

Short communication

Platanus acerifolia pollinosis and food allergy

Background: In Mediterranean areas, oral allergy syndrome (OAS) occurs independently of an associated birch pollinosis; moreover, on occasions it presents with no other associated pollinosis. The aim of this study was to assess the possible association of OAS with *Platanus acerifolia* pollinosis.

Methods: We evaluated consecutive patients seen for pollinosis in an allergy department. Seven hundred and twenty patients were selected on the basis of seasonal or perennial rhinitis, or asthma, or both. Respiratory and food allergies were studied in all patients. Clinical history was recorded and examinations and skin prick tests were performed with a battery of available common inhalant allergens and plant-derived food allergens. Specific IgE levels to *P. acerifolia* pollen extract and food allergens tested were measured. Molecular masses of the IgE-binding proteins and cross-reactivity among the *P. acerifolia* pollen and different food extracts were also determined.

Results: Of the 720 patients evaluated, 61 (8.48%) were sensitized to *P. acerifolia* pollen. Food allergy was observed in 32 (52.45%) of the 61 patients sensitized to *P. acerifolia* pollen. Food allergens most frequently implicated were hazelnuts, peach, apple, peanuts, maize, chickpea and lettuce. Enzyme allergosorbent (EAST)-inhibition showed high inhibition values when *P. acerifolia* pollen extract was used as free phase. On the contrary low inhibition was observed when plant-derived food allergens were used as free phase and *P. acerifolia* pollen extract as solid phase.

Conclusions: Cross-reactivity was observed among *P. acerifolia* pollen and plant-derived foods. OAS in these patients may have been caused by primary respiratory sensitization.

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Key words: oral allergy syndrome; plant-derived food allergy; *Platanus acerifolia* pollinosis.

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The association of pollinosis with allergic reactions to plant-derived foods has been defined as 'oral allergy syndrome' (OAS) (1). Patients with OAS exhibit a broad spectrum of symptoms related to direct contact of the plant food with oral mucosa, including oral itching, lip swelling, labial angioedema, glottis edema and, occasionally, systemic reactions (2). This association has been described implicating mainly birch, ragweed, mugwort and grass pollens (3–5).

The sensitizing allergen source in OAS is controversial, but most of the cross-reactive structures are expressed higher in pollen than in plant-derived food tissues. In north and central Europe birch pollen uptake through the respiratory system could induce, maintain and boost the production of cross-reactive IgE antibodies, which are responsible for plant food allergy in patients with OAS (6). However, in areas without birch trees, such as Mediterranean countries, allergy to these plant-derived foods is not always associated with pollinosis and is predominantly elicited by lipid transfer proteins (LTP), suggesting that sensitization may occur through the oral route (2, 7, 8).

P. acerifolia is a hybrid tree of *Platanus occidentalis* and *Platanus orientalis*. It is one of the trees most frequently used in most western European cities as street and shade trees, and its pollen is one of those most frequently found in the atmosphere over their cities, such as Barcelona, Spain (9).

The aim of the present study was to assess the prevalence of *P. acerifolia* pollinosis in our city and to check the role of this pollen in OAS syndrome in our region.

Material and methods

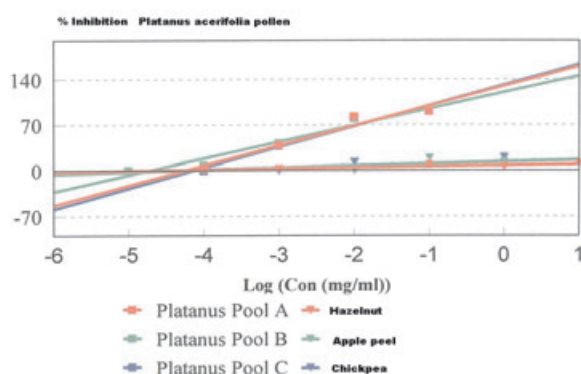
Patients

The study population was selected from 720 consecutive outpatients referred because of pollinosis to the Allergy Department of the Institut Universitari Dexeus in Barcelona.

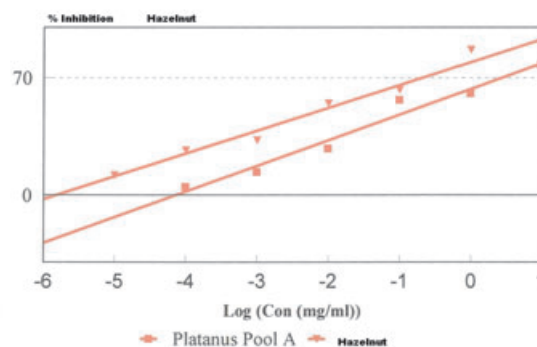
Skin prick test

Skin prick tests (SPT) were performed according to standard procedure with a panel of inhalant allergens containing the main allergens prevalent in our environment: mites, molds, cockroach, cat

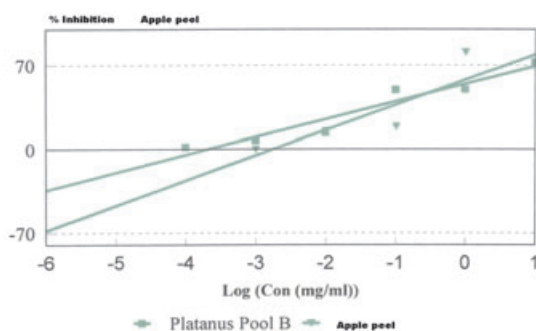
Platanus acerifolia



Hazelnut



Apple peel



Chickpea

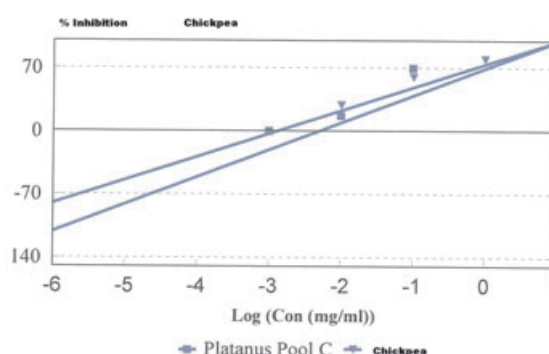


Figure 1A. EAST-inhibition assays: *Platanus acerifolia*: *P. acerifolia* pollen extract is used in solid phase. (Pool A: pool of serum samples from hazelnut-allergic patients. Pool B: pool of serum samples from apple peel-allergic patients. Pool C: pool of serum samples from chickpea-allergic patients). Hazelnut: hazelnut extract is used in solid phase. Apple peel: apple peel extract is used in solid phase. Chickpea: chickpea extract is used in solid phase.

and dog latex and pollens such as grass, *Parietaria judaica*, *Artemisia vulgaris*, *Plantago lanceolata*, *Chenopodium album*, *Mercurialis annua*, *Olea europea*, *Cupressus arizonica*, *Corylus avellana*, *Pinus* spp., and *P. acerifolia*. SPT with the vegetables suspected of causing allergy according to clinical history were performed with commercially available extracts (Leti, Barcelona, Spain) and by the prick-prick method. Histamine dihydrochloride 10 mg/ml and phosphate-buffered saline (PBS) solution served as positive and negative controls, respectively. The SPT was considered positive if the wheal area was greater than 7 mm² (diameter > 3 mm) 15 min after testing.

Specific IgE

The specific IgE was measured using an enzyme allergosorbent test (EAST) with the allergens coupled to BrCN-activated paper discs (0.4 mg/disc) (Bial-Aristegui); values equal to or greater than 0.35 PRU/ml were considered positive.

SDS-PAGE and SDS-PAGE immunoblotting

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the discontinuous method described by Laemmli in 12.5% polyacrylamide gel, with parallel application of a reference standard of proteins with known molecular masses. 20 µg protein, according to Bradford were applied per lane.

After electrophoresis, gels were stained in 0.1% Coomassie Brilliant Blue R-250 dissolved in methanol/acetic/distilled water (4:1:5). To prepare the samples under reducing conditions, they were dissolved in 0.125 M HCl-Tris buffer, pH 6.8, and were dissociated with SDS and 5% β-mercaptoethanol at 100°C for 5 min. For non-reducing conditions the β-mercaptoethanol was omitted in the sample buffer.

Separated protein bands were electrophoretically transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Milford, MA). After the Immobilon membrane was blocked with PBS-Tween-20 0.05% (v/v) (1 h at 25°C), immunodetection was performed using a chemiluminescent detection reagent (Lumigen PS-3) following the manufacturer's instructions (ECL+ Plus Western blotting detection reagents. Amersham Pharmacia Biotech, Amersham, UK).

IgE inhibition experiments

The cross-reactivity between *P. acerifolia* pollen extract and plant-derived food allergens was studied by quantitative measurements of EAST inhibitions. Plant-derived food allergens from different sources such as hazelnut, apple peel, chickpea, lettuce, peanut and maize were used. These experiments were performed with those allergens coupled to BrCN-activated paper discs (0.4 mg/disc) (Bial-Aristegui). Heterologous and homologous extracts were added as inhibitor agents in the liquid phase. Different pools of sera were used

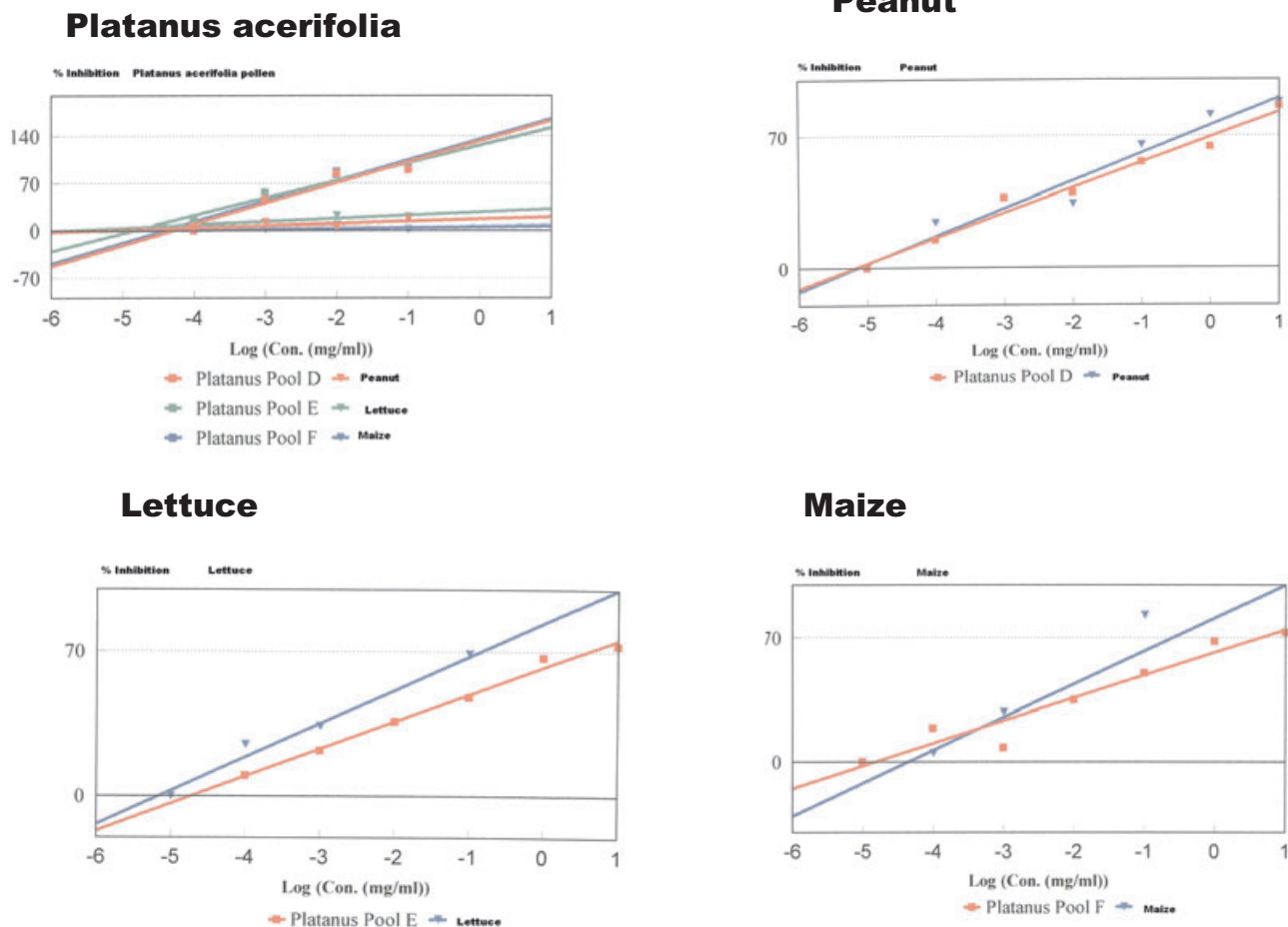


Figure 1B EAST-inhibition assays: *Platanus acerifolia*: *P. acerifolia* pollen extract is used in solid phase. (Pool D: pool of serum samples from peanut allergic patients. Pool E: pool of serum samples from lettuce allergic patients. Pool F: pool of serum samples from maize allergic patients). Peanut: peanut extract is used in solid phase. Lettuce: lettuce extract is used in solid phase. Maize: maize extract is used in solid phase..

for each of the EAST-inhibition assays, but the patient serum selected always had a specific IgE value higher than 0.70 PRU/ml to the food extract studied. In this way 14 serum samples were pooled for hazelnut experiments, 14 for apple peel, 14 for chickpea, 11 for peanut, nine for lettuce, and nine for maize.

Results

Evaluation of patient data

Of 720 patients evaluated, 61 (8.48%) were sensitized to *P. acerifolia* pollen. Rhinitis and rhinoconjunctivitis were the most frequent symptoms and only 22% reported asthma. Almost 25% of these patients were monosensitized to *P. acerifolia* pollen. Food allergy was observed in 32 (52.45%) of the 61 patients sensitized to *P. acerifolia* pollen. Related symptoms were anaphylaxis in 34%, oral pruritus in 35% and urticaria-angioedema in 19%; the remaining symptoms were nonspecific as gastrointestinal disorders (Table 1).

Food allergens most frequently implicated were

hazelnuts, fruits (peach, apple, melon and kiwi), peanuts, maize, chickpea, and vegetables such as lettuce and green beans (Table 1).

Detection of cross-reacting allergens in *Platanus* pollen and food by EAST-inhibition

In EAST-inhibition assays 90% inhibition was obtained when extracts of food as apple, chickpea, lettuce, corn, peanut or hazelnut were used in solid phase and *P. acerifolia* pollen extract was used as inhibitor phase whereas in the inverse situation (in the other way round) only a 20% inhibition was obtained (Fig. 1A, 1B).

Identification of allergens by immunoblotting

When *P. acerifolia* pollen extract was studied by SDS-PAGE immunoblotting, different IgE-binding proteins with molecular masses ranging from 14 to 70 kDa could be detected (Fig. 2). A different pattern of IgE-binding proteins was observed when the sample was incubated with two different pools of sera: a pool of plant-derived

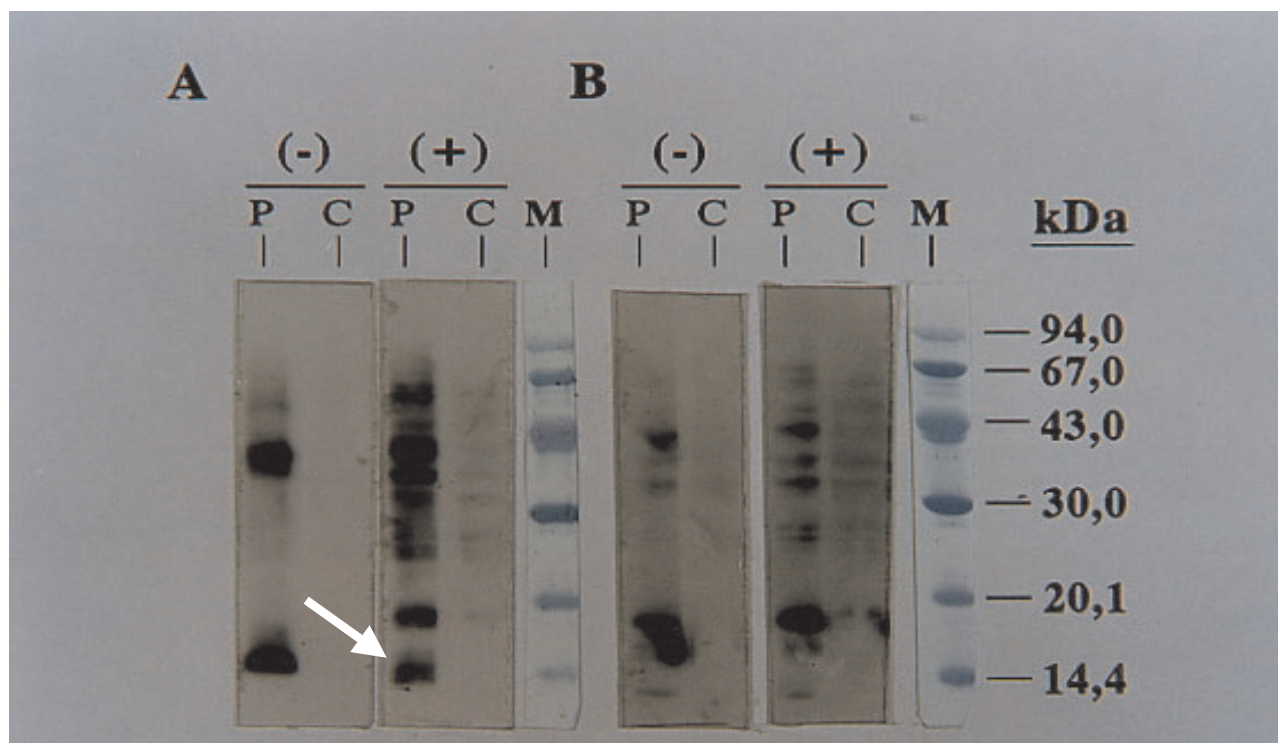


Figure 2. SDS-PAGE immunoblotting of *P. acerifolia* pollen extract. (A): Pool of food allergy patients' sera. (B): Pool of nonfood allergy patients' sera. Lane P: pool of patients' sera; Lane C: pool of nonatopic subjects' sera. (–) sample without β -mercaptoethanol (under non-reducing conditions); (+) sample with β -mercaptoethanol (under reducing conditions).

food allergy patients' sera and a pool of nonfood allergy patients' sera. An intense IgE-binding band with a molecular mass of 14–15 kDa was detected only when the extract was incubated with the food allergy patients' pool (marked with an arrow in Fig. 2).

Discussion

In Barcelona, approximately 8% of patients with pollinosis are sensitized to *P. acerifolia* pollen, two-fold the prevalence found by Valero (9); however, no great differences were observed in the clinical behavior of *P. acerifolia* pollinosis and the pattern of IgE-binding proteins on immunoblotting (10). Nevertheless, we found a higher percentage of patients (almost 55%) sensitized to *P. acerifolia* pollen with plant-derived food allergy. This clinical association and the results of EAST-inhibition studies suggest the existence of cross-sensitivity between *P. acerifolia* pollen and plant food allergens and that OAS as well as more severe symptoms of food allergy in these patients may have been caused by primary respiratory sensitization to *P. acerifolia* pollen.

Birch pollen-associated food allergy is a well known clinical phenomenon in central and northern Europe. A high percentage of patients allergic to birch pollen have OAS to plant-derived foods and, less frequently,

experience systemic reactions such as anaphylaxis. By contrast, in the Mediterranean area, OAS occurs independently of an associated birch pollinosis (8); moreover, it often occurs without associated pollinosis (7). In both cases, in Mediterranean countries it seems that these plant-derived food-allergic patients are sensitized to a cross-reactive LTP, and the fact that these proteins are highly resistant to digestive enzymes indicates a potential primary sensitization to LTP through the digestive route and systemic reactions may be elicited (7, 8).

P. acerifolia pollen is one of the main allergens in many towns in the Mediterranean area. Air concentrations of this allergenic pollen peak in early spring (9). In this study, a strong association was observed between *P. acerifolia* pollinosis and food allergy and a great number of systemic reactions occurred. However, the respiratory route seems to be the first sensitization route in these patients, as indicated by the fact that IgE binding to food extracts was inhibited by *P. acerifolia* pollen extract, but not vice versa, demonstrating a higher affinity of the cross-reacting IgE to pollen allergens.

The 14 kDa allergen identified in *P. acerifolia* pollen extract only by IgE antibodies from pool of food allergy patients' sera could be the reason of such cross-reactivity observed. Profilin have a molecular weight

Table 1. Clinical characterization of food allergy patients

Patient			Allergies			Symptoms	
No.	ID	Age (years)	Pollen	Food	Other	Pollen	Food
1	PSL	42	t,g,w,mu	N,K,Ce		RC	A
2	TNA	28	t,w,mu	N,P,Ce,M,V,L	ct	RC	U
3	NCV	25	t,g,w	N,V	ct	R	A
4	JSV	38	t,g,w,mu	M,MI		RC	OP
5	AVS	37	g,m	Pe		R	AE
6	CGV	32	t,g,w,mu	N,Pe,MI		R,As	OP
7	NJF	26		N	mi	R,As	OP
8	AFC	33	t,g,w,mu	N,Ce,P,M,V,L,Ro		RC,As	A
9	PSR	43		MI		RC,As	OP
10	MMG	29		N,P,V,Ro,MI		RC,As	A
11	XVX	24	t,w,mu	N,P,K	mi,ct,d	RC	AE
12	FGT	26		N,P	mi	RC	A
13	GGG	36		N		RC	OP
14	FFH	32		Pe,MI,K		RC	OP
15	FAC	30		N,Pe,MI,Ro		RC	AE
16	JMD	29	t,w	Pe		RC	U
17	RPR	36		MI,K		RC,As	OP
18	AMA	27	w,mu	N		RC	A
19	CGT	32	mu	N,P,M,V	ct	RC	A
20	EDP	24		MI,Ro	mi	RC	OP
21	JMG	25	t,g,w,mu	N,P,M,L	mi,ct,d	RC,As	U
22	GVC	24	w,mu	N,P,M,Ce,V,K	mi,ct	R	AE
23	LPC	33		K	mo	RC	OP
24	RGG	33	t	N,Ce,K	mi	R,As	A
25	CSL	28	t,g,w	N,P,Ce,M,V	mi	R	AE
26	ACJ	43	t,g,w,mu	N	ct,d	R,As	OP
27	SGR	32	w,mu	N	mi	R	A
28	MMM	20	t,g	N,Pe		RC	A
29	BAS	37	t,g,w,mu	Pe,Ro,L	mi	RC	OP
30	MCHM	36	t,g,w,mu	N,P,M,Pe,Ro		RC,As	A
31	JMO	63	t	K,B		RC	A
32	AAC	38	t,g,w,mu	Pe,MI	mi	RC	OP

Pollen allergies: t, trees; g, grass; w, weed; mu, mugwort. Food allergies: N, nuts; K, kiwi; Ce, cereals; P, peach; M, maize, V, vegetables; L, legumes; MI, melon; Pe, peanut; Ro, Rosaceae fruits; B, banana. Other allergies: ct, cat dander; mi, mites; d, dog dander; mo, mold. Pollen symptoms associated with *P. acerifolia*: RC, rhinoconjunctivitis; R, rhinitis; As, asthma. Food symptoms: A, anaphylaxis; U, urticaria; OP, oral pruritus; AE, angioedema.

of approximately 14 KDa and are known to be a cross-reacting allergen between some pollens and many plant-derived foods (11). However, they have rarely been described as a cause of anaphylaxis.

In conclusion, our data show that *Platanus acerifolia* is an important source of pollinosis in our environment,

and cross-reactivity with plant-derived food allergens has been found in a high percentage of patients with this pollinosis. Further studies are required to identify the cross-reacting structures and their importance for eliciting food allergy in patients allergic to *P. acerifolia* pollen.

References

- JOHANSSON SGO, O'B HOURIHANE J, BOUSQUET J, BRUIJNZEEL-KOOMEN C, DEBRORG S, HAAHTELA T, KOWALSKI ML, MYGIND N, RING J, VAN CAUWENBERGE P, VAN HAGE-HAMSTEN M, WÜTHRICH B. Position paper. A revised nomenclature for Allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001;**56**:813–824.
- ORTOLANI C, ISPANO M, PASTORELLO E, BIGI A, ANSALONI R. The oral allergy syndrome. *Ann Allergy* 1988;**61**:47–52.
- LAHTI A, BJÖRKSTEN F, HANNUKSELA M. Allergy to birch pollen and apple, and cross-reactivity of the allergens studied with the RAST. *Allergy* 1980;**35**:297–300.
- ANDERSON LB, DREYFUS E, LOGAN J, JOHNSTONE D, GLASER J. Melon and banana sensitivity coincident with ragweed pollinosis. *J Allergy* 1970;**45**:310–319.
- VALLIER P, ECHAMP C, VIAL O, DEVILLER P. A study of allergens in celery with cross-sensitivity to mugwort and birch pollens. *Clin Allergy* 1988;**18**:491–500.

6. KAZEMI-SHIRAZI L, PAULI G, PUROHIT A, SPITZAUER S, FRÖSCHL R, HOFFMANN-SOMMERGRUBER K, et al. Quantitative IgE inhibition experiments with purified recombinant allergens indicate pollen-derived allergens as the sensitizing agents responsible for many forms of plant food allergy. *J Allergy Clin Immunol* 1999;**105**:116–125.
7. FERNÁNDEZ-RIVAS M, VAN REE R, CUEVAS M. Allergy to *Rosaceae* fruits without related pollinosis. *J Allergy Clin Immunol* 1997;**100**:728–733.
8. SCHEURER S, PASTORELLO, E, WANGORSCH KÄSTNER M, HAUSTEIN D, VIETHS S. Recombinant allergens Pru av 1 and Pru av 4 and a newly identified lipid transfer protein in the in vitro diagnosis of cherry allergy. *J Allergy Clin Immunol* 2001;**107**:724–731.
9. VALERO AL, ROSELL E, AMAT J, SANCHO J, ROIG J, PIULATS J, et al. Hipersensibilidad a polen de *Platanus acerifolia*: detección de las fracciones alergénicas. *Alergol Inmunol Clin* 1999;**14**:220–226.
10. ASTURIAS JA, IBARROLA I, OJEDA I, MALET A, MARTÍNEZ A. Purification and characterization of Pla a 1, a major allergen of *Platanus acerifolia* pollen (Abstract). *Allergy* 2001;**56**:273.
11. VALENTA R, DUCHÊNE M, EBNER C, VALENT P, SILLABER C, DEVILLER P, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;**175**:377–385.