

Birch pollinosis and atopy caused by apple, peach, and hazelnut; comparison of three extraction procedures with two apple strains

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This study aimed, first, to study the prevalence in The Netherlands of atopy caused by apple, peach, and hazelnut in patients with tree pollinosis, and, second, to compare three extraction procedures for skin prick testing with two different apple strains. Skin prick tests and RAST were performed on 79 consecutive patients with tree pollinosis, visiting the department of allergology during spring 1995. In skin prick tests, we used three different extracts (juice, freeze-dried extract, and low-temperature acetone powder extract) of two apple strains, Golden Delicious and Granny Smith. Case histories for apple, peach, and hazelnut were positive in 35 (44.3%), 23 (29%), and 35 (44.3%) patients, respectively. More than two-thirds of the patients had symptoms characteristic of oral allergy syndrome. Skin prick tests for apple, peach, and hazelnut were positive in 51 (64.6%), 61 (77.2%), and 71 (89.9%) patients, respectively. Granny Smith showed more positive skin reactions and a better agreement with clinical history than Golden Delicious, and juice was superior to the two other extraction procedures for both apple strains. RAST for apple, peach, and hazelnut was positive in 53 (68.8%), 13 (16.9%), and 31 (40.3%) patients, respectively. Concordance between skin prick test and case history was found in 77%, 52%, and 54%, for apple, peach, and hazelnut, respectively. We found a high percentage of concurrence of clinical allergy to birch pollen and apple, peach, and hazelnut, confirmed by both skin prick testing and RAST. Approximately half of these patients had symptoms (especially oral allergy syndrome) after eating these products. We also found an easy extraction procedure (juice extract) suitable for apple skin prick testing, superior even to freeze-dried extraction or the low-temperature acetone powder technique.

Key words: apple; birch pollen; cross-reactive IgE; hazelnut; peach; RAST; skin prick test.

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Cross-reactivity between inhalant allergens and food allergens has been known for several years; for instance, between ragweed pollen and banana/melon (1); mugwort pollen and spices/celery/carrot (2–4); latex and kiwi/avocado/banana/chestnut (5); caddis fly and crab/shellfish (6); and the so-called bird-egg syndrome (7, 8). The oral allergy syndrome after consumption of fruits of the Rosaceae family (e.g., apple, peach) or nuts in patients suffering from birch pollinosis was described as early as 1948 by Juhlin-Dannfeldt (9). Cross-reactive IgE, the cause of these symptoms, can be directed against the major birch pollen allergen, Bet v 1, also present in fruits and nuts; against profilin, a

ubiquitous and highly conserved protein in pollen and vegetable foods; and against carbohydrate epitopes on glycoproteins (reviewed by van Ree & Aalberse [10]).

In this study we investigated first the prevalence of atopy caused by apple, peach, and hazelnut in a large group of patients with tree pollinosis in The Netherlands. For this purpose, consecutive patients with tree pollinosis were asked about symptoms after eating fruits and nuts; subsequently, food skin prick tests and RAST were performed. The second aim of our study was to compare three extraction procedures with two different apple strains in skin prick testing.

Material and methods

Subjects

Patient group. From February until July 1995, 79 consecutive patients of the Department of Allergy (University Hospital Dijkzigt, Rotterdam, The Netherlands), suffering from tree pollinosis were enrolled in the study. Patients had a positive case history, i.e., seasonal rhinoconjunctivitis, sometimes associated with bronchial asthma, confirmed by a clearly positive intracutaneous test with birch pollen and/or alder pollen. Of these 79 patients, 59 were female and 20 male; their mean age was 33.4 years (range 15–53 years).

Control group. Eight nonatopic volunteers (seven women and one man; mean age 34.0 years; range 26–45 years) were skin tested to detect irritative, nonspecific reactions. They had a negative history of birch pollinosis or food allergy, confirmed by negative skin test with tree pollen extract.

Allergens

Granny Smith and Golden Delicious apples, hazelnuts, and peaches were purchased at a local food store. Apple extracts were prepared from small pieces of ripe apples with peel, but without core, by three different methods.

Extract 1 (E1). Pieces of apple were homogenized in a food processor, the slurry was filtered, and the fluid was subsequently passed through a 0.22- μ m Millex GS filter (Millipore, The Netherlands).

Extract 2 (E2). Pieces of apple were lyophilized overnight at -70°C . After mechanical homogenization, a 10% (w/v) extract was prepared in phosphate-buffered saline (PBS), pH 7.4, containing 0.03% human serum albumin (HSA) and 0.5% phenol. After centrifugation for 10 min at 2000 g, the supernatant was passed through a 0.22- μ m filter.

Extract 3 (E3). The modified low-temperature acetone powder method, as described by Vieths et al. (11), was used. Apple was homogenized in acetone/dry ice at -60°C , and the slurry was equilibrated overnight at -25°C . After removal of the acetone fraction, the precipitate was freeze-dried overnight at -70°C and extracted in 0.01 M PBS, pH 7.4. After centrifugation (30 min, at 20000 g, 0°C) and dialysis against distilled water, the extract was lyophilized overnight at -70°C . Subsequently, a 1% extract (w/v) was prepared in distilled water.

Peach extract was prepared from small pieces of

ripe peaches without peel or core. The pulp was homogenized in a food processor, the slurry was filtered, and the fluid was subsequently passed through a 0.22- μ m filter.

Hazelnuts were homogenized mechanically, ground with a mortar, defatted by ether extraction, and subsequently air-dried. A 10% (w/v) extract in PBS was centrifuged for 10 min at 2000 g, and the supernatant was passed through a 0.22- μ m filter.

All the extracts were stored in appropriate aliquots at -20°C until use in skin tests. Extracts were defrosted for 1 h before use in the skin test and centrifuged for 45 s at 3200 g. Protein concentrations, determined by the method of Watanabe et al. (12), using pyrogallol red molybdate complex, are shown in Table 1.

Skin test

Skin tests were performed intracutaneously with 0.02 ml of 10-fold dilutions of birch pollen extract and alder pollen extract (ALK Benelux, Groningen, The Netherlands). Dilutions were made in phosphate buffer containing 0.3% HSA and 0.5% phenol. After 20 min, wheal-and-flare reactions were measured and graded by the system of standardized plus signs devised by Norman (13). Skin prick tests (SPT) were performed with apple, peach, and hazelnut by application of one drop of allergenic extract to the skin of the volar side of the forearm. Subsequently, the dermis was punctured with a standardized skin test needle, and results were read after 20 min. Reactions were expressed as mean wheal diameter (mm) (adding the longest diameter to the orthogonal diameter measured at one-half the longest diameter and dividing by two). A reaction of 3 mm or more was considered positive. Dilution buffer was used as negative control, and histamine chloride 10 mg/ml as positive control. All skin tests were performed by the same skilled nurses.

RAST

For routine RAST with apple, peach, and hazelnut, the Pharmacia Cap RIA (with allergens f49 [*Malus sylvestris*], f95 [*Prunus persica*], and f17 [*Corylus avellana*], respectively) was used according to the manufacturer's instructions.

Statistics

The results of different skin prick tests were analyzed by calculating the Spearman's rank correlation coefficient (r) with the SPSS statistical package. A P value of 0.05 or less was considered statistically significant.

Table 1. Protein concentrations (g/l), determined by method of Watanabe et al. (12)

Allergen	Extract	Concentration (g/l)
Golden Delicious	E1 (juice)	1.07
Golden Delicious	E2 (freeze-dried)	0.24
Golden Delicious	E3 (acetone-powder)	0.21
Granny Smith	E1 (juice)	0.83
Granny Smith	E2 (freeze-dried)	0.56
Granny Smith	E3 (acetone-powder)	0.12
Hazelnut	10% (w/v)	28.90
Peach	Juice	0.80

Table 2. Case histories (number of patients with symptoms) in 79 consecutive patients with tree pollinosis

Symptom	Apple	Peach	Hazelnut
Oral allergy	24	18	27
Urticaria/angioedema	11	6	6
Dyspnea/wheezing	5	3	7
Gastrointestinal tract	2	0	1
Eczema	3	0	1
Rhinitis	1	0	0
Total number of patients with signs	35	23	35

Results

Clinical history

Symptoms after eating fruits and nuts for all consecutive patients with tree pollinosis are shown in Table 2. Eating apple and hazelnut caused symptoms in 44%, and eating fresh peach in 29%, of the patients. Most symptoms were typical of oral allergy syndrome.

Skin test and RAST

Comparisons between different apple extracts in skin prick tests are shown in Figs. 1A–F. Prick tests with juice extract of Granny Smith compared with peach and hazelnut are shown in Figs. 2A and B.

Percentages of positive skin prick tests and RAST are shown in Table 3. Juice extract of Granny Smith apple was positive in 65% of the patients, comparable with the Pharmacia CAP-RAST. For the other apple extraction procedures, lower percentages of positive skin prick test were found. Prick test with hazelnut and peach extract was far more often positive than RAST. Concordance between clinical history, skin prick test, and RAST is summarized in Table 4. The best concordance between skin prick test and RAST or case history was achieved with juice extract of Granny Smith. For hazelnut and peach extract, we found a high percentage of positive skin prick tests, with negative RAST and case history.

Discussion

We investigated the prevalence of atopy caused by apple, peach, and hazelnut in a large group of patients with tree pollinosis in The Netherlands. Case histories for apple, peach, and hazelnut atopy were positive in 44.3%, 29%, and 44.3% of the patients, respectively. More than two-thirds of the patients had symptoms characteristic of oral allergy syndrome. Neither respiratory tract symptoms nor eczema was reported frequently. No case of

anaphylaxis was mentioned. The prevalence of cross-sensitization between tree pollinosis and fruits and nuts has been studied previously (14–18). With regard to apple, peaches, and hazelnut, positive case histories have been found in 47–78%, 34–46%, and 53–79%, respectively, of tree-pollen-allergic patients. The differences between the frequencies can be explained by differences in study population – children (14, 18) as opposed to young adults, in our study – by the severity of tree pollinosis and the high level of exposure to tree pollen in Scandinavian countries (14, 16–18), and, finally, by inclusion criteria: e.g., in our study, consecutive patients with tree pollinosis as opposed to patients undergoing immunotherapy with tree pollen extract (18).

We found a much higher percentage of positive reactions to apple, peach, and hazelnut in RAST and more pronounced reactions in skin prick tests than might be expected from the case histories. This discrepancy could not be explained by nonspecific irritative reactions, as skin test results were negative in nonatopic controls. The positive skin prick tests may have been caused by a low titer and/or a lower affinity of cross-reactive IgE, originally directed against the Bet v 1 epitope of birch pollen, or by the presence of cross-reactive IgE directed against Bet v 2 (profilin) and/or carbohydrate epitopes on glycoproteins (10). These antibodies are demonstrated by skin prick test, but possibly have no clinical relevance in most birch-pollen-allergic patients. Finally, mild symptoms after eating fruits or nuts may be ignored by the patient. A double-blind, placebo-controlled oral provocation test as a standard test of this hypothesis was not included in the routine investigations. Moreover, these challenge tests are not suitable, as oral contact with a high concentration of fresh food allergens is highly conducive to development of symptoms of oral allergy syndrome (19).

The second aim of our study was to compare three extraction procedures with two different apple strains in skin prick testing. The preparation of stable and reproducible allergen extracts from

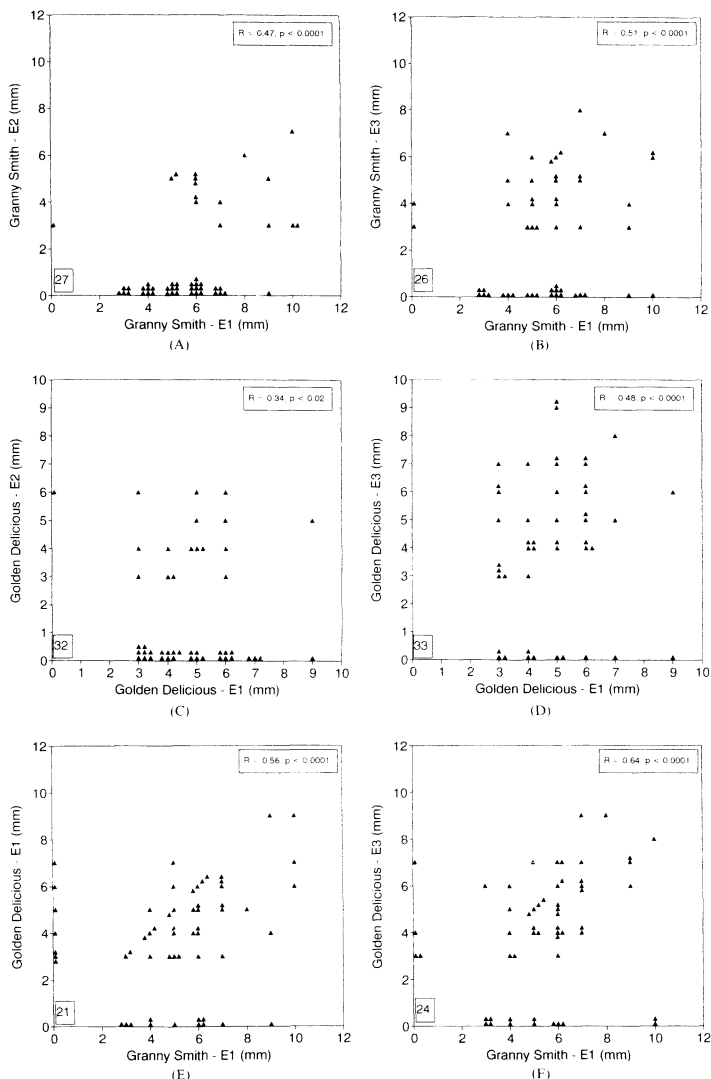


Fig. 1A-F. Comparison between different apple extracts in skin prick tests, expressed in mean wheal diameter (mm). Box shows number of patients with negative skin prick test results for both extracts. E1 = juice extract; E2 = freeze-dried extract; E3 = low-temperature acetone powder extract.

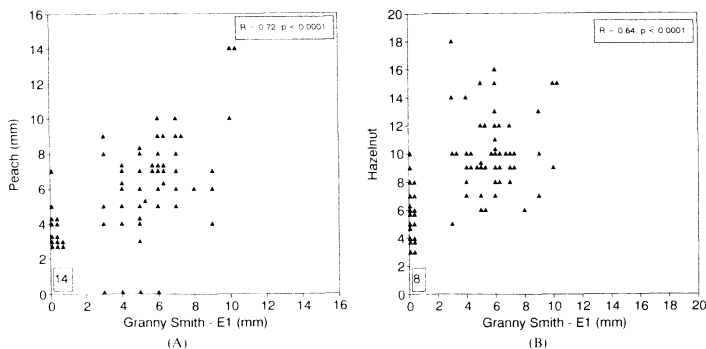


Fig. 2 Comparison of Granny Smith juice extract with peach extract (A) and with hazelnut extract (B) in skin prick tests, expressed in mean wheal diameter (mm). Box shows number of patients with negative skin prick test results for both extracts.

Table 3. Percentage of positive skin prick tests and RAST in 79 consecutive patients with tree pollinosis

Allergen extract	SPT	RAST
Golden Delicious E1	58.2	—
Golden Delicious E2	21.5	—
Golden Delicious E3	53.2	—
Granny Smith E1	64.6	—
Granny Smith E2	20.3	—
Granny Smith E3	36.7	—
Green apple	—	68.8
Peach	77.2	16.9
Hazelnut	89.9	40.3

apple fruit tissue is difficult. Enzymatically oxidized plant phenols interact with the apple allergen, leading to rapid denaturation of the proteins. Consequently, the reliability of skin prick tests with commercial preparations is very poor (14, 20–24). Using fresh fruit for skin testing is better, because deterioration of allergenic material is negligible (21). Dreborg & Foucard investigated the various skin test techniques using fresh fruit (14). A simple prick test technique using a lancet to pierce apple peel just before pricking the skin was shown to be the most practical and reproducible method (prick-prick). However, using fresh fruit is difficult in daily practice; furthermore, allergen potency is not constant in different apple strains and maturation stages (24).

There are several methods to overcome the loss of allergen activity due to phenol contamination during the extraction procedure. The first is accomplished by shortening the extraction procedure and subsequent lyophilization of the apple extract (20). The second is accomplished by adding enzyme

Table 4. Concordance (shown in percentage) between skin prick test, RAST, and case history for apple, hazelnut, and peach in 79 consecutive patients with tree pollinosis

	SPT	RAST	Allergen
RAST	73	—	GS – juice
CH	77	66	
RAST	62	—	GS – acetone
CH	70	66	
RAST	61	—	GD – juice
CH	66	66	
RAST	71	—	GD – acetone
CH	71	66	
RAST	48	—	Hazelnut
CH	54	71	
RAST	34	—	Peach
CH	52	71	

SPT: skin prick test; CH: case history; GS: Granny Smith; GD: Golden Delicious.

inhibitors to the extraction medium, as described by Björkstén et al. (25). Preparing extracts in the presence of polyvinylpyrrolidone and chelators resulted in allergen extracts that remained stable for at least 1 month when stored at -20°C . Thirdly, by the low-temperature acetone powder technique, as investigated extensively by Vieths et al., interaction of plant phenols with proteins can be avoided without addition of potentially irritant chemicals (11). Recently, Rudenschko et al. reported a fourth, technically easier extraction procedure for apple (26). Extracts prepared by precipitation in organic solvents at -20°C retained high allergen activity and were stable, especially when stored at -20°C in lyophilized state (27).

In this study, we compared lyophilization of the apple extract and the low-temperature acetone

powder technique with a simple technique, i.e., preparing juice from fresh apple in a rapid procedure including storage immediately afterward at -20°C . In this way, we tried to mimic the prick-prick test with fresh fruit, as devised by Dreborg & Foucard (14). The juice extract gave better results, in terms of positive skin reactions and concordance between skin prick tests and case history or RAST, than the low-temperature acetone powder extract, especially for the Granny Smith apple. In addition, juice extract of the Granny Smith apple elicited larger skin reactions than juice extract of Golden Delicious apple (Fig. 1E), suggesting that the former extract is more potent. We therefore decided to use juice extract of Granny Smith in our routine food allergy testing. However, the quality of an allergenic extract also depends on stability. The stability of this extract will be studied in the future. The comparisons between different extraction procedures and apple strains yielded low to moderate correlation coefficients ranging from 0.34 to 0.64. This emphasizes the considerable influence of extraction procedures and choice of apple strains in diagnosing patients allergic to apple.

In conclusion, a high percentage of cross-sensitization was found between birch pollen and apple, peach, and hazelnut, both in the skin prick test and RAST. Approximately 50% of patients allergic to birch pollen have symptoms (especially oral allergy syndrome) after eating these products. In addition, we found an easy extraction procedure (juice extract) suitable for apple skin prick testing, superior even to freeze-dried extraction or the low-temperature acetone powder technique.

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References

- Anderson LB, Dreyfuss EM, Logan J, Johnstone DE, Glaser J. Melon and banana sensitivity coincident with ragweed pollinosis. *J Allergy* 1970;45:310-19.
- Pauli G, Bessot JC, Dietemann-Molard A, Braun PA, Thierry R. Celery sensitivity: clinical and immunological correlations with pollen allergy. *Clin Allergy* 1985;15:273-9.
- van Toorenbergen AW, Dieges PH. Demonstration of spice-specific IgE in patients with suspected food allergies. *J Allergy Clin Immunol* 1987;79:108-13.
- Wüthrich B, Stager J, Johansson SGO. Celery allergy associated with birch and mugwort pollinosis. *Allergy* 1990;45:566-71.
- Blanco C, Carrillo T, Castillo R, Quirarte J, Cuevas M. Latex allergy: clinical features and cross-reactivity with fruits. *Ann Allergy* 1994;73:309-14.
- Koshte VL, Kagen SL, Aalberse RC. Cross-reactivity of IgE antibodies to caddis fly with Arthropoda and Mollusca. *J Allergy Clin Immunol* 1989;84:174-83.
- Mandallaz MM, de Weck AL, Dahinden CA. Bird-egg syndrome. *Int Arch Allergy Appl Immunol* 1988;87:143-50.
- van Toorenbergen AW, Gerth van Wijk R, van Dooremalen G, Dieges PH. Immunoglobulin E antibodies against budgerigar and canary feathers. *Int Arch Allergy Appl Immunol* 1985;77:433-7.
- Juhlin-Dannfeldt C. About the occurrence of various forms of pollen allergy in Sweden. *Acta Med Scand* 1948;26:563-77.
- van Ree R, Aalberse RC. Pollen-vegetable food cross-reactivity: serological and clinical relevance of cross-reactive IgE. *J Clin Immunossay* 1993;16:124-30.
- Vieths S, Schöning B, Brockmann S, Aulepp H. Untersuchungen zur Allergie gegen Lebensmitteln pflanzlicher Herkunft: Herstellung und Charakterisierung von Obst- und Gemüseextrakten für serologische Untersuchungen. *Dtsch Lebensm Rundschau* 1992;88:239-43;124-30.
- Watanabe N, Kamei S, Onkubo A, et al. Urinary protein as measured with pyrogallol red molybdate complex, manually and in Hitachi 726 automated analyser. *Clin Chem* 1986;32:1551-4.
- Norman PS. Skin testing. In: Rose NR, Friedman H, editors. *Manual of clinical immunology*. 2nd ed. Washington, DC: American Society for Microbiology, 1980:789-93.
- Dreborg S, Foucard T. Allergy to apple, carrot and potato in children with birch pollen allergy. *Allergy* 1983;38:167-72.
- Ebner C, Birkner T, Valenta R, et al. Common epitopes of birch pollen and apples - studies by Western and Northern blot. *J Allergy Clin Immunol* 1991;88:588-94.
- Eriksson NE, Formgren H, Svenonius E. Food hypersensitivity in patients with pollen allergy. *Allergy* 1982;37:437-43.
- Lahti A, Björkstén F, Hannuksela M. Allergy to birch pollen and apple, and cross-reactivity of the allergens studied with the RAST. *Allergy* 1980;35:297-300.
- Möller C. Effect of pollen immunotherapy on food hypersensitivity in children with birch pollinosis. *Ann Allergy* 1989;62:343-5.
- Ortolani C, Ispino M, Pastorello E, Bigi A, Ansaloni R. The oral allergy syndrome. *Ann Allergy* 1988;61:47-52.
- Löwenstein H, Eriksson NE. Hypersensitivity to foods among birch pollen-allergic patients. Immunochemical inhibition studies for evaluation of possible mechanisms. *Allergy* 1983;38:577-87.
- Ortolani C, Ispino M, Pastorello E, Bigi A, Ansaloni R, Magri GC. Comparison of results of skin prick tests (with fresh foods and commercial food extracts) and RAST in 100 patients with oral allergy syndrome. *J Allergy Clin Immunol* 1989;83:683-90.
- Rosen JP, Selcow JE, Mendelson LM, Grodofsky MP, Factor JM, Sampson HA. Skin testing with natural foods in patients suspected of having food allergies: is it a necessity? *J Allergy Clin Immunol* 1994;93:1068-70.
- Vieths S, Jankiewicz A, Schöning B, Aulepp H. Apple allergy: the IgE-binding potency of apple strains is related to the occurrence of the 18-kDa allergen. *Allergy* 1994;49:262-71.
- Vieths S, Aulepp H, Schöning B, Tschirnrich R. Untersuchungen zur Apfelallergie bei Birkenpollenallergikern. Hauttestergebnisse und Immunoblot-Analysen mit Tief-temperatur-extrakten unterschiedlicher Apfelsorten. *Allergologie* 1995;18:89-97.
- Björkstén F, Halmepuro L, Hannuksela M, Lahti A. Extraction and properties of apple allergens. *Allergy* 1980;35:671-7.

26. Rudenschko O, Fahlbusch B, Henzgen M, Schlenvoigt G, Herrmann D, Jäger L. Optimization of apple allergen preparation for *in vivo* and *in vitro* diagnostics. *Allergy* 1995;50:262-8.
27. Rudenschko O, Fahlbusch B, Henzgen M, et al. Investigation of the stability of apple allergen extracts. *Allergy* 1995;50:575-80.

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