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Cypress Pollinosis: from Tree to Clinic

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Abstract Cypress (*Cupressus* sp.pl) is a genus within the Cupressaceae family. This family covers all of the Earth's continents except for Antarctica, and it includes about 160 species. The most important taxa for allergic diseases belong to five different genera: *Cupressus*, *Hesperocyparis*, *Juniperus*, *Cryptomeria*, and *Chamaecyparis*. Cupressaceae species share a common pollen type that can even include the genus *Taxus* (Taxaceae) when this plant is also present. As *Juniperus oxycedrus* pollinates in October, *Cupressus sempervirens* in January and February, *Hesperocyparis arizonica* (prev. *Cupressus arizonica*) in February and March, and *Juniperus communis* in April, the symptomatic period is long-lasting. Due to global warming, the pollination period tends to last longer, and there is a trend for Cupressaceae bioclimate niches to migrate north. In Mediterranean areas, *C. sempervirens* (Italian cypress or Mediterranean cypress) is by far the most common pollinating species. It accounts for half of the total pollination level. The group 1 major allergens belong to the pectate-lyase family, and members share 70 to 97% sequence homology within the different Cupressaceae. Group 2 allergens correspond to the polygalacturonase protein family, while group 3, a minor allergen, belongs to the family of "thaumatin-like proteins," a pathogenesis-related protein 5. Group 4 allergens are Ca^{++}-binding protein (4 EF-hands). Aside from these four groups, about 15 other allergens have been reported. Prominent among these is a basic low-molecular mass cross-reactive allergen that was identified recently, and which is suspected to be involved in pollen food syndromes which are common with peach and citrus. The prevalence of cypress allergy in the general population ranges from 0.6 to 3%, depending on the degree of exposure to the pollen. Depending on the geographic area and the studied population, 9 to 65% of outpatients consulting an allergist may have sensitization to cypress pollen. Repeated cross-sectional studies performed at different time intervals have demonstrated a threefold increase in the percentage of cypress allergy around the Mediterranean area. Risk factors include a genetic predisposition and/or a strong exposure to pollen, and the natural history of cypress allergy allows identification of a subgroup of patients as allergic rather than atopic. Concerning the clinical expression, rhinitis is the most prevalent symptom, while conjunctivitis is the most disabling. Pharmacological treatment of cypress allergies is not different from that of other seasonal allergies. Immunotherapy has been used, initially by subcutaneous injections, but currently mostly through the sublingual route. Although clinical trials have included only a limited number of patients, it has proven effective and safe. Avoidance can be implemented at the individual level, as well as at the

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community level, through the use of alternative plants, low-pollinating cypresses, or by trimming hedges before pollination.

Keywords Cypress pollen · Allergens · Aerobiology · Epidemiology · Botanic, clinic

Abbreviations

MM	molecular mass
pI	isoelectric point
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
IUIS	International Union of Immunological Societies
LTP	lipid transfer protein
PG	polygalacturonase
BP14	basic protein 14 kDa
TLP	thaumatin-like proteins
PR	pathogenesis-related proteins
IFR	isoflavone reductase
CBP	calcium-binding proteins
GRP	gibberellin-regulated protein
CPLL	combinatorial peptide ligand library
PBS	phosphate buffer saline
HSP	heat shock protein
PFS	pollen food syndrome
API	annual pollen index
P/m^3	pollen grains per cubic meter of air
SEM	scanning electron microscopy

Introduction

Cypress is a general name given to five Cupressaceae genera, one of which is *Cupressus*. The name *Cupressus* is derived from Latin where it specifies the botanical genus. A Greek origin has also been proposed: "*kuparissos*" which also means cypress. Some authors claim that it comes from Cyprus in relation to the island of Cyprus from which it may be derived. A search for the origin of the cypress leads back to several ancient Greek mythology legends that tell the story of a man named Cyparissus who, as a result of an ill-timed javelin throw, killed his favorite tamed stag, an animal with a golden horn that was protected by the nympheas. Overwhelmed with grief, the young man, beloved of Apollo, asked the silver bow God for authorization to cry forever. He was therefore transformed into a cypress and became a symbol of mourning, i.e., a tree for the dead. Plato assigned to the cypress virtues of eternity, while the Persians believed the cypress was a symbol of heaven. It was planted in front of temples and palaces. In France, it is not only found in cemeteries, but since *Cupressus sempervirens* became the symbol for the French region Provence, it is often found as a decorative plant in gardens or flower pots. It is widely used for windbreak hedges on account of its fast growth, low water needs, and low cost. However, despite this millennial promiscuity, the first acknowledgment of allergy to cypress pollen occurred very late in France and Europe. At the end of the 1950s, French allergists [1, 2], working in the South of France, questioned why the huge quantity of pollen grains released into the atmosphere during the pollinating season was not correlated with high sensitization and allergic symptoms. The very first report on cypress pollen allergy dates back to 1929 in the US (Texas and New Mexico) [3] and the early 1960s in Europe [4]. Cypress pollinosis is reported in several locations worldwide: Japan [5], Australia [6], Iran [7], South Africa [8], the USA, and with special emphasis around the Mediterranean basin [9–15] (Fig. 1). As per allergies in general, the prevalence of sensitization to cypress pollen has increased in the last few decades. Some of the reasons are (*i*) a higher exposure of individuals due to an overuse and planting of trees in cities, in private gardens, or as windbreak hedges [16]; (*ii*) an impact of atmospheric pollutants (particulate or gaseous) increasing the allergenicity of pollen grains [7, 17–21]; (*iii*) an underestimation of the pollinosis because of the concomitant occurrence of pollinating season with winter illnesses [22] and the poor diagnostic efficiency of the first pollen extracts [23, 24]; and (*iv*) the effect of global warming inducing a re-distribution of allergenic species such as the Cupressaceae towards northern areas, leading to the sensitization of more individuals.

This review provides an update on various aspects of cypress pollen allergy with, first, a botanical and palynological presentation of cypress; second, the various cypress pollen allergens involved; third, data on epidemiology; fourth, data on clinical aspects; and fifth, the management of cypress pollen allergic patients.

Botanical and Palynological Aspects of Cypress Pollen

Cupressaceae Classification/Distribution/Phylogeny

Among the seven conifer families, Cupressaceae is the most widely distributed worldwide, with the exception of Antarctica which is free of any woody species. Cupressaceae is the most well-known gymnosperm family that produces allergenic pollen. Other gymnosperms such as Pinaceae and Ginkgoaceae have been associated with a lower incidence [25]. The Cupressaceae family is divided into seven subfamilies, approximately 30 genera, and about 160 species [26, 27] whose current distribution reflects the evolution of Cupressaceae over the geological time scale until common ancestors became disseminated across the Pangea continent [27]. The differentiation among the subfamilies would have

Fig. 1 Worldwide distribution of reported Cupressaceae pollen allergy

occurred before the breakup of Pangea, with the exception of the Cupressoideae and Callitroideae subfamilies whose differentiation co-occurred with the separation of Laurasia and Gondwana in the late Jurassic period. Consequently, these two subfamilies are distributed throughout the Northern hemisphere and, in a more restricted manner, the Southern hemisphere, respectively. 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 [28] (see below the description of the respective allergens in the section "Cypress Pollen Allergens").

Cupressaceae species grow in diverse habitats: in natural or poorly managed areas for most of the *Juniperus* species; in forests and under cultivation for *Cupressus* and *Cryptomeria*; and in cropland or suburban areas for *Cupressus* sp., which is used as windbreaks or in urban areas as ornamental trees. Some species grow at low and middle altitudes (e.g., *C. sempervirens*) while others grow at high altitudes up to the limit of vegetation (e.g., *Juniperus communis*).

A classification has been proposed based on allergen molecule diversity instead of botanical relationships [29]. Comparison of the structural and functional aspects of the different pollen tree allergens contributes to the molecule-based profiling of allergic sensitization and to the understanding of cross-reactivity [28]. The major Cupressaceae allergen has been identified as pectate lyase, whose pectolytic activity may play a role in the growth of the pollen tube [30] (Table 1). These allergens in different species exhibit a high degree of homology, up to 97% between *Hesperocyparis arizonica* (Cup a 1) and *C. sempervirens* (Cup s 1), although recent molecular studies led to a split of the two species into two different genera, *C. arizonica* being assigned to the newly created *Hesperocyparis* genus [31]. The high level of cross-reactivity among species within the Cupressaceae genus is generally attributed to their botanical proximity. Indeed, cross-reactivity with allergens of the same type (glycosylated pectase lyases) but produced by botanically distant plants, such as ragweed, appears to be very limited [32, 33] (Table 2).

Pollination: Phenology and Pollen Features

Due to the large number of Cupressaceae species producing allergenic pollen, and the wide distribution range of the plants in this family, humans are exposed to the pollen over an extended period that covers autumn, winter, and spring. In Mediterranean regions, pollen from early pollinating species (e.g., *Juniperus oxycedrus*) is produced in October or November [34–36], while pollination by late pollinating species (e.g., *C. sempervirens*) can occur up to late April or May [37]. The diversity, floral phenology, and distribution of the Cupressaceae species in the Iberian Peninsula were reported in 1999 [38]. All Cupressaceae species produce spherical pollen grains very similar in appearance (Fig. 2a). In the *Cupressus* genus, the mean size of hydrated pollen grains varies from 25 to 40 μm (Fig. 2b), and they differ based on their geographical origins. Thus, they are small for Mediterranean species (except for *C. dupreziana* which produces diploid pollen [39]), intermediate for New World species, and larger still for Asian species [40]. The Cupressaceae pollen type is usually described as inaperturate, although a faint circular pore can be seen in fresh material. It is also characterized by a very thin exine (outer membrane), whereby some granules or orbicules (Ubish bodies) appear to be scattered (Fig. 2a), and there is a very thick intine (inner membrane). The pollen grains are not

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

Groups	*Proteins*	*Cupressus sempervirens* Italian cypress	*Hesperocyparis arizonica* Arizona cypress	*Cryptomeria japonica* Japanese cedar	*Juniperus ashei* Mountain cedar	*Juniperus other*	*Chamaecyparis obtusa* Japanese cypress	*Taxodium disticum* Bald cypress	*Thuja* 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			
	OTHER	Cup s BP14 14 kDa	Cup a BP14 14 kDa	Cry j BP14 14 kDa	Jun a BP14 14 kDa		Cha o 3* 63 kDa		
		ß-galactosidase 46-50 kDa	ß-galactosidase 46-50 kDa	Chitinase 27 kDa					
		Profilin (Cup s 8) 14 kDa	LTP 14 kDa	CJP8 (LTP) 17 kDa					
		Phenylcoumaran reductase 33 kDa		Isoflavone réductase 35 kDa					
		Rab-like protein 18 kDa		Aspartic protease 42 kDa					
		Sigma factor regulation protein 29 kDa		Serine protease subtilisin-like 79 kDa					
		Cytochrome c 12 kDa							
		SOD 15 kDa							
		Lactoyl glutathione lyase 32 kDa							
		Malate deshydrogenase 31 kDa							
		Triosephosphate isomerase 33 kDa							
		Glucanase 37 kDa							
		HSP104							

Jun c: *Juniperus communis*	*Common juniper*	Thu oc: *Thuja occidentalis*	*Eastern white cedar*
Jun o: *Juniperus oxycedrus*	*Prickly juniper*	*: *referenced in IUIS*	
Jun r: *Juniperus rigida*	*Temple juniper*	SOD: Superoxide dismutase	
Jun v: *Juniperus virginiana*	*Eastern red cedar*	LTP: Lipid Transfer Protein	
Thu p: *Thuja plicata*	*Western red cedar*	HSP: Heat Shock Protein	

Table 2 Percent sequence identity within five groups of Cupressaceae allergens. Gray background represents a theoretical limit of cross-reactivity (<55% identity)

Reference sequence	Allergen name and % sequence identity with reference sequence											
								pectate lyase				
	Cup a 1	**Cup s 1**	**Jun a 1**	**Cry j 1**	**Cha o 1**	**Jun o 1**	**Jun v 1**	**Arabidopsis**	**Zinnia**	**Fragaria a.**	**Medicago s.**	**Amb a 1**
Cup a 1	100	96	91	75	81	97	95	51	50	46	46	45
Cup s 1		100	96	80								
									polygalacturonase			
	Cup a 2	**Cup s 2**	**Jun a 2**	**Cry j 2**	**Cha o 2**	**Pla a 2**	**Ole e PG**	**Phl p 13**	**Cucumis s.**	**Vitis v.**	**Solanum l.**	
Jun a 2	97	97 *	100	71	82	35	36	33	47	45	43	
	Cup a 3	**Cup s 3**	**Jun a 3**	**Cry j 3**	**Jun v 3**	**Pru av 2**	**Mal d 2**					
Jun a 3			100			45						
Cup s 3	97	100	96									
Cup a 3	100		95	81	94	45	45					
	Cu p a 4	**Jun o 4**				**Ole e 8**						
Cup a 4	100	90				37						
Cryptomeria japonica	**LTPs**		Peach	Hazelnut	Parietaria							
	Medicago	**Picea abies**	**Pru p 3**	**Cor a 8**	**Par j 1**	…						
CJP8 (LTP)	44	47		(very weak)								

: 97% sequence identity within 32 peptides representing 66% of the sequence coverage

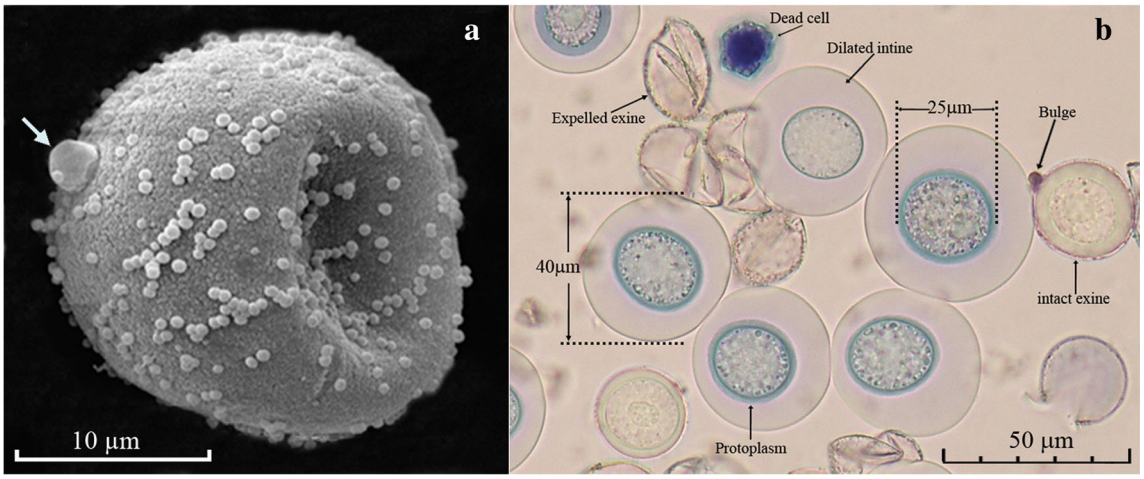

Fig. 2 **a** *Hesperocyparis arizonica* pollen grains (scanning electron microscopy from Shahali et al. [17]) collected in Tehran, Iran (2225× magnification). The *arrow* shows the presence of a bulge on the external surface of the exine serving as a valve for regulating the entrance of fluids at the beginning of pollen hydration [72]. 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 (100× magnification). The various elements are indicated

only uniform within the family but also very similar to those of the Taxaceae family. Thus, where plants from both families are abundant, it is common to refer to the Cupressaceae/Taxaceae pollen type. Cupressaceae pollination is anemophilous, and although the pollen grains do not have balloon-like wings as *Pinus* pollen, they can become airborne and hence be transported over long distances. Cupressaceae species generally produce huge quantities of pollen. The number of pollen grains per male cone is thought to average 400,000, and production by individual trees has been estimated to be 276,000 million [41, 42]. Cupressaceae pollen frequently predominates in the winter period, but is also present throughout the remainder of the year (Fig. 3). In Mediterranean regions, *Cupressus*, together with *Olea*, produces the largest amount of allergenic tree pollen [43]. Cupressaceae/Taxaceae pollen is one of the 12 most abundant aeroallergenic pollens in Europe [44]. *Cupressus* pollen accounts for 40% of the total annual pollen counts in Marseille, in southern France [45], 38% in Antalya [46], and 35% in Istanbul, Turkey [47], 25% in Thessaloniki, Greece [48], 23 and 24% in Toledo and Cuenca, Spain [34, 49], 18% in Nicosia, Cyprus [50], 17% in Palma de Mallorca, Balearic Islands, Spain [35], and 14% in Nerja, southern Spain [36]. Cupressaceae pollen is also abundant or present outside of the Mediterranean region: Northern Europe, 8% for Cupressaceae and Taxaceae in Munster, Germany [51], South America, 30% in Bahia Blanca, Argentina [52], North America, 18% in Mexico [53], 5 to 10% on the east coast of USA [54, 55] and up to 3872 pollen grains/m^3 in January in Tulsa, Oklahoma, central USA where *Juniperus ashei* is predominant [56, 57], Asia, 19% in Yunnan, China [58] and 60% in Japan because of the huge presence of *Cryptomeria japonica* [59], and finally 3% in Santa Cruz de Tenerife, Canary Islands, Spain [60].

Aerobiology

In the last decades of the twentieth century, the study of airborne pollen diversity shows that concentrations in the air have progressed steadily, and common sampling methods [61] that help to provide comparative data have been developed. Other methodologies have also been used [62].

The criteria used to describe the dynamics and patterns of airborne pollen are as follows: (*i*) the mean daily pollen concentration, expressed as the number of pollen grains per cubic meter of air (P/m^3); (*ii*) the annual pollen index (API), which equates to the sum of the mean daily pollen concentration for each year; (*iii*) the time corresponding to the beginning and end of the pollination; and finally (*iv*) the duration of the pollen season.

Various methods have been used to calculate the dates for the beginning and end of pollination. 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. Although summer can be considered a period free of this pollen type, in the Mediterranean area, days without any cypress pollen are rare.

This variability makes it difficult to establish dates for the beginning and end of pollination. The only way to obtain a date would be to use a percentage method, i.e., to assume that pollination begins when a given percentage, e.g., 2.5%, of the API, has been reached and that it ends when it reaches the complementary percentage of the API, e.g., 97.5%. This method poses problems, however, because there are substantial variations in the API for consecutive years. The 2.5% (or another percentage adopted) can account for very substantial quantities of pollen in years with high APIs and lesser quantities when the API is low.

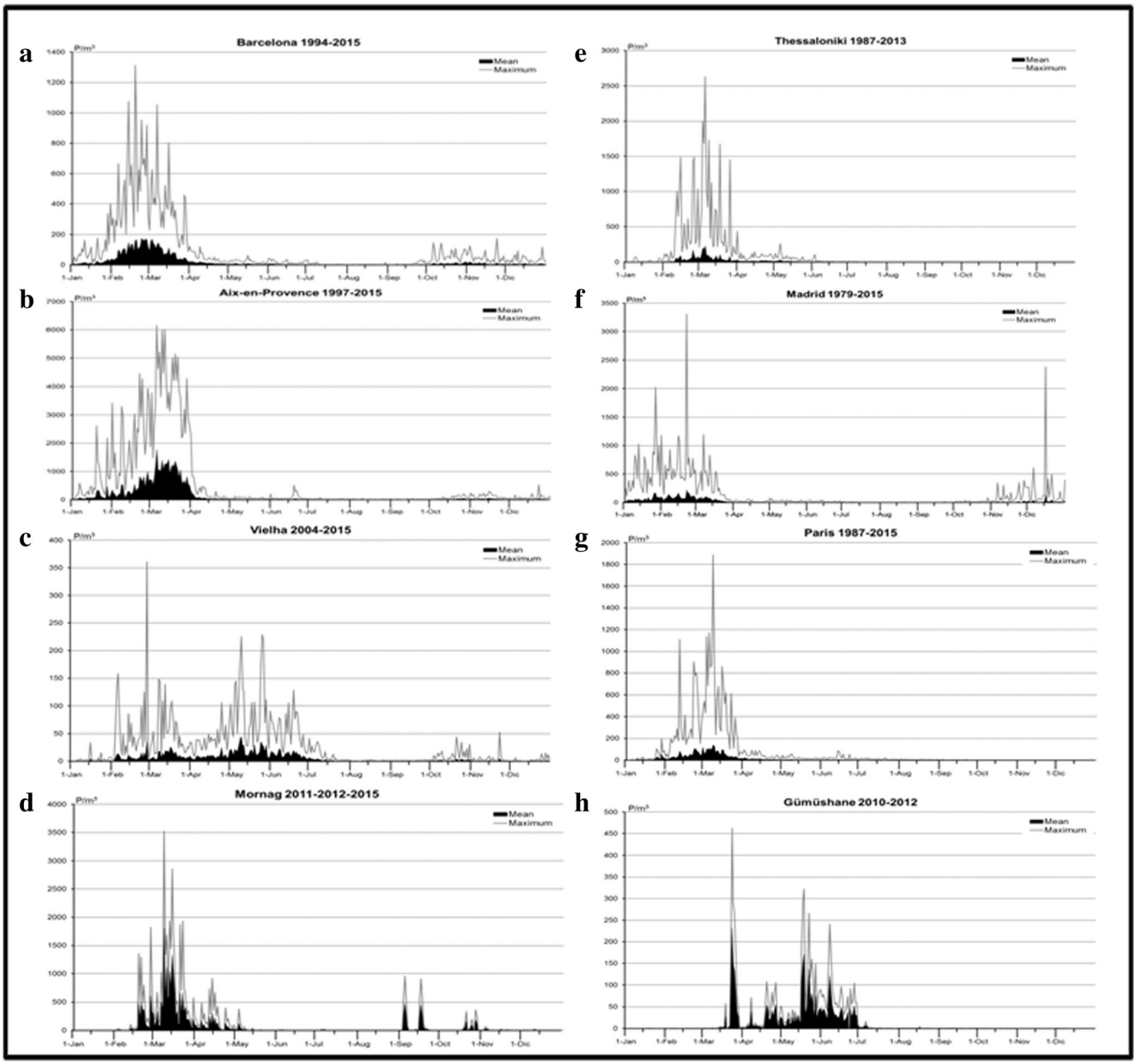

Fig. 3 Cupressaceae pollen dynamics over the course of the year in the Mediterranean area. **a** Barcelona (Spain), **b** Aix-en-Provence (France), **c** Vielha (Spain), **d** Mornag (Tunisia), **e** Thessaloniki (Greece), **f** Madrid (Spain). Outside Mediterranean area: **g** Paris (France) and **h** Gümüshane (Turkey). Mean daily (*thick black line*) and maximum daily (*thin black line*) pollen concentrations are indicated for the period

In the current literature, in Montpellier (1977–2001) and Lyon (1982–2001), using the Cour method, the Cupressaceae APIs exhibited pronounced differences from 1 year to the other, and they showed increasing trends [63], while the dates for the onset of pollination tended to occur earlier, by 18 and 28 days, respectively. In Thessaloniki (Greece), using the Hirst method, Cupressaceae APIs for the period 1987–2005 also exhibited a significant increasing trend [64]. In Catalonia (NE Iberian Peninsula), using the Hirst method, Cupressaceae APIs for the period 1994–2015 also exhibited increasing trends that were significant in four out of eight sampling sites. Two of these API trends, for Barcelona and Vielha, are shown in Fig. 4 together with the trends in other localities around the Mediterranean. No information could be found, however, regarding the end of the pollination period and the duration of the pollination. Ziello et al. [65] analyzed the APIs between 1977 and 2009 of 23 taxa from 13 European countries (97 sites, using the Hirst method). Cupressaceae pollen exhibited an increasing and highly significant trend in the yearly amount of airborne pollen, as well as in terms of the highest median levels. As the plot of the proportional annual change of Cupressaceae APIs against local temperature trends from January to April did not show a significant correlation, the increase in the API was explained by the extensive use of this

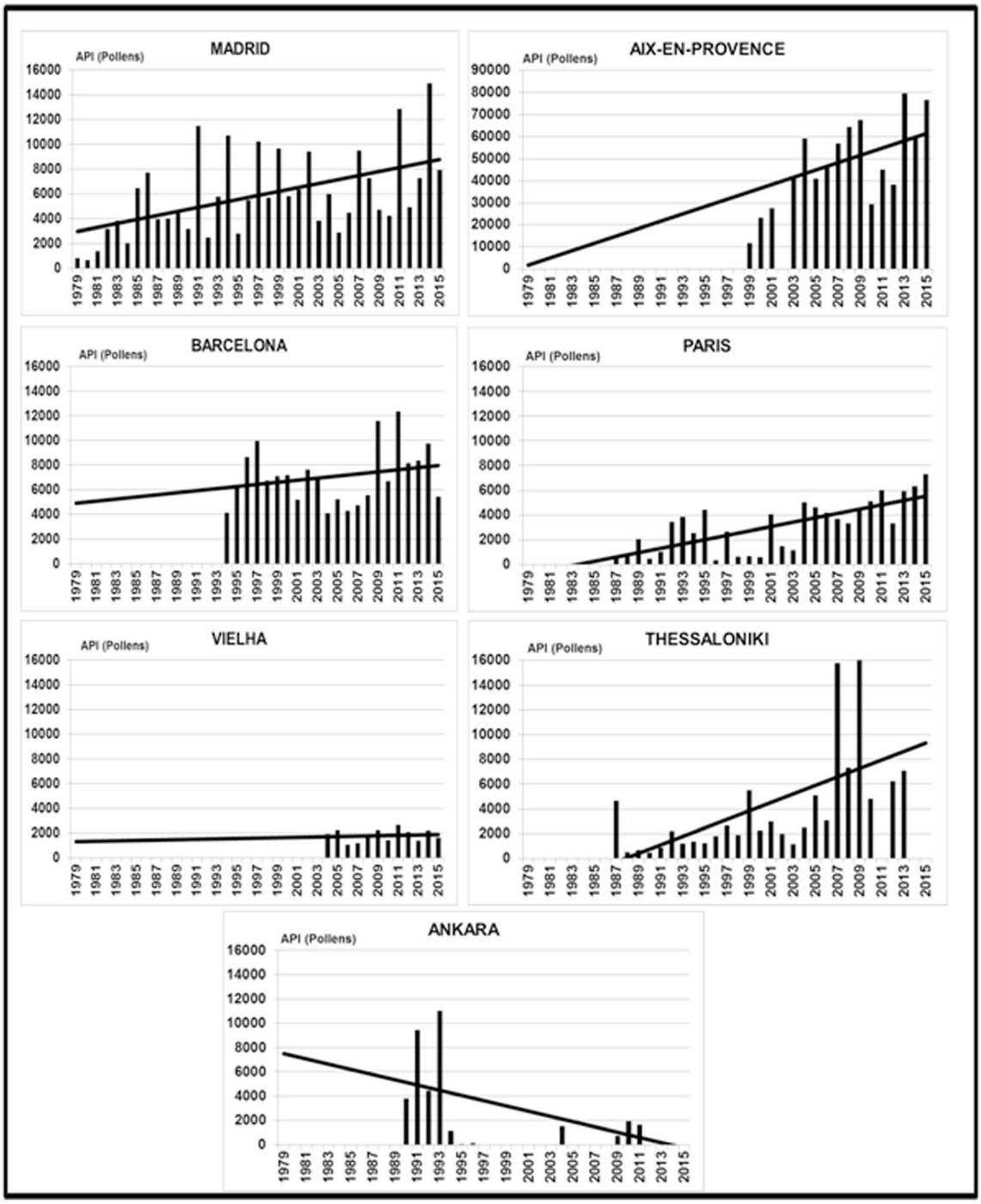

Fig. 4 Cupressaceae annual pollen index (API) and trends at the localities with the longest continuous data series

family as ornamental plants in the cities that were analyzed. Ariano et al. [66] have, however, attributed to climate change a possible role in variations in pollen seasons and allergic sensitizations.

Here, we report recent data obtained from Hirst traps and expressed as P/m^3 and APIs (Fig. 3). At all studied sites, the pollen was in the air throughout the entire year, although the highest concentrations occurred between February and the end of March, except for in Gümüshane (Turkey) where the highest concentrations occurred between April and the end of May. Daily concentrations varied significantly between urban areas (e.g., Aix-en-Provence, Madrid, Barcelona) and the countryside (e.g., Vielha), where they were lower. It is common for pronounced peaks of pollen to occur. These values, which are due to very favorable conditions for plant pollination or long-range pollen transport, must also be taken into

consideration when predicting the possible risk of allergy, since, although exceptional, if they have occurred once, they can occur again. Figure 4 shows the trend of the APIs in the localities studied. In six of the seven stations, the annual sum of Cupressaceae pollen tended to increase, with statistical significance in Aix-en-Provence and Paris (France), Madrid (Spain), and Thessaloniki (Greece), and without significance in Barcelona and Vielha (Spain). In Ankara, the APIs tended to decrease.

The daily pollen concentration threshold levels required to elicit allergic symptoms in patients are very difficult to establish, as they vary from person to person and with the environmental conditions. Moreover, a general agreement on how to define them has not been reached which makes comparison difficult. For instance, at present in Israel, it is considered that the symptoms start between 10 and 50 P/m^3. In France, different thresholds of symptom risk have been established for the Mediterranean area (designated as low, when 7–13 P/m^3, moderate when 14–141 P/m^3, and high >141 P/m^3, respectively), and for the north and center of France (designated as low when 70–141 P/m^3 and moderate when >141 P/m^3 [67]). The level designated high risk only exists in the Mediterranean area. The Catalan Network of Aerobiology defined the risk of allergy as being low when concentrations are <20 P/m^3, moderate for 20–50 P/m^3, high for 50–100 P/m^3, and very high when >100 P/m^3. It is also known that the risk of allergy is altered by exposure to outdoor air pollutants, especially $PM_{2.5}$ and suspended particulate matter, which increase the link with daily consultations for pollinosis [68].

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 [69]. Three years later, cross-reactivity was shown between *C. japonica* and *C. sempervirens* pollen allergens [70], and in 1991, 17 IgE binding proteins with molecular masses ranging from 14 to 96 kDa were reported in *C. sempervirens* pollen [24]. Interest in *H. arizonica* pollen increased, and Di Felice et al. described six IgE binding proteins from this pollen in 1994 [71]. All research groups deciphering allergens from the various cypress species reported a 42–43 kDa protein as being the major cypress allergen: Cry j 1 for *C. japonica*, Cup a 1 for *H. arizonica*, Cup s 1 for *C. sempervirens*, etc. (see Table 1). Water-soluble extracts of cypress pollen grains generally contain low concentrations of proteins as compared to grass, olive, or ragweed pollen treated under the same conditions. This particular feature may be related to the high sugar content of the intine, which swells rapidly when the pollen grain is suspended in water, and this might delay or prevent the release of proteins [72] (Fig. 2b).

Allergenicity of Cypress Pollen

This pollen is considered to be highly allergenic and is mentioned as such on 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 several factors such as the degree of exposure, a value related to the total pollen amount in the atmosphere (from intact or fragmented grains), the phenological conditions in the considered area, and other cofactors such as temperature, hygrometry, and air pollution. With regards to cypress pollen, the exposure is high because its production in the pollination season is huge, making this pollen the most represented in the atmosphere (up to 40% of total pollen counts around Marseille in the south of France). Not only is the load high but also the spreading, since rather small pollen, can be transported by the wind over long distances. Moreover, the pollen grain carries, as said in the "Pollination: Phenology and Pollen Features" section, some particles on its surface, orbicules. These orbicules contain allergens, at least from the group 1 of Cupressaceae [73–75], and group 2 allergens were also found in intracytoplasmic sub-particles, starch granules from *C. japonica* [76]. Furthermore, by using surface plasmon resonance analysis, it has been shown that Cry j 1 and Cry j 2 are released by pollen-derived fine particles smaller than 1.1 μm [77]. Interestingly, rainfall and an experimental in vitro treatment of cypress pollen grains with NO_2, a gas frequently found in gaseous pollutants, are sufficient to release free orbicules from the surface of the grain [74, 78]. Because of their small size (300 to 600 nm), these pollen sub-particles might be able to penetrate deeper in the bronchial tract and differentially sensitize individuals, as was shown in a rat model [79]. No data are yet available on the existence of such a sub-particle-sensitizing mechanism in situ, i.e., in real conditions, because free atmospheric orbicules have never been measured. Besides these characteristics, and in addition to protein allergens, some biogenic intrinsic adjuvant molecules may contribute to the allergenicity of cypress pollen [80–82] by favoring Th2 immune responses and/or triggering innate immune responses. For instance, NADPH oxidase is an enzyme that generates reactive oxygen species (ROS) and is reported to be involved in inflammation [83, 84]. This enzyme, which is required for pollen tube growth, is intrinsically expressed at different levels in the pollen grain of various plant species [85, 86]. Cypress pollen is one of the tree pollens containing the highest level of redox activity as compared to 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 [81]. PALMs are also able to activate

eosinophils and neutrophils and decrease IL12 production from dendritic cells, therefore, favoring Th2 biased immune responses [87–91].

Four Groups of Allergens

Currently, four groups of allergens have typically been described in cypress pollens, 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). Furthermore, several additional allergens have been reported in the three most studied pollens, *C. japonica*, *H. arizonica*, and *C. sempervirens* (Table 1).

Group 1: Pectate Lyase

Identified in all species of cypress, these allergens include the number "1" in their IUIS reference names (e.g., Cry j 1, Cup s 1, Cup a 1, Jun a 1) and are considered to be the major allergens of Cupressaceae, sensitizing almost 100% of patients who are allergic to cypress [92–94].

Their molecular masses (MMs) are around 43 kDa, with several acid-neutral glycosylated isoforms [92]. They are members of the pectate lyase protein family, which are enzymes that cleave galacturonic acid-containing polysaccharide chains. In pollen grain, they are involved in modeling tissue and pollen tube growth. Group 1 allergens are highly cross-reactive between Cupressaceae species, sharing 75 to 97% sequence identity (Table 2). While Cup s 1, Cup a 1, and Jun a 1 are the most similar, Cry j 1 is the most distant. Pectate lyase allergens are also found in some fruits and other pollen, such as short ragweed and mugwort pollen sharing only 45 to 49% sequence identity with cypress pectate lyase; no cross-reactivity has therefore been observed. Pectate lyase pollen allergens can be clustered, and as expected, the sensitization pattern of the different studied cohorts (e.g., Austrian, Canadian, Italian, and Japanese) was found to be related to pollen exposure [95]. In Europe, Cup a 1 is in fact the reference allergen for diagnosis of allergy to cypress pollen [96].

Group 2: Polygalacturonase

Group 2 allergens belong to the polygalacturonase (PG) protein family [97, 98]. Because sensitization occur in more than 80% of cypress pollen allergic patients, it can be considered as a major allergen [99]. PGs are enzymes involved in the maturation of the pollen grain and pollen tube growth. Their MMs are generally around 43 kDa, although some are also 56–60 kDa. They are glycosylated, and as for group 1 allergens, the oligosaccharide moieties elicit the production of specific IgEs that can induce some cross-reactivities [75, 92, 100–103]. They are basic proteins with an isoelectric point (pI) around 9.0. PGs from different species of cypress exhibit 71 to 97% sequence identity [104]. Besides Cupressaceae, members of this protein family have been reported to be allergenic in several pollens [105]. For instance, PG constitutes the group 13 of grass pollen allergens, sharing only 33 to 36% homology with Cupressaceae PGs [106]. A slightly higher sequence identity is found with fruit PGs (40–47%), suggesting that they might be good candidates for cross-reactivities that are sometimes observed with fruits (peach, orange, fig, etc.) and suspected with tomato [107]. They are able to interact in molecular complexes with lectins and other proteins such as non-specific lipid transfer protein (nsLTP) [108], which is a very well known family of allergens involved in the definition of a "Mediterranean allergy pattern." This characteristic could be important since PGs are abundant in Cupressaceae pollen. So far, no data has become available regarding their ability to interact with LTPs from fruits that are widely consumed in regions where Cupressaceae are abundant. Such molecular interactions might condition a preferred co-sensitization of cypress allergens with fruit allergens because allergenic molecules from the two different sources can, in a molecular complex, concomitantly interact with antigen presenting cells.

Group 3: Thaumatin-Like Protein

Group 3 allergens are considered to be at the frontier between major and minor allergens and sensitize 40 to 60% of cypress pollen allergic patients. Their MMs are 23–34 kDa and their pIs around 4.9–5.4. They are members of the thaumatin-like proteins (TLPs) and belong to the group 5 pathogenesis-related proteins (PR-5) that possess anti-fungal activity. Sequence identity between different members of the group 3 allergens in Cupressaceae is high (85–95%), but lower when compared to other TLPs from fruit (45–50%) [109, 110]. Their basal expression level is low although it has been reported that Cup a 3 can be up-regulated when pollen is exposed in situ to air pollution [19, 109]. Up to 63% of patients allergic to cypress may express IgEs directed against Cup a 3. Interestingly, some allergens of the TLP family of proteins were described as cross-reactive between pollen and food [111] and thus might be a good candidate for some of the fruit/cypress syndromes that have been described.

Group 4: Ca-Binding Protein

Like calmodulin, group 4 Cupressaceae allergens belong to the family of calcium-binding proteins (CBPs) and have four binding sites for calcium (4 EF-hand) [112]. Sensitization is reported in 10 to 15% of cypress pollen allergic patients, and thus, group 4 Cupressaceae allergens should be considered as minor allergens of MM 17–18 kDa and pI of about 4.3. Identity between Cup a 4 and Jun o 4 is 90% but is not yet known for Cup s 4. A 4 EF-hand CBP is found in olive and ash pollen (Ole e 8 and Fra e 8, respectively). The percent

identity of Cup a 4 with Ole e 8 is, however, as low as 37%, therefore precluding cross-reactivity. The majority of CBPs in other pollen, such as group 7 of grass allergens, have only two binding sites for calcium.

Other Allergens

BP14

In an in-depth analysis of *C. sempervirens* pollen allergens using more than 200 patients sera, a very strong IgE reactivity against a basic 14 kDa protein (named BP14) was discovered in 17 to 37% of the patients diagnosed with allergy to cypress [97, 113–115]. The observed reactivity was independent of the recognition of higher MM allergens (group 1 and/or 2 allergens), and BP14 sensitization did not correlate with age, gender, date of symptoms onset, severity of the symptoms, or the level of total IgE.

Extraction of BP14 from pollen grains requires either detergent or a minimum concentration of salt, such as the 0.15 M NaCl found in phosphate buffer saline (PBS), up to a concentration of 1 M. This suggests that BP14 is a complex in pollen grains with a water insoluble (or polar) protein that needs to be dissociated with detergent or with high salt conditions in order for it to be extracted. BP14 IgE epitopes are resistant to heat and are peptide. They are also conformational since treatment with a reducing agent completely abolished IgE recognition, in agreement with a highly folded structure. A homologous protein is found in *C. japonica* and *J. ashei*, but the level of BP14 is very low in *H. arizonica* pollen (Fig. 5).

Considering several physico-chemical properties including basic isoelectric point, low MM, heat resistance, and a folded structure, BP14 was suspected to be an LTP. This working hypothesis was supported by the fact that an LTP was reported in *C. japonica* pollen based on a molecular biology analysis [116] and also in *H. arizonica* pollen using immunochemical methods [117]. However, experimental biochemical procedures specifically designed to purify LTPs from fruit failed when applied to *C. sempervirens* pollen, and anti-LTP antibodies specifically directed against Pru p 3, the LTP from peach, or against Tri a 14, the LTP from wheat, did not bind to BP14. Mass spectrometry analysis of the reduced spotted BP14 band run in one-dimensional gel electrophoresis revealed that BP14 shares amino acid sequence homology with two proteins: peamaclein, described as the allergen Pru p 7 in peach (*Prunus persica*), and a gibberellin-regulated protein (GRP) similar to the one described in *Arabidopsis thaliana* (Table 3). Both proteins belong to the same Snakin/GRP family of proteins, which are widely distributed among plant species and are involved in several aspects of plant development including pollen maturation, plant responses to biotic or abiotic stress, participation in hormone crosstalk, and redox homeostasis [118]. GRP is a 108 amino acid protein with an MM

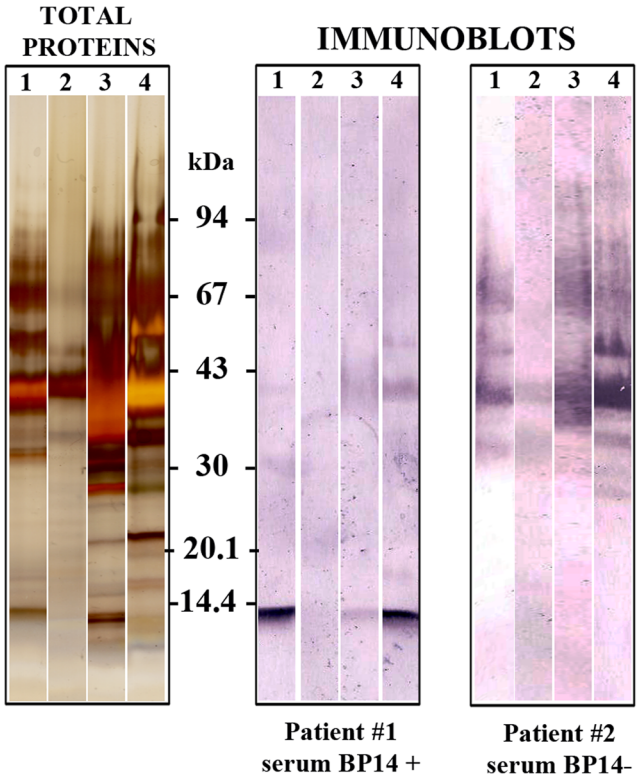

Fig. 5 BP14 is mainly expressed in *Cupressus sempervirens* and *Juniperus ashei*. Proteins extracted from pollen of (1) *Cupressus sempervirens*, (2) *Hesperocyparis arizonica*, (3) *Cryptomeria japonica*, and (4) *Juniperus ashei* were separated by 8–18% SDS-PAGE and either silver stained (*Total protein*) or blotted onto a nitrocellulose membrane and then incubated with serum from cypress pollen allergic patient #1 sensitized to BP14 (*Immunoblot left part*) or serum from cypress pollen allergic patient #2 sensitized only to high-molecular weight allergens (>30 kDa) (*Immunoblot right part*). IgE binding was revealed with alkaline phosphatase coupled goat antibodies directed against human IgE (ε chain specific) followed by addition of an appropriate substrate

of 11.4 kDa and a pI of 10.1, while peamaclein, the recently described Pru p 7 allergen in peach pulp and peel, corresponds to the 63 amino acid C-terminal end of GRP, with an MM of 7 kDa, a pI of 8.97, and with the 12 highly conserved cysteines [119–121] characteristic of the Snakin/GRP protein family. Peamaclein was shown to interact strongly with peach LTP, and according to Tuppo et al. [121], most natural Pru p 3 preparations are probably contaminated by Pru p 7. This contamination explains the preferential association of BP14 sensitization with a co-sensitization with "Pru p 3" using a multi-array allergen chip, for which the Pru p 3 preparation was probably not pure as it contained some Pru p 7. This is why our experimental data consistently failed to show cross-reactivity between BP14 and peach LTP [113]. In conclusion, BP14 is not an LTP.

Additional Allergens

Besides these four IUIS certified groups of Cupressaceae pollen allergens, several additional allergens have been reported

Table 3 Identification of BP14 as a member of the gibberellin-regulated protein family by liquid chromatography-quadrupole-time-of-flight-mass spectrometry analysis (LC/Q-TOF-MS)

Mr. exp. (kDa)	Accession	Protein	Species	Mr theo (kDa)	pI theo	Total ion score	#Peptides identified	% Cov
12	P86888	Peamaclein (Pru p 7)	*Prunus persica*	6.9	8.6	76.0	2	17.5
	O82328	Gibberellin-regulated protein 7	*Arabidopsis thaliana*	11.4	10.1	57.1	1	9.3

The proteins extracted from *Cupressus sempervirens* (*Cups*) pollen were separated by 8–18% acrylamide gradient SDS-PAGE under reducing conditions. The low-molecular mass protein was excised from the gel after staining with colloidal Coomassie blue. This allergen was then submitted to LC/Q-TOF-MS analysis, and the results of data bank queries are shown. The identified proteins in the *Viridiplantae* database are presented together with data related to accession number, protein names, the plant species in which the optimal homology was found (NCBInr, SwissProt), the coordinates as relative molecular mass (Mr), experimental (exp) or theoretical (theo) and isoelectric point (pI), the total ion scores, the number of peptides identified, and also the percentage coverage (% Cov)

in studies conducted with the aim of deciphering cypress pollen allergens, or in studies using cypress extract as a control pollen. Because reactivities were studied on a small number or a selection of cypress pollen allergic patients, no prevalence is available.

A chitinase [122], an aspartic protease [123], a plant subtilisin-like serine protease [124], an LTP [116], and an isoflavone reductase (IFR) [125] have been reported as allergens in *C. japonica.* β-Galactosidases in *H. arizonica* and *sempervirens* were also found to be allergenic [126]. Using an immunoblot inhibition assay, an allergenic LTP appears to be present in *H. arizonica* [117]. *C. sempervirens* pollen was shown to contain a cross-reactive IFR [127] and a profilin [128]. Profilin is very rarely detected as an allergen in Cupressaceae. The latter article [128] is the only indirect report of the involvement of a member of the profilin family in the allergenicity of Cupressaceae pollen. A cellulase (glycosyl hydrolase) from *Chamaecyparis obtusa* pollen, listed under the name Cha o 3, has been entered into the IUIS allergen databank. The sequence was recorded in GenBank in 2012, and the protein exhibited IgE binding in 14 out of 16 Japanese subjects allergic to cypress pollen. Moreover, three out of four tested patients exhibited a positive basophil activation test, thus demonstrating some degree of clinical relevance for Cha o 3 [129].

By sequential extraction of *C. sempervirens* pollen protein, followed by a combinatorial peptide ligand library (CPLL) treatment [130] to select low-abundance proteins and allergens, 10 additional IgE binding proteins were identified. Although not reported as allergens in any other allergenic sources, three out of 10 corresponded to isoforms of a rab-like protein, a chaperone heat shock protein HSP104, and a sigma factor sigB regulatory protein. The seven other allergens that were identified had already been described as allergens in other allergenic sources such as pollen, molds, insects, fruits, vegetables, latex, seafood, and seeds (http://www.allergome.org): cytochrome c, superoxide dismutase (Cu-Zn) , lactoylglutathione lyase, phenylcoumaran reductase (of the same protein family as IFR, NADPH-dependent aromatic alcohol reductase), malate dehydrogenase, triosephosphate isomerase, and glucanase-like protein [131]. This proteomic analysis also revealed that five additional putative allergens were identified in *C. sempervirens* pollen by CPLL technology: HSP70, enolase, methionine synthase, subtilase family protein, and thioredoxin (Table 1).

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. However, as previously stated, profilins have very rarely been detected as an allergen in cypress pollen. Some cross-reactivities were observed with *Podocarpus gracilor* of the Pinales order [132], although other authors did not find any cross-reactivity of *C. sempervirens* with pine pollen [133]. *Parietaria judaica*, *Lolium perenne*, and *Olea europaea* pollen were shown to exhibit some degree of cross-reactivity [134]. These cross-reactivities were mainly studied by inhibition in immunoblotting assays, and the nature of the allergens involved very often remains unknown.

Pollen/Food

Associations of cypress pollinosis with food allergies have been reported. In general, up to 60% of food allergies are associated with an inhalant allergy [135]. So-called pollen food syndrome (PFS) has been described for birch, mugwort, grass, ragweed, olive, plane, and cypress pollen. An oral allergy syndrome has been reported in patients allergic to Japanese cedar following consumption of specific vegetables and fresh fruits (e.g., melon, apple, peach, and kiwi) [136]. The cypress/peach syndrome has received the most attention [137, 138]. An uncharacterized (putative Cup s 1 or Cup s 2) allergen of 45 kDa has been proposed to be the cross-reactive

allergen. In another study, the LTP was proposed to be the cross-reactive allergen between cypress pollen and peach [117].

As indicated in Table 1, some cypress allergens are prone to being cross-reactive with allergenic food sources, by different molecules: glucanase [139], IFR [127], LTP [140], and polygalacturonase. The latter has been suspected of being the cross-reactive allergen in tomato/Japanese cedar syndrome [107]. In support of a cross-reactive molecule between these two allergenic sources, allergen immunotherapy using cedar pollen extract in Japanese patients also sensitized to tomato induced a degree of tolerance to further ingestion of tomatoes [141]. As it belongs to the GRP family of proteins, BP14 might also be a member of the cross-reactive Cupressaceae allergens, since an association of sensitization with BP14 was observed with sensitization to peach [113] and also to citrus allergenic sources [142, 143].

Preliminary experiments revealed a cross-inhibition between BP14 and a low MM protein from citrus [143]. In both the peach and citrus allergenic sources, GRPs have been reported to be allergens [119, 120, 144]. This Snakin/GRP protein family should, therefore, be added to the list of pollen-food cross-reactive allergens. This is the first report of a cross-reactive GRP between pollen and food. Indeed, besides peach and citrus, Snakin/GRP proteins are also found in strawberries, tomatoes, potatoes, French beans, rice, gerbera, beechnuts, maize, soybeans, and bell pepper.

Conclusion on Allergens

Numerous allergens have been identified in Cupressaceae pollen following improvements in analytical methods and procedures to extract recalcitrant cypress pollen proteins. The quality of the pollen extract is a key aspect in determining the total repertoire of studied allergens, whether water-soluble, insoluble, or low-abundance allergens. Allergen quantity and quality are also dependent on the environment, as demonstrated by the group 3 allergens, since the pollen, whether on the tree or released in the atmosphere, is subject to pollution and the various effects of global warming [78]. For many allergens, another point that remains to be improved is the assessment of the clinical relevance of unraveled IgE-binding proteins.

This is particularly the case for the recently identified BP14, which seems more specifically involved in PFS, an allergic reaction to multiple allergens that affects 5 to 8% of patients who are allergic to pollen [145]. PFS cases are expected to increase because they are related to the two main reasons for the increase in allergies: lifestyle (such as dietary habits) and environmental change. Knowledge of this diversity of allergen content in pollen is therefore crucial to understanding the molecular basis of associated allergies and to discriminating between co-sensitization, conditional sensitization, and cross-reactivity. This is important for the diagnosis and design of subsequent allergenic immunotherapy.

Epidemiology of Cypress Pollen Allergy

Prevalence of Sensitization and Allergy to Cypress Pollen in Non-selected Populations

There are consistent correlations between exposure to Cupressaceae pollen and the presence of sensitization and allergy. A Japanese ecological survey found a positive association between cedar and cypress pollen counts and the prevalence of rhino-conjunctivitis, but not asthma, in children [146]. In the early 1990s in southeastern France, a large survey was undertaken in the general population (18–65 years) who resided in two neighborhoods with contrasting exposures to cypress pollens. In the more exposed area, 2.4% of the subjects had seasonal symptoms and positive skin tests to cypress pollen, whereas only 0.6% of the population was positive for symptoms and SPT (skin prick test) in the less exposed community [147]. In the same area, 2500 schoolchildren (9–11 years) residing in two communities with different exposures to cypress pollen were investigated. Almost 10% of the children in the more exposed community were sensitized to cypress pollen, compared to 2.7% in the less exposed community [148]. Using the findings obtained from the Italian part of the European Community Respiratory Health Survey [149] (ECRHS), a representative sample of adults (20 to 44 years of age) revealed a 3.6% incidence of cypress pollinosis based on suggestive symptoms and positive skin tests. Asymptomatic positive skin test was found in 4.9% of the sample.

In Japan, a nationwide survey was carried out regarding allergy to Japanese cedar. This cedar belongs to the Taxodiaceae family, which is very close to the Cupressaceae family. A postal questionnaire was sent to a representative sample of 10,000 individuals [150]. The response rate was slightly over 50%. The prevalence of allergy to that pollen revealed pronounced geographical variations. After controlling for potential confounding factors, it was determined to be equal to 13.1%.

Prevalence of Sensitization and Allergy to Cypress Pollen in Outpatients

In an Israeli survey, 24 to 32% of patients attending an allergy clinic had an allergy to cypress pollen [11] according to their exposure. In Cagliari (Sardinia), 17% of 500 children at a pediatric allergy clinic had a positive skin test to cypress pollen [151]. In Turkey at a pediatric clinic, 21% of the children were sensitized to cypress pollen [152] and 14% of the adults were sensitized, although the nasal challenge test was positive for only one of the 37 monosensitized patients [14]. 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) [96]. In a cross-sectional study, 400 outpatients in Montpellier were investigated for allergic sensitization, and sensitization to cypress pollen (20.7%) ranked after sensitization to *Dermatophagoides farinae* (37%) and *pteronyssinus* (43%) allergens [153].

Increase in the Prevalence of Cypress Pollen Allergy

The published findings of several cross-sectional surveys carried out repeatedly over time show 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 [22], while rising from 9.3 to 30.4% between 1994 and 1999 in the area around Rome [154], and rising from 7.2 to 22.0% between 1995 and 1998 in Italy's Latium area [155].

Interestingly, in a recent study in the southern region of Italy, cypress pollen sensitization almost doubled from 2005 (17%) to 2010 (29%) [156]. An Italian survey of 3057 outpatients selected in 12 study centers demonstrated that 18% were sensitized to cypress or taxodiaceae pollen [157]. 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 amounted to 32.7, 62.9, and 16.1%, respectively [158]. Ariano et al. [159] evaluated trends in cypress pollen sensitization in western Liguria. They demonstrated an upward trend, whereas pollen counts remained unchanged. 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 [22]. On the other hand, a gradual increase in pollen load [68], pollen allergenicity [19], and interaction in the patient between air pollutants and cypress allergens [146] are clear indications of a genuine increase (see below "Air Pollution: a Risk Factor for Cypress Pollen Allergy").

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

1 - 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. In consequence, the proximity of pollen sources has drastically changed. Formally, Cupressaceae were planted in agricultural zones, far from dwellings. Today, they encircle the dwellings which they visually insulate by their dense hedges.
2 - at the same time, a decrease in farming allowed for the extensive proliferation of *Juniperus* in the countryside.
3 - lastly, in urban areas, air pollution interacts with pollen to increase the allergenicity [19, 109, 110] (see details of these interactions discussed below).

Air Pollution: a Risk Factor for Cypress Pollen Allergy

The association between Cupressaceae pollen allergy and air pollutants was first noticed by Ishizaki et al. [5] in the 1980s, who found that people living near Japanese cedar trees in urban areas tended to be more affected by this allergy than people living near these trees in rural areas. During the same years in Japan, Muranaka et al. [160] revealed the adjuvant effect of diesel exhaust particles on IgE reactivity to Japanese cedar pollen in mice. These results partly explained the rising prevalence of these pollen allergies observed between 1987 and 1991 (from 17 to 25%) in Japanese mountainous areas. Concomitantly, the late 1980s saw a drastic increase in the diesel vehicle fleet [161]. These chemical changes led to the weakening of the pollen outer layer (exine) and crack formation on the hose material and could help explain the prevalence in some Mediterranean countries of cypress pollinosis [9], which in certain regions is emerging as a source of morbidity in young adults [162].

Since then, Cupressaceae pollen grains have frequently been used as a model to study the interrelationship between air pollutants and pollen allergies [78, 163]. The effects of pollution on the molecular and developmental biology of Cupressaceae pollen have been the subject of several studies which reveal the accumulation of numerous inorganic elements such as sulfur, copper, aluminum, and iron on pollen grains [17, 20] and the acidification of pollen surfaces by the adsorption of acid gases such as nitric and citric acids in polluted areas [20]. It has been shown that major urban gaseous pollutants such as NO_2, SO_2, and NH_3 may be adsorbed on Japanese cedar pollen grains in urban areas [20]. Japanese studies also demonstrated that pollutants, by attaching to the exine surface, modify the morphology and ionic composition of pollen grains [17, 74, 164, 165], thereby facilitating the release and dispersion of pollen-derived particles smaller than pollen into the atmosphere [74]. This fact may help explain the increased level of particle matter ($PM_{2.5}$) and suspended particle matter during the Japanese cedar pollination period [166] and the negative impacts of this increase on the respiratory health of allergic patients [68]. In Barcelona and Madrid [19], the exposure of Arizona cypress pollen to air pollutants promotes the production and release of an allergenic protein (Cup a 3) of the pathogenesis-related family 5 (PR-5 protein). In light of these results, urban air pollution could alter the allergenic content of cypress pollen grains. Interesting in vitro exposure studies [167] demonstrate that levels of adsorption of

gaseous pollutants vary greatly among different plant species, and cypress pollen seems to be one of the most impacted. According to Visez et al. [167], the kinetics of NO_2 uptake by cypress pollen is two and six times that of grass and birch pollen, respectively. Furthermore, and as previously stated, exposure of cypress pollen to NO_2 releases allergen-carrying free orbicules [78]. These recent physicochemical experiments open the way to more comprehensive and experimentally designed studies on the interrelationship between pollen, air pollution, and respiratory allergies.

Natural History of Cypress Pollen Allergy

The relevance of exposure to pollen is exemplified by a Japanese survey that found large differences in the prevalence of allergy to Japanese cedar in various geographic areas [150], as well as by surveys undertaken in southeastern France [147, 148]. In Japan, a recent survey underscores the importance of early exposure to pollen for sensitization and severe pollinosis [68].

In regard to the mountain cedar, which belongs to Cupressaceae family, Ramirez [168] studied the risk factors for this allergy in 80 patients monosensitized to this pollen compared to 154 patients with an allergenic polysensitization. He showed that this monosensitization was associated with a late onset of allergic symptoms around age 40, no family history of atopic diseases, a low total IgE level, and with a place of birth outside of the area. Bousquet et al. [169] investigated these same factors in a group of 26 patients who were monosensitized to cypress pollen, compared to 63 patients who were polysensitized. They found that the monosensitized patients were older at the time of onset of allergic symptoms and that they had a lower total IgE level. Comparison of a group of patients who were allergic to cypress pollen and a group of patients allergic to grass pollen [170] led to the conclusion that females and males are equally susceptible to cypress pollen allergy, whereas the sex ratio was equal to two for patients allergic to grass pollen. Furthermore, for patients allergic to cypress pollen, the onset of allergic symptoms occurred at a late age, as was also mentioned in Bousquet et al. [169] and Boutin-Forzano et al. [170], and they coughed more often during pollination, compared to patients allergic to grass pollen [171]. These results suggest that a subgroup of patients allergic to cypress pollen behave as allergic more than atopic patients [170]. It could be inferred that while some patients were not inherently prone to becoming sensitized, sensitization nonetheless occurred due to heavy and sustained exposure to cypress pollen. This is quite similar to occupational allergies towards low molecular weight agents that develop specifically toward agents encountered in an occupational setting, such as platinum salts, for example [171]. This observation also means that essentially the entire population is at-risk of developing an allergy to cypress pollen allergy, should they become exposed to massive and sustained quantities.

Clinical Aspects

According to the Japanese survey [150], and a subsequent study from Europe [170], rhinitis is more common than conjunctivitis. Conjunctivitis is, however, the most disabling symptom, occurring in 72% of patients allergic to cypress pollen versus 26% of patients allergic to grass pollen. Moreover, based on a visual analog scale used by 4025 patients visiting their general practitioner for allergic rhinitis, allergy to cypress pollen was more disabling than other pollen allergies [172]. In this study [172], asthma symptoms during the pollen season were equally prevalent in patients allergic to grass and cypress pollens, although the occurrence of a chronic cough was much more frequent with cypress pollen allergy.

In a case series from Montpellier, asthmatic symptoms were reported by 18% of patients allergic to cypress pollen [173]. A dry cough could signify eosinophilic bronchitis [174] rather than asthma. Sposato et al. have suggested that patients monosensitized to cypress pollen have rhino-conjunctivitis, while polysensitized patients may also have asthma [158]. Compared to Japanese cedar pollinosis, patients suffering from Japanese cypress pollinosis reported more itchy throat sensation symptoms [175]. A Spanish study [176] suggests that asthma symptoms in patients suffering from allergic rhinitis are encountered more frequently with pet allergens or other pollens than with cypress pollens. Cutaneous symptoms during pollination were recorded in 7.7% of the patients in the Montpellier series [173]. Cross-reactivity to food allergens has recently been reported, mainly to peach allergens in Marseille [138] and Montpellier [177], but not in Italy [178]. For the patients from Montpellier, sensitization to peach extracts occurred in 8% of the patients allergic to cypress pollens. Among them, half had an overt allergy to peach [173]. More recently, an association between citrus food allergy and pollen allergy has also been demonstrated [142, 143] (see the description of allergens involved in the section "Cypress Pollen Allergens/Cross-Reactivities").

The diagnosis is first based on the clinical history, which is usually highly suggestive because most cypresses pollinate in wintertime when no other airborne pollens are present. Skin tests, using either a mixture of *C. sempervirens* and *C. arizonica* or extracts from *J. ashei*, support the diagnosis. However, in a few cases, despite the convincing medical history, skin tests are negative in 36% of patients and 15% have no IgE against Cup a 1, the major cypress allergen [179]. The allergist can ask for specific IgE measurement. Measurement of specific IgE to *J. ashei* has proven to be more sensitive than IgE directed towards *Cupressus* allergens [180]. In this study, measurement of IgE against recombinant allergens Cup a 1

provided very similar results to IgE to *J. ashei*. Because of possible local allergy demonstrated in the field of grass pollen and house-dust mite allergy [181], specific nasal provocation tests could be performed in those instances, but so far, no such data in cypress pollen allergic patients have been published.

Management of Cypress Allergic Patients

Pharmacological Treatment

There are no specific pharmacologic treatments for this condition. A clinical trial using a non-sedating anti-histamine drug suffered from a high dropout rate due to limited efficacy [182, 183]. Early administration of steroids has, however, been shown to be effective in controlling symptoms of seasonal allergies to Japanese cedar/cypress pollen, although the difference between the treated and control groups was only noticeable after 2 weeks of treatment [184].

Immunotherapy

Several clinical trials have addressed this issue, although they only included a limited number of patients (see Charpin et al. for articles published up to 2003 [63]). Four additional studies were subsequently published: Ariano et al. used a monomeric allergoid [185], Di Renzo et al. employed a high dose of *J. ashei* extract [186], Moriguchi et al. used a Japanese cedar pollen extract [187] for immunotherapy, and Ventura et al. studied the combined effect of sublingual and subcutaneous immunotherapy [188]. All of them were able to demonstrate a benefit in terms of symptoms, quality of life, use of on-demand medications, late cutaneous response to allergen, and specific nasal hyperactivity. Larger clinical trials including longer treatments and longer follow-up periods are, however, clearly needed.

Individual Avoidance Procedures

While all of these procedures are based on common sense, they have not been clinically validated. An example of this can be found on the Swiss Federal Meteorological and Climatic Department website (www.pollenundallergie.ch). It includes information on pollen counts and pollen forecasts, advice such as rinsing one's hair before going to bed [189], wearing protective sunglasses outdoors, not to exercise outdoors, to close windows and doors [190], and to avoid outdoor drying of linen [191]. All these pieces of advice are applicable when the taxon to which an individual is sensitized is present.

Collective Strategies

Due to their substantial increase in recent decades, allergies including cypress pollinosis are currently responsible for a significant change in health and significant costs related to medical treatments. In addition to medical care and desensitization, integrated strategies have to be developed to prevent cypress pollen allergy, and the reduction in individual exposures to pollen is the upstream component of this strategy. It has been observed that pollinosis is more frequent in urban areas, although airborne pollen concentrations should be lower than in rural areas [192]. Future urban planning needs to take into account the allergenic features of ornamental plants that are used in urban green spaces, parks, and gardens [193]. Non-allergenic species and/or insect-pollinated species should be chosen over allergenic wind-pollinated species. In order not to aggravate their impact on allergy sufferers, the use of the latter species should be reduced, even if cultural and historical reasons often make this a difficult choice.

An index integrating the different components of the allergenicity risk (e.g., tree size, type of pollen dispersal type, flowering period, etc.) was used to generate a "Database of Urban Tree Potential Allergenic Values." Index summations for all of the individual trees produced an estimate of the allergenicity of Urban Green Zones [194, 195].

Forecasting of pollen emissions based on phenological modeling of pollination may help people with allergies limit their exposure to pollen. During the pollination period, they should avoid spending time in areas with high levels of airborne pollen, while also reducing passing through areas with high densities of Cupressaceae taxa. The penetration of pollen into dwellings must also be minimized. Although pollen is much more abundant in winter, pollen penetration in summer was estimated to be 100 times higher than in winter, and this was directly attributed to the opening of doors and windows in the summer time [196]. Especially since the allergenic potency of cypress pollen was shown to persist over at least a 10-month period in an indoor environment [197].

A complementary strategy consists in reducing the amount of pollen produced by Cupressaceae trees. The trimming of isolated trees or hedges before the pollination period can significantly reduce pollen production [198]. Selection of low pollen producing varieties would represent an efficient medium- to long-term way to reduce atmospheric pollen loads without the need to eradicate the Cupressaceae species in urban areas. For the few monoecious species, female cultivars are preferable. With other species, low pollen cultivars should be selected either in natural populations or breeding populations, as for *C. japonica* [199]. For this species, the use of pollen-specific fungal infection has also been proposed as an approach to prevent pollen dispersal [200]. For *Cupressus*, the production of haploid lines from *C. dupreziana* surrogate mothers offers the opportunity to produce sterile cultivars [201].

Conclusion

Cypress pollen allergy is a widely distributed, highly prevalent and severe pollinosis. The molecular allergens involved in the sensitization process are increasingly well documented. It can occur in individuals who are not predisposed to become allergic and represent a public health threat. However, compared to ragweed or grass pollen allergy, it should be controllable because cypress trees rarely spontaneously reproduce. Accordingly, provision of information to the general public and policy makers is of the utmost importance in controlling this expanding allergy.

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Compliance with Ethical Standards

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Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval Human sera used for immunochemical studies (Allergens section) correspond to residues from biological analysis for diagnosis.

Informed Consent Not applicable.

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