Cypress pollen does not cross-react to plant-derived foods

Some pollen allergens, such as the major birch allergen, Bet v 1, and the plant pan-allergen, profilin, are well known to cross-react to homologous allergens in vegetable foods causing the so-called oral allergy syndrome. In contrast, the existence of cross-reactivity between specific cypress pollen allergens and plant-derived foods is poorly defined. In recent years, some authors reported cross-sensitisation to tomato, banana and apple in patients allergic to Juniperus ashei or Cryptomeria japonica pollen (1-5), and a French study observed potential cross-reactivity between Cypress pollen and peach (6). Further, in France the berry of Juniperus oxycedrus is present in several dishes, but data about potential cross-reactivity with the corresponding pollen are missing. We assessed whether cross-reactivity between Cupressus semprevirens and peach and other fruits or between Juniperus oxycedrus pollen and Juniperus oxycedrus berry occurs.

Seventy-two patients diagnosed as being monosensitized to Cupressus semprevirens at the outpatients allergy departments of Marseille, France, (n=39) and Bordighera, Italy, (n=33) were studied. The diagnosis was based on a clinical history of rhinoconjunctivitis with or without asthma from November to the end of March, confirmed both by positive SPT and increased levels of IgE specific for Cupressus semprevirens. All patients were thoroughly interviewed about adverse food reactions (including oral allergy syndrome, urticaria/angioedema, gastrointestinal symptoms immediately after the ingestion of specific foods). All patients underwent skin prick tests with our routine panel of aeroallergens (house dust mites, pellitory, grass, olive, Cupressus semprevirens, Betula alba, Alternaria, Cockroaches, Cat and Dog). Further, Juniperus oxycedrus fruit was tested by the prick-prick technique. IgE to peach, tomato and apple were measured by Immuno-CAP (Padia, Uppsala, Sweden) in all subjects. Thirty patients allergic to pollens other than cypress were used as controls. All skin tests were carried out using Lofarma extracts (Milan, Italy; 1/20 w/v), and were performed and read following established methods. Juniperus oxycedrus pollen (Jo) and desiccated Jo berries underwent 5% (w/v) aqueous extraction in 0.125 M NH4HCO3 for 4 h at 4°C under stirring at 4°C. The suspensions were centrifuged at 20,000 g for 1 h at 20°C and supernatants were extensively dialyzed against distilled water. Protein content of samples was assessed by BioRad assay. Specific IgE to Juniperus oxycedrus pollen or berry extracts were measured by ELISA using a pool of sera from patients allergic to Cupressus semprevirens pollen. A pool of 5 sera from non-atopic individual was used as control. To this purpose, 5µg of Jo pollen and berry extracts in 100 µl of buffer (15 mmol/L Na2CO3 and 35 mmol/L NaHCO3, pH 9.6) per well of 96-microtitre plates (Maxisorp Nunc, Roskilde, Den-
were used in the coating phase. After washings with 0.15 M phosphate-buffered saline, pH 7.4 (PBS) and 0.05% Tween 20 (Sigma, Milan, Italy), wells were saturated with 2% bovine serum albumin (BSA) in PBS (saturation and dilution buffer) for 2 hours at room temperature. Subsequently, after further washing, 100 µl of positive or negative pool diluted 1:2 in dilution buffer were added to the wells and incubated for 2 hours at room temperature. Wells were washed, and bound specific IgE was detected by peroxidase-conjugated anti-human IgE from goat (diluted 1:1500, Biopacific, Emeryville, CA, USA); a colorimetric reaction was induced using tetramethyl-benzidine/H₂O₂ as substrate. The enzyme reaction was stopped after 20 minutes by the addition of 1 mol/L HCl. Absorbance values were read at 450 nm by spectrophotometer.

In inhibition studies, patients’ sera positive to both pollen and berry extracts were pooled before pre-absorption for 2 hours at room temperature with different concentrations of Jo pollen or berry extracts (40 µl of sera and 80 µl of inhibiting extract diluted 1:1, 1:4 or 1:16). Subsequently, 100 µl of such solutions were added to Jo-coated wells and ELISA performed as before performing ELISA as described before. IgE levels were expressed as optical density units (OD). Based on the mean ± 2SD of IgE levels found in normal controls, values less < 0.100 OD were considered negative.

No patient allergic to *Cupressus sempervirens* reported clinical allergy or showed either in-vivo or in-vitro hypersensitivity to peach. Similarly, none out of 33 patients reported clinical allergy or immunological sensitivity to tomato or apple. Only 2/72 patients scored positive on SPT with Jo berry. The pool of sera used in the study showed strong IgE reactivity to *Juniperus oxycedrus* (O.D.: 1.024UA) (Tab. 1). As a difference from patients, 25% of 30 control subjects with pollen allergy reported oral allergy syndrome following the ingestion of vegetable foods. SPT with *Juniperus oxycedrus* pollen extract scored positive in 90% of patients, thus confirming the cross-reactivity between Cupressus and Juniperus pollen. No cross-reactivity between Juniperus pollen and berry was observed as shown by the lack of any inhibition of IgE reactivity to pollen pre-adsorption sera with Jo berry extract (Tab. 1). Altogether, our findings clearly rule out an association between *Cupressus sempervirens* pollen allergy and hypersensitivity to plant-derived foods. The two cases of skin reactivity to Juniperus berry in French subjects are probably the result of dietary habits, and we weren’t able to find any cross-reactivity between *Juniperus oxycedrus* pollen and berry extracts.

### Acknowledgements

The pollen of *Juniperus oxycedrus* was a kind gift of Prof. Dr. Paolo Raddi – Istituto di Patologia delle Piante, CNR, Sesto Fiorentino (FI).

### References


### Table 1 - ELISA inhibition of Jo pollen extract using pollen or fruit Jo extracts or *J. oxycedrus* pollen extract as inhibitors

<table>
<thead>
<tr>
<th>Inhibitor dilution</th>
<th><em>Juniperus oxycedrus</em> pollen extract</th>
<th><em>Juniperus</em> fruit extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UA</td>
<td>% inhibition</td>
</tr>
<tr>
<td>1:1</td>
<td>0.05</td>
<td>100</td>
</tr>
<tr>
<td>1:4</td>
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<td>100</td>
</tr>
<tr>
<td>1:16</td>
<td>0.128</td>
<td>88</td>
</tr>
<tr>
<td>no inhibition</td>
<td>1.024</td>
<td>0</td>
</tr>
</tbody>
</table>

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