



# *Ligustrum* pollen: New insights into allergic disease

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## ABSTRACT

Respiratory allergies are important medical conditions because they affect nearly 20% of the population worldwide, with higher prevalence in industrialized cities. Aeroallergens such as pollen are responsible for up to 40% of respiratory allergies. The pollen from *Ligustrum* (privet hedge) is a great source of inhalant allergens associated with allergic respiratory diseases around the world. However, it has been underestimated as a sensitization factor. Interestingly, over the last few years a number of novel allergens have been identified from *Ligustrum* using immunoproteomics technologies. Cross-linking of IgE and *Ligustrum* allergens could lead to the rapid release of inflammatory mediators by mast cells and basophils. These will promote a late response characterized by activation of T cells and overproduction of Th2 cytokines such as IL-4, IL-5, IL-9, and IL-13. These inflammatory changes cause respiratory diseases like asthma and allergic rhinitis in sensitized subjects. Here, we review *Ligustrum* pollen allergens and focus on their clinical and immunological significance in allergic disease as well as the use of hypoallergenic derivatives in personalized therapy.

**Keywords:** *Ligustrum*, Allergens, Epitopes, Proteomics, Allergy

## INTRODUCTION

The genus *Ligustrum* (privet) is a tree and shrub which pollen grains have been reported as allergens,<sup>1-4</sup> and comprises about 50 species including *Ligustrum vulgare*, *L. lucidum*, *L. sinense*, *L. japonicum*, *L. quihoui*, etc. They are deciduous or evergreen trees and hedges with entire, coriaceous, lanceolate or oval-shaped leaves. Their little white flowers are gathered in terminal clumps that bloom beautifully. Flowers have both male and

female parts, with white corollas and a whitish-tan to grey bark. Flowering takes place between May and July, and its pollination could be either entomophilous or anemophilous. Wild *Ligustrum* is cultivated worldwide and used extensively in landscape architecture thanks to its large ecological amplitude and fast growth, besides its resistance to pollution, pests, and diseases. *Ligustrum* is highly adaptable, growing in several soil conditions and found in different sizes, from shrubs to 10 meter high trees.<sup>5</sup> Different species of privet such as *L. vulgare*, *L. lucidum*, and *L. robustum* are commonly used as ornamental bushes around the world. Currently, it is distributed worldwide through Europe, Asia, North America, and South America, being a risk for sensitized patients. Some countries have banned its plantation because it can overgrow native species, where it is considered a plague.<sup>5,6</sup> Fig. 1 shows the global distribution of *L. vulgare* and *L. Lucidum*: potential risky areas for allergy sensitization.

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Fig. 1 Global distribution of *Ligustrum vulgare* and *Ligustrum lucidum*

*Ligustrum* is a member of the Oleaceae plant family which includes *Fraxinus excelsior* (ash), *Olea Europaea* (olive), and *Syringa vulgaris* (lilac). The production of allergens by their pollen grains is a shared feature. Among the Oleaceae family members, *O. europaea* pollen is considered the most allergenic, and it has been associated with allergic disease through extensive areas of Europe, North America, South America, South Africa, and Australia.<sup>7-9</sup> This is relatable to areas used for olive plantations that will release a great amount of pollen into the atmosphere at similar times and specific areas. In contrast, *Ligustrum* trees are dispersed in different areas and will release the pollen at different rates. Cross-reactivity between *Ligustrum* and other Oleaceae allergens is a feature of this plant family. This cross-reactivity is mainly due to high amino acid sequence homology between them *Lig v 1* and other major allergens of the Ole e 1-like protein family, including Ole e 1 (olive), Fra e 1 (ash), and Syr v 1 (lilac) present between 82% and 95% amino acid sequence homology. Interestingly, these four allergens bear the same N-glycosylation site at Asn-111. Moreover, Ole e 9 and Fra e 9 from olive and ash, share immune-dominant epitopes and IgE cross-reactivity despite their structural differences.<sup>10</sup> In this paper, we review the role of *Ligustrum* pollen in allergic disease. This allergenic pollen has

been underestimated in the past. However, recent reports place *Ligustrum* allergens as an important cause of allergic disease. The present study was based on bibliographic search and on the experience of the authors when performing a narrative review. A literature search was conducted in Pubmed and Scopus under the topics "*Ligustrum*- and/or *Privet* allergy" and "hypoallergenic Ole" The search was limited to articles published in English in the last two decades.

## LIGUSTRUM AND ALLERGIC DISEASE

The role of the species of *Ligustrum* on allergy has been largely underestimated because the large size of its pollen grains impedes long-distance dispersion, and the prevalence of allergic disease caused by its allergens has been poorly investigated. For example, *L. vulgare* (privet) pollen has been reported as a potential cause of allergic disease in the city of Cordoba, Spain,<sup>2</sup> and a high number of this pollen has also been reported in Huelva, Spain.<sup>11</sup> Similarly, Pendino et al. identified *L. lucidum* as the most prevalent cause of allergic pollen sensitization (61.5%) in Argentina affecting children aged 5-10 years.<sup>12</sup> The prevalence of *L. vulgare* allergy in Mexico City was found to be 36.6%, assessed

with skin tests,<sup>8</sup> although co-sensitization with *Fraxinus* allergens cannot be excluded as this tree is highly abundant in Mexico City. These findings can be explained because its allergens can be easily dispersed by other modes even though *Ligustrum* airborne pollen concentrations are low, causing allergic sensitization in susceptible subjects. These methods include: 1) pollen fragments produced after pollen rupture by osmotic shock during thunderstorms,<sup>13</sup> and 2) protein release under high humidity conditions<sup>14</sup> which could facilitate allergen deposition in the lungs.<sup>14-17</sup> Also, these particles could enable *Ligustrum* allergens to reach longer distances or last longer in the atmosphere than the pollen count suggests. On the other hand, the frequent use of privet trees as ornamentals in urban areas increases the allergen exposure for the population near these trees. Indeed, the pollen concentrations at human height can be up to one hundred times higher than those detected by the aerobiological samplers<sup>2</sup> facilitating allergen deposition and airway inflammation. Cariñanos et al. have proposed that cities such as Cordoba can become an urban island of biological pollution, in which pollen grains emanating from local sources are trapped in long, narrow avenues leading to high concentrations of particles including pollen grains.<sup>2</sup> On the other hand, De Linares et al. suggested that when *Ligustrum* pollen falls to the soil, it could be fragmented releasing its protein load, which could then be resuspended into the environment.<sup>18</sup> Co-sensitization between *Ligustrum* and other members have been shown in patients suffering allergic rhinitis and asthma.<sup>9</sup> A study conducted in New Zealand showed that airway responsiveness (PD20 histamine) was greater during the *Ligustrum* flowering season. Symptom scores and bronchodilator use were high, and peak expiratory flow rates low, during the Privet flowering season.<sup>19</sup> In this study, 17 subjects underwent *L. vulgare* bronchial challenge. However only 6 patients showed late asthmatic responses (there were no isolated early responses) suggesting that 35% of patients were sensitized to this pollen.

The cross-reactivity between *Ligustrum* and the other Oleaceae members has also major clinical implications in allergic disease. Batanero et al.

were the first to demonstrate that the cross-reactivity was mediated by Lig v 1.<sup>20</sup> In this study, the pool of sera from allergic patients to olive tree pollen recognized Lig v 1. More recently, Vara et al. proposed that people sensitized to Oleaceae pollen (including *Ligustrum pollen*) could develop allergic reactions throughout the entire year due to the cross-reactivity between those seasonal allergens.<sup>21</sup> They conducted the study in North-Western Spain and demonstrated that ash flowered during winter, olive in spring, and privet at the beginning of summer. Interestingly, levels of Ole e 1-like proteins including Ole e 1, Fra e 1 and Lig v 1 allergens coincided with their airborne pollen concentrations. Thus, the allergic reaction induced by olive and *Fraxinus* allergens during winter or spring could be aggravated as a result of *Ligustrum* allergens during summer promoting chronic symptoms in sensitized patients.

## LIGUSTRUM ALLERGENS

A proper identification of allergic proteins in any biological source is essential for accurate diagnosis and therapy. Early attempts to identify *Ligustrum* allergens were made in 1992: blotting experiments allowed identifying proteins around 18-19, 20, 40, and 70 kDa.<sup>22</sup> However, it was not until 1996 when Batanero et al. isolated Lig v 1 as an IgE binding protein from *L. vulgare*.<sup>20</sup> More recently, 7 different allergens have been found in *L. lucidum* extract using immunoproteomics.<sup>23</sup> Some of these allergens match other proteins reported as allergens, as listed below. The term "immunoproteomics" involves different techniques such as gels, array-based, mass spectrometry, and *in silico* studies of their interactions with the immune system:<sup>24</sup> the assessment of the allergens starts with allergen detection by serum from mono-sensitized patients followed by the characterization of the recognized proteins using mass spectrometry. Novel allergens identified in *Ligustrum* pollen are discussed below.

### Lig v 1

The characterized major allergen Lig v 1 is a homologue of other related species like Ole e 1, Fra e 1, Syr v 1 from *O. europaea*, *F. excelsior*, and *S. vulgaris*, respectively.<sup>20,25-28</sup> It is a

heterogeneous protein with two variants, one glycosylated (20 kDa) and one non-glycosylated (18.5 kDa) with 145 amino-acids.<sup>26</sup> It is part of the Ole e 1-like protein family, with conserved epitopes like E<sup>55</sup>, V<sup>56</sup>, G<sup>57</sup>, Y<sup>58</sup>, T<sup>59,28</sup> and K<sup>137</sup>, L<sup>138</sup>, G<sup>139</sup>, Y<sup>141</sup>, P<sup>142</sup> as well as a C-terminal region that is considered important for IgE-binding.<sup>29</sup> Furthermore, the larger loop of these proteins has been proposed as a T cell epitope.<sup>30</sup> Lig v 1 has also low IgE cross-reactivity with homologues from non-related species: these allergens include Lol p 11 (*Lolium perenne*), Che a 1 (*Chenopodium album*), Phl p 11 (*Phleum pratense*), and Pla l 1 (*Plantago lanceolata*). Several studies have highlighted the importance of these Lig v 1 homologues in allergic diseases.<sup>31,32</sup>

### Profilin

Previously, we reported the detection of one profilin within the *L. lucidum* extract.<sup>23</sup> These proteins are present in mammals, animal cells, plants, and viruses which share highly conserved amino acid sequences: some of them exhibit over 75% identity.<sup>33,34</sup> They are small proteins with the ability to bind actin and are involved with cell motility in eukaryotic cells. In plant cells, they play a role in cytokinesis, cytoplasmic streaming, cell elongation as well as the growth of pollen tubes and root hairs.<sup>35</sup> Profilins are pan-allergens produced by different species with highly conserved sequences. They have been identified as allergens from trees, grasses, weeds, plant-derived foods, and latex, sharing epitopes recognized by allergic patients, and they show cross-reactivity among food and respiratory allergens.<sup>36</sup> In our study, we identified a 15 amino acid fragment which was found to correspond to a predicted barley profilin (23). Future studies will define the role of this novel profilin as it has not been associated with allergic disease previously. Profilin allergens have been reported in Oleaceae family members including Ole e 2 in olive and Fra e 2 in ash.<sup>37,38</sup> The sequence VERLGD was recognized as profilin epitope shared by sunflower (*Helianthus annuus*) and olive.<sup>39</sup>

### Enolase

Members of the enolase family have been identified in various sources, from bacteria to

higher vertebrates. They play a key role in glycolysis, heat shock, and growth control, among other functions.<sup>30-42</sup> Some allergens have been reported to share IgE epitopes with the use of immunoproteomic techniques.<sup>43,44</sup> In addition, enolase epitopes have been found to be present in the protein structure of fungi allergens.<sup>45</sup> Enolases have been identified in *Hevea latex* (Hev b 9) and molds (Cla h 6 and Alt a 5).<sup>46</sup> The enolase Hev b 9 protein displays 62% identity with the enolase of the mold *Cladosporium herbarum* (Cla h 6), and 60% identity with the enolase of the mold *A. alternata* (Alt a 5): using IgE inhibition ELISA, Hev b 9 was shown to display cross-reactivity with both allergens. It has been proposed that they may account for the possible existence of a Latex mold syndrome.<sup>47</sup> The finding that an enolase is found in *Ligustrum* pollen place this allergen as a potential cause of allergy disease.<sup>23</sup> The IgE-binding enolase we have identified corresponds to a predicted polypeptide of 444 amino acids with a molecular weight of 47.38 kDa which was isolated as an enolase-encoding cDNA clone from *Brassica campestris* ssp (Chinese cabbage) by Zhao et al.<sup>48</sup>

### Fra e 9.01 allergen

One of the proteins detected by patient serum in *L. lucidum* pollen extract showed high similarity with Fra e 9.01 allergen.<sup>23</sup> Fra e 9.01 allergen is an endo- $\beta$ -1,3-glucanase produced by *F. excelsior* pollen grains.<sup>10</sup> It belongs to the glycosyl hydrolase family 17 (GHF 17), which is formed by proteins with heterogeneous characteristics, including molecular properties and biological roles.<sup>10</sup> One of the most common functions is to bind laminarin, and it is also believed to play a role in germination and pollen tube growth.<sup>10</sup> The recognized allergens from this protein family are Hev b 2 (from Latex), Ole e 9 (olive), and Fra e 9 (ash).<sup>10,49,50</sup> where the last two are respiratory allergens. The shared IgE epitopes present in latex (Hev b 2) and banana glucanases can elicit the latex-fruit syndrome.<sup>51</sup> Among Oleaceae allergens, Fra e 9 has 39% similarity with Ole e 9 from olive. The N-terminal domain from Fra e 9 showed 200-fold higher hydrolytic activity than its olive counterpart.<sup>10,49</sup> Even though glucanases have variable allergenicity, four epitopes for IgE were proposed for C-terminal

domain-Ole e 9.<sup>52</sup> The demonstration that a homologue of Fra e 9 is produced by *L. lucidum*<sup>23</sup> suggests that this allergen may contribute to the development of allergic symptoms in sensitized patients.

### Pollen-specific polygalacturonase

The polygalacturonase family is composed of proteins around 43-kDa which catalyze the degradation of highly polymeric galacturonate, a major component of pectin in plant cell walls, into individual galacturonic acid residues. Polygalacturonases are involved in cell elongation, organ abscission, microspore release, and pollen tube growth. There are a variety of polygalacturonases recognized as allergens, and they share IgE epitopes that promote cross-reactions between respiratory and food allergens.<sup>53</sup> For example, Pla a 2 is a major polygalacturonase allergen from *Platanus acerifolia* (plane tree) which is involved in the allergic responses of 84% of patients with plane tree-induced pollinosis.<sup>54</sup> Pla o 2 has also been found in *Platanus orientalis* (oriental plane) with 27% prevalence in sensitized patients.<sup>55</sup> Other allergenic polygalacturonidases include Cha o 2, Cry j 2, and Jun a 2 from *Chamaecyparis obtusa* (Japanese cypress), *Cryptomeria japonica* (Japanese cedar), and *Juniperus ashei* (mountain cedar), respectively,<sup>53</sup> and they share sequence identities between 71% and 82%.<sup>56</sup> The polygalacturonase allergens reported for *Oleaceae* up until now are Ole e 13 from olive raw fruit<sup>55</sup> and a polygalacturonase detected in *Ligustrum* pollen extract.<sup>23</sup> Interestingly, a polygalacturonase with low IgE activity has been identified in olive pollen and named "Ole e 14".<sup>57</sup> More recently, Cari p 1 was detected as an IgE reactive protein from papaya pollen as well as fruit proteome.<sup>58</sup> Using an immunoproteomic approach we obtained a protein sequence for pollen-specific polygalacturonase from *L. lucidum*, which has 88% homology to multiple allergens, including olive allergen Ole e 13.01<sup>23</sup> which suggests that this novel allergen may be involved in the *Ligustrum*-induced allergic reaction.

### Alanine aminotransferase

The alanine aminotransferase is an enzyme catalyzing the reversible transfer of the amino

group from glutamate to pyruvate to form 2-oxoglutarate and alanine.<sup>59</sup> Thanks to immunoproteomic techniques, this enzyme was detected as a possible allergen in *L. lucidum*<sup>23</sup> and maize<sup>60</sup> extracts.

### ATP synthase

This enzyme is a membrane-bound protein essential for metabolism, related to the sterility of the pollen grains.<sup>61</sup> Various peptides corresponding to ATP synthase beta subunits were detected as potential allergens in *Ligustrum* pollen grains,<sup>23</sup> *Cannabis*,<sup>62</sup> and *Ambrosia*,<sup>43</sup> as well as the ATP complex from bovine dandruff.<sup>63</sup> In our case, the recognition of 2 spots as ATP synthase suggests the presence of 2 isoforms in *L. lucidum* pollen grains.<sup>23</sup>

## DIAGNOSIS AND THERAPY

In this review, we have highlighted the importance of *Ligustrum* allergy in view of the finding that novel allergens were released by *L. lucidum* pollen.<sup>23</sup> Skin prick test is the most common diagnostic method used to detect sensitization to airborne allergens in the clinic. For example, a natural extract of *L. Lucidum* is placed on the skin and then punctured, and within 15-20 minutes a wheal and redness will indicate allergy sensitization to pollen allergens. Limitations of natural extracts are that their composition may be problematic with regard to their quality and variabilities regarding contents and biological activity, and sometimes important allergens are not even present in the extracts. Recombinant Lig v 1 has been used for *in vitro* allergy diagnosis. In fact, due to the extensive cross-reactivity of *Ligustrum*, it was proposed that *O. europaea* extract could be used in the diagnosis of *Oleaceae* pollen allergy.<sup>9</sup> However, the finding that *Ligustrum* pollens produce novel allergens suggests that they may also cause allergic disease, which raises some concerns about have been found in olive previously. Thus, the development of recombinant *Ligustrum*-derived allergens may allow distinguishing the true *Ligustrum* allergens from the cross-reactive molecules deriving from olive.

Currently, treatment for allergy includes corticosteroids, anti-histamines, bronchodilators, and

Company	Testing	Treatment
Stallergenes Greer	SPT <sup>a</sup>	Glycerinated, Aqueous
ALKAbello	SPT	Glycerinated, Aqueous
Hollister-Stier Labs	SPT	Glycerinated

**Table 1.** Ligustrum allergen extracts for diagnostics and immunotherapy. a. Skin prick test.

the use of monoclonal antibodies. However, allergen immunotherapy (AIT) remains as the only disease-modifying and curative treatment for allergic diseases. Privet AIT is achieved with either *Ligustrum* or *Olea europaea* extracts, because of their high similarity.<sup>23</sup> *Ligustrum* natural extracts are commercially available for diagnostics and immunotherapy (Table 1). To date, however, there are no studies showing the efficacy of *Ligustrum* immunotherapy. Limitations for the use of natural extracts include batch-to-batch differences from pollen-raw material containing allergen varieties growing in different cultivars and new sensitizations to extract components. Recombinant hypoallergenic allergens have been proposed as a replacement for whole allergen extracts in AIT. While there are not *Ligustrum* hypoallergenic derivatives being developed, Ole e 1 mutants have been investigated.<sup>28,64</sup> In 2006, Marazuela et al. identified 3 different Ole e 1 mutants, where the 135D10 variant showed very weak IgE-binding activity while maintaining the same T cell reactivity.<sup>65</sup> Mice immunization with this hypoallergenic variant induced IgG1 antibodies for the recognition of ash, lilac, and privet allergens. Researchers proposed the 135D10 Ole e 1 mutant as a candidate for immunotherapy of olive pollen allergy and to allergies to pollen containing Ole e 1-like allergens.<sup>65</sup> Another interesting approach is the use of peptide immunotherapy: allergen-specific regulatory T- and B-cells induced by AIT suppress the Th2 response via the cytokines IL-10 and TGFβ, and allergen-specific IgG4 antibodies are produced which inhibit IgE-mediated antigen presentation and block mediator release from mast cells.<sup>65</sup> Using microarray technology Calzada et al. showed that 2 hypoallergenic peptides (aa11-22 and peptide aa22-33), regulate the expression of 51 genes in allergic patients, including DNMT1, DAB2, LGMN, EB13, TREM1, CD84, FPR3, ALOX5, and other cytokines and chemokines.<sup>66</sup> While

these results open new ways to research the allergy regulation to-date, none of the hypoallergenic developed so far has entered the market. Development of recombinant allergen products has been delayed because the requirements differ for marketing natural extracts and recombinant products which makes it unlikely to have recombinant allergen molecules for *in vivo* testing in the near future.

## CONCLUSIONS

There is increasing evidence that *Ligustrum* pollens may play an important role in allergic disease. *Ligustrum* is an exotic plant distributed across the world, and it is used as ornamental bushes in urban areas where its pollen numbers can be very high and affect sensitized subjects. In the past, the *Ligustrum* allergenicity was attributed to Lig v 1 which is a homologue of Ole e 1, Fra e 1, Syr v 1 (they exhibit 82%-91% amino acid sequence identity). However, it has recently been shown that *Ligustrum* pollens produce a number of additional allergens including a profilin, an enolase, a Fra e 9.01, a pollen-specific polygalacturonase, an alanine aminotransferase, and 2 isoforms ATP synthase, suggesting that *Ligustrum* allergy is more complex than originally believed. Future studies which may develop recombinant *Ligustrum*-derived allergens may allow the distinguishing of true *Ligustrum* allergens from cross-reactive molecules deriving from other members of the Oleaceae family. Moreover, engineering *Ligustrum* hypoallergenic derivatives could have future applications in AIT. We encourage further studies about *Ligustrum* allergens because of their potential use in diagnosis and therapeutics.

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**Authors' contributions**

All three authors contributed to this work.

**Declaration of Competing Interest**

The authors declare that they have no competing interests.

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