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Original article

Prevalence of sensitization to the predatory mite *Amblyseius cucumeris* as a new occupational allergen in horticulture

Background: Protection against thrips, a common pest in bell pepper horticulture is effectively possible without pesticides by using the commercially available predatory mite *Amblyseius cucumeris* (Ac). The prevalence of sensitization to Ac among exposed greenhouse employees and its clinical relevance was studied.
Methods: Four hundred and seventytwo employees were asked to fill in a questionnaire and were tested on location. Next to RAST, skin prick tests (SPTs) were performed with common inhalant allergens, the storage mite *Tyrophagus putrescentiae* (Tp) which serves as a temporary food source during the cultivation process and Ac. Furthermore, nasal challenge tests with Ac were carried out in 23 sensitized employees.
Results: SPTs positive to Ac were found in 109 employees (23%). Work-related symptoms were reported by 76.1%. Sensitization to Tp was found in 62 employees of whom 48 were also sensitized to Ac. Immunoglobulin (Ig)E-mediated allergy to inhalant allergens appeared to be an important risk factor for sensitization to Ac. Employees with rhinitis symptoms showed a significantly higher response to all Ac doses during the nasal challenge test compared with employees without rhinitis symptoms.
Conclusions: The predatory mite Ac is a new occupational allergen in horticulture which can cause an IgE-mediated allergy in exposed employees. It is biologically active on the mucous membranes of the nose and therefore clinically relevant for the development of work-related symptoms.

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Key words: Amblyzeius cucumeris; bell pepper; greenhouse employees; horticulture; occupational allergy; predatory mite.

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The Netherlands count approximately 1150 hectares of sweet bell pepper horticulture. One of the major pests of this greenhouse crop is thrips. The most common thrips species are *Frankliniella occidentalis* and *Echinothrips americanus* (1). Especially the Frankliniella can cause tremendous damage to the plants, by feeding and as a consequence of transmission of viruses. Chemical control proved to be undesirable for environmental reasons and because of its interference with the biological control of other pests. Hence, an effective biological control agent of this thrips species was needed. From the various groups of natural enemies that can be used, the predatory mite *Amblyseius cucumeris* (Ac) appeared to be very successful (2, 3). This predatory mite was introduced in bell pepper greenhouses in 1985 and their use for year-round biological control has been stimulated ever since. In the past few years, an increasing number of allergic complaints seem to have appeared among employees of bell pepper greenhouses. A comprehensive study among 472 employees revealed that work-related symptoms in bell pepper horticulturists are highly prevalent (53.8%) and strongly associated with exposure to the bell pepper pollen (MS in preparation). However, not all symptoms could be explained by an IgE-mediated response to this occupational allergen and the question remains what may have caused the work-related symptoms in nonsensitized employees. Very few reports on sensitization to Ac or an antigenic relation between the common house dust mite *Dermatophagoides pteronyssinus* and the predatory mite Ac are found in scientific literature. However, it can be hypothesized that an elevated exposure to Ac as currently observed in bell pepper greenhouses might lead to sensitization as well as subsequently to work-related symptoms in sensitized employees.

Ac belongs to the order of mites (Acari), the suborder Mesostigmata, the family of the Phytoseidae, and the genus of Neoseiulus. It represents its own species. Taxonomically, the Ac mite is very different from the house dust mites or storage mites (Table 1). Four generation stages, from eggs to the adult animals pass through during the cultivation process which takes place together with the *Tyrophagus putrescentiae* (Tp). This storage mite, belonging to the Acaridae family, serves as a food source for nymphs and adult animals until the

first weeks after their introduction into the crop. This usually happens a few weeks after planting. When the Tyrophagus mites are no longer available, Ac starts actively to search for thrips. The predator population can be maintained throughout the year without reintroductions. This persistence, which occurs even in absence of thrips, may be attributed to the presence of bell pepper pollen as an alternative food source. The microclimate of the leaf surface of the bell pepper plant is mostly of such quality that low air humidity during frost periods and on bright summer days does not affect their predation rate. The species used nowadays shows a total absence of diapause, so that year-round effective biological control is now provided.

The aim of this study was to estimate the prevalence of sensitization to Ac and the clinical relevance of sensitization.

Material and methods

Study design

A comprehensive cross-sectional study was carried out from March 1999 to February 2000. Bell pepper 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 (smoking cigarettes in the year of the study), job and job activities, work history, symptoms at work, and atopic complaints. Symptoms were considered to be work-related if they were reported by the subject as being provoked by contact with the bell pepper plants containing Ac during work in the greenhouse. They comprised five categories: redness, itching and/or eczema of the skin, urticaria/angiedema, rhinitis, conjunctivitis and asthma (shortness of breath and/or coughing and/or wheezing). During the second visit sensitization to Ac was determined by means of a SPT, performed according to international guidelines (4). At the same time SPTs were performed in this study group with the storage mite Tp, home-made extracts of the bell pepper pollen and plant, common inhalant allergens and *Botrytis cinerea* as one of the moulds common in greenhouses. One parameter was used as indicator of IgE mediated allergy (5): the presence of a positive SPT result (defined as a wheal size of 3 mm or more) to at least one of the common inhalant allergens. Between the two visits it was not permitted to use antihistamines orally, with the exception of acrivastine 8 mg (as escape medication). This medication was also withdrawn 3 days before the SPTs. Blood samples were taken to evaluate sensitization by RAST and nasal challenges with Ac were performed to determine the clinical relevance of sensitization. The study was approved by our Hospital Medical Ethical Committee. Confidentiality was maintained.

Reference group

As controls, five nonallergic volunteers and five patients allergic to *Dermatophagoides pteronyssinus*, who had never been in contact with predatory mites or bell pepper pollen were skin tested and challenged nasally with Ac to detect irritation or nonspecific reactions.

Allergens

Predatory mites (Ac) were kindly supplied by Koppert Biological Systems (Berkel en Rodenrijs, the Netherlands). Killing of the mites was achieved by freezing at -60°C for 10 min. After defrosting, a 10% (w/v) extract was prepared in phosphate buffered saline (PBS) pH 7.4, containing 0.03% human serum albumin (HSA) and 0.5% phenol at 4°C. The extract was centrifuged for 10 min at 2000 g and the supernatant was filtered through a 0.22-µm filter. The protein concentration, determined by the method of Iwata and Nishikaze (6) with benzethoniumchloride, was 1.05 g/l. Pollen from flowers of the bell pepper plants were collected in a greenhouse in the period February – March. The flowers were in full blossom and biological control by Ac was not yet used. An 10% (w/v) extract was prepared in PBS pH 7.4, containing 0.03% HSA and 0.5% phenol (PBS). In the same period a fresh bell pepper plant was supplied by a bell pepper gardener. Stems and leaves were collected and a 25% (w/v) extract in PBS was prepared. Stamens from the bell pepper flowers were collected in August. We prepared a 25% (w/v) extract in PBS. The extracts of pollen and plant were centrifuged for 10 min at 2000 g, and supernatants were passed through a 0.22-µm Millex GS filter (Millipore, Bedford, MA, USA). A red bell pepper (inside flesh and seeds were removed) was homogenized in a food processor, the slurry was filtered, and the fluid was subsequently passed through a 0.22 µm filter. Protein concentrations of the 25% extract of stem, leaf and stamen were 0.28 g/l, 0.35 g/l and 1.01 g/l, respectively. The protein concentration of the 10% pollen extract was 0.59 g/l. All extracts were stored in appropriate aliquots at -20°C until use in skin tests. Before use, extracts were defrosted for 1 h before the skin test and were subsequently mixed. In addition, SPTs were performed with *Tyrophagus putrescentiae* (SQ 505), *Botrytis cinerea* (SQ 412) and six common inhalant allergens from ALK Abelló (Nieuwegein, the Netherlands): *Dermatophagoides pteronyssinus* (SQ 503), tree mix (SQ 108), grass mix (SQ 293), mugwort (SQ 312), dog dander (SQ 553) and cat dander (SQ 555).

Rast

Allergen-specific IgE was determined by RAST with use of agarose beads as allergen support, as described by Adkinson et al. (7). An amount of 10 mg of Ac mites was extracted with 2 ml coupling buffer (0.1 mol/1 NaHCO3 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 100 mg of CNBr-activated Sepharose 4B (Sigma Chemical Co. St. Louis, USA), according to the manufacturer's instructions. An amount of 1 mg per test of Ac mites–Sepharose preparation was incubated overnight with 0.050 ml patient serum. After four washes, radioiodinated rabbit antihuman IgE antibodies (Pharmacia & Upjohn, Uppsala, Sweden) were added. After an overnight incubation and four washes, the percentage of bound radioactivity was measured. Data are expressed as percentage-bound radioactivity. More than 2% bound radioactivity above the negative control sera was regarded as a positive RAST result.

Nasal challenge with Ac

To determine the clinical relevance of sensitization to Ac, nasal challenges with Ac were performed in accordance with the methods described by de Graaf-in 't Veld (8). Twenty-eight employees with a positive SPT result only to Ac or to Ac and the Tyrophagus mite, and four employees who were very strongly sensitized to Ac (SPT result $>$ 10 mm) were asked to participate. The nasal challenge tests were performed on location with the exception of the employees who were also sensitized to bell pepper pollen and/or plant. Those employees were invited to come to our department to avoid hyper-reactivity of the nose because of exposure to pollen in the greenhouses. Symptomatic medication for rhinitis was withdrawn, nasal corticosteroids 3 weeks and antihistamines 3 days before the start of the study. Employees with nasal surgery less than 3 months before and nasal infection during the 2 weeks preceding the nasal challenge were excluded. Before starting the nasal challenges, the employees waited for 30 min in order to give the nasal mucosa time to acclimatize. Nasal challenges were performed with four increasing doses of Ac extract (0.001%, 0.01%, 0.1%, and 1%)

at 10 min intervals after sham-challenge with PBS, containing HSA 0.03% and benzalkonium chloride 0.05% (ALK Abelló). The Ac extract was sprayed into each nostril by means of a nasal pump spray delivering a fixed dose of 0.125 ml solution. The nasal response was measured 10 min after each challenge. Nasal responsiveness was monitored by the number of sneezes, the amount of secretion collected and a symptom score according to Lebel et al. (9). The symptom scores sneezes, anterior rhinorrhoea, posterior rhinorrhoea, difficult nasal breathing, number of nostrils blocked, pruritus of the nose and/or palatum or ear and conjunctivitis were graded in points (total score ranges from 0 till 11 points). The areas under the curve (AUC) of symptom scores during the nasal challenge with the four concentrations of Ac were used in the statistical analysis. The study was performed in the month of February. This period was chosen to minimize occupational exposure to Ac and bell pepper pollen.

Statistical analyses

In the statistical analyses differences between continuous variables were tested with the unpaired student's *t*-test. The differences between frequencies of categorical variables were tested with the chi-square test (χ^2). A generalized log-linear model with a binominal 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 expresses the ratio of the subjects who have a disease over the total number of subjects at risk for this disease. The PRR is a better approximation of the relative risk than the often used odds ratio in situations where the disease prevalence is high (10). The statistical analysis was executed using the SAS computer package. In view of the small number of subjects tested in the nasal challenge procedure, we used the nonparametric Mann–Whitney *U*-test to determine whether various groups were significantly different or not. Results were expressed as median. A P value of < 0.05 was considered significant.

Results

Population characteristics

Out of the 110 greenhouses approached, 79 participated in the study. Moreover, six additional greenhouses participated spontaneously. Reasons for refusal to participate were lack of time and/or lack of interest because of absence of work-related symptoms, fear of losing (hard to find) employees with allergic complaints and other individual causes. The invited group of workers in 85 greenhouses comprised 487 employees of which 472 participated (response rate 96.9%). Regular workers were full-time and year around and were exposed to the bell pepper pollen and predatory mites in the greenhouses. Seasonal workers were employed during holidays and periods with an extensive workload in the greenhouses. They were working in the bell pepper horticulture for approximately 2–4 months each season.

The greenhouses in the study together cover an area of about 10% of the total bell pepper horticulture in the Netherlands. A SPT result positive to Ac was seen in 109 of the 472 tested employees (23%). Population characteristics of this subgroup and characteristics of the participating greenhouses, concerning the number of regular and seasonal employees and the area, are given in Table 2. Work-related symptoms were rather highly

Table 1. Classification of *Amblyseius cucumeris* and its relation to the storage mite *Tyrophagus putrescentiae* and the house dust mite *Dermatophagoides pteronyssinus*

Order	Acari (mites)		
	↓	↓	
	Parasitiformes	Acariformes	
	↓	↓	
Suborder	Mesostigmata	Astigmata	
	↓	↓	↓
Superfamily	Ascoidea	Pyroglyphoidea	Acaroidea
	↓	↓	↓
Family	Phytoseiidae	Pyroglyphidae	Acaridea
	↓	↓	↓
Genus	Neoseiulus	Dermatophagoides	Tyrophagus
	↓	↓	↓
Species	*N. cucumeris* (= *A. cucumeris*)	D. pteronyssinus	T. putrescentiae

prevalent among these sensitized employees. One or more symptoms were reported by 83 employees (76.1%) of which 68 (62.4%) individuals (71.6%) noted a substantial improvement or complete regression during weekends and holiday. Complaints consisted of rhinitis in 78 individuals (71.6%), conjunctivitis in 53 individuals (48.6%) and redness, itching and/or eczema of the skin in 29 individuals (26.6%). Asthmatic symptoms were mentioned by 28 employees (25.7%), always simultaneously with rhinitis symptoms, and urticaria and/or angiedema by only 14 employees (12.8%). A positive SPT result to one or more common inhalant allergens was found in 81 employees (74.3%) of this subgroup. Of these inhalant allergens sensitization to the house dust mite *D. pteronyssinus* was most prevalent (58.7%). Positive skin responses to the storage mite Tp were seen in 62 employees of which 48 were sensitized to Ac (77.4%). Furthermore, sensitization to other occupational allergens was also highly prevalent in this subgroup as shown in Table 3. Eighty employees (73.4%) appeared to have a concomitant sensitization to the bell pepper pollen and/or plant. In addition, all control subjects showed negative responses to the SPT.

RAST results

Ac-specific IgE could be demonstrated in 63 out of 109 employees. There was a good agreement between Ac sensitization measured by skin prick testing and IgE analysis. Of the 63 IgE-positive employees 85.7% also had a positive SPT result to Ac, whereas 49.5% of the employees positive to the SPT were also RAST positive. In nine Ac-sensitized employees a serum sample was unfortunately not available. The other 46 Ac-sensitized employees were RAST negative as were all the other individuals with negative SPT results to Ac.

Nasal challenge tests with Ac

Out of the 32 invited employees 23 participated and underwent nasal challenge tests with Ac (response rate 71.9%). Eleven employees of this group had a positive SPT result only to Ac whereas nine employees were

Table 2. Characteristics of the bell pepper greenhouses and their employees sensitized to Ac ($n=109$)

	Mean	SD	Range
Age (year)	34.2	9.1	15.8–59.4
Duration of employment (year)	9.1	6.3	1.0–31.0
Regular employees (year)	6.2	2.6	1.0–31.0
Seasonal employees (year)	3.5	2.9	0.3–13.0
Area of the greenhouse (m^2)	25003	9868	6500–49000
	N	%	
Sex male	95	87.2	
Sex female	14	12.8	
Smoking of cigarettes	32	29.4	
Job classification	N	%	
Owner	31	28.4	
Supervisor	4	3.7	
Fulltime employee	65	59.6	
Part-time employee	8	7.3	
Sorter	1	0.9	

Table 3. Prevalence of work-related symptoms and results of the skin prick tests in employees sensitized to Amblyseius cucumeris ($n=109$)

	N	%
Symptoms at work		
Skin (itching/redness/eczema)	29	26.6
Urticaria and/or angiedema	14	12.8
Rhinitis	78	71.6
Conjunctivitis	53	48.6
Asthma	28	25.7
SPT + with inhalant allergens		
Dermatophagoides pteronyssinus	64	58.7
Tree pollen	28	25.7
Grass pollen	41	37.6
Mugwort	17	15.6
Cat dander	24	22.0
Dog dander	37	33.9
SPT + with occupational allergens		
Tyrophagus putrescentiae	48	44.0
Botrytis cinerea	15	13.8
Stamen bell pepper plant	72	66.1

sensitized to Ac and Tp. Three employees were strongly sensitized to Ac as well as to bell pepper pollen and, to a lesser extent, also to Tp. The characteristics of the participating employees were: mean age 38.3 years (range 20–59), mean exposure time in horticulture 12.1 years (range 2–31). The challenged employees were divided into two groups according to the presence of work-related rhinitis symptoms. Figure 1 shows the nasal response to Ac assessed with the symptom score. Employees with rhinitis symptoms showed a significantly higher response to all Ac doses than employees without rhinitis. An increased response was also found when the reaction to Ac was expressed as the AUC nasal score. In the control group, no nasal responses were seen to any of the Ac challenges. In Fig. 2 the responsiveness to Ac expressed as skin reactivity and as nasal reactivity is compared for employees with rhinitis and without rhinitis symptoms. There was no difference in skin reactivity to Ac in both groups (mean wheal diameter 5.0 mm in rhinitis- and rhinitis+ group) whereas the nasal response to Ac, expressed as AUC, was significantly higher in the rhinitis + group than in the rhinitis – group ($P=0.014$).

Discussion

The predatory mite Ac appears to be an important occupational allergen in bell pepper horticulture next to the bell pepper pollen. As many as 23% of the greenhouse employees in this study showed a positive SPT result to this mite. In these sensitized employees work-related symptoms were highly prevalent (76.1%), the main symptoms being rhinitis and conjunctivitis in 71.6% and 48.6%, respectively. Local dermatitis and asthma, the most serious manifestation of an occupational allergy, were reported to a lesser extent, in 26.6% and 25.7%, respectively. As in the case of high-molecular-weight agents, rhinoconjunctivitis is often more pronounced and may precede the onset of symptoms of the lower airways in exposed subjects (11). The prevalence rate of sensitization to Ac in this study was in accordance with previous recent studies on occupational allergies caused by mites: the citrus red mite (*Panonychus citri*), a common pest in citrus trees, was found positive in 16.5% of 181 citrus farmers (12) while the European red mite (*Panonychus ulmi*) and the two-spotted spider mite (*Tetranychus urticae*), common pests in apple orchards, were positive in 23.2% and 16.6%, respectively, of 725 apple-cultivating farmers (13). To our knowledge, this is the second study in which work-related symptoms are related to mites that are deliberately introduced into the working environment. A recent preliminary report by van Hage-Hamsten *et al.* revealed that cucumber-cultivating greenhouse workers, who use predatory mites for biological crop protection, may be at risk for occupational allergy to these mite species (14).

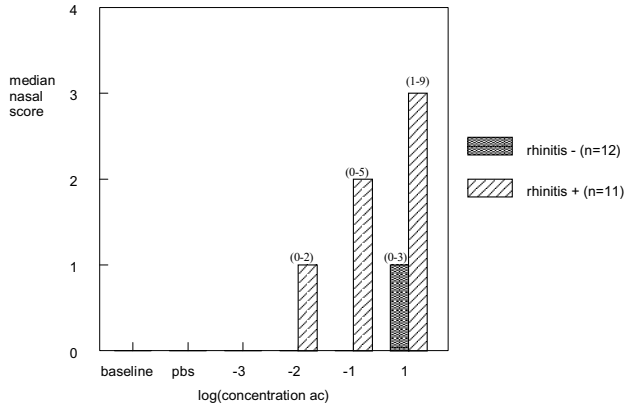

Figure 1. Nasal response to increasing doses of *Amblyseius cucumeris* (Ac) extract in 23 bell pepper greenhouse employees sensitized to Ac. For each Ac dose the data of the two groups were compared. Data are presented as medians (ranges in parenthesis).

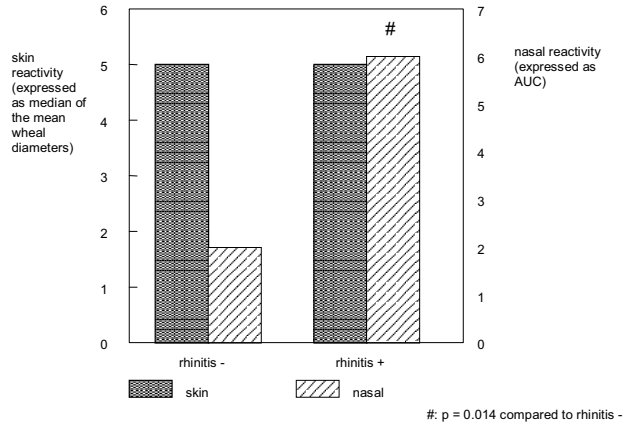

Figure 2. The response to Ac in 23 sensitized employees expressed in skin and nasal reactivity. The responses of both groups were compared. Data are presented as medians. # $P = 0.014$.

Sensitization to high-molecular-weight allergens is known to occur at a higher rate among atopic individuals (15–18). In this study 81 (74.3%) of the 109 employees sensitized to the Ac mite were also sensitized to one or more common inhalant allergens. The sensitization rate to Ac was significantly higher in atopic employees than in nonatopic employees, illustrating a clear association between sensitization to Ac- and IgE-mediated allergy to inhalant allergens (PRR 4.82; 95% confidence interval (CI) 3.27–7.10). Of all common inhalant allergens sensitization to the house dust mite and grass pollen were most prevalent in our study population as well as in our subgroup of Ac-sensitized employees. The association between sensitization to house dust mite and sensitization to Ac (PRR 4.08; CI 2.96–5.62) was stronger than the association between grass pollen and Ac (PRR 2.56; CI 1.87–3.50). Although sensitization to the house dust mite was most prevalent and more marked with sensitization to Ac than the other inhalant allergens, the positive skin reactions do not necessarily reflect cross-sensitization to Ac. The house dust mite is known to be one of the most common sensitizing allergens in the Netherlands. Moreover, there is no close taxonomic relationship between the two mites, which also reduces the probability of cross-allergy. Sensitization to Tp was in most cases associated with sensitization to Ac (77.4%, PRR 5.20). This storage mite only serves as a temporary food source for Ac, is normally not common in greenhouses and also has no close taxonomic relation with Ac. It can be supposed that the positive SPT results to Tp found in this study might be owing to the presence of Tp in commercially available Ac mites. Furthermore, sensitization to Tp was also strongly associated with sensitization to house dust mite (PRR 8.20). Cross-reactivity between these two mites has been described in a previous study (19). Of the 64 employees sensitized to Ac and house dust mite, 38 (59.4%) showed a concomitant sensitization to Tp. Because of the fact that all three mites are closely associated to each other, it is not possible to draw any further conclusions. Although from our investigations an independent sensitization to Ac might be suspected, crossreactivity between Ac and Tp and between Ac and *D. pteronyssinus*, respectively, cannot be excluded. Further investigation by means of RAST inhibition tests and immunoblot analyzes is necessary and under way.

The biological activity of Ac on human mucous membranes, in particular those of the nose, and the consequent clinical-allergological relevance of sensitization could be confirmed by nasal challenge tests. When comparing the clinical response in employees with and without rhinitis complaints, the former showed a significantly higher clinical response at all Ac concentrations. Furthermore, there was a significant difference in the nasal reactivity between the rhinitis+ and the rhinitis– group, with a higher response in the rhinitis+ group. This difference was not revealed by the skin reactivity to Ac in the SPT, which implies that a nasal challenge test with Ac is a better and more sensitive test for discrimination between sensitized employees with and without rhinitis.

It is difficult to single out the specific effect of Ac in an occupational population with a high exposure and consequent sensitization to bell pepper pollen and/or plant. Hence, it is also difficult to predict whether intervention strategies focused on the contribution of bell pepper pollen and plant will be more beneficial than those aimed at reduction or elimination of the use of Ac. This is, however, important to know when possible solutions for exposure intervention are considered and should therefore be a subject for further research. The described results of this study may have consequences for the extensive use of biological control in horticulture nowadays. In the knowledge that indoor as well as outdoor mites are a frequent cause of allergic diseases, the use of predatory mites as biological control agents should be critically evaluated. Furthermore, the benefits of biological control should be weighed carefully against the increased risk of employees developing an occupational allergy.

In conclusion, the predatory mite Ac is a new occupational allergen in horticulture which cause an IgE-mediated allergy in exposed employees. It is biologically active on the mucous membranes of the nose and therefore clinically relevant for the development of work-related symptoms.

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