

Peach tree pollen and Pru p 9 may induce rhinoconjunctivitis and asthma in children[†]

To the editor

Pollen allergens elicit respiratory symptoms in children and adolescents, with the most important clinical entities being rhinitis and rhinoconjunctivitis, though asthma also occurs.¹ Pollen can be anemophilous or entomophilous, the former being more relevant in allergy. Fruit plant and tree pollens, including peach tree, are preferably entomophilous² with symptoms more usually occurring in farmers. Children sensitized to pollens can also be allergic to fruits because of panallergens such as Bet v 1 present in birch pollen, Mal d 1 in apple and Pru p 1 in peach and others³. Due to the homology of sequences, these allergens have common epitopes sharing specific IgE antibodies with cross-reactivity.

Peach tree pollen can be an important inducer of allergy in regions of peach tree cultivars.⁴ Children may visit orchards and become sensitized during the flowering period, experiencing symptoms after exposure (direct contact). Alternatively, children whose parents work in the cultivars can be exposed indirectly to peach tree pollen since it adheres to clothes and can be released at home (indirect contact). Studies have shown that in areas where these children live, there is high sensitization to peach tree pollen, affecting persons both directly and indirectly exposed.⁵ Indeed, this pollen is the most relevant after olive tree and grass, with a prevalence of 20%.

Although little evidence of sensitization to fruit tree pollens has been reported, studies in the United States, India and elsewhere have shown these pollens to be relevant and amongst the most prevalent allergens responsible for allergic manifestations.^{6,7} We describe a small group of 10 children with respiratory symptoms after either direct or indirect contact with peach trees during the period the pollen is in the air (late February to early June). These children were allergic to peach tree pollen and to a recently identified allergen, Pru p 9.⁸

We undertook a detailed clinical history plus skin prick testing (SPT) with inhalant and food allergens: *Phleum pratense*, *Olea europaea*, *Platanus acerifolia*, *Artemisia vulgaris*, *Parietaria judaica*, *Salsola kali*, *Cupressus arizonica*, *Dermatophagoides pteronyssinus*, *Alternaria alternata*, cat and dog dander, apple, banana, peanut, almond, nut, tomato, kiwi, melon, mustard and pineapple (ALK Abello®, Spain).

Pru p 9 was purified from peach tree pollen (Iberpolen®, Spain). Briefly, it was purified from defatted mixture and extracted with PBS buffer (0.1 mM sodium phosphate, pH 7.4, 1.5 M NaCl; 1:5 (w/v);

1 hour at 4°C. The extract was dialyzed against H₂O, freeze-dried and fractionated by anion-exchange chromatography on a Waters AccellTM Plus 7 QMA Sep-PakR cartridge (Waters Corp). Elution was carried out with 20 mM ethanolamine, pH 9.0, and the retained material was then eluted with 0.75 mM NaCl in the same buffer (1 mL/min). Purified Pru p 9 was quantified by means of the commercial bi-cinchoninic acid (BCA) test (Pierce, Cheshire, UK), and purity was measured by SDS-PAGE, mass spectrophotometric analysis with a Biflex III Spectrometer (Bruker-Franzen Analytik) and fingerprinting after tryptic digestion, using standard methods.

Skin prick testing with peach tree pollen, Pru p 9 and Pru p 3 was carried out as reported.⁹ Nasal provocation test (NPT) was performed with peach tree pollen and Pru p 9. Changes in nasal volume were measured by acoustic rhinometry, and the symptom score was calculated as described.¹⁰

Table 1 shows the clinical and allergological data. The oldest case was a 14-year-old boy and the youngest involved two females aged 8 years. All the children, except case 3, had a positive SPT to different pollens and four to other allergens such as pet dandruff and alternaria. Case 3 was only sensitized to peach tree pollen. Four of the cases were SPT positive to peach tree pollen and Pru p 9, with the wheal being bigger with peach tree pollen in cases 2, 3 and 4. We found no association between exposure to peach tree pollen (direct or indirect) and sensitization to Pru p 9. In most subjects, the clinical symptoms started two or more years before this study was undertaken. Rhinitis or rhinoconjunctivitis was referred by all, with one also referring asthma after pollen exposure.

Figure 1 shows the NPT results. In all cases challenged with peach tree pollen, a positive response appeared within 15 minutes, normalizing at 60 minutes, except case 3 in whom it persisted for 24 hours. A tendency to a higher decrease in nasal volume was observed in those positive to both peach tree pollen and Pru p 9 (cases 1-4). Table 2 shows the symptom score: in the left column, the symptoms induced by peach tree pollen and those induced by Pru p 9 in the right. Only one case had cough without any other respiratory symptoms, with no decrease in the FEV₁. Some cases also experienced ocular manifestations. Subjects with negative SPT to Pru p 9 also had negative NPT (cases 5-10). The symptom score was higher in those with larger wheals after SPT.

A recent study in Spain has shown that peach tree is an important inducer of respiratory allergy, including asthma, in subjects working in orchards.⁴ Several allergens have been identified which are present in leaves and small branches, although peach tree pollen was not studied.⁴

[†]Correction added on 09 July 2019, after first online publication on 03 May 2019 : A comment on Table 2 in the 8th paragraph has been updated in this version.

TABLE 1 Clinical characteristics of the study cases. Cases 1-4: subjects sensitized to both peach tree pollen and Pru p 9; cases 5-10: subjects only sensitized to peach tree pollen, cases 11-22: subjects matched by age and gender to cases 1-10, sensitized to different pollen, peach tree pollen but not to Pru p 9

Case	Age	Gender	+SPT pollen	+SPT other allergens	SPT peach tree pollen	SPT Pru p 9	Dx	T	E	Case	Age	Gender	+SPT pollen	+SPT other allergens	SPT peach tree pollen	SPT Pru p 9	Dx	T	E
1	14	M	G, O, S	CD	110	110	R, C	3	D	12	14	M	G, O, S	DD	200	0	R, C	1	D
2	12	F	G, O, C, S	CD, AA	400	300	R, C	1	ID	13	12	F	G, O, S, P	No	100	0	R, C	3	ID
3	13	M	No	No	500	250	R, C	2	ID	14	13	M	G, O, S, P, C	CD	120	0	R, C	3	ID
4	8	F	O, C, S	CD	250	110	R, C, A	3	D	15	8	F	G, O, S, C	No	110	0	R, C	2	ID
5	13	M	G, O, C, S	No	100	0	R	1	ID	16	13	M	G, O	No	200	0	R, C	2	D
6	13	M	G, O, C, P, S	DD	120	0	R, C	2	D	17	13	M	G, O, S	No	400	0	R	1	D
7	13	F	O	No	100	0	R, C	2	ID	18	13	F	G, O, S, P, C	No	200	0	R	2	ID
8	13	M	G, O, C, S	No	110	0	R	1	D	19	13	M	G, O, S	CD	250	0	R, C, A	3	D
9	8	F	O	No	100	0	R, C	2	D	20	8	F	G, O, S	No	500	0	R, C	1	ID
10	12	M	G, O, C, P, S	No	100	0	R, C	7	ID	21	12	M	G, O, S, P	CD, AA	100	0	R, C	4	D
11	12	F	G, O, S	No	120	0	R, C	2	ID	22	14	M	G, O	No	110	0	R, C	6	D

Note: SPT is shown as the percentage of the wheal area of histamine given the histamine value of 100.

Abbreviations: +, positive; A, asthma; AA, A; alternata; C, conjunctivitis; C, cypress; CD, cat dander; D, directly exposed (subjects who visit family orchards); DD, dog dander; Dx, clinical symptoms; E, exposure to peach tree pollen; F, female; G, grass; ID, indirectly exposed (no visits or no family orchards); M, male; O, olive tree; P, plane tree; R, rhinitis; S, *S. kali*; SPT, skin prick test; T, time of evolution in y.

Our cases were SPT positive to more than two pollens as well as other allergens, except in one case, showing that they were atopic.

The SPT in a control group of 12 children sensitized to pollens, matched for age and gender with our cases, was positive to peach tree pollen and negative to Pru p 9, indicating that this is a specific protein of peach tree pollen. However, in our study group, six cases were also positive to peach tree pollen and negative to Pru p 9, suggesting that other allergens are involved (see Table 1).

In our study, all cases were negative to Pru p 3 and subjects had oral tolerance to peach and other fruits as well as peanuts.

The NPTs showed that symptoms could be induced by the pollen extract and Pru p 9 in sensitized persons, confirming the role of this allergen in the induction of symptoms. In these cases, the SPT response was higher with peach tree pollen than with Pru p 9. This is in line with results from a large group of adults sensitized to both peach tree pollen and Pru p 9, in which a poor correlation of wheal sizes exists.

Peach is one of the most important fruits cultivated worldwide, with Spain, China and the United States being the greatest producers.¹¹ Although our study presents a small number of subjects, increasing the sample size will probably result in more cases appearing, as occurs with the adult population.⁵ Similar cases are likely to be detected in other European countries, such as France, Italy and Greece, where peach trees are widely cultivated, as well as in other countries such as the United States and China. We are currently evaluating how far this pollen, or microparticles carrying fragments of pollen, can be aero-transported.

We present here for the first time a group of children sensitized to peach tree pollen who developed symptoms after exposure with almost half of them also being allergic to a new allergen (Pru p 9). This indicates that allergy to peach tree pollen and Pru p 9 must be evaluated in exposed children. The identification of other allergens is currently in progress.

FIGURE 1 Peach tree pollen and Pru p 9 nasal provocation tests (NPT). Cases 1-4: positive NPT with peach tree pollen and positive NPT with Pru p 9. Cases 5-10: positive NPT with peach tree pollen and negative NPT with Pru p 9

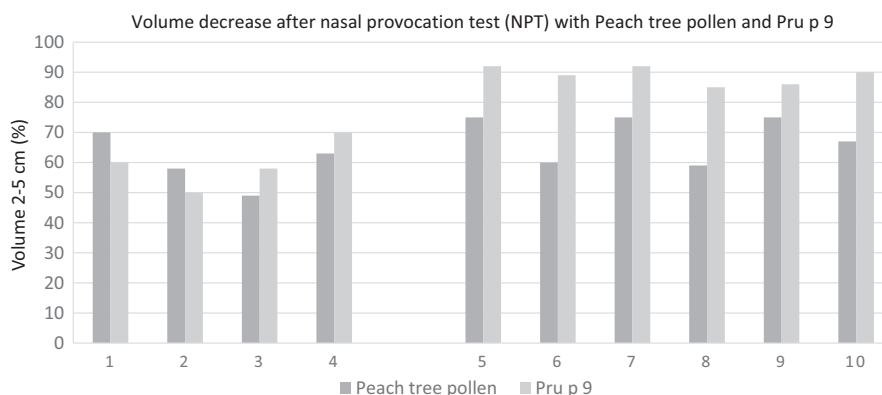


TABLE 2 Symptoms developed after nasal challenge (NPT) with peach tree pollen and Pru p 9

Case	Symptoms of NPT with peach tree pollen	Index Score	Symptoms of NPT with Pru p 9.	Index Score
1	Nasal and ocular itching, rhinorrhoea, sneezing, nasal obstruction.	9	Nasal and ocular itching, rhinorrhoea, sneezing and nasal obstruction.	8
2	Nasal itching with obstruction, followed by rhinorrhoea and itching in the eyes appearing 10 min after challenge. Oxymetazoline administration was required.	11	Nasal itching with rhinorrhoea, sneezing and nasal obstruction followed by eye redness.	10
3	Nasal itching with rhinorrhoea, sneezing and nasal obstruction followed by eye redness persisting for 24 h. Oxymetazoline administration was required.	12	Nasal itching with obstruction, followed by rhinorrhoea and itching in the eyes appearing 15 min after challenge.	9
4	Nasal and ocular itching, rhinorrhoea, sneezing and nasal obstruction.	10	Nasal itching with rhinorrhoea, sneezing and nasal obstruction followed by eye redness.	8
5	Nasal itching, rhinorrhoea, sneezing and nasal obstruction.	9	None.	0
6	Nasal and ocular itching, rhinorrhoea, sneezing, nasal obstruction and cough. Oxymetazoline administration was required.	11	None.	0
7	Nasal itching with rhinorrhoea, sneezing and nasal obstruction followed by eye redness.	8	None.	0
8	Nasal itching, rhinorrhoea, sneezing and nasal obstruction. Oxymetazoline administration was required.	11	None.	0
9	Nasal itching with obstruction, followed by rhinorrhoea and itching in the eyes appearing 15 min after challenge.	9	None.	0
10	Nasal and pharyngeal itching followed by rhinorrhoea, sneezing and obstruction 10 min after challenge.	10	None.	0

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

Maria Luisa Somoza contributed to evaluation of cases, undertook nasal provocation tests, made statistical analysis and elaborated the manuscript. María Garrido-Arandia contributed to allergen extract preparation, purification of allergens and manuscript elaboration. Laura Victorio and José Damián López-Sánchez involved in recruitment of cases, evaluation of cases, sample processing and elaboration of manuscript. Natalia Blanca-López designed the manuscript and involved in the critical discussion. Elisa Haroun-Díaz designed the manuscript, selected the negative controls and made the critical discussion. Alejandro Romero Sahagún involved in purification and sequencing of the allergens and in the critical discussion. Araceli Díaz-Perales involved in allergen extract preparation, purification of allergens and manuscript elaboration. Gabriela Canto contributed to elaboration of manuscript, selection of negative cases and critical discussion. Miguel Blanca involved in patients' recruitment and evaluation,

skin testing molecular allergens, data management and elaboration of manuscript.

FUNDING INFORMATION

This study was supported by grants FIS PI17/00615, RIRAAF RD12/0013/0005 and ARADyAL RD16/0006/0024.

ETHICAL APPROVAL

The study was approved by our institutional Ethics Committee.

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DOI: 10.1111/pai.13068

Antenatal stress and illness in mothers of children with food allergy

To the Editor,

Allergic diseases are common and increasing, and food allergy affects 3%-6% of children. A better understanding of the developmental origins of atopic disease is essential to guide effective prevention.

Anxiety and depression are common psychological morbidities in pregnancy. Antenatal psychological stress affects over 75% of urban women.¹ Pregnancy is a particularly vulnerable time, due to profound hormonal and physiological changes. Antenatal stress is linked to the development of atopic conditions, primarily through activation of the maternal hypothalamic-pituitary-adrenal (HPA) axis, with increased production of glucocorticoids. Cortisol crosses the placenta and leads to long-term changes in the foetus' own HPA axis.² This alters gene expression and skews the developing immune system's Th1/Th2 balance. Stress is also associated with aberrant infant gut colonization, impairing establishment of a healthy neonatal microbiome.³

Maternal stress in pregnancy is associated with childhood wheeze and asthma (OR: 1.56; 95% CI: 1.36-1.80).⁴ There is a paucity of evidence exploring the association of antenatal stress with food allergy. We aimed to test the hypothesis that mothers of food-allergic children have more stressful pregnancies (as measured by

a higher number of stressful life events or higher perceived stress score) than mothers of children who do not develop food allergy. Secondary objectives included establishing correlations between specific stressful events during pregnancy and food allergy in the offspring.

We recruited 32 mothers of children with food allergy (cases) and 40 mothers of children without food allergy (controls). The children were all under 6 years of age and attending specialist paediatric allergy clinics or general paediatric clinics in south-east London. The mothers were sent a questionnaire by post, prior to their clinic appointment, and also interviewed in clinic. As this was a pilot study, no formal power calculations were undertaken. Statistical analysis was carried out in SPSS version 24 (IBM). Research Ethics Committee approval was obtained (16/WM/0090).

Children in the food allergy group were slightly older than controls, mean age 2.9 years vs 2.0 years ($P = 0.019$) (Table 1). There was a higher incidence of emergency lower segment Caesarean section (LSCS) in the food allergy group (25% vs 7.5% in the control group) ($P = 0.048$). Children with food allergy had a higher incidence of eczema (75% vs 35% of controls, $P = 0.002$) and a positive family history of atopy (90.6% vs 62.5% of controls, $P = 0.006$).