

Short communication

Prevalence of occupational allergy to *Chrysanthemum* pollen in greenhouses in the Netherlands

Background: An increasing number of allergic complaints appear to have occurred among *Chrysanthemum* greenhouse employees. The aim of this study was to estimate the prevalence of work-related allergic symptoms and the prevalence of sensitization to pollen of different members of the *Chrysanthemum* family.

Methods: We studied 104 employees who were invited to answer an extensive questionnaire and to complete a rhinitis quality of life questionnaire. In addition, they were skin prick tested on location with inhalant allergens and home-made pollen extracts of seven different members of the *Chrysanthemum* family. Radio-allergo-sorbent tests were performed to confirm IgE-mediated reactions.

Results: Work-related symptoms were reported in 56.7% of all cases, with the main symptom being rhinitis. Sensitization to *Chrysanthemum* pollen was found in 20.2% of the employees without one member of the *Chrysanthemum* family in particular being most prevalent. Sensitization to *Chrysanthemum* pollen was considered to be an important risk factor for the occurrence of work-related symptoms of the upper airways. Furthermore, inhalant atopy as well as sensitization to common airborne pollen including mugwort were closely associated with sensitization to *Chrysanthemum* what might be suggestive for cross-sensitization.

Conclusions: There is a high prevalence of work-related symptoms in *Chrysanthemum* greenhouses. In one-third of the employees these symptoms were caused by an IgE-mediated allergy caused by the pollen of the flowers. Inhalant atopy appeared to have a great impact on the development of such a sensitization. Measurements to reduce the pollen exposure are necessary to prevent a further increase of this occupational allergy.

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Common airborne pollen are known to be one of the most frequent triggers for allergic sensitization and clinical symptomatology. IgE-mediated allergy (1) caused by exposure to pollen of ornamental flowers, especially of the Compositae family, is rather common in the general atopic population and particularly among growers of these flowers (2,3).

Chrysanthemum flowers originate from Japan and were imported to Europe some 200 years ago. Since then they have been cultured all over the world. *Chrysanthemum* cultivation in the Netherlands has become an important branch of horticulture under glass with a size of approximately 820 hectares, divided over 650 greenhouses, and with a workforce of about 2500 people. *Chrysanthemum* flowers are now one of the most important cut flowers for export, and the annual turnover is growing every year. At the same time, however, the number of allergic complaints also appear to have increased among the workers. Up to the present, only a

few studies have been published in the literature on IgE-mediated occupational allergy to *Chrysanthemum* pollen (2,4–6). A broad investigation, however, among employees in Dutch *Chrysanthemum* greenhouses with different kinds of *Chrysanthemum* pollen has never been performed. The following study was conducted with the aim to investigate the prevalence and determinants of work-related allergic symptoms among this population at risk.

Material and methods

A cross-sectional study was carried out in March and April 2000. *Chrysanthemum* greenhouses in the western part of the Netherlands were approached at random by telephone and asked to participate in the study. The investigators paid two visits to each participating greenhouse. During the first visit the volunteers gave informed consent and were asked questions concerning age, sex, medication use, smoking habit, job and job activities, work history, symptoms at work, and atopic diseases such as hay fever, allergic asthma or the atopic eczema/dermatitis syndrome (1). Symptoms present after occupational exposure comprised five categories: itching, redness and/or eczema of the skin, urticaria/angioedema, rhinitis (sneezing, rhinorrhoea, itching, obstruction), conjunctivitis (redness, itching,

Abbreviations. QOL: Quality of life, PRR: Prevalence Rate Ratio, CI: Confidence Interval.

watery eyes), and asthma (wheezing, coughing, shortness of breath). Exacerbations during the work week and regression on weekends and holiday were considered exemplary for work-relatedness. Furthermore, the volunteers completed a rhinitis quality of life (QOL) questionnaire. During the second visit sensitization to the pollen of seven different members of the *Chrysanthemum* family and a *Chrysanthemum* pollen mix was determined by means of skin prick tests. Blood samples were taken to evaluate sensitization by the radio-allergo-sorbent test. The study was approved by our Hospital Medical Ethical Committee. Confidentiality was maintained.

Rhinitis QOL questionnaire

Quality of life was measured using the rhinitis QOL questionnaire originally developed by Juniper (7,8) and translated into Dutch and validated by de Graaf in 't Veld (9). This questionnaire describes more precisely the effect of rhinitis on different areas of the employees' day-to-day lives. It consists of 28 questions subdivided into the following domains: activities ($n = 3$), sleep ($n = 3$), non-rhinitis symptoms (washed-out; thirst; less output/less productive; tiredness; less ability to concentrate; headache; exhausted), practical problems (disability to carry handkerchiefs always; the need to rub the nose or eyes; the discomfort always to blow one's nose), nasal symptoms (blocking; rhinorrhoea; sneezing; post nasal drip), eye symptoms (itching; tears; painful eyes; swollen mucosae) and emotions (frustrated; restless; irritable; ill at ease having complaints). For each item, they were asked how much they were troubled as a result of their nasal problems during the previous 2 weeks. The score ranged from 0 (not troubled) to 6 points (extremely troubled). For the items concerning activities, the employees were asked to identify the activities that were limited because of their nasal symptoms. If more than three activities were identified, employees were asked to choose the three most significant. The investigators instructed the employees according to the guidelines defined by the designers of the questionnaire. All employees filled in their questionnaires in the canteen of the greenhouse in the presence of the investigators. In the analysis the mean within-employee score of each QOL domain was used (these mean domains scores were measured by calculating the mean of the items within each domain). Furthermore, the total score of the means of the seven domains was used to calculate the mean QOL score.

Prick tests

Prick tests were performed by application of 1 drop of allergenic extract to the skin of the volar side of the forearm. Subsequently, the skin was punctured with a standardized skin test needle and the results were read after 20 min. Reactions were expressed (mm) of 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 (10). Dilution buffer was used as a negative control, and histamine chloride 10 mg/ml as a positive control.

Allergens

Skin prick tests were performed with *Botrytis cinerea* (SQ 412) as one of the moulds found in greenhouses (11) and six common inhalant allergens: *Dermatophagoides pteronyssinus*, tree mix, grass mix, mugwort, dog dander, and cat dander (ALK Abelló, Nieuwegein, the Netherlands). Seven of the most frequent cultivated *Chrysanthemum* varieties in the Netherlands (Stallion, Biarritz, Reagan, Regoltime, Euro, Tiger and Klondike) were purchased from several greenhouses. The flowers had to be in full bloom. In some cases, collecting pure pollen from the flowers was not possible, as, for instance, when the flowers were very small or did not produce enough pollen. In those cases, a small part of the heart of the flower

was taken, with the intention of taking as much pollen as possible. An 25% (w/v) extract was prepared as described by de Jong et al. (6). The protein concentration of the 25% extract of Biarritz pollen, determined by the method of Iwata and Nishikaze (12), was 0.25 g/l, of Stallion and Reagan pollen 0.30 g/l and of Euro pollen 0.38 g/l. The protein concentrations of the 25% pollen extract of Regoltime, Tiger and Klondike were 0.64 g/l, 0.66 g/l and 1.16 g/l, respectively. In addition, a *Chrysanthemum* pollen mix consisting of different genotypes of breeding material (protein concentration 0.34 g/l) was obtained from the Fides Research Office (De Lier, the Netherlands).

Reference group

As controls, 10 non-atopic volunteers without allergic complaints, and five patients with a grass pollen atopy who had never been in close contact with *Chrysanthemum* flowers, were skin tested to detect irritative, non-specific reactions on our home-made occupational allergen extracts. All skin tests performed in this group were negative.

Specific IgE-determination

Allergen-specific IgE against Klondike pollen and *Chrysanthemum* pollen mix was determined by radio-allergo-sorbent test by the use of agarose beads as allergen support, as described by Adkinson et al. (13). An amount of 20 mg of pollen was extracted with 2 ml coupling buffer (0.1 mol/l NaHCO_3 and 0.5 mol/l NaCl, pH 8.5) for 1 h at room temperature. After centrifugation for 10 min at 1400 g, protein in the supernatant was coupled to 200 mg of CNBr-activated Sepharose 4B (Sigma Chemical Co. St. Louis, USA), according to the manufacturer's instructions. An amount of 2 mg per test of *Chrysanthemum* pollen Sepharose preparation was incubated overnight with 0.05 ml patient serum. After four washes, radio-iodinated rabbit antihuman IgE antibodies (Pharmacia & Upjohn, Uppsala, Sweden) were added. After overnight incubation and four washes, the percentage of bound radioactivity was measured.

Statistical analysis

In the statistical analyses differences between continuous variables were tested with the unpaired Student t -test. The differences between frequencies of categorical variables were tested with the chi-squared test (χ^2). A generalized log-linear model with a binomial distribution was used to present associations between work-related risk factors and respiratory symptoms. Prevalence Rate Ratios (PRR) were estimated as a measure of association between risk factors and respiratory symptoms. The PRR is a better approximation of the Relative Risk than the often used Odds Ratio in situations where the disease prevalence is high (14). Since age appears to strongly influence the probability of respiratory symptoms, it was included in each logistic model, regardless of the level of significance. For the initial selection of variables in multivariate log-linear models a significance level of $P < 0.10$ was used. In the final models only variables with a P -level below 0.05 were retained. The statistical analysis (especially PROC REG AND PROC GENMOD) was executed using the SAS computer package.

Results

Population characteristics

Of the 35 greenhouse owners and managers approached by telephone, 20 participated in the study. Reasons for refusal to participate were lack of time and/or interest,

Table 1. Characteristics of the *Chrysanthemum* greenhouses and their employees ($n = 104$)

	Mean	Range
Age (years)	38.8	14–71
Duration of employment (years)	13.4	0.3–50
Regular employees	5.6	2–14
Seasonal employees	3.2	0.3–25
Area of the greenhouse (m ²)	23 681	7000–46 000
	<i>n</i>	%
Sex male	72	69
Sex female	32	31
Smoker	40	39
<u>Job Classification</u>		
Owner	32	31
Supervisor	1	1
Full-time employee	44	42
Part-time employee	23	22
Sorter	4	4

change of flower cultivation and other individual causes. The invited group of workers in 20 greenhouses comprised 109 employees of whom 104 participated (response rate of 95%). The greenhouses in the study together cover an area of 2 462 800 square metres, nearly 30% of the total *Chrysanthemum* cultivation in the Netherlands. Population characteristics and characteristics of the participating greenhouses are given in Table 1. Symptoms at work were highly prevalent among the greenhouse workers. One or more symptoms were reported by 59 employees (57%) of whom 55 (93%) notified a substantial improvement or complete regression on weekends and holidays. Complaints consisted of rhinitis in 50 individuals (48%) and of conjunctivitis in 27 individuals (26%). Redness, itching and/or eczema of the skin were mentioned by 15 individuals (14%), shortness of breath by 10 individuals (9%) and urticaria and/or angioedema by 10 individuals (9%). In subsequent analyses work-related rhinitis and conjunctivitis are analysed together as work-related symptoms of the upper airways (55 employees, 53%) and compared with work-related symptoms of the lower airways (8 employees; 8%). Symptoms of the lower airways are considered to be the most serious manifestation of an occupational allergy. One parameter was used as indicator of atopy: the presence of a positive skin prick test result (defined as a wheal size of 3 mm or more) to at least one of the common inhalant allergens. This was found in 35 employees (34%). Sensitization to occupational allergens was also highly prevalent. Of all employees 21 (20%) were sensitized to the pollen of one or more different members of the *Chrysanthemum* family as shown in Table 2.

Symptoms vs. sensitization

In order to investigate associations between respiratory symptoms and sensitization the study population was divided into four subgroups based on the presence of

Table 2. Results of the skin prick tests with common inhalant allergens and occupational allergens ($n = 104$)

	<i>n</i>	%
<u>Inhalant allergens</u>		
<i>Dermatophagoides pteronyssinus</i>	20	19
Tree pollen	10	10
Grass pollen	16	15
Mugwort pollen	16	15
Dog dander	16	15
Cat dander	8	8
<i>Botrytis cinerea</i>	4	4
<u><i>Chrysanthemum</i> pollen</u>		
Pollen mix	17	16
Stallion	7	7
Biarritz	12	12
Reagan	13	13
Regoltime	13	13
Euro	7	7
Tiger	12	12
Klondike	13	13

work-related symptoms and sensitization to occupational allergens. The majority of the employees sensitized to *Chrysanthemum* pollen appeared to have work-related symptoms (81%), whereas only 29% of the employees with work-related symptoms were sensitized to *Chrysanthemum* pollen. There was not much difference between the number of positive skin reactions to the different members of the *Chrysanthemum* family, although sensitization to the pollen mix was slightly more prevalent. When the characteristics of sensitized and non-sensitized employees were compared, all symptoms with exception of asthma were relatively more prevalent in sensitized employees. In addition, the average age of sensitized employees was higher, they had worked for longer periods with *Chrysanthemum* (19 years vs. 12 years) and more than 85% were atopic. Sixteen out of 21 workers had a positive skin test to mugwort pollen.

Determinants of work-related symptoms

There was one significant determinant associated with work-related symptoms of the upper airways: sensitization to *Chrysanthemum* pollen. This was found in the univariate (PRR 1.48; 95% confidence interval (CI) 1.04–2.10) as well as in the multivariate analysis (PRR 1.51; CI 1.07–2.13). There was, however, no association between sensitization to *Chrysanthemum* pollen and work-related symptoms of the lower airways, nor between inhalant atopy and work-related symptoms of both upper and lower airways. Several other determinants were tested but none of them was significantly associated with work-related symptoms of the upper airways: age (PRR 1.02), sex (PRR 1.01), job classification (PRR 1.13), duration of employment (PRR 1.01), hours per week (PRR 0.98), size of the greenhouse (PRR 0.94) and smoking (PRR 0.84). Addition of any of these variables to the multivariate model did not change the results. Furthermore, inhalant

atopy appeared to be an important determinant of sensitization to *Chrysanthemum* pollen with a highly significant prevalence rate ratio (PRR 11.83; CI 3.74–37.45). Next to a significant association between sensitization to grass pollen and sensitization to *Chrysanthemum* pollen (PRR 7.33; CI 3.71–14.48), respectively, tree pollen and *Chrysanthemum* pollen (PRR 5.78; CI 3.29–10.46), there was also a significant association between mugwort and *Chrysanthemum* pollen (PRR 3.90; CI 3.90–3.90).

Rhinitis QOL questionnaire

A total of 95 employees completed their questionnaire correctly and data of these employees were used. Rhinitis symptoms were reported by 44 of them. We analysed the effect of rhinitis symptoms on the seven domains of the rhinitis QOL and the mean rhinitis QOL. The presence of rhinitis symptoms was significantly correlated with most QOL domains with the exceptions of sleep and emotions (Table 3). In addition, a significant negative effect of rhinitis on the mean rhinitis QOL ($P < 0.005$) was also found. The influence of rhinitis symptoms was obviously most pronounced for the domains nasal symptoms, practical problems and activities, respectively, with a magnitude of the median of 1.00 or more.

Specific IgE determination

Specific IgE against *Chrysanthemum* pollen from the pollen mix and the Klondike *Chrysanthemum* was demonstrated in 11 employees, ranging from 0.59 to 28 E/ml. Of this group 10 employees had a positive skin prick test result to both kinds of pollen, indicating that in 48% of the employees sensitized to *Chrysanthemum* pollen the presence of specific IgE to this pollen could be confirmed by radio-allergo-sorbent test.

Discussion

In this study a high prevalence of work-related symptoms among employees of *Chrysanthemum* greenhouses was found (57%), with the main symptoms being rhinitis and conjunctivitis. It was striking that symptoms of the skin,

often mentioned as an important manifestation of an occupational allergy to *Chrysanthemum* (15) were reported to a lesser extent (14%). In addition, symptoms of the lower airways, which are considered to be the most serious manifestation of an occupational allergy, were found in 9%. Sensitization to *Chrysanthemum* pollen, confirmed by skin prick testing and the radio-allergo-sorbent test, appeared to be an important determinant for the occurrence of work-related symptoms of nose, eyes, and skin. This association was logically not found for symptoms of the lower airways because there were only two sensitized employees with symptoms of such a kind. The impression that greenhouse employees with work-related rhinitis were impaired in their day-to-day lives was supported by the results of the rhinitis QOL questionnaires. The negative effect of rhinitis was obviously most pronounced for the domains practical problems, activities and nasal symptoms, which seem to be closely related. Of the seven tested members of the *Chrysanthemum* family there was not one kind in particular to which the employees were sensitized mostly and therefore most suitable to screen for sensitization to *Chrysanthemum*. The prevalence rate of work-related symptoms in this study was in accordance with a previous study among 75 flower growers by Goldberg et al. (2) in which 45% reported respiratory, nasal, or ocular symptoms after their work. The frequency of positive SPT responses to ornamental plants in their study (52%) was however, much higher than our prevalence rate of sensitization (20%). This might be due to the fact that in this study six other flowers of the *Compositae* family were also tested. The allergenicity of the pollen of these other members, for example *Solidago*, might be stronger than the allergenicity of the *Chrysanthemum* pollen. This possibility is supported by the fact that *Solidago* has previously been mentioned as most suitable to screen for sensitization to the *Compositae* family (6).

It was striking that sensitization to mugwort was only found in employees sensitized to *Chrysanthemum* pollen. This sensitization pattern suggest a strong cross-sensitization to *Chrysanthemum* and mugwort, which was also suggested by de Jong et al. (6). In addition, cross-reactivity between *Helianthus*, also a member of the *Compositae* family, and mugwort pollen has been described earlier by Fernandez et al. (16). Although by our investigations cross-reactivity between *Chrysanthemum*

Table 3. Quality of life in employees with and without rhinitis

QOL domains	No rhinitis (n = 51) Median (25/75 percentiles)	Rhinitis (n = 44) Median (25/75 percentiles)	P-value
Activities	0.00 (0.00/0.00)	1.00 (0.00/1.33)	< 0.005
Sleep	0.00 (0.00/0.00)	0.00 (0.00/0.58)	0.08
Non-rhinitis symptoms	0.00 (0.00/0.29)	0.29 (0.00/0.93)	< 0.005
Practical problems	0.00 (0.00/0.67)	1.17 (0.00/1.92)	< 0.005
Nasal symptoms	0.00 (0.00/0.50)	1.25 (0.50/2.88)	< 0.005
Eye symptoms	0.00 (0.00/0.00)	0.00 (0.00/0.50)	0.02
Emotional	0.00 (0.00/0.25)	0.00 (0.00/0.50)	0.17
Mean rhinitis QOL	0.00 (0.00/0.38)	0.77 (0.24/1.35)	< 0.005

pollen and grass and/or tree pollen, respectively, *Chrysanthemum* pollen and mugwort in particular might be suspected, an independent sensitization to *Chrysanthemum* pollen cannot be excluded. Further investigation by means of RAST inhibition and immunoblot analyses is necessary to answer this question and, in the case of cross-reactivity, whether employees are primarily sensitized by grass, tree and/or mugwort pollen or by *Chrysanthemum* pollen.

Although work-related rhinoconjunctivitis in *Chrysanthemum* greenhouses is obviously associated with contact with the pollen of the flowers, not all symptoms could be explained by an IgE-mediated response to this allergen. In this study 57% of the employees reported work-related allergic symptoms, but in only 29% of these workers could IgE-sensitization to *Chrysanthemum* pollen be demonstrated. The question is what may have caused the work-related symptoms in the group of employees without sensitization? A possible explanation might be first of all that our home-made extracts may have failed to detect sensitization in some individuals, although the method used was comparable to a previous study on flowers performed by our research group, and the radio-allergo-sorbent tests in these employees were negative. Second, the employees involved might be specifically sensitized to another kind of *Chrysanthemum*, not tested in this study, or to other occupational allergens (like other moulds) not identified yet. Non-specific hyperreactivity, inducing complaints on exposure to *Chrysanthemum*, or to the humid and warm environment in greenhouses, or to pesticides is probably a third (additional) explanation.

The described relationships may have important consequences for healthcare in this occupational group. It provides important evidence of causation and suggest that work-related symptoms are to some extent preventable by reducing exposure levels to pollen. Flower

cultivation is not very labour-intensive when compared with other crops (17). The most important activity for employees during the cultivation process is to gather the flowers. This happens by pulling them out of the ground, causing a release of pollen from the flowers. Inhalation of this pollen may cause immunological sensitization with subsequent allergic symptoms in sensitized workers. Personal contact with employees in *Chrysanthemum* greenhouses confirmed that the gathering of flowers often initiated the onset of symptoms or aggravated already existing symptoms. There is, however, no visible pollen release, unlike the case in other crops, for example bell pepper plants (17). For this reason the level of pollen exposure is expected to be less high, which might explain the absence of symptoms of the lower airways. In addition to individual medical guidance and personal protection of allergic employees, possible solutions to reduce the release of pollen should be considered. Measurements which can be recommended are first, irrigation of the flowers just before gathering. Secondly, the gathering of flowers should be taken place as early as possible in the morning, because of the circadian day and night rhythm of the flowers. Finally, the greenhouse should be kept as clean as possible to prevent pollen from the remaining plants from accumulating on the floor, thereby increasing the exposure to pollen.

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