

Original article

Brassica oleracea pollen, a new source of occupational allergens

Background: Vegetable pollen is a rare source of occupational allergens. Occupational allergy has only been described in the case of paprika pollen and tomato pollen. We describe a new source of occupational pollen allergy.

Aim: To study the incidence and the impact of broccoli and cauliflower pollen allergy in employees involved in classical plant breeding.

Methods: Fifty-four employees of five companies working with cauliflower (*Brassica oleracea botrytis*) and broccoli (*B. oleracea italica/cymosa*) pollen were eligible for complete evaluation. Allergy to cauliflower and broccoli pollen was evaluated by questionnaire and determination of sensitization by radioallergosorbent test (RAST) and skin-prick tests (SPT). SPT and RAST were performed with a panel of commercial and homemade extracts from cauliflower and broccoli pollen.

Results: Work-related symptoms such as rhinitis, conjunctivitis, asthma and urticaria caused by *B. oleracea* pollen were reported by 44% of the participants (24/54), of whom all but one had positive SPT for cauliflower- and/or broccoli-pollen/flower extracts and 58% (14/24) had positive RAST results. Symptoms had developed within the first 2 years in 33% of the patients. Six patients had to stop or change work.

Conclusions: *Brassica oleracea* pollen is a new source of occupational allergen with strong allergenic potential leading to symptoms in almost half of the exposed employees.

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Cauliflower (*Brassica oleracea botrytis*) is one of the most important vegetable crops in the Netherlands. Both cauliflower and broccoli (*B. oleracea italica/cymosa*) belong to the Brassicaceae family (Cruciferae). Flowers of *Brassica* plants develop after the crop and are pollinated by insects. They produce small amounts of airborne pollen of 19–24 µm diameter.

Shoots of *B. oleracea* develop out of a full-grown crop and give rise to yellow or white flowers. During growth and production of cauliflower and broccoli as vegetables, the workers do not come into contact with the flowers because the vegetables are harvested before flowering. However, in plant improvement and seed production activities there is scope for close contact. Classical plant breeding has become very important in horticulture in the Netherlands. For crossbreeding of different species, pollen of the filament of one flower is placed on the pistil of another, requiring the close contact of the employees with the flowers. An IgE-mediated inhalant allergy can be expected, as has been described for paprika pollen (1).

A visit of an employee, working in *B. oleracea*, to our outpatient department with progressive rhino-conjunctival symptoms and shortness of breath in the last 3 years, raised the question about the prevalence of work-related

symptoms to cauliflower and broccoli pollen. As the employee had positive SPT to cauliflower and Brussels sprouts pollen, a type I allergy was likely.

Allergy to vegetable pollen has been reported only rarely. In a study of sweet bell pepper workers in a greenhouse, symptoms were reported in 54% (1). The only other case is a patient with tomato pollen allergy (2). The aim of this study was to evaluate the allergenic potential of *B. oleracea* pollen among classical plant breeders and to analyse possible risk factors.

Material and methods

Study design and patient selection

A cross-sectional study was carried out from December 2004 to April 2005. The inclusion criterion was that a person should have worked with *B. oleracea* pollen for at least one season. Continuous use of antihistamines and presence of cholinergic urticaria were the exclusion criteria. The local ethics committee approved the study. All participants gave written informed consent. Thirteen companies working with *B. oleracea* species were approached. Six companies with 68 volunteers participated. Seven companies could not participate because there were no breeding activities, no *B. oleracea* plants during the study period or there was no interest in participation. Participation forms were sent to a contact person who distributed

them among regular breeder employees working with *B. oleracea* pollen. The laboratory employees and the seasonal workers were not approached. Approximately 30–87% of the regular employees who worked with *B. oleracea* pollen (senior and assistant breeders, pollinators and vegetation providers) were willing to participate.

During the first visit, cauliflower and broccoli flowers, as only flowering *B. oleracea* species present, were collected to make extracts for the skin-prick test (SPT). During the following visits, all the participating subjects underwent SPT and blood samples were collected for radioallergosorbent (RAST) tests. Nine of 68 persons who filled in the participation form could not participate in the study for different reasons such as illness, lack of interest, holiday, exclusion criteria or not fulfilling the inclusion criterion. One company with a total of five persons willing to participate was not visited because of the long distance. In total, 54 persons belonging to five different companies in the Netherlands were eligible for complete evaluation of cauliflower and broccoli pollen allergy, using history, SPT and RAST.

Questionnaire

The questionnaire involved general questions concerning gender, age, smoking habit, medication use, common inhalant allergies and atopic history. Specific questions dealt with job activities, working history and work-related symptoms that were divided into four categories: nose, eye, lower airway and skin symptoms. Work-related symptoms were considered likely if symptoms started during the flowering season of cauliflower and broccoli and diminished or disappeared during weekends or holidays.

Skin-prick test

Skin-prick test was performed according to Dreborg and Frew (3) and reactivity was calculated using computer scanning (4). SPT was considered positive when the quotient of the wheal area of the allergen extract and that of the histamine control was at least 0.25 (5). Atopy was defined by one of the following criteria: the presence of allergic rhinitis, conjunctivitis, allergic asthma or eczema and positive SPT to the common inhalant allergens, or a convincing history of seasonal rhino-conjunctivitis or asthma without sensitization as was the case in four patients (4/54).

Allergen extracts

Extracts from cauliflower and broccoli flowers were prepared using whole flowers including the pollen, according to de Jong et al. (6). In short, flowers were collected from a glasshouse in December 2004 and dried using a water absorber, in a closed box for 2 weeks. The flowers were subsequently suspended in phosphate-buffered saline (PBS) (pH 7.4), containing 0.03% human serum albumin (HSA) and 0.5% phenol (PBS). The extracts were centrifuged for 10 min at 2000 g before and after dialysing against distilled water for 5 days. Despite this rather long dialysis period, there was no allergen degradation because the SPT extract performed extremely well. After centrifugation, the extracts were freeze-dried overnight and stored at 4°C until further use for a maximum of 4 weeks. To prepare the SPT extracts, freeze-dried material was dissolved in the negative control solution and the supernatants were sterilized through a 0.22-µm Millex GS filter. The SPT extracts were prepared in three concentrations: 0.05, 0.50 and 5.00 mg/ml, a day to 1 week before use, and stored at 4°C. The highest concentration was used for further evaluation of the results. Commercial inhalant allergens including birch pollen, grass pollen, mugwort pollen, and house dust mite were purchased from ALK-ABELLO (Nieuwegein, the Netherlands).

Radioallergosorbent test

Specific IgE levels for broccoli and cauliflower pollen were determined by radioallergosorbent test (RAST) as described previously (7, 8). Calculation was performed by means of a standard curve that was obtained by RAST with a dilution series of a chimeric monoclonal IgE antibody against the major house dust mite Der p 2 and sepharose-coupled recombinant Der p 2 (9). For statistical analysis, a result >0.33 IU/ml was regarded as positive.

Statistical analysis

Various variables were tested by statistical analysis, e.g. individual characteristics, work history and symptoms. Possible risk factors like smoking and atopy were evaluated by the Fisher's exact test. Results were considered to be significant when $P < 0.05$.

Results

Participant characteristics

All 54 participants were working with Brassicaceae flowers for a mean period of 13 seasons (range 1–31). The mean age was 44 years (range 22–61), and 48% were male (26/54). All participants were regular employees working full time or part time.

Participants were divided into two groups: those with work-related symptoms and those without work-related symptoms. No significant age differences were seen between both groups. Sex distribution (female vs male) was 10 vs 14 in the group with work related symptoms and 18 vs 12 in the group without work related symptoms (Table 1).

Work-related symptoms and duration of exposure

Symptoms at work caused by *B. oleracea* pollen were reported by 44% of the employees (24/54). Rhinitis was the main work-related symptom and was reported by 96% (23/24) followed by conjunctivitis in 75% (18/24). Airway and skin symptoms were reported in 38% (9/24) and 42% (10/24), respectively (Table 2). There was no relation between the specific job and the chance of developing work-related symptoms (data not shown).

Table 1. Participants' characteristics ($n = 54$)

Characteristics	Yes ($n = 24$)	No ($n = 30$)	Total ($n = 54$)
Work-related symptoms			
Male	14	12	26
Various symptoms caused by <i>B. oleracea</i> pollen	24	2	26
Atopy	16	11	27
Smoking	7	2	9
Sensitization			
Cauliflower and/or broccoli pollen	23	4	27
Cauliflower and/or broccoli (vegetables)	10	3	13

Table 2. Characteristics of participants with work-related symptoms, results of the SPT and RAST to cauliflower (ca) and broccoli (b) pollen/flower extracts

Participants	Sex	Age	Exposure	Development of symptoms (years)	Still working	Symptoms	Atopy	SPT						RAST	
								Birch	Grass	Mugwort	<i>D. pteronyssinus</i>	Cauliflower	Broccoli	Cauliflower	Broccoli
1	F	52	ca, b, oth	3	+	c, r, d, w, co, e, u, i	+	0.71	0.75	0.48	—	2.12	0.92	17.1	19.17
2	M	49	ca, b, oth	1	+	c, r, d, w, co, s, i	+	—	0.39	—	—	0.73	0.54	8.85	6.48
3	F	47	ca, b, oth	2	0	r, c, co, e, u, i	+	0.91	0.93	—	—	0.92	0.63	0.45	0.65
4	M	38	ca, oth	0	0	c, r, a, u, i	+	—	0.97	—	—	0.46	0.37	—	—
5	F	48	ca, b, oth	6	+	r, c, co, d, w, s, as, i, u	+	0.82	0.92	0.43	—	1.62	0.84	5.0	6.01
6	M	34	ca, b, oth	1	+	c, r	+	—	—	—	0.63	0.79	0.91	0.65	1.01
7	M	35	oth	0	+	r	+	0.66	0.96	—	1.84	0.55	0.65	—	—
8	M	37	ca, b, oth	3	+	c, r, d, w, er, i	+	0.85	—	—	0.55	1.61	1.70	2.86	2.99
9	F	60	ca, b, oth	5	+	c, r	+	—	0.27	0.79	0.29	0.98	1.27	1.89	1.89
10	M	28	b, oth	0	+	r	+	—	0.38	0.94	1.46	0.37	0.41	2.00	2.81
11	F	42	ca, b, oth	1	+	c, r	+	—	—	—	—	0.51	0.35	—	—
12	M	46	ca, b, oth	1	0	r, c, co	0	—	—	—	—	2.01	1.74	2.11	2.35
13	F	22	ca, b, oth	3	+	c, r, u	+	—	—	—	0.56	1.28	2.14	60.37	86.91
14	F	27	ca, b, oth	3	+	r	+	—	—	—	0.32	1.07	1.08	0.46	—
15	M	55	ca, b, oth	21	+	r, c, co	+	0.31	—	—	—	—	0.33	—	—
16	M	44	oth	10	+	r	+	—	—	—	—	0.64	—	—	—
17	M	29	ca, b, oth	3	+	c, r, er, i	0	—	—	—	—	0.75	0.49	9.99	9.92
18	F	38	ca, b, oth	8	+	c, r, i, er	+	—	—	—	—	1.28	1.12	4.84	7.88
19	F	55	ca, b, oth	0	+	c, r	0	—	—	—	—	1.44	0.69	1.32	2.39
20	M	55	ca, b, oth	15	0	c, r	0	—	—	—	—	0.5	0.56	—	—
21	F	57	?	6	0	c, r	0	—	—	—	—	0.65	0.54	—	—
22	M	48	ca, b, oth	3	0	c, r, i, er, co, s	0	—	—	—	—	0.29	—	—	—
23	M	46	ca, b, oth	20	+	d, w	+	—	—	—	0.64	—	—	—	—
24	M	38	ca, b, oth	±5(?)	+	r	0	—	—	—	—	0.56	0.52	—	—

+, yes; 0, no; —, <0.25 by SPT or <0.33 by RAST.

ca, cauliflower; b, broccoli; oth, other *B. oleracea* species: Brussels sprouts; kale; red cabbage; savoy cabbage; green cabbage; white cabbage.

as, asthma; c, conjunctivitis; co, cough; d, dyspnoe; e, eczema; er, erythema; i, itching; r, rhinitis; s, sputum; u, urticaria; w, wheezing; *D. pteronyssinus*, *Dermatophagoides pteronyssinus*.

?, unknown.

Specific work-related symptoms developed after an average of 4 years (range 0–21); and in 33% of the participants (8/24), they developed within the first 2 years. Four participants had to change to other jobs within the same companies. Two other persons limited their contact with *B. oleracea* pollen to a minimum.

Eating (raw) cabbages did not cause symptoms in any of the participants. Part of the symptomatic employees had positive SPT to broccoli and/or cauliflower as vegetables, indicating the presence of common allergens both in flowers and vegetable (data not shown).

Risk factor

Of the study population, 50% (27/54) was atopic. Of these 27 atopic participants, 59% (16/27) had work-related symptoms and were sensitized to cauliflower and/or broccoli pollen. One person had work-related symptoms but was not sensitized, in contrast to 27 non-atopic participants of whom 26% (7/27) had work-related symptoms and were sensitized to cauliflower and/or broccoli pollen. A clear association was found between atopy and work-related symptoms. Atopy appeared to be a significant risk factor ($P = 0.027$). In contrast, smoking was not a risk factor.

Sensitization

Of all participants, 39% (21/54) were found to be sensitized by SPT to cauliflower and broccoli pollen/flower extract, 4% (2/54) had positive SPT for broccoli pollen only, and 4% (2/54) for cauliflower pollen only. RAST for both broccoli and cauliflower pollen was positive in 28% (15/54) of the participants, whereas 6% (3/54) had only specific IgE to cauliflower. RAST values varied from 0.32 to 60 IU/ml.

Of the symptomatic breeder employees ($n = 24$), 96% (23/24) had positive SPT to broccoli and/or cauliflower pollen and 58% (14/24) had a positive RAST, in contrast to the group without work-related symptoms ($n = 30$) of which 14% (4/30) had positive SPT or RAST. Sensitization appeared to be long-lasting. Of the four participants who stopped working 5–20 years earlier, all were still found to be sensitized by SPT and one also by RAST.

Discussion

In this study, we found a high percentage (44%) of allergy to cauliflower and broccoli pollen. A positive history with rhinitis as the main work-related symptom followed by

conjunctivitis was supported by sensitization to cauliflower and/or broccoli pollen by SPT (96%) and RAST (58%). The high percentage found in this study could be an overestimation because employees with symptoms might be more willing to participate. On the other hand an underestimation is also possible, as most of the workers in this industry are seasonal workers who tend to change their job when symptoms occur. Groenewoud et al. (1) reported an even higher percentage of 54% (254/472) of allergy to sweet bell pepper pollen.

Allergy caused by vegetable pollen seems to be very rare. The only other example in the literature was occupational asthma by a woman working in a tomato glasshouse (2). Occupational asthma among greenhouse employees working with (flowering) vegetables is more frequently reported, but in relation to allergy for predatory mites or thrips (10–12). In our study, three persons reported the presence of mildew (*Erysiphe cruciferarum*) or false mildew (*Peronospora parasitica*) as a cause of their symptoms. All three however were sensitized to cauliflower and/or broccoli pollen as well and two of them reported symptoms around the flowering season. So the symptoms are most probably the result of exposure to *B. oleracea* pollen, although a role of mildew cannot be fully excluded.

Differences between the severity of symptoms caused by various varieties of *B. oleracea* pollen (white cabbage, Savoy cabbage, Brussels sprouts) were only reported in a minority of cases. Symptoms caused by cauliflower and broccoli pollen were very similar. It seems therefore likely that *B. oleracea* pollen of various varieties have a more or less similar allergenic potential.

Skin-prick test was highly sensitive in contrast to the RAST. Of the 24 persons with work-related symptoms, 96% (23/24) had positive SPT for broccoli and/or cauliflower pollen and 58% (14/24) had a positive RAST. Therefore, homemade extracts for SPT are a reliable alternative when no commercial extract is available (1).

The low correlation between the SPT and RAST results can be explained by the fact that the amount of allergens present in the extracts was overestimated due to residual HSA, which in turn led to the detection of lower allergen content in the RAST.

Atopy appeared to be a risk factor for allergy to *B. oleracea* pollen. This was previously described for other *Brassica* species pollen (13). This high prevalence of respiratory allergy caused by *B. oleracea* pollen has major consequences for the health of the employees. Some were forced to search for a different job because of progressive respiratory symptoms. Others were able to reduce symptoms by avoidance measures (1). Theoretically, by using gloves and dust masks the exposure can be reduced. One employee reported relief of symptoms by using a dusk mask and gloves, whether others mentioned a decrease of symptoms by restricting the contact with the flowering plants. However, in practice, such measures are not very easy to realize. Antihistamines can improve rhino-conjunctivitis. Sensitization to *B. oleracea* pollen tends to be persistent for many years. Often, especially in case of asthma not responding to medication, the search for another profession is the best solution.

In conclusion, we have demonstrated that *B. oleracea* pollen is a highly potent occupational allergen causing work-related symptoms in almost half of the exposed employees.

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