Chapter

Respiratory Allergy to Conifers

Denis Charpin, Hélène Sénéchal and Pascal Poncet

Abstract

The conifers, from the latin meaning "cone carrier," include about 650 species distributed in seven families. They are found all over the world, and the most known conifers are the cypresses, the junipers, the yews, the larches, the firs, or the pines. The most allergenic pollen is emitted by the Cupressaceae/Taxaceae family with mainly five different genera: *Cupressus, Hesperocyparis, Juniperus, Cryptomeria*, and *Chamaecyparis*. The symptomatic period starts in November and ends in April. In Mediterranean areas, *Cupressus sempervirens* is the most common pollinating species. Five main cypress allergens have been thoroughly described. Depending on the geographic area and the studied population, the prevalence of cypress allergy in the general population ranges from 0.6% to 3%, and 9–65% of outpatients consulting an allergist are sensitized to cypress pollen. This prevalence is increasing likely to be due to the modifications of the environment. Rhinitis is the most prevalent clinical symptom, while conjunctivitis is the most disabling. Clear-cut improvements of the quality of life are observed upon an effective and safe specific immunotherapy. Associations with food allergy based on molecular allergen cross-reactivities were described resulting in sometimes severe symptoms. Pollens from Pinaceae family, especially pines or firs, although abundant, do not demonstrate a significant clinical impact.

Keywords: cypress pollen, pine pollen, allergens, aerobiology, epidemiology, botanic, clinic

1. Introduction

Respiratory allergic diseases are among the most prevalent chronic disease, affecting 20–25% of the general population. Allergy reactions at large encompass several mechanisms, but allergy reactions to pollen are considered as a " type-1 " or " immediate-type " or " IgE-dependent " hypersensitivity reaction involving mast cells and basophiles as effectors cells. Those cells are responsible for releasing inflammatory and immune mediators leading to ocular, nasal, and bronchial symptoms. Pathophysiology of these reactions allows the use of skin tests and/or measurement of serum specific IgE as powerful diagnostic tools.

The prevalence of allergy is increasing whatever is the allergenic source, pollen, food, animals. Pollen grains are the main inducers of respiratory allergies, and conifers play a major role around the Mediterranean basin, in North America, or in Japan. According to a phylogenetical classification, the conifers consist of one class, Pinopsida, and seven families have been described: Araucariaceae, Podocarpaceae, Sciadopityaceae, Cupressaceae/Cephalotaxaceae/Taxaceae, and Pinaceae. No extensive studies were reported on the allergenicity of the pollen grains from Araucariaceae and Sciadopityaceae, but a huge amount of data are published for

Cupressaceae/Taxaceae (also reviewed in [1]) and Pinaceae pollen (see below). Cephalotaxaceae are sometimes included in Taxaceae and Araucariaceae and might be assimilated to pine because of the Wollemi pine discovered in Australia. A few data are available on the allergenicity of Podocarpaceae pollen [2, 3].

This review provides an update on various aspects of the highly allergenic family of conifer, i.e., Cupressaceae (Chapter 2) with, first, a botanical and palynological presentation of cypress followed by the various cypress pollen allergens involved; second, data on epidemiology; and third, the clinical aspects together with the management of cypress pollen allergy. Chapter 3 is devoted to the poorly allergenic conifer family, Pinaceae.

2. Cupressaceae

2.1 Trees, pollen, and allergens

2.1.1 Trees

Cupressaceae corresponds to a family of the order Pinales. According to a phylogenetical classification, the family includes about 140–160 species with 27–30 genera. Cupressaceae is the most widely distributed conifer worldwide, except Antarctica devoid of any trees (**Figure 1**). Cupressaceae, commonly named cypress, is the most well-known gymnosperm family that produces allergenic pollen. Two main contributors to cypress pollen allergies belong to Cupressoideae by species from the *Cupressus*, *Juniperus,* and *Thuja* genera and to Taxodioideae by species from *Cryptomeria* and *Taxodium* genera [4] (see below the description of the respective allergens in the section "Allergens").

Besides botanical and phylogenetical classification, a classification was proposed based on the functional and structural aspects of allergens (**Table 1**) [4, 5]. These allergens in different species exhibit a high degree of homology, up to 97% between *Hesperocyparis arizonica* (Cup a 1) and *Cupressus sempervirens* (Cup s 1), although molecular studies led to a split of the two species into two different genera, *C. arizonica* being assigned to the newly created *Hesperocyparis* genus [6]. Botanical proximity is responsible for cross-reactivities. The same molecular-type allergen produced by botanically distant plants appears very limited [7, 8].

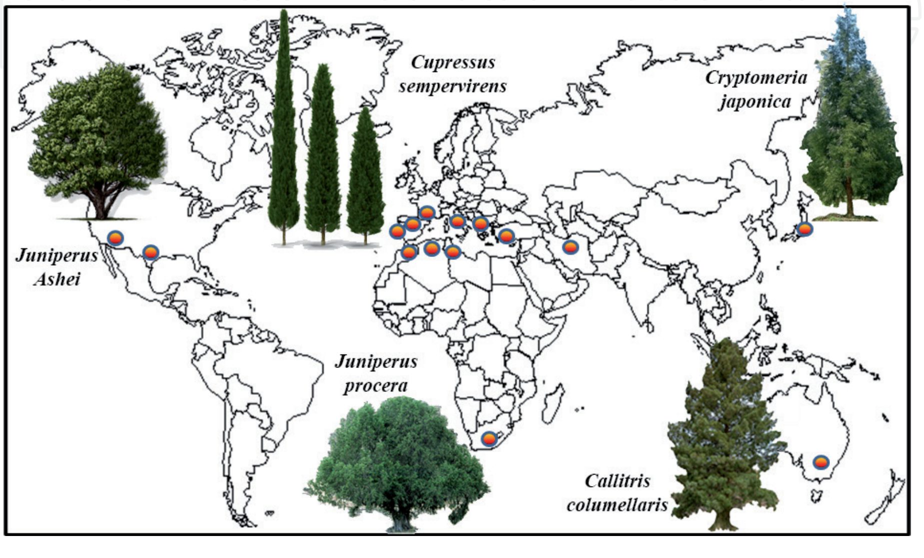

Figure 1.
Worldwide distribution of reported Cupressaceae pollen allergy (orange dots).

Groups	Proteins	Cupressus sempervirens	Hesperocyparis arizonica	Cryptomeria japonica	Juniperus		Chamaecyparis obtusa	Taxodium disticum	Thuja
					ashei	*other*			
		Italian cypress	Arizona cypress	Japanese cedar	Mountain cedar		Japanese cypress	Bald cypress	cedar
Group 1	Pectate lyase (40-45 kDa)	Cup s 1*	Cup a 1*	Cry j 1*	Jun a 1*	Jun c 1, o 1, v 1*	Cha o 1*		Thu p 1
Group 2	Polygalacturonase (43-60 kDa)	Cup s 2*	Cup a 2	Cry j 2*	Jun a 2*		Cha o 2*	Tax d 2	
Group 3	Thaumatin-like protein PR-5 (24-34 kDa)	Cup s 3*	Cup a 3	Cry j 3	Jun a 3*	Jun r 3, v 3*			Thu oc 3
Group 4	Ca-Binding protein (17-29 kDa)		Cup a 4	Cry j 4		Jun o 4*, v 4			
Group 5	Gibberellin-regulated protein (8kDa)	Cup s 7*	Cup a 7	Cry j 7*	Jun a 7*				
OTHER		ß-galactosidase 46-50 kDa	ß-galactosidase 46-50 kDa	Chitinase 27 kDa			Cha o 3* 63 kDa		
		Profilin (Cup s 8) 14 kDa	LTP 14 kDa	CJP8 (LTP) 17 kDa					
		Phenylcoumaran reductase 33 kDa		Isoflavone reductase 35 kDa					
		Rab-like protein 18 kDa		Aspartic protease 42 kDa					
		Sigma factor regulation protein 29 kDa		Serine protease subtilisin-like 79 kDa					

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Groups	**Proteins**	**Cupressus sempervirens**	**Hesperocyparis arizonica**	**Cryptomeria japonica**	**Juniperus**		**Chamaecyparis obtusa**	**Taxodium disticum**	**Thuja**
					ashei	*other*			
		Italian cypress	Arizona cypress	Japanese cedar	Mountain cedar		Japanese cypress	Bald cypress	cedar
		Cytochrome c 12 kDa							
		SOD 15 kDa							
		Lactoyl glutathione lyase 32 kDa							
		Malate dehydrogenase 31 kDa							
		Triosephosphate isomerase 33 kDa							
		Glucanase 37 kDa							
		HSP104 104 kDa							

**referenced in IUIS/WHO database; Jun c:* Juniperus communis *(Common juniper) ; Jun o:* Juniperus oxycedrus *(Prickly juniper); Jun r:* Juniperus rigida *(Temple juniper); Jun v:* Juniperus virginiana *(Eastern red cedar); Thu p:* Thuja plicata *(Western red cedar); Thu oc:* Thuja occidentalis *(Eastern white cedar); SOD: Superoxide dismutase; LTP: Lipid transfer protein; HSP: Heat shock protein.*

Table 1.
Cupressaceae allergens. Name, protein function, and molecular masses (kDa) are indicated.

2.1.2 Pollen

2.1.2.1 Pollination: phenology and pollen features

Within a species, the pollination period is usually short. However, because of the different species in Cupressaceae, the pollination periods do not overlap, and this contributes to extend the exposition duration to Cupressaceae pollen from autumn to spring. In Mediterranean regions, pollen from early pollinating species (e.g., *Juniperus oxycedrus*) is produced in October or November [9–11], while pollination by late pollinating species (e.g., *C. sempervirens*) can occur up to late April or May [12]. Belmonte *et al* reported the diversity, floral phenology, and distribution of the Cupressaceae species in the Iberian Peninsula in 1999 [13].

All Cupressaceae species produce spherical pollen grains very similar in appearance (**Figure 2A**) [14]. In the *Cupressus* genus, the mean size of hydrated pollen grains varies from 25 to 40 micrometers (**Figure 2B**). However some variations can occur. Pollen grains are small for Mediterranean species (except for *C. dupreziana*, which produces diploid pollen [16], intermediate for New World species, and larger for Asian species [17]. The Cupressaceae pollen is inaperturate, although a faint circular pore blocked with a bulge can be seen in fresh material. The exine (outer membrane) is very thin and covered with scattered granules or orbicules (Ubish bodies, 300–600 nm) (**Figure 2A**). The intine (inner membrane) is very thick, and hydration unblocks the bulge leading to the swelling of the intine until the exine cracks (**Figure 2B**).

Cupressaceae trees are anemophilous, and pollen grains can be wind-transported over long distances because of their small size. Cupressaceae species generally produce huge quantities of pollen. The number of pollen grains per male inflorescence average 400,000, and production by individual trees has been estimated to be 276,000 million [18, 19]. Cupressaceae pollen predominates in the winter period, but can also be present all year long (**Figure 3**). In Mediterranean regions, *Cupressus,* together with *Olea,* produces the largest amount of allergenic tree pollen [20]. Cupressaceae/Taxaceae pollen is one of the 12 most abundant aero-allergenic pollens in Europe [21]. *Cupressus* pollen can account as much as 40% of the total annual pollen counts in Marseille, in Southern France [22], 38% in Antalya [23], and 35% in Istanbul, Turkey [24], 25% in Thessaloniki, Greece [25], 23% and 24% in Toledo and Cuenca, Spain [9, 26], 18% in Nicosia, Cyprus [27], 17% in

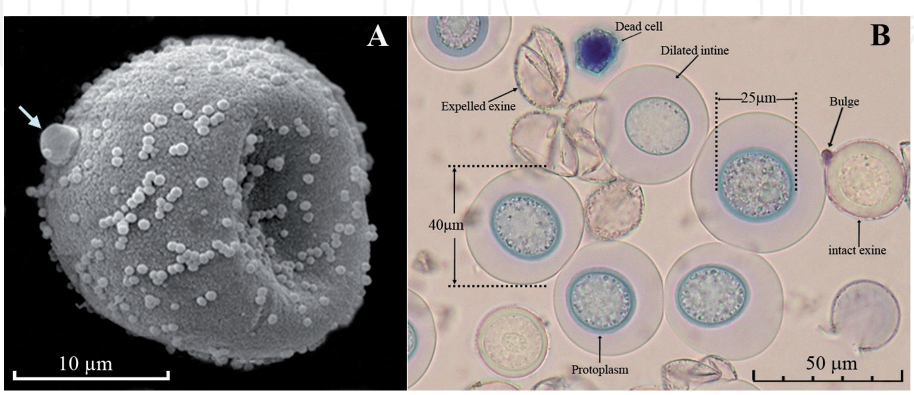

Figure 2.
A: Hesperocyparis arizonica *pollen grains (scanning electron microscopy from Shahali et al. [14]) collected in Tehran, Iran. (2225x magnification). The arrow shows the presence of a bulge on the external surface of the exine serving as a valve for regulating the entrance of fluids at the beginning of pollen hydration [15]. Numerous submicronic orbicules (300–600 nm) are visible on the pollen surface. B:* Hesperocyparis arizonica *pollen hydrated for 5 min in phosphate buffer saline. Optical light microscopy observation after viable trypan blue staining (100x magnification). The various elements are indicated.*

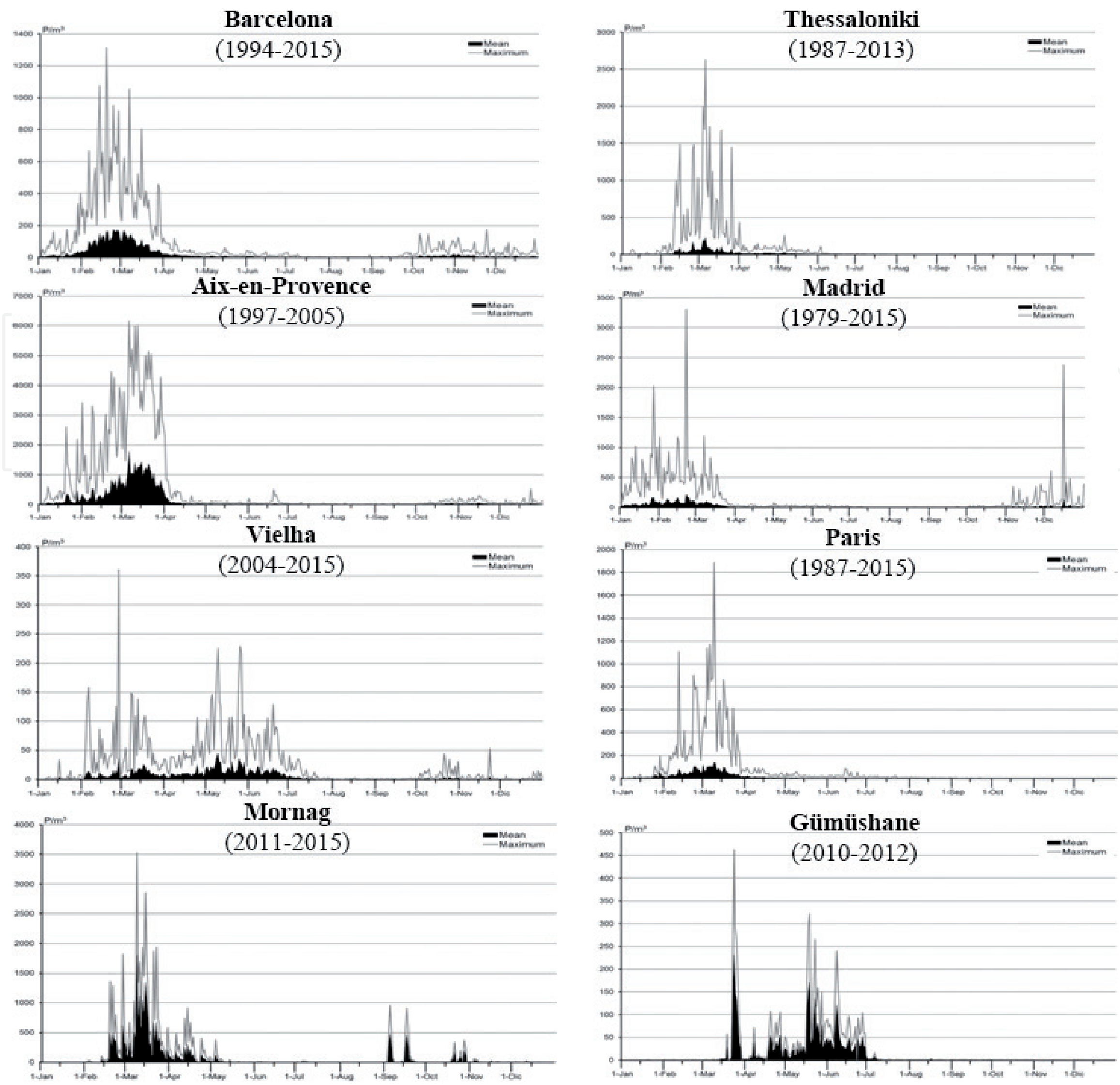

Figure 3.
Cupressaceae pollen dynamics over the course of the year in the Mediterranean area: Barcelona (Spain); Aix-En-Provence (France); Vielha, (Spain); Mornag (Tunisia); Thessaloniki (Greece); Madrid (Spain) and outside Mediterranean area: Paris (France); and Gümüshane (Turkey). Mean daily (thick black line) and maximum daily (thin black line) pollen concentrations are indicated for the period.

Palma de Mallorca, Balearic Islands, Spain [10], and 14% in Nerja, southern Spain [11]. Cupressaceae pollen is also abundant or present outside of the Mediterranean region: Northern Europe, 8% for Cupressaceae and Taxaceae in Munster, Germany [28], South America, 30% in Bahia Blanca, Argentina [29], North America, 18% in Mexico [30], 5–10% on the east coast of the United States [31, 32], and up to 3872 pollen grains/m^3 in January in Tulsa, Oklahoma, central United States where *Juniperus ashei* is predominant [33, 34], Asia, 19% in Yunnan, China [35], and 60% in Japan because of the huge presence of *Cryptomeria japonica* [36], and finally 3% in Santa Cruz de Tenerife, Canary Islands, Spain [37].

2.1.2.2 Aerobiology

Comparative sampling methods developed during the last decades of the twentieth century showed that concentrations of airborne pollen diversity have steadily progressed [36–39].

At least four indices exist to characterize the dynamics and patterns of airborne pollen: *(a):* the mean daily pollen concentration, expressed as the number of pollen grains per cubic meter of air (P/m^3); *(b):* the annual pollen index (API), which corresponds to the sum of the mean daily pollen concentration for each year); *(c):* the dates corresponding to the beginning and end of the pollination; and finally *(d):* the duration of the pollen season.

In the case of cypress pollen, the pollination period (except in mountainous and in very cold sites) begins in autumn and lasts until the end of the following spring. However, in the Mediterranean area, days without any cypress pollen are rare. Therefore a percentage method was proposed. The season is considered to begin when 2.5% of the API has been reached and finished when 97.5% was reached. This method is not totally satisfactory because of substantial year-to-year variations of API.

Using pollen collectors mainly located in urban areas, the Cupressaceae API showed increasing trends in Mediterranean countries. This was shown in Southern France with an early pollination onset [40], in Greece [41] or in Catalonia (NE Iberian Peninsula). Two of these API trends, for Barcelona and Vielha, are shown in **Figure 4** together with the trends in other localities around the Mediterranean. These trends were confirmed for 23 taxa from 13 European countries (97 sites) [42]. Authors did not find any correlation with variation of temperature and rather proposed, as an explanation, the extensive use of Cupressaceae as ornamental plants in the cities. Ariano *et al.* [43] have, however, attributed to climate change a possible role in variations in pollen seasons and allergic sensitizations.

The daily pollen concentration threshold levels required to elicit allergic symptoms in patients remain a crucial question, and no general agreement has been reached. For instance, in Israel, the threshold is considered to be between 10 and 50 pollen grains/m^3, whereas in France, different thresholds of symptom risk have been established for the Mediterranean area (designated as low, when 7–13 pollen grains/m^3, moderate when 14–141 pollen grains/m^3, and high >141 pollen grains/m^3, respectively), and for the north and center of France (designated as low when 70–141 pollen grains/m^3 and moderate when >141 pollen grains/m^3 [44]). The Catalan Network of Aerobiology defined the risk of allergy as being low when concentrations are <20 pollen grains/m^3, moderate for 20–50 pollen grains/m^3, high for 50–100 pollen grains/m^3, and very high when >100 pollen grains/m^3. Furthermore the risk to develop allergy symptoms was shown to be increased by airborne pollutants, especially $PM_{2.5}$ and suspended particulate matter [45].

2.1.2.3 Allergenicity of cypress pollen

The cypress pollen is considered to be highly allergenic (see, for instance, the website of the French National Network of Aerobiological Surveillance, RNSA,"Réseau National de Surveillance Aérobiologique", www.pollens.fr). The allergenic potential of specific pollen depends on the following:

- the degree of exposure, related to the total pollen amount released in the atmosphere (from intact or fragmented grains);
- the phenological conditions in the considered area;
- temperature, hygrometry, photoperiod, ...;
- air pollution.

The exposure to cypress pollen is high because of an abundant production of pollen (see pollen chapter), making of this pollen the most represented in the atmosphere (up to 40% of total pollen counts around Marseille in the south of

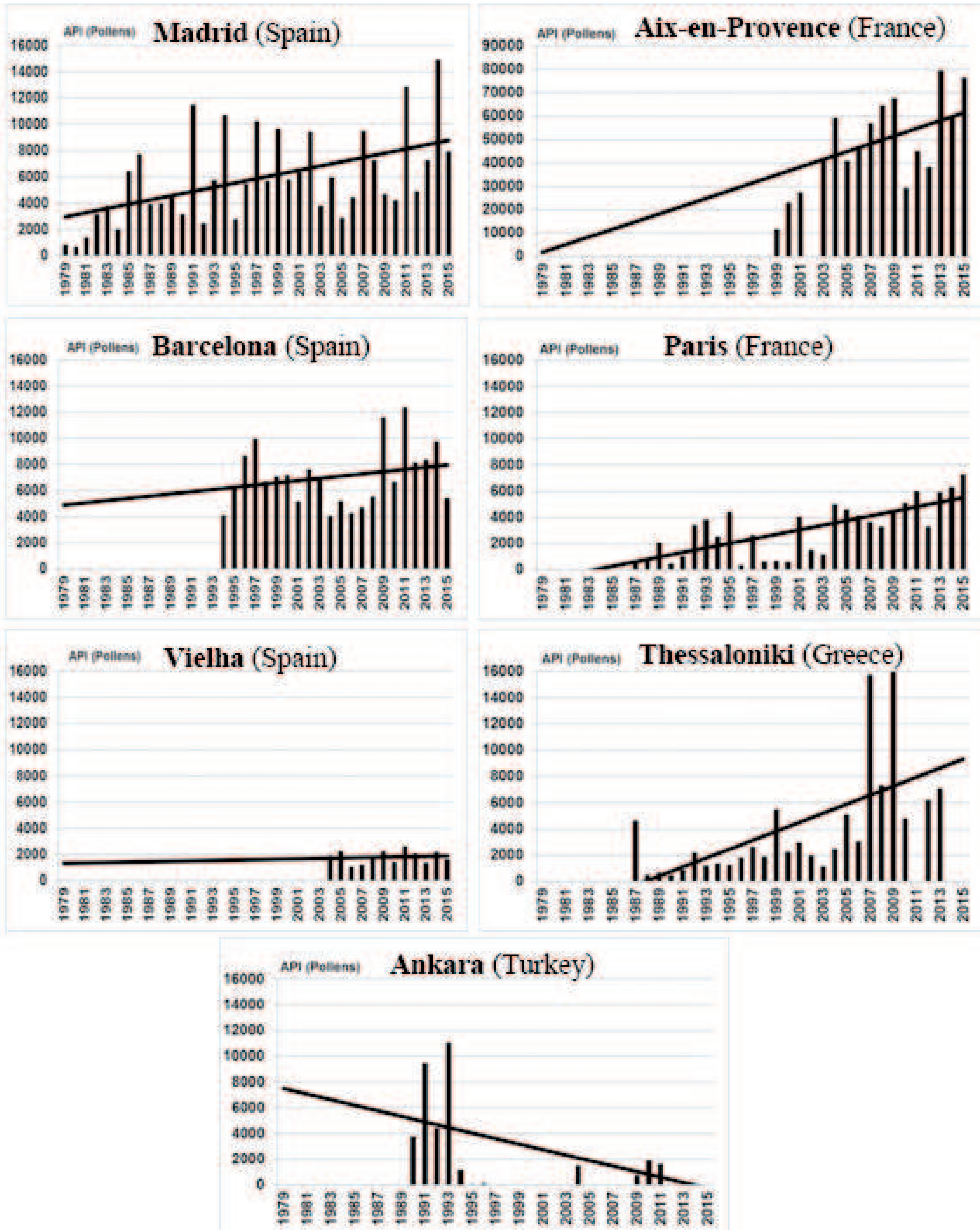

Figure 4.
Cupressaceae annual pollen index (API) and trends at the localities with the longest continuous data series. Madrid (Spain), Barcelona (Spain), Vielha, (Spain), Aix-En-Provence (France), Paris (France), Thessaloniki (Greece), and Ankara (Turkey).

France). Not only is the load high but also the spreading, since rather small pollen, can be wind-transported. Moreover, the pollen grain carries sub-micronic particles named orbicules on its surface (**Figure 2A**). These orbicules were shown to contain allergens from the groups 1 and 2 [46–50] (see below for the definition of groups of allergens) and to be easily released upon rainfall and an experimental *in vitro* treatment of cypress pollen grains with NO_2, a gas frequently found in gaseous pollutants [46, 51]. Because of their small size (300–600 nm), orbicules might be able to penetrate deeper in the bronchial tract and sensitize individuals to induce asthma, as was shown in a rat model [52]. However, free airborne orbicules have never been evaluated; therefore such an orbicule-sensitizing mechanism, in real conditions, was not as yet demonstrated. Besides these characteristics, and in addition to protein allergens, some biogenic intrinsic adjuvant molecules may contribute to the allergenicity of cypress pollen [53–55] by favoring Th2 immune responses and/or triggering innate immune responses. For instance, NADPH oxidase, an enzyme that generates reactive oxygen species, is reported to be involved in inflammation [56, 57]. This enzyme, required for pollen tube growth, is intrinsically expressed at different levels in the pollen grain of various plant species [58, 59]. Cypress pollen is one of the tree pollens containing the highest level of redox activity as compared with other pollen and in particular to the poorly allergenic pine pollen, which expresses the lowest redox activity. Moreover, other biogenic cofactors, pollen-associated lipid mediators (PALMs), play a role in pollen allergenicity. Studies performed on cypress pollen show that lipids of the pollen membrane interact with CD1+ dendritic cells to activate CD1-restricted T cells with the Th0/Th2 phenotype [54]. PALMs are also able to activate eosinophils and neutrophils and decrease IL12 production from dendritic cells, therefore, favoring Th2-biased immune responses [60–64].

2.1.3 Allergens

2.1.3.1 Cypress pollen allergens

C. japonica was the first cypress pollen studied at the level of allergen content, and in 1983 Cry j 1 (previously called SBP, for Sugi Basic Protein) was reported to be its major allergen [65]. Several other allergens were then described in *C. sempervirens* and *Hesperocyparis arizonica* [66–68]. All research groups deciphering allergens from the various cypress species reported a cross-reactive 42–43 kDa protein as being the major cypress allergen: Cry j 1 for *C. japonica*, Cup a 1 for *Hesperocyparis arizonica*, Cup s 1 for *C. sempervirens*, etc… (see **Table 1**). As compared with grass, olive, or ragweed pollen, the extraction of proteins from cypress pollen is difficult, yielding few proteins. This particularity may be related to the high sugar content of the intine quickly swelling when the pollen grain is hydrated; this might delay or prevent the release of proteins [15] (**Figure 2B**). One way to circumvent this difficulty is to grind the pollen in suspension with silica microbeads.

Up to now, five groups of allergens have been described in cypress pollen, although all allergen members for each species have yet to be referenced in the International Union of Immunological Societies (IUIS) allergen data bank (www.allergen.org): group 1: pectase lyase, group 2: polygalacturonase, group 3: thaumatin-like protein, group 4: Ca-binding protein, group 5: Gibberellin-regulated protein. Furthermore, about 20 additional allergens have been reported in the three most studied pollens, *C. japonica*, *Hesperocyparis arizonica*, and *C. sempervirens* (**Table 1**). More details on cypress allergens are reported in Charpin et al. [1] and Poncet et al. [69].

2.1.3.2 Cross-reactivities

- Pollen/pollen

Cross-reactivities between pollen are common because proteins may belong to families of panallergens, such as Ca^{++}-binding proteins or profilins. Some cross-reactivities were observed with *Podocarpus gracilior* of the Pinales order [70], although other authors did not find any cross-reactivity of *C. sempervirens* with pine pollen [71]. *Parietaria judaica*, *Lolium perenne*, and *Olea europaea* pollens were shown to exhibit some degree of cross-reactivity [72] although the nature of the involved allergens remains unknown.

- Pollen/food

Like for pollen from birch, mugwort, grass, ragweed, olive, plane, cypress pollen sensitization was shown to be associated to food allergies. In general, up to 60% of food allergies are associated with an inhalant allergy [73]. A pollen food allergy syndrome (PFAS) has been described, including mainly an oral allergy syndrome. As soon as 2000, Ishida *et al* reported PFAS in patients allergic to Japanese cedar pollen following consumption of specific vegetables and fresh fruits (e.g., melon, apple, peach, and kiwi) [74]. The cypress/peach syndrome was mostly studied [75, 76]. Symptoms might, in some conditions, with cofactors, be more severe than an oral syndrome, up to an anaphylactic shock [77]. An uncharacterized (putative Cup s 1 or Cup s 2) allergen of 45 kDa was proposed to be the cross-reactive allergen [76], and more recently the allergens from the Gibberellin-regulated protein family (group 5 allergens) were shown to be cross-reactive with peach, Japanese apricot, citrus, and pomegranate [77–80]. Prevalence of sensitization to Pru p 7, the GRP from peach, coincides with the prevalence of sensitization to cypress pollen in France [81].

2.2 Epidemiology of cypress pollen allergy

Cypress pollen allergy was reported for the first time in 1929 in the United States (Texas and New Mexico) [82] and in the early 1960s in Europe [83]. Cypress pollinosis is also reported in several locations worldwide: Japan [84], Australia [85], Iran [86], South Africa [87], the United States, and with special emphasis around the Mediterranean basin [88–94] (**Figure 1**).

2.2.1 Prevalence of sensitization and allergy to cypress pollen in non-selected populations

There are consistent correlations between exposure to Cupressaceae/Taxaceae pollen and the presence of sensitization and allergy [95]. Studies performed in the general population are scare. In southern France and in Italy, two studies performed in children [96, 97] and one study in young adults [98] concluded that around 2–4% might suffer from cypress pollen allergy. A study performed in Japan led to a number of *Cryptomeria* pollen allergy of 13% [97–99].

2.2.2 Prevalence of sensitization and allergy to cypress pollen in outpatients

In surveys performed in Mediterranean countries, 14–32% of patients attending an allergy clinic had an allergy to cypress pollen [1]. In a larger Italian study from Rome, 23,077 outpatient sera were studied. The presence of specific IgEs against 75 allergens was investigated, and 42.7% of the subjects exhibited specific IgEs against cypress pollen. In this survey, cypress allergy was the leading cause of sensitization in adults over 35 years of age (in children, house-dust mite allergy was the leading cause) [100]. In Montpellier, a cross-sectional study performed in 400 outpatients concluded that cypress pollen sensitization (20.7%) ranked third, after sensitization to *Dermatophagoides farinae* (37%) and *pteronyssinus* (43%) allergens [101].

2.2.3 Increase in the prevalence of cypress pollen allergy

Several cross-sectional surveys carried out repeatedly over time showed an increase in the proportion of cypress allergy among outpatients consulting for allergic rhinitis: rising from 9.9% in 1991 to 24.5% in 1993, then to 35.4% in 1994 in central Italy [102], from 9.3 to 30.4% between 1994 and 1999 in the area around Rome [103], and from 7.2 to 22.0% between 1995 and 1998 in Italy's Latium area [104]. A recent study in the southern region of Italy showed that cypress pollen sensitization almost doubled from 2005 (17%) to 2010 (29%) [105]. Eighteen were sensitized to cypress or Taxodiaceae pollen in an Italian survey of 3057 outpatients selected in 12 study centers [106]. The sensitization rate was higher in southern Italy (20.1%) and central Italy (28.2%) than in northern Italy (9.2%). In a more recent survey, the geographical hierarchy was unchanged, but the prevalence figures went up to 32.7%, 62.9%, and 16.1%, respectively [107]. A study performed in western Liguria demonstrated an upward trend, whereas pollen counts remained unchanged [108, 109]. This study, like the one by Mari *et al.*, suggests that confounding factors, such as the quality of allergenic extracts, might at least partially explain these discordances [102]. On the other hand, a gradual increase in pollen load, pollen allergenicity [1, 110], and interaction in the patient between air pollutants and cypress allergens [95] are clear indications of a genuine increase.

The rationale for such a rapid increase in prevalence mainly lies with the fact that:

- millions of cypresses were planted in the 1970s and 1980s in the suburbs and around private houses and blocks of flats to offer a degree of privacy. Therefore the proximity of pollen sources has drastically changed: whereas Cupressaceae were traditionally planted in agricultural zones, away from dwellings, nowadays they are planted as hedges to as visual barriers.

- at the same time, a decrease in farming allowed for the extensive proliferation of *Juniperus* in the countryside.

- lastly, in urban areas, air pollution interacts with pollen to increase the allergenicity [51] (see below).

2.2.4 Risk factors for developing cypress pollen allergy

2.2.4.1 Repeated and heavy exposure

In contrast to other respiratory allergic diseases, part of cypress pollen allergic patients have no personal or familial history of allergic diseases, and in this subgroup, the onset of symptoms occurs at an older age [111, 112]. Therefore, even non-atopic individuals repeatedly and heavily exposed during many years to cypress pollens can develop this allergic condition. In high exposure areas, the general population may become allergic to this pollen.

2.2.4.2 Air pollution

Ishizaki et al. first noticed in the 1980s the association between Cupressaceae pollen allergy and air pollutants [84]. They found that living near Japanese cedar trees in urban areas tended to increase the allergy risk compared with living near these trees in rural areas. Concomitantly in Japan, Muranaka *et al.* demonstrated the adjuvant effect of diesel exhaust particles on IgE reactivity to Japanese cedar pollen in mice [113]. The rising prevalence of these pollen allergies observed between 1987 and 1991 (from 17 to 25%) in Japanese mountainous areas could then be partly explained by a drastic increase in the diesel vehicle fleet [114]. Japanese cedar pollen grains in urban areas may adsorb major urban gaseous pollutants such as NO_2, SO_2, and NH_3. Besides, Japanese studies also demonstrated that, on the exine surface, pollutants may be attached, thereby modifying the morphology and ionic composition of pollen grains [115, 116]. This phenomenon could facilitate the release and dispersion of pollen-derived particles smaller than pollen into the atmosphere [46]. During the Japanese cedar pollination period, the level of particle matter ($PM_{2.5}$) and suspended particle matter is therefore increased [117], which induce a negative impacts of this increase on the respiratory health of allergic patients [45].

Since then, Cupressaceae pollen grains have frequently been used as a model to study the interrelationship between air pollutants and pollen allergies [51, 118]. The effects of pollution on the molecular and developmental biology of Cupressaceae pollen has been exemplified by several studies. In polluted areas, the accumulation of numerous inorganic elements such as sulfur, copper, aluminum, and iron on pollen grains and the acidification of pollen surfaces by the adsorption of acid gases such as nitric and citric acids were demonstrated. The natural exposure of Arizona cypress pollen to air pollutants in Barcelona and Madrid promotes the production and release of an allergenic protein (*Cup a* 3) of the pathogenesis-related family 5 (PR-5 protein) [110, 119]. Therefore, the allergenic content of cypress pollen grains could be modified by urban air pollution. Interestingly, levels of adsorption of gaseous pollutants vary greatly, in *in vitro* exposure studies, among different plant species, and cypress pollen seems to be one of the most impacted. The kinetics of NO_2 uptake by cypress pollen is two and six times that of grass and birch pollen, respectively [120]. Furthermore, allergen-carrying free orbicules are generated following exposure of cypress pollen to NO_2 [51]. More comprehensive and experimentally designed studies on the interrelationship between pollen, air pollution, and respiratory allergies should derive from these recent physicochemical experiments.

2.3 Clinical and management aspects

2.3.1 Symptoms and diagnosis

According to the Japanese survey [99], and a subsequent study from Europe, rhinitis is more common than conjunctivitis. The latter is, however, the most disabling symptom, occurring in 72% of patients allergic to cypress pollen, versus 26% of patients allergic to grass pollen [111]. In this study, the occurrence of a chronic cough was much more frequent with cypress pollen allergy, whereas asthma symptoms during the pollen season were equally prevalent in patients allergic to grass and cypress pollens. Besides, allergy to cypress pollen was more disabling than other pollen allergies, according to a visual analog scale used by 4025 patients visiting their general practitioner for allergic rhinitis [121].

The diagnosis of cypress pollen allergy mostly relies on the clinical history, which is usually highly suggestive because most cypresses pollinate in wintertime when no other airborne pollens are present. The diagnosis is supported by skin tests, using either a mixture of *C. sempervirens* and *Hesperocyparis arizonica* or extracts from *J. ashei*. However, in a few cases, despite the convincing medical history, skin tests are negative. [122]. The allergist can ask for specific IgE measurement. Measurement of specific IgE to *J. ashei* has proven to be more sensitive than IgE directed toward *Cupressus* allergens [123]. In few cases, the patient is indeed sensitized to cypress pollen, but the relevance of this sensitization in the clinical picture is questionable. Then, measurement of serum recombinant Cup a 1, which evaluates antibodies directed to the major allergen, can be useful [122].

2.3.2 Management of cypress allergic patients

2.3.2.1 Pharmacological treatment

Compared with other allergic diseases, no specific pharmacologic treatments are given for this condition.

2.3.2.2 Immunotherapy

Although they only included a limited number of patients, several clinical trials have addressed this issue [1]. A benefit in terms of symptoms, quality of life, on-demand medications, late cutaneous response to allergen, and specific nasal hyperactivity was demonstrated in all trials. Clearly, larger clinical trials including longer treatments and longer follow-up periods are required.

2.3.2.3 Individual avoidance procedures

While all of these procedures are based on common sense, they have not been clinically validated [124]. This paper demonstrated that four recommendations are provided by most scientific committees and organizations: avoiding outdoor activities, consulting pollen forecasts, avoiding drying laundry outdoors, and wearing pollen protective glasses and mask when outdoors. All these pieces of advice are applicable when the taxon to which an individual is sensitized is present.

2.3.2.4 Collective strategies

Integrated strategies have to be developed to prevent cypress pollen allergy, in addition to medical care and desensitization. The reduction in individual exposure to pollen is the upstream component of this strategy. Pollinosis is more frequent in urban areas, although airborne pollen concentrations should be lower than in rural areas [125]. Therefore, the allergenic features of ornamental plants that are used in urban green spaces, parks, and gardens should be taken into account in future urban planning [126]. Should be chosen over allergenic wind-pollinated species non-allergenic species and/or insect-pollinated species the use of the latter species should be reduced in order not to aggravate their impact on allergy sufferers, even if cultural and historical reasons often make this a difficult choice.

A "Database of Urban Tree Potential Allergenic Values," integrating the different components of the allergenicity risk (e.g., tree size, type of pollen dispersal type, flowering period, etc.), has been generated for all of the individual trees producing an estimate of the allergenicity of Urban Green Zones [127, 128].

People with pollen allergies could limit their exposure to pollen through consulting forecast of pollen emissions based on phenological modeling of pollination. They should avoid spending time in areas with high densities of Cupressaceae taxa. Because pollen penetration in summer was estimated to be one hundred times higher than in winter and although pollen is much more abundant in winter, the penetration of pollen into dwellings must also be minimized by avoiding the opening of doors and windows in the summer time [129]. This is all the more true that cypress pollen allergenic potency was shown to last over at least a 10-month period in an indoor environment [130].

Trimming of isolated trees or hedges before pollination represent a complementary strategy to reduce the amount of pollen produced by Cupressaceae trees. It can significantly reduce pollen production [131]. An efficient medium- to long-term way to reduce atmospheric pollen loads without the need to eradicate the Cupressaceae species in urban areas could be to select low pollen producing varieties. Female cultivars are preferable for the few monoecious species. Low pollen cultivars should be selected for other species, either in natural populations or breeding populations, as for *C. japonica* [132]. For this latter species, an approach to prevent pollen dispersal lies in the use of pollen-specific fungal infection [133]. For *Cupressus*, sterile cultivars can be produced through the production of haploid lines from *C. dupreziana* surrogate mothers [134].

3. Pinaceae

As stated in the introduction, Cupressaceae/Taxaceae and Pinaceae are the three families of conifers studied at an allergy point of view. Pinaceae is mentioned as poorly allergenic in the RNSA data bank despite a huge amount of pollen produced. Eleven genera were described distributed in four subfamilies and 220–240 species. Two genera are presented below, *Pinus* and *Abies*.

3.1 Pinus

From the family Pinaceae, the genus *Pinus* includes about 120 species. The main species studied at an allergy point of view are *Pinus pinea, halepensis, radiata, sylvestris,* and *nigra.*

3.1.1 Trees and pollen

Pine trees are evergreen, conifer trees with leaves as needles bundled in clusters called fascicles. Pines are mostly with male and female cones on the same tree. The male cones are mainly present in spring, falling after pollen shedding. The female cones have numerous spirally arranged scales, with two seeds per scale. Some pine seeds (pine nuts) are edible and have been reported to induce allergies. Pine pollen grains are 40–80 μm diameter, are heavy, and harbor a waxy hydrophobic coat. They are easily distinguishable under microscope observation because of two balloons filled with air. This particularity does not help the pollen to float in the air but rather to float on a water surface. The tree is anemophilous, and pollination is abundant generating the so-called “sulfur rain” during pollinating season [135, 136].

3.1.2 Allergenicity

Despite the sometimes widespread pine forest and the abundance of pollen grains, the allergenicity of pine pollen was considered very poor if not nonexistent by some authors [137–144]. The involvement of pine pollen in seasonal allergic reactions has been evaluated in some studies and has generally been considered of little clinical significance. For example, Harris and German, in 1985, evaluated 200 patients during the pine pollen season [145]. Among them, only five had a positive skin test to pine pollen (*Pinus radiata*), i.e., about 2%. Kalliel and Settipane reported 6% using *Pinus strobus* pollen [146], and Armentia *et al* described three cases with *P. pinea* including one patient also sensitized to pine nuts [147]. Cross-reactions were reported with ray-grass, but some genuine sensitization to pine pollen could also be demonstrated in *P. radiata* [71, 148, 149]. In another study involving 826 patients in northern Arizona [150], only 12 (1.5%) had a positive skin test to pine pollen (*Pinus ponderosa*). Among them, eight reported a rhino-conjunctivitis during the pollen season while four had perennial symptoms. However, a paper originating from an area with high exposure to pine trees (north-west of Spain) described a series of 10 patients sensitized to pine pollen (*Pinus pinaster* and *radiata*) with symptoms during the pine-pollen season, among whom eight were mono-sensitized [151]. As well, in Canada, an increase in pollen from Pinaceae (pine, fir, spruce), Tsuga (hemlock), and Larix (larch, tamarack) was shown to play a role in increase of daily hospitalization for asthma [152]. These studies did not result in the description of specific pine pollen allergens. Allergens were only reported in pine nuts and correspond to storage proteins, 7S vicilin, 2S albumin, and a 17 kDa protein [153–155].

3.1.3 Hypotheses for low allergenicity

There are several hypotheses to account for this low apparent clinical significance, which is at variance with the heavy pollen exposure in areas densely covered with pines. Firstly, there might be an underestimation of the sensitization rate because protein extraction from pine pollen is difficult [156]. In comparison to classical extraction protocols such as soft incubation in aqueous solution, grinding of the pollen grains together with 1 mm silica beads results in 20–50 times more extracted proteins amount (**Figure 5**) [157]. The improvement of the extraction is not only quantitative but also qualitative. Interestingly Pasaribu *et al*, using adapted extractions protocols showed sequence homologies between oleosins from pine nuts and pine pollen [158]. Oleosins have been reported to be allergenic in sesame, peanut, and hazelnut, but classical protocols do not allow the extractions of these hydrophobic proteins. Secondly, the pine pollen might have a low allergenic potency because intrinsic compounds, which have been shown to play a role in enhancing a Th2 immune response via innate immunity, are deficient. For instance, the enzyme NADPH oxidase, proteases, and PALM (pollen-associated lipid mediators) contents are low in pine pollen [56, 159, 160]. NADPH oxidase leads to generation of reactive oxygen species, and PALM boosts Th2-type allergic reactions [161]. Finally, similarly to other airborne allergenic sources, pollution and climatic change have an impact on the allergenicity of pollen grain, and allergenicity of pine pollen was indeed shown to be affected by O_3 [162].

3.2 Abies

3.2.1 Trees and pollen

From the family Pinaceae, the genus *Abies* includes 46 species. They originated from temperate and north hemisphere, and fir is the most represented. They are

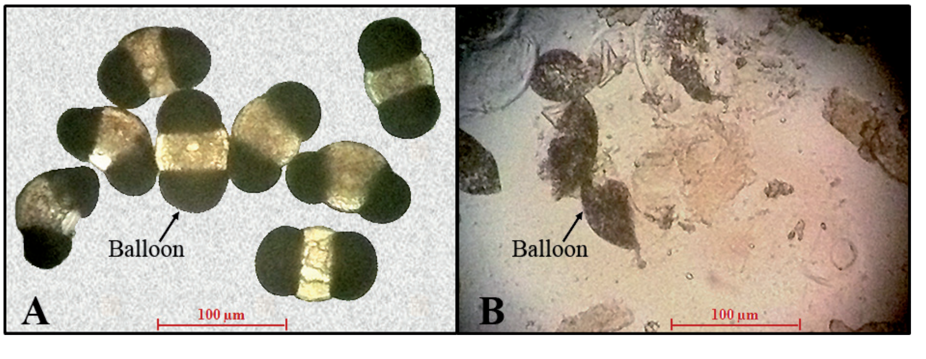

Figure 5.
*Pine pollen (*Pinus halepensis*) observed under optical light microscopy, (200x magnification) from Brazdova et al. [157]. A: Intact pollen grains. B: Grinded pollen using a multidirectional grinder (fast-prep 24-5G, cool prep, MPBiomedicals) in the presence of 1 mm silica beads. The disruption of pollen grains results in qualitative and quantitative enriched protein extraction.*

found in North and Central America, Europe, Asia, and North Africa, occurring mostly in mountains. They are large trees, reaching heights of 10–80 m tall when mature. Firs can be distinguished from other members of the pine family by the way in which their needle-like leaves are attached singly to the branches with a base resembling a suction cup and by their cones, which stand upright on the branches like candles and disintegrate at maturity. The leaves are significantly flattened with an upper surface uniformly green and shiny. Fir trees produce very large amounts of pollen annually in the spring and early summer. The pollen grains are large (160 μm) and similar to the pine pollen grains exhibiting two balloons filled with air.

3.2.2 Allergenicity

Abies pollen is considered barely allergenic, and only one study mentioning fir pollen together with other Pinaceae pollen has been carried out so far in Canada (see above [152]). No prevalence is reported and no allergens are described.

Fir is present in many homes during Christmas time, and there are a few reports of rhinitis and conjunctivitis occurring during and following Christmas tree exposure [163]. However, authors concluded that these symptoms were not pollen-dependent but rather caused by volatile organic compounds emitted by the tree since fir pollen grains have disappeared at Christmas time. One of these compounds was identified as colophonium shown to be able to sensitize allergic patient to induce dermatitis [164]. Another confounding and misleading factor could be mold spores contaminating the Christmas tree [165]. Mold spores such as *Aspergillus* or *Cladosporium* are well-known allergenic sources.

4. Conclusion

Out of the seven families described in conifers, obviously the Cupressaceae/Taxaceae was the most studied precisely because its wide distribution and the powerful allergenic potential of its pollen giving rise to a high prevalence where Cupressaceae/Taxaceae is implanted. Furthermore numerous associations with food allergy were reported inducing not only respiratory but also food allergy symptoms from the oral syndrome to more severe outputs such as systemic anaphylaxis or urticaria. Sensitization can occur lately, in non-atopic individuals and thus, represents a public health threat. However, compared with ragweed or grass pollen allergy, Cupressaceae trees rarely spontaneously reproduce, and their expansion could then be controlled by policymakers.

Pinaceae pollen allergy was also studied, though to a lower extent, because pollination is huge despite a very low prevalence. Therefore it does not represent an important health issue. The question of food cross-reactivity was also addressed, especially with the edible pine seeds, but no convincing data were published.

However, the climatic change and polluted environment might result in a general trend to increase allergenicity of airborne allergenic sources, including pollen. Therefore an immunosurveillance and health monitoring should be maintained for all pollen species.

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Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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