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Plant Foods for Human Nutrition

ISSN 0921-9668

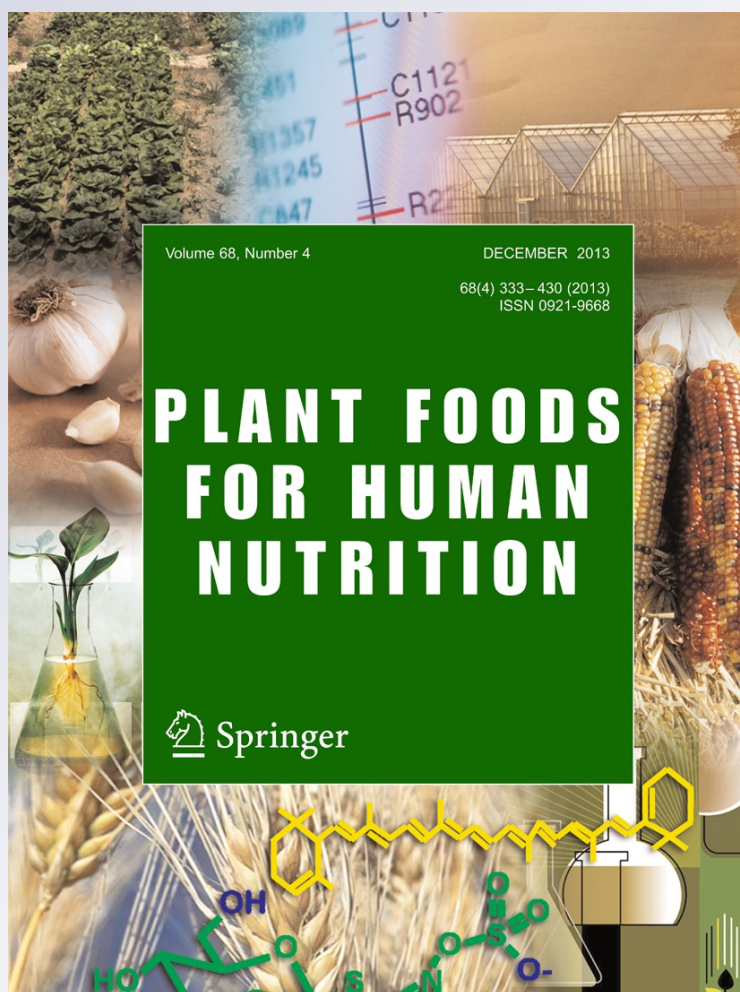
Volume 68

Number 4

Plant Foods Hum Nutr (2013)

68:391-395

DOI 10.1007/s11130-013-0385-6



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Published online: 15 September 2013
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Abstract Patients with Oral Allergy Syndrome (OAS) to fresh apple may tolerate low allergenic apple cultivars. We aimed to investigate if the low allergenic properties of Elise and Santana, as previously identified in a Dutch population, could be generalised within North West Europe within the birch pollen region with regard to both the prevalence and degree of sensitization. Prick-to-prick tests (PTP) were performed in eighty-five adult patients with OAS to fresh apple in Great Britain, Switzerland and Northern Italy, before the birch pollen season, using the putatively low allergenic apple cultivars Elise, Santana, Granny Smith, Modi and McIntosh, as well as the putatively high allergenic apple cultivars Golden Delicious and Kanzi. No significant differences in percentages of negative responses of PTPs were found

between the three countries. Negative responses did not differ from negative responses to the different apple cultivars we previously found in 2006/2007 in the Netherlands. The size of the PTPs of all apple cultivars tested were correlated to the size of the skin prick tests with birch pollen. These results add to the indications for the low allergenic properties of the low allergenic apple cultivars Santana and Elise, as the number of negative responses were reproducible in three countries within the birch pollen region and were similar to previous results in the Netherlands. These results justify oral challenge studies with Elise and Santana within the birch pollen region, to establish the low allergenic properties for the benefit for apple allergic consumers for definite conclusions.

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Keywords Oral allergy syndrome · Prick-to-prick test · Apple allergy

Abbreviations

OAS	oral allergy syndrome
PTP	prick-to-prick test
SPT	skin prick test
CV	coefficient of variation

Introduction

Patients having oral allergy syndrome (OAS) to fresh apple experience itching and swelling of the tongue, buccal mucosa and throat when eating fresh apple, resulting in reduced consumption of fresh apples. In North West Europe, this common kind of apple allergy is largely caused by sensitization to one of the major apple proteins and allergens, Mal d 1, due to concomitant birch pollen allergy and cross-reactivity with the major birch pollen allergen Bet v 1. Patients with OAS to fresh apple may also be allergic to other fresh fruits of the rosacea family such as pear, cherry and peach, but may tolerate low allergenic apple cultivars [1–4]. As apple is a frequently consumed source of fruit rich in flavonoids and dietary fibre OAS to multiple fruits may have a negative impact on a healthy diet [5, 6].

Prick-to-prick tests (PTPs) with material from fresh apples are widely used as a quick and easy method in everyday clinical practice to screen patients for sensitization to apple [7]. PTPs with fresh apple are performed by pricking into the apple with a 1 mm sharp lancet and subsequently in the skin of the arm of the patient. Patients who are sensitised to apple have positive PTP responses with fresh apple. However, it has been demonstrated that in food allergy there is no association between the size of PTPs and the severity of the clinical reactivity [8].

In previous studies, the apple cultivars Elise and Santana were identified as low allergenic in Dutch adults with OAS to fresh apple, yielding the highest percentages of negative responses in PTPs and, most importantly, significantly milder symptoms in oral food challenges as compared to other cultivars [1–3]. In contrast to PTPs with fresh apple, the oral food challenge test with apple, administering fresh apple in increasing amounts to the patient, is a suitable test to compare the allergenicity of apple cultivars.

A consumer's survey showed that approximately 50 % of Dutch patients with OAS to fresh apple tolerate the Santana apple [4]. This might be true for other apple allergic consumers in North West Europe. However, studies have shown that the degree and, to some extent, the prevalence of sensitization to apple may be influenced by the prevalence of sensitization to birch pollen [7, 9].

To investigate if the low allergenic properties of Santana and Elise may be generalised to other patients with OAS to

fresh apple within the birch pollen region of North West Europe and justify further oral challenge studies for definite conclusions, we aimed to study the prevalence and degree of sensitization to these apple cultivars in different North West European regions. Therefore, in three countries in which birch pollen allergy is highly prevalent [7, 9], we performed skin prick tests (SPTs) with birch pollen extract and PTPs with Elise and Santana and five other cultivars for comparison, in a large study population of patients with OAS to fresh apple.

Materials and Methods

Study Design and Study Population

The study was undertaken in February and March 2009 in Great Britain, Switzerland and Northern Italy, before the birch pollen season started. Participating centers were Royal Brompton & Harefield NHS Trust-London, Universitäts-Hospital, Zürich and the University of Bologna, respectively. For each participating center, approval of the local ethical committee was obtained.

Fresh apples of the cultivars Elise, Santana, Granny Smith, Modi, McIntosh, Golden Delicious and Kanzi were used for the PTPs. As it is known that different apple cultivars yield different PTP results, we used cultivars with different allergenicity [1–3, 10]. *Santana* and *Elise* were selected because of their confirmed low allergenic properties [1–4]. Modi was one of the cultivars that yielded a high number of negative PTP results in our previous study in the Netherlands [2]. Granny Smith was included because allergic consumers experience it as being low allergenic [4]. McIntosh was included because it is one of the ancestors of Santana and also a relatively unknown cultivar [11]. Golden Delicious was included as this cultivar is a widely accepted reference for high allergenicity. As shown in a previous study, Golden Delicious is particularly rich in Mal d 1 [12]. Kanzi was selected as a second high reference cultivar, having a percentage of negative responses (8 %) similar to Golden Delicious in our previous study and because it is a popular cultivar with consumers [2].

Adult patients with symptoms of OAS to fresh apple were included. Patients with systemic reactions to fresh apple were excluded. Oral allergy to fresh apple was confirmed by an inclusion challenge with Golden Delicious, as previously described [2] before the PTPs. The study participants all gave signed informed consent.

PTPs, SPTs and Apple Cultivars

PTPs with the fresh cultivars Elise, Santana, Granny Smith, Modi, McIntosh, Golden Delicious and Kanzi were performed in duplicate in all participating countries by the same

dedicated allergy nurse from the University Medical Center Groningen.

PTPs with fresh apple, skin prick tests (SPTs) with standardised birch pollen extract (*Betula verrucosa*, 50,000 SBE/ml) (ALK-Abello, Hørsholm, Denmark), a positive control (histamine dihydrochloride 10 mg/ml) and a negative control (SPT diluent) were performed on the volar side of the forearm according to the method of Dreborg et al. [13], using the Alk-Abello 1-mm-tipped lancet (Hørsholm, Denmark). For the PTPs, the unpeeled apple was pricked in the upper 2 cm area around the stalk [14] and through the peel, so that peel and pulp material was used [15]. The skin tests were expressed as the average of the largest and corresponding perpendicular diameters of the wheal in mm. The PTPs and SPTs were regarded as negative when the mean diameter of the wheal was <3 mm [13, 16]. Antihistamines were stopped 72 h prior to the PTPs. For SPT and PTP duplicates the mean value (mm) of the PTPs were calculated.

The cultivars Elise, Santana, Granny Smith, McIntosh, Golden Delicious and Kanzi were supplied by the experimental station of Applied Plant Research of Wageningen-UR (PPO), The Netherlands. Modi apples were supplied by the Research Center for Agriculture & Forestry Laimburg South-Tyrol, Italy.

The apple cultivars were harvested 1 to 2 weeks before full maturity and stored mechanically at 2–3 °C in normal atmosphere at the facilities of PPO. Apple cultivars were shipped by courier to Italy on 4th February 2009, to the United Kingdom on 9th February 2009 and 3rd March 2009, and to Switzerland on 17th February 2009.

Statistics

All statistical analyses were performed using SPSS software, 18th edition, SPSS Inc. (Chicago, IL, USA). The allergenic properties of cultivars were assessed and compared by the percentages of negative PTP responses. Differences in numbers of negative PTP responses were calculated by Chi-square test. To investigate the reproducibility of SPTs and PTPs in duplicate, within-subject coefficients of variation (CVs) were calculated. Within each cultivar, differences in median CV between countries were analysed by the Kruskal-Wallis test. Correlations between SPTs and PTPs to birch and the apple cultivars were calculated by Spearman's rho. *P* values <0.05 were considered significant.

Results

Within Cultivar Differences in CVs, SPTs and PTPs Between Countries

Eighty-five participants were included: Twenty-eight participants in Great Britain, 30 in Switzerland, and 27 in Northern

Italy (Table 1). For histamine, birch pollen extract and within each cultivar there were no significant differences in the CVs or percentages of negative SPT and PTP responses between the three countries (Table 1) (data for CVs not shown). For all cultivars PTP results in Switzerland yielded the highest percentages of negative responses. For Modi, PTP results in Switzerland were significantly higher than in Italy ($p=0.046$). For comparison, negative responses to the different apple cultivars we previously found in 2006/2007 in the Netherlands (2) are shown in Table 1. Within each cultivar, no significant differences in negative responses were found between all three countries together and the Netherlands.

Differences in SPT Responses Between Cultivars

For all three countries together, Golden Delicious and Kanzi yielded the lowest number of negative responses (9/85 and 12/85, respectively), while McIntosh yielded the highest number of negative responses (20/85) (Table 1), followed by Santana and Modi (17/85). Only the difference between Golden Delicious and McIntosh was statistically significantly ($p=0.025$).

Correlation Between SPT of Birch and the PTPs of the Different Cultivars

Almost all ($n=79$, 93 %) of our patients had a positive skin test with birch pollen extract. The degree of sensitization to birch did not differ among the three countries. The size of the birch SPT correlated moderately but statistically significant (r between 0,51 and 0,63) with the PTPs of the seven apple cultivars as shown in Fig. 1.

Discussion

For all cultivars, PTP results performed in duplicate by one dedicated nurse, were reproducible, as no significant differences in percentages of negative responses of PTPs nor median PTPs were found between three countries in the birch pollen region in North West Europe. Additionally, percentages of negative PTPs were not significantly different from previous PTP results in Dutch adults. Thus, these results add additional indications for the low allergenic properties of Santana and Elise, as the number of negative responses and median PTPs were reproducible in three countries within the birch pollen region and were similar to previous results in the Netherlands. It will be of clinical practice for patients with OAS to fresh apple if they can expand their diets with new low allergenic apple cultivars.

Note that there is no association between the size of PTPs and the severity of the clinical reactivity [2, 8]. Thus, the results of this study reflect the prevalence and degree of sensitization to fresh apple, but not the severity of OAS. This

Table 1 Study population and results of SPTs and PTPs, expressed as numbers and percentages of negative SPTs and PTPs responses in the three European countries in 2009, and in the Netherlands in 2006/2007

	Great Britain <i>N</i> =28 ^a	Switzerland <i>N</i> =30 ^b	Italy <i>N</i> =27 ^c	All countries <i>N</i> =85 ^d	Netherlands 2006/2007 <i>N</i> =49
Histamine	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Birch pollen	3 (11 %)	3 (10 %)	0 (0 %)	6 (7 %)	1 (2 %)
Elise	3 (11 %)	6 (20 %)	4 (15 %)	13 (15 %)	9 (18 %)
Santana	5 (18 %)	9 (30 %)	3 (11 %)	17 (20 %)	9 (18 %)
Granny Smiths	4 (14 %)	6 (20 %)	2 (7 %)	12 (14 %)	–
Modi	4 (14 %)	10 (33 %)*	3 (11 %)*	17 (20 %)	10 (20 %)
McIntosh	7 (25 %)	8 (27 %)	5 (19 %)	20 (24 %)	–
Golden Delicious	2 (7 %)	5 (17 %)	2 (7 %)	9 (11 %)	4 (8 %)
Kanzi	4 (14 %)	5 (17 %)	3 (11 %)	12 (14 %)	4 (8 %)

**p*=0.046^a Great Britain: 4 (14 %) male; median age 40, 5 years (range 20–70 years); 25 (89 %) sensitised to birch^b Switzerland: 7 (23 %) male; median age 34 years (range 17–55 years); 27 (90 %) sensitised to birch^c Italy: 9 (33 %) male; median age 31 years (range 18–55 years); 27 (100 %) sensitised to birch^d All countries: 20 (24 %) male; median age 34 years (range 17–70 years); 79 (93 %) sensitised to birch

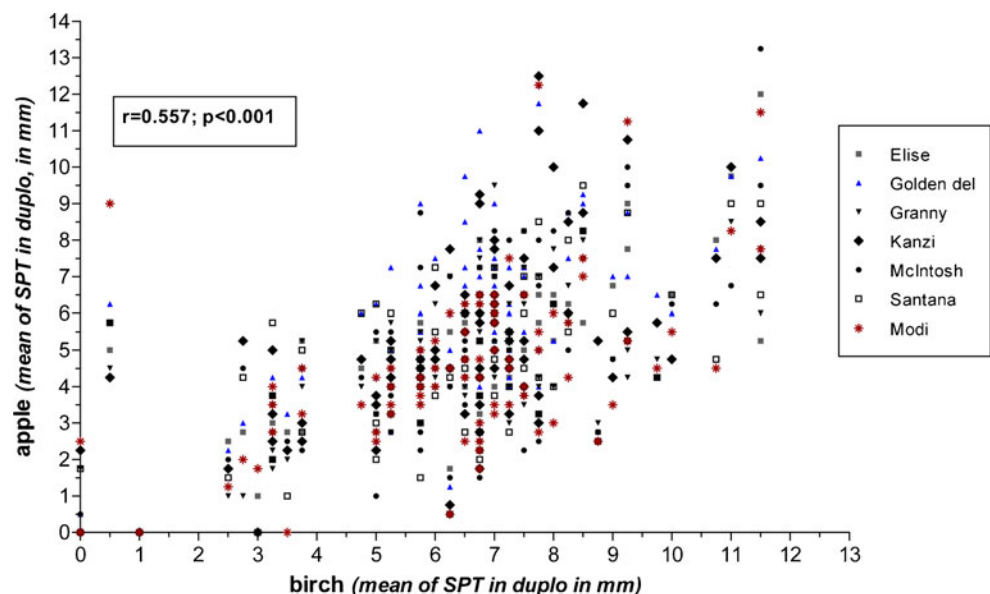
study provides justification for further oral challenge testing with Elise and Santana in different countries in North West Europe other than the Netherlands for definite conclusions on the low allergenic properties of these cultivars. This conclusion may not apply to Scandinavia, as it was recently shown that the high sensitization rate to apple in these countries may be influenced by the high exposure and sensitization to birch pollen, although this effect seems to be limited [9]. Indeed, we found that the sizes of the PTPs of all apple cultivars tested were correlated to the size of the SPT with birch pollen (as is shown in Fig. 1), both in this study as in our previous study on allergenic properties of apple cultivars in the Netherlands [2].

Also Modi and McIntosh yielded high percentages of negative responses, but we have no data on oral challenges

with these cultivars and may thus be worth further studying by oral challenge testing.

One of the strengths of this study was that the PTPs were performed by the same dedicated allergy nurse, ensuring that differences in PTPs cannot be explained by operator variability. Another strength is that the PTPs were performed in duplicate, so that the reproducibility of the PTP results could be demonstrated.

It may be argued that this study had no control group to calculate sensitivity and specificity of the PTPs. However, the purpose of this study was not to establish the diagnostic value of PTPs with different apple cultivars, but to investigate whether these numbers of negative PTP responses of Elise and Santana were reproducible and comparable in countries within the birch pollen region with regard to the prevalence

Fig. 1 Correlation between SPTs with birch (mm) and PTPs with apple cultivars

and degree of sensitization and whether these results justify further oral challenge studies.

Apple protein consists of several major allergens, such as Mal d 1, Mal d 2, Mal d 3 and Mal d 4. We did not determine sensitization patterns in blood serum to these individual apple proteins in these three countries. This might have been of interest, as in Central and Northern Europe, apple allergy may not only be caused by sensitization to Mal d 1, which is cross-reactive with the major birch pollen allergen Bet v 1 [10, 15–17], but also by sensitization to the apple profilin Mal d 4, which is associated with a different risk profile. Recently, sensitization to profilin (Mal d 4) was shown to be clinically relevant in patients with OAS to apple in Italy [18]. In contrast, in a study in Austrian birch-pollen allergic patients, with or without related OAS to food, it was shown that the oral allergy reactions could be attributed to sensitization to Bet v 1 and not to Bet v 2 (the birch pollen profilin) [19]. Additionally, in a recent study it was shown that sensitization to Mal d 1 did not discriminate between birch pollen allergic patients with and without OAS [20]. However, we did determine sensitization to the apple proteins Mal d 1, Mal d 2, Mal d 3 and Mal d 4 in the previous 28/49 Dutch study population described in Table 1. All but one patient were sensitised to Mal d 1, only 8/28 to Mal d 2, 3/28 to Mal d 3 and only 7/28 to Mal d 4 (data not shown). Based on these results we cannot exclude that a proportion of the Italian patients in our study was co-sensitised or mono-sensitised to profilin. However, if this would have been true, this did not lead to differences in PTPs between countries.

Acknowledgments Walter Guerra of the Research Center for Agriculture & Forestry Laimburg South-Tyrol - Italy is acknowledged for supplying fruit of Modi and Gondy Peppelman and Marc op 't Hof of DLO-PPO Wageningen-UR, The Netherlands are acknowledged for supplying fruit of all the other examined cultivars.

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

Conflict of Interest None.

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