

# Identification of low allergenic apple cultivars using skin prick tests and oral food challenges

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## Keywords

food allergy; immunologic tests

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## Abstract

**Background:** As oral allergy syndrome (OAS) symptoms to apple are frequent, we aimed to identify low allergenic apple cultivars and to validate the prick-to-prick skin prick test (SPT) as a suitable screening method.

**Methods:** Sixty-eight apple cultivars were tested by SPTs in 33 Dutch adults with OAS, before and during the birch pollen season in 2006 and 2007, respectively. Three cultivars yielding the largest number of negative SPTs (Elise, Santana and Pink Lady®) and one reference cultivar (Golden Delicious) were subsequently tested by single-blind oral food challenges (SBFC) just after picking in fall 2007 (fresh) and in spring 2008 (stored), outside the birch pollen season and preceded by SPTs. In spring, Santana was replaced by Modi®.

**Results:** In fresh apples, OAS symptoms of Elise, as measured by cumulative scores on a Visual Analogue Scale VASt, were significantly lower than those of Santana, Pink Lady and Golden Delicious ( $P = 0.021$ ; 0.040 and 0.005, respectively). VASt scores of Santana were significantly lower than those of Golden Delicious ( $P = 0.049$ ). In stored apples, VASt scores of Elise were significantly lower than that of Golden Delicious ( $P = 0.038$ ). VASt scores of fresh apples did not differ significantly from stored apples, except in Golden Delicious (spring < fall:  $P = 0.021$ ). The SPTs did not predict the severity of OAS.

**Conclusion:** SPTs are not useful to assess the allergenicity of apple cultivars. By using SBFC, Elise and Santana were identified as low allergenic apple cultivars in patient with OAS. Our data on the effect of storage are inconclusive.

Mild oropharyngeal symptoms, known as oral allergy syndrome (OAS) to fresh apple, are a common phenomenon in north-west Europe (1, 2). OAS is primarily because of cross-reactivity between Bet v 1, the major allergen in birch pollen, and its homologue protein in apple, Mal d 1 (3). As apple is a frequently consumed source of fresh fruit in north-west Europe, the availability of low allergenic apple cultivars, which can be tolerated by apple allergic consumers, is desirable to meet nutritional needs and to diminish dietary restrictions because of OAS. To date, there have been clinical studies on the identification of apple cultivars with reduced allergenicity for patients suffering from OAS (4–7). This

resulted in the identification of Santana, a cultivar that is tolerated by approximately 50% of apple allergic consumers (5, 8). The performance of oral food challenges with fresh apple is considered to be the most reliable method of diagnosing apple allergy. However, it is not a feasible method for screening large numbers of apple cultivars in large numbers of patients.

Therefore, the aim of this study was (i) to identify low allergenic apple cultivars that can be tolerated by patients with OAS by SPTs and (ii) to validate the SPT as a suitable screening method by examining the allergenicity of a large variety of apple cultivars in an adult study population with

OAS by oral food challenge tests with cultivars yielding the highest percentages of negative SPT responses.

This study was performed as part of ISAFRUIT (LK5-CT-2002-01492), an integrated EU-funded multi-centre project.

## Methods

### Study design

To select putatively low allergenic apple cultivars, SPTs were performed with 68 different apple cultivars. SPTs were performed in two test series: outside the birch pollen season, shortly after picking (fall 2006, September until November) and during the birch pollen season, after several months of storage (spring 2007, April and May). Cultivars yielding the largest percentages of negative SPT responses were selected for further study. In the following year, these cultivars were tested in apple allergic patients by single-blind oral food challenges (SBFCs) to assess apple-related allergic symptoms shortly after picking and after several months of storage. These challenges were performed outside the birch pollen season.

### Study population

Adult participants with reported symptoms of OAS to fresh apple were recruited from the Allergy Outpatient Clinic of the University Medical Centre Groningen (UMCG) and by local advertisements. Participants with confirmed OAS symptoms to fresh apple by open food challenge with Golden Delicious were included in the study.

Patients with a history of more extensive systemic reactions to fresh apple, using beta-blockers, undergoing immunotherapy, or currently pregnant, were excluded from the study. The study protocol was approved by the medical ethics committee of UMCG.

### Apple material

The 68 apple cultivars that were selected for SPTs in fall 2006 and spring 2007 are shown in Table 1. Cultivars grown in different orchards, such as Golden Delicious Poland and Golden Delicious Italy Vinschau, were considered as separate cultivars. All apples were harvested at the usual degree of maturity. After harvest, apples were stored at 4°C in normal atmosphere at the facilities of Applied Plant Research (PPO) at Wageningen University and Research Centre, the Netherlands. Shortly before the onset of each of the two test series, apples were transported to UMCG where they were stored at 3°C until use.

### Skin prick test procedure

Because of the large number of apples to be skin tested, all cultivars were tested in several skin test sessions. Forty-nine cultivars were tested in both the fall of 2006 and the spring of 2007. Twelve cultivars were only tested in fall and replaced

by seven different cultivars in spring, because they could not be stored until spring. In the fall of 2006, SPTs were performed no longer than 70 days after the harvest date of the cultivars (Table 1). Each test for individual participants consisted of 22–25 different apple cultivars, birch pollen extract (*Betula verrucosa*, 50.000 SBE/ml), a positive control (histamine dihydrochloride 10 mg/ml) and a negative control (SPT diluent). Santana and Golden Delicious were tested in all sessions in fall and spring, as putatively low and high allergenic reference cultivars, respectively (4, 5) and to assess the reproducibility of the SPT.

SPTs were performed using apple material obtained from the stem region of the apples. Antihistamines were stopped 72 h prior to the SPTs. The SPT was regarded as positive when the diameter of the wheal was  $\geq 3$  mm (9). Of all 68 cultivars tested, the percentage of negative SPT responses ( $< 3$  mm) for fall and spring was calculated.

To select cultivars tolerated by the largest proportion of patients, mean percentages of negative SPT responses for fall and spring were calculated and ranked for 50 cultivars that were tested both in fall and spring. Putatively low allergenic cultivars yielding the largest mean percentages of negative SPT responses were selected for oral challenge testing.

To study the influence of the birch pollen season and the effect of storage on the SPT values, the percentages of negative SPT responses of the 50 cultivars tested in both seasons were compared.

### Oral food challenges

Three putatively low allergenic apple cultivars (Elise, Santana and Pink Lady®) and one putatively highly allergenic cultivar (Golden Delicious) were tested in random order in two series of single-blind oral food challenges (SBFC) just after picking in November 2007 (“fresh”) and in February and March 2008 after a few months of storage (“stored”). All challenges took place outside the birch pollen season. As Santana did not meet consumption requirements because of a limited storability (which is different each year), in spring, Santana was replaced by Modi®.

Preceding the oral challenge tests, SPTs were performed with the apple cultivars used in the challenge tests as well as with birch pollen extract, histamine and negative control.

The food challenges were performed in the Allergy Outpatient Department of the UMCG.

Loss of allergenicity of the apples was minimized by allowing the participants to bite into the unpeeled apples directly. Sensory perception of the participants was limited using a nose clip and blind fold, and by blinding the participants to the order and kind of apple cultivars tested. Subjective symptoms were scored on a Visual Analogue Scale (VAS) at baseline, and after 1, 5, 10 and 15 min after each challenge dose (5).

For each apple cultivar, the SBFC consisted of up to three doses with increasing amounts of unpeeled apple: one small bite (approximately 15 g) in an intact apple, followed by a piece of 30 and 100 g cut from an intact apple. The challenge was stopped at the lowest dose that induced clear symptoms of OAS and when the participant wanted to stop the

**Table 1** Details of apple cultivars used in SPTs in the fall of 2006 and the spring of 2007\*

| Cultivar                     | Mother                  | Father                     | Source of fruit† | Harvest date | Test series |       |
|------------------------------|-------------------------|----------------------------|------------------|--------------|-------------|-------|
|                              |                         |                            |                  |              | '06‡        | '07§  |
| Alkmene                      | Dr. Geheimrat Oldenburg | Cox Orange Pippin          | WUR-PPO          | 30-Aug       | 1           | –     |
| Ambrosia                     | Golden Delicious        | Starking Delicious         | LFV-L            | 3-Oct        | 2           | 2     |
| Ariane                       | Florina × Prima         | Golden Delicious × Unknown | KG               | 25-Sep       | 2           | 2     |
| Astramel                     | Roter Astrachan         | James Grieve × Melba       | JKI              | 4-Aug        | 1           | –     |
| Autento®                     | Delgollune              | Cox Orange Pippin          | WUR-PPO          | 3-Oct        | 2           | 2     |
| AW 106                       | RubINETTE (Rafzubin)    | Fuji                       | KG               | 27-Oct       | 3           | –     |
| Algot                        | Unknown                 | Unknown                    | SLU              | 29-Aug       | 1           | –     |
| Breaburn                     | Lady Hamilton           | Unknown                    | LFV-L            | 4-Oct        | 2           | 2     |
| Cameo®                       | Golden Delicious        | Delicious                  | LFV-L            | 12-Oct       | 3           | 3     |
| Collina                      | Priscilla               | Elstar                     | Fleuren          | 17-Aug       | 1           | –     |
| Cortland                     | McIntosh                | Ben Davis                  | RIPF             | 28-Sep       | –           | 3     |
| Cox Orange Pippin            | Ribston Pippin          | Blenheim Orange            | WUR-PPO          | 12-Sep       | 3           | 1     |
| Sundowner®                   | Golden Delicious        | Lady Williams              | LFV-L            | 20-Sep       | 3           | 3     |
| Dalisco®                     | Elstar                  | X3191                      | WUR-PPO          | 20-Sep       | 3           | 3     |
| Delcorf                      | Jongrimes               | Golden Delicious           | WUR-PPO          | 23-Aug       | 1           | –     |
| Discovery                    | Worcester Pearmain      | Beauty of Bath             | WUR-PPO          | 14-Aug       | 1           | –     |
| Dr. Geheimrat Oldenburg      | Unknown                 | Unknown                    | JKI              | 6-Sep        | 1           | 1     |
| Ecolette                     | Elstar                  | Prima                      | WUR-PRI          | 6-Oct        | 3           | 3     |
| Elise                        | Septer                  | Cox Orange Pippin          | WUR-PRI          | 28-Sep       | 2           | 2     |
| Elstar                       | Golden Delicious        | Ingrid Marie               | WUR-PPO          | 24-Aug       | 1           | 1     |
| Fiesta                       | Cox Orange Pippin       | Idared                     | WUR-PRI          | 28-Aug       | 1           | 1     |
| Fuji                         | Ralls Janet             | Delicious                  | WUR-PRI          | 13-Oct       | 3           | 3     |
| Gala                         | Kids Orange Red         | Golden Delicious           | WUR-PPO          | 13-Sep       | 1           | 1     |
| Gloster                      | Weise Winter            | Delicious                  | WUR-PRI          | 6-Oct        | 3           | 3     |
|                              | Glockenapfel            |                            |                  |              |             |       |
| Gold Chief                   | Starkrimson             | Golden Delicious           | LFV-L            | 12-Oct       | 3           | 3     |
| Golden Delicious-It-Laimburg | Grimes Golden           | Unknown                    | LFV-L            | 12-Sep       | 2           | 2     |
| Golden Delicious-It-Vinschau | Grimes Golden           | Unknown                    | LFV-V            | 26-Sep       | 2           | 2     |
| Golden Delicious-NI-PPO      | Grimes Golden           | Unknown                    | WUR-PPO          | 16-Oct       | 3           | 1,2,3 |
| Golden Delicious-PI          | Grimes Golden           | Unknown                    | RIPF             | 3-Oct        | 2           | 2     |
| Goldrush                     | Coop-17                 | Golden Delicious           | WUR-PPO          | 30-Oct       | 3           | 3     |
| Gravensteiner**              | Unknown                 | Unknown                    | JKI              | 21-Aug       | 1           | –     |
| Greenstar®                   | Granny Smith            | Golden Delicious           | WUR-PPO          | ?-Oct        | 3           | 3     |
| Holsteiner Cox's             | Cox Orange Pippin       | Unknown                    | JKI              | 8-Sep        | 2           | 2     |
| Honeycrisp                   | Keepsake                | Unknown                    | WUR-PPO          |              | 1           | 1     |
| Idared                       | Jonathan                | Wagnerapfel                | WUR-PRI          | 6-Oct        | 3           | 3     |
| Ingrid Marie                 | Cox Orange Pippin       | Unknown                    | WUR-PRI          | 26-Sep       | 2           | 2     |
| Jamba                        | Unknown                 | Unknown                    | JKI              | 18-Aug       | 1           | –     |
| James Grieve                 | Cox Orange Pippin       | Unknown                    | WUR-PRI          | 28-Aug       | 1           | –     |
| Jazz®                        | Royal Gala              | Braeburn                   | LFV-L            | 12-Oct       | 3           | –     |
| Jonagold**                   | Jonathan                | Golden Delicious           | WUR-PPO          | 11-Oct       | 3           | 3     |
| Jonathan                     | Esopus Spitzenburg      | Unknown                    | WUR-PRI          | 28-Sep       | 2           | 2     |
| Junami®                      | Idared × Maigold        | Elstar                     | WUR-PPO          | 11-Oct       | 3           | 3     |
| Kanzi®                       | Gala                    | Braeburn                   | WUR-PPO          | 11-Oct       | 3           | 3     |
| La Flamboyante (Mairac®)     | Gala                    | Maigold                    | LFV-L            | 11-Sep       | 2           | 2     |
| Ligol                        | Linda                   | Golden Delicious           | RIPF             | 5-Oct        | 2           | 2     |
| Lobo                         | McIntosh                | Unknown                    | RIPF             | 12-Sep       | –           | 3     |
| Maigold                      | Frau Rotacher           | Golden Delicious           | RIPF             | 9-Oct        | 3           | 3     |
| Modi®                        | Gala                    | Liberty                    | LFV-L            | 8-Sep        | 1           | 1     |
| Mutsu                        | Golden Delicious        | Indo                       | RIPF             | 5-Oct        | 3           | 3     |
| Pink Lady®                   | Golden Delicious        | Lady Williams              | LFV-L            | 26-Oct       | 3           | 3     |
| Prima                        | PRI-14-510              | NJ-123249                  | WUR-PPO          | 11-Sep       | 1           | –     |
| Priscilla-NL                 | Unknown                 | Unknown                    | WUR-PRI          | 21-Aug       | 1           | 1     |
| Raika                        | Sampion                 | Unknown                    | WUR-PRI          | 26-Sep       | 2           | 2     |

**Table 1** (Continued)

| Cultivar              | Mother                                     | Father                | Source of fruit† | Harvest date | Test series |         |
|-----------------------|--------------------------------------------|-----------------------|------------------|--------------|-------------|---------|
|                       |                                            |                       |                  |              | '06‡        | '07§    |
| Red Delicious         | Mutant of Delicious                        |                       | WUR-PPO          | 2-Oct        | 2           | 2       |
| Rubens®               | Gala                                       | Elstar                | WUR-PPO          | 20-Sep       | 2           | 2       |
| Rubin                 | Lord Lambourne                             | Golden Delicious      | RIPF             | 21-Sep       | 2           | 2       |
| RubINETTE (Rafzubin®) | Golden Delicious                           | Cox Orange Pippin     | WUR-PRI          | 26-Sep       | 2           | 2       |
| Santana               | Elstar                                     | Priscilla             | WUR-PPO          | 7-Sep        | 1,1,2,3     | 1,1,2,3 |
| Sonya®                | Gala                                       | Red Delicious         | LFV-L            | 3-Oct        | 2           | 2       |
| Starking Delicious-It | Mutant of Red Delicious                    |                       | LFV-L            | 6-Sep        | 1           | 3       |
| Starking Delicious-Pl | Mutant of Red Delicious                    |                       | RIPF             | 12-Oct       | 3           | 1       |
| Stayman-It            | Winesap                                    | Unknown               | LFV-L            | 12-Oct       | 3           | 3       |
| Stayman-Pl            | Winesap                                    | Unknown               | RIPF             | 9-Oct        | 3           | 3       |
| Topaz                 | Rubin                                      | Vanda                 | WUR-PPO          | 4-Oct        | –           | 1       |
| Wellant               | (Golden Delicious × Ingrid Marie) × Elstar | Elise                 | WUR-PPO          | 5-Oct        | –           | 1       |
| 90025-159             | Elise                                      | Prima × Breeding line | WUR-PRI          | 4-Oct        | –           | 1       |
| 92028-125             | Elise                                      | Priscilla             | WUR-PRI          | 5-Oct        | –           | 1       |
| 87052-131             | Melrose                                    | Ecolette              | WUR-PRI          | 4-Oct        | –           | 1       |

ACW: Agroscope Changins Wädenswil, Swiss; Fleuren: Dutch nursery; JKI: Julius Kühn Institut Dresden and Jork, Germany; KG: Kompetenzzentrum Gartenbau, RheinBach, Germany; LPV Land und Forstwirtschaftliches Versuchszentrum Laimburg and Vinschgau, It; RIPF: Research Institute of Pomology and Floriculture; SLU: Swedisch Agricultural University.

\*Apple cultivars were selected based on good growing characteristics in NW-Europe, being used in previous clinical studies, or occurring in the ancestry of previously reported low-allergenic cultivars.

†WUR-PPO, Randwijk, The Netherlands.

‡Dates of transport from WUR-PPO to UMCG: 1: 26-9; 2: 13-10; 3: 10-11.

§Dates of transport from WUR-PPO to UMCG: 1: 4-4; 2: 18-4; 3: 4-5.

\*\*Triploid.

challenge. Each participant underwent SBFC with all four apple cultivars in one challenge session. The next dose or apple cultivar was administered when symptoms had completely resolved. As dose 1 was administered to all participants, the cumulative VAS score (VAST) of dose 1 was computed for each participant. Participants were asked whether they would eat the apple cultivar in question after the study at the end of each challenge session, for each of the apple cultivars tested.

### Statistics

All statistical analyses were performed using SPSS software, 16th edition, SPSS Inc, Chicago, IL, USA). SPTs to histamine, Golden Delicious, Santana and birch were repeated in every session (Golden Delicious in spring only). To investigate the reproducibility of these measurements between sessions, within-subject coefficients of variation (CVs) were calculated. Differences in mean wheal sizes between sessions or seasons were tested for statistical significance in a random-effect model to account for within-subjects dependencies. A random-effects model was also used to study whether the SPT wheal sizes to apple cultivars were associated with the SPT wheal size to birch. Statistical calculations for oral challenge tests were performed for the participants who participated in both fall and spring ( $n = 30$ ).

Random-effects models were used to study whether mean cumulative total VAS scores (VAST) of dose 1 were different

between apple cultivars or between seasons. For these analyses, VAST scores were logarithmically transformed to obtain a normal distribution of the residuals. To study whether differences in mean VAST scores between apple cultivars could be explained by the SPT wheal sizes to apple or birch, these variables were added to the model. Differences in percentages were tested by chi-square tests.

### Results

Sixty-eight cultivars were tested by SPTs in 33 (fall 2006) and 31 participants (spring 2007), respectively. Ten (30%) and 9 (29%) participants were men in fall and spring, respectively. The mean age of the participants in both fall and spring was 25 years (range 18–52). All participants except one were sensitized to birch. Twenty-seven participants participated in all test series.

### Reproducibility of the skin testing

Within the seasons, SPTs (mm) for histamine were very reproducible with a median CV of 5.5%. For the SPTs with birch pollen extract, the CV varied from 8.8% in autumn to 13.4% in spring. However, the highest variation (20%) was found in the SPTs with Golden Delicious in spring. Thus, repeated testing confirmed the reproducibility of SPT with histamine, which was high, the reproducibility of birch pollen extract, which was moderate, and the reproducibility of

**Table 2** Percentage of negative SPT responses (<3 mm) of all apple cultivars tested. Cultivars were ranked in alphabetical order

| Apple cultivar               | % of negative SPT's in fall 2006 | % of negative SPT's in spring 2007 |
|------------------------------|----------------------------------|------------------------------------|
| Alkmene                      | 16.1                             | n.a.                               |
| Ambrosia                     | 6.5                              | 3.3                                |
| Arianne                      | 6.5                              | 6.7                                |
| Astramel                     | 6.7                              | n.a.                               |
| Autento®                     | 6.5                              | 6.7                                |
| AW 106                       | 6.1                              | n.a.                               |
| Algot                        | 9.7                              | n.a.                               |
| Breaburn                     | 12.9                             | 10.0                               |
| Cameo®                       | 9.1                              | 10.3                               |
| Collina                      | 25.8                             | n.a.                               |
| Cortland                     | n.a.                             | 3.3                                |
| Cox Orange Pippin            | 6.5                              | 10.0                               |
| Sundowner®                   | 9.1                              | 10.0                               |
| Dalinco®                     | 3.0                              | 6.7                                |
| Delcorf                      | 12.9                             | n.a.                               |
| Discovery                    | 12.9                             | n.a.                               |
| Dr. Geheimrat Oldenburg      | 6.5                              | 10.0                               |
| Ecolette                     | 15.2                             | 3.3                                |
| Elise                        | 16.1                             | 20.0                               |
| Elstar                       | 19.4                             | 10.0                               |
| Fiesta                       | 12.9                             | 6.7                                |
| Fuji                         | 15.2                             | 3.3                                |
| Gala                         | 19.4                             | 3.3                                |
| Gloster                      | 6.1                              | 16.7                               |
| Gold Chief                   | 15.2                             | 6.7                                |
| Golden Delicious It-Laimburg | 6.5                              | 6.7                                |
| Golden Delicious It-Vinschau | 9.7                              | 3.3                                |
| Golden Delicious-NI-PPO      | 12.9                             | 3.4                                |
| Golden Delicious -PI         | 9.7                              | 10.0                               |
| Goldrush                     | 9.1                              | 6.7                                |
| Gravensteiner                | 6.5                              | n.a.                               |
| Greenstar®                   | 6.1                              | 10.0                               |
| Holsteiner Cox's             | 6.5                              | 3.3                                |
| Honeycrisp                   | 12.9                             | 10.0                               |
| Idared                       | 12.1                             | 13.3                               |
| Ingrid Marie                 | 6.5                              | 3.3                                |
| Jamba                        | 6.5                              | n.a.                               |
| James Grieve                 | 16.1                             | n.a.                               |
| Jazz®                        | 9.1                              | n.a.                               |
| Jonagold                     | 3.0                              | 10.0                               |
| Jonathan                     | 6.7                              | 3.3                                |
| Junami®                      | 3.0                              | 3.3                                |
| Kanzi®                       | 6.1                              | 10.0                               |
| La Flamboyante (Mairac®)     | 6.5                              | 6.7                                |
| Ligol                        | 9.7                              | 6.7                                |
| Lobo                         | n.a.                             | 17.6                               |
| Maigold                      | 15.2                             | 6.7                                |
| Modi®                        | 25.8                             | 13.3                               |
| Mutsu                        | 9.1                              | 6.7                                |
| Pink Lady®                   | 12.1                             | 30.0                               |
| Prima                        | 10.0                             | n.a.                               |
| Priscilla-NI                 | 16.1                             | 10.0                               |
| Raika                        | 13.3                             | 3.3                                |

**Table 2** (Continued)

| Apple cultivar        | % of negative SPT's in fall 2006 | % of negative SPT's in spring 2007 |
|-----------------------|----------------------------------|------------------------------------|
| Red Delicious         | 16.1                             | 3.3                                |
| Rubens®               | 3.2                              | 3.3                                |
| Rubin                 | 16.1                             | 6.5                                |
| RubINETTE (Rafzubin®) | 9.7                              | 3.3                                |
| Santana (mean)        | 19.4                             | 17.2                               |
| Sonya®                | 6.5                              | 6.7                                |
| Starking Delicious-It | 22.6                             | 10.0                               |
| Starking Delicious-PI | 24.2                             | 10.0                               |
| Stayman               | 3.0                              | 13.3                               |
| Stayman PI            | 21.2                             | 3.3                                |
| Topaz                 | n.a.                             | 3.3                                |
| Wellant               | n.a.                             | 6.7                                |
| 90025-159             | n.a.                             | 3.3                                |
| 92028-125             | n.a.                             | 3.3                                |
| 87052-131             | n.a.                             | 6.7                                |

prick-to-prick skin testing with Golden Delicious, which was relatively low.

#### Skin prick tests

Table 2 shows the percentages of negative SPT responses for fall and spring of all 68 cultivars tested. In Fig. 1, the cultivars yielding the 10 highest and 10 lowest mean percentages of negative responses for fall and spring are shown. The putatively high allergenic reference cultivar Golden Delicious is also shown. Overall, there was no significant difference in the percentage of negative SPT responses between fall and spring ( $P = 0.25$ ). However, the overall mean SPT diameter was 1 mm smaller in fall than in spring ( $P < 0.001$ ).

Three putatively low allergenic cultivars were selected for SBFC: Pink Lady®, Santana and Elise. Santana was replaced by Modi® in the spring. Modi® is a recently introduced cultivar that can be stored for a much longer period than Santana.

Birch pollen SPTs in spring were significantly greater than in fall ( $P = 0.04$ ). The wheal sizes of the apple SPTs were significantly associated with the wheal size of the birch SPT with similar associations in fall and spring. With every millimetre increase in diameter of the birch SPT, the diameter of apple SPT increased with 0.89 mm (95% CI: 0.53–1.25) in fall and 0.82 mm (95% CI: 0.61–1.02) in spring.

#### Outcome of oral food challenge tests and correlation with SPTs

Thirty-one participants were tested in the first series of SBFCs in the fall of 2007, and 32 in the second series of SBFCs in the spring of 2008. Thirty participants were tested in both series, of whom 23 had also participated in the SPTs in the previous year.

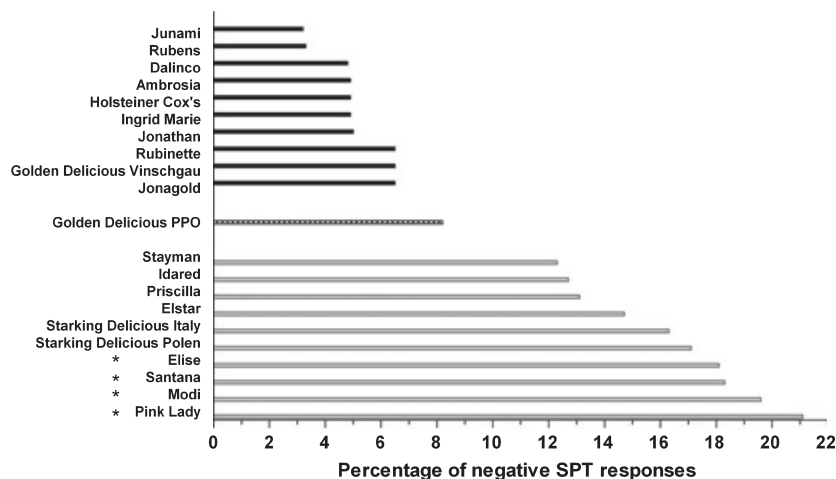


**Figure 1** Cultivars yielding the 10 highest and 10 lowest mean percentages of negative SPT responses for the fall of 2006 and the spring of 2007. Additionally, our standard apple cultivar Golden Delicious is shown.

For all cultivars, SPT results preceding the oral challenges were not associated with VASt scores (Fig. 2). The percentages of negative SPT responses varied from 3.3 to 6.6%, which were much lower than found during the first year of this study.

There was no significant difference between any of the cultivars in the percentage of participants given all three doses (6–16%), the percentage of participants remaining completely asymptomatic after the three doses (6–16%) or the percentages of participants stating that they would be willing to consume the cultivar in the future (38–48%), although these percentages were highest for Santana and Elise.

As dose 1 was administered to all participants, calculations for VASt scores were performed for this dose. In Fig. 3, differences in VASt score between cultivars are shown. In the fresh apples, VASt scores of Elise were significantly lower

than those of Pink Lady® ( $P = 0.04$ ), Santana ( $P = 0.02$ ) and Golden Delicious ( $P < 0.001$ ). Also, VASt scores of Santana were again significantly lower than those of Golden Delicious ( $P = 0.05$ ). In the stored apples, only the VASt scores of Elise were significantly lower than those of Golden Delicious ( $P < 0.001$ ).

To study whether SPT sizes to birch or apple cultivars were associated with VASt scores and whether this could (partly) explain the observed differences in VASt scores between apple cultivars, we added these variables to the random-effects model. This did not change the associations.

#### Effect of storage of apple cultivars on VASt scores

Overall, VASt scores for dose 1 were 27% lower (95% CI: 2%–45%) in stored apples than in fresh apples. In contrast, the oral challenge tests were more frequently stopped after the first dose in stored than in fresh apples (86% vs 69%,  $P < 0.001$ ). The data on storage effects are thus inconclusive.

#### Discussion

The primary aim of this study was to identify apple cultivars of low allergenicity. We found that in freshly picked apples, Elise yielded significantly milder responses in SBFCs than other cultivars, and milder responses than Golden Delicious in stored apples. Also, Santana showed milder responses in freshly picked apples, although less than Elise. However, a nonallergenic cultivar could not be identified. These findings apply to patients with OAS to fresh apple because of primary sensitization to birch pollen, but not for patients with systemic reactions to apple and sensitized to Mal d 3.

Based on previous studies with Santana (4, 5), Santana has been marketed as an apple cultivar with reduced allergenic properties in the Netherlands since 2006. In a consumer survey among 295 Dutch apple allergy sufferers, the low allergenic properties of Santana were established as 52% of the respondents with mild allergic symptoms tolerated the Santana apples (8). In our study, Elise gave even less allergic

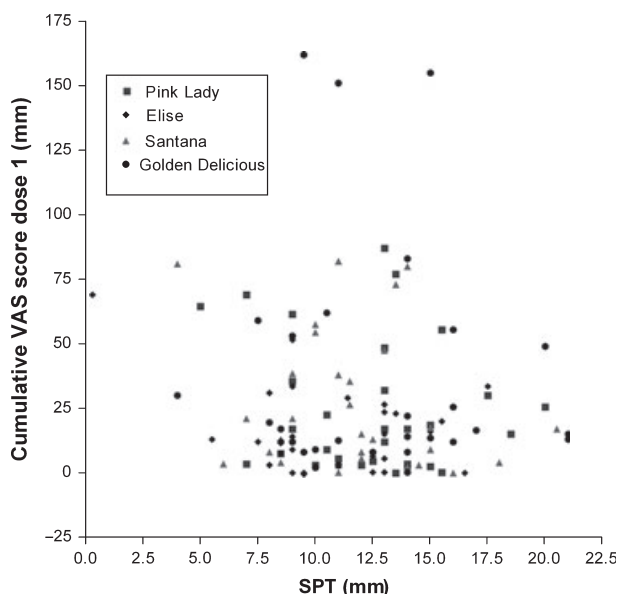


**Figure 2** SPT (mm) versus VASt (mm) of dose 1 of all cultivars tested in fall 2007 and spring 2008 ( $n = 240$ ).

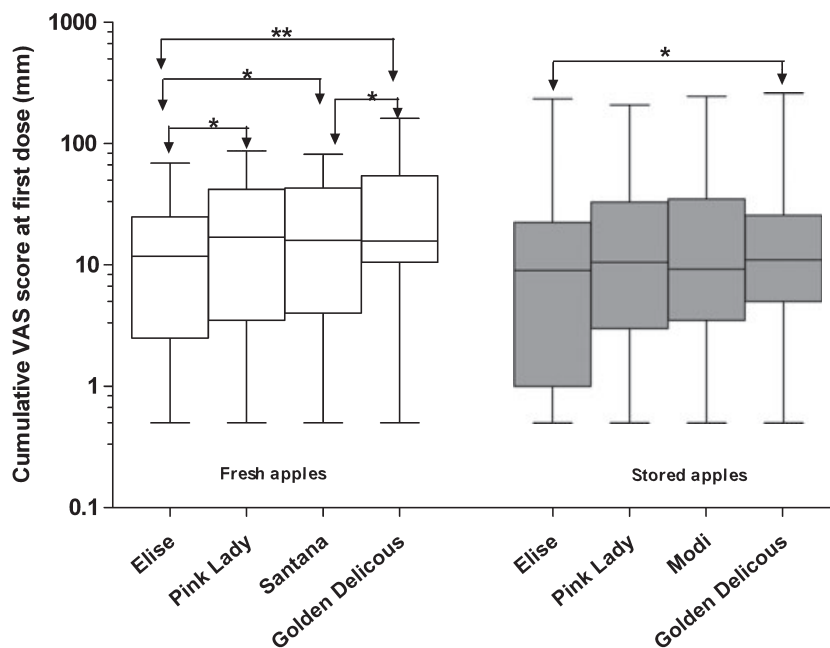


**Figure 3** Differences in total VAS scores (VAsT) of dose 1 as observed in SBFCs between cultivars in August/September 2007 (fresh) and February/March 2008 (stored). All challenges were performed outside the birch pollen season. \* $P < 0.05$ ; \*\* $P < 0.01$ .

responses than Santana. Moreover, Elise can be stored for a longer period than Santana and tastes differently. Therefore, Elise may increase the choice of low allergenic apple cultivars for consumers with OAS to fresh apple and can also be marketed as a cultivar with reduced allergenic properties.

Although we succeeded in selecting two apple cultivars (Elise and Santana) with significantly lower allergenic properties than the reference cultivar of high allergenicity (Golden Delicious), patients did not remain asymptomatic significantly more frequently following challenge with apple cultivars than they did to Golden Delicious, nor did they indicate that they would be willing to eat such apple cultivars more often than Golden Delicious. This is in contrast to the study by Kootstra et al. (5), where significantly more participants remained asymptomatic after oral challenge (53%) and more participants (73%) indicated that they would be willing to eat the Santana apple than Golden Delicious and Topaz. These differences may be attributed to intra-cultivar differences, but may also be attributed to investigator-driven effects. Therefore, we consider the VAsT score as a more valid outcome measure.

Comparing the results of different studies using oral challenges, agreement is generally seen as to which apple cultivars cause the least allergic symptoms. Bolhaar et al. (4) divided apples into those of low-, moderate- and high allergenicity, based on SPTs. Santana, Topaz, Elise and Braeburn were considered to be of low allergenicity, which is comparable to our results. In their study, the reduced allergenicity of Santana when compared to Golden Delicious was first established by DBPCFCs (in five patients), later confirmed by Kootstra et al. in 15 patients (5) and currently by our study results.

Asero et al. (6) performed SPTs with 8 different cultivars and subsequent SBFCs in nine, respectively, seven patients with OAS to apple. They found a large inter-patient, inter-apple and intra-apple variability. Although our findings con-

firm these results, the large intra-apple variability as found by Asero et al. (6) may be attributed to large variations in Mal d 1 content of the cultivars (10–13), the small study population used, as well as to a nonstandardized way of pricking location of the apple (14). Also, no data are provided on storage conditions and possible seasonal influences (15, 16).

The second aim of our study was to validate the SPT as a suitable screening method for the selection of low allergenic apple cultivars. We found no correlation between SPTs and VAsT scores. Thus, the percentage of negative skin test responses was not able to predict the severity in oral symptoms in allergic individuals or the percentage of patients that remained without symptoms. As has been observed previously, there is poor correlation between the size of the skin test and the intensity of symptoms during oral challenge in apple allergic individuals (5, 6, 17). The lack of correlation between skin test results and clinical reactivity to foods is a general phenomenon. (18). Indeed, a previous study demonstrating such a relationship was based on double-blind, placebo-controlled challenges with only two apple cultivars in only five patients (4). Additionally, we found significant differences between test series in Santana and in Golden Delicious (large CVs), as well as between two subsequent years, and in Golden Delicious between different locations (large variation in percentage of negative responses) (Table 2). This illustrates the intra-cultivar variability and could contribute to the lack of correlation between SPTs and oral challenge test results. Moreover, an unequal distribution of allergens over the apple may contribute to lack of correlation, as SPT evaluates only a minuscule fraction of the entire apple (10–13).

Overall, VAsT scores in freshly picked apples were significantly higher than in stored apples. These results were unexpected, as it has been shown that Mal d 1 expression and

SPT responses increase with longer storage duration (4, 15, 19). However, overall, the number of doses ingested decreased, but this parameter may be less valid and more investigator dependant than scoring of severity of symptoms on a VAS-scale by study participants. The effect of storage on oral symptoms is thus uncertain and may be more complicated than anticipated from these studies.

In conclusion, skin prick testing is not a useful test to assess the allergenicity of apple cultivars or to select apples that may cause few or no symptoms following oral exposure. By using single-blind oral food challenges, Elise and Santana could be identified as low-allergenic apple cultivars. Our data on the effect of storage are inconclusive.

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