Occupational allergy in horticulture

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Beroepsallergie in de glas- en tuinbouw

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‘ Voor wie weet te wachten opent de tijd alle deuren’

(*Chinees gezegde*)

Voor mijn ouders

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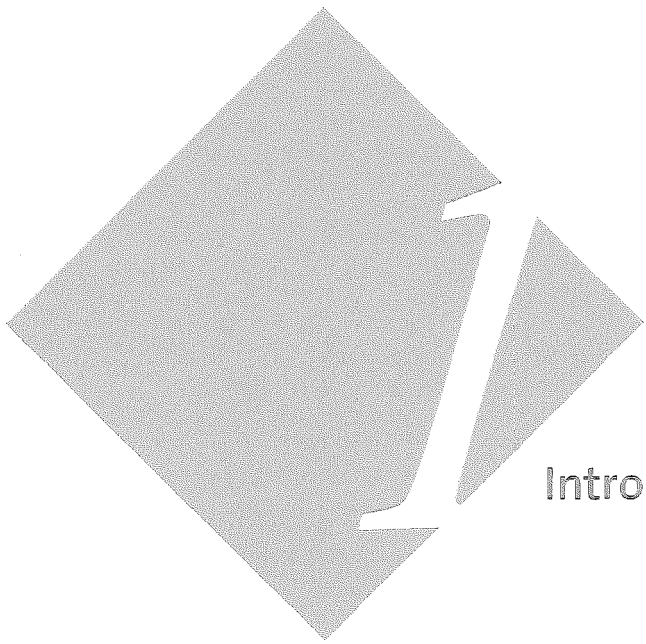

Introduction

Allergic diseases are a worldwide health problem with an increasing incidence. An allergic reaction is the result of an exaggerated response of the immune system to external agents. These agents may induce clinical symptoms such as rhinitis, conjunctivitis, asthma and urticaria, through a type one hypersensitivity reaction with the production of specific IgE antibodies. Offending agents encompass a broad spectrum of natural and synthetic chemicals found in a diverse range of materials and processes.[1] Common airborne and indoor environmental allergens, both perennial and seasonal, are the most frequent triggers for allergic sensitization and clinical symptomatology. However, agents from the working environment may also be involved.

The influence of the working environment on the onset of allergic symptoms was recognized a long time ago, first by Ramazzini in the early 18th century and later on by Jack Pepys who continued the ongoing interest in this condition.[2] As a result of improvements in methodology and the use of immunologic techniques, there have been considerable advances in the field of occupational allergies in the past two decades, through identification of new agents and knowledge of pathophysiology. Nowadays it is well known that work-related symptoms can be due to three different mechanisms. First, as the result of an immunologic response which can be subdivided into IgE mediated (allergic) reaction and non-IgE mediated reaction types, according to whether the production of specific IgE antibodies is induced by the causative agent. Secondly, symptoms may be the result of a non-immunologic response as irritating agents and non-specific stimuli can provoke symptoms in employees with hyperreactivity of upper and lower airways. Direct toxic injury may be the third cause of work-related symptoms. However, pre-existing airway symptoms may also be aggravated in the work environment by irritants or physical stimuli and an occupational rhinitis or asthma may develop in an employee with pre-existing rhinitis or asthma after workplace exposure. When studying work-related symptoms in an occupational setting it is important to distinguish the different possible categories. In this thesis we focus on IgE-mediated occupational allergy.

Certain occupational groups are known to be at particularly high risk of developing occupational allergy. Workers in horticulture are one such group, because exposure to a variety of respiratory sensitizers and irritants such as pollens, plant antigens, molds and mites is inevitable. The low level of dust and endotoxin found inside greenhouses by Monsó [3] did not suggest that these substances are clinically significant as triggers of symptoms reported by horticulture workers. Although this particular working environment may contain many (potential) allergenic agents, up till now only a limited number are well known and described in the literature. Previous studies revealed some important sources and an overview of known occupational agents in horticulture up to the present is shown in Table 1.

Table 1 Occupational agents in horticulture

Agents	Year [ref]	Occupation
Flowers:		
Ageratum	1998 [4]	gerbera grower, florist, floriculturist, gardener
Alstroemeria	1998 [4]	gerbera grower, florist, floriculturist, gardener, student florist
Amaryllis	1996 [5]	amaryllis greenhouse employee
Anemone	1998 [6]	flower grower
Antirrhinum	1998 [6]	flower grower
Asclepias	1998 [4]	gerbera grower, florist, gardener, student florist
Aster	1998 [4]	florist, gardener
Chrysanthemum	1975 [7]	-
	1989 [8]	chrysanthemum greenhouse employee
	1994 [9]	florist
	1998 [4]	florist, floriculturist, gardener, greenhouse-employee
	1998 [6]	flower grower
Dianthus	1998 [4]	gerbera grower, florist, floriculturist, gardener
	1999 [10]	carnation cultivation
Euphorbia	1998 [4]	gerbera grower, florist, gardener
Eustoma	1998 [4]	gerbera grower, florist, floriculturist, gardener
Freesia	1984 [11]	flower greenhouse employee
	1989 [8]	freesia greenhouse employee
	1994 [9]	gardener
	1998 [4]	gerbera grower, florist, floriculturist, gardener student florist
Gerbera	1989 [8]	gerbera greenhouse employee
	1998 [4,6]	gerbera grower, florist, flower grower, gardener
Helianthus	1998 [4,6]	gerbera grower, florist, floriculturist, student-florist
Lilium	1998 [6]	flower grower
Lisianthus	1998 [6]	flower grower
Matricaria	1998 [4]	florist, floriculturist, gardener
Narcissus	1998 [4]	gerbera grower, floriculturist, student florist
Pelargonium	1998 [4]	floriculurist
Saintpaulia	1998 [4]	gerbera grower, florist, floriculturist, student-florist
Senecio	1998 [6]	flower grower
Solidago	1998 [4,6]	gerbera grower, florist, floriculturist, gardener, greenhouse employee
Solidaster	1998 [6]	flower grower
Stephanotis	1999 [12]	greenhouse employee
Tulip	1994 [9]	florist, gardener
Verbersina	1998 [6]	flower grower
Mites:		
H. miles	2003 [13]	greenhouse employee vegetables,plants,herbs
L. destructor	2000 [14]	farmers, grain elevator
P. citri	1999 [15]	citrus farmers
P. ulmi	1992 [16]	fruit growers
	1999 [17]	apple-cultivating farmers
P. persimilis	2003 [13]	greenhouse employee vegetables,plants,herbs
T. urticae	1996 [18]	tomato greenhouse employee
	1996 [19]	carnation greenhouse employee

Table 1 Continued

Agents	Year [ref]	Occupation
	1999 [17]	apple-cultivating farmers
	1999 [20]	-
	2000 [21]	greenhouse employee agricultural cooperative
T. putrescentiae	1997 [22]	farmers
Plants:		
Christmas cacti	1999 [23]	workers cactus nursery
Easter cacti	1999 [23]	workers cactus nursery
Ficus Benjamina	1986 [24]	plantkeepers
Vegetables:		
Bell pepper pollen	1984 [11]	bell pepper greenhouse employee
	1989 [8]	bell pepper grower
Tomato pollen	1989 [8]	tomato greenhouse employee

Symptoms and causes of occupational complaints

Occupation related complaints may be caused by an allergic reaction to occupational allergens, hyperreactivity of nose and airways, and by direct toxic effects of irritating agents.

Occupation related rhinitis

Occupation related rhinitis is caused by an IgE-mediated allergy to inhalant allergens from the occupational environment. Allergic symptoms occur immediately after exposure and exposure level and/or duration of exposure determine the risk of developing an occupational allergy to a large extent. Repeated exposure to allergens may lead to a so-called priming effect in the nose: the sensitivity of the mucosa to a certain amount of allergen increase with time. In an occupational setting this priming effect can result in increasing complaints at the end of the week and a decrease of complaints in the weekend.

Occupation related asthma

The definition of allergic occupational asthma is asthma which is induced immunologically by exposure to an allergen or agents from the occupational environment. However, it can also develop because of a short period of high exposure to known occupational allergens during leisure activities. Two types of occupational asthma can be distinguished, according to whether there is a latency period. [1,2] Occupational asthma with latency is the most common type and comprises all instances of immunologic asthma. After immunologic sensitization due to an occupational agent (so-called "inducer"), work-related complaints appear after a variable time of a few weeks to several years. Occupational asthma without a latency

period follows acute exposure to high concentrations of irritant gases, fumes, or chemicals on one or several occasions.[1,2,25] This non-immunologic type is often called irritant-induced asthma or reactive airways dysfunction syndrome (RADS). Furthermore, a distinction can be made between asthma caused by high molecular weight agents and by low molecular weight agents. High molecular weight agents act as complete antigens and induce the production of specific IgE antibodies. The inhaled occupational sensitizer can bind to these antibodies on the surface of mastcells and basophils, and possibly macrophages and eosinophils, leading to a cascade of events that results in an influx of inflammatory cells into the airway and the release of inflammatory mediators. Many low molecular weight agents do not consistently induce specific antibodies, and if they are found their pathogenic significance is unclear.[2]
Because of non-specific bronchial hyperresponsiveness there might also exist an intolerance towards irritating agents such as cigarette smoke, fragrances, fog and temperature changes (so-called "inciters"). These kinds of stimuli also frequently occur outside the working environment and asthmatic symptoms may become more prominent.

Transition of rhinitis to asthma

In the general population rhinitis is three times more prevalent than asthma and tends to occur three times more frequently in occupational settings.[26] Although rhinitis symptoms are as prevalent in the cases of high molecular weight (HMW) and low molecular weight (LMW) agents, they are more marked in the case of HMW agents. Besides, there appears to be a tendency of a more serious course of rhinitis in HMW induced asthma. A possible explanation for this might be the physical nature of the agent. HMW agents generally exist as dry or liquid aerosols, whereas LMW agents are more generally vapours. Aerosols may more readily deposit in the upper airways and cause symptoms. Additionally, subjects with nasal obstruction are more prone to mouth breathing, implying an increased risk for inhaling agents directly into the lower airways without passing the nasal filter. Rhinitis symptoms generally appear at the same time as symptoms of asthma in the case of LMW agents, whereas they either precede or accompany symptoms of asthma for HMW agents.[27] Karjalainen demonstrated that employees with occupationally induced rhinitis have a high risk of asthma [28], so occupational rhinitis may be a marker of the likelihood of developing occupational asthma in the case of HMW agents. However, the predictive value of having such a symptomatology and having developed IgE-dependent sensitization for the development of occupational asthma remains to be assessed.

Hyperresponsiveness of the airways

Hyperreactivity is the most important diagnostic consideration next to occupational allergy. Increased sensitivity to non-specific stimuli is a general feature of rhinitis or asthma. Patients with an allergic rhinitis to known inhalant allergens as well as

patients with a non-allergic (idiopathic) rhinitis may show symptoms on exposure to non-specific stimuli. Such patients are more easily hampered by occupational stimuli. Although allergy and hyperreactivity give rise to the same kind of symptoms, differentiation between them is important. In the case of an occupational allergy a process of inflammation is started by contact with allergens. Continuation of exposure will only lead to more inflammation and possible irreversible damage. Non-specific stimuli will not induce significant inflammation.

Direct toxic effects of irritating agents

Many chemical products are able to cause mucosa deviations. Loss of cilia, hyperplasia and metaplasia have been described after long-lasting exposure to solvents, formaldehyde, chromium, woodpulp, copper, nickel and leather. The pathophysiological mechanism, however, is unknown. Forming of crusts, epistaxis, hyposmia and anosmia occur more frequently compared to an average rhinitis population. If atrophical rhinitis is seen frequently in a certain group of employees, non-allergic occupational problems should be considered. It is well known that one short but major exposure to an irritating agent may cause persisting asthma and hyperreactivity (RADS).[26] It may also affect the upper airways and give rise to chronic rhinitis which has been defined as the reactive upper airways dysfunction syndrome (RUDS). However, literature references about, or related to this subject were not found.

Diagnosis of occupational allergy

Since the contraction of an occupational allergy carries significant social and financial consequences, it is important to confirm the diagnosis by means of objective testing. The diagnosis needs to be made on firm grounds, as the consequences of either diagnosing occupational allergy when absent or missing the diagnosis when present, are substantial. The different steps involved in the investigation of occupational allergy are respectively clinical history, quality of life assessment, immunological tests, monitoring of peak expiratory flow (PEF) and bronchial responsiveness, and specific nasal and bronchial challenges (Figure 1).

Clinical history

To underpin the diagnosis of occupational allergy a good occupational history, not only of the current job and exposure, but also of past jobs and exposures is required. Here, the questionnaire is the basic, essential tool used in most epidemiological surveys and all individual assessments. The main point of the history is to establish the temporal link between occupational environment and clinical symptoms.[25,29] Moreover, there is a latent interval between first exposure to an occupational sensitizing agent and the onset of allergic symptoms. Such an interval varies widely with individual agents. Potent sensitizers such as laboratory animals may sensitize workers within a few months [30] whereas sensitization to wheat flour and α-amylase

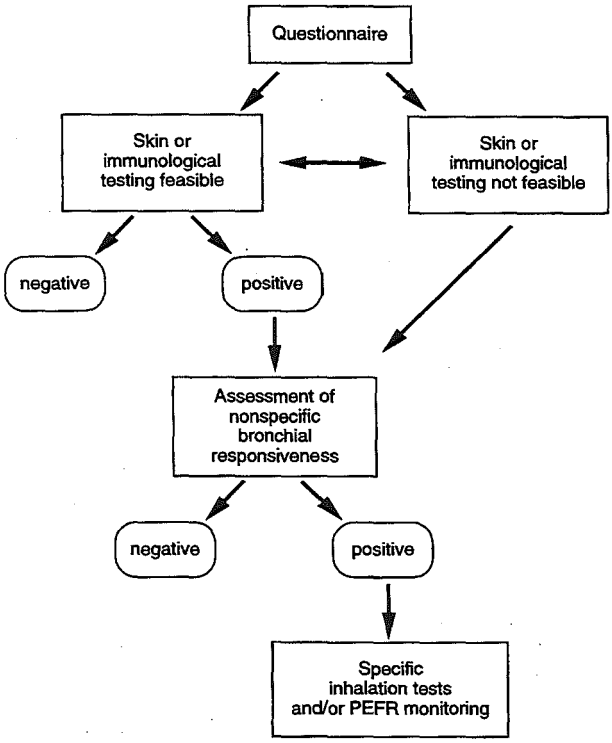

Figure 1 Epidemiological investigation of occupational asthma (figure adapted from ref. 1)

in bakers may appear after several years.[31] The classical history of occupational allergy is one of an employee displaying allergic symptoms while at work with improvement over weekends and/or holidays. However, in many cases this pattern is absent, as symptoms are also usually present outside the workplace, being triggered by exposure to irritants. Symptoms may even be more severe at home, and weekends may not be long enough to allow for recuperation. However, a history suggestive of an occupational allergy, even in an employee exposed to a known occupational agent, is not sufficient to make the diagnosis. A questionnaire is a sensitive, but non-specific tool. Even in the hands of experienced physicians, the predictive value of a positive questionnaire was only 63% while the predictive value of a negative questionnaire was 83%. [25] A thorough assessment of workplace exposure is also important. This should include detailed information on specific job duties and work processes, as well as the qualitative frequency and intensity of relevant exposures, and possible exposures to peak concentrations of potential agents.

Quality of life assessment

The importance of quality of life issues in health care practice and research is steadily growing. In the field of allergy it has also been recognized that allergic diseases comprise more than the classical signs and symptoms which are part of physical disorders, such as allergic rhinitis and asthma.[32] Allergic diseases may impair a person's day-to-day functioning, not only at home but also at work.[33] Therefore, assessment of health-related quality of life (HRQL) is not only important in the case of perennial or seasonal allergies but also when an occupational allergy is involved. However, little is known about the relative impact of an occupational allergy on daily life and until now, only a few studies have been published on this topic.[34,35] Quality of life can be categorized into four domains namely: physical status and functional abilities, psychological status and well-being, social functioning and economic and/or vocational status. As the true quality of life value cannot be measured directly, one has to resort to questionnaires to measure these four domains indirectly. For this purpose, HRQL instruments have generally been constructed.[32] Several instruments (questionnaires) are currently available which can be broadly classified as either generic or disease-specific. The generic instruments are general questionnaires and can be applied to all medical conditions, measuring physical, psychological and social domains, irrespective of the underlying disease. Disease-specific questionnaires are used to evaluate quality of life in a particular disease state such as asthma and rhinoconjunctivitis. They are particularly useful because they have been developed by asking patients about the disease-related problems which they experience and which bother them most.[36] These questionnaires comprise questions which address symptoms specific to the condition. They can detect small but clinically important changes in peoples' problems, and additionally focus on specific problems which can be targeted for specific intervention. Juniper and colleagues have developed several questionnaires for measuring HRQL in adults with rhinitis which can easily be used in allergy research in health care practice as well as in research. These provide a method for quality of life assessment which has been profoundly tested and accepted in terms of reliability, responsiveness and validity.[37]

Immunological tests

The demonstration of immediate skin reactivity and specific IgE antibodies to occupational agents can be achieved by the means of a skin prick test and radio-allergo-sorbent test (RAST) respectively. Skin prick tests are performed by the application of an allergenic extract to the skin of the volar side of the forearm. Subsequently, the dermis is punctured with a standardized skin test needle and the results are read after twenty minutes. Reactions with a mean weal diameter of three millimeters or more are considered positive [38] (Figure 2).
Allergen-specific IgE can be determined by RAST using agarose beads as allergen support, as described by Adkinson et al.[39] A positive reaction to these tests may reflect exposure and/ or sensitization; on the other hand, a negative test result cannot

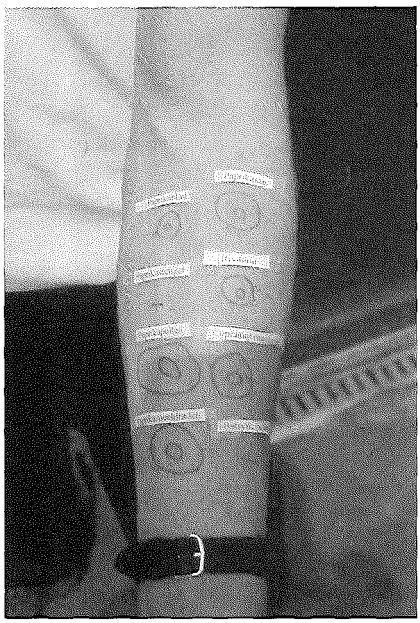

Figure 2 Results of a skin prick test with occupational allergens (read downwards: bell pepper leaf, stem, pollen and stamen (on the left side), bell pepper juice, *Amblyseius cucumeris*, *Tyrophagus putrescentiae* and *Botrytis cinerea* (on the right side)).

entirely exclude the diagnosis occupational allergy, but does make it unlikely. The employee involved may be sensitized to another (unknown) agent found in the work environment or to another component of the offending agent. It is also possible that an employee is sensitized to allergens which are not included in the available standardized skin test reagents and antigens. This lack of commercially available extracts hampers the use of immunologic tests in the diagnosis of occupational allergy and underlines the importance of home-made extracts.[2] If the method of preparation is accurate and reproducible, it may be a reliable way of confirming an IgE-mediated occupational allergy.[4] However, these tests are limited to specialized centres with laboratories and analysts at their disposal.

Monitoring of peak expiratory flow (PEF) and bronchial responsiveness

The diagnosis of occupational asthma can be confirmed by the presence of reversible airway obstruction. However, most employees investigated for occupational asthma have normal spirometry when seen in a clinic. Pre and post shift monitoring of forced expiratory volume in one second (FEV_1) has not proved sensitive or specific enough to be a useful tool.[25] The availability of portable, simple and inexpensive devices allows monitoring of peak expiratory flow (PEF) at work and away from work, and this method is used frequently now to assess airway calibre. Although PEF is less sensitive than FEV_1 in assessing the late asthmatic response, Weytjens demonstrated that PEF is as satisfactory as FEV_1 for detecting a significant immediate asthmatic

reaction after exposure to an occupational agent.[40] The sensitivity and specificity of PEF monitoring is optimal when PEF is measured every two hours from waking to sleeping for a period of four weeks.[29] Less frequent readings are sometimes acceptable but will miss acute reactions at work. Beta$_2$-agonists should be taken on demand only, while inhaled steroids and theophylline should be continued during monitoring. Reduction of medication upon return to work may be associated with reduction of PEF which may be mistaken as diagnostic of occupational asthma. Although serial PEF monitoring is a useful tool, it is time consuming and the results depend on the employee's compliance and honesty. Collaboration of the employee is not always obtained due to fear of losing his job or malingering in order to receive compensation benefit.

There are no uniformly accepted criteria for the interpretation of PEF recordings. It is best to plot the daily maximum, mean and minimum peak flows. The daily mean peak flow is calculated starting with the first reading at work, continuing for the next 24 hours, so that the first reading after waking is included with the previous day's work exposure. In this way the record gives an idea of diurnal variation as well as a measurement of the daily mean. The graphs can be inspected visually to see if there is deterioration during the week at work and improvement during weekends. Records with an increased diurnal variation and deterioration at work, and improvement away from work, clearly show occupational asthma.[29] Experience is required to read these records which are often evaluated subjectively.

Specific nasal and bronchial challenges

These tests are still considered to be the gold standard in the confirmation of the diagnosis of occupational allergy.[2,25] By exposing the nose or lung gradually to increasing doses of the suspected allergen, work exposure is mimicked and reproduction of clinical disease can be achieved. Challenge tests are obviously more conclusive when performed in a controlled, laboratory setting, with known and available substances, and specially equipped facilities. They are more difficult when dealing with an unknown agent, multiple agents, dangerous agents or when specific testing facilities are not available. In these instances, a workplace challenge is recommended. Challenges should always be carried out under close supervision of an expert physician. In most subjects, the tests can be carried out on an outpatient basis or in the work environment, restricting hospitalization to subjects who have severe late reactions.

A nasal challenge test is an objective method for evaluating occupational rhinitis. However, the methodology is not standardized and there are several methods for assessing nasal physiological responses. Secretions can be collected for evaluation of secretory activity in terms of weight by using measures such as weighing tissue papers.[26] This technique is useful when nasal responses are characterized by strong secretions, such as after high-dose allergen challenge. Secretions can also be rinsed off the nasal mucosa by the additional means of nasal lavage for collection. This

method does not allow quantification of secretion weight, but is useful where the quantity of secretions is not high as is the case in most real-life situations. Whatever the means of collection, the secretions can be analysed for measures of markers of inflammation, mediators and cells. Objective evaluation of nasal congestion can be done by rhinomanometry as well as by acoustic rhinometry.[26] Rhinomanometry measures resistance to flow by simultaneously measuring pressure and flow, using a pressure transducer placed in the anterior nostril or posteriorly, in the oro- or nasopharynx. On the other hand, acoustic rhinometry uses a piezoelectric spark to generate a three-dimensional image of the nasal passages, which allows measurement of nasal volume and the cross sectional area. Finally, nasal responses can easily be assessed by using compound symptoms scores, such as the scoring system according to Lebel et al.[41]

A bronchial challenge test is the final test to prove the specific cause of occupational asthma. In all cases, spirometry (FEV_1 and forced vital capacity (FVC)) should be monitored on a control day to ensure stability of airway caliber. Depending on the nature of the agent, the challenges can be performed by exposing the employee to a fine dust or by nebulizing an aerosol. Exposure is increased progressively for up to 2 hours with intermediate functional assessments. As on the control day, spirometry is performed immediately and 10 minutes after each period of exposure. A significant reaction is defined as a 20% fall in FEV_1.[25] An immediate reaction is maximal 10 to 30 minutes after exposure, with complete recovery within 1-2 hours. Although usually readily reversible by inhaled $beta_2$-agonists, these reactions are actually the most dangerous, as they can be severe and unpredictable. Late reactions develop slowly, either 1-2 hours or 4-8 hours after exposure, generally responding well to inhaled $beta_2$-agonists.

A false-negative response may occur if the wrong agent is used, if the exposure conditions are not comparable with those in the workplace, or if the employee has been away from work for a long time.[1] Specific challenge tests are most useful when a new agent is suspected of causing occupational allergy.[5]

High-risk occupations in relation to occupational allergy

Certain occupational groups are known to be at particular risk of developing occupational allergy. A national health and nutrition examination survey from 1988 to 1994 in the United States revealed eight main industries identified at risk for work-related asthma: entertainment industry, agriculture, forestry and fishing, construction, electrical machinery, repair services and lodging places, respectively.[42] In the UK, the Surveillance of Work-related and Occupational Respiratory Disease (SWORD) project has provided a consistent and reliable estimate of the incidence and pattern of occupational respiratory disease. Between 1990 and 1997 over 24,000 cases were reported of which 27% involved occupational asthma. Identification of occupational groups at highest risk included spray painters, plastics processors and

manufacturers, chemical processors, bakers, laboratory workers, metal treatment workers and electrical assemblers.[43] Latex was first reported as a suspected cause of occupational allergy and has since become increasingly prominent. Sensitization rates are high among laboratory workers and various occupations in health services, such as nurses, operating room personnel and dentists.[44,45] From recent literature, besides bakery workers [31,46] and laboratory animal workers[30], workers in horticulture seem to be especially at risk. Occupational allergy has been described for plant keepers (weeping fig, Christmas and Easter cacti, Stephanotis floribunda),[12,23,24] florists and flower growers and employees of tomato and bell pepper greenhouses.[3-11,18]

Aim of study

The Commodity Board of Horticulture in the Netherlands provided important information on an increasing number of allergic complaints among workers in bell pepper and in *Chrysanthemum* greenhouses. Both cultivations have become important branches of horticulture under glass in the Netherlands (Figure 3). The presence of work-related allergic symptoms among bell pepper horticulturists has been described in two case reports by Gerth van Wijk and by van Toorenenbergen.[8,11] In 1993 and 1996 two inventory questionnaire surveys were performed among bell pepper gardeners and their employees, showing respectively that 15% and 22% of the workforce attributed their complaints to the work environment in the greenhouses.[47] As a possible explanation for these results the high pollen concentrations in the greenhouses were mentioned. The relation, however, between exposure to pollen and the reported symptoms was not further analysed and until now no studies have been published on work-related symptoms among bell pepper horticulturists.

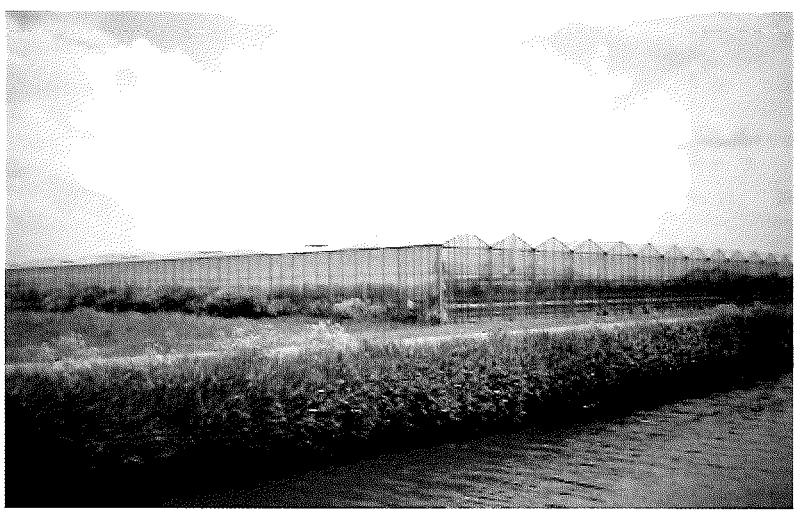

Figure 3 Greenhouses in the western part of the Netherlands ("Westland" or "glass city").

Pollens from cut flowers and ornamental plants mostly do not give rise to allergic symptoms because these pollens are spread by insects and not by the wind. However, in the case of high exposure in an occupational setting, sensitization might occur. Up to the present, only a few studies have been published in the literature on IgE-mediated occupational allergy to *Chrysanthemum* pollen.[4,6-9] A broad investigation, however, among employees of several *Chrysanthemum* greenhouses with different kinds of *Chrysanthemum* pollen has never been performed.
Therefore, new studies should focus on the prevalence of work-related symptoms and determinants of specific sensitization related to exposure as risk factor. The demonstration of a relation between exposure and work-related symptoms is not only an important element in establishing causality in epidemiological studies, it also offers prospects of environmental control and possible solutions to prevent the development of occupational allergies.

Of course, the presence of other possible occupational allergens in greenhouses should be considered. During the collection of pollen for our pollen extract, the presence of large amounts of predatory mites in the flowers of the plants was noticed. The predatory mite *Amblyseius cucumeris* was introduced into bell pepper greenhouses in 1985 as an effective biological control agent for common thrips species[48,49], the most important pest in bell pepper horticulture (Figure 4). It has been used for year-round biological control ever since. Reports on sensitization to these predatory mites or an antigenic relation between the common house dust mite and the predatory mite are scarcely present in the scientific literature. However, the relation between allergic complaints and sensitization to mites in general is well known. An increased exposure to predatory mites, as currently observed, might very well lead to sensitization and subsequently to work-related symptoms. Therefore, the role of the predatory mite should be evaluated as well.

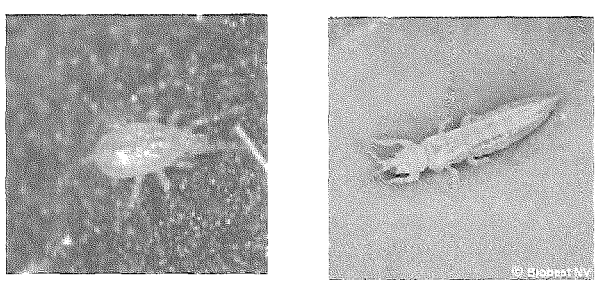

Figure 4 The predatory mite *Amblyseius cucumeris* and its food source (thrips).

Not everyone develops occupational allergy given the same degree of exposure to occupational allergens. Atopy, an inherited tendency to produce IgE antibodies to inhaled allergens, has been found to be an important risk factor for developing work-related symptoms in several other studies on occupational allergy[30,31,44,45]. Therefore, the question can be addressed whether employees sensitized to common inhalant

allergens, in particular to pollen, are more likely to become sensitized to bell pepper and/or *chrysanthemum* pollen compared to non-atopic employees, and whether there exist cross-reactivities between the different pollens.
Finally, an occupational allergy may impair employees' day-to-day functioning and because of this, it may place a great economic burden on both the individual and occupational group as a whole. For this reason, estimation of the influence of sensitization to different occupational allergens on the quality of life of greenhouse employees seems relevant.

The objectives of this thesis:

1. To determine the prevalence of rhinoconjunctivitis and asthma among employees of bell pepper and *Chrysanthemum* greenhouses.
2. To assess the prevalence of type I allergy to bell pepper and *Chrysanthemum* pollen among employees of greenhouses in relation to exposure.
3. To investigate the role of predatory mites as the cause of occupational complaints in bell pepper greenhouses.
4. To evaluate whether there is a connection between atopic constitution, especially to other pollen, and the prevalence of an occupational allergy to bell pepper and *Chrysanthemum* pollen.
5. To study the effect of an occupational allergy on the quality of life of greenhouse employees.

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Prevalence of occupational allergy to bell pepper pollen in greenhouses in the Netherlands

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Abstract

Background

An increasing number of allergic complaints appear to have occurred among bell pepper greenhouse employees.

Objective

The aim of this study was to estimate the prevalence of work-related allergic symptoms and the prevalence of sensitization to specific occupational allergens and its determinants.

Methods

We studied 472 employees who were invited to answer an extensive questionnaire and to be tested on location with inhalant allergens and home-made extracts of the bell pepper plant. In addition, peak expiratory flow monitoring and RASTs were performed.

Results

Work-related symptoms were reported in 53.8% of all cases. Sensitization to the bell pepper plant was found in 35.4%. Positive reactions to leaf, stem and/or juice, however, were associated in nearly 90% with sensitization to pollen, which appeared to be most important allergen of the plant. Sensitization to the bell pepper plant and inhalant atopy were considered the most important risk factors for the occurrence of work-related symptoms of the upper airways (PRR 2.63, CI 2.11- 3.25 and PRR 2.25, CI 1.82 - 2.79) as well as of the lower airways (PRR 4.08, CI 2.38 - 7.00 and PRR 3.16., CI 1.87 -5.33).

Conclusion

There is a surprisingly high prevalence of work-related respiratory symptoms (53.8%) in bell pepper horticulture. In two-thirds of the employees, symptoms at work were associated with an IgE-mediated allergy due to the high and chronic exposure to bell pepper pollen. Complaints at work without specific sensitization to bell pepper pollen can be caused by non-specific hyperreactivity or atopy to other occupational allergens. The extent of this occupational allergy has important consequences for the health care of this large, still growing occupational group.

Introduction

Bell pepper cultivation in the Netherlands has increased enormously during the past few years. It has become the main branch of horticulture under glass, with a size of 1150 hectares, divided over approximately 1500 greenhouses, and a workforce of more than 4000 people. However, at the same time an increasing number of allergic complaints appear to have occurred among the workers. The presence of work-related allergic symptoms among bell pepper horticulturists has been described in two case reports by Gerth van Wijk and by van Toorenenbergen[1,2]. It was already mentioned at that time that the handling of bell peppers or contact with the pollen of the plant may cause an IgE-mediated allergy, with symptoms ranging from rhinoconjunctivitis to asthma. Recently, it was demonstrated that major IgE binding occurred to allergens of about 30 and 64 kDa in bell pepper pollen extract[3]. In 1993 and 1996 two inventory questionnaire surveys were performed among bell pepper gardeners and their employees, showing that 15% and 22%, respectively, of the workforce attributed their complaints to the work environment in the greenhouses. As a possible explanation for these results the high pollen concentrations in the greenhouses were mentioned[4]. Unfortunately, the relation between exposure to pollen and the reported symptoms was not further analysed and until now no studies have been published on work-related symptoms among bell pepper horticulturists. The prevalence of work-related symptoms and determinants of specific sensitization remain largely unknown. The described observations raised concern about health problems within this large occupational group. As a result we were asked by the Commodity Board for Horticulture to start a broad investigation among employees in bell pepper greenhouses. The aim of this study was to estimate the prevalence and determinants of work-related allergic symptoms among this population at risk, with a special focus on bell pepper pollen.

Methods

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, job and job activities, work history, symptoms at work, and atopic complaints. Symptoms present after occupational exposure comprized five categories: itching, redness and/or eczema of the skin, urticaria/angioedema, rhinitis (sneezing, rhinorrhoea, itching, obstruction), conjunctivitis (redness, itching, 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. During the second visit sensitization to occupational allergens (substances of the bell pepper plant and pollen) was determined by means of skin prick tests. Blood samples were taken to evaluate sensitization by RAST.

To study possible work-related asthmatic reactions peak expiratory flow (PEF) monitoring was performed during the 2 weeks following the medical survey. For reasons of feasibility bronchial provocation tests with histamine were not performed during the visit on site. However, the decline in PEF over a working day or between days away from and at work can also be used as a tool to detect occupational asthma[5]. Each participant was given a mini-Wright peak flow meter (Glaxo Wellcome, Zeist, the Netherlands) and they were asked to record PEF twice a day: before and just after work during working days and during the weekend or days away from work on waking and at bedtime. On each occasion they were asked to blow after maximal inspiration three times into the peak flow meter; the highest of the three attempts was used for analysis. On each day the use of medication and symptom scores (rhinitis and asthma symptoms) on a four-point scale (0 = no symptoms, 1-2 = moderate symptoms, 3 = severe symptoms) were noted by the participant in a diary. For each day the mean PEF and the absolute difference between morning and afternoon were calculated. In the statistical analysis the two indices used were the average PEF (PEFMEAN), calculated as average over the mean PEF on 14 days, and the average PEF difference, calculated as average over the PEF difference on 14 days (PEFDIF). The study was approved by our hospital medical ethical committee. Confidentiality was maintained.

Reference group

Ten non-atopic volunteers without allergic complaints who had never been in contact with bell pepper plants were skin tested to detect irritative, non-specific reactions to our home-made occupational allergen extracts.

Prick tests

Prick tests were performed by application of one drop of allergenic extract to the skin of the volar side of the forearm. Subsequently, the dermis was punctured with a standardized skin test needle and the results were read after 20 min. Reactions were expressed (mm) of mean weal diameter (adding the longest diameter to the orthogonal diameter and dividing it by 2). A diameter of 3 mm or more was considered positive[6]. Dilution buffer was used as a negative control, histamine chloride 10mg/mL as a positive control.

Allergens

Skin prick tests were performed with *botrytis cinerea* (SQ 412) as one of the moulds found in greenhouses 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). 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 predatory mites *(Amblyseius cucumeris)* was not used yet. A 10% (w/v) extract was prepared in phosphate-buffered saline pH 7.4, containing 0.03% human serum albumin 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. Stamen from the bell pepper flowers were collected in August. We prepared a 25% (w/v) extract in PBS. All extracts were centrifuged for 10 min at 2000 *g* and supernatants were passed through a 0.22-μm Millex GS filter (Millipore, Bedford, MA, USA). To prepare bell pepper juice, 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 were determined by the method of Watanabe et al. with pyrogallol red molybdate complex[7]. The protein concentrations of the 25% extract of the stem, leaf and stamen were 0.28 g/L, 0.35 g/L and 1.01 g/L, respectively, whereas 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 skin test and mixed.

RAST

Pollen from flowers of the bell pepper plant were obtained from a greenhouse. Allergen-specific IgE was determined by RAST by the use of agarose beads as allergen support, as described by Adkinson et al.[8]. An amount of 10 mg of pollen was extracted with 2mL coupling buffer (0.1 mol/L $NaHCO_3$ and 0.5mol/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, MO, USA), according to the manufacturer's instructions. An amount of 2 mg per test of bell pepper 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-square test (χ^2). Multiple linear regression analysis was applied to study the influence of several individual characteristics, respiratory symptoms and work history on the peak flow outcomes. A generalized loglinear 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 is a better approximation of the relative risk than the often-used odds ratio in situations where the disease prevalence is high[9]. As age appears strongly to 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 loglinear 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 (esp PROC REG and PROC GENMOD) was executed using the SAS computer package.

Results

Population characteristics

Of the 110 greenhouses approached by telephone, 79 participated in the study. Moreover, six additional greenhouses announced they would take part. 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 of 96.9%). The greenhouses in the study together cover an area of 1 888 363 square metres, nearly 10% of the total bell pepper horticulture in the Netherlands. Population characteristics and characteristics of the participating greenhouses, concerning the number of regular and seasonal employees and the area, are given in Table 1.

Symptoms at work were highly prevalent among the greenhouse workers. One or more symptoms were reported by 254 employees (53.8%), of whom 208 (44%) notified a substantial improvement or complete regression on weekends and holidays. Complaints consisted of rhinitis in 233 individuals (49.4%) and of conjunctivitis in 143 individuals (30.3%). Redness, itching and/or eczema of the skin were mentioned by 83 individuals (17.6%), asthma by 63 individuals (13.3%) and urticaria and/or angioedema by 42 individuals (8.9%). In subsequent analyses work-related rhinitis and conjunctivitis are analysed together as symptoms of the upper airways (51.3%) and compared with work-related symptoms of the lower airways (13.3%), considered as the most serious manifestation of an occupational allergy.

One parameter was used as indicator of atopy: the presence of a positive skin prick

Table 1. Characteristics of the bell pepper greenhouses and their employees (n = 472)

	Mean	SD	Range
Age (yr)	36.6	11.5	13-79
Duration of employment (yr)	8.5	6.1	0-31
Regular employees (n)	6.0	2.6	1-13
Seasonal employees (n)	3.7	3.1	0-15
Area of the greenhouse (m^2)	24710.5	10425.9	6500-50000
	n	**%**	
Sex: male	387	82.0	
Sex: female	85	18.0	
Smoking	153	32.4	
Job classification			
Owner	133	28.2	
Supervisor	14	3.0	
Full-time employee	235	49.8	
Part-time employee	60	12.7	
Sorter	30	6.4	

test result (defined as a weal size of 3 mm or more) to at least one of the common inhalant allergens. This was found in 177 employees (37.5%). Sensitization to occupational allergens was also highly prevalent. Of all employees, 167 (35.4%) were sensitized to various substances of the bell pepper plant, 129 of them (27.3%) to the pollen as shown in Table 2. In addition, all control subjects showed negative responses in the skin prick test.

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 work-related symptoms and sensitization to occupational allergens (Table 3). The majority of the sensitized employees appeared to have work-related symptoms (84%), whereas only 55% of the employees with work-related symptoms were sensitized to the bell pepper plant (group A vs. group B). A high proportion of the employees in group A (128) were sensitized to bell pepper pollen and/or stamen (91.4%), which also contain a certain amount of pollen. The majority of them (93) also had positive reactions in the skin prick test to leaf, stem and/or juice of the plant. In group C 22 employees appeared to be sensitized to the pollen and/or stamen (81.5%) but only five of them were also sensitized to one of the other tested substances of the bell pepper plant.
Sensitization to those substances without sensitization to pollen was only seen in a relatively small number of employees: in group A eight employees showed an isolated positive reaction to bell pepper juice and one to the leaf of the plant, while one and two employees, respectively, were found positive to the leaf and bell pepper juice and to the stem and bell pepper juice. Five employees in group C were only sensitized to the bell pepper juice. A high proportion of the employees in group A were also sensitized to at least one of the common inhalant allergens (76%). The presence of specific IgE to common allergens was less prevalent in the other three groups, ranging from 32% and 33%, respectively, in group B and C to only 13% in group D.

Determinants of work-related symptoms

Tables 4 and 5 show the association between work-related respiratory symptoms and the significant determinants in the univariate as well as in the multivariate analysis. The two most important variables strongly associated with work-related symptoms were sensitization to substances of the bell pepper plant and inhalant atopy. These associations, however, were clearly more pronounced for symptoms of the lower airways. A third positive association was found between the age of the employee and work-related symptoms of the upper airways and although significant, the prevalence rate was much lower compared with the other two determinants. The number of reported work-related symptoms decreased by age (P =0.005), with the lowest prevalence for the group employees of 40 years and older (28.6% vs. 36% for the group of 30 years and younger).
Several other determinants were tested but none of them was significantly

Table 2. Prevalence of work-related symptoms and results of the skin prick tests

Symptoms	Symptoms at work		Symptoms at work, less or absent in weekends	
	n	%	n	%
Skin (itching/redness/eczema)	83	17.6	71	15.1
Urticaria and/or angioedema	42	8.9	38	8.1
Rhinitis	233	49.4	190	40.3
Conjunctivitis	143	30.3	124	26.3
Asthma	63	13.3	55	11.7
Skin prick test positive:				
Inhalant allergens				
Dermatophagoides pteronyssinus	122	25.8		
Tree pollen	53	11.2		
Grass pollen	90	19.1		
Mugwort	35	7.4		
Cat dander	41	8.7		
Dog dander	63	13.3		
Botrytis	24	5.1		
Occupational allergens				
Leaf bell pepper plant	74	15.7		
Stem bell pepper plant	52	11.0		
Pollen bell pepper plant	129	27.3		
Stamen bell pepper plant	139	29.4		
Bell pepper juice	97	20.6		

Table 3. Results of the skin prick tests of greenhouse employees grouped by work-related symptoms en sensitization to occupational allergens (proteins of the bell pepper plant and pollen) (n = 472).

Group	A	B	C	D	total
Work-related symptoms	+	+	-	-	
Sensitisation to occupational allergens	+	-	+	-	
n	140	114	27	191	
Positive skin prick test					
Bell pepper leaf	72	-	2	-	74
Bell pepper stem	51	-	1	-	52
Bell pepper pollen	116	-	13	-	129
Bell pepper stamen	120	-	19	-	139
Bell pepper juice	87	-	10	-	97
Inhalant allergens	107	36	9	25	177

Group A: symptoms at work and occupational sensitization
Group B: symptoms at work without occupational sensitization
Group C: asymptomatic workers with occupational sensitization
Group D: asymptomatic workers without occupational sensitization

associated with work-related symptoms of upper and lower airways: sex (PRR 0.97 and 1.01), job classification (PRR 0.91 and 1.38), years of education (PRR 0.99 and 1.01), hours per week (PRR 1.27 and 0.84), size of the greenhouse (PRR 0.99 and 1.02) and smokers (PRR 0.88 and 0.71). Addition of any of these variables to the multivariate model did not change the results as presented in Table 5. Furthermore, inhalant atopy appeared to be an important determinant of sensitization to the bell pepper plant, with a highly significant prevalence rate ratio (PRR 3.79, 95% CI 2.89 - 4.97). Of all common inhalant allergens, sensitization to grass pollen and house dust mite were most prevalent in our study population. However, as suspected, the association between sensitization to grass pollen and sensitization to bell pepper pollen (PRR 4.88, CI 3.76 - 6.32) was stronger than the association between the house dust mite and bell pepper pollen (PRR 2.27, CI 1.72 -3.00).

Peak flow results

Completed PEF readings and symptom diaries over 14 days were returned by 448 employees (94.9%). Twelve participants, however, had PEF records without any difference between morning and evening during 7 days or more. Those records were considered unreliable and as a consequence left out for analysis. Multiple regression analyses were performed with the two PEF indices as dependent variables. The PEFMEAN of the entire group (n=436) was 508.5 L/min (SD ± 27.00) and the PEFDIF, assessing PEF variability, -12.07 L/min (SD ± 37.8). Slight to moderate symptoms of the upper airways were mentioned by 306 employees and severe symptoms by 179 employees. Considering the lower airways, slight to moderate symptoms were reported by 198 subjects during the 2-week period of peak flow measurements, while 98 mentioned severe symptoms in the accompanying diary. Workers with complaints of the upper airways during the peak flow measurement period had similar PEFMEANs and PEFDIFs than those without these complaints. However, for subjects with lower respiratory complaints on at least 7 out of 14 days the PEFMEAN decreased by 52.4 L/s (10.3%) for those with moderate symptoms and 66.9 L/s (13.2%) for those with severe symptoms. Workers with asthmatic complaints in the baseline survey had a significantly lower PEFMEAN of -40.2 L/s (7.9%). Sensitization to substances of the bell pepper plant was not associated with the peak flow measurements.

RAST

Bell pepper pollen specific IgE was demonstrated in 88 employees, ranging from 0.36 to 80.7 E/mL. Of this group, 83 employees had a positive skin prick test result to pollen, indicating that in 64% (83/129) of the employees sensitized to bell pepper pollen the presence of specific IgE to these pollen could be confirmed by skin prick testing as well as by RAST. In the other five employees only the RAST test was found positive. Of nine employees with a positive skin prick test to pollen serum samples were not available. The other 37 employees with a positive prick test were RAST negative, as were all the other individuals with negative skin test results for bell pepper pollen.

Discussion

In this study a high prevalence of work-related respiratory symptoms among employees of bell pepper greenhouses was found (53.8%), the main symptoms being rhinitis and conjunctivitis in 49.4 and 30.3%, respectively. Local dermatitis and asthma were reported to a lesser extent: 17.6 and 13.3%, respectively. Asthma is considered the most serious manifestation of an occupational allergy, mostly preceded by rhinoconjunctivitis[10]. Sensitization to proteins of the bell pepper plant, confirmed by skin prick testing and RAST, appeared to be the most important and strongest determinant for the occurrence of work-related symptoms. This association was even more pronounced for asthma symptoms than for symptoms of nose, eyes and skin. Of all tested substances of the bell pepper plant, the pollen appeared to be the most important allergen. Pollen are not only responsible for a large proportion of the work-related complaints mentioned but probably also for the more severe symptoms. A higher percentage of workers with asthma complaints was found in the group of symptomatic employees with sensitization to pollen and/or stamen than among symptomatic employees without sensitization (30% vs. 18%).

The established association between sensitization to pollen and lower airway symptoms is also supported by the results of the PEF measurements. Work-related respiratory symptoms caused a 10% decrease of the average PEF. The fact that full-time working employees with a daily exposure to the pollen have an increased PEF might be due to a selection process: a considerable number of symptomatic employees with subsequently a lower PEF will change job or reduce their number of working hours. The prevalence rate of work-related respiratory symptoms in this study was surprisingly high compared with the previous surveys among bell pepper horticulturists in 1993 (15%) and 1996 (22%). The prevalence rate of sensitization to occupational allergens in this study was also high compared with previous epidemiological studies on occupational allergies: rat/mouse urinary allergens in laboratory workers were found positive in 18.2% and in 10.7%, respectively[5], wheat flour/α-amylase in bakers in 10% and in 7%, respectively[11], and natural rubber latex in operation room personnel in 14.1%[12].

Apart from sensitization to the bell pepper plant inhalant atopy was also considered a significant risk factor for symptoms of the lower airways. However, its effect on the development of work-related symptoms is less strong than the effect of sensitization to the bell pepper pollen and plant. Atopy has been identified as an important risk factor for work-related symptoms in several other studies[5,11-13]. In this study 116 (69.5%) of the 167 employees sensitized to the bell pepper plant were also sensitized to one or more common inhalant allergens, 71 to grass pollen. This illustrates a clear association between sensitization to the bell pepper pollen and inhalant atopy, in particular to grass pollen.

The age of the employee was also identified as a risk factor for the development of work-related rhinoconjunctivitis and/or local dermatitis. There was a significant decrease in the prevalence of these symptoms in different age groups, with the lowest prevalence of work-related symptoms in the group of 40 years and older. This might indicate a so-called healthy worker effect as a result of selection processes.

Greenhouse workers who develop severe symptoms due to exposure to the bell pepper plant have changed their jobs because of their health problems. Casuistic information from current employees confirmed this hypothesis. A better estimate of this selection bias can only be studied in a longitudinal survey over several years. Duration of employment, job classification and full-time vs. part-time are proxies for exposure that appear not to capture the relevant exposure characteristics for the occurrence of work-related symptoms.

Although work-related symptoms in bell pepper greenhouses are obviously associated with contact with the pollen of the plants, not all symptoms could be explained by an IgE-mediated response to this allergen. In this study 53.8% of the employees reported work-related allergic symptoms but in only 65.7% of these workers IgE-sensitization to the bell pepper plant and pollen could be demonstrated. The question is what may have caused the work-related symptoms in the group of employees without sensitization. During the collection of pollen for our pollen extract, the presence of large amounts of predatory mites in the flowers of the plant was noticed. The predatory mite *Amblyseius cucumeris* (Ac) was introduced in bell pepper greenhouses in 1985 as an effective biological control agent for common thrips species, the most important pest in bell pepper horticulture. It has been used for year-round biological control ever since. Reports on sensitization to Ac or an antigenic relation between the common house dust mite and the predatory mite Ac are hardly found in scientific literature. However, the relation between allergic complaints and sensitization to mites in general is well known. An increased exposure to Ac, as currently observed in bell pepper horticulture, might very well lead to sensitization and subsequently to work-related symptoms from the skin, nose, eyes and lower airways. The role of Ac is discussed in more detail in another publication.

However, there are other possible explanations for work-related symptoms without sensitization. First, our skin prick test may have failed to detect sensitization in some individuals, although the method used was comparable to other studies performed by our research group. Secondly, the involved employees might be specifically sensitized to other occupational allergens, not identified yet. A non-specific reaction to the humid and warm environment in greenhouses is probably a third (additional) explanation.

The described relationship may have important consequences for health care in this large, still growing occupational group. It provides important evidence of causation and suggests that work-related symptoms are to some extent preventable by reducing exposure levels to pollen. The continuing fertilization process of the self-pollinated bell pepper plant requires the maintenance of high pollen concentrations throughout the greenhouse during the season. Besides, the necessary daily activities during the cultivation process, like heading of the bell pepper plants, cause the release of large amounts of pollen from the flowers. These pollen, well-known as rather large and heavy particles, are falling down towards the ground, immediately causing a peak exposure for the employee who is working on the crop. To perform the work-task properly, it is necessary that the employee stands very close with his face towards the plant and so contact with the pollen is hardly avoidable. Inhalation

of these pollen may cause immunological sensitization with subsequent allergic symptoms in sensitized workers. Personal contact with employees in bell pepper greenhouses confirmed that the mentioned activity on the plants often initiate the onset of symptoms or aggravate existing symptoms.
Measures should be taken at different levels at the same time. First, personal protection of the employee to avoid inhalation of pollen. Secondly, new upgrading methods to create plants with a good setting of bell peppers with as little pollen as possible. Finally, exploring the possibilities of genetic engineering of the bell pepper plant to create flowers without pollen. We fully realize that these solutions are relatively time-consuming to develop. Furthermore, we would like to emphasize the need for follow-up (longitudinal) survey to see whether sensitized employees without work-related symptoms will develop allergic complaints and whether sensitized employees with rhinoconjunctivitis will develop asthma during continuation of their current job.
In conclusion, there is a surprisingly high prevalence of work-related respiratory symptoms and sensitization to bell pepper pollen in bell pepper horticulture. The extent of this occupational allergy has important consequences for health care in this large, still growing occupational group.

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

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Abstract

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.

Introduction

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.

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, 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 τ-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, 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.

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)	23681	7000 - 46000
	n	**%**
Sex male	72	69
Sex female	32	31
Smoker	40	39
Job Classification		
Owner	32	31
Supervisor	1	1
Full-time employee	44	42
Part-time employee	23	22
Sorter	4	4

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

	n	%
Inhalant allergens		
Dermatophagoides pteronyssinus	20	19
Tree pollen	10	10
Grass pollen	16	15
Mugwort pollen	16	15
Dog dander	16	15
Cat dander	8	8
Botrytis cinerea	4	4
Chrysanthemum pollen		
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

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 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).

Rhinitus 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.

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

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

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Abstract

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 seventy-two 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.

Introduction

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. 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.

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

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/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 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 hyperreactivity 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 prevalent among these sensitized employees. One or more symptoms were reported by 83 employees (76.1%) of which 68 (62.4%) individuals

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	
	n	%	
Job classification			
Owner	31	28.4	
Supervisor	4	3.7	
Full-time employee	65	59.6	
Part-time employee	8	7.3	
Sorter	1	0.9	

(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

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

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 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 Figure 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*)*.

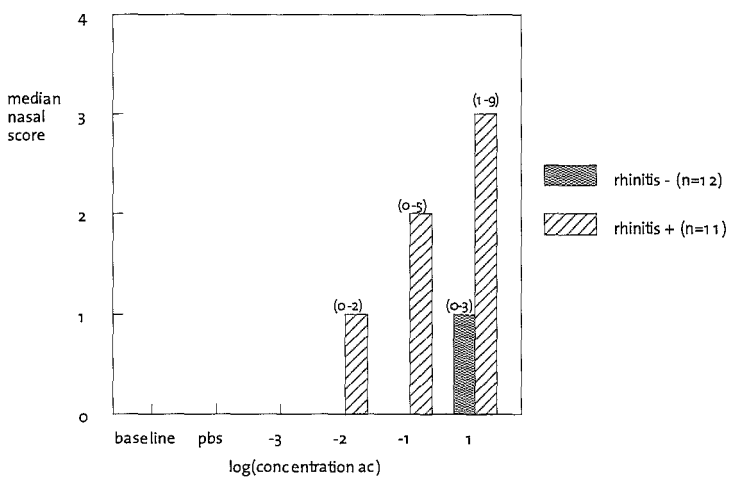

Figure 1 Nasal response to increasing doses of *Amblyseius cucumeris* (Ac) extract in 23 bell pepper greenhouse employees sensitized to Ac. For each Ac dose the data of the two groups were compared. Data are presented as medians (ranges in parenthesis).

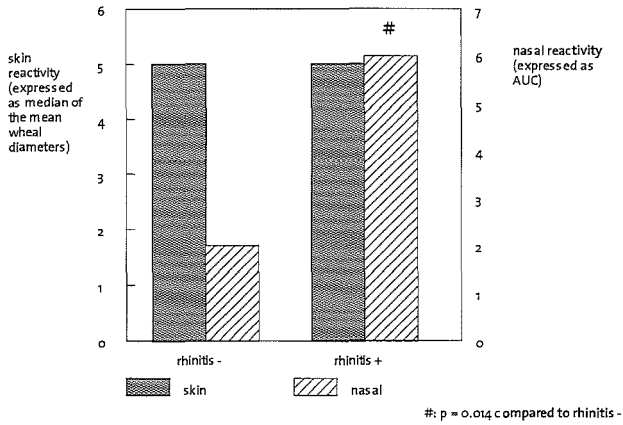

Figure2. The response to Ac in 23 sensitized employees expressed in skin and nasal reactivity. The responses of both groups were compared. Data are presented as medians. #*P*=0.014.

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].

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, cross-reactivity between Ac and Tp and between Ac and *D. pteronyssinus,* respectively, cannot be excluded. Further investigation by means of RAST inhibition tests and immunoblot analyses 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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Primary sensitization to sweet bell pepper pollen in greenhouse workers with occupational allergy

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Abstract

Background

In a previous investigation, a high prevalence of allergy to sweet bell pepper pollen was found among exposed horticulture workers. Allergy to plant-derived food is often the consequence of primary sensitization to common pollen allergens.

Objective

We therefore investigated the cross-reactivity between sweet bell pepper pollen and pollen from grass, birch or mugwort.

Methods

We selected 10 sera from greenhouse workers who had, besides specific IgE against sweet bell pepper pollen, also IgE to grass, birch or mugwort pollen. Cross-reactivity was tested by the inhibition of IgE binding to solid-phase coupled sweet bell pepper pollen extract. The 10 sera were also analysed for IgE binding to sweet bell pepper pollen by immunoblotting.

Results

With these sera, no or small inhibition of IgE binding to sweet bell pepper pollen extract was observed with grass, birch and mugwort pollen. With immunoblotting, major IgE-binding structures were seen at 14, 29 and 69 kDa in sweet bell pepper pollen extract.

Conclusion

The results of our study demonstrate that sweet bell pepper pollen contains allergens that have no or limited cross-reactivity with common pollen allergens. With sera from the 10 patients tested, sensitization to sweet bell pepper pollen was not the consequence of primary sensitization to common pollen allergens.

Introduction

About 10% of the greenhouse area in the Netherlands is used for growing sweet bell pepper fruit. The presence of IgE against sweet bell pepper pollen in greenhouse workers with symptoms of allergy upon contact with these plants has been described in a number of case reports[1-3]. Recently, the prevalence of occupational allergy to sweet bell pepper plants among 472 exposed horticulture workers was investigated. Skin prick tests with sweet bell pepper pollen extract were positive in one-third of the greenhouse workers[4]. A strong association was found between a positive skin test with grass pollen extract and sensitization to sweet bell pepper pollen[4].
IgE-mediated allergic reactions to plant-derived food are often a consequence of primary sensitization to common pollen allergens, like birch, grass and mugwort pollen[5,6].
In the present study, we addressed the question of whether occupational allergy to sweet bell pepper pollen is also a consequence of primary sensitization to these common pollen allergens. The results of our study indicate, however, that there is no or limited cross-reactivity between sweet bell pepper pollen and common pollen allergens.

Methods

Sera

From the group of 472 employees who participated in the prevalence study[4], sera with IgE antibodies to sweet bell pepper pollen were selected that came from patients with positive skin tests to birch, grass and/or mugwort pollen (Table 1). IgE antibody levels against these pollens are shown in Table 2. All 10 selected patients experienced work-related rhinitis; work-related asthma was not reported by these 10 patients. The study was approved by the Medical Ethical Committee of the University Hospital Rotterdam; all employees gave their written informed consent.

Allergens

Pollen from flowers of the sweet bell pepper plant and from flowers of tomato plants were collected in greenhouses. Pollen of grass *(Dactylus glomerata),* birch *(Betula pendula)* and mugwort *(Artemisia vulgaris)* were obtained from ARTU Biologicals BV (Lelystad, the Netherlands).

IgE antibody measurements

Specific IgE against sweet bell pepper, grass, birch and mugwort pollen was determined using agarose beads as allergen support, as previously described[1-3]; 10 mg of sweet bell pepper, grass, birch or mugwort pollen were extracted with 2mL coupling buffer (0.1 M $NaHCO_3$ 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 covalently coupled with 100 mg of CNBr-activated Sepharose 4B (Sigma Chemical Co., St Louis, USA). The sweet bell pepper, grass, birch and mugwort pollen

Table 1. Skin prick test results with GP: grass pollen, BP: birch pollen, MP: mugwort pollen and BPP: sweet bell pepper pollen extracts; the results are expressed as weal diameter, as described in[4]

	Skin prick test, weal (mm)			
Patient	GP	BP	MP	BPP
1	7	5	6	14
2	10	4	0	10
3	10	4	0	18
4	6	6	4	12
5	6	6	6	10
6	5	4	6	12
7	5	5	5	11
8	5	5	0	11
9	6	8	0	10
10	4	4	4	10

Table 2. Measurement of IgE against GP: grass pollen, BP: birch pollen, MP: mugwort pollen and BPP: sweet bell pepper pollen in sera from the 10 selected patients

	kUa/L				
Patient	GP	BP	MP	BPP	Total IgE
1	3.83	3.67	2.37	30.10	279
2	3.57	1.67	< 0.35	30.20	231
3	3.26	< 0.35	< 0.35	4.75	42
4	1.09	0.72	0.38	25.50	146
5	7.40	1.09	1.91	14.80	320
6	1.54	1.10	2.78	27.10	191
7	6.15	6.31	1.73	45.80	486
8	<0.35	4.04	<0.35	3.56	123
9	0.55	4.04	0.92	14.40	319
10	nd	nd	nd	8.73	223

sepharose conjugates were suspended in 25 mL RAST buffer (0.82% NaCl, 10 mM phosphate, 0.1 % NaN_3, 0.2% Tween 20, 0.3% bovine serum albumin, pH 7.4). In all, 2mg per test of sweet bell pepper, grass, birch or mugwort pollen sepharose conjugates, suspended in 0.25 mL RAST buffer, was incubated overnight with 0.05 mL patient serum. After four washes, the agarose beads were further incubated overnight with 0.05 mL radio-iodinated anti-IgE antibodies (Pharmacia & Upjohn, Uppsala, Sweden). After another four washes, bound radioactivity was measured and expressed in kUa/L when read from the Pharmacia CAP standard curve.

IgE inhibition

For IgE inhibition experiments, 20 mg pollen was extracted with 1 mL RAST buffer under rotation for 1 h. After centrifugation, 0.05 mL supernatant was incubated

overnight at room temperature with 0.05 mL serum (diluted in RAST buffer). Sera 3, 8 and 10 were incubated undiluted, sera 5 and 9 were 2 x diluted, sera 1, 2, 4 and 6 were 3 x diluted, and serum 7 was 4 x diluted. A volume of 0.05 mL of this mixture was incubated with pollen sepharose as described above.
For immunoblotting, sweet bell pepper pollen was extracted (10 mg/mL) in distilled water for 1 h at room temperature, under continuous rotation. After centrifugation, the supernatant was frozen in aliquots at -20°C. The protein content of this supernatant contained 40mg/L, when analysed with the benzethonium chloride method[7] on the Hitachi 911 routine clinical chemistry analyser, according to the manufacturer's instructions (Boehringer/Roche, Almere, the Netherlands). Before electrophoresis, this extract, diluted in sample buffer, including sodium dodecyl-sulphate and dithiothreitol, was placed at 100°C for 5 min.

Immunoblotting

SDS-PAGE (12% homogeneous gel 100 x 70 x 1 mm 5% stacking gel) and electrotransfer to nitrocellulose were performed with the Mini Protean II system (BioRAD Laboratories, Veenendaal, the Netherlands). After electrotransfer, the nitrocellulose strips (BioRAD Laboratories, Veenendaal, the Netherlands) were saturated at 37°C with RAST buffer for 1 h. Sera were diluted (1 : 2) with the same buffer and nitrocellulose strips were incubated with 0.5 mL diluted serum under continuous shaking overnight at room temperature. After the removal of unbound compounds by washing (PBS/0.2% Tween 20), radiolabelled anti-IgE (Pharmacia & Upjohn, Uppsala, Sweden) was added. Bound IgE was visualized by autoradiography.

Results

Whereas IgE binding to agarose-bound sweet bell pepper pollen extract could be largely inhibited by pre-incubation of the 10 selected sera with sweet bell pepper pollen extract, the extracts of tomato, grass, birch and mugwort pollen had no or only a small inhibitory effect (Figure 1). The amounts of grass, birch and mugwort pollen extract, used for the inhibition of IgE binding to agarose-bound sweet bell pepper pollen extract, were able to inhibit IgE binding to homologous UniCAP reagents (data not shown).
IgE binding to sweet bell pepper was further analysed by immunoblotting. With seven sera, strong IgE binding occurred to a component of about 14kDa. A heterogeneous pattern of IgE binding to other proteins was found with prominent IgE binding to 21 kDa (serum 4), 29 kDa (sera 1 and 2), 69 kDa (sera 5 and 9), and 56 and 43 kDa proteins (serum 9) (Figure 2).
Three of these 10 sera were pre-incubated with sweet bell pepper pollen extract, before they were added to agarose-bound grass, birch or mugwort pollen extract. Sweet bell pepper pollen extract could inhibit the IgE binding to grass and birch pollen (Figure 3). Not enough serum was available to incubate all 10 sera from Figure 1 with birch, grass or mugwort pollen sepharose, after pre-incubation with sweet bell pepper pollen extract.

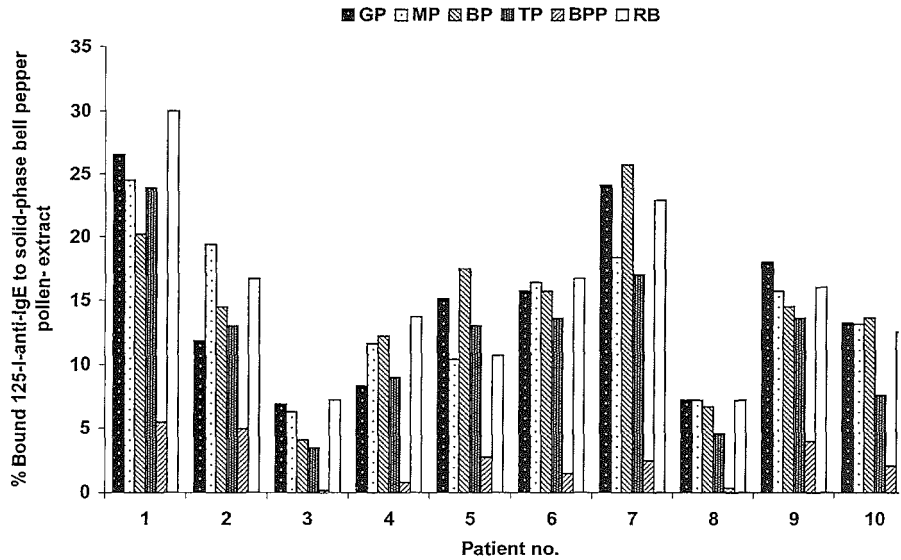

Figure 1 Inhibition of IgE binding to sepharose-bound sweet bell pepper pollen extract with – from left to right – extracts of grass pollen (GP), mugwort pollen (MP), birch pollen (BP), tomato pollen (TP) or sweet bell pepper pollen (BPP), and with RAST buffer (RB) as a control.

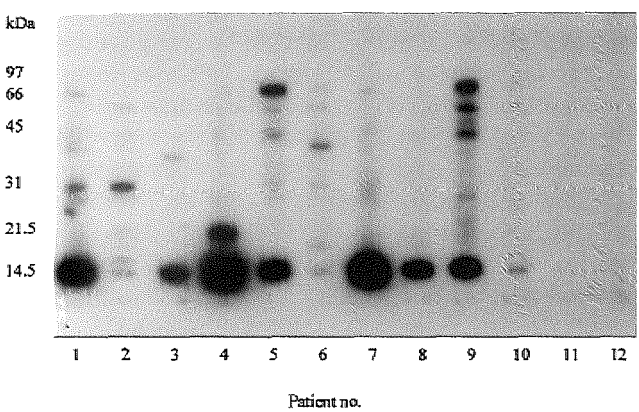

Figure 2 IgE binding to SDS-PAGE separated sweet bell pepper pollen extract after incubation with 10 sera from greenhouse workers with IgE against sweet bell pepper pollen (lanes 1–10), serum of a patient with no IgE against common pollen (lane 11) and buffer control (lane 12).

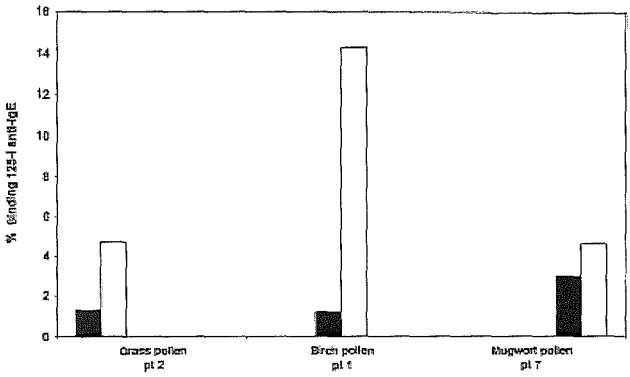

Figure 3 Inhibition of IgE binding to sepharose-bound birch, grass or mugwort pollen extract with an extract of bell pepper pollen. Three sera from Table 2 were tested. White columns: pre-incubation with buffer; black columns: pre-incubation with bell pepper pollen extract.

Discussion

Because of the strong association between positive skin tests to sweet bell pepper and grass pollen in our recent study[4], cross-reactivity between these pollens was expected. Further more, extensive IgE cross-reactivity between tomato fruit and grass pollen allergens was found by Petersen et al.[8]; sweet bell pepper and tomato both belong to the Solanaceae plant family. The results shown in Figure 1, however, indicate that most IgE-binding structures in sweet bell pepper pollen do not cross-react with allergens in grass, birch or mugwort pollen. In the 10 selected sera, the average IgE levels against grass, birch or mugwort pollen were at least seven times lower than the IgE levels against sweet bell pepper pollen (Table 2). The results in Figure 3 rather suggest that the IgE binding to common pollen extracts in sera with relatively low levels of IgE against these pollens can actually be caused by primary sensitization to sweet bell pepper pollen. Groenewoud et al.[4] found that 51 of 187 patients with positive skin tests to sweet bell pepper pollen were negative in skin tests with grass, birch or mugwort pollen extract. This further indicates that sweet bell pepper pollen contains IgE-binding structures that are not present in grass, birch or mugwort pollen. Pre-incubation of the 10 sera with tomato pollen extract showed no inhibition of IgE binding to solid-phase-coupled sweet bell pepper pollen extract (Figure 1). In a previous study[3], the absence of cross-reactivity between bell pepper and tomato pollen towards IgE from two greenhouse workers was also seen; one of these two patients had IgE against sweet bell pepper pollen, but no IgE against tomato pollen. In the same report[3], IgE from a greenhouse worker with symptoms of rhinitis and asthma upon contact with tomato plants displayed partial cross-reactivity between tomato and sweet bell pepper pollen: IgE binding to tomato pollen could only partially be inhibited by sweet bell pepper pollen extract. In contrast, pre-incubation of serum from this patient with tomato pollen extract showed complete inhibition of the IgE binding to solid-phase-coupled sweet bell pepper pollen extract.
In immunoblotting, major IgE binding was seen to a protein of about 14 kDa.

In a previous case report, no substantial IgE binding to a 14 kDa protein was seen[3]. Similar to these cases[3], sera 2 and 6 in Figure 2 also show more IgE binding to another protein than to the 14 kDa band. Yu et al.[9] isolated profilin cDNA from tomato pollen and calculated the molecular weight of the protein as 14.435 kDa. This may suggest that the 14 kDa band in the figure is a sweet bell pepper pollen profilin. Jensen-Jarolim et al.[10], who analysed IgE-binding structures in bell pepper fruit, could inhibit IgE binding to a 14 kDa band in SDS-PAGE separated and nitrocellulose blotted sweet bell fruit pepper extract by pre-incubation of sera from food-allergic patients with recombinant birch pollen profilin. Not enough serum was available to extend the experiments, shown in Figure 1, with sera, pre-incubated with recombinant birch, pollen profilin.

The lack of cross-reactivity between bell pepper pollen and common environmental pollen has practical consequences for the diagnosis of occupational allergy: a negative result of skin tests with, or IgE antibody measurements against, common inhalant allergens does not exclude the presence of an IgE-mediated occupational allergy. In her review on spice allergy, Niinimaki[11] quotes three case reports of inhalant allergy to powdered spices, all three with negative skin test results to birch, grass and mugwort pollen.

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Impact of occupational and inhalant allergy on rhinitis specific quality of life in employees of bell pepper greenhouses in the Netherlands

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Submitted

Abstract

Background

Rhinitis symptoms among bell pepper greenhouse employees can be caused by an allergy to occupational allergens such as bell pepper pollen and predatory mites (*Amblyseius cucumeris*) and common inhalant allergens.

Objectives

We attempted to estimate the influence of sensitization to these different allergens on rhinitis specific quality of life (QoL) in and outside the flowering period of the crop. In addition, it was assessed whether the QoL of sensitized bell pepper greenhouse employees is comparable to the QoL of *Chrysanthemum* greenhouse employees with rhinitis and to an average population-sample with perennial rhinitis, respectively.

Methods

233 employees with symptoms of rhinitis were invited to complete two rhinitis QoL questionnaires (RQLQ) and to be skin-prick tested on location with common inhalant allergens and occupational extracts. In 209/233 employees who completed the questionnaires, multiple regression analysis was used to estimate the impact of sensitization to the various occupational and common inhalant allergens on QoL.

Results

Sensitization to bell pepper pollen had a significant negative effect on all domains and the mean QoL. Tree pollen also affected the QoL except for the domains activities, non-rhinitis symptoms and emotions. The other allergens had no influence on the QoL. A significant decrease of all rhinitis scores was found outside the flowering period of the crop. Bell pepper greenhouse employees were more impaired in practical problems compared with *Chrysanthemum* greenhouse employees. There were no other relevant differences in the mean scores of the different domains for both occupational groups. Furthermore, greenhouse employees scored higher on limitations in activities and surprisingly much lower on emotional, sleeping and practical problems, compared to subjects with perennial rhinitis.

Conclusions

Bell pepper greenhouse employees with rhinitis are impaired in QoL because of their sensitization to bell pepper pollen whereas allergies to predatory mites or common inhalant allergens do not influence rhinitis specific QoL. These findings suggest that bell pepper pollen is the most important occupational allergen in greenhouse workers showing allergic symptomatology. Irrespective of the causative occupational allergen, the QoL of affected greenhouse employees is comparable with each other. A common allergy does not have more impact on a person's day-to-day life than an occupational allergy, however, there is a clear difference in the way in which an occupational group is hampered in daily life, when compared with a non-occupational group.

Introduction

In the last decade, the economic and social impact of chronic respiratory diseases in industrialised countries has become topical, especially because of their long-term effect on the quality of life (QoL) of affected subjects. However, this does not only include asthma or chronic obstructive pulmonary diseases but also allergic rhinitis, nowadays a rather frequent and persistent condition. Individuals with allergic rhinitis experience their complaints as troublesome and, consequently, consider their QoL as diminished compared to healthy subjects[1,2]. Allergic complaints, especially rhinitis, are a major health problem in bell pepper horticulture nowadays. The high prevalence of allergic symptoms among employees is not only caused by common inhalant allergens like house dust mite or tree pollen but mainly by occupational allergens. The pollen of the bell pepper plant and the predatory mite *Amblyseius cucumeris* (*Ac*), used for biological control, appeared to be the most important offending agents[3,4]. Employees with rhinitis experience not only nasal symptoms like sneezing, nasal itchiness, rhinorrhoea, and nasal blockage after exposure. They are also confronted with problems like whether to continue in their current job or to leave and retrain for a new job or to sell their greenhouse. These kinds of changes in lifestyle might also have impact on the QoL. Malo et al. assessed the QoL of subjects with occupational asthma[5]. However, the influence of occupational rhinitis in a large population-based sample of employees on the QoL in general has not been the subject of research yet. In this study, we investigated the possible effect of occupational allergic rhinitis on the QoL of employees in bell pepper greenhouses in the western part of the Netherlands. Apart from our interest in the effects of occupational allergy on quality of life, assessment of rhinitis with a disease specific QoL questionnaire provided us with a method profoundly tested and accepted in terms of reliability, responsiveness and validity[6], whereas other outcome measures to assess the severity of rhinitis such as daily symptom scores and visual analogue scales and objective measures of nasal airways resistance are neither standardised nor generally accepted[7]. The aim of the study was to answer the following questions: first, what is the relative influence of sensitization to bell pepper pollen, predatory mite Ac or common inhalant allergens on the QoL of employees with rhinitis and secondly, whether the QoL of the involved employees is increasing when the bell pepper plants are not in blossom. A third question was whether the QoL of symptomatic greenhouse employees of different crops is comparable to each other. Our research group performed a cross-sectional study among 20 Dutch *Chrysanthemum* greenhouses comprising 104 employees to investigate the prevalence and determinants of work-related allergic symptoms[8]. Of the 95 employees who completed their rhinitis QoL questionnaire correctly, 44 suffered from rhinitis symptoms. Because QoL was measured by using the same rhinitis QoL questionnaires as described below, data from both groups could easily be compared. A fourth question was whether the QoL of greenhouse employees with rhinitis is comparable to the QoL of a large population-based sample of subjects with perennial rhinitis. Unfortunately, this kind of research has never been performed in the Netherlands. In France, however, an extensive survey specifically designed to explore the QoL in a large population-based

sample of people with perennial rhinitis was carried out in 1995[2]. For this reason, our research data were compared with the results of a French study.

Methods

Study design

Employees took part in a large cross-sectional study to estimate the prevalence and determinants of work-related allergic symptoms in bell pepper horticulture. Bell pepper greenhouses in the western part of the Netherlands were approached at random by telephone and asked to participate. The investigators paid three visits to each participating greenhouse. The first two visits took place in the period March to August 1999, when the bell pepper plants were in full blossom and biological control was provided, the last visit took place in December 1999 / January 2000 when the bell pepper plants had been removed from the greenhouses. In this period the employees are not likely to be exposed to bell pepper pollen and predatory mites. During the first visit the volunteers were asked questions concerning age, sex, medication use, smoking habit, work history, work-related symptoms and atopic complaints. Furthermore, they completed a rhinitis QoL questionnaire. Two weeks after the first visit they underwent skin prick tests with common inhalant allergens and several occupational allergens. During the third visit, the employees completed a second rhinitis QoL questionnaire. The study was approved by our Hospital Medical Ethical Committee. All employees gave written informed consent and confidentiality was maintained.

Subjects

Four hundred and seventy-two employees of 85 different bell pepper greenhouses participated in the study. They were all exposed to the occupational allergens involved. During the first and second visit the use of antihistamines orally was not permitted, apart from Acrivastine (8 mg) as rescue medication. Acrivastine had also to be withdrawn 3 days before the skin prick test. Employees who did not have a thorough command of the Dutch language were excluded from filling in the rhinitis QoL questionnaire.
Two hundred thirty three employees were identified as having rhinitis by history[3] and included for further research to rhinitis related quality of life.

Skin prick tests

The skin prick tests were performed by application of 1 drop of allergenic extract to the skin of the volar side of the forearm. Subsequently, the dermis was punctured with a standardised skin test needle and the results were read after 20 minutes. 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[9]. Dilution buffer was used as a negative control, histamine chloride 10mg/mL as a positive control. The skin prick test were performed with *botrytis cinera* (SQ 412) as one of the moulds found in greenhouses and 6 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 (SQ553), and cat dander (SQ 555). In addition, prick test were performed with a home-made extract of the leaf, stem, pollen, stamen and juice of the bell pepper plant and a home-made extract of the predatory mite Ac[3,4].

Rhinitis QoL questionnaire (RQLQ)

Quality of life was measured using the approved Dutch translation of the RQLQ developed by Juniper et al[6,10]. This questionnaire more precisely describes the effect of rhinitis on different areas of the employees' day-to-day lives. It comprises 28 questions subdivided into the following domains: activities (n = 3), sleep (n = 3), non-rhinitis symptoms (n = 7), practical problems (n = 3), nasal symptoms (n = 4), eye symptoms (n = 4) and emotions (n = 4). In each item, they were asked how much they were troubled as a result of their nasal symptoms during the previous two weeks. The score ranged from 0 (not troubled) to 6 points (extremely troubled). In case of the items concerning activities, the employees were asked to identify 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. To ensure that all possible relevant items were considered, a list of 29 probe activities was provided as well. 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.

Statistical analysis

Of the 233 employees with rhinitis 24 could not participate in the study because Dutch was not their first language. The other 209 employees completed their questionnaire correctly and data of these employees were used. To test for statistical significance of explanatory variables with regards to the outcome variable (i.e. rhinitis QoL), the method of multiple regression analysis was applied. Not only the (relative) importance of explanatory variables but also possible confounding can be simultaneously tested in the regression model; both were co-variables in the model. In this study age, sex and smoking were considered to be confounding, therefore the importance of the explanatory variables were adjusted for age, sex and, smoking. The unstandardised regression coefficient of the co-variable (B) indicates the importance of the co-variable in the model, the corresponding standard error is an estimate of sampling fluctuation, and the corresponding *p* value gives the significance level. To gain insight into the relative importance, the standardised regression coefficient (ß) was estimated. This parameter ß enabled us to compare the importance of the co-variables with each other. The height of the ß-number reflects the height of the QoL score. A two-tailed *p* value of less than 0.05 was

considered significant. The t-test has been used for paired and unpaired observations for purposes of comparison with regard to the first and second QoL questionnaire of bell pepper greenhouse employees, the QoL questionnaires of bell pepper and *Chrysanthemum* greenhouse employees as well as for comparison of the QoL questionnaires of both greenhouse groups and French population respectively. The statistical analysis was executed using the SPSS 8.0 computer package.

Results

Population characteristics

The total number of 209 employees comprised 171 men and 38 females with an average age of 33.9 years (SD 10.5 yr., range 14.0 - 62.1 yr.). Of them 28.2 % were current smokers. The average duration of employment was 8.3 years (SD 5.7 yr.; range 0 - 31 yr.). A number of 166 (79.4%) employees notified a substantial improvement or complete regression of work-related symptoms on weekends and holidays. Complaints consisted of conjunctivitis in 115 employees (55.0%) and of redness, itching and/or eczema of the skin in 58 employees (27.8%). Asthmatic symptoms were mentioned by 54 employees (25.8%) and urticaria and/or angioedema by only 30 employees (14.4%).

Skin prick tests

A positive skin prick test result to one of the common inhalant allergens was found in 124 employees (59.3%). Sensitization to house dust mite, grass pollen and tree pollen were most prevalent and found in 83 (39.7%), 73 (34.9%) and, 45 (21.5%) employees respectively. Ninety-nine employees appeared to be sensitized to the pollen of the plant (47.4%) and 71 to the predatory mite Ac (34.0%). Positive skin reactions to the leaf, stem or juice of the plant were in most cases associated with sensitization to pollen and / or stamen, which also contain a certain amount of pollen.[3]

Rhinitis QoL questionnaires

Table 1 shows the mean quality of life scores and their standard deviations for all domains and for the mean rhinitis QoL. The 3 activities selected most frequently by the employees in which they feel limited by their symptoms appeared to be work-related activities, bicycling and, sports respectively.

With multiple regression we analysed the effect of duration of employment, sensitization to the three most prevalent inhalant allergens (house dust mite, tree pollen and grass pollen) and the occupational allergens bell pepper pollen and predatory mite Ac respectively on the 7 domains of the rhinitis QoL and on the mean QoL. These eight separate analyses were adjusted for age, sex and smoking. Sensitization to bell pepper pollen had a significant negative effect on all 7 domains ($p < 0.01$) as well as on the mean rhinitis QoL ($p < 0.01$). Sensitization to tree pollen was only significantly correlated with practical problems ($p = 0.01$) and eye symptoms

Table 1. Mean scores and standard deviation between parentheses of the RQLQ domains, reported by greenhouse employees (n=209) at the start of the study

Domains	Scores
Activities	1.39 (1.22)
Sleep	0.67 (1.15)
Non- rhinitis symptoms	0.89 (1.06)
Practical problems	1.83 (1.67)
Nasal symptoms	2.02 (1.59)
Eye symptoms	1.10 (1.41)
Emotional	0.70 (1.02)
Mean QoL	1.23 (1.05)

(p = 0.04) whereas sensitization to house dust mite had only a significant negative effect on non - rhinitis symptoms (p = 0.03). Figure 1 shows the relative impact of the different factors on mean rhinitis QoL expressed as standardised regression coefficient (ß). Duration of employment, sensitization to grass pollen and house dust mites respectively had no significant effect on the different domains and mean rhinitis QoL .The association between mean tree pollen sensitization and rhinitis QoL almost reached statistical significance (ß= 0.14;t=1.931;p=0.055), whereas bell pepper sensitization had a major effect on QoL (ß=0.349;t=4.49;p<0.001). In contrast, the effect of sensitization to predatory mites was a factor 4.59 less and not significant (ß=0.076;t=1.054;p=0.293).

The scores of the first QoL questionnaire were compared to the scores of the second QoL questionnaire (Figure 2). Both questionnaires were available from 161 employees. The other 48 employees were not present during the third visit on the greenhouses. Unfortunately, we have no detailed information upon the reasons of their absence. The possibility that employees with (severe) symptoms might have left the job cannot be excluded. A significant decrease of the rhinitis scores was found for all QoL domains and the mean rhinitis QoL score. This decrease was, however, most pronounced for the domains practical problems, nasal symptoms, activities and, eye symptoms respectively with a magnitude of change in score of more than 0.5 and for practical problems even more than 1.0.

The mean scores on the rhinitis QoL of the 209 bell pepper workers were also compared to the mean scores of 44 *Chrysanthemum* greenhouse workers with rhinitis. Although the employees of bell pepper greenhouses showed slightly higher scores on all domains, no significant differences were seen between the two groups with the exception of the domain practical problems in which bell pepper workers showed a higher score.

In addition, the mean scores on the rhinitis QoL of both greenhouse groups were compared to the scores of 1094 patients with perennial rhinitis (Figure 3). These subjects took part in a large-scale nation-wide population-based survey in France to assess the impact of perennial rhinitis on the QoL[2]. Our employees had an obviously higher score in the areas of nasal symptoms (bell pepper workers) and

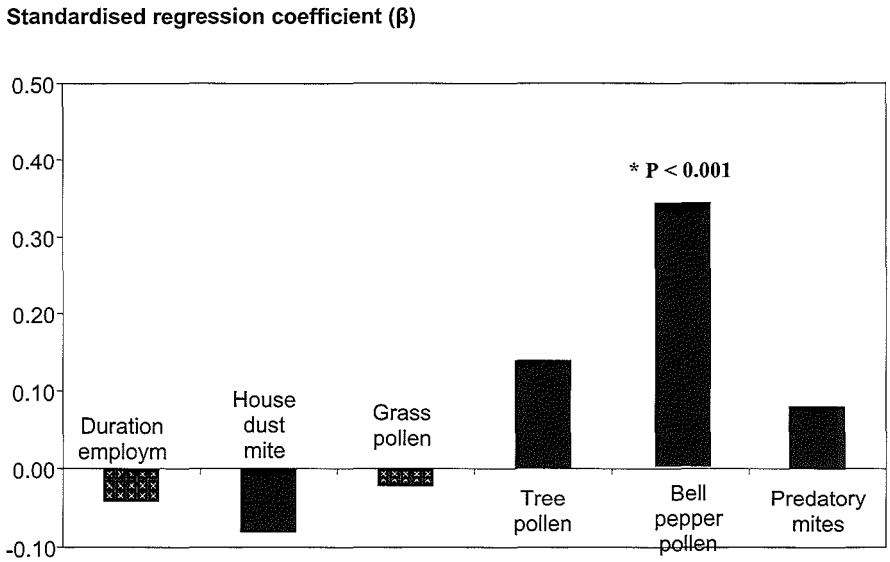

Figure 1. Standardised regression coefficients ß representing the relative impact of factors on mean rhinitis quality of life

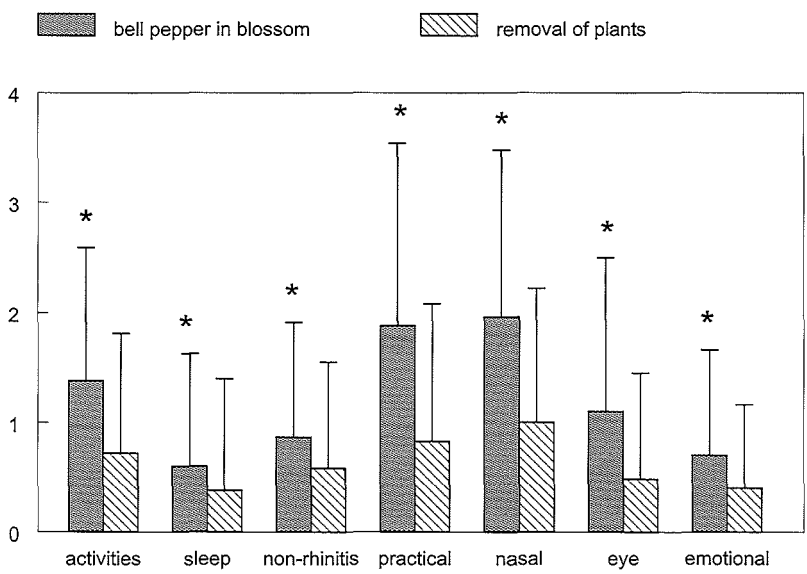

Figure 2. Mean scores of rhinitis quality of life domains at two occasions respectively during the blossom period of bell pepper plants and after removal of plants. Asterisks represent statistical significance ($p<0.05$)

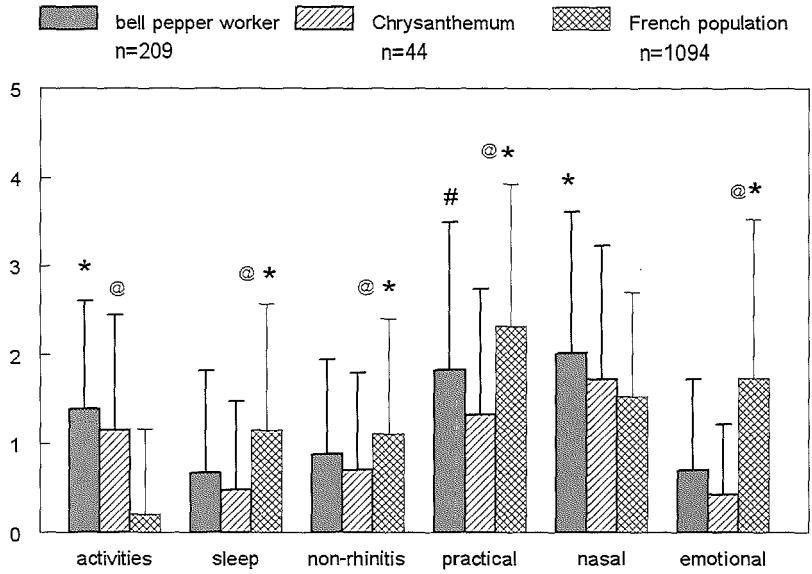

Figure 3. Mean scores of rhinitis quality of life domains, obtained from bell pepper greenhouse workers compared with data derived from a cross-sectional study among *chrysanthemum* greenhouses as well as from a French population study. The different asterisks represent statistical significance ($p<0.05$) between bell pepper greenhouse employees and the French population (*), between bell pepper and *chrysanthemum* greenhouse employees (#) and between *chrysanthemum* greenhouse employees and the French population (@) respectively

activities (both groups) whereas, on the other hand, the score in emotional problems was remarkable lower compared to the French population. For the domains sleep and practical problems the French subjects showed a much higher score although the difference between the bell pepper and the French group was just less than 0.5 on a 7-point scale.

Discussion

Subjects with rhinitis may have nasal symptoms that adversely affect their day-to-day lives. The main goal of this study was to estimate the impact of occupational and inhalant allergy on the QoL in bell pepper greenhouse employees. All rhinitis QoL domains and mean QoL score were strongly and significantly associated with the extent of sensitization to bell pepper pollen. This association indicates that employees with rhinitis symptoms caused by an occupational allergy to bell pepper pollen are impaired in QoL. Moreover, with multiple regression analysis we demonstrated that the pollen of the bell pepper plant is a more important occupational allergen compared to the predatory mite. Although 109 (23%) employees are sensitized to this mite[4], in these analyses sensitization to Ac did not affect rhinitis specific QoL at all. The reason might be that - in terms of prevalence - bell pepper allergy not only outweighs allergy to predatory mites (35.4% versus 23%),

but also that in the subjects sensitized to Ac 73.4% appeared to be co-sensitized to bell pepper allergens[3, 4]. Previously, we demonstrated that atopy, defined as skin reactivity to one or more inhalant allergens, is an independent risk factor for the presence of upper and lower airway symptoms[3]. However, the current analysis to the importance of sensitization to inhalant allergens separately identifies a relatively small contribution of tree pollen only. In addition, sensitization to house dust mite, most prevalent of all common inhalant allergens, did not affect rhinitis specific QoL, except for the domain "non-rhinitis symptoms" (probably a change finding). There was no effect of duration neither of employment nor of age on the QoL in general and the so-called healthy worker effect, found previously[3], was not clearly present in this analysis.

Juniper et al. demonstrated that the rhinitis QoL is a useful instrument in clinical studies to detect within-subject changes over time[6]. The significant improvement in all domains and mean scores of the RQLQ in December and January may be explained by the absence of exposure to bell pepper pollen. The mean magnitude of the difference in the four domains nasal symptoms, practical problems, activities and, eye symptoms between the two questionnaires varied from 1.04 to respectively 0.62. These changes are considered as clinically significant as changes of 0.5 on a 7-point scale represent the minimal important difference (MID)[11]. The MID can be defined as the smallest difference in score in the domain of interest which subjects perceive as beneficial and which would mandate a change in management. The change in score in domain practical problems (> 1.0) may be considered a moderate change in QoL.

Employees of bell pepper greenhouses were more impaired in practical problems compared with employees of *Chrysanthemum* greenhouses. There were no other important differences between the mean scores of both occupational groups, although their rhinitis symptoms are caused by different occupational allergens. The symptomatic greenhouse employees of both groups recorded strikingly higher scores in the domains of nasal symptoms, practical problems and activities than in the domains of sleep disorders, non-rhinitis symptoms and emotions. These findings fit into the comparison with the French population study. The employees showed a higher score in limitations of activities (difference of 1.18 respectively 0.94) and in nasal symptoms (difference of 0.49). On the other hand, they scored significantly lower with respect to sleep disorders, practical problems and in particular to emotional problems (latter difference of 1.03 respectively 1.30). This seems to be in contrast with each other. However, these results might be biased, first of all by transcultural differences. The perception of the degree of impairment caused by rhinitis symptoms might be different between Dutch and French subjects. Most importantly, greenhouse employees may not want to admit their impairment because of rhinitis symptoms as part of their professional attitude. Personal contact with employees confirmed the fact that in this occupational field one does not easily complain or seek medical attention unless the daily symptoms are severe or prevent one from performing his job in a proper way. Financial insecurity caused by

the necessity to retrain for another job, change of crop or perhaps to sell the greenhouse, may be partly responsible for this attitude.
Social desirability has long been viewed as a potential source of error variance in self-report measures. It has been suggested that social desirability masking effects can significantly increase the likelihood of Type II errors in sports medicine research that involves self-report measures[12]. We cannot exclude that such masking effects are involved when studying occupational allergy. An underestimation of the number and severity of rhinitis symptoms and therefore, more impairment in QoL is very well possible. The RQLQ but also other self-report measures such as visual analogue scales or daily symptom scores will not identify this kind of bias. Identification and exclusions of subjects with high social desirability response set scores is required to minimise this effect[12].

A second possibility for bias might have been the difference in the way of measurement. The greenhouse employees completed their questionnaires in the presence of the investigators whereas the French population received their questionnaires by mail and were kindly asked to return it after completing. This more anonymous way of measurement might encourage especially subjects who experience their symptoms as an emotional burden to complete and return the questionnaire.

Taking these restrictions into account, we conclude that bell pepper greenhouse employees with rhinitis are impaired in QoL because of their sensitization to bell pepper pollen whereas concomitant allergies to predatory mites or common inhalant allergens do not influence the rhinitis QoL substantially. These findings suggest that bell pepper pollen is the most important occupational allergen in bell pepper greenhouse workers showing allergic symptomatology. The QoL of these employees is comparable with the QoL of *Chrysanthemum* greenhouse employees with nasal symptoms. This may suggest that in horticulture the kind of causative occupational allergen does not have a substantial influence on the impact of an occupational allergy on a person's daily life. Furthermore, a common allergy does not have more impact on a person's day-to-day life than an occupational allergy, however, there is a clear difference in the way in which an occupational group is hampered in daily life, when compared with a non-occupational group. The results of this study underline the importance of QoL measures in occupational settings. Considering the health care of this important branch of horticulture, further studies are required to assess the impact of reducing pollen exposure levels in the work environment as well as individual management of employees with severe symptoms.

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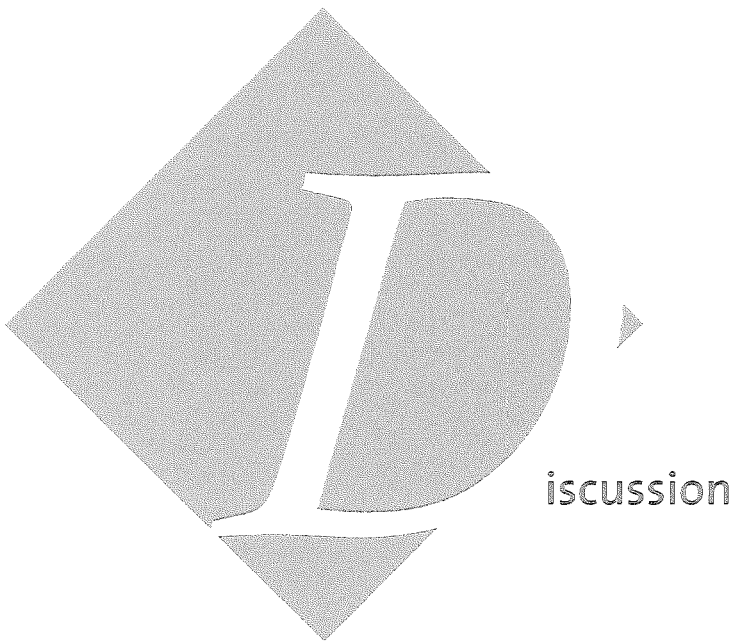
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Introduction

To our knowledge the study presented in this thesis is the largest cross-sectional study on occupational allergy in horticulture that has been performed to date. The main aim was to study the prevalence of work-related allergic symptoms and the prevalence of sensitization to specific occupational allergens and its determinants. The answers to the formulated research questions will be discussed in more detail in this chapter and our results will be compared with results of other large cross-sectional studies on occupational allergies reported in the literature. The findings presented in this thesis may have important consequences for the health care of this large, still growing occupational group. Therefore, the chapter concludes with possible solutions and suggestions for prevention.

Prevalence rate of occupational allergy in bell pepper and *Chrysanthemum* horticulture

Occupational allergy is a major health problem among employees working in bell pepper and *Chrysanthemum* greenhouses. In our study 53.8% of the employees of bell pepper greenhouses reported work-related symptoms. Among employees of *Chrysanthemum* greenhouses work-related symptoms were reported by 57%. In both groups the main symptoms were rhinitis (49.4% and 48%, respectively) and conjunctivitis (30.3% and 26%, respectively). Symptoms of the lower airways, which are considered to be the most serious manifestation of an occupational allergy, were reported to a lesser extent, in 13.3% and 9% respectively. In bell pepper horticulture, the pollen of the bell pepper plant appeared to be an important allergen (**chapter 2**). Of all employees, 35.4% were sensitized to various substances of the bell pepper plant, 27.3% of them to the pollen. Sensitization to proteins of the bell pepper plant and the occurrence of work-related symptoms correlated strongly: of all sensitized employees, 84% reported work-related symptoms. This association was also present in employees of *Chrysanthemum* greenhouses. Sensitization to *chrysanthemum* pollen was found in 20% of the employees, 81% of these sensitized workers suffered from allergic symptoms (**chapter 3**).

The fact that we found a high occurrence of rhinoconjunctivitis symptoms coupled to immunologic sensitization is in agreement with the results of a prospective study among apprentices exposed to laboratory animals. This study showed that the occurrence of occupational rhinoconjunctivitis was more than twice the occurrence of occupational asthma at all time points[1]. Occupational rhinitis is nowadays a common condition in subjects exposed to high molecular weight agents. It is usually considered to be a mild disease compared with occupational asthma. However, this condition is frequently associated with occupational asthma and seems to precede the latter condition in the case of high molecular weight allergens. Karjalainen et al, in a Finnish population study, recently reported that of 3,637 patients with occupational rhinitis, 11.6% received a diagnosis of asthma[2]. This finding suggests that occupational rhinitis carries a crude relative risk of asthma of 4.8 (95%

confidence interval, 4.3 to 5.4). In addition, Gautrin et al. found a positive predictive value of occupational rhinoconjunctivitis for the development of occupational asthma of 11.4%[1]. In this context, a large proportion of our study group may be at risk of occupational asthma if they remain in their current job. This underlines the importance of identifying employees with rhinoconjunctivitis in occupations at risk to detect occupational asthma at an early stage.

The prevalence rate of work-related symptoms in this study is surprisingly high compared with the previous surveys among bell pepper horticulturists in 1993 (15%) and 1996 (22%)[3]. The prevalence rate of work-related symptoms and sensitization to occupational allergens in this study is also high when compared with previous epidemiological studies on occupational allergy. In a cross-sectional study among bakers, sensitization to wheat flour and α-amylase was present in 10% and 7% respectively. In this group rhinitis was present in 21% and chest tightness in 7%[4]. In a large cross-sectional study among laboratory workers 18.8% of those working with rats reported allergic symptoms and sensitization to rat urinary allergens was present in 18.2%. Among employees working with mice allergic symptoms were found in 10.1% and 10.7% appeared to be sensitized to mouse urinary allergens[5]. A recently performed prospective study among animal-health apprentices demonstrated that 24% of all tested students suffered from rhinoconjunctivitis symptoms, and 9.6% had symptoms combined with skin sensitization to an animal-derived allergen[6]. The high prevalence of work-related symptoms in combination with a high prevalence of sensitization to bell pepper pollen seems to confirm the idea that bell pepper pollen allergy is a significant cause of allergic complaints among employees of bell pepper greenhouses. It can be argued that the high prevalence of sensitization to pollen in this occupational group is no proof of its contribution to the cause of allergic complaints. However, one could see this differently. Reversing the reasoning, the hypothetical finding of a low prevalence of sensitization to bell pepper pollen in these employees would have ruled out its importance as cause of the increasing number of allergic, work-related complaints. We showed, for the first time with adequate techniques, that this was not the case.
The prevalence rate of work-related symptoms among *Chrysanthemum* greenhouse employees is in accordance with a previous study among 75 flower growers[7], in which 45% reported respiratory, nasal or ocular symptoms. The frequency of positive SPT responses to ornamental plants in that study (52%) was however, much higher than our prevalence rate of sensitization. This might be due to the fact that in Goldberg et al's 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[8].

Selection and information bias in relation to the prevalence rate

We performed a cross-sectional study which allows assessment of a larger group of employees, including those with many years of exposure as well as relatively newly exposed employees. However, a cross-sectional study can have a bias, underestimating the true prevalence of disease due to the 'healthy worker effect'. Employees with work-related symptoms are more likely to exchange their work in greenhouses for another job without allergen exposure or may have left to other job functions with low allergen exposure. For example, a symptomatic owner can switch over to management tasks and symptomatic employees to sorting duties instead of working on the crop. This may result in a survivor population with a lower prevalence of work-related symptoms. In this study there was a significant decrease in the prevalence of work-related rhinoconjunctivitis in different age groups, with the lowest prevalence of these symptoms in the group of 40 years and older (**chapter 2**). This might indicate a so-called 'healthy worker effect' as a result of selection processes. Casuistic information from current employees confirmed this hypothesis. However, a better estimate of this selection bias can only be studied in a longitudinal (incidence) study over several years. Another factor that may contribute to the healthy worker effect is pre-employment selection. Persons with allergic respiratory symptoms may tend not to choose jobs that burden their respiratory system. There is, however, little published information about self-selection by allergic young adults into careers with less respiratory irritant or allergen exposure[9]. Although we have no information on these potential selection processes, this kind of selection bias does not seem very likely in this occupational group. First of all, a substantial number of the people working in greenhouses are (relatives of) the owners of the greenhouses who take this work for granted. Secondly, most employees have low education levels and it is often very difficult to find another, well-paid job. To our knowledge, in none of the greenhouses under study were pre-employment screening strategies applied to exclude atopic applicants.

Bias can also occur from errors in obtaining information. In our study, occupational allergy was assessed by subjective means, such as questionnaires, as well as by objective means such as skin prick tests, PEF measurements and specific IgE tests. When questionnaires are used to assess the presence of allergic symptoms or the degree of impairment because of allergic symptoms, overestimation as well as underestimation of symptoms may occur. The perception of the presence of work-related symptoms by employees might be different compared to patients who seek medical attention because of seasonal or perennial allergic symptoms. Employees may not want to admit their symptoms as part of their professional attitude or because of fear of losing their jobs. In addition, financial insecurity caused by the necessity to retrain for another job, may also be partly responsible for this attitude. On the other hand, the presence of work-related symptoms can also be exaggerated when adequate financial compensation and proper rehabilitation programs are provided by the government. Personal contact with employees in this study, however, confirmed the fact that in this particular occupational field one does not easily

complain or seek medical attention unless the daily symptoms are severe, or prevent one from performing the job properly. In this view, work-related allergic symptoms among these greenhouse employees may be even more pronounced and the prevalence rate may have been underestimated.

Discrepancies between work-related symptoms and sensitization

Although work-related symptoms in bell pepper and *Chrysanthemum* greenhouses are obviously associated with contact with the pollen of the plants, not all symptoms could be explained by an IgE-mediated response to these allergens. In 65.7% of the symptomatic employees of bell pepper greenhouses an IgE-sensitization to the bell pepper plant and pollen could be demonstrated, whereas in *Chrysanthemum* greenhouses only 29% of the symptomatic employees were sensitized to *Chrysanthemum* pollen. The question remains as to what may have caused the work-related symptoms in employees without sensitization. The predatory mite *Amblyseius cucumeris* appeared to be a second (additional) important occupational allergen in bell pepper horticulture (**chapter 4**). Sensitization to this mite was present in 23% of the employees and in these sensitized employees work-related symptoms were also highly prevalent (76.1%), the main symptoms being rhinitis and conjunctivitis again (71.6% and 48.6%, respectively). The biological activity of *Amblyseius cucumeris* 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. The specific effect of *Amblyseius cucumeris* on work-related symptoms, however, is difficult to single out in an occupational population with a high exposure and consequent sensitization to bell pepper pollen as well. The results of the rhinitis quality of life questionnaires showed that the greenhouse employees with rhinitis are impaired in quality of life because of their sensitization to bell pepper pollen and not because of sensitization to predatory mites (**chapter 6**), suggesting that bell pepper pollen is the most important occupational allergen in bell pepper greenhouse workers with allergic symptoms. In addition, the use of predatory mites has already been introduced in *Chrysanthemum* greenhouses although not yet to such an extent as in bell pepper horticulture.

There are other possible explanations for work-related symptoms without sensitization. It is possible that some of these symptoms can still be attributed to an IgE-mediated allergy to occupational allergens. First, our skin prick test may have failed to detect sensitization in some individuals, although the method used was comparable to other studies performed by this research group. A positive skin prick test result for bell pepper pollen in combination with a negative IgE-test was found in a small number of employees. Second, although bell pepper and *Chrysanthemum* pollens are considered as the most important occupational allergens in this branch of horticulture, other airborne agents in horticulture may be capable of inducing IgE-mediated allergic reactions in the respiratory tract. The low level of dust and endotoxin found inside greenhouses by Monsó[10] did not suggest that these

substances are clinically significant as triggers of symptoms. Although exposure to endotoxin in grain handlers has been associated with respiratory symptoms, this effect is related to inhaled dose and does not appear when employees are exposed to low concentrations. Most noticeable agents are probably other mites, biological control agents or molds[10]. These potential allergens have not been tested in our study and further research to the sensitizing capacities of other potential allergens in population studies is needed. A third reason might be non-specific airway hyperresponsiveness in symptomatic employees only sensitized to common inhalant allergens. These employees may also have symptoms outside the working-environment or pre-existing symptoms which exacerbate at work. A non-specific reaction to the humid and warm environment in greenhouses might be an additional explanation.

Cross reactivity

In several other studies on occupational allergy atopy has been identified as an important risk factor for work-related symptoms[4,5,8,11]. In this study 37.5% of the employees of bell pepper greenhouses appeared to be sensitized to at least one of the common inhalant allergens. Of the employees sensitized to the bell pepper plant, however, inhalant atopy was present in 69.5%, in particular to grass pollen. Among the employees of *Chrysanthemum* greenhouses sensitization to one or more common inhalant allergens was found in 34%. It was striking that sensitization to mugwort was only found in employees sensitized to *Chrysanthemum* pollen. These sensitization patterns suggest cross-sensitization between respectively, bell pepper and *Chrysanthemum* pollens, and common inhalant pollen. IgE-mediated allergic reactions to plant-derived food are often a consequence of primary sensitization to common pollen allergens, like birch, grass and mugwort pollens[12,13]. The question can be addressed whether occupational allergy to either bell pepper or *Chrysanthemum* pollen is also a consequence of primary sensitization to common pollen allergens.

By means of IgE inhibition and immunoblotting experiments however, we could demonstrate that most IgE-binding structures in bell pepper pollen do not cross-react with allergens in grass, birch, or mugwort pollens. Furthermore, the results described in **chapter 5** suggest that IgE binding to common pollen-extracts in sera with relatively low levels of IgE against these pollens, was actually caused by primary sensitization to bell pepper pollen. Therefore, sensitization to bell pepper pollen does not seem to be the consequence of primary sensitization to common pollen allergens. Indeed, 30.5% of the employees sensitized to bell pepper pollen had negative skin prick test with common environmental pollen, which supports these results. This lack of cross-reactivity may have important practical consequences for the diagnosis of occupational allergy: a negative response to common inhalant pollen in skin prick tests or specific IgE analysis does not exclude the presence of an IgE-mediated occupational allergy. Therefore, skin prick tests and RAST with common inhalant pollen cannot be used as screening tests for bell pepper pollen allergy in

medical surveillance programmes for at risk employees. In addition, pre-entry screening of applicants for horticulture work cannot be advised as a consequence of these results. However, they raise the question as to whether allergic young adults should be informed and educated by physicians or in school curricula regarding such increased risks to help them make informed career choices.

For employees sensitized to *Chrysanthemum* pollen, these experiments have not been performed. However, the results of the skin prick tests in these employees suggest a strong cross-reactivity between *Chrysanthemum* pollen and mugwort. This sensitization pattern was also found by de Jong et al.[8] who suggested that testing for sensitization to mugwort may have predictive value in the diagnosis of occupational allergy to flowers, as allergy to flowers is unlikely in the absence of sensitization to mugwort. In addition, cross-reactivity between *Helianthus*, also a member of the *Compositae* family, and mugwort pollen has been described earlier by Fernandez et al.[14] However, an independent sensitization to *Chrysanthemum* pollen cannot be excluded and further investigation by means of RAST inhibition and immunoblot analyses are required to answer this question.

Quality of life assessment

It has been acknowledged that rhinitis, a common chronic condition among persons of working age, is associated with impairment in peoples' functioning in day-to-day life. From information obtained as a result of the introduction of disease-specific questionnaires it is clear that people may be bothered by sleep disorders, emotional problems, impairment in activities and social functioning[15]. However, rhinitis may also have major quality-of-life and economic impacts through occupational disability. Occupational disability can take many forms, including decreased labour force participation rates, change of job or job responsibilities due to health reasons, loss of working days, or decreased effectiveness on the job because of illness. Blanc et al[16]. demonstrated that both asthma and rhinitis negatively affect work productivity. Those with asthma are less likely to be employed at all, while among those remaining on the job, rhinitis is a more potent cause of decreased work effectiveness. The definition of rhinitis used here is, however, broad and heterogeneous, subsuming allergic rhinitis (both perennial and seasonal) and non-allergic rhinitis. In this thesis we assessed the influence of rhinitis symptoms caused by different occupational allergens on the daily life of greenhouse employees in general (**chapter 6**). We showed that there is a clear difference in the way in which an occupational group is hampered in daily life with respect to a non-occupational group. Although we uncovered no information on the effect of an occupational rhinitis on work productivity in particular, it is possible that this aspect also plays an important role in our study population. In addition, there might also be a difference between a common allergy and an occupationally induced allergy with respect to work disability. However, little is known about the relative impact of an occupational allergy on work loss or decreased productivity and to our knowledge, no studies have been published about this subject.

Therapeutic interventions and preventive measures

This study did not include an exposure assessment measure other than aeropollinic sampling with a fixed pollen trap which was kindly provided by F. Spieksma (laboratory of Aerobiology, University Hospital Leiden). The recording pollen counting system is able to sample airborne inorganic and organic particles of a diameter from 1 to 10 micrometres. The sampler was placed in the centre of a bell pepper greenhouse with a surface of 25,000 square metres. To get an impression of the extent of exposure to bell pepper pollen in the micro-environment of employees, daily pollen sampling was carried out during three months of the flowering period of the plants. The owner of the greenhouse kept a diary, noting the daily activities on the crop, as well as the outside temperature and the weather type. We did not detect high pollen concentrations within the greenhouse, which is not unusual in the case of the self-pollinating bell pepper plants. However, these measurements provided information about a slight increase of pollen concentration during the day with additionally, peak release of pollen caused by work activities on the crop. The continuing fertilization process of these plants requires the maintenance of pollen concentrations throughout the greenhouse during the season. The necessary daily activities during the cultivation process, like heading of the bell pepper plants, cause the release of large amounts of pollen from the flowers. These pollens, well-known as rather large and heavy particles, fall down towards the ground, immediately causing a peak exposure for any employee working in the crop. Employees can only perform their work properly if they stand close to the plants, so contact with the pollen is hardly avoidable. Although a clear relation between the degree of exposure, work-related symptoms and sensitization to pollen cannot be demonstrated by these results, we presume that repeated moments of peak exposure might be sufficient to induce an immediate immunologic reaction with histamine release. Of course, in order to define the real extent of (peak) exposure in sensitized employees, portable personal samplers should be used for assessment. However, these samplers were not at our disposal at the time we performed our study. Nevertheless, the results and relationships described in this thesis provide important evidence of causation and suggest that work-related symptoms are to some extent preventable by reducing pollen exposure levels. Possible solutions to reduce exposure or, even better, to avoid inhalation of pollen might lie in the following measures. First, personal protection of the employees by means of facemasks and extraction fans above the plants. Secondly, new upgrading methods to create plants with a good setting of bell peppers with as little pollen as possible. Exploring the possibilities of genetic engineering of the bell pepper plant to create flowers without pollen might be a third option. We fully realize that the last two measures are relatively expensive and time-consuming to develop. Moreover, the feasibility and effectiveness of these possibilities can only be initialized and directed by the Commodity Board for Horticulture and not by individual greenhouse owners. The results of a recently performed study[17], however, suggest a fourth possible method to reduce pollen exposure in a much easier and cheaper way. This research demonstrated that the introduction of honeybees into bell pepper greenhouses significantly reduced the

pollen count in a dose-dependent way, through which a decrease of work-related rhinitis symptoms in allergic employees was achieved. Although this involved a pilot study, the results seem promising for the near future.

Flower cultivation on the contrary, is not very labour- intensive when compared with bell pepper cultivation. The most important activity for employees during the cultivation process is to gather the flowers by pulling them out of the ground, which may cause a release of pollen. This pollen release is, however, not obviously visible, as is the case for employees working on bell pepper plants. Therefore, possible peak exposure might be lower. This thought is supported by the proven absence of symptoms of the lower airways among employees in *Chrysanthemum* greenhouses. However, we did not perform any exposure measurements and therefore the supposition cannot be evaluated. Besides the use of personal protection, several measures to reduce pollen exposure can be recommended. First, irrigation of the flowers before gathering. Secondly, the gathering should take 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.
Future research may provide more insight into the question whether the measures described here may prevent sensitization in newly exposed employees or deterioration of allergic symptoms in employees who have already been sensitized.

Next to measures to reduce pollen exposure, individual medical guidance for symptomatic employees is important and necessary. It is known that rhinoconjunctivitis can be well managed with medications such as antihistamines and anti-inflammatory preparations which generally allow employees to remain at work[6]. Job retraining is usually an option only when rhinoconjunctivitis is accompanied by bronchial hyperresponsiveness, the worker is young and the possibility of the current employee avoiding further exposure is poor. Because occupational rhinoconjunctivitis might predispose or concomitantly appear with occupational asthma, it is important to make sure that employees have no lower airway involvement. In the case of occupational asthma, removal from exposure is the most ideal treatment. Continuing working is often not possible because workers with occupational asthma tend to react to extremely low concentrations of the causative agent. Pharmacologic treatment of employees with occupational asthma is similar to the treatment of patients with other forms of asthma. Although removal from exposure generally results in improvement, employees may continue to require medication and have airflow limitation or pharmacologically induced bronchial hyperresponsiveness for many months or years[18].

Furthermore, we would like to emphasize the need for a follow-up (longitudinal) survey to see whether sensitized employees without work-related symptoms will develop allergic complaints and whether sensitized employees with rhinoconjunctivitis will develop asthma during continuation of their current job. In addition, it would be interesting to determine the latency period for the development

of allergic symptoms and whether symptoms tend to increase or diminish with time when exposure exists. Our study shows a high prevalence of occupational allergic symptoms associated with allergic sensitization which, in our opinion, may justify the development of surveillance programs to identify employees at risk in an early stage.

Conclusions:

The results of this study lead to the following main conclusions:

1. In employees of bell pepper and *Chrysanthemum* greenhouses the prevalence of allergic symptoms, especially rhinoconjunctivitis, is high. These results suggest that work-related symptoms are indeed a major health problem in these two important branches of horticulture.

2. Sensitization to bell pepper pollen and *Chrysanthemum* pollen appeared to be the most important and strongest determinants for the occurrence of work-related symptoms.

3. The predatory mite *Amblyseius cucumeris* 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.

4. There is no cross-reactivity between bell pepper pollen and common inhalant pollen. Therefore, skin prick tests and RAST with common inhalant pollen cannot be used as screenings tests for bell pepper pollen allergy in medical surveillance programmes.

5. Bell pepper greenhouse employees with rhinitis are impaired in quality of life because of their sensitization to bell pepper pollen, which shows that bell pepper pollen is the most important occupational allergen in this professional group. There is no striking difference in the quality of life of *Chrysanthemum* and bell pepper greenhouse employees nor between employees with an occupational allergy and persons with a common, perennial allergy respectively.

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ummary

Chapter 1 provides a general introduction. It starts with a description of the background of an IgE-mediated occupational allergy. The symptoms and causes of occupational complaints are discussed as well as the different steps involved in the investigation comprising the clinical history, quality of life assessment, immunological tests, monitoring of peak expiratory flow, and specific nasal or bronchial challenges. The introduction continues with a description of the background of the study. In the Netherlands, bell pepper and *Chrysanthemum* cultivation have increased enormously during the past few years, becoming the main branches of horticulture under glass. At the same time, an increasing number of allergic complaints appear to have occurred among the greenhouse workers. Up to the present, little is known about IgE-mediated occupational allergy in horticulture. However, this particular working environment may contain many potential allergenic agents, such as pollens, plant antigens, mites and moulds. The study presented in this thesis was conducted with the aim to investigate the prevalence and determinants of work-related allergic symptoms among these populations at risk.

Chapter 2 presents the results of a cross-sectional epidemiologic survey among 472 employees of bell pepper greenhouses. In this chapter, the prevalence of work-related allergic symptoms and the determinants of specific sensitization were studied, with a special focus on bell pepper pollen. Sensitization to proteins of the bell pepper plant appeared to be the most important and strongest determinant for the occurrence of work-related symptoms. This association was most pronounced for asthma symptoms, although these symptoms were reported to a lesser extent (13.3%) when compared to rhinitis (49.4%) and conjunctivitis (30.3%) respectively. Of all the tested substances of the bell pepper plant, pollen appeared to be the most important allergen. Pollens are not only responsible for a large proportion of the work-related complaints mentioned but probably also for the more severe symptoms. A higher percentage of workers with asthma is found in the group of symptomatic employees with sensitization to pollen than among symptomatic employees without sensitization (30% versus 18%). Atopy was considered to be another significant risk factor for work-related symptoms of the lower and upper airways, however, its effect on the development of symptoms is less strong. Furthermore, the age of the employee was identified as a risk factor as there was a significant decrease in the prevalence of symptoms in different age groups. No relation was found between work-related symptoms and/or sensitization to occupational allergens and sex, job and job activities, size of the greenhouse and duration of employment. This may be due to the continuing and chronic exposure to pollens because of which a dose response-relationship cannot be found anymore. In 65.7% of the employees with work-related symptoms IgE sensitization to the bell pepper plant and pollen could be demonstrated. The question remains as to what may have caused the work-related symptoms in employees without sensitization. Sensitization to other occupational allergens, non-specific airway hyperresponsiveness or a non-specific reaction to the humid and warm environment in greenhouses might be additional explanations. The relationships described provide important evidence of causation and suggest that measures to reduce pollen exposure levels are necessary to prevent a further increase of this new occupational allergy.

In ***Chapter 3*** a cross-sectional study among 104 employees in Dutch *Chrysanthemum* greenhouses is described. In this study the prevalence of work-related allergic symptoms was investigated as well as the prevalence of sensitization to pollen of different members of the *Chrysanthemum* family. Also in this particular occupational group, work-related symptoms were highly prevalent (56.7%), with the main symptom being rhinitis. Symptoms of the skin and lower airways were hardly found. Sensitization to *Chrysanthemum* pollen was found in 20.2% of the employees and it appeared to be an important risk factor for the occurrence of work-related symptoms of the upper airways. Positive skin tests were found for all of the 7 members of the *Chrysanthemum* family tested. However, no preference was shown for any particular kind and therefore no individual family member could be considered as most suitable to screen for sensitization to *Chrysanthemum*. In addition, the results of the skin tests were independent of the individual *Chrysanthemum* family members, which were cultivated mainly in the greenhouses under study. This implies that sensitization develops against the "common allergens" of the *Chrysanthemum*. Furthermore, sensitization to common airborne pollens and *Chrysanthemum* pollens was also closely associated. It was striking that sensitization to mugwort was only found in employees sensitized to *Chrysanthemum* pollen. Although cross-sensitization might be suspected, an independent sensitization to *Chrysanthemum* pollen cannot be excluded. Not all work-related symptoms could be explained by an IgE-mediated response to *Chrysanthemum* pollen. Symptomatic employees might be specifically sensitized to another kind of *Chrysanthemum* or to other occupational allergens, not tested in this study. Non-specific hyperreactivity, inducing complaints on exposure to the flowers, or to the humid environment or to pesticides is another possibility. Besides medical guidance and personal protection, possible solutions to reduce the release of pollen should be considered.

In ***Chapter 4*** the prevalence of sensitization to the predatory mite *Amblyseius cucumeris* (Ac) among exposed greenhouse employees and its clinical relevance is studied. Protection against thrips, a common pest in bell pepper horticulture is nowadays effected without pesticides by using this commercially available predatory mite. The use of Ac for year-round biological control started in 1985 and has been stimulated ever since, resulting in an increased exposure, as currently observed. Positive skin prick tests to Ac were found in 109 of the 472 participating employees (23%). In employees sensitized to Ac work-related symptoms were highly prevalent (76.1%), the main symptoms being rhinitis (71.6%) and conjunctivitis (48.6%) respectively. IgE-mediated allergy to inhalant allergens, in particular to the house dust mite, appeared to be an important risk factor for sensitization to Ac. Sensitization to the storage mite *Tyrophagus putrescentiae* (Tp), which serves as a temporary food source for Ac during the cultivation process, was in most cases associated with sensitization to Ac (77.4%). Although there is no close taxonomic relation between the three mites, they are closely associated with each other and therefore, cross-reactivity cannot be excluded. The biological activity of Ac on human mucous membranes, in particular those of the nose, and the consequent clinical relevance of sensitization could be confirmed by nasal challenge tests. Employees

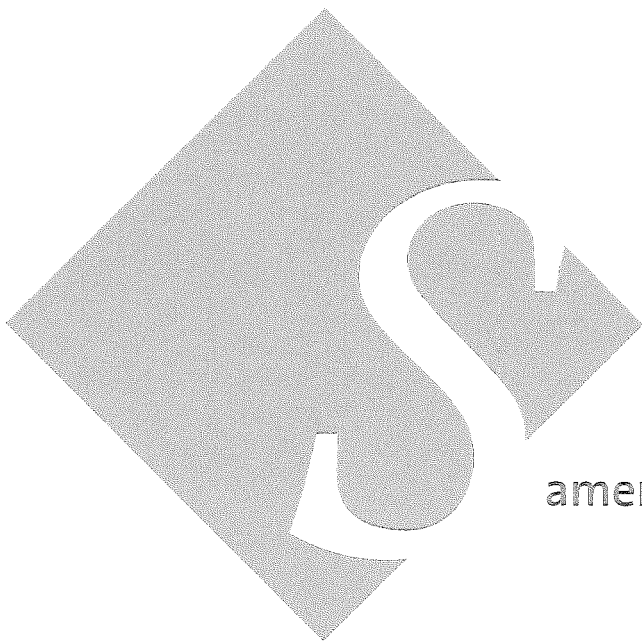
with rhinitis symptoms showed a significantly higher response to all Ac doses during the nasal challenge test compared with employees without rhinitis. This difference was not revealed by skin reactivity to Ac in the skin prick tests, which implies that a nasal challenge test with Ac is a better and more sensitive test than a skin prick test to discriminate between sensitized employees with and without rhinitis.

In ***Chapter 5*** cross-reactivity between bell pepper pollen and common pollen is investigated. Sera from 10 symptomatic greenhouse employees, sensitized to bell pepper pollen and having positive skin prick tests to birch, grass and /or mugwort pollens, were selected and analysed. Subsequently, IgE antibody measurements, IgE inhibition and immunoblotting experiments were performed. Little or no inhibition of IgE binding to bell pepper pollen extract was observed with grass, birch and mugwort pollens which suggests that bell pepper pollens contain allergens that have little or no cross-reactivity with common pollen allergens. Therefore, sensitization to bell pepper pollen does not seem to be the consequence of primary sensitization to common pollen allergens.

Rhinitis symptoms among employees of bell pepper greenhouses can be caused by an allergy to occupational allergens such as bell pepper pollen, predatory mites (Ac) and common inhalant allergens. In ***Chapter 6*** the influence of sensitization to these different allergens on rhinitis specific quality of life (QoL) is evaluated in and outside the flowering period of the crop. In addition, it was assessed whether the QoL of sensitized employees of bell pepper greenhouses is comparable to the QoL of *Chrysanthemum* greenhouse employees with rhinitis and to an average population sample with perennial rhinitis, respectively. Sensitization to bell pepper pollen had a significant negative effect on all domains and the mean QoL, whereas the other allergens had no influence on the QoL at all. Furthermore, a significant decrease of all rhinitis scores was found outside the flowering period of the bell pepper plants. These findings suggest that bell pepper pollen is the most important occupational allergen in greenhouse workers showing allergic symptomatology. Bell pepper greenhouse employees were more impaired in practical problems compared with *Chrysanthemum* greenhouse employees. There were no other relevant differences in the mean scores of the different domains for both groups. So irrespective of the causative occupational allergen, the QoL of affected greenhouse employees is comparable with each other. On the other hand, compared to subjects with perennial rhinitis, greenhouse employees scored higher on limitations in activities and much lower on emotional, sleeping and practical problems. A common allergy does not have more impact on a person's day-to-day life than an occupational allergy. There is, however, a clear difference in the way in which an occupational group is hampered in daily life, when compared with a non-occupational group.

Chapter 7 is a general discussion of the results described in chapters 2-6. It is concluded that the results and relationships presented in this thesis further define and elucidate the prevalence and determinants of work-related, allergic complaints which have emerged in bell pepper and *Chrysanthemum* horticulture.

Important information of causation is provided as well as the effects and impact of an occupational allergy on an individual day-to-day's life. It emphasizes the need for follow-up (longitudinal) surveys and warrants the concept of new studies on therapeutic interventions and preventive measures to prevent a further increase of this occupational allergy.



amenvatting

Hoofdstuk 1, de algemene inleiding, begint met het beschrijven van de achtergrond van een IgE-gemedieerde beroepsallergie. Vervolgens worden de symptomen en oorzaken van beroepsgebonden klachten besproken evenals de verschillende onderdelen van de diagnostiek, te weten de beroepsanamnese, kwaliteit van leven bepaling, immunologische testen, piekstroommetingen en neus of long provocaties. Hierna wordt de achtergrond van de studie beschreven. In de afgelopen jaren is zowel de paprika- als de chrysantenteelt in Nederland enorm toegenomen en zijn daarmee de belangrijkste gewassen geworden binnen de glas- en tuinbouw. Tegelijkertijd is ook het aantal allergische klachten onder de medewerkers fors toegenomen. Tot op heden is er echter weinig bekend over IgE-gemedieerde beroepsallergieën binnen de glas- en tuinbouw. Deze specifieke werkomgeving bevat echter vele mogelijke allergenen zoals pollen, plant antigenen, schimmels en mijten. De studie zoals beschreven in dit proefschrift werd uitgevoerd met als doel om de prevalentie en de determinanten van beroepsgebonden allergische klachten te onderzoeken in deze beroepsgroep met een mogelijk verhoogd risico op een beroepsallergie.

Hoofdstuk 2 laat de resultaten zien van een epidemiologisch dwarsdoorsnede onderzoek onder 472 medewerkers van paprikakassen. In dit hoofdstuk wordt de prevalentie van beroepsgebonden klachten en de determinanten van specifieke sensibilisatie bestudeerd met speciale aandacht voor de paprikapollen. Sensibilisatie voor de eiwitten van de paprikaplant bleek de belangrijkste en sterkste risicofactor te zijn voor het ontwikkelen van beroepsgerelateerde klachten. Deze associatie was het sterkst voor astma klachten hoewel deze veel minder vaak voorkwamen ten opzichte van rhinitis (49.4%) en conjunctivitis klachten (30.3%). Van alle geteste onderdelen van de paprikaplant, bleken de pollen het belangrijkste allergeen te zijn. De paprikapollen zijn niet alleen verantwoordelijk voor een groot deel van de werkgerelateerde klachten, maar ook voor de meer ernstige symptomen. In de groep van symptomatische werknemers met sensibilisatie voor paprikapollen werd een hoger percentage werknemers met astma gevonden ten opzichte van de groep symptomatische werknemers zonder sensibilisatie (30% versus 18%). Atopie was ook geassocieerd met het ontstaan van werkgerelateerde klachten van de onderste en bovenste luchtwegen. Het effect ervan op de ontwikkeling van klachten is echter minder sterk in vergelijking met sensibilisatie voor de paprikaplant en pollen. Verder bleek ook de leeftijd van een werknemer een risicofactor te zijn gezien het feit dat er een significante afname werd gevonden met betrekking tot de prevalentie van klachten in de verschillende leeftijdsgroepen. Er was geen relatie tussen het hebben van klachten en/of sensibilisatie voor beroepsallergenen en het geslacht, het soort werkzaamheden in de kas, de oppervlakte van de kas en het aantal jaren in de paprikateelt. Dit kan te maken hebben met de continue en chronische blootstelling aan pollen, zodat een dosis/respons relatie niet meer gevonden wordt. Bij 65.7% van de werknemers met werkgerelateerde klachten kon een IgE-gemedieerde allergie tegen de paprikaplant en pollen worden vastgesteld. Het is niet geheel duidelijk wat de oorzaak is van de klachten binnen de groep werknemers zonder sensibilisatie. Een allergische reactie tegen andere beroepsallergenen en niet-spe-

cifieke hyperreactiviteit ten gevolge van het warme en vochtige klimaat in de kassen lijken momenteel de meest plausibele verklaringen. Maatregelen om blootstelling aan pollen te verminderen lijken noodzakelijk om verdere toename van deze nieuwe beroepsallergie te voorkomen.

In **Hoofdstuk 3** worden de resultaten beschreven van een dwarsdoorsnede onderzoek onder 104 medewerkers van Nederlandse chrysantenkassen. In deze studie werd er gekeken naar de prevalentie van zowel werkgerelateerde allergische klachten als van sensibilisatie voor pollen van verschillende leden van de chrysantenfamilie. Ook in deze beroepsgroep bleken werkgerelateerde klachten veelvuldig voor te komen (56.7%), met rhinitis als belangrijkste klacht. Klachten van de huid en onderste luchtwegen werden nauwelijks gerapporteerd. Sensibilisatie voor chrysantpollen was aanwezig bij 20.2% van alle werknemers en dit bleek een belangrijke risicofactor te zijn voor het optreden van werkgerelateerde klachten van de bovenste luchtwegen. Er waren positieve huidtesten op alle 7 geteste soorten van de chrysantenfamilie. Er was echter niet een soort in het bijzonder die het meeste voorkwam en derhalve geschikt zou zijn voor screening op sensibilisatie voor de chrysant. Bovendien waren de testuitslagen onafhankelijk van het soort chrysant welke het meest gekweekt werd in de deelnemende kassen. Er lijkt dus sprake te zijn van een allergie gericht tegen chrysant specifieke eiwitten. Verder bleek er een nauwe associatie te bestaan tussen sensibilisatie voor "gewone" pollen en chrysantpollen. Het was opvallend dat sensibilisatie voor bijvoetpollen alleen aanwezig was bij werknemers die gesensibiliseerd waren voor chrysantpollen. Alhoewel er sprake zou kunnen zijn van kruissensibilisatie, kan een op zichzelf staande sensibilisatie voor chrysantpollen niet worden uitgesloten. Niet alle werkgerelateerde klachten konden door een IgE-gemedieerde allergie voor chrysantpollen worden verklaard. Symptomatische werknemers kunnen gesensibiliseerd zijn voor een specifieke chrysant soort of voor andere beroepsallergenen, niet getest in deze studie. Aspecifieke hyperreactiviteit, hetgeen klachten kan induceren bij blootstelling aan bloemen, pesticiden of aan een vochtig klimaat, is een andere mogelijkheid. Naast medische begeleiding en beschermende maskers moet er verder worden gezocht naar andere mogelijke oplossingen om de afgifte van pollen en daarmee de blootstelling eraan te reduceren.

Het voorkomen van sensibilisatie voor de roofmijt *Amblyseius cucumeris* (Ac) onder medewerkers van kassen en de klinische relevantie ervan wordt beschreven in **Hoofdstuk 4.** Bescherming tegen thrips, een veel voorkomende plaag in de paprikateelt, is tegenwoordig mogelijk zonder pesticiden door toepassing van de commercieel beschikbare roofmijt. Deze vorm van biologische bestrijding, die het gehele jaar toepasbaar is, werd geintroduceerd in 1985. Het gebruik ervan is sindsdien voortdurend gestimuleerd, hetgeen geleid heeft tot een toegenomen blootstelling hieraan zoals recentelijk naar voren is gekomen. Bij 109 van de 472 deelnemende werknemers werden positieve huidtesten voor Ac gevonden (23%). Werkgerelateerde klachten kwamen veelvuldig voor bij gesensibiliseerde werknemers (76.1%), met als belangrijkste symptomen rhinitis en conjunctivitis (71.6% versus 48.6%). IgE-gemedieerde allergie voor inhalatie allergenen, met name voor de huisstofmijt, bleek

een belangrijke risicofactor te zijn voor het ontwikkelen van sensibilisatie voor Ac. Sensibilisatie voor de voorraadmijt *Tyrophagus putrescentiae* (Tp), die als tijdelijke voedselbron dient voor Ac tijdens het kweekproces, was in de meeste gevallen geassocieerd met sensibilisatie voor Ac (77.4%). Hoewel er geen nauwe taxonomische relatie is tussen de drie mijtsoorten, zijn zij nauw aan elkaar gerelateerd en kan kruisreactiviteit niet worden uitgesloten. De biologische activiteit van Ac op humane slijmvliezen, met name het neusslijmvlies, en de klinische consequenties van sensibilisatie, kon worden aangetoond door middel van neus provocatietesten. Werknemers met rhinitis klachten toonden een significant hogere respons op alle Ac concentraties gedurende de neus provocatietesten in vergelijking met werknemers zonder rhinitis klachten. Dit verschil kon niet worden waargenomen wanneer er gekeken werd naar de mate van huidreactiviteit op Ac tijdens huidtesten. Dit betekent dat de neus provocatietest met Ac een gevoeligere en dus betere test is dan de huidtest ter onderscheiding van gesensibiliseerde werknemers met en zonder rhinitis klachten.

In **Hoofdstuk 5** is de mogelijkheid van kruisreactiviteit tussen paprika en gewone pollen onderzocht. Sera van 10 symptomatische kaswerknemers, gesensibiliseerd voor paprikapollen en met positieve huidtesten voor berk, gras en/of bijvoetpollen, werden geselecteerd en geanalyseerd. Vervolgens werden er IgE antilichaam metingen, RAST-remming en immunoblot- experimenten uitgevoerd. Met gras, berk en bijvoetpollen werd er geen of weinig remming gezien van IgE binding aan het paprikapollen extract. Dit suggereert dat paprikapollen allergenen bevatten die geen of beperkte kruisreactiviteit vertonen met gewone pollen allergenen. Sensibilisatie voor paprikapollen is daarom niet het gevolg van een primaire sensibilisatie voor gewone pollen allergenen.

Rhinitis klachten onder medewerkers van de paprikateelt kunnen worden veroorzaakt door een allergie voor zowel beroepsallergenen zoals paprikapollen en de roofmijt Ac als door gewone inhalatie allergenen. De invloed van sensibilisatie voor deze verschillende allergenen op de rhinitis specifieke kwaliteit van leven (KvL) binnen en buiten de bloeiperiode van het gewas wordt besproken in **Hoofdstuk 6**. Hiernaast werd er gekeken of de KvL van gesensibiliseerde medewerkers van paprikakassen vergelijkbaar is met de KvL van chrysantkas medewerkers met neusklachten, respectievelijk met een gemiddelde populatie met een chronische allergische rhinitis. Sensibilisatie voor paprikapollen had een significant negatief effect op de gemiddelde kwaliteit van leven en op alle zeven domeinen, te weten activiteiten, slaap, niet nasale klachten, praktische problemen, neus en oogklachten en emoties. De andere allergenen hadden daarentegen helemaal geen invloed op de kwaliteit van leven. Verder werd er een significante afname gezien van alle rhinitis scores buiten de bloeiperiode van de paprikaplanten. Deze bevindingen suggereren dat de paprikapollen het meest belangrijke beroepsallergeen is onder kasmedewerkers met allergische klachten. De medewerkers van paprikakassen werden meer gehinderd met betrekking tot praktische problemen dan chrysantmedewerkers. Er werden geen andere relevante verschillen gevonden in de gemiddelde scores van de verschillende

domeinen voor beide beroepsgroepen. Onafhankelijk van het oorzakelijke beroepsallergeen, is de kwaliteit van leven van symptomatische kasmedewerkers dus vergelijkbaar met elkaar. Daarentegen scoorden kasmedewerkers veel hoger met betrekking tot beperkingen in activiteiten en veel lager qua emotionele, slaap en praktische problemen in vergelijking met een gemiddelde populatie met een chronische allergische rhinitis. Een gewone allergie heeft dan ook niet meer invloed op het dagelijks leven van een persoon dan een beroepsallergie. Er is echter wel een duidelijk verschil in de manier waarop een beroepsgroep gehinderd wordt in het dagelijks leven ten opzichte van een niet-beroepsgroep.

Hoofdstuk 7 is een algemene discussie van de resultaten beschreven in hoofdstuk 2-6. Geconcludeerd wordt dat de resultaten en relaties zoals beschreven in dit proefschrift de prevalentie en determinanten van werkgerelateerde, allergische klachten die zijn ontstaan in de paprika- en chrysantteelt nader definiëren en ophelderen. Er wordt belangrijke informatie verschaft over de oorzaken van deze beroepsallergie alsmede het effect en de invloed ervan op het dagelijks leven van de individuele kasmedewerker. Het onderstreept het belang van vervolgonderzoek en rechtvaardigt de opzet van nieuwe studies naar therapeutische interventies en preventieve maatregelen om verdere toename van deze beroepsallergie te voorkomen.

Toelichting

Allergenen

Stoffen (eiwitten) die van buiten het lichaam afkomstig zijn en aanleiding kunnen geven tot een allergische reaktie bij daartoe gevoelige personen.

Atopie

Erfelijke aanleg voor allergische aandoeningen zoals hooikoorts, eczeem en astma.

Conjunctivitis

(seizoensgebonden) aandoening van de slijmvliezen van de ogen hetgeen leidt tot rode, jeukende en tranende ogen.

Hyperreactiviteit

toegenomen gevoeligheid voor aspecifieke (niet-allergische) prikkels.

IgE-gemedieerde allergie

het afweersysteem maakt na het binnendringen van een stof antistoffen aan, de zogenoemde immunoglobulinen van het type E, afgekort IgE. Normaal zijn deze antistoffen betrokken bij afweerreacties tegen schadelijke ziekteverwekkers (zoals parasieten); bij een allergie worden deze IgE-antistoffen aangemaakt tegen onschadelijke stoffen.

Immunoblot analyse

laboratoriumtechniek om eiwit structuren in allergeenextracten te scheiden.

Inhalatieallergenen

eiwitten die worden ingeademd en via contact met de slijmvliezen van ogen, neus, mondkeelholte en/of longen allergische klachten kunnen veroorzaken.

Kruisreactiviteit

antistoffen gericht tegen een inhalatieallergeen (zoals pollen) kunnen bepaalde onderdelen van eiwitten herkennen die in andere (niet verwante) inhalatie- of voedselallergenen voorkomen. Deze niet verwante allergenen kunnen dan een positieve uitslag geven in huidtest en bloedonderzoek, maar hoeven niet altijd aanleiding te zijn voor allergische klachten.

Provocatie

belasting van een bepaald orgaan (bijvoorbeeld de neus of de longen) met een allergene stof.

RAST

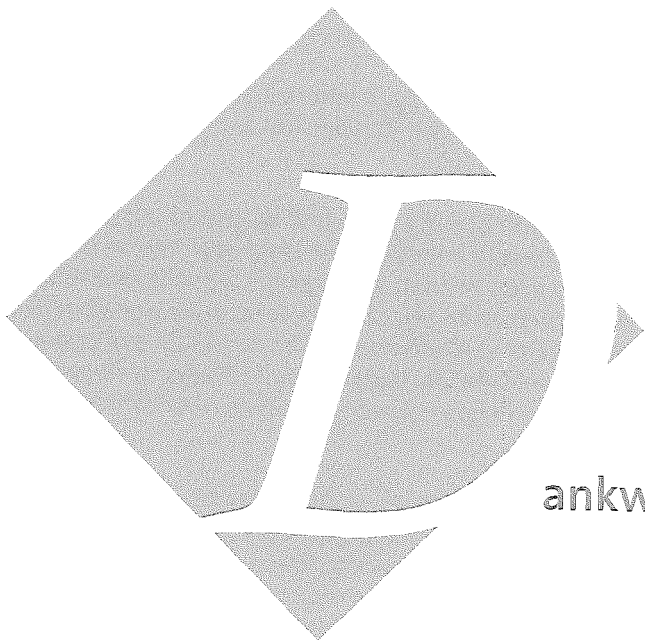
het aantonen van allergische (IgE-type) antistoffen in het bloed middels een Radio Allergo Sorbens Test.

Rhinitis

(seizoensgebonden) aandoening van de slijmvliezen van de neus welke gekenmerkt wordt door niezen, jeuk in de neus, waterige loopneus en neusverstopping.

Sensibilisatie

het ontwikkelen van allergische (IgE-) antistoffen gericht tegen bepaalde allergene stoffen.


ankwoord

Het is zover, het proefschrift is voltooid. Nu komt zowel het leukste als ook het moeilijkste gedeelte, het dankwoord!
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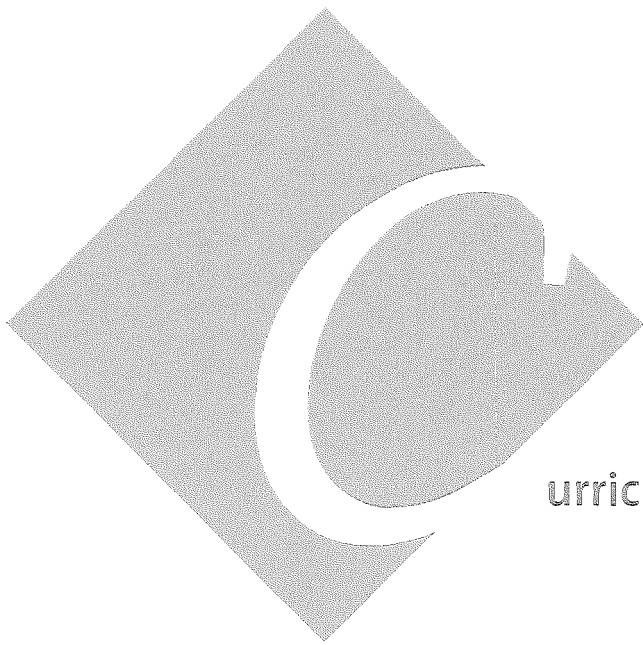

urriculum Vitae

De auteur van dit proefschrift werd geboren op 8 april 1969 te Beverwijk. In 1987 behaalde zij het gymnasium diploma aan het Stedelijk Gymnasium te s' Hertogenbosch. In hetzelfde jaar startte zij met de studie Geneeskunde aan de Universiteit Utrecht, waar in 1994 het artsexamen werd verkregen. Tijdens haar studie werkte zij als student-assistent bij de vakgroep pathologie en deed zij een stage gynaecologie/ obstetrie te Bratislava, Slowakije. Vanaf eind 1994 tot en met 1998 was zij werkzaam als assistent geneeskundige niet in opleiding binnen de interne geneeskunde (Jeroen Bosch Ziekenhuis te s'Hertogenbosch en het Slotervaart Ziekenhuis te Amsterdam) en de dermatologie (Antonius Ziekenhuis te Nieuwegein). Van 1999 tot en met 2000 werkte ze als arts-onderzoeker aan het wetenschappelijk onderzoek dat tot dit proefschrift heeft geleid bij de vakgroep allergologie in het Erasmus MC te Rotterdam onder leiding van Dr. R. Gerth van Wijk en Dr. H. de Groot.
In 2001 is zij begonnen met de opleiding tot internist. Het perifere gedeelte van de opleiding vond plaats tussen 1 januari 2001 en 1 september 2003 in het Albert Schweitzer Ziekenhuis, lokatie Dordwijk, te Dordrecht (opleider Dr. A.C.M. van Vliet). De opleiding werd voortgezet in het Erasmus MC te Rotterdam (opleider Prof.dr. H.A.P. Pols). In 2005 zal worden gestart met het aandachtsgebied medische oncologie in het Erasmus MC, lokatie Daniel den Hoed, te Rotterdam (opleider Prof.dr. G. Stoter).