ORIGINAL ARTICLE

Prevalence of sensitization to *Quercus ilex* pollen and its contribution to Pollen Food Allergy Syndrome in Central Spain

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Summary

Background. *Quercus ilex* is a major contributor to airborne pollen levels in Madrid (central Spain); however, its clinical importance as sensitizer remains unclear. This study aimed to evaluate the prevalence of *Q. ilex* pollen sensitization, its association with pollen food allergy syndrome (PFAS), and the frequency of sensitization to Que i 1 (PR-10) and Que i 2 (profilin) in a subgroup of *Q. ilex*-sensitized patients. **Methods.** We included 60 children and 80 adults with respiratory allergic disease during the spring season. Skin prick tests for spring aeroallergens were performed, and the presence of PFAS was assessed. A group of 41 sera from *Q. ilex*-sensitized patients were tested by ELISA to determine specific IgE to Que i 1 and Que i 2. **Results.** 41.7% of children and 53.8% of adults were sensitized to *Q. ilex*. The most frequent co-sensitizers were *P. pratense* and *O. europaea*. PFAS was present in 20% of adult and 15% of children. *Q. ilex* was the only pollen significantly associated with PFAS in both children and adults ($p = 0.0268$ and $p = 0.0288$, respectively). In the group of patients tested by ELISA, Que i 1 was detected in 48.78% and to Que i 2 in 51.22%. Double sensitization was significantly more frequent among PFAS-positive individuals ($p = 0.0014$). **Conclusions.** *Q. ilex* pollen represents a relevant

allergen source in central Spain, with sensitization rates near to 50% among patients with spring pollen allergy. Its significant association with PFAS emphasizes its role in allergy disease and supports its inclusion in diagnostic testing.

KEY WORDS

Quercus ilex pollen allergy; spring pollinosis; pollen food allergy syndrome; Que i 1; Pollen food allergy syndrome.

Impact statement: *Q. ilex* represents a clinically relevant allergen in central Spain, with high sensitization prevalence and significant association with PFAS.

Introduction

Pollen allergy is a significant clinical concern across Europe and is influenced by environmental and geographic factors that affect the distribution and intensity of allergen exposure.

Madrid is located in the center of the Iberian Peninsula and it is characterized by a broad spectrum of airborne allergenic sources. During the spring, from March to June, the most clinically relevant pollen types are derived from grasses, followed by *Olea europaea* (olive tree) and *Platanus acerifolia* (plane tree). Although these pollens are widely recognized as primary contributors to seasonal spring allergies, emerging evidence suggests that *Quercus ilex* (holm oak) should also be regarded as a relevant springtime sensitizer.

Q. ilex belongs to the Fagaceae family within the order Fagales, a taxonomic group with high levels of allergenic cross-reactivity among representative genera including birch, alder and hazel, among others (1). Previous studies performed in Madrid area reported that *Quercus spp.* exhibited the highest airborne presence in the spore traps during spring (2). In addition, current aerobiological monitoring indicates that *Q. ilex* pollen is present in the atmosphere of Madrid from March to June, with a cumulative rate of 10.310 grains/m3 recorded in 2024 (3) (Supplementary Fig.2).

Despite its abundance, this pollen has been largely overlooked based on early studies performed in the nineties, which reported low sensitization prevalence rates ranging from 3.5% to 14% (2,4). Recent

studies have documented a substantial increase in sensitization rates, reaching 22.9% in adults and 56.0% in pediatric patients with pollen allergy (5). Furthermore, the allergenic potential of *Q. ilex* pollen has been objectively confirmed through nasal provocation testing (5).

Likewise Que i 1 (belonging to Bet v 1 PR-10 family) has been described as a major allergen of *Q. ilex* linked to pollen food allergy syndrome (PFAS) in Spanish patients sensitized to PR-10 proteins but not exposed to birch pollen (6).

Due to the historical underestimation of the clinical relevance of this pollen, research on its role in pollinosis remains limited. To address this gap, we conducted this study to assess the prevalence of *Q. ilex* pollen sensitization, in both pediatric and adult patients, in relation with the sensitization to the other spring pollens from Madrid, in central Spain. Besides, we evaluated the association between spring pollens sensitization and the presence of PFAS among those patients. On the other hand, we described *Q. ilex* profilin, included as Que i 2 in allergen bank and studied the frequency of sensitization to Que i 1 and Que i 2 in a group of patients sensitized to *Q. ilex* pollen.

Material and methods

Study design and patients

We conducted a prospective, observational, and descriptive study involving pediatric patients (<16 years) and adult patients (≥16 years) who presented with symptoms suggestive of respiratory allergic disease (rhinoconjunctivitis and/or asthma) during the spring season. All patients were first-time referrals and were evaluated by an allergologist at the Allergy Department of a third level Hospital in Spain. The local Ethics Committee of the Hospital approved the study (Protocol Number PI-2243). Written informed consent was obtained from the patients or legal tutors.

A total of 100 children and 100 adults were consecutively recruited between January and May 2024. All patients underwent skin prick testing (SPT) using a standardized panel of respiratory aeroallergen extracts. Patients without allergic sensitization to spring pollens, as determined by SPT, were excluded

from the study. Ultimately, 60 children and 80 adults met the inclusion criteria and were confirmed as eligible participants (Supplementary Fig. 1A).

The presence of PFAS in these populations was assessed in relation to the implicated food. Diagnosis of PFAS was made according to routine clinical practice, which includes SPT with commercial extract of involved foods, prick-prick testing with fresh foods, sIgE determination to involved foods and/or panallergens.

Sera from 41 patients sensitized to *Q. ilex* were available for *in vitro* ELISA determination of Que i 1 (PR10) and Que i 2 (profilin) (Supplementary Fig. 1B).

Sensitization profile

All the patients underwent skin prick tests (SPT) with commercial extracts: *Platanus acerifolia* (ALK-Abelló), *Olea europaea*, *Quercus ilex,* and *Phleum pratense* (Roxall-Medicina). The SPT was performed according to European guidelines (7), considering it positive if the diameter of the wheal was 3 mm greater than that induced by the negative control.

Clinical outcomes

Demographic data, including sex and age, were collected. The presence of symptoms consistent with rhinoconjunctivitis and/or asthma was recorded, along with the months during which these symptoms occurred. Additionally, the presence of PFAS was assessed, as well as the specific fruit implicated in each case.

Cloning, expression and purification of Que i 1 and Que i 2 (profilin)

Que i 1 was obtained as described (6). The *Q. ilex* profilin sequence was identified by sequence similarity analysis, using Bet v 2 (accession no. AAA16522.1) and *Q. acutissima* profilin (accession no. QVU02258.1) as references to query the *Q. ilex* genome (8,9). A synthetic open reading frame (ORF) encoding *Q. ilex* profilin was designed, cloned, and expressed in the pET30a expression vector by

GenScript Biotech (Rijswijk, Netherlands). The resulting plasmid encodes a protein with an N-terminal hexahistidine tag, which was purified using nickel affinity chromatography (GenScript Biotech). Sequence similarity was analysed with an NCBI BlastP programme using the All non-redundant GenBank CDS translations + PDB+SwissProt + PIR + PRF and excluding environmental samples from WGS projects databases (10).

Determination of rQue i and rQue i 2 specific IgE by ELISA

Polystyrene 96-well plates (Costar 3590, Corning, NY) were coated with 100 µL of recombinant allergen (0.01 µg/µL in carbonate buffer, pH 9.6) for 2 hours at 37 °C. Wells were then blocked with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 30 minutes at 37 °C, followed by overnight incubation at room temperature with 100 µL of patient serum (1:4 dilution in 1% BSA, 0.05% Tween-20 in PBS). After two washes with 0.05% Tween-20 in PBS, wells were incubated for 2 hours at room temperature with peroxidase-conjugated anti-human IgE (SouthernBiotech, Birmingham, AL; 1:2000 dilution). Following additional washes, plates were developed with 100 µL of TMBturbo ELISA substrate (Thermo Scientific, Rockford, IL). The reaction was stopped after 30 minutes with 100 µL of 2 M H_2SO_4, and optical density was measured at 450 nm. Assays were performed in duplicate. Blocking buffer alone served as the negative control. To establish the cutoff value, sera from 25 healthy individuals were analyzed, and the threshold was defined as the mean optical density plus four standard deviations (positive values $\geq$ 0.15 absorbance units), as previously described (11).

Statistical Methods

Statistical analyses were performed using the R statistical program, version 4.3.3 (R Core Team, 2024). Qualitative variables were described using absolute frequencies and percentages and quantitative variables using the mean and standard deviation. We studied the normality of continuous variables using the Kolmogorov-Smirnov test. The association between qualitative variables was analyzed using the chi-square or Fisher's exact test. The individual effect of pollen sensitization on Pollen Food Allergy

Syndrome (PFAS) was studied using univariate logistic regression models. Multivariate logistic regression models have also been applied to analyze the combined effect of sensitizations. All statistical tests were considered two-sided, and p values lower than 0.05 were considered significant.

Results

A total of 60 children (45% female, 55% male; mean age: 11.2 years) and 80 adults (52.5% female, 47.5% male; mean age: 35.4 years) with sensitization to at least one spring pollen, as determined by skin prick testing (SPT), were included in the study (Supplementary Table 1). Allergic rhinitis was reported in 88.3% of children and 96.3% of adults, while allergic conjunctivitis was present in 80.0% and 91.3%, respectively. Asthma was diagnosed in 35.0% of children and 40.0% of adults. The most common months during which symptoms occurred in children were May (76.7%), April (71.7%) and June (71.7%) whereas symptoms were most frequent in May (78.8%) and June (72.5%) among adult patients (Supplementary Table 1).

Regarding PFAS, it was present in 15% of children and 20% of adult patients. The most common clinical manifestation was oral allergy syndrome, occurring in 88.9% of children and 68.8% of adults. No cases of anaphylaxis were recorded. There were no statistically significant differences between children and adults in terms of clinical or demographic characteristics (Supplementary Table 1).

Sensitization profiles by SPT and prevalence of Q. ilex sensitization

The 26.7% of children and 28.8% of adults with spring pollen respiratory allergy were sensitized to all four pollens evaluated (Table 1). Sensitization to *Q. ilex* was observed in 41.7% of children and 53.8% of adults. The most frequent causes of sensitization were *P. pretense* (grass) (81.7% in children and 72.5% in adults) and *O. europaea* (76.7% in children and 78.8% in adult), followed by *P. acerifolia* (58.3% children and 57.5% adults).

Within the subgroup of patients sensitized to *Q. ilex*, 64.0% of children and 53.5% of adults were co-sensitized to *Q. ilex* and three additional pollens, 20.0% of children and 27.9% of adults to *Q. ilex* and

two additional pollens, and 16.0% of children and 14.0% of adults to *Q. ilex* and one additional pollen. Notably, 4.7% of adults (n = 2) were monosensitized to *Q. ilex* (Fig.1).

Prevalence of PFAS and its association with pollen sensitization

PFAS were reported in 15% of children and 20% of adult patients with spring pollen allergy (Supplementary Table 1, Supplementary Fig. 1). Sensitization to Q. ilex was less frequent in the pediatric population, while in adults its prevalence was comparable to that observed for the other spring pollens. Nevertheless, it was the only pollen significantly associated with the presence of PFAS in the univariate logistic regression models in both children (OR 6.417; IC95%: 1.381, 46.225; p=0.0294, Supplementary Table 2) and adults (OR 4.911; IC95%: 1.420, 22.934; p=0.0207, Supplementary Table 2). Furthermore, this effect persisted when Multivariate models were applied to analyze the combined effect of sensitizations (Table 2).

The most common offending fruits among children with PFAS were melon (5/9), banana (3/9), apple (3/9) and peach (5/9). PFAS symptoms among adults were most frequently reported with melon (7/16), peach (6/16) and apple (4/16) (Suplementary Table 3).

Molecular cloning and expression of rQue i 2

The cloned sequence coded for 133 amino acids with a calculated mass of 14307.18 kDa and an isoelectric point of 4.90 (data not shown). This sequence was deposited into the GenBank database under accession No. PV388576 and was designated as Que i 2.0101 by the WHO/IUIS Allergen Nomenclature Committee. The translated sequence showed highest homology with *Q. acutissima* profilin (99.25% identity; QVU02258.1), followed by *Castanea sativa* profilin (98.50% identity; XP_075632987.1) (data not shown).

Comparing Que i 2 with the profilins from the pollens considered in this study, the percentages of identity are 84 % with Ole e 2, 80 % with Pla a 4, 78% with Phl p 12. However, considering conserved

amino acids, the percentage of similarity among the four profilins is 90-91% (sequence and alignment available in Supplementary Fig. 3).

Recombinant protein was produced with 7 additional amino acids including 6 histidine residues (MHHHHHH) at the N-terminus. The protein yield was 42 mg/L of bacterial culture in soluble form. The recombinant protein showed an apparent molecular weight of 15 kDa by SDS-PAGE, the approximate percentage purity of rQue i 2 according the SDS-PAGE was 95% (data not shown).

Association between PFAS and Que i 1/Que i 2 sensitization in *Q. ilex*-sensitized patients

Specific IgE (sIgE) to rQue i 1 (PR-10) and Que i 2 (profilin) were measured by ELISA in a group of 41 sera samples from sensitized *Q. ilex* patients (individual ELISA values are available in Supplementary Table 4). PFAS was diagnosed in 43.90% of those patients, while the remaining 56.09% exhibited pollen allergy without food-related symptoms (Table 3, Supplementary Fig. 1). Overall, sIgE to Que i 1 was detected in 48.78% of patients, and to Que i 2 in 51.22% (Table 3, Supplementary Fig. 1). The frequency of sensitization to each allergen alone was comparable between PFAS-positive and PFAS-negative groups. However, double sensitization to both Que i 1 and Que i 2 was significantly more common among PFAS-positive individuals (44.44%). Importantly, all PFAS-positive patients were sensitized to at least one of the two allergens, whereas 39.13% of PFAS-negative individuals showed no sensitization to either of the allergens studied (Table 3, Supplementary Fig. 1).

Discussion

Pollen is a major contributor to allergic sensitization worldwide. However, establishing an accurate etiological diagnosis in patients with pollen allergy remains challenging due to the wide diversity of pollen sources and the increasing prevalence of polysensitization (12). Identifying the primary sensitizing allergen through appropriate diagnostic testing and careful assessment of exposure allows for a more personalized and precise therapeutic approach. This includes tailored pharmacological

treatment, targeted environmental control measures, and/or allergen immunotherapy (AIT), ultimately leading to improved clinical outcomes and long-term disease management (13,14).

The first objective of this study was to assess the actual prevalence and of *Q. ilex pollen* sensitization, the most abundant tree belonging to *Quercus* genus in the region of Madrid, located in the center of Spain. Only patients who presented clinical respiratory symptoms in spring and positive SPT to at least one of the main spring pollen were included. Interestingly, although we initially recruited 100 patients in each group, we found that a considerable proportion of patients (40% of children and 20% of adults) who consulted an allergologist did not show sensitization to any spring pollens. This phenomenon was particularly notable among children, probably due to many different causes including local rhinitis, sensitization to non-spring allergens, or nonspecific symptoms resulting from other conditions (Supplementary Fig. 1).

The atmospheric pollen levels during spring in Madrid are high, originated from multiple sources. The most important allergenic pollens during springtime are grasses, *O. europae*a (olive tree) and *P. acerifolia* (plane tree); however, the overlapping among these pollens in the atmosphere is high (6). Usually, the first spring pollen to appear in atmosphere is *P. acerifolia*, followed by *Q. ilex* (holm oak), *O. europae*a and grasses. *Quercus* pollination season in Madrid generally starts from mid-April to mid-June, with the peak in mid-May (6). *Quercus* genus is characterized by high pollen production (15), in fact it exhibits one of the highest airborne concentrations in Madrid, measured in grains/m3, surpassing *O. europaea* and grasses (Supplementary Fig. 2).

The genus *Quercus* has been recognized as an allergenic source in different regions of the world (16,17). However, in central Spain, its role as a relevant pollen sensitization source has been largely overlooked due to early studies conducted in the nineties, which reported low prevalence of sensitization. Prados et al. found a 3.5% of sensitization rate to *Q. ilex* in Mérida area (Spain); they also performed nasal and conjunctival provocation test in sensitized individuals with negative results (4). In addition, Subiza et al. reported that although *Quercus* spp. had the highest airborne pollen levels in spring, only 14 % of patients with pollen positive SPT were sensitized to *Q. ilex* (2).

Q. ilex pollen has gained renewed attention due to growing evidence of its allergenic potential and associated clinical implications. The prevalence of *Q. ilex* sensitization among Spanish adult and pediatric patients in recent years has been published as 56% of children and 22% of adult pollen allergic patients (5). Furthermore, the clinical relevance of *Q. ilex* pollen has been objectively demonstrated through positive nasal provocation test results (5).

Our results show that the prevalence of sensitization was comparable between children and adults, although slightly lower in children (41.7%) compared to adults (53.75%). Besides, two adult patients (4,65%) were found to be monosensitized to *Q. ilex*, while no monosensitization was observed among children. Previous studies have reported a monosensitization of 0.9% in children (6) , whereas others have found no cases of monosensitization (4).

The current data reaffirm that *Q. ilex* constitutes a clinically relevant allergen source in our region and indicate a significant increase in sensitization since the first studies performed in the nineties. Subiza et al. performed evaluated the impact of climate change on ambient temperatures, pollen counts and pollen sensitization in Madrid over a 40-year period (1979-2018). They observed a 1.3ºC increase in temperature in Madrid over this period, associated with earlier onset of the pollen season and increase in atmospheric pollen levels. The pollens that have increased more are *Quercus, P. acerifolia* and *Cupresaceae* whereas *O. europaea* and grasses remain stable. The autors suggest possible impacts on the rates of allergic sensitization to *Cupressaceae*, *Platanus* and *Quercus* (18), which coincides with the results presented here.

According to the growing evidence, *Q. ilex* should be systematically included in the diagnostic workup of patients with suspected respiratory allergies during its pollen season, especially in regions with high *Quercus spp* pollen concentrations. This could be particularly relevant in clinical practice, where some patients undergoing allergen immunotherapy (AIT) show limited improvement despite molecular diagnostics. In this sense, *Q. ilex* belongs to *Fagales* order that includes relevant allergens with no homologs in other botanical orders (*ie* PR-10 allergens). One potential explanation of limited improvement after AIT could be the omission of those significant allergens in the AIT composition.

The second objective of this study was to investigate the association between PFAS and *Q. ilex* sensitization. PFAS is a syndrome of type I hypersensitivity to plant-based foods secondary to pollen sensitization (19). The underlying mechanism frequently involves panallergens, present across different pollens and many different foods including PR-10, profilin, LTP and other less studied allergens (20,21). PFAS prevalence varies across geographic regions, largely due to differences in local pollen exposure together with the different dietary habits. These epidemiological patterns are influenced by geographic and environmental factors, including the diversity of pollen species diversity and airborne concentrations (20,22). In Northern and Central Europe, birch (belonging to order *Fagales*) is the predominant trigger of seasonal allergic rhinitis and a frequent cause of PFAS.

The mechanism by which birch induces PFAS is well characterized, with sensitization to the major birch allergen, Bet v 1 (a PR-10 allergen), frequently identified among affected individuals (20).

Despite Madrid being a birch-free Mediterranean region, cases of pollen food allergy syndrome (PFAS) associated with PR-10 have been reported (6). A member of the PR-10 protein family, Que i 1, has been identified as major allergen of *Q. ilex* (6). In that first study, 19.2% of children exhibited PFAS, with a higher prevalence among those sensitized to *Q. ilex* compared to those who were not (24% *vs*. 13.5%). This was the first study to report a link between *Q. ilex* and its PR-10 allergen with PFAS in Southern Europe, however no comparison with other pollen sensitizations was made (6). In agreement with that first study, we have found PFAS in 15% of children and 20% of adults with spring respiratory allergies in Madrid. Noteworthy, despite the high percentage of patients sensitized to more than one spring pollen, Q. ilex pollen was the only one significantly associated with PFAS, in both children and adults. However, it is important to emphasize that this finding reflects an association rather than a causal relationship. Moreover, our findings should be interpreted with caution due to the small number of PFAS cases, which may limit the precision and stability of the logistic regression estimates.

Another panallergen frequently associated with PFAS is profilin (23). To investigate the frequency of IgE recognition of *Q. ilex* profilin, we cloned and produced the recombinant protein. This newly characterized allergen has been incorporated into the allergen database as Que i 2. It shows 90%

sequence homology with profilins from the other pollens analyzed, supporting a high degree of cross-reactivity among these proteins.

To determine the frequency of IgE recognition of rQue i 1 and rQue i 2 in *Q. ilex*–sensitized patients and to assess its association with the presence of PFAS, we evaluated sIgE responses to both recombinant allergens in a well-characterized patient cohort. The results indicate that dual sensitization to Que i 1 and Que i 2 is strongly associated with PFAS and may serve as a potential immunological marker for this condition. In contrast, monosensitization to either allergen does not reliably distinguish between PFAS-positive and -negative individuals, while the absence of sensitization to those allergens is predominantly observed in patients without PFAS. This results are in agreement with Högerle et al that described that patients with birch allergy recognizing both Bet v 1 (PR-10) and Bet v 2 (profilin) have more probabilities of suffering from an oral allergy syndrome and gastrointestinal symptoms (24). Our findings should be interpreted in light of certain limitations that include the monocentric nature of the study, which limits the generalizability of the results. In addition, oral food challenges were not performed in all patients to confirm PFAS. Moreover, results should be interpreted with caution due to the small sample size. Further studies involving larger and more diverse populations across multiple centers are needed.

In conclusion, *Q. ilex* sensitization was observed in over 40% of children and 50% of adults with spring pollinosis. Notably, it was the only pollen significantly associated with PFAS in both pediatric and adult populations, despite grasses and *O. europaea* being the most prevalent sensitizers. Among *Q. ilex*-sensitized patients, Que i 1 and Que i 2 showed similar IgE recognition frequencies. However, double sensitization to both allergens was strongly associated with the presence of PFAS, suggesting it may represent a marker of PFAS.

Funding Sources:

This study was funded by grant PI22/00221 from Instituto de Salud Carlos III (ISCIII) and co-funded by the European Union.

Contributions:

Coceptualization: RRP, JDO. Data curation: MCB, MLB, CGT, EPA, TNC, SQ. Formal analysis: RRP, MCB, ILG, MAC. Writing original draft: MCB, RRP. Writing review&editing: MLB, JDO, CGT, EPA, TNC, MAC, SQ.

Conflict of Interests:

The authors declare no conflict of interests.

Acknowledgements:

The authors would like to thank Elisa Pulido Lucas (IdiPAZ) and Alba Salinas (Alcala de Henares University) for their excellent technical support.

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Figure 1. Profile of sensitization to other pollens among patients sensitized to *Q. ilex*. (A) Venn diagrams illustrating differential sensitization profiles: yellow circles represent sensitization to *P. pratense*, green circles indicate sensitization to *O. europaea*, and purple circles denote sensitization to *P. acerifolia*. (B) Doughnut chart showing the percentage distribution of *Q. ilex*–sensitized patients according to the number of additional pollen sensitizations.

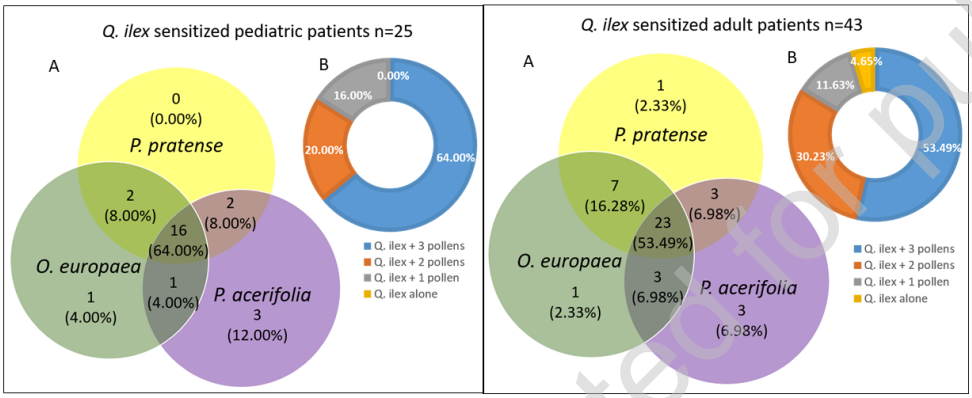


Table 1. Pollen sensitization patterns based on skin prick test results, with corresponding mean wheal diameters.

	Pediatric patients n=60			**Adult patients n=80**		
	n	**% (95%CI[a])**	Wheal size mean ± SD	**n**	**% (95%CI[a])**	Wheal size mean ± SD
Q. ilex	25	41.67 (29.07, 55.12)	4.2 ± 2.0	43	53.75 (42.24, 64.97)	5.6 ± 2.1
P. pratense	49	81.67 (69.56, 90.48)	5.6 ± 2.9	58	72.50 (61.38, 81.90)	7.6 ± 2.3
O. europaea	46	76.67 (63.96, 86.62)	7.6 ± 2.3	63	78.75 (68.17, 87.11)	9.7 ± 4.4
P. acerifolia	35	58.33 (44.88, 70.93)	4.2 ± 1.4	46	57.50 (45.94, 68.49)	7.1 ± 3.4
4 pollens	16	26.66 (16.07, 39.66)	-	23	28.75 (19.18, 39.95)	-

[a] 95%CI: 95% confidence interval

Table 2. Association between pollen sensitization and Pollen–Food Allergy Syndrome. Results from the multivariate model analysis; statistically significant associations are highlighted in bold.

Pediatric patients PFAS[a] +				
pollen	Initial model		Final model	
	OR[b] (95%CI[c])	p-value	OR (95%CI)	*p-value
Q. ilex	5.943 (0.995, 57.055)	0.0740	6.417 (1.381, 46.225)	**0.0294***
P. pratense	0.775 (0.111, 7.047)	0.8007	-	-
O. europaea	1.012 (0.161, 9.272)	0.9904	-	-
P. acerifolia	1.151 (0.150, 10.505)	0.8915	-	-
Adult patients PFAS +				
pollen	Initial model		Final model	
	OR (95%CI)	p-value	OR (95%CI)	*p-value
Q. ilex	5.581 (1.390, 30.377)	**0.0252***	4.911 (1.420, 22.934)	**0.0207***
P. pratense	0.690 (0.165, 3.052)	0.6088	-	-
O. europaea	2.794 (0.591, 21.352)	0.2428	-	-
P. acerifolia	1.025 (0.274, 4.004)	0.9704	-	-

[a] PFAS: Pollen Food Allergy Syndrome. [b] OR: odds ratio; [c] 95%CI: 95% confidence interval; *significant p-value (<0.05)

Table 3. Association between Pollen Food Allergy Syndrome (PFAS) and sIgE to Que i 1 and Que i 2. statistically significant associations are highlighted in bold.

	Q.ilex + patients n = 41		*p-value
	PFAS[a] + n=18 (43.90%)	PFAS - n=23 (56.09%)	
None	**0 (0.00%)**	**9 (39.13%)**	**0.0014***
Que i 1 (PR-10)	5 (27.78%)	6 (26.09%)	
Quei 2 (profilin)	5 (27.78%)	7 (30.43%)	
Que i 1+2	**8 (44.44%)**	**1 (4.35%)**	

[a] PFAS: Pollen Food Allergy Syndrome; *significant p-value (<0.05)

Supplementary Table 1. Demographic and clinical characteristics.

		Pediatric patients n=60	Adult patients n=80
Age (mean; SD)		11.2 (3.39)	35.4 (14.5)
Sex	**Female**	27 (45.0%)	42 (52.5%)
	Male	33 (55.0%)	38 (47.5%)
Symptoms	**Rhinitis**	53 (88.3%)	77 (96.3%)
	Conjunctivitis	48 (80.0%)	73(91.3%)
	Asthma	21(35.0%)	31(40.0%)
Month of symptoms	**March**	34 (56.7%)	44 (55.0%)
	April	43 (71.7%)	49 (61.3%)
	May	46 (76.7%)	63 (78.8%)
	June	43 (71.7%)	58 (72.5%)
PFAS[a]:		n=9 (15.0%)	n=16 (20.0%)
	Oral allergy syndrome	8 (88.9%)	11 (68.8%)
	Urticaria	1 (11.1%)	6 (37.5%)
	Anaphylaxis	0 (0.0%)	0 (0.0%)

[a] PFAS: Pollen food allergic syndrome.

Supplementary Table 2. Univariate model to test the association between pollen sensitization and Pollen Food Allergy Syndrome (PFAS). Statistically significant results are highlighted in bold.

Pediatric patients PFAS +		
pollen	OR[a] (95% CI [b])	*p-valor
Q. ilex	6.417 (1.381, 46.225)	**0.0294***
P. pratense	0.750 (0.149, 5.588)	0.7443
O. europaea	1.077 (0.223, 7.875)	0.9320
P. acerifolia	2.875 (0.622, 20.575)	0.2139
Adult patients PFAS +		
pollen	OR (95% CI)	*p-value
Q. ilex	4.911 (1.420, 22.934)	**0.0207***
P. pratense	1.174 (0.354, 4.627)	0.8024
O. europaea	2.143 (0.518, 14.630)	0.3476
P. acerifolia	1.823 (0.590, 6.340)	0.3129

[a] OR: odds ratio; [b] 95%CI: 95% confidence interval; *significant p-value (<0.05)

Supplementary Table 3. Pollen SPT sensitization in pediatric and adults patients with PFAS according to more frequent offending foods.

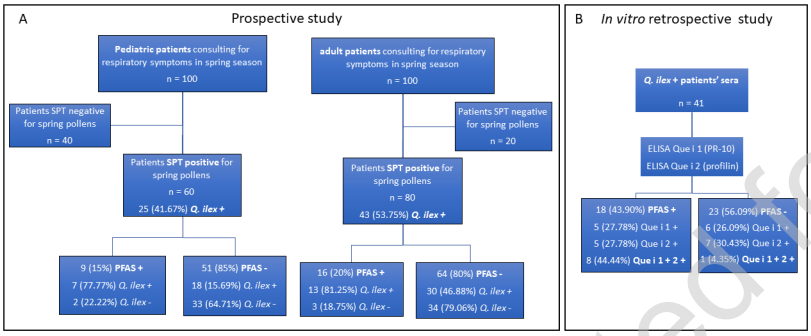
	Pediatric patients (n=9)			
	Q. ilex	***P. pratense***	***O. europaea***	***P. acerifolia***
Melon (n=5)	3 (60.0%)	5 (100%)	5 (100%)	3 (60.0%)
Banana (n=3)	3 (100%)	3 (100%)	3 (100%)	3 (100%)
Apple (n=3)	3 (100%)	2 (66.7%)	2 (66.7%)	3 (100%)
Peach (n=3)	2 (66.7%)	1 (33.3%)	1 (33.3%)	2 (66.7%)
Peanut (n=1)	1 (100%)	1 (100%)	1 (100%)	1 (100%)
	Adult patients (n=16)			
	Q. ilex	***P. pratense***	***O. europaea***	***P. acerifolia***
Melon (n=7)	6 (85.7%)	6 (85.7%)	6 (85.7%)	5 (71.4%)
Banana (n=1)	1 (100%)	1 (100%)	1 (100%)	1 (100%)
Apple (n=4)	3 (75.0%)	2 (50.0%)	3 (75.0%)	3 (75.0%)
Peach (n=6)	4 (66.7%)	3 (50.0%)	5 (83.3%)	4 (66.7%)
Peanut (n=2)	2 (100%)	1 (50.0%)	1 (50.0%)	1 (50.0%)

Supplementary Table 4. rQue i 1 and rQue i 2 sIgE in será of patients sensitized to *Q. ilex* determined by ELISA. Data in 450 nm Absorbance Units (AU) values ≥ 0.15 are considered positive (highlighted in bold).

Patient	Que i 1 (AU)	Que i 2 (AU)	PFAS
1	**0.28**	**2.12**	**yes**
2	0.11	0.07	no
3	0.01	0.09	no
4	**3.62**	0.09	no
5	**3.89**	0.03	no
7	**3.80**	0.06	**yes**
8	0.02	0.05	no
9	**1.75**	0.06	**yes**
10	**0.78**	0.00	**yes**
11	0.05	0.02	no
12	**3.87**	0.01	**yes**
13	0.02	**0.23**	**yes**
14	**3.87**	**0.97**	**yes**
15	0.02	0.09	no
16	0.03	**0.24**	no
17	0.04	**0.38**	no
18	0.01	**0.75**	yes
19	0.01	**0.44**	no
20	0.01	**1.40**	**yes**
21	0.02	**0.66**	no
22	0.01	**1.11**	**yes**
23	**0.67**	**0.91**	no
24	**3.89**	**3.20**	**yes**
25	**1.99**	**0.66**	**yes**
26	**3.56**	0.05	**yes**
27	0.02	0.04	no
28	0.01	**1.34**	**yes**
29	0.00	0.03	no
30	**2.57**	0.01	no
31	0.05	0.05	no
32	**3.89**	**1.65**	**yes**
33	0.09	**0.18**	no
34	0.04	**0.19**	no
35	0.05	0.08	no
36	**3.35**	0.05	no
37	**0.21**	0.06	no

38	0.13	**2.72**	no
39	**3.89**	**3.25**	**yes**
40	**3.89**	**3.24**	**yes**
41	**3.88**	**0.83**	**yes**
42	**0.81**	0.14	no

Supplementary Figure 1. Flowchart of the study design including the main findings related to Q. ilex sensitization. (A) Prospective in vivo study. (B) Serological in vitro study.



Supplementary Figure 2. Atmospheric concentrations of major spring pollens recorded between 2020 and 2024 by the Subiza Clinics pollen collector in Madrid (Central Spain)

(https://www.polenes.com/home).

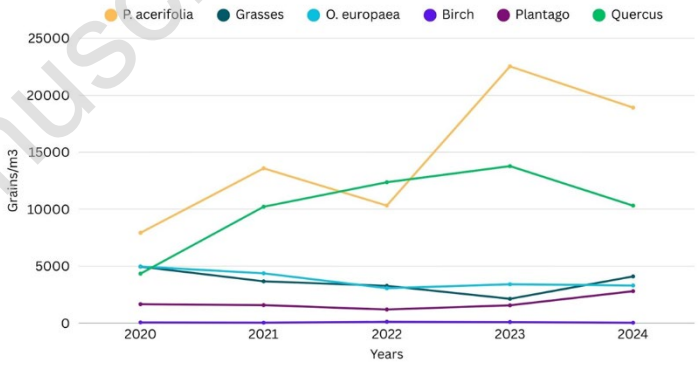


Figure 1. Atmospheric levels of the main spring pollens during 2020-2024. Available from: https://www.polenes.com

Supplementary Figure 3. Amino acid sequence alignment of allergenic profilins from the pollens investigated: Que i 2 (Q. ilex, PV388576), Ole e 2 (O. europaea, CAA73035), Pla a 4 (P. acerifolia, UYQ90947), and Phl p 12 (P. pratense, CAA70608). Symbols indicate the following levels of conservation: "*" identical residues fully conserved across all sequences; ":" residues with strongly similar properties; "." residues with weakly similar properties. Percentage identity represents the proportion of fully identical residues (*) relative to the total alignment length, while percentage similarity includes both identical and strongly similar residues ("*" and ":"). Alignments were generated using Clustal Omega (v1.2.4).

```
CLUSTAL O(1.2.4) multiple sequence alignment


Que i 2   MSWQTYVDEHLMCDIDGQGQH-LAAAAIIGHDGSVWAQSSNFPQFKAEEISDIMKDFEEP 59
Ole e 2   MSWQAYVDDHLMCDIEGHEDHRLTAAAIVGHDGSVWAQSATFPQFKPEEMNGIMTDFNEP 60
Pla a 4   MSWQTYVDDHLMCDLEGN---HLSHAAIIGHDGSVWAQSAAFPQFKPEEMTGIMNDFAEP 57
Phl p 12  MSWQTYVDEHLMCEIEGH---HLASAAILGHDGTVWAQSADFPQFKPEEITGIMKDFDEP 57
          ****:***:****:::*:    *: ***:****:*****: ***** **:.** ** **

Que i 2   GHLAPTGLHLGGTKYMVIQGEAGAVIRGKKGSGGVTVKKTSQALVFGIYEEPVTPGQCNM 119
Ole e 2   GHLAPTGLHLGGTKYMVIQGEAGAVIRGKKGSGGITIKKTGQALVFGIYEEPVTPGQCNM 120
Pla a 4   GSLAPTGLYLGGTKYMVIQGEPGAVIRGKKGSGGVTIKKTSLALIIGIYEEPLTPGQCNM 117
Phl p 12  GHLAPTGMFVAGAKYMVIQGEPGAVIRGKKGAGGITIKKTGQALVVGIYDEPMTPGQCNM 117
          * *****:.:.*:******** *********:**:*:***. **:.***:**:*******

                                      % Identity  % similarity
Que i 2   VVERLGDYLVDQGL       133      100
Ole e 2   VVERLGDYLVEQGM       134      84          91
Pla a 4   IVERLGDYLIDQGL       131      80          90
Phl p 12  VVERLGDYLVEQGM       131      78          91
          :*********::**:
```