult due to viscous nature and low protein content of aqueous extracts.

Ford *et al.* [111] identified in an extract of *C. sempervirens*, both on stained polyacrylamide gels and on the autoradiographs, one component approximately 42 kDa which reacted with IgE antibodies in the sera of 81.3% of the allergic subjects and, for each of the subjects, this component bound the greatest quantity of IgE.

Patients allergic to Cypress pollen usually suffer from conjunctivitis and sometimes rhinitis. Bronchial asthma is present in subjects with sensitization to other aeroaller-

gens also. In some patients with Cypress pollen allergy there is decreased skin reactivity to Cypress pollen allergen just before the start of the pollen season [112].

The diagnosis of allergy to Cypress is usually not difficult in Mediterraneans since the trees there have their pollination season in winter when other plants [except for *Corylus* (Hazel) and *Acacia* (Mimosa)] are not flowering.

Other allergenic plants

Among other allergenic plants in the Mediterranean area only *Artemisia* is responsible for some cases of allergic sensitization (% varying between 3 and 10%) with clinical symptoms from end of August and November [18, 113, 114, 121, 122].

In the interior of the northern Mediterraneans *Betula* [115] and ragweed [116–118] are present. Recently, *Mercurialis annua* pollen has been shown to be a new source of allergic sensitization and respiratory disease in the Mediterraneans [119, 120].

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