

Occupational Allergy in Strawberry Greenhouse Workers

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Key Words

Allergy · Nasal provocation tests · Occupational allergy · Greenhouse · Strawberry pollen

Abstract

Background: Employees in strawberry greenhouses are highly exposed to several (potential) allergenic agents. However, no occupational allergy in this branch has been described before. First, the presence of work-related allergic symptoms in strawberry workers was explored. Second, we aimed to prove the concept that an IgE-mediated allergy could be responsible for work-related symptoms. To test the possibility of an IgE response secondary to cross-reactivity to birch or grass pollen, inhibition experiments were performed. **Methods:** First, a questionnaire survey concerning work-related allergic symptoms among strawberry workers in the Netherlands was carried out. Second, 3 workers with work-related symptoms were investigated in detail. Skin tests, serum-specific IgE tests with home-made extracts of strawberry pollen and other possible allergenic agents of the strawberry greenhouse environment were executed. Furthermore, immunoblots and nasal provocations with strawberry pollen extract were performed. In addition, inhibition experiments were performed. **Results:** 29 of 75 questionnaire respondents (38.7%) reported work-related symptoms. Sensitization to strawberry pollen was found in skin

tests in all 3 employees with work-related symptoms. ELISA and immunoblotting with strawberry pollen showed positive results in 2 employees. Birch and grass pollen failed to inhibit IgE binding to strawberry pollen in 1 of 2 employees. Partial inhibition was seen in the second employee. Nasal provocation validated clinically relevant allergy to these pollens in 2 of 3 subjects. **Conclusions:** Allergic symptoms attributable to the workplace are present among a proportion of strawberry greenhouse employees. An IgE-mediated occupational allergy to strawberry pollen may contribute to these symptoms.

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Introduction

The prevalence of occupational allergy has increased over the past few decades [1]. Pollen from flowers and bell pepper plants cultivated in greenhouses are known causes of this type of allergy [2–6].

In the last 10 years, strawberry cultivation in the Netherlands shifted from open-field horticulture towards greenhouses. In 2006, the size of strawberry horticulture was estimated at 245 ha, divided among 710 greenhouses. The workforce consists of approximately 3,500 people.

Strawberry plants grow in rows in boxes at 150 cm height. Work activities comprise pulling blossoms of a

plant towards the pathway to improve exposure to light and to facilitate harvesting. These activities take place from April to June and from October to December. While performing these particular activities, large amounts of pollen are released. Though bumblebees and honeybees used for pollination reduce exposure [7], workers are likely to inhale a substantial amount of pollen. Employees are also exposed to the predatory mite *Amblyseius cucumeris*, which serves as biological pest control agent [4]. Furthermore, greenhouse humidity favors exposure to moulds, such as *Botrytis cinerea* [4, 8].

Despite potential allergen exposure, no occupational allergy in this branch of horticulture has been described before.

As some strawberry greenhouse workers reported symptoms suggestive of an occupational allergy to their occupational physician, an investigation of these symptoms and possible causes was carried out. First, we tried to estimate the prevalence of work-related symptoms in strawberry greenhouses using questionnaires. Second, we aimed to prove the concept that an IgE-mediated allergy could be responsible for work-related symptoms. Therefore, we tried to assess an IgE response to strawberry pollen in a few selected employees. In addition, we used a nasal provocation test, the golden standard in the diagnosis of occupational rhinitis [9], to test the clinical relevance of sensitization to strawberry pollen.

At this stage, we considered a positive challenge in a few patients would be sufficient to confirm the existence of a clinically relevant allergy. To test the possibility of an IgE response secondary to cross-reactivity to birch or grass pollen, additional inhibition experiments were performed. Confirmation of a new IgE-mediated allergy to strawberry pollen would be the starting point of future research to the contribution of this allergy to work-related symptoms.

Material and Methods

Questionnaire

A questionnaire survey was conducted among strawberry greenhouse employees in Brabant, a region in the south of the Netherlands, from October until December 2004. Strawberry growers from a list of addresses were contacted at random by telephone by trained personnel and asked to participate in the study. When an owner agreed upon participation, he or she was asked to record the number of employees enlisted who mastered the Dutch language. Subsequently, the questionnaires were sent by surface mail and distributed by the owners among all potential participants. In an attached letter, the aim of the study was explained, employees were asked for participation, and instruc-

tions were given on how to fill in the questionnaire. A response envelope was enclosed to return the questionnaire afterwards. The contents of the self-administered questionnaires were derived from an authorized practice guideline 'management of workers with asthma or COPD' issued by the Netherlands Society of Occupational Medicine [10] and supplemented with strawberry cultivation-specific questions. Questions concerned gender, age, smoking habit, job (characteristics), work history, symptoms at work, atopic symptoms, (chronic) airway symptoms, and family history of atopy. Symptoms present after occupational exposure comprised the following categories: itching, redness and swelling of the skin, rhinitis (sneezing/rhinorrhea/itching/obstruction), conjunctivitis (redness/itching/watery eyes), and asthma (wheezing/coughing/sputum/shortness of breath). Symptoms or exacerbation of symptoms during work were assumed to be related to work. Furthermore, regression upon homecoming, on weekends and holidays were considered to strengthen the possible relation with work. Confidentiality was maintained.

Statistical Analysis

Data were saved and handled statistically using Statistix for Windows, version 8.0 (Microsoft, The Netherlands). In statistical analysis, differences between continuous variables were tested with the unpaired Student's t test. Differences between frequencies of categorical variables were tested with the χ^2 test. The significance level α was 0.05.

In the second part of the study, 3 strawberry greenhouse employees, selected by the occupational physician because of a strongly suggestive history of an occupational allergy, were studied in detail. These 3 employees were included because of symptoms of rhinitis (i.e., nasal blockage, sneezing and watery discharge) during work and regression of these symptoms while being away from work. The medical history of these employees was recorded and allergy tests were performed.

Medical History

Patient A, a 46-year-old female joint owner of a strawberry greenhouse, with a history of respiratory symptoms after contact with dogs, was referred with work-related rhinoconjunctivitis, asthma and itchiness of the air-exposed skin. She had been performing production activities in the greenhouse since 1994, but because of the aforementioned symptoms she discontinued her occupation from 1998 until 2004. After returning to work, she switched to cleaning up the office and canteen while wearing a mask. The symptoms subsided and she scarcely needed antiallergic medication.

Patient B, a 52-year-old female employee, has been performing production activities since 1999. In 2003, she developed rhinoconjunctivitis on working days and itchiness of the skin upon contact with a strawberry plant. Symptoms partially improved when using antihistamines, which she obtained from the drugstore. Additionally, she mentioned oral allergy when eating strawberries and mild conjunctivitis due to grass.

Patient C, a 50-year-old woman who has been an employee since 1993, presented with an 8-year history of work-related rhinoconjunctivitis, red itchy macula of air-exposed skin and rarely symptoms of coughing at work, but no dyspnea. Treatment with antihistamine tablets, prescribed by her family doctor, gave no clear improvement.

Both patient B and C mentioned having work-related symptoms during periods of pulling blossoms, i.e. from April to June and from October to December.

Allergy Tests

Skin Tests

Skin tests were carried out according to international guidelines [11, 12] and performed by skilled allergy nurses. Test results were scanned with a standard scanning device and the wheal size in square millimeters was calculated by a software application [13]. Skin reactivity was expressed as the ratio between allergen and histamine wheal size (histamine-equivalent wheal size). Histamine-equivalent wheal size >0.22 in skin prick test (SPT) and >0.55 in intracutaneous test was regarded as positive. This classification is a modification of the grading system described by Niemeijer et al. [14]. In addition, reactions were expressed in mean wheal diameter (adding the longest diameter to the orthogonal diameter and dividing it by 2). A diameter of 3 mm or more was considered positive [15]. The skin test had to be positive using both methods.

Allergen Extracts

Pollen from strawberry plants were collected in a greenhouse, and since this activity was highly labor-intensive, the essential material was collected on several occasions. Strawberry pollen extracts were made in phosphate-buffered saline (PBS), pH 7.4, containing 0.03% human serum albumin and 0.5% phenol [2]. To prepare strawberry juice, strawberries were homogenized in a food processor, the slurry was filtered, and the fluid was subsequently passed through a 0.22- μ m filter (Millex GS, Millipore, The Netherlands). Protein concentrations were determined by the method of Watanabe et al. [16]. The concentrations of the strawberry pollen extract and strawberry juice were 0.25 and 1.1 g/l, respectively.

Predatory mites (*A. cucumeris*) were kindly supplied by Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands) and extracted according to Groenewoud et al. [4]. Appropriate aliquots were stored at -20°C , defrosted 1 h before skin test and mixed. Protein concentration was 1.05 g/l. All home-made extracts were skin prick tested.

In addition, skin tests were performed with *B. cinerea* (SPT: SQ 412), 1 of the moulds found in greenhouses and with 6 common inhalant allergens (intracutaneous test) from ALK Abelló (Nieuwegein, The Netherlands). The common inhalant allergens comprised *Dermatophagoides pteronyssinus*, pollen from grass and birch, mugwort and animal dander (dog and cat). Dilution buffer served as a negative control, histamine chloride (10 mg/ml) as a positive control.

Control Group

Ten control subjects allergic to grass pollen (9 of whom were also allergic to birch pollen), who had never been in contact with strawberry plants, were skin tested with strawberry pollen extract to investigate possible nonspecific irritative reactions of the strawberry pollen extract or cross-reactivity of strawberry pollen with grass or birch pollen.

ELISA (Inhibition)

Allergen-specific IgE to strawberry pollen was determined by ELISA.

100 mg strawberry pollen were incubated for 2 h at room temperature with 1 ml PBS. After 10 min centrifugation at 1,700 g, the supernatant was frozen at -20°C . The protein content of this supernatant was 2.18 g/l. 0.1 ml of a 1/100 dilution of this extract in coating buffer (50 mM carbonate buffer, pH 9.5, 0.1% NaN_3) was incubated in wells of a microtiter plate for 48 h at 4°C . After 3 washes with PBS, 0.05% Tween 20 (PBST), 0.1 ml of PBST, 1% bovine serum albumin, was added to each well. After 1 h incubation at room temperature, and 3 subsequent washes with PBST, 0.1 ml of the patient sera was added. After 2 h incubation at room temperature under rotation, and 3 subsequent washes with PBST, 0.1 ml alkaline phosphatase-labeled anti-IgE (BD Science San Diego, Calif., USA), diluted 1/1,000 in PBST, 1% bovine serum albumin, was added to each well. After 2 h incubation at room temperature under rotation, and 3 subsequent washes with PBST, 0.1 ml *p*-nitrophenyl phosphate substrate was added. After 2 h incubation in the dark, the absorbance was read at 405 nm. For ELISA-inhibition, birch pollen and *Dactylis glomerata* grass pollen (Allergon, Sweden) were extracted as described above for strawberry pollen. The protein content of these extracts was 0.69 and 0.71 g/l, respectively. For inhibition of the strawberry pollen ELISA, the 3 extracts were diluted in PBST to a final concentration of 0.1 g/l. 0.05 ml of these extracts was incubated for 90 min under continuous rotation at room temperature with 0.05 ml serum; 0.1 ml of this mixture was tested in the strawberry pollen ELISA as described above.

Ten sera with diverse levels of IgE against birch and/or grass pollen (as determined by CAP-RAST) were also tested in the strawberry pollen ELISA. A number of wells of the microtiter plate were not coated with strawberry pollen extract but with birch pollen extract. These wells were incubated with serial dilutions of a serum with a known level of specific IgE against birch pollen, quantitated by the Immuno CAP system. This dilution curve was used as standard curve, and made it possible to convert the ELISA signals into kilo-units per liter.

RAST

Allergen-specific IgE to *A. cucumeris* was determined by RAST by use of agarose beads as allergen support, with a modification of the procedure described previously [17].

CAP-RAST

Total IgE and specific IgE to common inhalant allergens, *B. cinerea* and strawberry juice were determined by the Immuno-CAP system (Phadia, Uppsala, Sweden).

Immunoblotting

Immunoblotting with strawberry pollen extract (100 mg extracted/ml PBS) was performed with a modification of the procedure as described previously [17]. Bound IgE was visualized with alkaline phosphatase-labeled anti-human IgE antibodies (BD Science, San Diego, Calif., USA) and BCIP/NBT substrate (Sigma Biochemicals, Zwijndrecht, The Netherlands).

For immunoblot inhibition, 4-fold dilutions of serum of both patients B and C in PBS were mixed with an equal volume of the pollen extracts that we used in ELISA (i.e., strawberry pollen, birch pollen and grass pollen), diluted in PBS to a final concentration of 0.1 g/l. Diluted sera were incubated for 90 min at room temperature under continuous rotation. Subsequent immunoblotting was performed as described above.

Nasal Provocation with Strawberry Pollen Extract

To determine the clinical relevance of sensitization to strawberry pollen, nasal provocations with strawberry pollen were performed as described by de Graaf in 't Veld [18]. Medication for rhinitis was withdrawn: nasal corticosteroids 3 weeks and antihistamines 3 days before the provocation. Nasal provocations were performed with 8 increasing doses of strawberry pollen extract (0.0000001–1%) at 10-min intervals after sham challenge with PBS containing human serum albumin 0.03% and benzalkonium chloride 0.05% (ALK Abelló). The strawberry pollen extract was sprayed into each nostril with a nasal pump spray delivering a fixed dose of 0.125 ml solution. The nasal response was measured 10 min after each step in the challenge by a symptom score according to Lebel et al [19]. This scoring system was graded in points and the total score ranged from 0 to 11 points. The various items were scored in the following manner: sneezing (0–2 times sneezing: 0 points; 3–4 times sneezing: 1 point; >5 times sneezing: 3 points), rhinorrhea (anterior rhinorrhea: 1 point; posterior rhinorrhea: 1 point; both anterior and posterior rhinorrhea: 2 points), difficulty in breathing (difficulty in breathing: 1 point; 1 blocked nostril: 2 points; both nostrils blocked: 3 points), and pruritus of nose (1 point), palate or ear (1 point) and conjunctivitis (1 point).

A score ≥ 3 (progressive) above PBS provocation was considered positive.

Three nonatopic volunteers, who had never been in contact with strawberry plants, were challenged intranasally with strawberry pollen extract to rule out an irritative reaction.

Results

Questionnaire

Population Characteristics

13/19 approached greenhouses (68%) agreed to participate. Reasons for refusal were lack of interest and absence of work-related symptoms. With 75/149 dispatched questionnaires returned, the response rate was 50.3%. Fifty-two of these subjects (69.3%) were female and the mean age was 43.7 years (range 13–74). The majority (80%) of workers was engaged as employee and the mean duration of employment was 8.6 years, with a mean working week of 24 h. Twenty-nine of 75 respondents (38.7%) reported 1 or more symptoms attributable to the workplace. Population characteristics between this group and the group without these symptoms were not significantly different, except for the mean hours of work per week, which were 17.3 and 28.4 h, respectively ($p < 0.001$).

Presence of Work-Related Allergic Symptoms

Work-related symptoms consisted of rhinitis (30.7%), conjunctivitis (23.3%), asthma (4.2%) or skin symptoms (16.7%). 77.8% noted an improvement while being away from work. As a possible cause of symptoms, the straw-

berry plant, strawberry pollen, sulfur, or biological control agents were mentioned by the respondents. Pulling blossoms of a strawberry plant towards the pathway and picking strawberries were 2 of the activities held responsible for symptoms.

Determinants of Work-Related Allergic Symptoms

The variables seasonal or perennial rhinoconjunctivitis were statistically associated with work-related symptoms ($p < 0.001$). Smoking, (chronic) airway symptoms, family history of atopy, on the other hand, were not significantly associated with work-related symptoms.

Skin Tests

All 3 employees who were studied in detail in the second part of the investigation showed positive reactions in SPTs with home-made strawberry pollen extract. Histamine-equivalent wheal size and mean wheal diameter were 1.54, 0.33, and 1.62 and 9, 5, and 8 mm in patient A, B, and C, respectively. SPTs with strawberry pollen were negative in all control subjects.

ELISA and Immunoblotting

Strawberry pollen-specific IgE could be demonstrated by ELISA in 2 cases (patients B and C) (fig. 1). Nine of 10 sera with diverse levels of IgE against birch and/or grass pollen (range 0.1–96.9 kU/l in the grass pollen CAP-RAST, range 0.1–54.9 kU/l in the birch pollen CAP-RAST) scored less than 0.35 kU/l in the strawberry pollen ELISA; 1 serum with <0.1 kU/l IgE against grass pollen, 7.08 kU/l against birch pollen and a total IgE of 71 kU/l scored 0.50 kU/l in the strawberry pollen ELISA. When quantifying the ELISA measurements, sera of patients A, B, and C were found to contain, respectively, <0.10 , 1.21 and 6.67 kU/l of specific IgE against strawberry pollen. The sera of patient B and C revealed a positive IgE response to strawberry pollen in immunoblotting with molecular weight bands between 18 and 50 kDa (fig. 2). ELISA and immunoblotting were negative in the control sera (fig. 1, 2, respectively).

ELISA Inhibition (fig. 3). IgE binding to strawberry pollen in serum from both patients B and C could almost completely be inhibited by preincubation with strawberry pollen extract. In serum from patient C, no inhibition was observed after preincubation with grass or birch pollen, whereas preincubation of serum from patient B with grass and birch pollen extract gave more than 50% inhibition of IgE binding.

Immunoblot Inhibition. Whereas the IgE binding to serum of patient C could be completely inhibited after

Fig. 1. IgE binding to strawberry pollen in ELISA. From left to right in duplo, respectively. Blank; patient A (total IgE 17 kU/l); patient B (total IgE 187 kU/l); patient C (total IgE 670 kU/l); negative control subject 1 (total IgE 14 kU/l); negative control subject 2 (total IgE 209 kU/l); negative control subject 3 (total IgE 785 kU/l) 3. The absorbance was read at 405 nm.

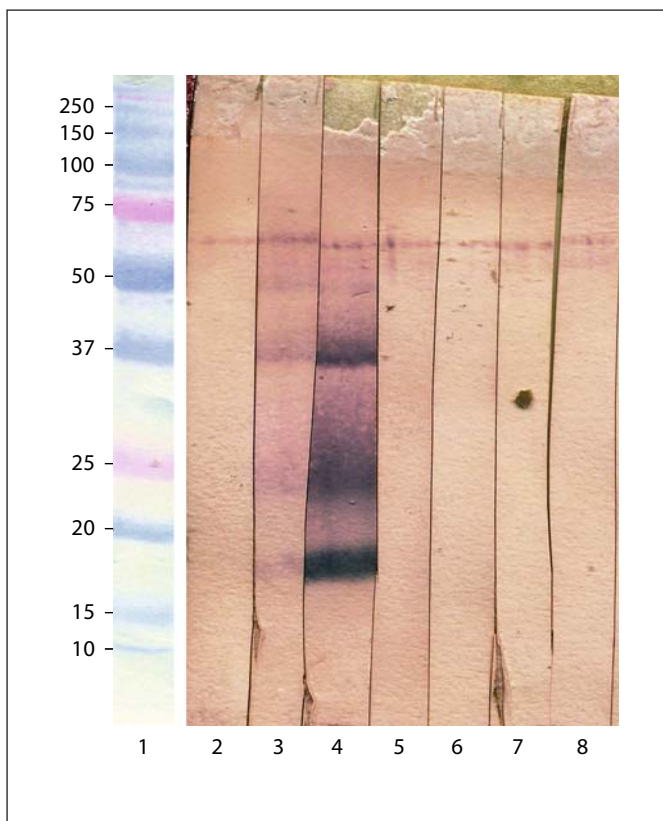
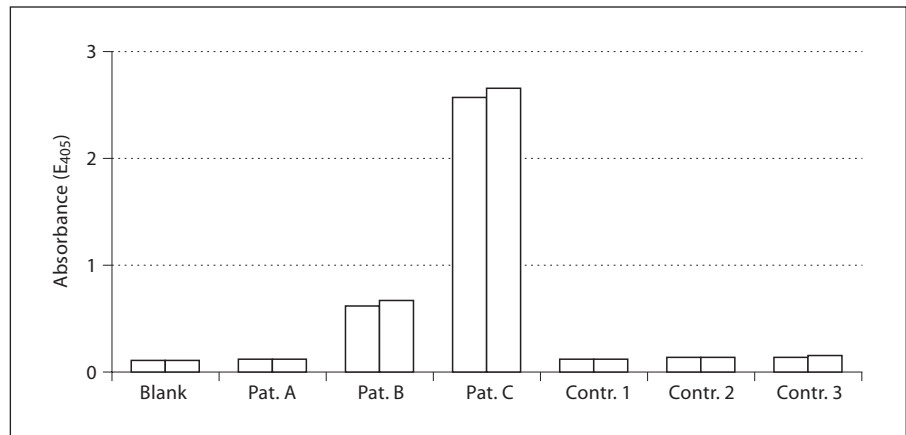


Fig. 2. Immunoblotting with strawberry pollen extract. Molecular weight bands are shown to the left (kDa); lane 1: molecular weight standard; lane 2: serum of patient A (total IgE 17 kU/l); lane 3: serum of patient B (total IgE 187 kU/l); lane 4: serum of patient C (total IgE 670 kU/l); lanes 5–8: serum of 4 subjects without an occupational history in strawberry horticulture and with total IgE scores of 18, 153, 1,586, 1,670 kU/l in patient 5, 6, 7, 8, respectively.

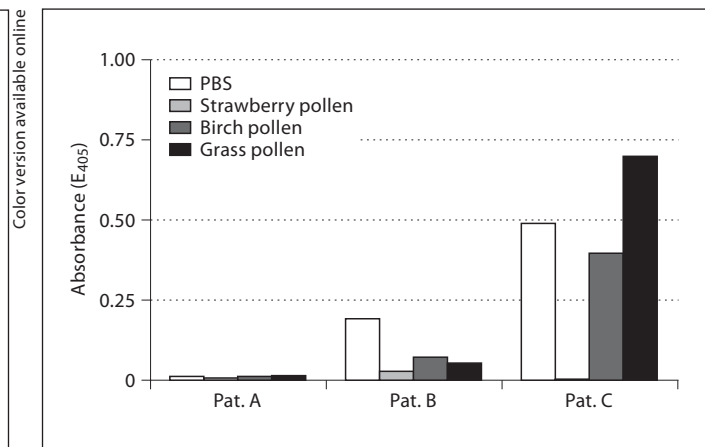


Fig. 3. ELISA inhibition experiments. Inhibition experiments are shown from left to right in patient A–C, respectively. The sera were inhibited with PBS, strawberry pollen extract, birch pollen and grass pollen extract. The absorbance was read at 205 nm.

preincubation of this serum with strawberry pollen extract, no inhibition was seen after preincubation with grass and birch pollen extract (fig. 4). Immunoblot inhibition experiments with serum of patient B, which gave a rather weak binding in the strawberry immunoblot, were inconclusive (data not shown).

Nasal Provocation. Nasal provocation with strawberry pollen was positive in 2 employees (fig. 5; patients A and C). Symptoms during provocation were comparable with symptoms at work. Nasal allergen provocation was negative in 3 nonatopic volunteers (fig. 5; control 1–3).

All 3 employees were atopic; 1 of the employees exhibited a positive IgE response to dog dander (patient A), whereas the other 2 employees were sensitized to grass

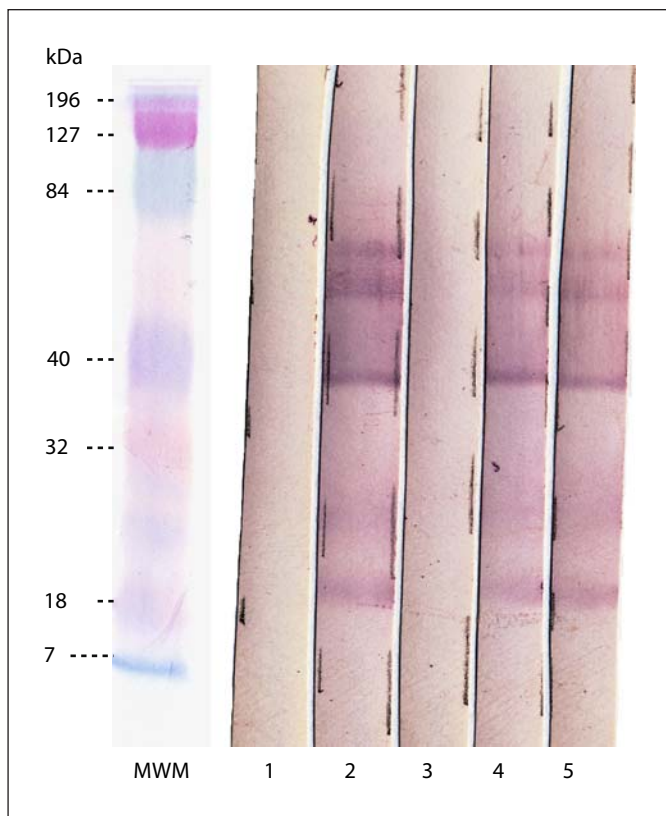


Fig. 4. Immunoblot inhibition experiment in patient C. Molecular weight bands are shown to the left (kDa); lane 1: PBS; lanes 2–4: serum from patient C with immunoblotting with strawberry extract after preincubation with PBS (2), strawberry pollen extract (3), birch pollen extract (4) and grass pollen extract (5), respectively. MWM = Molecular weight marker.

pollen (patient C) or to both grass and birch pollen (patient B). Additionally, patient B and C revealed a positive response to strawberry juice in both SPT and CAP-RAST (2.36 and 1.93 kU/l, respectively). No sensitization to *A. cucumeris* or *B. cinerea* was found.

Lung Function Tests

Spirometry and histamine bronchial challenge were performed in all 3 employees, and no bronchial obstruction or airway hyperresponsiveness was found.

Discussion

Due to a shift from open-field horticulture towards greenhouses, employees in strawberry horticulture are highly exposed to several (potential) allergenic agents

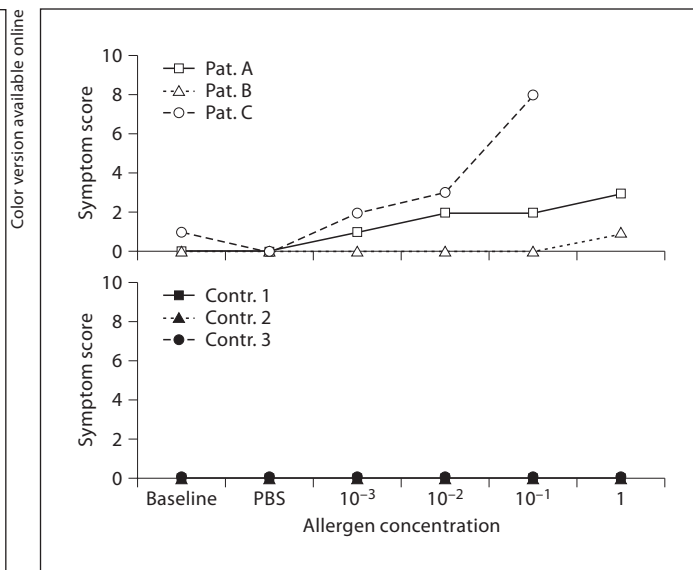


Fig. 5. The upper part shows the response to nasal provocation with increasing doses of strawberry pollen in patients A–C and the lower part shows the response of 3 nonatopic volunteers; control 1, 2 and 3. Eight increasing doses of strawberry pollen extract (10^{-7} to 10^0) were used.

[20]. However, besides 2 case-reports on contact urticaria in strawberry pickers [21, 22], no occupational allergy in this branch of crop-growing has been described before.

The strawberry plant (*Fragaria ananassa*) is a member of the Rosaceae family. Allergic symptoms to another member of this family, i.e. rose (and its pollen), among villagers cultivating roses have been described by Demir et al. [23]. Food allergy to related fruits, for example apple and peach, is better known [24].

This study concerned the initial research phase regarding a possible occupational allergy in the strawberry greenhouse environment. It comprised two parts; first, the questionnaire survey demonstrated that work-related symptoms suggestive of an occupational allergy occur among employees. The allergy tests, especially the nasal provocation tests, in the second part of the study, showed that symptoms can be caused by a strawberry pollen allergy.

Another research phase is required to determine disease prevalence and incidence by means of a larger cross-sectional study and a longitudinal study among apprentices, and to demonstrate pollen in the air of the strawberry greenhouse environment.

Although questionnaires concerning occupational (allergy and) asthma have not been validated yet [1, 25],

questionnaires in general are a fundamental tool to determine health outcomes and exposure to external agents in epidemiologic studies [26]. In the epidemiological part of the study, 38.7% of respondents mentioned work-related allergic symptoms, and insight into the strawberry greenhouse environment with its possible allergenic agents was provided as well.

Thus, the questionnaire shows that work-related allergic symptoms possibly due to an occupational allergy are present among strawberry greenhouse workers in the Netherlands. On the basis of these data, it is, however, not possible to estimate the true prevalence of these symptoms. First, the response rate of the questionnaire was 50.3%, and therefore nonresponse bias could have occurred. Not only the low response rate, but also the cross-sectional design of the study can cause selection bias. In contrast to a longitudinal study, symptoms can be underestimated [1]. Furthermore, the sensitivity of medical questionnaires (in general) is high, whereas the specificity is low and false-positive results can occur [1, 25, 27].

Symptoms of the upper airway system are more pronounced than complaints of the lower airway system. This matches with findings of earlier reports in the literature [3, 6, 28, 29]. A possible explanation for the finding that workers with symptoms work significantly less hours per week than those without is that the former group shortens the workweek to avoid exposure thereby reducing the symptoms. Further, atopic people have an increased risk of developing work-related symptoms while being exposed to high-molecular-weight antigens [3, 30]. The association between 'complaints of seasonal or perennial rhinoconjunctivitis' and work-related complaints supports this statement. However, occurrence of seasonal or perennial rhinoconjunctivitis does not differentiate between manifestation of atopy or work-related complaints, and therefore caution is required when drawing a conclusion from this finding.

In the second part of the study, sensitization to strawberry pollen in all 3 strawberry greenhouse employees with work-related allergic symptoms was demonstrated with SPTs. Furthermore, ELISA and immunoblotting showed positive results in 2 out of 3 employees. Moreover, the intensity of the bands corresponded with the levels of specific IgE.

Since none of the birch pollen-allergic and grass pollen-allergic control subjects showed a positive reaction in SPTs with strawberry pollen, irritative reactions were ruled out and cross-reactivity was considered unlikely. Furthermore, 9 of 10 sera with IgE against birch and/or

grass pollen were negative in the strawberry pollen IgE ELISA; 1 serum was borderline positive.

In patient C, neither the ELISA nor the immunoblot were inhibited by grass and or birch pollen, indicating that the sensitization to strawberry pollen in this specific patient was not caused by a sensitization to birch or grass pollen originally but that there is a true primary sensitization to the strawberry pollen. In patient B, the ELISA shows partial inhibition and partial cross-reactivity may be conceivable in this patient. Taken together, the experiments point at primary sensitization to strawberry pollen. This is also underlined by the absence of skin reactions to strawberry pollen in control subjects sensitized to birch or grass pollen and by the absence of IgE binding to strawberry pollen in sera with IgE against birch/and or grass pollen. On the other hand, partial cross-reactivity with other pollens in some subjects might be possible.

The intra- and interindividual differences in different allergy tests could possibly be partly explained by the fact that standardized strawberry extracts were not available. Pollens from flowers of strawberry plants were obtained from a greenhouse on several occasions. During 1 of these visits, collecting pollen for subsequent testing was hampered by the fact that climate conditions in the greenhouse were suboptimal, i.e., greenhouse temperature was below normal and humidity was high.

Measurements of airborne pollen were not performed. Symptoms were, however, specifically mentioned during periods with pollen release, i.e. from April to June and from October to December.

Two patients in our study exhibited positive IgE responses to strawberry juice, and this sensitization was clinically relevant in 1 patient, as she mentioned symptoms of oral allergy while eating strawberries (patient B). The same patient was also sensitized to birch pollen. Musidłowska-Persson et al. [31] described homology between Bet v 1 and Fra a 1 allergen in strawberry juice. Since no RAST inhibition experiment with Bet v 1 and strawberry juice was performed, cross-reactivity between birch pollen and strawberry juice cannot be ruled out in this particular patient.

Because of the differences in sensitization route, an inhalation allergy presumably leads to a different sensitization pattern than food allergic reactions [3, 17]. Corecognition between strawberry pollen and strawberry juice is, however, conceivable. Zuidmeer et al. [32] demonstrated that strawberry also contains allergens with high molecular weights (20–28, 40–80 kDa).

The strawberry pollen immunoblots in our patients showed comparable weight bands. Since inhibition studies have not been performed, no conclusion can be drawn on cross-reactivity between strawberry pollen and juice.

In conclusion, we demonstrated that work-related symptoms are clearly present among strawberry greenhouse workers. An IgE-mediated occupational allergy to strawberry pollen may contribute to these symptoms.

Acknowledgements

We sincerely thank Theo Roovers, allergologist, Department of Allergology, St. Elisabeth Hospital, Tilburg, The Netherlands for performing one of the nasal provocation tests in his clinic. Gerard Klein Heerenbrink, laboratory technician from the Department of Clinical Chemistry, Erasmus MC, Rotterdam, The Netherlands, is also gratefully acknowledged for performing the immunological tests.

References

- Gautrin D, Newman-Taylor AJ, Nordman H, Malo JL: Controversies in epidemiology of occupational asthma. *Eur Respir J* 2003;22: 551–559.
- de Jong NW, Vermeulen AM, Gerth van Wijk R, de Groot H: Occupational allergy caused by flowers. *Allergy* 1998;53:204–209.
- Groenewoud GC, de Jong NW, van Oorschot-van Nes AJ, Vermeulen AM, van Toorenenbergen AW, Mulder PG, Burdorf A, de Groot H, Gerth van Wijk R: Prevalence of occupational allergy to bell pepper pollen in greenhouses in the Netherlands. *Clin Exp Allergy* 2002;32:434–440.
- Groenewoud GC, de Graaf in 't Veld, vVan Oorschot-van Nes AJ, de Jong NW, Vermeulen AM, van Toorenenbergen AW, Burdorf A, de Groot H, Gerth vWijk R: Prevalence of sensitization to the predatory mite *Amblyseius cucumeris* as a new occupational allergen in horticulture. *Allergy* 2002;57:614–619.
- Kronqvist M, Johansson E, Kolmodin-Hedman B, Oman H, Svartengren M, Hage-Hamsten M: IgE-sensitization to predatory mites and respiratory symptoms in Swedish greenhouse workers. *Allergy* 2005;60:521–526.
- Hermanides HK, Lahey-de Boer AM, Zuidmeer L, Guikers C, van Ree R, Knulst AC: *Brassica oleracea* pollen, a new source of occupational allergens. *Allergy* 2006;61:498–502.
- de Jong NW, van der Steen JJ, Smeekens CC, Blacquiere T, Mulder PG, Gerth van Wijk R, de Groot H: Honeybee interference as a novel aid to reduce pollen exposure and nasal symptoms among greenhouse workers allergic to sweet bell pepper (*Capsicum annuum*) pollen. *Int Arch Allergy Immunol* 2006;141: 390–395.
- van Hage-Hamsten M, Kolmodin-Hedman B, Johansson E: *Phytoseiulus persimilis* and *Amblyseius cucumeris*, used for biological crop protection, cause sensitization among greenhouse workers. *Allergy* 2000;suppl:55–30.
- Moscato G, Vandenplas O, Gerth van Wijk R, Malo JL, Quirce S, Walusiak J, Castano R, de Groot H, Folletti I, Gautrin D, Yacoub MR, Perfetti L, Siracusa A: Occupational rhinitis. *Allergy* 2008;63:969–980.
- NVAB: 'Management of workers with asthma or COPD.' Authorized Practice Guideline (in Dutch). Hulshof, CTJ, NVAB (Netherlands Society of Occupational Medicine), 2003.
- Dreborg S: The skin prick test in the diagnosis of atopic allergy. *J Am Acad Dermatol* 1989;21:820–821.
- Norman PS, Rose NR, Friedman H: Skin testing; in *Manual of Clinical Immunology*. Washington, American Society for Microbiology, 1980, pp 789–793.
- de Jong NW, Hoorn E, de Groot H, Gerth van Wijk R: Estimation of HEIC-index of ICT and SPT reactions with a scanning program. *Analyse* 2003;9:261–265.
- Niemeijer NR, Fluks AF, de Monchy JG: Optimization of skin testing. II. Evaluation of concentration and cutoff values, as compared with RAST and clinical history, in a multicenter study. *Allergy* 1993;48:498–503.
- Dreborg S, Frew A: Allergen standardization and skin tests. Allergy position paper, European Academy of Allergology and Clinical Immunology, 1993;48(suppl):49–75.
- Watanabe N, Kamei S, Ohkubo A, Yamanaoka M, Ohsawa S, Makino K, Tokuda K: Urinary protein as measured with a pyrogallol red-molybdate complex, manually and in a Hitachi 726 automated analyzer. *Clin Chem* 1986;32:1551–1554.
- van Toorenenbergen AW, Waanders J, Gerth van Wijk R, Vermeulen AM: Immunoblot analysis of IgE-binding antigens in paprika and tomato pollen. *Int Arch Allergy Immunol* 2000;122:246–250.
- de Graaf in 't Veld, Garrelds IM: Nasal Hyperreactivity and Inflammation in Perennial Allergic Rhinitis; thesis, Rotterdam 1995.
- Lebel B, Bousquet J, Morel A, Chanal I, Godard P, Michel FB: Correlation between symptoms and the threshold for release of mediators in nasal secretions during nasal challenge with grass-pollen grains. *J Allergy Clin Immunol* 1988;82:869–877.
- Monso E: Occupational asthma in greenhouse workers. *Curr Opin Pulm Med* 2004; 10:147–150.
- Grattan CE, Harman RR: Contact urticaria to strawberry. *Contact Dermatitis* 1985;13: 191–192.
- Weltfriend S, Kwangskuthith C, Maibach HI: Contact urticaria from cucumber pickle and strawberry. *Contact Dermatitis* 1995;32: 173–174.
- Demir AU, Karakaya G, Kalyoncu AF: Allergy symptoms and IgE immune response to rose: an occupational and an environmental disease. *Allergy* 2002;57:936–939.
- Crespo JF, Rodriguez J, James JM, Daroca P, Reano M, Vives R: Reactivity to potential cross-reactive foods in fruit-allergic patients: implications for prescribing food avoidance. *Allergy* 2002;57:946–949.
- Moscato G, Malo JL, Bernstein D: Diagnosing occupational asthma: how, how much, how far? *Eur Respir J* 2003;21:879–885.
- Weisel CP, Weiss SH, Tasslimi A, Alimokhtari S, Belby K: Development of a Web-based questionnaire to collect exposure and symptom data in children and adolescents with asthma. *Ann Allergy Asthma Immunol* 2008;100:112–119.
- Nicolson PJ, Cullinan P, Newman Taylor AJ, Burge PS, Boyle C: Evidence-based guidelines for the prevention, identification and management of occupational asthma. *Occup Environ Med* 2005;62:290–299.
- Bousquet J, Vignola AM, Demoly P: Links between rhinitis and asthma. *Allergy* 2003; 58:691–706.
- Malo JL, Lemièrre C, Gautrin D, Labrecque M: Occupational asthma. *Curr Opin Pulm Med* 2004;10:57–61.
- Petsonk EL: Work-related asthma and implications for the general public. *Environ Health Perspect* 2002;110(suppl):569–572.
- Musidlowska-Persson A, Alm R, Emanuelsson C: Cloning and sequencing of the Bet v 1-homologous allergen Fra a 1 in strawberry (*Fragaria ananassa*) shows the presence of an intron and little variability in amino acid sequence. *Mol Immunol* 2007;44:1245–1252.
- Zuidmeer L, Salentijn E, Rivas MF, Mancebo EG, Asero R, Matos CI, Pelgrom KT, Gilissen LJ, van Ree R: The role of profilin and lipid transfer protein in strawberry allergy in the Mediterranean area. *Clin Exp Allergy* 2006; 36:666–675.