Occupational and food allergy:

focus on allergen extracts

Cover illustration: Levien Willemse, Rotterdam
Printed by: Optima Grafische Communicatie, Rotterdam

ISBN 90-77595-73-2

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Beroeps - en voedselallergieën:

aandacht voor allergeen extracten

Proefschrift

Ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
Rector magnificus

Prof.dr. S.W.J. Lamberts

en volgens besluit van het College voor promoties.

De openbare verdediging zal plaatsvinden op
Woensdag 17 November 2004 om 15.45 uur

door

Nicolette Wilma de Jong

Geboren te Rotterdam

Promotiecommissie

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Financial assist for the printing of this thesis from the following companies is gratefully acknowledged:
ACM Ooms Allergie BV, ALK Abelló BV, DPC Nederland BV, HAL Allergy BV, Koppert BV, Mead Johnson, Pharmacia Diagnostics AB, Precision bijscholingen BV, Schering- Plough BV

Aan mijn ouders

Table of contents

Abbreviations used

Ac	*Amblyseius cucumeris*
As	*Acarus siro*
BBP	bell pepper pollen
CCD	crossreactive carbohydrate determinants
CI	confidence interval
CH	case history
CV	coefficient of variation
DBPCFC	double-blind, placebo-controlled food challenge
Dp	*Dermatophagoides pteronyssinus*
FEV1	forced expiratory volume in 1 second
FS	field sting
FVC	forced vital capacity
HC	hospital sting challenge
HBV	honeybee (*Apis mellifera*) venom
HEIC	histamine equivalent intracutaneous test
HEP	histamine equivalent prick test
HMW	high molecular weight
IHR	in house reference
ICT	intracutaneous test
ICC	intra class correlation
IL	interleukin
IQR	inter Quartile Range
LAS	work-related lower airway symptoms
Ld	*Lepidoglyphus Destructor* (Ld)
LMW	low molecular weight
LR	local reaction
ND	not done
NRL	natural rubber latex
OAS	oral allergy syndrome
PBS	phosphate buffered saline (pH 7.4, 0.03% human serum albumin, 0.5% phenol)
PBS – A	phosphate buffered saline, with 10 mM EDTA and 0.3% BSA
PBS – T	phosphate buffered saline, with 0.1 % NaN3, 0.2% Tween
PBS – AT	phosphate buffered saline, with 0.1 % NaN3, 0.2% Tween, 0.3% BSA
PEF	peak expiratory flow
PR	pathogenesis related
PRR	prevalence rate ratio
QOL	quality of life
RAST	radio allergo sorbent test
RIA	radioimmunoassay
SE	standard error
SD	standard deviation
SPT:	skin prick test
SDS PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SR	systemic reaction
Th	T-helper
Tp	*Tyrophagus putrescentiae*
UAS	work related upper airway symptoms
UV-B	ultraviolet B light
VAS	visual analogue scale
VIT	venom immunotherapy
YJV	yellow jacket (*Vespula germanica*) venom

Preface

Allergic disorders are common in the northern part of Europe. Besides the well known house dust mite, grass and tree pollen as causative agents many other allergens such as insects, foods, biological agents, and latex, can elicit allergic symptoms as well. An increase in prevalence of allergic disorders has also been observed. An increasingly accepted explanation for this is provided by the hygiene hypothesis (Smith et al. 2004) This hypothesis suggests that the increasing prevalence of allergy is due to a diminished exposure to immune stimulants such as viruses, bacteria and endotoxins, in particular in the early childhood. As an underlying mechanism for this hypothesis, it is proposed that due to the lack of microbial stimulation either a misbalance in T helper type responses or a misbalance in regulatory mechanisms develop.

When antigens enter the body, the immune system produces T-helper (Th) cells. These lymphocytes produce high levels of cytokines in order to protect the body against the antigens. Based on their unique cytokine production profiles these Th cells can be classified into subsets with different functional properties: Th1 and Th2 cells. Th1 cells play an important role in the protecting against inter cellular infections, e.g. viruses, whereas Th2 cells are essential in the clearance of extra cellular parasites e.g. bacteria. A dysregulation of the immune system can cause an increased production of Th1 or Th2 cells as a result of the absence of a network of anti-inflammatory regulatory cells (Tr-cells). An increased production of Th1 cells is seen in type I, e.g. diabetes mellitus and, rheumatoid arthritis. Characteristic of allergic patients is the excessive and persistent maturation of T-cell immune response into Th2 direction. The Th2 cells produce high levels of cytokines e.g. interleukin (IL) 4 and IL 13, which favours the production of specific IgE by B-cells. This specific IgE binds to mast cells and basophils, leading to mediator release after contact with an allergen.

Allergic reactions can be distinguished in four different types: IgE mediated (Type I) and non-IgE mediated (Type II, III and IV). Type I is the immediate type (reactions within 15 minutes), and type IV is called the delayed (reactions after 48-72 hours) type.

Type of Reaction	Antibody or Lymphocytes induced	Clinical Manifestations
I	IgE	Urticaria, rhinitis, conjunctivitis, asthma, Anaphylaxis, oral symptoms, Gastrointestinal symptoms
II	IgM, IgG	Haemolytic anaemia Blood transfusion reactions
III	IgG	Serum sickness, Glomerulonephritis
IV	Th cells	Contact dermatitis

The diagnosis of IgE mediated allergy (Type I) largely depends on the clinical history (CH). Consequently, the clinical history needs to be confirmed by other methods. The skin prick test (SPT) or the intracutanous test (ICT) with an extract of the suspected allergen is the first diagnostic procedure to be applied. The Radioallergosorbent test (RAST) is used for the detection of allergen specific IgE. This determination of IgE is now widely performed and generally correlates with other diagnostic procedures. Furthermore, an allergen provocation with the responsible allergen is possible. In the case of a food allergy, the double blind placebo controlled food challenge (DBPCFC) is considered to be ‘the golden standard’. In case of inhalant allergies a lung provocation or nose provocation is possible.

The most common inhalant allergen extracts for SPT, ICT and RAST are commercially available and standardised. On the contrary, the diagnostic work up of the uncommon allergies caused by relatively new allergens, e.g. certain food allergens, occupational allergens, is more difficult. Commercial extracts are not generally available, the allergens are often unknown and in vitro assays often give unpredictable responses. Consequently, the need for good standardised extracts to confirm these uncommon allergies is increasing.

Not only the prevalence of allergic diseases is increasing; also the spectrum of sensitivities in allergic subjects seems to extend. There is an increasing awareness that patients may be sensitised to a series of related allergens. Likewise, pollen allergic patients are frequently co-sensitised to different plant foods. This phenomenon is not limited to plant foods. Many examples are described and can be explained by cross-reactive IgE antibodies directed to homologous allergens. In the diagnosis of the allergic subjects, physicians should pay attention to these co-sensitisations. Again, standardised extracts being not available, makes it difficult to examine co-sensitisation.

The treatment of allergic disorders comprises allergen avoidance, pharmacotherapy, and -in selected cases only- immunotherapy. In case of food allergy, avoidance of the offending food is warranted. In case of occupational allergy, change of profession might be the only solution.

This thesis presents the results of some diagnostic and therapeutic studies, on occupational - and food allergies. Furthermore, research has been done on cross-reactivity between inhalation mutual and between inhalation and food allergens. Because standardised commercially available extracts were not available, ‘in house manufactured’ extracts have been used in most studies. Finally we examined the quality of ‘in house manufactured’ extracts.

Part one

Chapter 1

General introduction

1 Occupational allergy

Introduction

Occupational diseases are well-recognised problems. They comprise rhinitis, asthma and eczema. Occupational asthma (OA) is the most prevalent lung disease in industrialised countries. Rhinitis is mostly related to OA. Eczema is mainly based on contact allergy and will not be discussed in this thesis. The diagnosis and treatment of occupational rhinitis and asthma is complex. The causative agents are high molecular weight (HMW) compounds (>5000 Da) e.g. animal and plant proteins and low molecular weight (LMW) compounds e.g. isocyanates, aspecific stimuli e.g. cleaning products or irritantia e.g. desinfectantia. In addition, toxic agents or non-specific stimuli may lead to upper and lower respiratory symptoms.
Since occupational rhinitis and asthma have important medical, social and economic consequences, a correct diagnosis is mandatory. The diagnosis of occupational asthma is generally established on the basis of a suggestive history of a temporal relationship between exposure and the onset of symptoms and objective evidence that these symptoms are related to airflow limitation. In many cases the exposing agents and airborne levels of allergens are unknown. There is a critical need for the development of methods to quantify the airborne levels of sensitising agents at the workplace. (Galdi 2002) In the absence of this information, a thorough taking of the case history and a visit at the workplace of the patient can give a rough information on the possible agents: HMW and/or LMW.

In the case of HMW agents SPT, RAST and provocation tests can be used to confirm an IgE mediated occupational allergy. This is much more difficult when LMW agents are involved.
In the Netherlands occupational allergies are found among e.g. bakers (Visser 2001) and greenhouse employees (Groenewoud et al. 2002a, 2002b). In some environments the exposure is totally unknown. For instance, in glasshouses in the Netherlands many airborne sensitising agents from unknown origin may be present, apart from the pollen from the growing plants.
The number of horticulture under glass enterprises has increased enormously during the past few years. Concomitantly, the number of patients with occupational allergic complaints visiting our clinics has risen. Our hospital is close to one of the biggest glasshouse areas in the Netherlands called “the Westland”, and serves as a referral centre.

In recent years the Erasmus MC and adjacent hospitals encountered increasing occupational problems in health care workers. It appeared that health care workers experienced symptoms of rhinitis and conjunctivitis on exposure to airborne latex from gloves.

The increasing number of consultations and requests from individuals and professional organisations warranted studies to investigate the prevalence of occupational allergies in greenhouse employees and in employees working with latex.

1.1 Pollen

One of the triggers of respiratory occupational symptoms is the exposure to airborne pollen. Contact with these airborne pollen may cause type I allergic disorders. A rising incidence of allergic complaints appear to occur among greenhouse employees in the Netherlands. The presence of work-related allergic symptoms among bell pepper horticulturists has been described in case reports by Gerth van Wijk et al. (1989) and by van Toorenenbergen et al. (1984). Recently Groenewoud at al. (2002) found 53.8% of work-related symptoms, e.g. rhinitis conjunctivitis, in a prevalence study among 472 bell pepper employees.

Figure 1. Bell pepper greenhouse.

Furthermore the pollen of ornamental flowers, especially those of the Compositae family e.g. *Chrysanthemum* are well-known sensitisers. *Chrysanthemums* are now one of the most important cut flowers for export. Therefore, we hypothesised that sensitisation to *Chrysanthemum* might lead to increasing occupational problems in employees. Until now, however, only a few studies focussed on IgE-mediated allergy to *Chrysanthemum* pollen have been published. (Piirilä et al. 1994, Suzuki et al. 1975). An extensive investigation, among employees exposed to different kinds of C*hrysanthemum* pollen in Dutch *Chrysanthemum* greenhouses has never been performed. The pollen of other flowers from the C*hrysanthemum* family e.g. sunflower (*Helianthus annuus*), chamomile (*Matricaria chamomilla*), golden rod (*Solidago virgaurea*) can induce allergic symptoms as well. (Fernandez et al. 1993). Different flowers causing type I allergy have been described by several authors. (Bousquet et al. 1985, Eriksson et al. 1987) In recent years occupational allergy against flowers among florists, floriculturists, and gardeners has been reported more often in the Netherlands.
Since individuals working in these industries may be exposed to a variety of airborne pollen, there was a need to study the allergenic characteristics of flowers more in detail beside Chrysanthemum.

1.2 Latex

The first anaphylactic reaction in a patient, on exposure to rubber during a medical procedure, was reported in 1979. Soon after, respiratory allergy to natural rubber latex in medical personnel was described. Natural rubber latex is a biologically complex mixture of substances, many of which may be altered during the process of collection of the material and its fabrication into useful products. Nowadays, immediate reactions to natural rubber products (latex) are recognised as an important health problem. This is a consequence of increased use of latex gloves among health care personnel. Latex is and probably will be the most common material surgical and examination gloves are made of, because of its excellent tensile strength and high elongation at break as well as its good barrier properties due to its film forming ability. One of the major problems in characterising latex gloves is the large variety in allergen content between brands, even between batches of the same brand. Moreover, the prevalence of latex sensitisation differs considerably in populations with different latex exposure (Turjanmaa et al. 1987, 1996) In addition, investigators found that the cornstarch powder on latex products is an allergen carrier. (Swanson et al. 1996, Tomazic et al. 1994). In the Netherlands occupational latex allergy has only rarely been described (Meeren et al. 1988).

As a basis for a policy in latex allergy associated problems in our hospital, a study was conducted to investigate the prevalence of type IV and type I allergy to natural rubber latex in a population at risk among laboratory employees with regular contact with powdered latex gloves in the Netherlands.

2 Food allergy

The prevalence of food allergies (i.e. IgE mediated food allergies) has doubled over the last 25 years. Although this development cannot be fully explained, important changes in the western life style and consumption patterns e.g. environmental factors, novel foods, and modern agricultural practice may have contributed. Recently, Zuberbier et al. (2004) performed a prevalence study on 4000 inhabitants of Berlin, Germany. They found IgE mediated adverse reactions to food in 2.5% of the adult population. Moreover, Burks et al. (1998) found that 8% of the children of a general population have allergic reactions to food. Young children are mainly affected by primary food allergy e.g. to peanuts, cows milk, eggs, whereas adults use to develop IgE mediated food allergy as a consequence of an inhalant sensitisation e.g. allergy to fruits and vegetables in subjects allergic to birch-pollen. This type I allergy comprises the oral allergy syndrome (OAS), urticaria, angioedema, rhinitis, asthma and systemic anaphylaxis. (Bruijnzeel- Koomen et al. 1995) The more common known food allergens are nuts (hazelnut, almond, walnut, pistachio), fruits (peach, apple, kiwi, banana), spices (coriander, curry, celery), seeds (sesame seeds, poppy seed), peanut and wheat. Novel foods, containing proteins to which the population has not been exposed before, (i.e. tropical fruits) gain access to world markets at an, from historical perspective, unrivalled pace. Taken together, not only the number of food allergic patients is increasing, the variety of food allergens is expanding as well. At present, the diagnostic instruments for investigation of food allergy are not satisfying. (Ortolani et al. 1998, 1999) There is a great need for safe and reliable extracts to confirm an IgE mediated food allergy.

3 Diagnostic procedures

3.1 Occupational allergy

Firstly, an extensive questionnaire to provide a good case history is warranted. This questionnaire comprises itching, redness and/or eczema of the skin, urticaria/ angioedema, rhinitis, (sneezing, rhinorrhoea, itching, obstruction), conjunctivitis, (redness, itching, watery eyes), and asthma (weezing, coughing, shortness of breath). Moreover, the work history and an analysis of the relationship between symptoms and labour are essential. Work related symptoms are defined when the worker notices a substantial improvement or complete regression on weekends and holidays and exacerbation during a workweek. Furthermore, to study possible asthmatic reactions peak expiratory flow (PEF) can be performed.

Secondly, a possible IgE mediated occupational allergy should be confirmed by SPT with an extract of the suspected allergen. Unfortunately, commercial and standardised extracts of these uncommon and new allergens are not available. If a bell pepper pollen allergy is suspected (Groenewoud et al. 2002), an extract of the pollen should be made. The same goes for the flower pollen e.g. *chrysanthemum*. In our prevalence studies we manufactured extracts of the pollen of the flowers of the plants. Besides investigating the prevalence of allergy in the employees, we consequently compared the results of the CH, SPT and RAST in sensitised and non-sensitised patients in order to examine the quality of these extracts.
For the diagnosis of IgE mediated occupational *latex* allergy, extracts for SPT have been described by Turjanmaa et al. (1987, 1996). This method is still widely used and reliable. In our study we made allergen extracts for SPT of the 4 most used gloves in our hospital. It was of great importance to investigate which latex glove extract is best used to confirm this allergy. Moreover, this extract could be used in a larger study on prevalence study among operation room personnel in Rotterdam. (Bijl et al. 1999) Furthermore, the occupational allergy to a biological agent *A.cucumeris* (Ac) was examined. During the bell pepper study we found large amounts of this biological agent and it was likely that the

employees were sensitised. Allergen extracts were not available. For this prevalence study an extract of *A.cucumeris* was made for SPT.

Thirdly, in the case of a possible occupational allergy a RAST and the recently developed Capture Allergen Protein-RAST (CAP-RAST) can be performed. This Radio allergo sorbent test involves blood measurement of the minute quantities of IgE antibody specifically directed at particular antigens. The advantage of this method of evaluating specific allergic sensitivities is that a trained technician is not needed to apply the test, use of antihistamines will not interfere with the results, and there is no risk of adverse reactions. To determine specific IgE against the occupational allergens in our studies, we used agarose beads as allergen support and made extracts of the allergens. In this thesis RAST results are used to compare with CH and SPT.

Finally, to determine the clinical relevance of sensitisation, challenges with the suspected occupational allergen can be performed in specialist centres. In general, the history, evidence of bronchial hyperresponsiveness, serial peakflow measurements and immunological tests to sensitising agents are sufficient to support the diagnosis of occupational asthma (Malo et al.2001). Specific challenge tests are useful when there is a doubt about the diagnosis of occupational asthma or in the treatment of a patient with occupational asthma, but it is necessary for their management to confirm the identity of the causative agent. Specific challenge testing is also useful when a new agent is suspected of causing occupational asthma. (Jansen et al. 1996)

Unfortunately, these tests are unsuitable for large epidemiological studies as they are time consuming, uncomfortable for the patient and difficult to interpret. Nevertheless it can be used in certain cases. For instance, to examine the biological activity of an extract.
We used ‘in house manufactured’ *Amblyseius cucumeris* (Ac) extracts in SPT. We were quite unsure about the clinical relevance of a positive SPT with this extract. Therefore, we additionally carried out nasal challenge tests with Ac-extract (Groenewoud et.al. 2002) and compared the results of CH, SPT, RAST and challenge tests.

3.2 Food allergy

As in occupational allergy, the sequence of diagnostic procedures is first history, then skin tests and RAST tests, and finally oral challenge tests. The presence of food allergen specific IgE antibodies does not always correlate with clinical symptoms after exposure to the respective food. For this reason oral provocation tests (ideally double blind placebo controlled food challenges) are much more important in the diagnoses of food allergy than inhalation provocation tests in inhalant allergy. However, oral provocation tests are practically cumbersome and could include a specific risk for the allergic patient. Furthermore, Vlieg-Boerstra et al. (2004) showed that the challenge material must be standardised and validated and that the use of professional tasters is recommended.
Acknowledging the major role of provocation tests in the diagnosis of food allergy, SPT should identify potentially important allergens. If possible they should lead to a plausible diagnosis and eventually the SPT results should be helpful in the selection of allergens for provocation tests. Unfortunately, the specificity and sensitivity of commercial food extracts are unknown because, among other things, the source and allergen content differs between batches. (Skamstrup et al. 2001,Osterballe et al. 2003, Akkerdaas et al. 2003)
Therefore, we developed new in house manufactured allergen extracts for the diagnosis of IgE mediated food allergy. We developed a set of extracts from the most common foods, considered likely to cause a food allergy. This set of extracts appeared to comprise frequently sensitising allergens as can be seen from table 1. This table shows the number of positive SPT of 228 consecutive patients with symptoms of possible food allergy, seen at the allergology clinics of the Erasmus MC in 2002.

Table 1. Positive SPT in 2002 with in house manufactured extracts tested in 228 consecutive patients.

Allergens	Positive SPT (n=)
Bell pepper juice	58
Bell pepper powder	42
Celeriac	68
Coriander	**62**
Curry powder	69
Hazelnut	**109**
Peach juice	**88**
Peanut	77
Sesame seed	**39**
Shrimp	28
Tomato juice	36
Wheat	45

The reproducibility, stability and dose response relationships in these in house manufactured food extracts is unknown. We therefore chose four different food extracts to address these issues: coriander, hazelnut, peach and sesame seed. Choices were made on numbers of positive SPT and diversity of food source.

4 Cross-reactivity

Allergic proteins originate from a great variety of sources (pollen, mites, moulds, venom, animal products foods and latex) and are able to induce the immune system to produce high-affinity immunoglobulin E (IgE) antibodies and/or to trigger allergic symptoms in a sensitised individual. The phenomenon of allergen cross-reactivity occurs when IgE antibodies originally raised against one allergen binds or recognises a similar protein from another source. The clinical relevance of cross-reactivity seems to be influenced by a number of factors including the host (the immune response against the allergen), exposure and the allergen (Aalberse et al. 2000, Ferreira et al. 2004). Several cross-reactive patterns have been detected e.g. birch pollen-apple, peach, hazelnut (van Ree et al. 1993, Breiteneder et al. 2000), birch- mugwort- cellery, spice (Wutrich et al. 1990, Bauer et al. 1996) latex- fruits, (Blanco et al. 1994, 2003) and mites (van der Heide 2000).

Many researchers have extensively studied these cross-reactive patterns and clinical relevance. Individuals with birch pollen allergy often have IgE against plant-derived foods. This can be due to cross-reactive IgE against Bet v 1, the major birch pollen allergen (from *Betula verrucosa*) and homologues. Furthermore, profilins often referred as cross-reactive plant pan-allergens, are present in all eukaryotic cells. Finally, cross-reactive carbohydrate determinants (CCD's) are present throughout the plant kingdom, in insect venom, molluscs and parasites) (Aalberse et al. 1981, van der Veen et al.1997).
Recently, Wensing et al. (2002) demonstrated that Bet v 1 has a more limited spectrum of cross-reactivity than profilin, but Bet v 1 frequently gives rise to clinically relevant cross-reactivity to food. Furthermore, in analogy to anti carbohydrate IgE, cross-reactive IgE against food profilins were shown to have no or very limited clinical relevance.
Besides the well-known cross-reactive pollen-food patterns, cross-reactivity between inhalation allergens is also possible. Van der Heide et al. (2000) found cross-reactivity between *Dermatophagoides pteronyssinus* (Dp) and *Acarus siro* (As)*, Tyrophagus putrescentiae* (Tp) and *Lepidogluphus Destructor* (Ld), whereas Asero et al.(2004) found that Parietaria profilin showed limited cross-reactivity with birch and grass profilins.

In the Netherlands several prevalence studies of allergy to e.g., birch pollen, flower pollen, latex, bell pepper pollen (Groenewoud et al. 2002), *chrysanthemum* pollen*, Amblyseius cucumeris* (Groenewoud et al. 2002) have been performed in consecutive patients and greenhouse employees. Most of these studies are presented in this thesis.

In these prevalence studies cross-reactivity is most likely:

Between several inhalation allergens:

A.cucumeris	Mites e.g. *D. pteronyssius*
Bell pepper pollen	grass and/ or birch pollen
Chrysanthemum pollen	Mugwort pollen
Flower pollen	flower pollen of the same family and Mugwort

Between inhalation and food allergens:

Birch pollen	Fruits (apple, peach) and hazelnut
Latex	Fruits (kiwi, banana, avocado), *Ficus benjamina*

Figure 2. Latex cross- reactive fruits

To establish the diagnosis in patients suspected of occupational and food allergy it is of great importance to examine the possible cross-reactive allergens involved and the clinical relevance of cross-reactivity.

5 Intervention/ treatment

5.1 Avoidance

Avoidance or elimination of the offending allergen is the mainstay of therapy. Further treatment consists of pharmacotherapy and in selected cases, immunotherapy can be effective.
Complete avoidance of allergens is, in most cases, impossible. For instance, avoidance of pollen is impossible. On the other hand avoidance or delayed contact with certain food allergens during childhood is feasible and in some cases advisable. Unfortunately, most patients with occupational allergy have no other alternative than giving up their job. This very difficult decision has a huge impact on the life of the patient.
Therefore, it is important to search for other solutions. One solution could be to accomplish a low as possible exposure to the responsible allergen during work. (Gore et al. 2004, Smith et al. 2004) For instance, in case of a latex allergy, it is possible to work in a latex free environment. When focussing on occupational pollen allergies in greenhouses a reduction of pollen exposure is desirable. Therefore we started a prospective intervention study to reduce the pollen exposure in greenhouses. The aim of this study was to create an environment in which allergic greenhouse workers could keep their job.

Figure 3. Honeybee discarding pollen from the bell pepper flower

5.2 Immunotherapy

Immunotherapy is a well-known treatment with pollen, mites and even animal dander.
For at least 40 years this treatment has been used with insect venom as well.
Allergy to insects is a well-known problem all over the world. In most cases the patient is allergic to the sting of a wasp (*Vespula germanica*) or honeybee (*Apis mellifera*). A sting anaphylaxis can evoke urticaria, angioedema, asthma, conjunctivitis, and even anaphylactic shock. For these patients specific immunotherapy has been successful (Muller et al. 1993)
It is less well known that insect allergy may present itself as a form of occupational allergy. Although bumblebees under natural conditions are usually not aggressive and seldom cause trouble by stinging, the use of these insects for pollination in greenhouses has increased the number of sting induced anaphylactic reactions in the Netherlands. Besides, the bumblebees are bred in a bumblebee farm. The employees in this farm are most likely to get stung many times a day. The bumblebees are more aggressive because they live in an abnormal environmental situation. Since the presence of bumblebee venom-specific IgE has been shown in a number of patients, immunotherapy is probably the treatment of choice, as in other *Hymenoptera* venom-induced problems. De Groot et al. (1995) described immunotherapy in three case reports with purified bumblebee venom. Therefore, we found it necessary to investigate the safety and efficacy of immunotherapy in more patients with occupational bumblebee-venom anaphylaxis.

Aims of the study:

-To develop safe and reliable methods for the diagnosis of occupational and food allergy.

-To investigate cross-reactivity in occupational and food allergic patients.

-The management and treatment of occupational allergic disorders.

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Part two

Occupational pollen allergy

Chapter 2

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

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Allergy 2002;57:835-40

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 7 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 in particular being most prevalent. Sensitization to *chrysanthemum* pollen was considered to be an important risk factor for the occurence 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 due to 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] due to exposure with pollen of ornamental flowers, especially of the *Compositae* family, is rather common in the general atopic population and particulary 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 a workforce of about 2500 people. *Chrysanthemums* are now one of the most important cutflowers for export and the annual turnover is still 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 literature on IgE-mediated occupational allergy to *Chrysanthemum* pollen [2, 4-6]. A broad investigation, however, among employees in dutch *Chrysanthemum* greenhouses with different kinds of *chrysanthemum* pollen has never been performed. The following study was conducted with the aim to investigate the prevalence and determinants of work-related allergic symptoms among this population at risk.

Material and Methods

A cross-sectional study was carried out in March and April 2000. *Chrysanthemum* greenhouses in the Western part of the Netherlands were approached at random by telephone and asked to participate in the study. The investigators paid two visits to each participating greenhouse. During the first visit the volunteers gave informed consent and were asked questions concerning age, sex, medication use, smoking habit, job and job activities, work history, symptoms at work, and atopic diseases such as hayfever, 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 examplary for work-relatedness. Furthermore, they completed a rhinitis quality of life (QOL) questionnaire. During the second visit sensitisation to pollen of 7 different members of the *chrysanthemum* family and a *chrysanthemum* pollenmix was determined by means of skin prick tests. Blood samples were taken to evaluate sensitisation by 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 in Dutch and validated by de Graaf in 't Veld [9]. This questionnaire more precisely describes 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; painfull eyes; swollen mucosae) and emotions (frustrated; restless; irritable; ill at ease having complaints). In each item, they were asked how much they were troubled as a result of their nasal problems 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 are limited because of their nasal symptoms. If more than three activities are 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 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 [10]. Dilution buffer was used as a negative control, 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 6 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* 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 posssible, 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* pollenmix consisting of different genotypes of

breeding material (protein concentration 0.34 g/L) was obtained from Fides Research Office (De Lier, the Netherlands).

Reference group
As controls, ten 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, nonspecific 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* pollenmix 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 NaHCO3 and 0.5 mol/l NaCL, pH 8.5) for 1 h at room temperature. After centrifugation for 10 min at 1400 g, protein in the supernatant was coupled to 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, radioiodinated rabbit anti-human 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-square test (χ^2). A generalised 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 [14]. Since age appears to influence strongly 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 35 greenhouses 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 which 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 which 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 analyzed 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 (yr)	38.8	14-71
Duration of employment (yr)	13.4	0.3-50
Regular employees	5.6	2-14
Seasonal employees	3.2	0.3-25
Area of the greenhouse (m²)	23681	7000-46000
	N	**%**
Sex male	72	69
Sex female	32	31
Smoking	40	39
Job Classification	**N**	%
Owner	32	31
Supervisor	1	1
Fulltime employee	44	42
Parttime 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 versus 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 pollenmix was slightly more prevalent. When the characteristics of sensitized and non-sensitized employees are compared, all symptoms with exception of asthma are relatively more prevalent in sensitized employees. In addition, the average age of sensitized employees is higher, they have worked more years with *chrysanthemum* (19 years versus 12 years) and more than 85% is 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 a significant association between mugwort and *chrysanthemum* pollen (PRR 3.90; CI 3.90 - 3.90) as well.

Rhinitis QOL questionnaire
A total of 95 employees completed their questionnaire correctly and data of these employes were used. Rhinitis symptoms were reported by forty-four of them. We analysed the effect of rhinitis symptoms on the 7 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 by 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 rhinitis 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 pollenmix 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 these pollen could be confirmed by radio-allergo-sorbent test.

Discussion

In this study a high prevalence of work-related symptoms among employees of *chrysanthemum* greenhouses was found (57 %) with the main symptoms being rhinitis and conjunctivitis. It was striking that symptoms of the skin, often mentioned as an important manifestation of an occupational allergy to *chrysanthemum* [15] were reported to a lesser extent (14 %). In addition, symptoms of the lower airways which are considered to be the most serious manifestation of an occupational allergy, was found in 9 %. Sensitization to *chrysanthemum* pollen, confirmed by skin prick testing and radio-allergo-sorbent test, appeared to be an important derminant 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 2 sensitized employees with symptoms of such a kind. The impression that greenhouse employees with work-related rhinitis are 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 7 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 6 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* is mentioned before as most suitable to screen for sensitization to the *Compositae* family [6].
It was striking that sensitization to mugwort was only found in employees sensitized to *chrysanthemum* pollen. This sensitization pattern suggest a strong cross-sensitization to *chrysanthemum* and mugwort which was also suggested by de Jong et al. [6]. Besides, 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 crossreactivity 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 are 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 IgE-sensitization to *chrysanthemum* pollen could 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 involved employees might be specific 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 pestizids is probably a third (additional) explanation.
The described relationships may have important consequences for the 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 proces is to gather the flowers. This happens by pulling them out of the ground, causing a release of pollen from the flowers. Inhalation of these pollen may cause immunologic sensitization with subsequent allergic symptoms in sensitized workers. Personal contact with employees in *chrysanthemum* greenhouses confirmed that the gathering of flowers often initiate the onset of symptoms or aggrevate already existing symptoms. There is, however, no visible pollen release what may be the case by work on other crops, for example on bell pepper plants [17]. For this reason the level of pollen exposure is expected to be less high what might explain the absence of symptoms of the lower airways. Next 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 recommanded 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 that pollen of the flowers are left behind on the floor, causing an extension of exposure.

Acknowledgments

The professional advice of H. van Gennip, Van Zanten Middelburg B.V., Maasdijk, the Netherlands, is gratefully acknowledged. Furthermore we are thankful for the participation of M. Gijsbers and the workers in the greenhouses. Financial support was provided by the Commodity Board for Horticulture, Zoetermeer, the Netherlands.

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Chapter 3

Occupational allergy caused by flowers.

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Allergy 1998;53:204-9

Chapter 3

Abstract

We describe 14 consecutive patients with complaints due to the handling of flowers. The symptoms varied from allergic rhinoconjunctivitis and asthma to urticaria. Most patients had professions in the flower industry. SPT's were performed with home-made pollen extracts from 17 different flowers known to be the most commonly grown and sold in the Netherlands. RASTs against Mugwort, *Chrysanthemum* and Solidago were performed.
The diagnosis of atopy against flowers was based on work related symptoms due to the handling of flowers, positive SPT with flower extracts and positive RASTs. Concordance between SPT and case history was 74%, and between SPT and RAST was 77%. Extensive cross sensitization was seen to pollen of several members of the Compositae family (e.g. *Matricaria*, *Chrysanthenum*, Solidago) and to pollen of the Amaryllidacae family (*Alstroemeria and Narcissus*). Home-made flower extracts can be used to confirm an IgE-mediated flower allergy. Mugwort can be used as a screening test for a possible flower allergy. With most patients the allergy led to a change of profession.

Introduction

Flowers have been cultured all over the world for hundreds of years. The handling or smelling of flowers, or contact with the flower pollen, can cause type I allergic reactions like rhinoconjunctivitis, asthma and urticaria. In recent years occupational allergy against flowers among florists, floriculturists, growers and gardeners has been seen more often in the Netherlands. Case reports of occupational allergy to flowers can hardly be found in the literature. Up to the present 13 different flowers causing type I allergy have been described by several authors. These case reports are summarised in Table 1. The increasing number of patients with an occupational flower allergy in the Netherlands has raised our interest in the questions of which flowers are responsible for this allergy, and whether there is any cross reactivity between different flowers from the same family. The main purpose of the investigation described in this paper is to provide the answers to these questions. For this purpose we selected 17 of the most common flowers in the Netherlands and made pollen extracts for skin prick testing.
Our report demonstrates, firstly, that patients who have complaints caused by the handling of only one or two different flowers are usually sensitive to more flowers. Secondly, we report an extensive cross sensitization to different members of flower families. Finally we describe a reliable way confirming an IgE-mediated flower allergy by SPT with home-made flower extracts.

Materials and methods

Patients
From November 1994 till December 1996 we selected 14 consecutive patients who had complaints caused by the handling of flowers. The complaints varied from allergic rhinoconjunctivitis and asthma to urticaria. A list of the patients is given in Table 2. All patients were asked to fill in a questionnaire concerning their complaints caused by the handling of flowers. The list comprised 5 categories: rhinitis, conjunctivitis, asthma, urticaria, no complaints or 'no contact with'. Control group: 3 non-atopic volunteers were skin tested to detect irritative, non-specific reactions.

Allergens
Seventeen of the most common flowers in the Netherlands were purchased from a local florist. The flowers had to be in full bloom. In some cases collecting pure pollen from the flowers was not possible, for instance, when the flowers were very small or the flowers did not produce enough pollen. In those cases we took a small part of the heart of the flower, with the intention of taking as many pollen as possible.We prepared a 25 % (w/v) extract in phosphate-buffered saline, pH 7.4, containing 0.03 %

human serum albumin and 0.5 % phenol. After a 10 minute centrifugation at 2,000 g, the supernatant was passed through a 0.22 ìm filter (Millipore). All extracts were stored in appropriate aliquots at -20°C until use in skin tests. Before use, extracts were defrozen for 1 hour before the skin test and centrifuged for 45 seconds at 3,200 g. Selected flowers and protein concentrations of the extracts (determined by the method of Watanabe (24), using Pyrogallol Red Molybdate complex) are shown in Table 3.

Table 1: Flower allergy reports published previously (n = number of patients)

Flower name	Latin name	Author	n=	ref.
Amaryllis	*Amaryllis hippeastrum*	Jansen	1	9
Baby's breath	*Gypsophila Paniculata*	Schroeckenstein	1	17
		Twiggs	1	22
Chrysanthemum	*Chrysanthemum*	Blamoutier	1	3
		Gerth van wijk	1	7
		Piirilä	2	14
		Suzuki	6	19
Freesia	*Freesia tubergenii*	Gerth van wijk	1	7
		Piirilä	1	14
		Toorenenbergen	2	20
Gerbera	*Gerbera Jamesonii*	Gerth van wijk	1	7
Sunflower	*Helianthus annuus*	Bousquet	1	4
		Fernández	2	6
Lilac	S*yringa vulgaris*	Sainza	1	16
Lilium	*Lilium longiflorum*	Lahti	1	12
Mimosa	*Acacia floribunda*	Ariano	29	2
Spathe flower	*Spathiphillum Wallisii*	Kanerva	1	10
Statice	*Limonium sinuatum*	Ueda	3	23
	" *tataricum*	Quirce	1	15
Tulip	*Tulipa*	Krüsman	1	11
		Lahti	1	12
		Tupasela	1	21

Table 2: Examined patients with complaints caused by the handling of flowers

Nr	sex	age	relation to the allergy
1	M	28	Gerbera- grower
2	F	23	florist
3	F	28	floriculturist
4	M	22	florist
5	F	38	hobby: gardener
6	F	50	florist
7	F	19	student at a florist school
8	F	25	florist
9	F	25	florist
10	F	48	hobby: gardener
11	F	36	hobby: gardener
12	F	28	floriculturist
13	F	53	Matricaria, Chrysanthemum and Solidago grower
14	F	49	florist

Skin test
Skin tests were performed intracutanously (ICT) with 0.02 ml of Mugwort (*Artemisia Vulgaris*) pollen extract (ALK Benelux, Groningen, The Netherlands). After 20 minutes wheal and flare reactions were measured and graded by the system of standardized plus signs devised by Norman (13).
Skin prick tests (SPT) with flowers were performed by application of one drop of allergenic extract to the skin of the volar side of the fore-arm. Subsequently the dermis was punctured with a standardized skin test needle, and results were read after 20 minutes. Reactions were expressed in mm of mean wheal diameter (adding the longest diameter to the othogonal diameter measured at one half of the longest diameter and dividing it by two). A diameter of 3 mm or more was considered positive. Dilution buffer was used as negative control, histamine chloride 10 mg/ml as positive control. All skin tests were performed by the same skilled nurses.

RAST (radioallergosorbent test)
For routine RAST with Mugwort and Solidago the Pharmacia Cap RIA (with allergens w6 [*Artemisia Vulgaris*] and w12 [*Solidago Virgaurea*], respectively) was used according to the manufacturer's instructions. Total IgE was measured also with the Pharmacia Cap RIA. Chrysanthemum pollen were obtained from Laboratorium Diephuis, ALK, Groningen, the Netherlands. Allergen specific IgE was determined by RAST by the use of agarose beads as allergen support as described by Adkinson (1). One hundred milligrams of pollen were extracted with 2 ml. coupling buffer (0.1 mol/L $NaHCO_3$ and 0.5 mol/L NaCl, pH 8.5) for one hour at room temperature. After centrifugation for 10 minutes at 1,400 g, protein in the supernatant was coupled to 100 mg of CNBr-activated Sepharose 4B (Pharmacia, Uppsula, Sweden) according to the manufacturer's instructions. Two milligrams per test of Chrysanthemum pollen-Sepharose preparation was incubated overnight with 0.05 ml patient serum. After four washes, radiodinated rabbit antihuman IgE antibodies (Pharmacia) were added. After an overnight incubation and four washes the percentage of bound radioactivity was measured.

Table 3: Selected flowers with their family name, latin name and protein concentrations

Family name	Flower name	Latin name	Protein g/L.
Amaryllidacae	Daffodil	*Narcissus*	0.05
	Peruvain lily	*Alstroemeria*	0.41
Asclepiadacae	Asclepias	*Asclepias*	0.40
Cazyophyllacae	Carnation	*Dianthus*	0.13
Compositae	Ageratum	*Ageratum Houstanium*	0.34
"	Chamomile	*Matricaria Chamomilla*	0.32
"	Chrysanthemum	*Chrysanthemum*	0.36
"	Golden rod Michaelmas	*Solidago Virgaurea*	0.16
"	Daisy	*Aster*	0.36
"	Sunflower	*Helianthus Annuus*	0.29
"	Transvaal daisy	*Gerbera*	0.33
Euphorbiacae	Poinsettia	*Euphorbia Pulcherrimma*	0.34
Gentianacae	Lisianthus	*Eustoma Russellianum*	0.50
Geraniacae	Zonal geranium	*Pelargonium Zonale*	0.05
Gesneriacae	African violet	*Saintpaulia*	0.46
Iridiacae	Freesia	*Limonium*	0.11

Table 4: Flower-specific case histories

Flower	Patient no													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Narcissus	NA	-	+	-	-	+	NA	+	+	NA	-	-	NA	+
Alstroemeria	NA	-	+	-	+	NA	NA	+	+	NA	-	+	NA	+
Asclepias	NA	-	+	-	+	NA	NA	-	+	NA	-	-	NA	+
Dianthus	NA	-	+	-	-	-	NA	-	+	NA	-	-	NA	-
Ageratum	NA	+	+	+	-	+	NA	-	+	-	+	+	NA	-
Matricaria	NA	+	+	+	+	+	NA	+	+	+	+	+	+	+
Chrysanthemum	NA	+	+	+	+	+	NA	+	+	+	+	+	+	+
Solidago	NA	+	+	+	+	+	NA	+	+	+	+	+	+	+
Aster	NA	-	+	-	-	NA	NA	+	+	+	-	+	NA	-
Helianthus	NA	-	+	+	-	+	NA	-	+	+	-	NA	NA	-
Gerbera	+	-	+	+	+	NA	NA	-	+	NA	+	+	NA	-
Euphorbia	NA	+	+	-	+	NA	NA	-	+	+	-	NA	NA	-
Eustoma	NA	-	+	-	+	+	NA	-	+	+	-	+	NA	+
Pelargonium	NA	+	+	+	-	NA	NA	-	+	NA	-	NA	NA	-
Saintpaulia	NA	-	NA	+	-	NA	NA	-	+	NA	-	NA	NA	-
Freesia	NA	+	+	-	+	NA	NA	-	+	+	-	NA	NA	-
Limonium	NA	-	NA	-	-	NA	NA	-	+	NA	-	-	NA	-

\+ = complaints (rhinitis, conjunctivitis, asthma and / or urticaria)
\- = no complaints
NA = no contact with, or no flower- specific complaints

Table 5: Skin Prick test results.

Flower	Patients no													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Narcissus	8	ND	4	-	-	-	8	-	-	-	-	-	-	-
Alstoemeria	4	-	3	5	4	-	3	-	3	-	-	-	-	-
Asclepias	4	-	-	3	5	-	5	-	-	-	-	-	-	-
Dianthus	3	-	3	5	3	-	-	-	3	-	-	-	-	-
Ageratum	5	5	9	17	-	4	-	-	9	-	7	-	-	-
Matricaria	-	7	5	8	3	3	-	-	4	6	3	5	4	4
*Chrysanthe*mum	-	8	4	13	3	3	-	-	5	-	-	-	3	*3*
Solidago	4	5	7	13	3	4	-	3	4	-	6	5	3	4
Aster	-	-	-	3	-	-	-	-	-	3	-	-	-	-
Helianthus	8	ND	10	4	-	-	3	5	7	-	-	-	-	-
Gerbera	6	ND	-	6	3	-	-	-	-	-	-	-	-	3
Euphorbia	4	ND	-	4	4	-	-	-	-	-	-	-	-	-
Eustoma	5	-	4	5	3	-	-	-	3	4	-	-	-	3
Pelargonium	-	ND	-	-	-	-	-	-	-	-	-	3	-	-
Saintpaulia	5	-	4	8	-	-	6	-	4	-	-	-	-	-
Freesia	6	-	5	4	4	3	4	-	-	-	-	-	-	-
Limonium	-	-	-	ND	-	-	-	-	-	-	-	-	-	-

SPT's are expressed in mm
ND = not done

Chapter 3

Results

Case History
Most complaints manifested themselves during the patients'work as florist or grower or while gardening. In most cases the patients'complaints increased during their work with a peak at the end of the week and disappeared in the weekends. 14/14 had rhinoconjunctivitis, 10/14 had asthma and 5/14 had complaints of urticaria. These complaints were reported by all patients for *Matricaria, Chrysanthemum and Solidago*. Three patients were not capable of giving a flower-specific case history. All case histories are shown in Table 4. With regard to the inhalant atopy of these patients, it needs to be mentioned that patient number 2,12 and 13 were monosensitized to Mugwort pollen, while the other patients were also sensitized to Grass pollen and Birch pollen.

Skin prick tests
The results of SPT are shown in Table 5. A positive SPT was seen in 11/14 patients with Matricaria and 12/14 with Solidago from the Compositae family. A positive SPT with Ageratum from the same family was seen in 7/14 patients. Most positive SPT with flower extracts from other families were seen with *Alstroemeria* and *Eustoma*: 6/14 and 7/14 respectively.The ICT with Mugwort pollen extract was in all cases positive. Three nonatopic volunteers had a negative SPT with all pollen extracts.

RAST
Rast's against Mugwort, Chrysanthemum and Solidago were in most cases positive. The results of the RASTs and Total IgE are shown in Table 6.

Table 6: RAST results in patients studied

Patient no.	Mugwort	Chrysanthemum	Solidago	Total IgE
1	1.38	2.27	1.16	185
2	4.55	4.70	0.69	52
3	ND	ND	ND	ND
4	17.0	20.1	20.6	251
5	6.57	ND	ND	260
6	4.28	4.10	5.97	200
7	17.8	31.7	17.8	441
8	2.62	11.4	3.15	208
9	17.4	34.5	7.00	267
10	5.82	5.54	8.02	202
11	1.58	<0.35	1.14	155
12	4.01	6.69	0.97	23
13	2.27	3.49	1.00	46
14	ND	ND	ND	ND

RAST results are expressed in kU_A/L.
Total IgE is expressed in kU/L.
ND= not done

Discussion

This investigation describes 14 consecutive patients with complaints caused by flowers. Allergy to flowers was based on questionnaires describing work-related symptoms, positive SPT with flower extracts and RASTs. Questionnaires obtained from 2 patients were not eligible for analysis.
Evaluation of the remaining 12 flower-specific case histories resulted in a satisfactory concordance of

74% compared with the SPTs. In spite of the fact that home-made extracts are not standardized and therefore less reliable, the absence of commercially available flower extracts made us produce extracts to confirm an IgE-mediated flower allergy. In 6% of the cases the SPT was positive with a negative case history. Discrepancies due to irritative skin-reactions were excluded in 3 nonatopic controls, who did not show any reaction. Cross reactive IgE, originally directed against an epitope of, for instance, Mugwort pollen, might also explain this discrepancy, as these antibodies do not necessarily have clinical relevance (8). Finally, incorrect identification of the flower could cause this discrepancy.
In 20% of all cases the SPT was negative with a positive case history. Again, an incorrect case history might explain this kind of discrepancy. Secondly, the quality of the extract could influence skintest results. In a few cases we took a small part of the heart of the flower to make an extract, as we could not gather enough pollen. It will be clear that this may possibly result in a less potent extract as extracts of pollen yields better SPT results (7,23). Lastly, nonspecific hyperreactivity could induce complaints on exposure to flowers (5).
Although a challenge test - the golden standard of allergy diagnosis - may elucidate these discrepancies, the time-consuming character and the risk of anaphylactic reactions (9,10) with non-standardized extracts dissiduaded us from performing these tests.The reliability and specificity of the flower extracts was supported by the high concordance of 77% for both Chrysantemum and Solidago SPTs and RASTs.The pattern of sensitization to flowers is suggestive for a strong cross sensitization to different members of the Compositae family. Most patients demonstrated positive skinreactivity to the *Matricaria* and Solidago. Moreover, all patients were sensitized to mugwort pollen, which may imply strong cross sensitization between mugwort pollen and pollen from flower families. However, cross reactivity has to be established by RAST-inhibition and immunoblots, experiments which are currently in preparation. Cross reactivity between Helianthus and Mugwort pollen has been described earlier by Fernandez (6) and Subiza (18). Neither their studies nor our experiments, however, can address the question whether patients are primarily sensitized by mugwort of by flower pollen.
As patients were sensitized mostly to Solidago and *Alstroemeria*, these flowers appear to be suitable to screen for sensitization to the Compositae and Amaryllidicae family, respectively. 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 conclusion, we demonstrated an IgE-mediated occupational allergy to flowers in 14 patients. Home-made extracts may be reliable in the diagnosis of occupational allergy when standardized extracts are not available. The sensitization pattern in these patients suggests a strong cross sensitizati-on to several members of flower families and Mugwort. There-fore, Mugwort skintest and RAST may be used as screening tests for flower allergy.

Acknowledgements

We thank A.W. van Toorenenbergen (PhD, Laboratory of Allergology, Rotterdam, the Netherlands), who reviewed the manuscript. We are also very grateful to A. Breedveld and D. Tieman (Flowershop 'Dijkzigt') for supplying the flowers.

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Part three

Occupational latex allergy

Chapter 4

Prevalence of natural rubber latex allergy (types IV and I) in laboratory workers in The Netherlands

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Contact Dermatitis 1998;38:159-163

Abstract

The objective of the study was the prevalence of type IV and type I allergy to natural rubber latex (NRL) in a population at risk in the Netherlands. Laboratory workers regularly using gloves were invited to complete a questionnaire and to be tested. We performed epicutaneous tests with standard contact allergens, rubber additives, glove powder and pieces of four gloves; skin prick tests with inhalant allergens, glove extracts, glove powder and fruit extracts; and RASTs. Glove-related hand dermatitis was reported in 36.9 % of the individuals interviewed. A positive patch test result for rubber additives was seen in only 6.6 %. Glove-related urticaria, rhinoconjunctivitis and/or asthma were reported in 24.6 % of all cases. Confirmation of an IgE-mediated reaction was achieved in 8.3 % by skin prick test with glove extracts and 5.0 % by RAST. No reaction to glove powder was noticed in patch testing or in prick testing. A high prevalence rate of glove-related symptoms and NRL type I allergy was found in laboratory workers exposed to rubber gloves. Surprisingly, there was no co-existence of type I and type IV allergy in this population.

Introduction

NRL allergy is a growing health care problem. Due to increasing use of rubber gloves, more workers in hospitals and laboratories are exposed to latex allergens. Latex hypersensitivity can induce local symptoms after glove use (usually caused by type IV allergy to rubber additives), as well as more serious type I allergic reactions ranging from urticaria and rhinoconjunctivitis to asthma and even anaphylactic shock.The prevalence of latex sensitization differs considerably in populations with different latex exposure (as reviewed in 1). In the Netherlands occupational latex allergy has been described only rarely (2). Therefore, we decided to study the prevalence of type IV and type I allergy to natural rubber latex in a population at risk, i.e. laboratory workers with regular contact with latex gloves. After informed consent and ethical approval an extensive questionnaire concerning contact allergy, inhalant atopy and glove-related symptoms was filled in by the volunteers. To study the prevalence of type IV allergy, epicutaneous tests were performed with standard contact allergens, rubber additives, gloves and glove powder. Skin prick tests and RAST were done in order to study type I-related sensitization to inhalant allergens, latex and latex-related fruits.

Materials and Methods

Volunteers
All workers (n = 98) at the Department of Immunology, Erasmus University, Rotterdam, were asked to participate in the study. The volunteers gave informed consent and were asked to fill in an extensive questionnaire concerning age, sex, time in current occupation, smoking habit, use of rubber gloves, symptoms related to glove use, signs of contact allergy and atopic complaints. Subsequently, blood was drawn for RAST and skin tests (prick and epicutaneous) were performed. The answers to the questionnaire were evaluated with each volunteer by the investigator (HdG). The study was conducted according to the World Medical Association Declaration of Helsinki, September 1989 and approved by our Hospital Medical Ethical Committee. Confidentiality was maintained.

Patch tests
The standard European screening tray (including thiuram mix, mercaptobenzothiazole, black rubber mix) was purchased from Van der Bend BV (Brielle, the Netherlands). Patch tests were also performed with an additional rubber additive (carbamate mix), absorbable dusting powder (10 % w/v) and 1 cm^2 pieces of the four gloves. The patch tests were applied to the upper back with Van der Bend Square Chambers and removed 48 hours later. Sites were inspected for erythema and dermatitis 48 and 72 hours after application. All patch tests were performed by the same investigator (LG).

Prick Test

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 results were read after 20 minutes. Reactions were expressed in mm of mean wheal diameter (adding the longest diameter to the orthogonal diameter measured at one half of the longest diameter and dividing it by two). A diameter of 3 mm or more was considered positive (3). Dilution buffer was used as a negative control, histamine chloride 10 mg/ml as the positive control. All skin tests were performed by the same nurse (ED).

Allergens
The latex allergens for prick testing were prepared by extracting pieces, cut from a pair of gloves, in phosphate-buffered saline (PBS), pH 7.4, containing 0.03% human serum albumin and 0.5% phenol during 72 hours at 4^{o}C. After centrifugation at 3,000 g, 15 min, supernatants were collected and dialysed with a 3,500 d molecular weight cutoff membrane (Molecularpouros Dialyses Membrane, Spectrum, California) against deionized water for 72 hours. The dialysate was centrifuged at 13,000 g for 15 minutes, and the supernatants were sterilized with a 0.22 ìm Millex GS filter (Millipore, the Netherlands) and freeze-dried. A 1% solution in PBS was made of the freeze-dried material (4).
Four types of gloves were used for skin testing:
-Sterile Latex Surgeon's Gloves (powdered), Baxter Healthcare Corporation, Valencia, USA.
-Latex Examination Gloves (powdered), Romed, Omnilabo, the Netherlands.
-Hypoallergenic Latex Examination Gloves (unpowdered), Savacare, Van der Bend BV, Brielle, the Netherlands.
-Non-sterile synthetic medical gloves (powdered), Elastyren, ECI Medical Technologies Inc. Nova Scotia, Canada.
The protein content was determined by the method of Watanabe using Pyrogallol Red Molybdate complex (5). The protein concentrations of the four gloves extracts (1 % w/v), used in skin prick testing, were in the range of 5.5 - 7.4 g/L.

From the glove powder (Absorbable Dusting Powder, Keoflo 7136P-USP, van der Bend BV, Brielle, the Netherlands), a 10% extract (w/v) was made in PBS. After 1 hour the extract was centrifuged for 10 minutes at 2000 g and supernatant was passed through a 0.22 ìm filter.
Avocado, kiwi, and banana extracts were prepared from small ripe pieces of the fruits without peel or core. The pulps were homogenized in a food processor and the slurries were passed through a 0.22 ìm filter. All extracts were stored in appropriate aliquots at -20^{o}C until use in skin prick tests. Before use, extracts were defrozen during 1 hour before the skin test and centrifuged for 45 seconds at 3200 g. Finally prick tests were performed with common inhalant allergens from ALK Benelux (Groningen, the Netherlands): grass mix (SQ 293), tree mix (SQ 108), Dermatophagoides pteronyssinus (SQ 503), cat dander (SQ 555) and dog dander (SQ 553).

RAST
For the RAST, the Pharmacia Cap RIA (with allergen k82 Hevea braziliensis) and total IgE were measured according to the manufacturer's instructions.

Results

Clinical history
Of the 98 individuals invited, 66 participated in the study (67.3 %). Reasons for failure to attend were irregular (part-time) working schedules, lack of interest because of absence of work-related symptoms, and other individual causes. Demographic characteristics of the volunteers and data from the questionnaire are shown in Table 1.
Glove-related symptoms were reported by 28 individuals (43.1 %); complaints consisted of local dermatitis in 36.9 % and of urticaria, rhinoconjunctivitis and/or asthma in 24.6 %

Table 1. Results from the questionnaire concerning natural latex allergy in laboratory workers.

	n	%
number of participants	66	
age: range (yr)	19-56	
avg (yr)	33.1	
sex F/M	42/24	
smoking/non-smoking	10/55	
duration of exposure		
0-5 yr	37	56.1
5-10 yr	24	36.4
> 10 yr	5	7.6
glove use: hrs/day		
range	0-6.5	
average	1.2	
glove use: hrs/week		
range	0-30	
average	5.0	
glove use: hrs/month		
range	0-100	
average	21.0	
glove-related complaints		
itching	22	33.8
redness	19	29.2
eczema	6	9.2
urticaria	9	13.8
angioedema	4	6.2
rhinoconjunctivitis	10	15.4
asthma	2	3.1
inhalant atopy	25	41.7

Patch tests

Patch tests with standard contact allergens were positive in 42.6 % of the 61 individuals tested (Table 2). Positive test results for nickel were found frequently, viz. in 14 women with complaints about jewellery. The rubber additives were positive in only 4 individuals, 3 of them with complaints of glove-related itching and redness. The positive patch test with the Romed glove was seen in 3 individuals with a very strong prick test result for the latex gloves. Patch tests with the rubber additives, however, were negative in these patients. Glove powder extract gave no positive reaction in any of the individuals tested.

Table 2. Positive patch test results (61 individuals tested)

allergen	n	%
Nickel	14	23.0
Colophony	4	6.6
Fragrance mix	4	6.6
Thiuram mix	3	4.9
Balsam of Peru	3	4.9
Romed glove	3	4.9
Cobalt chloride	2	3.3
Formaldehyde	1	1.6
Kathon CG	1	1.6
Carba mix	1	1.6
Baxter glove	1	1.6

Prick test
Atopy for inhalant allergens was demonstrated in 25 out of 60 individuals tested (41.7 %). Prick tests were positive for Dermatophagoides pteronyssinus in 26.7 %, for grass pollen in 25.0 %, for tree pollen in 15.0 %, for cat dander in 20.0 % and for dog dander in 11.7 % of the volunteers. In 5 individuals there was a positive skin prick test with the glove extracts. These cases are shown in Table 3. All were atopic and used rubber gloves very often. Complaints consisted of glove-related dermatitis in 5, urticaria in 4, and rhinoconjunctivitis in 3 individuals. Latex-associated foodsensitization was demonstrated in 4 out of 5 latex-positive individuals and in 5 out of 55 latex-negative individuals. There was no positive reaction to glove powder extract or to the non-latex glove extract (Elastyren).

Table 3. Positive prick test results

case no.	L05	L15	L21	L31	L43
age (yr)	26	29	29	30	23
sex	F	M	F	F	F
years of occupation	0-5	6-10	0-5	0-5	0-5
glove use					
hrs/day	2	2	4	1	2
hrs/month	35	40	80	20	30
atopy	+	+	+	+	+
glove-related complaints					
itching	+	+	+	+	+
redness	+	-	+	+	+
urticaria	+	+	+	+	-
angioedema	-	-	+	-	-
rhinoconjunctivitis	+	+	+	-	-
asthma	-	-	-	-	-
Skin prick test (mm)					
glove powder	0	0	0	0	0
Baxter triflex	9	10	9	0	6
Romed	17	21	14	4	9
Savacare	8	13	8	0	4
Elastyren	0	0	0	0	0
avocado	4	5	0	4	8
banana	4	6	0	0	5
kiwi	0	8	0	5	0
RAST latex (kU_A/L)	2,79	9,29	8,62	<0,35	<0,35
Total IgE (kU/L)	208	60	534	368	128
Patch test positive	Ro	Ro	Ro Ni	-	-

(Ro = Romed glove; Ni = Nickel)

RASTs
Natural rubber latex-specific IgE could be demonstrated in 3 cases (Table 3), with strong positive skin prick test results for glove extracts. The other 2 cases with moderate positive skin prick tests were RAST negative, as were all other individuals with negative skin test results for glove extracts.

Table 4. Relation between inhalant allergy (positive case history and positive SPT to common inhalant allergens) and the diagnosis of type I NRL-allergy

	total number	with NRL-allergy	%
atopic	25	5	20
non-atopic	35	0	0

Discussion

Latex sensitization is seen frequently as an "allergic disease" in our daily practice. Health care workers in particular are at risk due to their frequent contact with rubber gloves. Prevalence rates of latex atopy (i.e. positive skin prick test) are ranging from 2.8 to 16.9 % for hospital personnel in general (6-9); from 5.0 to 17.6 for ward nurses (8-10); from 5.4 to 14.4 for O.R. personnel (7,11-15); and finally from 7.4 to 9.9 for physicians (7,9,16). Regarding laboratory workers two studies provide information about the existence of type I NRL allergy. In a children's hospital in the USA positive skin prick tests were found in 17.1 % of 41 laboratory workers (9). In a small primary care hospital in Belgium the prevalence was 5.0 % in 36 laboratory workers (8). In the Netherlands only one report regaring latex allergy in health care workers was found (2). All surgeons, gynaecologists and urologists practising in the Netherlands were sent a questionnaire on the incidence of glove-related symptoms. The response rate was 64.0 % Glove-related dermatitis was reported in 9.5 %, of which 1.1 % consisted of contact urticaria. None mentioned rhinoconjunctivitis or asthma-like symptoms. No confirmatory tests like patch test or skin prick test were performed.

We investigated the prevalence of atopy for natural rubber latex in a population at risk in the Netherlands. For this purpose we studied laboratory workers of the Department of Immunology, Erasmus University, Rotterdam. Approximately 40.000 pairs of powdered NRL gloves were used in 1996 at this department and workers were exposed frequently. Glove-related dermatitis was reported by 37 % (n = 25) of the individuals, the main symptoms being itching and redness of the skin. In only 4 of these 25 individuals, a positive patch test with rubber additives confirmed the diagnosis of "allergic contact dermatitis". Overall a high prevalence of positive patch tests with standard contact allergens was seen. The allergens most frequently found were nickel, colophony and fragrance mix.

Regarding immediate type allergy also, a high percentage of atopic individuals was seen; inhalant allergens most frequently found were Dermatophagoides pteronyssinus (27 % of individuals tested), grass pollen (25 %), cat dander (20 %) and tree pollen (15 %). Glove-related rhinoconjunctivitis was reported by 15 % (n = 10) of the participants; in 5/10 cases type I allergy for latex gloves was confirmed by skin prick testing, in 3/10 by latex RAST. All 5 individuals were atopic for inhalant allergens, but unaware of the cause of the glove-associated complaints. The relation of inhalant atopy to the diagnosis of NRL allergy is summarized in Table 4.

None reported symptoms of dyspnoea or wheezing, although in 2 individuals we measured lung function (17) and showed in 1 case a bronchial hyperreactivity suggesting asymptomatic occupational asthma. This was reported earlier by Vandenplas, who found a positive bronchial response to latex inhalation challenge in nurses without a history of occupational asthma (8).

As shown in Table 3, "hypo-allergenic" gloves also caused strong positive skin reactions in 4/5 NRL atopic cases. Therefore these gloves are no alternative for NRL atopic individuals, in accordance with previous research (18).

Non-latex glove extract and glove powder extract were negative in all cases in patch tests and prick tests. Patch tests with rubber additives, like thiuram mix and carba mix, were also negative in all NRL-atopic persons and had no predictive value for type I allergy. This was confirmed by the case history: all 5 cases with type I NRL allergy had complaints of itching and/or redness of the skin within 30 minutes glove use. Only 1 case reported chronic handeczema (with negative patch test results for rubber additiva).

In summary we found, in accordance with previous investigations in other countries, a high prevalence of glove-related symptoms and NRL type I and type IV allergy in laboratory workers exposed to rubber gloves. Furthermore we showed that regular contact with NRL gloves resulted in two distinct disease groups: a type I allergic population, all atopics and with a high prevalence of coexisting tropical fruit

allergy; and a type IV allergic population without evidence of type I allergic reactions to inhalant allergens, fruit or NRL. Most individuals were not aware of the existence of latex allergy. Therefore we conclude that there is a compelling need for education and active search for "patients" in at-risk populations like health care workers. In this way we can prevent further sensitization to latex allergens and worsening of atopic complaints by means of individual as well as work-related measures (19,20).

Acknowledgments

We sincerely thank Pharmacia & Upjohn BV (Woerden, the Netherlands) and Van der Bend BV (Brielle, the Netherlands) for kindly providing materials for CAP-RASTs and epicutaneous tests, respectively.

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Part four

Food allergy

Chapter 5

Reproducibility and stability of 'in house manufactured' extracts used in the diagnosis of IgE mediated allergy.

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submitted

Abstract

Background. Allergic reactions to food are common in the northern part of Europe. Skin Prick Test (SPT) is usually the first diagnostic procedure applied when food allergy is suspected. Unfortunately standardised food allergen extracts for SPT are not commercially available. In the absence of these extracts researchers are searching for a safe, practical and reliable method to diagnose IgE mediated food allergy.

Objectives The aim of our study was to examine the reproducibility, stability and dose response quality of, in house manufactured food extracts. Although not the main issue in this study, we additionally focussed on the association between SPT, CH and RAST.

Methods We performed SPT in 21 food sensitised patients with 4 different food allergens: coriander, hazelnut, peach and sesame seed. Dose response, batch to batch and a stability studies were done. Allergen specific Ch was recorded. Area of the wheal of SPT was measured by planimetry and consequently HEP was used for statistical analysis. RAST's were done.

Results Overall pair wise comparisons of dose response were significant in all four extracts. Batch to batch studies in coriander, hazelnut, peach and sesame seed gave coefficients of variation of 39%, 33%, 37% and 26% respectively. Stability studies gave no significant differences between fresh, 3 months and 6 months old extracts. Mean concordance between SPT and CH, SPT and RAST and between CH and RAST was 72%, 29% and 37% respectively

Conclusion 'In house manufactured extracts' are characterised by good reproducibility and stability in skin tests; moreover they yield a high concordance between skin test results and clinical history. In the absence of well-standardised extracts, they may be a reliable alternative for both commercially available preparations and fresh food applied by prick-to-prick test. Further investigation is necessary to examine these and other in house manufactured extracts on sensitivity and specificity.

Introduction

Allergic reactions to food are common in the northern part of Europe. Skin Prick Test (SPT) is usually the first diagnostic procedure applied when food allergy is suspected. Consequently, the test must be highly sensitive. To perform a SPT the quality of the extract is most important. The extracts should be highly sensitive, specific, standardised and technically optimised to confirm an IgE mediated food allergy. Unfortunately, SPT with commercial food extracts are not standardised and the diagnostic performance is limited (1). To overcome these problems prick-to-prick test with fresh food has been used, particularly in case of heat-labile allergens such as fruits. Although this method appears to be most sensitive, it is impracticable for some allergens. Moreover, it cannot be ruled out that the use of fresh food introduces a source of variation, which may negatively affect the reproducibility of the test. Ideally, the stability of standardised inhalant extracts and the reproducible test results obtained with these extracts should be combined with diagnostic performance of fresh foods. This made us manufacture new in house allergen extracts for the diagnosis of IgE mediated food allergy. Previous investigations proved that these extracts are very sensitive (2-6) and although they are not standardised either, few discrepancies were found between SPT and case history of the patients. To compare extracts, the size of the allergen-induced wheal by SPT has been evaluated. A quantitative approach is essential, and highly precise determinations of SPT reactions are crucial for research purposes. Therefore, we measured the area of the wheal by planimetry and consequently used HEP (Histamine Equivalent Prick) for further calculation. The aim of our study was to examine the reproducibility, stability and dose response quality of, in house manufactured food extracts. Although not the main issue in this study, we additionally focussed on the association between SPT, CH and RAST.

Material and methods

Study design

The study comprised three different parts. The first one was performed in March, the second in June and the third in September 2003. The same patients underwent allergy tests at the three different occasions.The first part comprised a dose-response study, in which three different concentrations of each extract were used applied in duplicate within each subject. The second part was a batch-to-batch study. We made five different batches from each extract in one concentration. The concentration chosen for this study was the same as we normally use in our daily practice. The third part was a stability study. We used fresh extracts and extracts of three months and six months old, again with the same concentrations as used in the second study. Calculations were made concerning positive SPT (HEP > 0.15) seen in patients visiting our outpatient clinic in 2002. From a total of 225 food batteries tested on the patients the following extracts were most positive: bell pepper juice: 58, celeriac: 68, coriander seed: 62, curry powder: 69, hazelnut: 109, peach juice 88, peanut: 77, sesame seed: 39, shrimp: 28, tomato juice: 36 and wheat: 45. Dependent on the number of positive SPT-s and the extraction method we chose four different food allergens: hazelnut, coriander, peach, and sesame seed. Moreover, apart from peach, these foods do not lend themselves very well for the prick to prick test method. An allergen specific CH was recorded and graded as positive or negative for four categories: skin complaints (itching, redness and/or eczema, urticaria/angioedema), rhinitis (sneezing, rhinorrhoea, itching, obstruction), OAS (itching, swelling of the lips and/or the tongue), gastro-enteric complaints. The local ethics committee approved the study and all subjects gave written informed consent before entering the study.

Patients

From January 2002 until December 2002, 81 consecutive patients of the Department of Allergology, with a positive SPT, > 7 mm^2 (7) for 3 out of the 4 selected food allergens, were approached. This selection was irrespective of the symptoms of the patients. None of the patients had undergone immunotherapy previously or was undergoing immunotherapy during the study. As infants and small children have a good prognosis for outgrowing their food allergy (8), only adults or adolescents were selected. Of the 81 patients approached, 21 participated in the study. Reasons for refusal to participate were lack of time and/or lack of interest. Another reason for not participating was the fact that some patients could not stop their anti-allergic medication three day's before SPT. The patients (8 males, 13 females) had a mean age of 35 (range 19-51 years). Nineteen subjects reacted to skin tests with inhalant allergens (obtained from ALK/Abello, Denmark). Most of the patients were pollen allergic, e.g. treepollen: 90%, grasspollen: 86%. Symptoms to food allergens comprised skin problems (itching/redness/eczema), rhinitis, symptoms fitting in the oral allergy syndrome (OAS) and gastro-intestinal complaints. Eleven subjects (47%) reported symptoms to coriander, 19 (90%) to hazelnut, 18 (83%) to peach and 12 (55%) to sesame seed. Skin symptoms and OAS symptoms were most frequently encountered (coriander 42 and 26 % respectively; hazelnut: 50 and 75%; peach: 33 and 67%; sesame seed 30 and 33%). Five patients experienced food-induced anaphylaxis. In two cases the anaphylaxis was induced by hazelnut, in the other patients the exact allergen was unknown. In these cases it was difficult to find out which allergen was responsible, as these patients appeared to have an extensive food allergy: e.g. nuts, fruits, seeds and spices. One of the five patients suffered from an exercise-induced anaphylaxis.

SPT

Prior to SPT, medication was discontinued according to the EAACI guidelines (9): short-acting antihistamines at least 3 days before. SPT was performed by application of one drop of allergenic extract to the skin of the back of the patient. Subsequently the dermis was punctured with a standardised skin test needle, and results were read after 20 minutes. Dreborg et. al (10). Dilution buffer was used as negative control. Every battery of SPT-s was accompanied by 8 histamine chloride 10 mg/ml as positive control and used for statistics, e.g. calculating HEP index. All SPT-s were performed by trained nurses and done in duplicate. The contours of the wheal were encircled after 15 min. with a fine-tip pen and transferred to a record sheet by means of translucent tape. Area determination with a scanning program was used. (11, 12)

Area scanner
The computer program is an internal development and is designed for all Microsoft Windows operating systems. It uses any flatbed scanner (we used a Hewlett Packard 2400c). An Access database is used for storage of all patient data and SPT results.The computer program runs on hardware Windows 95 compatible. For scanning, a HP scanjet 2400c (Hewlett-Packard Belgium S.A./N.V.) was used. The area, which was calculated by the program, was without the outline. If the outline of the area did not form a close figure, this was indicated on the screen. The program is used to calculate the HEP (histamine equivalent Prick). The area scanner counts the pixels within the area of the wheal. The most important reason for using this new method is the accuracy and high reproducibility of the scanner.

Extracts
The raw materials for each food allergen were carefully screened to select the material that is best at representing allergen. Hazelnuts (fresh, not roasted, organic nuts) were homogenised mechanically, ground with a mortar, defatted by ether extraction, and subsequently air-dried. This pre-treated material was stored at -20°C until use for further preparation. For the batch to batch study this process was repeated 4 times. The following preparations were done on the day the patient was tested. Dilutions were made depending on the current moment in the study. The pre-treated hazelnut material was defrosted and a 20%, 10%, or 5% (w/v) extract in PBS (phosphate-buffered saline pH 7.4, containing 0.03% human serum albumin and 0.5% phenol) was made. Peach juice was prepared from small pieces of ripe peaches with peel, without core. The pulp was homogenized in a food processor, the slurry was filtered, and the fluid was subsequently passed through a 0.22 µm filter. The juice was used undiluted, and a 50% and 25% (w/v) dilution in PBS was made. Sesame seed and coriander seed were homogenized mechanically. A 20%, 10% or 5% (w/v) extract was prepared in PBS. All extracts were centrifuged for 10 min at 2000 g, and supernatants were passed through a 0.22-um filter (Millex GS, Millipore, the Netherlands). From all extracts appropriate aliquots were stored at -20°C for the stability study and for further investigations. In these cases, the extracts were defrosted for 1 hour before skin test and mixed. Protein concentrations were analysed with the benzethonium chloride method (13) on the Hitachi 911 routine clinical chemistry analyser, according to manufacturer instructions (Boehringer/Roche, Almere, the Netherlands). The concentrations were in hazelnut 10% (w/v): 2.98 g/L, in coriander 20% (w/v): 3.78 g/L, in peach juice 2.18 g/L, and in sesame seed 20% (w/v): 1.08 g/L.

RAST
Specific IgE was determined by the CAP-RAST system (Pharmacia, Uppsala, Sweden). RIA with coriander, hazelnut, peach and sesame seed (with allergen f317 *[Coriandrum sativum],* fl7 *[Corylus avellana],* f95 *[Prunus persica],* and flO *[Sesamicum indicum],* respectively was used according to the manufacturer's instructions. Total IgE was measured with the same method.

Statistical Analysis
All calculations were done with geometric areas measured by area scanner.
In calculations a RAST class >1, SPT HEP>0,15 and CH 1/4 possibilities were regarded as positive results. Significance is supposed to be reached if the p-value is smaller than 0.05. Wheal areas were analysed after natural logarithmic transformation because of positive skewness. A dose response relationship of each extract was estimated using mixed ANOVA, given the three within-subject concentrations of the extract applied in duplicate to each subject. Differences in response between the doses were expressed as ratios of geometric mean wheal areas. Pairwise comparisons of the concentrations were only tested if the overall p-value fell below 0.05. A similar analysis was done for three age categories of the allergen extracts. Reproducibility of the measurements was expressed by means of the intra class correlation coefficient (ICC) and by means of the within subject standard deviation (SD) of replicated observations. As the observations are on a natural logarithmic scale, the SD can be approximately interpreted as the coefficient of variation (CV). For the histamine control eight within subject replicate observations were available. For the other extracts five within subject replicate observations of mean dose were available.

Results

Control group
As controls, ten nonatopic volunteers were skin tested to detect irritative, non-specific reactions. They were all found negative in SPT.

Safety
Three adverse reactions were registered after SPT with the allergen extracts. These complaints were only reported during the first study. One patient reported an itchy throat, another patient reported an itchy throat and coldness 20 minutes after SPT. Furthermore, one patient reported early and late phase reactions comprising sneezing, tiredness and throat tightness. This reaction was regarded as serious. Those three patients experienced food-induced anaphylaxis before. Symptoms were treated with antihistamine tablets.
In the second and third study no adverse reactions were seen. In a few cases medication was given after SPT, as precaution for reactions happened in earlier studies. During the whole study a few patients developed a local, late-phase skin reaction after SPT with fresh food.
The control group did not report any adverse events during SPT.

Histamine
The reproducibility of the histamine response was determined from the eight replicate observations per subject. Geometric mean area was 97.7 (IQR {Inter Quartile Range} 32-172). The ICC was 72%. The Coefficient of variation was 25%.

Dose response test (first study)
Geometric mean areas (mm2), (CI95% interval) of the different extracts with the different concentrations are shown in table 1. In table 2 the results of the assessed dose-response are presented by means of ratios of the effect on geometric mean wheal area, with 95% confidence interval and overall p-value. As the overall p-values of all four extracts are significant also all pairwise comparisons are tested.

Table 1. Geometric mean area of wheals and inter quartile range by dose and allergen in the group of patients studied.

Allergen	concentration	geometric mean area Mm2	(IQR)
Coriander	5%	10.1	(7.3-44.6)
	10%	16.8	(4.3-39.4)
	20%	25.8	(9.5-39.6)
Hazel	2.5%	8.0	(0-31.4)
	5%	11.5	(3.1-24.4)
	10%	19.2	(8.1-42.8)
Peach	25%	6.9	(4.0-11.1)
	50%	11.2	(5.9-25.5)
	undiluted	21.8	(9.9-29.7)
Sesame seed	5%	7.8	(3.8-17.1)
	10%	8.7	(3.7-14.4)
	20%	11.8	(5.8-21.0)

Table 2. Pair wise comparisons of dose effects on geometric mean wheal area in terms of ratios of the effect of a higher concentration relatively to a lower concentration.

Allergen	concentration comparison	ratio	CI 95%	p-value	overall p-value
Coriander	10%-5%	1.66	1.24-2.22	0.001	
	20%-10%	1.53	1.14-2.07	0.006	
	20%-5%	2.55	1.89-3.44	<0.001	<0.001
Hazel	5%-2.5%	1.45	1.05-2.00	0.025	
	10%-5%	1.66	1.21-2.30	0.003	
	10%-2.5%	2.42	1.75-3.34	<0.001	<0.001
Peach	50%-25%	1.61	1.24-20.9	0.002	
	und.-50%	1.96	1.50-2.54	<0.001	
	und.-25%	3.15	2.42-4.09	<0.001	<0.001
Sesame seed	10%-5%	1.13	0.92-1.38	0.241	
	20%-10%	1.35	1.10-1.65	0.005	
	20%-5%	1.52	1.24-1.86	0.001	0.001

Table 3.
Geometric mean areas stability (Skin prick test)

Allergen	age	geometric mean (CI 95%) Mm2	effect on area p-value
Coriander	fresh	24.4 (13.9-42.8)	
10%	3 months	18.8 (10.7-33.0)	
	6 months	16.9 (9.6-29.7)	0.254
Hazelnut	fresh	20.3 (10.5-39.6)	
5%	3 months	13.3 (6.8-25.8)	
	6 months	17.1 (8.8-33.2)	0.102
Peach	fresh	18.4 (11.1-30.5)	
undiluted	3 months	21.2 (12.9-35.2)	
	6 months	22.4 (13.5-37.1)	0.1404
Sesame seed	fresh	13.7 (7.9-23.7)	
10%	3 months	11.5 (6.7-19.9)	
	6 months	11.6 (6.7-20.1)	0.3227

Batch-to-batch (second study)
Intra class correlation coefficient (ICC) (reliability index) of batch-to-batch for coriander (10%), hazelnut (5%), peach (undiluted) and sesame seed (10%) were 0.81, 0.87, 0.82 and 0.88 respectively. Coefficients of variation were 39%, 33%, 37% and 26% respectively.

Stability (third study)
Geometric mean areas (mm2), (CI 95%), with p-values of the fresh, 3 months old and 6 months old extracts of the four allergens are shown in Table 3.

Concordancy
The mean concordance between SPT and CH, between SPT and RAST, and between CH and RAST was 72% (52%-84%), 29% (16%-42%) and 37% (11%-53%) respectively.
Correlation between SPT (mean HEP batch-to batch), RAST and Case history are shown in Table 4. For these calculations the mean HEP results from the batch to batch study were used.

Table 4.

Correlation between SPT, RAST and Case history

		SPT		RAST		
No. of patients		Neg	Pos	Neg	Pos	Allergen
Case history	Neg	1	9	8	0	coriander
	Pos	1	10	9	2	
	Neg	0	2	2	0	hazelnut
	Pos	3	16	13	4	
	Neg	0	3	1	2	peach
	Pos	1	17	15	1	
	Neg	3	6	5	2	sesame seed
	Pos	0	12	7	5	

RAST
RASTs were done in 19-21 sera with coriander, hazelnut, peach and sesame and found positive in 4,2,3 and 7 cases respectively. Mean total IgE was 218.4 (15.7-1192) kU/L.

Discussion

The number of patients visiting our outpatient clinic with food allergic complaints is growing. To diagnose these IgE mediated food allergies we were searching for a method, which is practical, safe and reliable. Many researchers have attempted to optimise diagnostic procedures, but many practical problems have to be overcome. Although DBPCFC has been accepted as the gold standard of food hypersensitivity diagnosis (14) and this procedure has been recommended by the European Academy for Allergy and Clinical immunology as the only conclusive evidence of a food allergy, this method is very time consuming and in case of some allergens impracticable. Moreover, besides conceivable ethical reasons routine challenge of multi-sensitised subjects is not easy to organise. Apart from logistic problems, research to optimal doses has been performed for only a few allergens (15). Standard protocols for DBPCFC need to be developed further. For the diagnosis of food allergy (16) skin tests have also been used extensively.Unfortunately, the allergenic activity of extracts now commercially available varies, and results obtained with extracts from different manufacturers or even with various batches from the same producer differ. (17,18) Recently Akkerdaas et al. (1) found significant differences in SPT between various products. Although there is a strong need for standardised allergen extracts for in vivo and in vitro diagnosis, only very few attempts have so far been carried out to apply biological standardisation on food allergen extracts as has been done for inhalation allergen extracts for many years (19). An alternative for allergen extracts is the use of fresh

foods (20,21). Fresh fruits and vegetables may be used with the 'prick to prick' test; that is pricking the food to be tested and then pricking the skin of the patient being evaluated. This prick to prick method is usually more sensitive and reproducible than SPT with commercially available extracts. The prick to prick test may be impracticable in case of large patient groups sensitised to multiple allergens. Also, some food allergens such as sesame seed may be less suitable to be used in prick-by-prick test. Ideally, food preparations for skin testing should be characterised by the biological activity of fresh food and by the stability and reproducibility of commercially available inhalant allergen extracts. Therefore, we aimed to evaluate the stability and reproducibility of 'in house' prepared extracts from fresh food. We demonstrated that 'in house manufactured extracts' are stable, can be stored during at least 6 months and may yield reproducible skin test results over time, thereby using different batches. Before accepting these findings several issues should be addressed. First, this study was not designed to assess the positive and negative predictive value of the tests, since only patients with a known sensitisation to food and healthy subjects were included and DBPCFC were not performed. As five out of twenty-one patients experienced anaphylactic reactions to food, DBPCFCs were not considered. Although the study was not designed to assess sensitivity and specificity of the extracts, the good concordance between skin test results and clinical history suggests that the extracts are useful in daily clinical practice, in particular taking the much lower concordance between RAST and clinical history taking into account. Standard allergen concentrations in skin prick tests should identify subjects characterised by a low degree of sensitisation to allergen, however without eliciting adverse reactions in highly sensitised patients. Therefore, we chose for the second and third study the middle concentration from the dose response study for hazelnut, coriander and sesame seed. Firstly, because the highest concentration in this study gave three anaphylactic reactions and secondly because the pair wise comparisons of the dose responses gave significant results in all cases. For peach we chose the undiluted juice, as this is the same concentration as used in prick-to- prick. We therefore conclude that these concentrations may be suitable for clinical practice. Although the batch to batch study coefficients of variation of our extracts are somewhat higher compared to histamine the intra class correlation coefficient for histamine is comparable with the coefficients for allergen extracts. Importantly, the variation of the histamine of 25% may suggest that one skin tests with histamine is not inadequate to calculate HEP. The coefficient of variation for the SPT histamine is comparable with the result found by Niemeyer et al (22): 21.2% Stability of the test extracts appeared to be strikingly high. Significant differences between the fresh and six months old extracts could not be demonstrated. We assume that the short time of preparing the extracts, e.g. the peach juice is made in a few minutes and frozen in small aliquots, contributes to the stability of the extracts. Neither difference in varieties of fruits and vegetables, nor seasonal variation appeared to influence SPT results, as described previously (Skamstrup 21). In conclusion, 'in house manufactured extracts' are characterised by good reproducibility and stability in skin tests; moreover they yield a high concordance between skin test results and clinical history. In the absence of well-standardised extracts, they may be a reliable alternative for both commercially available preparations and fresh food applied by prick-to-prick test. Further investigation is necessary to examine these and other in house manufactured extracts on sensitivity and specificity.

Acknowledgements

We thank Ellen Duijster-Kunst, Marina Meijer, and Rinia Maris-Nelstein, allergy nurses, Department of Allergology, ErasmucMC, Rotterdam, the Netherlands, for their skilful technical support in this study.

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Chapter 6

Birch pollinosis and atopy for apple, peach and hazelnut; comparison of three extraction procedures with two apple strains

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updated version, adapted from Allergy 1996;51:712-8.

Abstract

Aim of the study: First, to study the prevalence in the Netherlands of atopy for apple, peach and hazelnut in patients with tree pollinosis. Second, to compare three extraction procedures for skin prick testing with two different apple strains.
Methods: In 79 consecutive patients with tree pollinosis, visiting the department of Allergology during the spring of 1995, skin prick tests and RAST were performed on the same occasion. In skin prick testing we used three different extracts (juice, freeze-dried extract, and low-temperature acetone powder extract) of two apple strains, Golden Delicious and Granny Smith.
Results: Case histories for apple, peach and hazelnut were positive in 35 (44.3 %), 23 (29 %), and 35 (44.3 %) patients, respectively. More than two-thirds of the patients had symptoms characteristic of an oral allergy syndrome. Skin prick tests for apple, peach and hazelnut were positive in 51 (64.6 %), 61 (77.2 %), and 71 (89.9 %) patients, respectively. Granny Smith gave more positive skin reactions and a better agreement with clinical history than Golden Delicious, and juice was superior to the two other extraction procedures for both apple strains. RASTs for apple, peach and hazelnut were positive in 53 (68.8 %), 13 (16.9 %), and 31 (40.3 %) patients, respectively. Concordance between skin prick test and case history was found in 77 %, 52 %, and 54 %, for apple, peach and hazelnut, respectively.
Conclusions: We found a high percentage of concurrence of clinical allergy to birch pollen and apple, peach and hazelnut, confirmed by both skin prick testing and RAST. Approximately half of these patients have symptoms (especially oral allergy syndrome) after eating these products.
Also, we found an easy extraction procedure (juice extract) suitable for apple skin prick testing, even superior to freeze-dried extraction or the low-temperature acetone powder technique.

Introduction

Cross-reactivity between inhalant allergens and food allergens has been known for several years, for instance between: ragweed pollen and banana/melon (1); mugwort pollen and spices/celery/carrot (2-4); latex and kiwi/avocado/banana/chestnut (5); caddisfly and crab/shellfish (6); and the socalled bird-egg syndrome (7,8). The oral allergy syndrome after consumption of fruits of the Rosaceae family (e.g. apple, peach) or nuts in patients suffering from birch pollinosis was described as early as 1948 by Juhlin-Dannfeldt (9). Cross-reactive IgE, the cause of these observations, can be directed against the major birch pollen allergen, Bet v 1, also present in fruits and nuts, against profilin, an ubiquitous and highly conserved protein in pollen and vegetable foods and against carbohydrate epitopes on glycoproteins (10-12).
In this study we investigated first the prevalence of atopy for apple, peach and hazelnut in a large group of patients with tree pollinosis in the Netherlands. For this purpose consecutive patients with tree pollinosis were asked about symptoms after eating fruits and nuts; subsequently, food skin prick tests and RAST were performed. The second aim of our study was to compare three extraction procedures with two different apple strains in skin prick testing.

Materials and Methods

Human subjects
Patient group: from February till July 1995, 79 consecutive patients of the Department of Allergology (ErasmusMC, Rotterdam, the Netherlands), suffering from tree pollinosis were enrolled in the study. Patients had a positive case history, i.e. seasonal rhinoconjunctivitis, sometimes associated with bronchial asthma, confirmed by a clearly positive intracutaneous test with birch pollen and/or alder pollen. Of these 79 patients 59 were females and 20 males; their mean age was 33.4 years (range 15-53 years).
Control group: 8 non-atopic volunteers (7 females and 1 male; mean age 34.0 years; range 26-45 years) were skin tested to detect irritative, non-specific reactions. They had a negative history of birch pollinosis or food allergy, confirmed by negative skin test with tree pollen extract.

Allergens

Granny Smith and Golden Delicious apples, hazelnuts and peaches were purchased at a local food store. Apple extracts were prepared from small pieces of ripe apples with peel, but without core, by three different methods.

Extract 1 (**E1**): pieces of apple were homogenised in a food processor, the slurry was filtered and the fluid subsequently passed through a 0.22 µm Millex GS filter (Millipore, the Netherlands).

Extract 2 (**E2**): pieces of apple were lyophilized overnight at -70 °C. After mechanical homogenization, a 10 % (w/v) extract was prepared in phosphate-buffered saline, pH 7.4, containing 0.03 % human serum albumin and 0.5 % phenol. After centrifugation for 10 minutes at 2000 g, supernatant was passed through a 0.22 µm filter.

Extract 3 (**E3**): the modified low temperature acetone powder method, described by Vieths et al (13) was used. Apple was homogenized in acetone/dry ice at -60 °C and the slurry was equilibrated overnight at -25 °C. After removing of the acetone fraction, the precipitate was freeze-dried overnight at -70 °C and extracted in 0.01 M phospate-buffered saline, pH 7.4. After centrifugation (30 minutes, at 20.000 g, 0 °C) and dialysing against distilled water, the extract was lyophilized overnight at -70 °C. Subsequently, a 1 % extract (w/v) was prepared in distilled water. Peach extract was prepared from small pieces of ripe peaches without peel or core. The pulp was homogenized in a food processor, the slurry was filtered and the fluid subsequently passed through a 0.22 µm filter. Hazelnuts were homogenized mechanically, ground with a mortar, defatted by ether extraction and subsequently air-dried. An 10 % (w/v) extract in phosphate-buffered saline was centrifuged 10 minutes at 2000 g and supernatant was passed through a 0.22 µm filter. All the extracts were stored in appropriate aliquots at -20 °C until use in skin tests. Before use, extracts were defrosted 1 hour before the skin test and centrifuged for 45 seconds at 3200 g. Protein concentrations, determined by the method of Watanabe (14), using Pyrogallol Red Molybdate complex, are shown in table 1.

Table 1: Protein concentrations, determined by the method of Watanabe (14), in gram per Liter

Allergen	extract	[ ] in g/l
Golden Delicious	E1 (juice)	1.07
Golden Delicious	E2 (freeze-dried)	0.24
Golden Delicious	E3 (acetone-powder)	0.21
Granny Smith	E1 (juice)	0.83
Granny Smith	E2 (freeze-dried)	0.58
Granny Smith	E3 (acetone-powder)	0.12
hazelnut	10 % (w/v)	28.90
peach	juice	0.80

Skin test

Skin tests were performed intracutaneously with 0.02 ml of tenfold dilutions of birch pollen extract and alder pollen extract (ALK Benelux, Groningen, the Netherlands). Dilutions were made in phosphate buffer containing 0.3 % HSA and 0.5 % phenol. After 20 minutes wheal and flare reactions were measured using the grading system of standardized plus signs, devised by Norman (15). Skin prick tests (SPT) were performed with apple, peach and hazelnut 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 results were read after 20 minutes. Reactions were expressed in mm of mean wheal diameter (adding the longest diameter to the orthogonal diameter measured at one half of the longest diameter and dividing by two). Three mm or more was considered positive. Dilution buffer was used as negative control, histamine chloride 10 mg/ml as positive control. All skin tests were performed by the same skilled nurses.

RAST

For the routine RAST with apple, peach and hazelnut, the Pharmacia Cap RIA (with allergens f49 *Malus sylvestris*, f95 *Prunus persica*, and f17 *Corylus avellana*, respectively) was used according to the manufacturer's instructions.

Statistics
The results of different skin prick tests were analysed by calculating the Spearman's rank correlation coefficient (R) using the SPSS statistical package. A P value of 0.05 or less was considered statistically significant.

Results

Clinical history
Symptoms after eating fruits and nuts of all consecutive patients with tree pollinosis are shown in table 2. Eating apple and hazelnuts caused symptoms in 44 %, eating fresh peaches in 29 % of the patients. Most symptoms were typical of oral allergy syndrome.

Table 2: Case histories (number of patients with symptoms) in 79 consecutive patients with tree pollinosis

Symptom	apple	peach	hazelnut
oral allergy	24	18	27
urticaria/AO	11	6	6
dyspnoe/wheezing	5	3	7
G.I. tract	2	0	1
Eczema	3	0	1
Rhinitis	1	0	0
Total number of patients with signs	35	23	35

Table 3: Percentage of positive skin prick tests and RAST in 79 consecutive patients with tree pollinosis

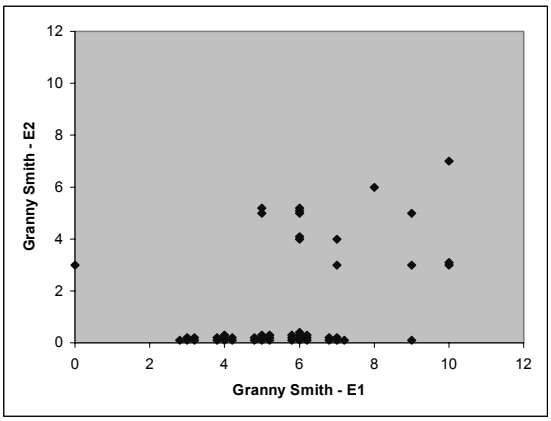
Allergen extract	SPT	RAST
Golden Delicious E1	58.2	-
Golden Delicious E2	21.5	-
Golden Delicious E3	53.2	-
Granny Smith E1	64.6	-
Granny Smith E2	20.3	-
Granny Smith E3	36.7	-
green apple	-	68.8
peach	77.2	16.9
hazelnut	89.9	40.3

Skin test and RAST
Comparisons between different apple extracts in skin prick tests are shown in figure 1A-F. Prick tests with juice extract of Granny Smith compared with peach and hazelnut are shown in figure 2A and B.

Percentages of positive skin prick tests and RAST are shown in Table 3. Juice extract of Granny Smith apple was positive in 65 % of the patients, comparable with the Pharmacia CAP-RAST. For the other apple extraction procedures, lower percentages of positive skin prick test were found. Prick test with hazelnut and peach extract was far more often positive than RAST. Concordance between clinical history, skin prick test and RAST is summarized in table 4. The best concordance between skin prick test and RAST or case history was achieved with juice extract of Granny Smith. For hazelnut and peach extract, we found a high percentage of positive skin prick tests, with a negative RAST and case history.

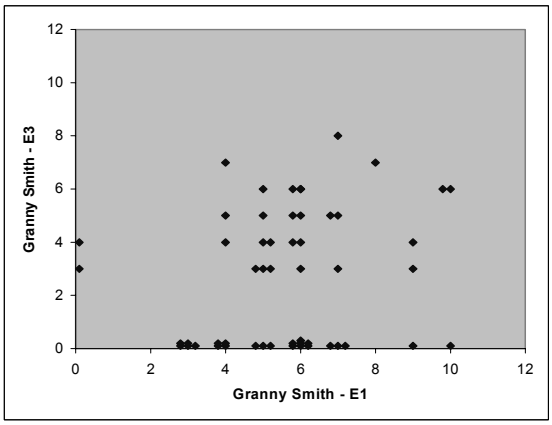
Figure 1 A-F. Comparison between different apple extracts in skin prick tests, expressed in mm of mean wheal diameter. E1 = juice extract; E2 = freeze-dried extract; E3 = low-temperature acetone powder extract.

Figure 1A.



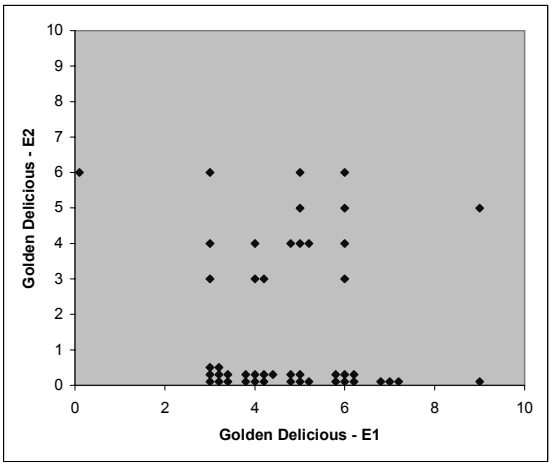
No. of patients with negative SPT results for both extracts: 27 R = 0.47, p < 0.0001

Figure 1B



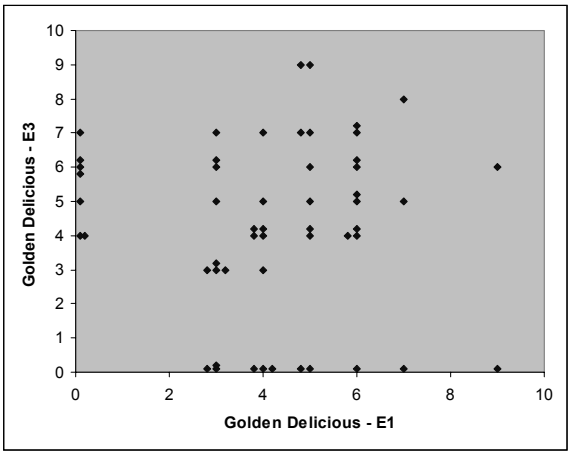
No. of patients with negative SPT results for both extracts: 26 R = 0.51, p < 0.0001

Figure 1C.



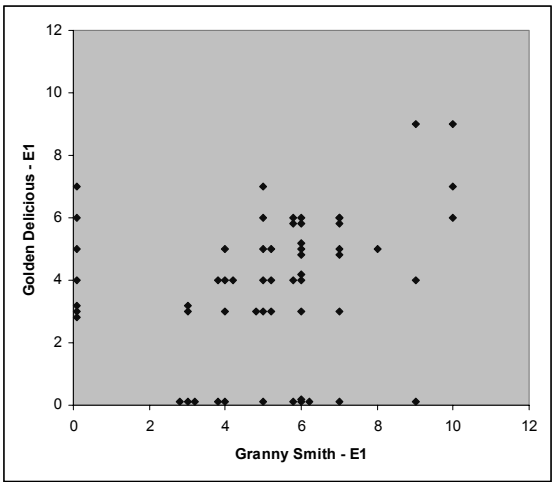
No. of patients with negative SPT results for both extracts: 32 R = 0.34, p < 0.02

Figure 1D.



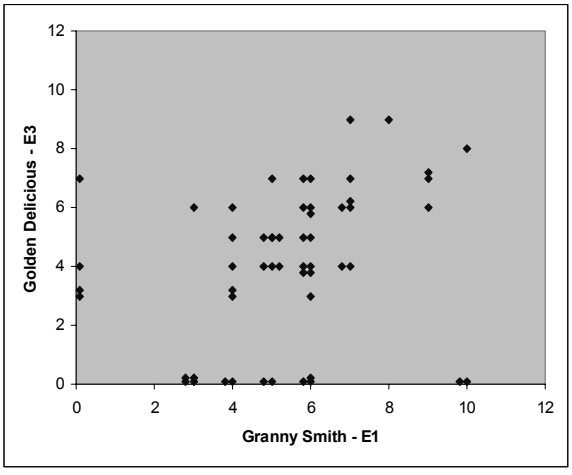
No. of patients with negative SPT results for both extracts: 26 R = 0.51, p < 0.0001

Figure 1 E.



No. of patients with negative SPT results for both extracts: 21 R = 0.56, p < 0.0001

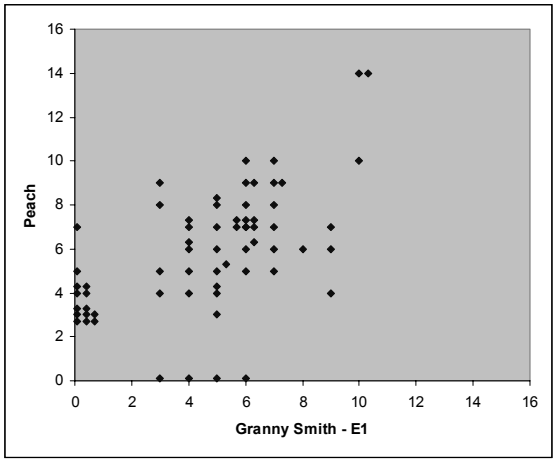
Figure 1F.



No. of patients with negative SPT results for both extracts: 24 R = 0.64, p < 0.0001

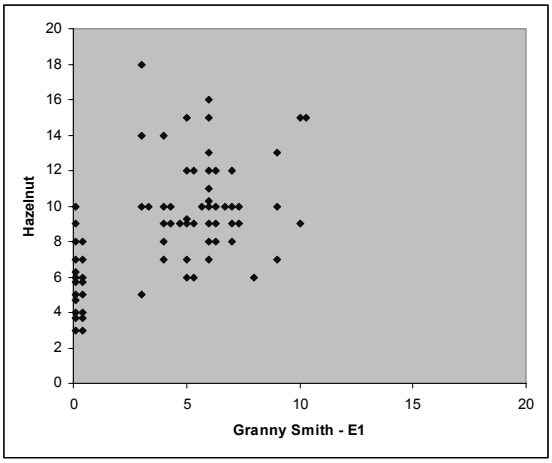
Figure 2. Comparison between Granny Smith juice extract with peach extract (**A**), and hazelnut extract (**B**) in skin prick tests, expressed in mm of mean wheal diameter.

Figure 2A.



No. of patients with negative SPT results for both extracts: 14 R = 0.72, p < 0.0001

Figure 2B



No. of patients with negative SPT results for both extracts: 8 R = 0.64, p < 0.0001

Table 4: Concordance (shown in %) between skin prick test, RAST and case history for apple, hazelnut and peach in 79 consecutive patients with tree pollinosis

	SPT	RAST	Allergen
RAST	73	-	GS - juice
CH	77	66	
RAST	62	-	GS - acetone
CH	70	66	
RAST	61	-	GD - juice
CH	66	66	
RAST	71	-	GD - acetone
CH	71	66	
RAST	48	-	Hazelnut
CH	54	71	
RAST	34	-	Peach
CH	52	71	

SPT: skin prick test; CH: case history; GS: Granny Smith; GD: Golden Delicious

Discussion

We investigated the prevalence of atopy for apple, peach and hazelnut in a large group of patients with tree pollinosis in the Netherlands. Case histories for apple, peach and hazelnut atopy were positive in 44.3 %, 29 %, and 44.3 % of the patients, respectively. More than two-thirds of the patients had symptoms characteristic of an oral allergy syndrome. Respiratory tract symtoms or eczema were not reported frequently. No case of anaphylaxis was mentioned. The prevalence of cross-sensitization between tree pollinosis and fruits and nuts has been studied previously (16-20). With regard to apple, peaches and hazelnut, positive case histories have been found in 47-78 %, 34-46 %, and 53-79 %, respectively, of tree pollen allergic patients. The differences between the frequencies can be explained by differences in study

population: children (16,20) as against young adults in our study, by the severity of tree pollinosis and the high level of exposure to tree pollen in Scandinavian countries (16,18-20), and finally by inclusion criteria, e.g. consecutive patients with tree pollinosis in our study as against patients for immunotherapy with tree pollen extract (20).

We found a much higher percentage of positive reactions for apple, peach and hazelnut in RAST and more pronounced reactions in skin prick tests than might be expected on the basis of the case histories. This discrepancy could not be explained by non-specific irritative reactions, as skin test results were negative in nonatopic controls. Mild symptoms after eating fruits or nuts may be ignored by the patient. A double-blind placebo-controlled oral provocation test as golden standard to test this hypothesis was not included in the routine investigations. Moreover, these challenge tests are not suitable as oral contact with a high concentration of fresh food allergens is strongly conductive to development of symptoms of oral allergy syndrome (21).

The positive skin prick tests may have been caused by a low titer and/or a lower affinity of cross-reactive IgE, originally directed against the Bet v I epitope of birch pollen, or by the presence of cross-reactive IgE directed against Bet v II (profilin) and/or carbohydrate epitopes on glycoproteins (10). These antibodies are demonstrated by skin prick test, but possibly have no clinical relevance in the majority of birch pollen allergic patients. In recent years new insights about the nature and biological function of the crossreactive allergens have become available. Ferreira mentioned 28 major groups of cross-reactive proteins from various sources (12). Allergens of six of these groups belong to some families of pathogenesis related (PR) proteins from plants. They are induced in response to infections by pathogens (fungi, bacteria and viruses), by wounding or other stress including drought, flooding, freezing temperature, ozone and ultraviolet B light (UV-B). With regard to the allergens involved in birch pollen allergic patients two groups can be mentioned.

Firstly the fagales pollen group – group 1 (PR-10, plant steroid hormone transported) e.g. Bet v, *Bettula verrucosa* (Birch) 1, Cor a, *Corylus avellana* (Hazel) 1, Aln g, *Alnus glutinosa* (Alder) 1. This major pollen allergen from Fagales trees exists as multiple isoforms showing a high degree of sequence similarity. Sensitisation to these isoallergens frequently leads to cross-reactions with homologues proteins in e.g. apple, cherry, hazelnut, and peach. The IgE cross-reactivity of Bet v 1 and its homologous proteins is one of the main causes of the so-called Pollen-Food-Syndrome for patients allergic to pollen of the Fagales trees order. These pollen allergic patients often display adverse reactions after ingestion of these fruits, nuts and even of soy, *Glycine max* (Gly m).

Secondly, the actin binding protein, profilin can cause cross-reactivity in birch pollen allergic patients. Profilin is now considered as a ubiquitous cross-reactive plant allergen and sensitised patients typically react to a broad range of pollen and food sources. To these pollen groups belong the allergenic molecules, e.g. Bet v 2, Cor a 2, Fra e, *Fraxinus exelsior* (Ash) 2. But also allergenic molecules from diverse grass pollen and weeds. This profilin is furthermore found in a large scale of fruits, vegetables, nuts, seeds and latex. Despite this extensive cross-reactivity among plant profilin and to the human homologue as well, it seems that a large proportion of IgE reactivities to profilin is clinically irrelevant. Recently, Mellon et al. found that the destruction of the IgE-binding epitopes of profilin by chimase might thus hinder further mast cell activation and limit the allergic responses to profilin in sensitised individuals (22).Wensing et al. concluded that IgE antibodies against Bet v 1 have a more limited spectrum of cross-reactivity than those against profilin, but they frequently give rise to clinically relevant cross-reactivities to food (11). Furthermore, profilin was associated with a higher number of positive RAST result to plant derived foods than Bet v 1. In contrast, Bet v 1 was associated with more positive skin prick test responses and more food-related symptoms. Sensitisation to Bet v 1 was associated with IgE against apple, hazelnut and peach.

The second aim of our study was to compare three extraction procedures with two different apple strains in skin prick testing. The preparation of stable and reproducible allergen extracts from apple fruit tissue is difficult. Enzymatically oxidized plant phenols interact with the apple allergen, leading to rapid denaturation of the proteins. The reliability of skin prick tests with commercial preparations is consequently very poor (16,23-27). Using fresh fruit for skin testing is better, because deterioration of allergenic material is negligible (24). Dreborg investigated the different skin test techniques using fresh fruit (16). A simple prick test technique with a lancet piercing an apple peel just before pricking the skin was shown to be the most practical and reproducible method (prick-prick). However, using fresh fruit is difficult in daily practice, furthermore the allergen potency is not constant in different apple strains and

maturation stages (27). There are several methods to overcome the loss of allergen activity due to phenol contamination during the extraction procedure. First, by shortening the extraction procedure and subsequent lyophilization of the apple extract (23). Second, by adding enzyme inhibitors to the extraction medium, as described by Björkstén (28). Preparing extracts in the presence of polyvinylpolypyrrolidone and chelators resulted in allergen extracts that remained stable for at least 1 month when stored at -20 ^{o}C. Third, by using the low-temperature acetone powder technique as investigated extensively by Vieths, interaction of plant phenols with proteins can be avoided without any addition of potentially irritant chemicals (13). Recently, Rudenschko et al reported a fourth and technically easier extraction procedure for apple (29). Extracts prepared by precipitation in organic solvents at -20 ^{o}C retained a high allergen activity and were stable, especially when stored at -20 ^{o}C in lyophilized state (30).
In this study, we compared lyophilization of the apple extract and the low-temperature acetone powder technique with a simple technique, i.e. preparing juice from fresh apple in a rapid procedure including storage immediately afterwards at -20 ^{o}C. In this way we tried to mimick the prick-prick test with fresh fruit, as propagated by Dreborg (16). The juice extract gave better results in terms of positive skin reactions and concordance between skin prick tests and case history or RAST compared with the low-temperature acetone powder extract, especially for the Granny Smith apple. In addition, juice extract of the Granny Smith apple elicited larger skin reactions compared with juice extract of Golden Delicious apple (figure 1E), suggesting that the former extract is more potent. We therefore decided to use juice extract of Granny Smith in our routine food allergy testing. The quality of an allergenic extract, however, also depends on stability. The stability of this extract will be studied in the future. The comparisons between different extraction procedures and apple strains yielded low to moderate correlation coefficients ranging from 0.34 - 0.64. This emphasizes the considerable influence of extraction procedures and choice of apple strains in diagnosing allergic patients with an allergy to apple.
In conclusion, a high percentage of cross-sensitization was found between birch pollen and apple, peach and hazelnut, both in skin prick test and RAST. Approximately 50 % of patients allergic to birch pollen have symptoms (especially OAS) after eating these products. In addition, we found an easy extraction procedure (juice extract) suitable for apple skin prick testing, even superior to freeze-dried extraction or the low-temperature acetone powder technique.

Acknowledgements

We gratefully acknowledge the contribution of A.W. van Toorenenbergen (PhD, Laboratory of Allergology, Rotterdam, the Netherlands) who critically read the manuscript.

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Part five

Cross-reactivity

Chapter 7

Immunoblot and RAST-inhibition studies of allergenic cross-reactivity of the predatory mite Amblyseius cucumeris with the house dust mite Dermatophagoides pteronyssinus.

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Ann. Allergy Asthma Immunol. 2004;93:281-7

Abstract

Background: In 1999, an extensive study among bell pepper growers showed that a predatory mite, *Amblyseius cucumeris*, is a potentially relevant source of occupational allergens, since 23 % of the population was SPT-positive. We want to investigate whether cross-reactivity between *Amblyseius cucumeris* (Ac) and *Dermatophagoides pteronyssinus* (Dp) is responsible for co-sensitisation to both mite species found in 58.7 % of the *A. cucumeris*-sensitised greenhouse workers.
Methods: Fifteen sera from greenhouse workers with work-related inhalant allergy and a positive RAST to *A. cucumeris* and/or *D. pteronyssinus* were selected for immunoblot analysis with extracts of both mites. A subselection (n=5) was used for RAST- and immunoblot-inhibition to investigate potential cross-reactivity.
Results: On immunoblot two distinct patterns were observed: one pattern with common protein bands in *A. cucumeris* blot and *D. pteronyssinus* blot suggestive of cross-reactivity between *A. cucumeris* and *D. pteronyssinus*, and one pattern showing no shared protein bands.
D. pteronyssinus-RAST inhibition with *A. cucumeris*-extract was low in 4 sera (< 25 % inhibition) and nearly absent in 1 serum. *A. cucumeris*-RAST inhibition with *D. pteronyssinus*-extract was high in 1 serum (75 % inhibition), low in 2 sera (35 % and < 15% inhibition) and absent in 2 sera. These results were confirmed by immunoblot inhibition experiments.
Conclusions: *Amblyseius cucumeris,* a new occupational allergen, has species specific antigens as well as common antigens cross-reactive with the house dust mite, *Dermatophagoides pteronyssinus.*

Introduction

Allergens from mites are well known sensitisers in allergic diseases. One of the most important allergenic mite species is the house dust mite, *Dermatophagoides pteronyssinus*. Moreover, other mite genera, such as spider mites [1-4] and storage mites [5-9] have been shown to be important inducers of allergic reactions as well. In 1999, an extensive study in bell pepper workers showed that predatory mites can also cause allergic disorders [10]. The predatory mite *Amblyseius cucumeris* is used as protection of crops against thrips in this branch of horticulture [11,12]. Thrips are one of the major pests in greenhouses and commonly belong to the species Frankliniella occidentalis and Echinothrips americanus. Numerous studies have shown cross-reactivity between different mites of closely related taxonomy [5-9]. Although *A. cucumeris* is only distantly related (table 1) to *D. pteronyssinus*, still there was a high percentage (58.7 %) of co-sensitisation to *D. pteronyssinus* in these *A. cucumeris*-sensitised greenhouse workers [10]. The aim of this study was to evaluate the allergenic cross-reactivity between *A. cucumeris* and *D. pteronyssinus*. Cross-reactivity patterns can help elucidating to what extent occupational exposure to *A. cucumeris* is an independent risk factor for the development of allergic disorders.
Because the greenhouse workers in the Netherlands are mainly exposed to the predatory mites as the new biological control agent, we focussed primarly on this mite in this study. Future experiments are planned regarding the *Tyrophagus putrescentiae* sensitisation, also found in a high number of greenhouse workers.

Table 1. Taxonomic position of the mites

	Class	Subclass	Suborder	Family	Genus	Species
					Acarus	A. siro
				Acaridae	Aleuroglyphus	
	Insects				Tyrophagus	T.putrescentiae
		Scorpione			Lepidoglyphus	
Arthropods				Glycyphagidae	Glycyphagus	
		Araneae				
				Chortoglyphidae	Chortoglyphus	
	Arachnida					
			Astigmata		Dermatophagoides	**D.pteronyssinus**
		Acari		Pyroglyphidae		
		(mites and Ticks)			Euroglyphus	
			Prostigmata	Tetranychidae	Tetranychus	T. urticae
					Panonychus	P. ulmi
						P. citri
			Mesostigmata	Phytoseiidae	Neoseiulus	**A.Cucumeris**

Material and Methods

Patients

For this study, sera were selected from a cohort of greenhouse workers who had previously participated in a study in the bell pepper horticulture [10]. This investigation included 472 employees, of which 109 were sensitised to *A. cucumeris* as demonstrated in skin prick test. Sixty-three of these workers had a positive RAST to *A. cucumeris* (concordance of 58 %). Sera of 15 of these *A. cucumeris*-sensitised patients (mean age 38.6 years, range 25-62 years) were selected on the basis of a positive SPT (> 3 mm diameter), positive RAST (≥ class 1), positive case history to *A. cucumeris* and enough material for further immunoblot and IgE-inhibition studies (Table 2).

Skin prick tests

Skin prick tests (SPT) were done in employees with work-related symptoms. The symptoms were defined as work-related when the employees noticed a substantial improvement or complete regression of their complaints during weekends and holidays. SPT was 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 standardised ALK prick test lancet (ALK, Horsholm, Denmark), and results were read after 20 minutes. Reactions were expressed in mm of mean wheal diameter (adding the longest diameter to the orthogonal diameter measured at one half of the longest diameter and dividing it by two). A diameter of 3 mm or more was considered positive [5]. Dilution buffer was used as negative control, histamine chloride 10 mg/ml as positive control. The same investigator performed all skin tests.

Table 2. Characteristics of the patients, results of the SPT with inhalant allergens and serum IgE values to *Amblyseius cucumeris (Ac)* and *Dermatophagoides pteronyssinus (Dp)*.

					Mites					Pollen			Animals	
					Ac		Dp		Tp	BBP	TP	GP	Cat	Dog
					SPT	RAST	SPT	RAST	SPT	SPT	SPT	SPT	SPT	SPT
Serum No.	Age	M/F	UAS	LAS	mm		kU/L	mm	kU/L	mm	mm	mm	mm	mm
1	62	M	+	+	10	1.6	12	13.8	0	0	0	0	0	4
2	44	M	+	-	11	4.71	4	<0.35	5	11	0	0	0	0
3	31	M	+	+	6	4.82	4	2.45	5	6	4	4	0	4
4	47	M	+	-	10	3.89	8	29.5	5	9	0	0	4	6
5	25	M	+	-	18	14	7	63.6	4	7	6	10	10	8
6	28	F	+	+	5	10.5	0	<0.35	0	14	5	0	0	0
7	37	M	+	+	6	10.9	8	79.9	0	11	5	10	7	4
8	41	M	+	-	8	46.2	6	18	0	8	5	7	0	0
9	26	M	+	-	9	8.04	9	7.12	0	5	8	10	0	4
10	60	M	+	-	8	2.25	0	2.42	0	6	5	4	6	0
11	34	M	+	-	10	28.3	0	<0.35	4	0	0	0	0	0
12	28	M	+	+	11	10.2	5	2.26	3	0	0	0	0	0
13	29	F	+	-	7	4.79	0	0.51	0	0	0	0	0	0
14	47	F	+	+	4	22.9	4	>100	0	8	0	8	0	4
15	40	M	+	+	8	30.7	4	93	5	3	0	0	9	0

UAS = work related upper airway symptoms
LAS = work-related lower airway symptoms
M = Male
F = Female
BBP = bell pepper pollen
Ac = *Amblyseius cucumeris*
Dp = *Dermatophagoides pteronyssinus*
Tp = *Tyrophagus putrescentiae*
TP = tree pollen
GP = grass pollen

Allergens for SPT
For SPT living predatory mites (Amblyseius cucumeris) were kindly supplied by Koppert Biological Systems (Berkel en Rodenrijs, the Netherlands). A 10% (w/v) extract was prepared in phosphate buffered saline pH 7.4, containing 0.03% human serum albumin and 0.5% phenol at 4˚C.
Pollen from flowers of the bell pepper plants were collected in a greenhouse. The flowers were in full bloom. A 25% (w/v) extract was prepared in PBS-AT (0.82 % NaCl, 10mM Phosphate, 0.1 % NaN3, 0.2% Tween, 0.3% bovine serum albumin, pH 7.4). The extracts were centrifuged for 10 minutes at 2000g, and supernatants were passed through a 0.22 µm Millex GS filter (Millipore, the Netherlands). All extracts were stored in appropriate aliquots at -20˚C until use in skin tests. Before use, extracts were defrosted for 1 hour before skin test and mixed. In addition, skin prick tests were performed with *T. putrescentiae* (SQ 505), and 5 common inhalant allergens from ALK Abelló (Nieuwegein, the Netherlands): Dermatophagoides pteronyssinus (SQ 503), tree mix (SQ 108), grass mix (SQ 293), dog dander (SQ 553) and cat dander (SQ 555).

Allergens for RAST, RAST inhibition and immunoblotting
The Research Station for Floriculture and Glasshouse Vegetables (Naaldwijk, the Netherlands), kindly supplied the *A. cucumeris* mites. A cultivating process to obtain a minimum of contamination with other

mites was applied for this study. Usually the mites are cultivated on other mites. For this project the *A. cucumeris* mites were cultivated on a selection of flower pollen. *D. pteronyssinus* mite bodies were obtained from Commonwealth Serum Laboratories (Melbourne, Australia).
The protein concentration was analysed with the benzethonium chloride method [13] on the Hitachi 911 routine clinical chemistry analyser, according to manufacturers instructions (Boehringer/Roche, Almere, the Netherlands), in the *D. pteronyssinus* and *A. cucumeris* supernatant. After extraction 20 mg/ml in PBS-T (0.82 % NaCl, 10mM Phosphate, 0.1 % NaN3, 0.2% Tween, pH 7.4), for 1 hour at room temperature, the protein concentration was 1.34 and 0.250 mg/ml respectively.

IgE antibody measurements
IgE antibodies to *A. cucumeris* and *D. pteronyssinus* were measured by RAST, as described by Adkinson et al [14]. Briefly, an amount of 10 mg of *A. cucumeris* and *D. pteronyssinus* bodies was extracted with 2 ml coupling buffer (0.1 mol/L NaHCO3 and 0.5 mol/L NaCL, pH 8.5) for 1 h at 4 °C. After centrifugation for 10 min at 1400 g, protein in the supernatant was covalently coupled to 100 mg of CNBr-activated Sepharose 4B (Sigma chemical Co. St.Louis, USA), according to the manufacturer's instructions. Sepharose (4 mg/test)-coupled *A. cucumeris* and *D. pteronyssinus* was incubated overnight with 50 µl serum in a final volume of 550 µl PBS-AT. After four washes with PBS-T, radioiodinated rabbit anti-human IgE antibodies (Pharmacia, Uppsala, Sweden) were added. After overnight incubation and four washes, the percentage of bound radioactivity was measured and expressed in kU/L when read from the Pharmacia CAP standard curve obtained simultaneously, using the same batch of radiolabeled anti-IgE antibodies.

IgE inhibition experiments
For IgE inhibition experiments, 10 mg *A. cucumeris* and *D. pteronyssinus* were extracted in 1 ml PBS-AT under rotation for 1 hour at room temperature. After centrifugation, the supernatant was vortexed and 50 µl supernatant was incubated overnight at 4 ^{o}C with 50 µl of diluted sera. Dilutions of the sera in PBS-AT were made to obtain a concentration of specific IgE of approximately 5 – 10 kU/l. For *D.pteronyssinus* inhibition the sera were diluted as follows: serum no. 4 four times, serum no. 7 twenty times, serum no. 8 two times and serum no. 15 ten times. For *A.cucumeris* inhibition, serum nos. 4,7 and 9 were incubated undiluted, serum no. 8 seven times and serum no. 15 four times diluted. All experiments were done with the same freshly made extract. 50 µl of the mixture was incubated with Sepharose-coupled *A. cucumeris* and *D. pteronyssinus*. Sera were also incubated with PBS-AT and grass pollen extract (Dactylus glomerata, ARTU Biologicals BV, Lelystad, the Netherlands,10 mg/ml RAST-AT buffer) as control extract.

Immunoblotting and immunoblot-inhibition experiments
Mite extracts (20 µg/cm) were separated by SDS-PAGE according to Laemmli's method [15]. Gradient gels (10-20% acrylamide) were prepared using SDS-Polyacrylamide Gel System protocol from GIBCO BRL (Research Products Life Technologies, Inc.,Gaithersburg, MD, USA) and silver stained using the protein silver staining procedure as described by the instruction manual 80-1310-00 (Pharmacia LKB Biotechnology, Uppsala, Sweden).
Western blotting was performed by transferring the proteins after separation (semi-dry) to nitrocellulose (PROTRAN, Nitrocellulose Transfer Membrane; pore size 0.2 µm, Schleicher & Schuell, Dassel, Germany)on a Novablot electrophoretic transfer apparatus, according to the protocol of the manufacturer (Invitrogen). After blocking with PBS-A (phosphate buffered saline, 10 mM EDTA, 0.3% BSA) for a minimum 10 minutes, the blots were cut into 3 mm wide strips. For inhibition, serum was allowed a 2 hours pre-incubation with 150 µl of 20 mg/ml mite extracts. Incubation with 150 µl PBS-AT served as control. Immunoprobing of the blotstrips took place overnight with 150 µl serum (+/- inhibitor) in 3 ml of PBS-AT. After washing extensively away unbound serum with PBS-T, strips were air-dried. Radiolabeled sheep antibodies against human IgE (Sanquin, Amsterdam, The Netherlands) were used for detection of bound IgE. Blots were exposed to X-ray film (Eastman Kodak Company, Rochester, NY, USA) 14 days at –70 ^{o}C, with the exception of the strip incubated with serum no. 15 (1 night), because of the high IgE level.

Figure 1.

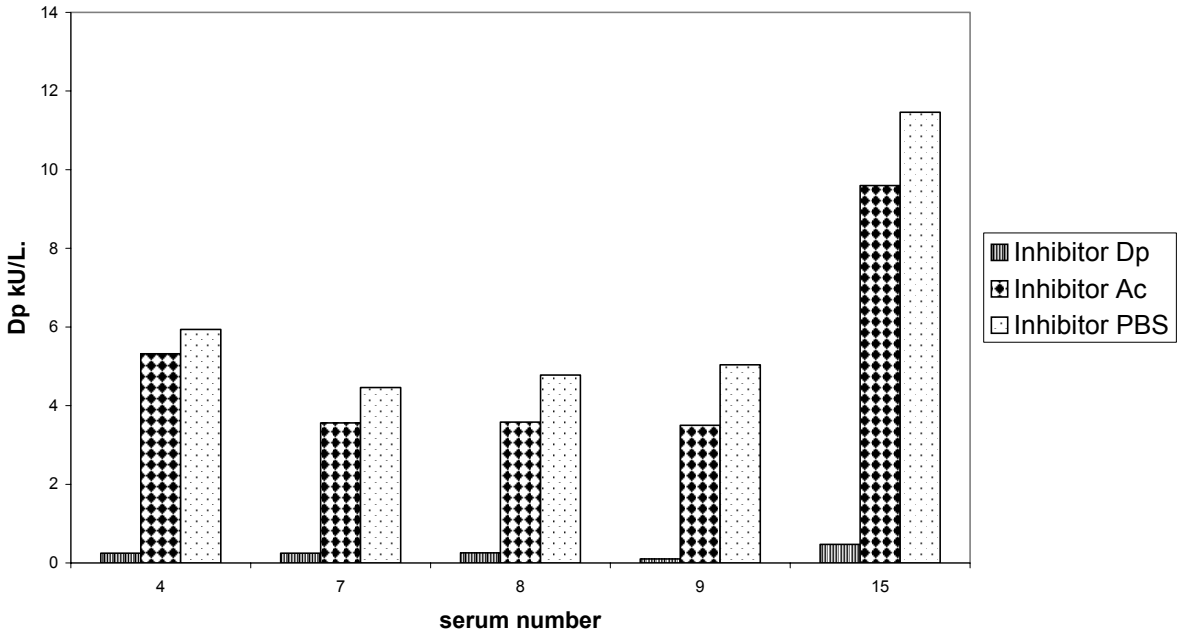

Figure 2.

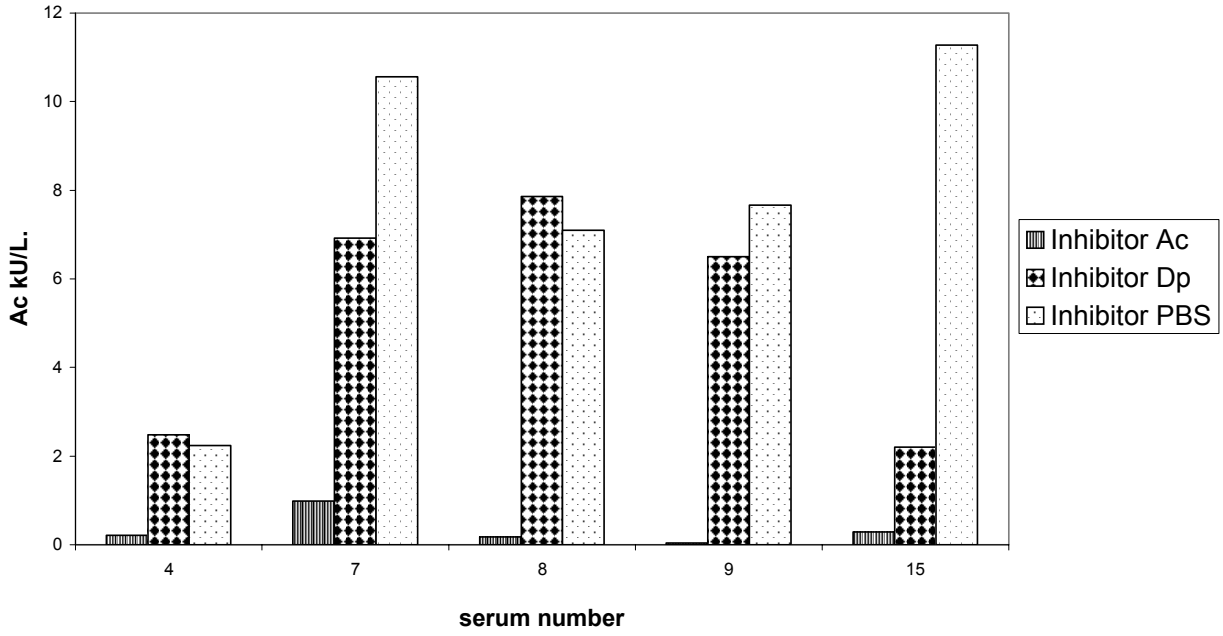

Figure 1.
IgE-inhibition of *D.pteronyssinus* antibodies with *D.pteronyssinus, A.cucumeris,* and buffer. Data are expressed in kU/L.
Figure 2.
IgE-inhibition of *A.cucumeris* antibodies with *A.cucumeris, D.pteronyssinus* and buffer. Data are expressed in kU/L.

Results

IgE antibody measurements

Of the 15 selected sera with IgE antibodies against *A. cucumeris*, 12 had IgE antibodies against *D. pteronyssinus* (table 2). Half of these sera demonstrated a stronger IgE recognition of *D. pteronyssinus* than *A. cucumeris* (greater by a factor 2 and up to almost a factor 10). Three sera had similar IgE response to both mites (within a factor 2). For the remaining six sera (including 3 *D. pteronyssinus*-negative), IgE responses to *A. cucumeris* were stronger than to *D. pteronyssinus* (greater by a factor 2 and up to at least a factor 25).

Inhibition of IgE binding in RAST

For RAST-inhibition, sera were selected from each of the three categories: *D. pteronyssinus* > *A. cucumeris* (serum 4, 7 and 15), *A. cucumeris* ~ *D. pteronyssinus* (serum 9) and *A. cucumeris* > *D. pteronyssinus* (serum 8). *D. pteronyssinus*-RAST inhibition with *A. cucumeris*-extract (Figure 1) was ≤ 25% in 4 sera (serum 7, 8, 9 and 15) and nearly absent in 1 serum (serum 4). *A. cucumeris*-RAST inhibition with *D. pteronyssinus*-extract (Figure 2) was about 75% in case of serum 15, 35 % for serum 7, and < 15 % for serum 9. For serum 4 and 8 no inhibition of the *A. cucumeris*-RAST with *D. pteronyssinus* extract was observed. The homologue RAST-inhibition was effective in all cases (> 85%). The results of the RAST's with inhibitor PBS were comparable with the uninhibited RAST results in table 2. No inhibition of IgE- binding to *A. cucumeris* and *D. pteronyssinus* was seen after preincubation of the diluted sera with grass pollen extract (data not shown).

Blotting Experiments

All fifteen sera were tested on *A. cucumeris* and *D. pteronyssinus* immunoblots (Figure 3,4). No obvious similarities in recognition profiles between *D. pteronyssinus* and *A. cucumeris* were observed. Sera from the *D. pteronyssinus* > *A. cucumeris* group all showed a similar pattern on *D. pteronyssinus* blot with prominent bands at the major allergens Der p 2/5 (~14 kDa) and Der p 1/7 (~25 kDa) [16]. In contrast, on *A. cucumeris* blots most sera recognised several high-molecular weight bands (between 50 and 100 kDa). In addition, the majority of the sera also recognised a band of approximately 7 kDa. In the range of 10 to 50 kDa, recognition of *A. cucumeris* was highly variable.

To identify allergens involved in the possible cross-reactivity between *A. cucumeris* and *D. pteronyssinus,* blotinhibition experiments were performed with 4/5 serum samples (7, 8, 9 and 15), also used in RAST-inhibition experiments (Figure 5).

In accordance with the partial cross-reactivity observed by RAST-inhibition, *D. pteronyssinus* extract clearly inhibited IgE-binding of serum 7 (*D. pteronyssinus*>*A. cucumeris*) to several allergens of *A. cucumeris*. *A. cucumeris* did not inhibit binding of IgE to *D. pteronyssinus* blot. Serum 8 (*A. cucumeris*>*D. pteronyssinus*) showed no cross-inhibition on immunoblot as was the case by RAST-inhibition. For serum 9 (*A. cucumeris*~*D. pteronyssinus*) almost complete inhibition by *D. pteronyssinus* of low-molecular-weight bands on *A. cucumeris*-blot (5-14 kDa) was observed. Prominent IgE-binding around 25 kDa and between 50 and 100 kDa was unaffected. This partial inhibition was in line with the results obtained by RAST-inhibition (~15%). *A. cucumeris* did not affect binding to *D. pteronyssinus*. Finally, serum 15 (*D. pteronyssinus*>*A. cucumeris*) demonstrated complete inhibition by *D. pteronyssinus* of binding to *A. cucumeris* blot, which was even more efficient than the homologous inhibition. As expected, *A. cucumeris* did not affect binding on *D. pteronyssinus* blot.

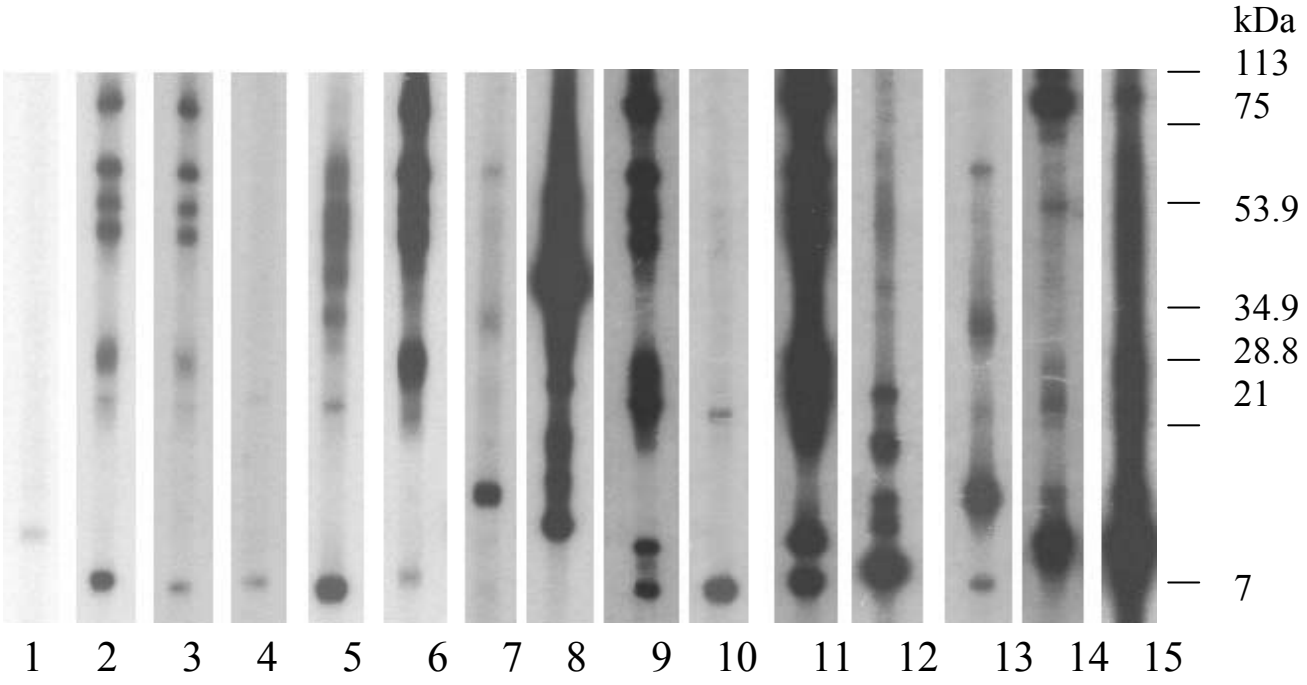

Figure 3.
Western immunoblotting using 15 sera (# 1-15) on Ac-mite extracts. Binding was visualized with radiolabeled sheep antibodies against human IgE.

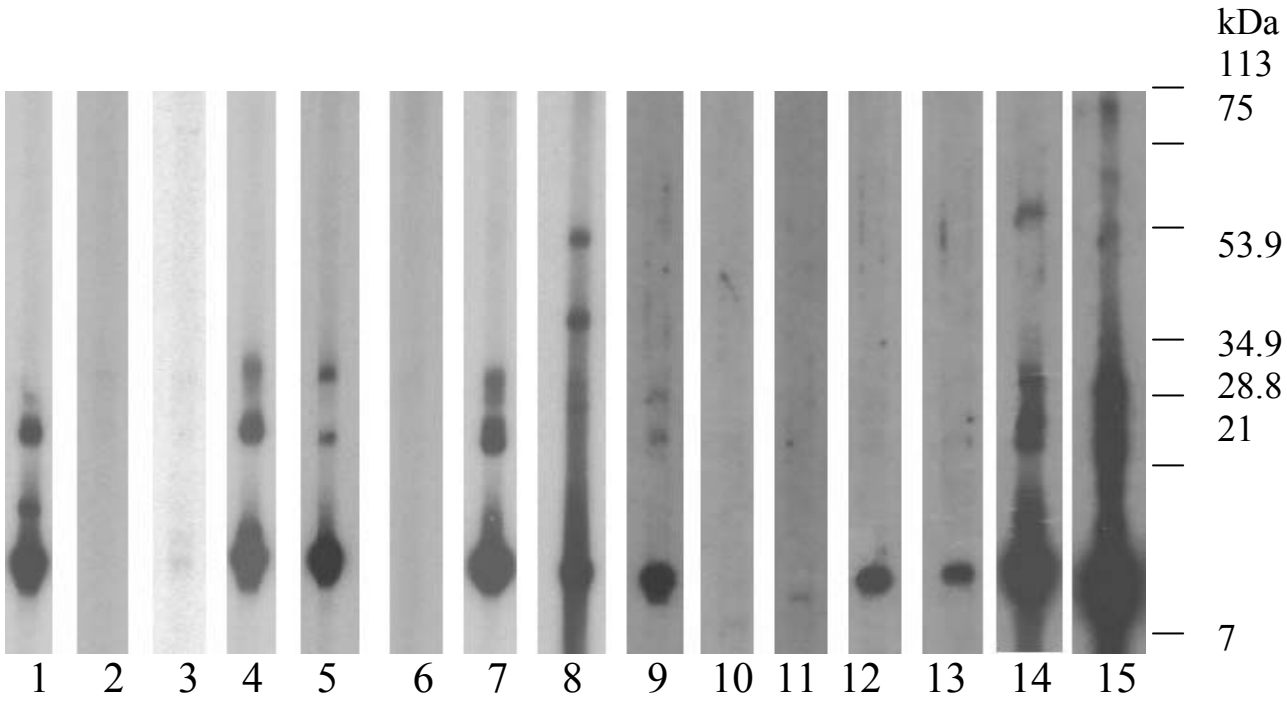

Figure 4.
Western immunoblotting using 15 sera on Dp-mite extracts. Binding was visualized with radiolabeled sheep antibodies against human IgE.

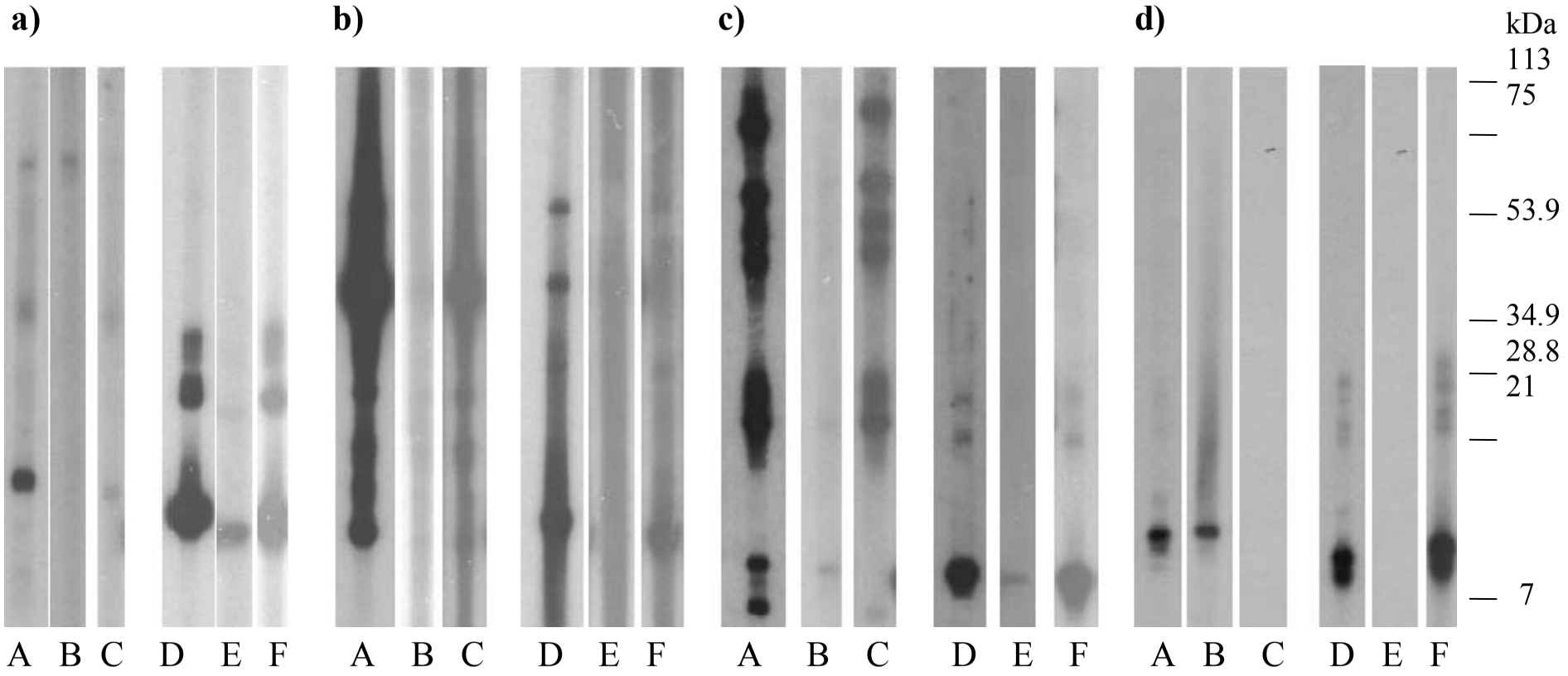

Figure 5.
Blotinhibition of IgE binding to *A.cucumeris (*Ac) and *D.pteronyssinus* (Dp). Serum # 7 was used in (**a**), serum # 8 in (**b**), serum # 9 in (**c**) and serum # 15 in (**d**). Lane A: Ac blot without inhibition, lane B: Ac blot after homologues inhibition, lane C: Ac blot after inhibition with Dp, lane D: Dp blot without inhibition, lane E: Dp blot after homologues inhibition, lane F: Dp blot after inhibition with

Discussion

In a recent study performed by our clinic, 109 out of 472 (23%) employees in bell pepper greenhouses were shown to be skin prick test positive to the predatory mite *A. cucumeris* [10]. The predatory mite *A. cucumeris* is a relatively new allergen in horticulture. Until now only one other study, by Van Hage-Hamsten *et al*.[6], described IgE-mediated sensitisation to these mites among green house workers. They reported four patients with IgE-binding to *A. cucumeris* extract.

In the bell pepper study 81 (74.3%) of the 109 employees sensitised to the *A. cucumeris* mite were also sensitised to one or more common inhalant allergens [17]. The sensitisation rate to *A. cucumeris* was significantly higher in atopic employees than in non-atopic employees, illustrating a clear association between sensitisation to *A. cucumeris* and IgE mediated allergy to inhalant allergens (Prevalence Rate Ratio 4.82; 95% confidence interval (CI) 3.27 - 7.10). Of all common inhalant allergens sensitisation to the house dust mite and grass pollen were most prevalent in our study population as well as in the subgroup of *A. cucumeris* sensitised employees. The association between sensitisation to house dust mite and sensitisation to *A. cucumeris* (PRR 4.08; CI 2.96 - 5.62) was stronger than the association between grass pollen and *A. cucumeris* (PRR 2.56;CI 1.87 - 3.50).

Earlier studies have shown cross-reactivity of *D. pteronyssinus* and storage mites like *Tyrophagus putrescentiae*, *Acarus siro*, *Lepidoglyphus destructor* [5-8]. These mite species are, however near relatives to *D. pteronyssinus* compared to the predatory mites in our study (Table 1). *D. pteronyssinus* belongs to the suborder *Astigmata,* whereas the *A. cucumeris* belongs to the suborder *Mesostigmata.* Comparative studies between mites of different families are rare. Kim *et al.* [1] studied cross-reactivity between spider mites (*Prostigmata*) and house dust mites (*Astigmata*). Kim suggested that common allergens exist in these distantly related mite species. In this study, we demonstrated that *A. cucumeris*-sensitisation is largely *D. pteronyssinus*-independent. Moreover, three *A. cucumeris*-positive sera were negative for *D. pteronyssinus*. Cross-reactivity between both mites exists in some co-sensitised patients, but is of secondary importance. Only for one patient inhibition studies revealed that most IgE against *A. cucumeris* was cross-reactive to *D. pteronyssinus* (serum 15). The nature of the specific and cross-reactive allergens of *A. cucumeris* was not revealed in this study. Certainly, the recognition profile on *A. cucumeris*-immunoblot was completely different from that observed for *D. pteronyssinus*. In general, the recognition of *D. pteronyssinus* allergens was normal, i.e. recognition of allergens around 14 kDa (Der p 2 and possibly Der p 5) and around 25 kDa (Der p 1, 7 and the serine-proteases Der p 3, 6 and 9). In contrast, several *A. cucumeris* allergens appeared to be of higher molecular weight (> 50 kDa), although also allergens between 5 and 50 kDa were recognised. There was one patient (8) with strong binding to a 36-kDa allergen, which could point towards tropomyosin. This muscle protein has been considered an important cross-sensitising invertebrate panallergen [18]. This allergen has been found in several organisms, among them the house dust mites *D. pteronyssinus* (Der p 10) and *D. farinae* (Der f 10). A RAST for tropomyosin did not confirm the identity of this allergen as tropomyosin (not shown). Immunoblot-inhibition showed that cross-reactive structures are mainly found among the low-molecular weight allergens (serum 7, 9 and 15). The nature of these allergens with molecular weights between 7 and 12 kDa remains to be established.

In summary, *Amblyseius cucumeris* is a new occupational allergen, responsible for work-related complaints in greenhouse workers. Sensitisation to this mite is not simply a cross-reactive phenomenon related to house dust mites. The results of this study may have consequences for the extensive use of biological control in horticulture. 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.

Acknowledgement

We thank Pierre Ramakers, entomologist at the Research Station for Floriculture and Glasshouse Vegetables (Naaldwijk, the Netherlands) for kindly supplying *Amblyseius cucumeris*.

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Part six

intervention

Chapter 8

Honeybees as an aid in reducing occupational pollen allergy in sweet bell pepper (*Capsicum annuum*) greenhouses

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Submitted

Abstract

Background. In 1999, an extensive study among bell pepper growers showed that 35% was sensitised to bell pepper pollen. Since a few years bee researchers experiments with bees to discard pollen from bell pepper flowers.
Methods. To investigate whether bees can reduce the pollen exposure in bell pepper greenhouses and whether this reduction results in a decrease of allergic complaints in the greenhouse workers, 18 greenhouses were selected for the study. The investigators paid three visits to each greenhouse, complaints during work were asked for, skin prick tests were performed, nasal Visual Analogue Scales were obtained and spirometry was done. In each greenhouse, pollen were measured. In 6 and 3 of the greenhouses high (2 colonies of 10.000 bees per 10.000 m2, respectively low numbers of honeybees (1 colony per 10.000 m2) were placed throughout the pollen season of the sweet bell pepper plant. Nine greenhouses were kept without honeybees.
Results. 44 of the 133 employees invited had work related symptoms. Bees reduced pollen amount in a dose-dependent way. Also, a significant trend relationship between Visual Analogue Scale in nasal symptoms and the number of colonies of bees was seen. Although the changes in lung function corresponded with the ordering of numbers of bees, statistical significance was not reached.
Conclusions. The interference of bees in bell pepper greenhouses significantly reduces the pollen amount. Reduction of pollen amount decreases the work-related rhinitis symptoms in allergic greenhouse workers. This intervention study demonstrated very clearly that allergic work-related complaints of green house workers, sensitised to bell pepper pollen, are caused by occupational exposure to these pollen in the greenhouse.

Introduction

Occupational respiratory complaints are well recognised problems (1-6). In the Surveillance of Work-related and Occupational Respiratory Disease (SWORD) project (1,2) the causal agents were classified in 44 categories and a third of the suspected causes of asthma were organic. Studies addressing risk factors for occupational allergy among green house workers are hardly found in literature [3-5].
In 1999, an extensive study in bell pepper culture in the Netherlands among 472 employees showed that 53.8% had work-related respiratory symptoms. Sensitisation to the bell pepper pollen plant was found in 35.4%. Association between positive skin prick test reactions with the pollen and complaints appeared to be 90% [7].
Inhalation of the pollen during work is likely because the workers stand very close with their face towards the plants. Contact with the pollen is hardly avoidable and personal protection is proved to be inconvenient and impractical. One solution to protect the employees from sensitisation is to reduce the pollen output from the bell pepper plant. Honeybees as well as bumblebees can be used to pollinate flowers [8]. However, application is not common practice, since the bell pepper plant is a rather good self-pollinator under normal conditions. Furthermore, it is unknown how effective the use of bees is, in reducing respiratory complaints. Since a few years the Applied Plant Research, unit Bee Research experiments with bees to discard the pollen from the flowers. When honeybees will be helpful to reduce the pollen load in the greenhouse air, it would lead to a reduction of allergy symptoms.
The aim of this prospective intervention study was to investigate whether bees can reduce the amount of pollen in sweet bell pepper greenhouses. Secondly, whether this reduction has a notable effect on the work-related complaints caused by bell pepper pollen in allergic employees.

Material and methods

Bell pepper greenhouses in the western part of the Netherlands were approached at random by telephone and asked to participate in the study. 18 greenhouses were included and divided into three groups with distinct numbers of honeybees throughout the flower season of the sweet bell pepper plant. Group I comprised a control group of 9 greenhouses without bees (total area 308.500 m^2, total number of employees 72, number of allergic employees 22). Group II consisted of 3 greenhouses with a low number of bees (total area 61.000 m^2, total number of employees 17, number of allergic employees 8). Group III was a group of 6 greenhouses with a high number of bees (total area 126.500 m^2 , total number of employees 44, number of allergic employees 14).
Employees were considered eligible for the follow-up study if they had at least one of the following symptoms at work: rhinitis, conjunctivitis or asthmatic complaints. The investigators paid three visits to each greenhouse. The first visit took place in January / February (measurement 1, no bees were placed yet), the second in May / June (measurement 2) and the last in September / October (measurement 3). During the first visit the volunteers gave informed consent and were asked questions concerning age, sex, medication use, symptoms at work, and allergic complaints. The symptoms comprised five categories: redness, itching and/or eczema of the skin, urticaria / angioedema, rhinitis, conjunctivitis, and asthmatic complaints (asthma was defined as shortness of breath and/or coughing and/or wheezing). Sensitisation was determined by means of a skin prick test performed according to international guidelines with homemade extracts of the bell pepper pollen, and common inhalant allergens [9]. During the first, second and last visit spirometry values and V.A.S. (Visual Analogue Scale) were determined. On each visit the use of medication during the period was asked and noted by the investigator. The Hospital Medical Ethical Committee approved the study. Confidentiality was maintained.

Prick tests
Skin prick tests (SPT) were done in employees with work-related symptoms. The symptoms were defined as work-related when the employees notified a substantial improvement or complete regression of their complaints during weekends and holidays. SPT was 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 standardised skin test needle, and results were read after 20 minutes. Reactions were expressed in mm of mean wheal diameter (adding the longest diameter to the orthogonal diameter measured at one half of the longest diameter and dividing it by two). A diameter of 3 mm or more was considered positive [9]. Dilution buffer was used as negative control, histamine chloride 10 mg/ml as positive control. The same investigator performed all skin tests.

Allergens
Pollen from flowers of the bell pepper plants were collected in a greenhouse. The flowers were in full bloom. A 25% (w/v) extract was prepared in phosphate-buffered saline pH 7.4, containing 0.03% human serum albumin and 0.5% phenol (PBS). The extracts were centrifuged for 10 minutes at 2000g, and supernatants were passed through a 0.22 μm Millex GS filter (Millipore, the Netherlands). All extracts were stored in appropriate aliquots at -20˚C until use in skin tests. Before use, extracts were defrosted for 1 hour before skin test and mixed. In addition, skin prick tests were performed with 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 (SQ 553) and cat dander (SQ 555).

Honeybees
In January 2002 all greenhouses were visited. After the first visit, honeybees were placed in the selected greenhouses. All colonies originated from the experimental bee stand of Applied Plant Research, Unit Bee Research (Hilvarenbeek, the Netherlands). The colonies were healthy, and had recently not been used for experiments that might influence their development. During the experiments all colonies were fed with Apifonda sugar.
Approximately 1 colony of 10.000 bees per 10.000 m2 were used in group II, approximately 2 colonies of bees per 10.000 m2 were used in group III. The Bee Research Unit paid visits every three weeks to inspect whether the size of the colonies was large enough to warrant good foraging and to cheque whether the colonies were healthy.
In every greenhouse where bees were placed an Epipen® autoinjector was delivered, to use in case of bee sting anaphylaxis, and instructions were given to each employee.

Visual Analogue Scale
To score the severity of the rhinitis complaints, as experienced by the employees, we used visual analogue scale. The V.A.S. is a continuous horizontal line of 100 mm where 0 mm indicates no complaints; 100 mm indicates severe complaints (totally hampered by nose complaints throughout the week). The patient is asked to evaluate his or her complaints experienced over the past two weeks during work in the greenhouse concerning itching of the nose, sneezing, running nose, blocked nose and total nose complaints. These complaints are represented by a single, vertical line on this continuous horizontal line for each item [10].

Spirometry
To collect spirometry data (including expiratory Flow/Volume, Volume/Time curves) a Micro DL spirometer was used. (Micro Medical Limited, Kent, England). This instrument is a compact, and fully portable data recording spirometer. For each individual patient age, height and sex had to be entered and these data were used to calculate the predicted values for FEV1, FVC, and PEF. A personal identifier was added as well. When performing a spirometry test the user inserts a disposable mouthpiece into the holder of the spirometer. The employees had to stand up, inhale as deeply as possible, seal their lips around the mouthpiece and exhale as strong as possible until no more air could be exhaled. This was repeated three times and the best test results were stored in the instrument. Spirometry was done before presence of the bees (measurement 1), after 3 months (measurement 2) and 6 months (measurement 3) of interference with bees.

Pollen measurements
To investigate if and how many pollen from the flowers were collected by the bees a new method was developed. In this method pollen of 10 flowers in full bloom were collected by tapping three times against each flower. The pollen fell into a petri dish. Six dishes with pollen were collected in every greenhouse. In the laboratory, 1 ml. PBS was added to each dish and after one hour shaking a droplet of the substance was put into a Kova^{r} Glasstic Slide (Hycor Biomedical Inc., Garden Grove, California, USA) and the number of pollen grains were counted. The coefficient of variation (c.v.) of the variability across the six petri dishes was 27.7 %. For this reason we used the average of six dishes for further calculations.

Statistical Analysis
The CV across the six petri dishes was estimated using a variance-components analysis after natural logarithmic transformation of the pollen counts.
Three groups of employees are considered, depending on the greenhouse in which they used to work: greenhouses without bees (group I), greenhouses with a low number of bees (group II) and greenhouses with a high number of bees (group III). The three categories of bee amounts were randomly allocated to the greenhouses.
The various VAS-scores were log-transformed (ln (x+1)) before being analysed using mixed model ANOVA. In this analysis greenhouse was considered to be the subject as greenhouses

represent the experimental units that were randomised. Two measurements in time were involved (at months 3 and 6 after baseline). A dose-response trend relationship is modelled of VAS-scores across the three ordered randomisation groups according to the number of bees in the greenhouses (0 = no bees; 1 = low dose of bees; 2 = high dose of bees). Adjustment was made for the covariables gender, age, smoking and baseline VAS-score of the underlying individual employees. Also month (with two levels: 3 and 6) was included in the model. The within-greenhouse covariance structure was defined as compound symmetry.
The effects are presented as percent changes in VAS-score from one group to the next higher group. Lung function variables were analysed at 3 and 6 months as from the start of the trial using analysis of covariance (ANCOVA), where the three groups of employees are compared given the baseline measurement (measurement 1) of the outcome variable at hand. Interest is in a trend relationship of the lung function level with the ordering of the three employee groups. Significance is supposed to be reached if the p-value is smaller than 0.05.

Results

Employees
Of the 35 greenhouses approached by telephone, 18 participated in the study. The total area of the greenhouses was 496.000 m^2. Reasons for refusal to participate were lack of time and/or lack of interest. Another reason for not participating was the use of honeybees or bumblebees. The invited group comprised 133 employees. Of this total group, 44 employees appeared to be eligible for the follow-up study according to the predetermined criteria. In 43 employees SPT's were done. Characteristics of these 44 selected employees and results of the skin prick tests with occupational allergens and inhalant allergens are shown in table 1. The presence of a positive skin prick test with bell pepper pollen extract was found in 39 of 43 employees. 24 employees used anti-allergy medication. 22 of the 44 employees used medication for their rhinitis and 11 workers used medication for asthma. During the whole study there was no important change in medicine use by the workers, with the exception of a few employees who temporarily used an antihistamine.

Honey bees
During the first two weeks of placement in the greenhouses the colony strength (number of bees) generally decreased, probably because they get lost in the greenhouse or outside (open vents). Also the brood area decreased during the first weeks, but then remained constant. In most cases after 10 weeks the number of bees decreased, due to a lowered brood production under greenhouse circumstances. Subsequently, the colonies had to be replaced. In all colonies sweet bell pepper pollen was found in the cells of the beehive. Pollen collecting behaviour of the bees was generally higher during the morning than during the afternoon. An interference of crop protection measures with bee activity was observed: after application of Admire®, an insecticide toxic for honeybees, the bees remained inactive for at least a week. During applications of crop protection substances, the closed bee colonies were placed in a cool place outside the greenhouse. After re-entering the greenhouse the activity only slowly re-established. In September and October the bees were remarkably less active in all greenhouses. A possible cause of this phenomenon may be that the flowers produce less pollen in the late season. The plants produce less fruits and November is the end of the season for this cultivation.
The workers were not hampered by the presence of the bees, only a few were stung with only local reactions. Therefore, the Epipen® autoinjector was not used throughout the study.

Table 1. Characteristics of the included employees

Employees (n = 44)	
Mean age (years)	36 (21-50)
Male	35 (80 %)
Medication use	24 (55 %)
Smoking	9 (20 %)
Symptoms at work (n = 44)	
Skin (itching/ redness/ eczema)	17 (39 %)
Rhinitis	44 (100 %)
Conjunctivitis	33 (75 %)
Asthma	20 (45 %)
Spt positive with Bell pepper pollen extract (n = 43)	
Bpp extract	39 (91 %)
Spt+ with inhalant allergens (n = 39)	
Dermatophagoides Pt.	15 (38 %)
Tree pollen	10 (26 %)
Grass pollen	14 (36 %)
Mugwort pollen	3 (8 %)
Cat dander	5 (13 %)
Dog dander	9 (23 %)
Any inhalant allergen	25 (64 %)
Baseline lung function (n=44) Mean (SD), percentage predicted	
FVC	96 (20)
FEV1	110 (17)
PEF	98 (16)

Pollen counts

Pollen counts range from 25 to 1127 across the 18 greenhouses and three measurement times, with a mean of 414 and a SD of 277. Because the distribution of pollen counts is skewed to the right, the median of this distribution is taken as the appropriate location measure. The median of percentage pollen, expressed as percentage of the base line (measurement 1), in the group without bees was 101 % for measurement 2 versus baseline, and 69 % for measurement 3 versus baseline. The median of percentage pollen in the group with bees (low numbers) in measurement 2 versus baseline, and 3 versus baseline was 33% and 29% respectively. The median of percentage pollen in the group

with bees (high numbers) in measurement 2 versus baseline, and 3 versus baseline was 12% and 18% respectively. All results are shown figure 1.

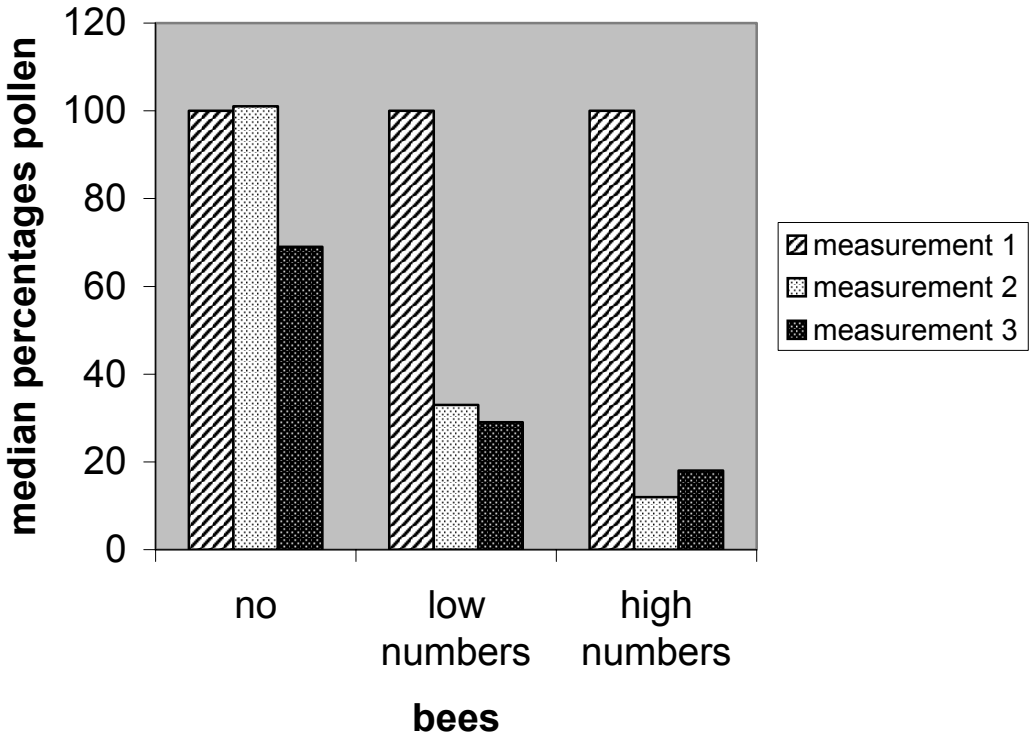

Figure 1. Pollen measurements (mean of 6 petri dishes) in green houses before intervention and after 3 and 6 months, respectively, of introduction of honeybees, in low and high numbers.

VAS

The effects are presented as percent changes in VAS-score from one group to the next higher group. Significant p-values were found for itching (p= 0.023), sneezing (p = 0.053), droplet (p = 0.011) and total nose (p = 0.017) score. All results are presented in Table 2.

Table 2: Percent changes in VAS-score from one group to the next higher group with 95 % confidence interval (CI) and P-value.

VAS-score	Percent change	95 % CI	P-value
Itching	-20	-34 to -3	0.023
Sneezing	-19	-35 to 0	0.053
Droplet	-25	-39 to -7	0.011
Stuffed	-4	-25 to +23	0.728
Total nose	-22	-37 to -5	0.017

Spirometry
All estimated relationships are summarised in table 3. No significant effects were seen although the ordering of the differences in lung function corresponded with the ordering of the number of bees in the greenhouses.

Table 3. Estimated relationship of lung function with the three ordered treatment groups.

a. Trend relationship

The effect (in percent points) is the estimated difference between two consecutive groups, assuming that the difference between groups I and II is the same as the difference between groups II and III.

Lung function variable (percentage predicted)	Effect (SE)	p-value
FVC	2.71 (2.26)	0.24
FEV1	3.33 (2.41)	0.18
PEF	2.53 (1.82)	0.17

b: Nominal relationship

The effect (in percent points) is the difference of the respective groups II and III with group I.

Lung function variable (percentage predicted)	Effects (SE) group II- group I	group III - group I	overall p-value
FVC	3.84 (4.91)	5.36 (4.60)	0.49
FEV1	3.62 (5.37)	6.64 (4.91)	0.41
PEF	0.56 (4.25)	5.27 (3.71)	0.35

SE = standard error

Discussion

The high percentage of bell pepper pollen sensitised workers (35.4%) in bell pepper culture forced us to consider possible solutions. The most logical solution is to reduce the pollen output. Since honeybees proved to pollinate flowers [8], we decided to investigate whether bees can be used to reduce complaints caused by bell pepper pollen in these occupational allergic employees. The results of pollen counts proved that the bees could certainly remove pollen from the flowers. The third measurement in the group with high numbers of bees even showed a decrease of 82% of pollen. Comparing the groups with low numbers and high numbers of bees, results in a dose response in pollen counts. The more bees were used the less pollen were found. Remarkable is the decrease of pollen in the group without bees at the third measurement. Probably the bell pepper plant is not so active in the late season and produces less pollen.
The effect on the nose symptoms by lowering the pollen output is satisfactory. A dose response relationship was demonstrated and in several items (itching, sneezing, droplet and total nose), significance was reached. No significant dose response relation in stuffed nose scores was seen. An explanation could be that changes in chronic nasal blockage are less well perceived by patients than changes in symptoms as sneezing and rhinorrhea.

In trend- and nominal relationships of lung function and bee numbers significance was not reached . However, it has to be taken into account that the diagnosis of asthma was assessed by questionnaire and not by the inclusion of a test for bronchial hyperreactivity. In addition, the baseline values were in the normal predicted range, so there was less room for improvement. Nevertheless, FVC, FEV1 and PEF slightly improved. Moreover, the percentage predicted values in the group employees with high numbers of bees differed 6.64 % in FEV1 compared with the group employees without bees. Comparing the three groups a trend relation was found, although not significant.
Explanations for not reaching significance in some items of nose complaints and lung function may be found in a perhaps variable functioning of the bees. They were placed in a more or less unnatural environmental situation. As earlier mentioned the number of bees decreased after 10 weeks. Furthermore, sometimes the bees were placed outside the greenhouse and only re-established slowly after re-entering. Nevertheless, great effort was put in achieving a constant number of bees throughout the study.
A second reason may be that this exploratory study was underpowered. Therefore further investigation is necessary is with larger groups to obtain more results and to decrease possible distortion caused by selection of workers and greenhouses.
Finally, in these experiments with workers, flowers and bees, we must not forget in which complex biological and environmental conditions we are interfering. It is likely that other, not yet known factors play a role in the complaints of these bell pepper employees. Moreover, concomitant allergies in a part of the employees may give complaints at work as well
In conclusion, the interference of bees in bell pepper greenhouses can significantly reduce the pollen output. This reduction decreases the work-related rhinitis symptoms in allergic greenhouse workers. Furthermore, we found a dose response relation in the number of bees and a decrease of rhinitis symptoms of the workers. Therefore, this intervention study demonstrated very clearly that allergic work-related complaints of green house workers, sensitised to bell pepper pollen, are caused by occupational exposure to this pollen in the greenhouse.

Acknowledgements

We thank H. van der Woude, Department of General Practition (Erasmus MC, Rotterdam, the Netherlands), for kindly supplying the Micro DL spirometer. Furthermore we acknowledge Dr. Ir. A. Burdorf, Associate Professor in Occupational health, Department of Public health (Erasmus MC, Rotterdam, the Netherlands), for critically reading the manuscript.

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Chapter 9

Allergy to bumblebee venom. III. Immunotherapy follow-up study (safety and efficacy) in patients with occupational bumblebee venom anaphylaxis.

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Allergy 1999;54:980-4.

Chapter 9

Abstract

Background: to analyse whether venom immunotherapy with bumblebee venom (BBV) is safe and effective.
Methods: eleven patients with severe occupational anaphylaxis, caused by stings of bumblebees were studied. Sensitisation to bumblebee venom was confirmed using skin tests and RAST Immunotherapy was started using bumblebee venom extract by semi-rush procedure, because these patients showed a primary sensitisation to bombus venom, and a low or absent degree of crossreactivity with honeybee venom. IgE-titer and skin tests with bumblebee venom were performed yearly. Efficacy was evaluated by means of in-hospital sting challenge and/or occupational field stings with bumblebees.
Results: all patients reached maintenance dose in 6 weeks without severe side effects. During the follow-up period (1.5 – 5 years) 3 systemic reactions in two patients were seen in 20 bumblebee stings. However, these reactions were milder compared to the index sting.
Conclusions: immunotherapy with bumblebee venom is safe and effective, comparable with honey bee and yellow jacket venom immunotherapy.

Introduction

In contrast to honeybee or yellow jacket venom allergy, immediate type allergies to bumblebee (Bombus terrestis) venom are rare. However, the growing use of bumblebees for the pollination of vegetable flowers in The Netherlands accounts for the increasing number of anaphylactic reactions to bumblebee stings.
The efficacy of venom immunotherapy (VIT) for the treatment of patients with allergic systemic reactions to Hymenoptera stings is established (1). It was our objective to analyse whether VIT with bumblebee venom could also be as safe and effective as well.
We started immunotherapy with purified BBV extract in-patients with anaphylactic reactions to bumblebee venom using a semi-rush protocol.
This paper describes the results of the follow-up study, regarding side effects, skin tests, RAST's, in hospital sting challenge (HC) and field stings (FS) on these 11 patients with BBV immunotherapy after reaching 1.5 to 5 years of maintenance dose.

Materials and methods

Patients
We describe 11 patients with severe occupational anaphylaxis, caused by stings of bumblebees (Table 1). Apart from a large local reaction in some patients, only patient Bo experienced an anaphylactic reaction to a previous bumblebee sting (grade IV, according to the Mueller classification (2)).

Immunotherapy
As earlier described by de Groot et al.(3), patient Ko and Rij showed no evidence of cross-reactivity between bumblebee venom and honeybee venom by SPT and Rast. Therefore immunotherapy was started very carefully in these two patients with very low doses of purified bumblebee venom extract. Maintenance dose was reached after 8 weeks, without any side effect (3). The following patients reached maintenance dose in approximately 5 weeks using a modified semi-rush protocol: week 1 - 0,001/0,01/0,1 microgram; week 2 – 1/5/10 microgram; week 3 – 20/30 microgram; week 4 – 50/50 microgram; week 5,6 and next every 4 weeks 100 microgram of bumblebee venom.

Follow-up
Follow-up was performed yearly: skin test, Rast with BBV; registration of reactions caused by occasional field stings; side effects of the immunotherapy. This registration contained severity, anaphylactic grade of

the reaction and rescue medication used. Hospital sting challenge in the first two patients were performed as previously described (3).

Table 1. Characteristics of 11 patients with bumblebee venom allergy

Patient	Sex	Age	Job	Anaphylaxis grade(2)	Previous stings by bumblebees
Ko	M	48	Tomatogrower	IV	7
Rij	M	48	Tomatogrower	IV	25
Mu	F	49	Gardenworker	IV	1
Mi	M	47	Tomatoworker	II	3
Wu	F	44	Tomatogrower	III	15
Bo	M	49	Tomatogrower	IV	15
Ke	M	35	Bumblebee farm	III	4
Kjr	M	25	Tomatogrower	IV	2
Bu	M	35	Bumblebee farm	III	15
Vi	M	47	Tomatogrower	IV	2
Sc	M	31	Tomatogrower	II,III	5

Allergens
Bumblebee venom (Bombus terrestis) extract for skin tests (1 µg/ml) and venom for immunotherapy (100 µg/ml.) were obtained from ALK Benelux, Groningen, the Netherlands. This venom was obtained by electric stimulation.

Skin test
Skin tests were performed intracutaneously with 0.02 ml. of 10-fold dilutions of bumblebee venom. After 20 min, wheal-and-flare reactions were measured with the grading system of standardised plus signs devised by Norman et al.(4). The lowest concentration resulting in a whealdiameter of 5 mm was qualified as positive in endpoint titration. The negative control wheal with dilution buffer only was considered valid if the wheal disappeared or was less than 2 mm. The positive control test was histamine diphosphate (0.1 mg/ml).

RAST (radioallergosorbent test)
Agarose beads as allergen support were used by RAST to determine allergen specific IgE. Threehundred microliters of bumblebee venom (ALK Denmark), 100 µg per milliliter, were coupled to 300 mg of CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) according to the manufactures instructions.

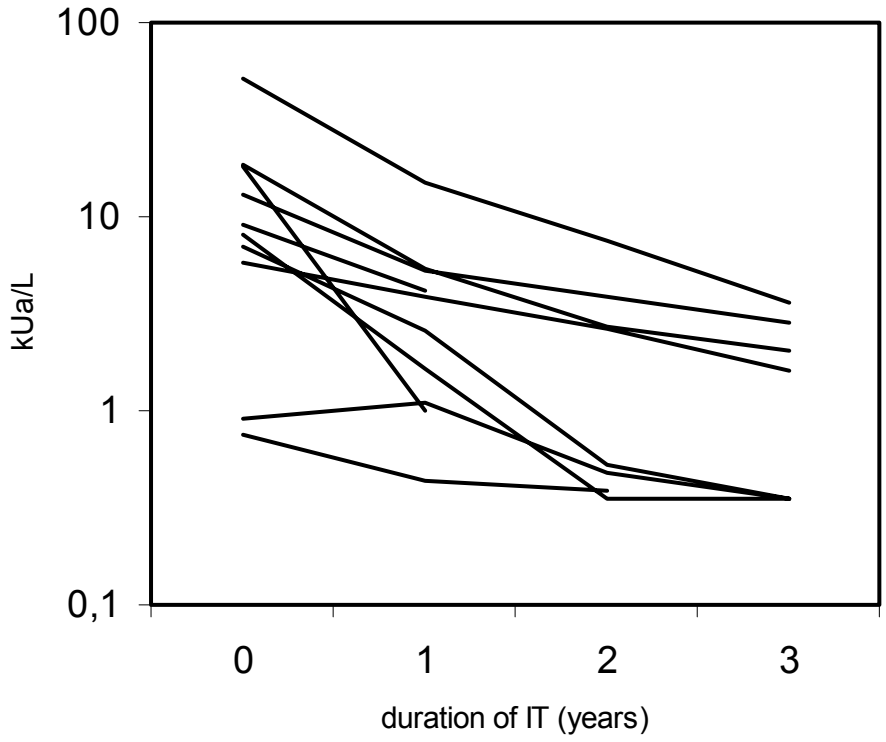
Results

Side effects
During induction phase (194 injections) we registered 12 (6.2%) local reactions (5-10 cm), and two (1%) systemic reactions, consisted of shortness of breath appearing 15 minutes after the injection. The asthmatic reactions resolved after the administration of B-sympaticomimetica and an antihistamine tablet. These reactions occurred in patient Kjr. who had an instable asthma caused by a housedustmite allergy. After stabilising the bronchial hyperreactivity with inhalant corticosteriods no additional problems were seen.

During maintenance dose (393 injections) we registered 11 (2.8%) local reactions and no systemic reactions.

Sensitisation
All patients showed a 10-100 fold decrease in skin test reactivity after 1 year of immunotherapy. During the next 3-4 years of immunotherapy a continuing but slight reduction was seen. These data were confirmed by RAST, as visualised in figure 1. Both skin test and RAST data are comparable with the IgE-response during immunotherapy with HBV and YJV (1).

Figure 1. RAST with bumble bee venom



Efficacy
We started immunotherapy with patient Ko and Rij in 1993. After one year we performed an in-hospital sting challenge which caused in both only a local reaction. The following nine patients who started immunotherapy with purified bumblebee venom experienced 18 fieldstings (see table 2). Most reactions were only local reactions, in 2 patients 3 anaphylactic reactions occurred, however milder compared to the index field sting. Two patients were anxious when stung and used the Epipen® after approximately 20 minutes, although they did not have any signs of anaphylaxis.

Discussion

Immunotherapy with insect venom is already known as a safe and effective therapy (1,5). In the Netherlands we see an increasing number of patients with insect allergy to bumblebee venom as an occupational disease. As bumblebees and honeybees are from the Apidae family strong crossreactivity was supposed, as indeed proven by Hoffman et al.(6,7). As BBV was in those days not yet commercially available for diagnostic and therapeutic purposes, Kochuyt et al.(8) performed immunotherapy with HBV successfully in patients with BBV allergy. Nevertheless earlier RAST-inhibition experiments showed in our patient group a low level of crossreactivity, which can be explained by the fact that these patients are primarily stung and sensitised by bumblebees (9). This explains the experiences of Stern et al.(10) who

reported two patients with occupational allergy to BBV which were hyposensitized with purified HBV. Both patients developed a severe anaphylactic reaction after an incidental sting. This suggests that immunotherapy with HBV is not effective in these BBV patients. Therefore, immunotherapy with purified BBV was started in our patient group as the degree of systemic reaction and the risk of re-exposure was high. We did not have any data about the spontaneous prognosis of patients with BBV anaphylaxis, so patients were advised to change their profession or start immunotherapy.

Table 2. Outcome of sting reactions during immunotherapy with purified BBV

Patient	months after starting IT	sting	outcome	therapy
Ko	12	HC	LR	-
Rij	12	HC	LR	-
	40	FS	-	-
	48	FS	-	-
	60	FS	LR	-
Mu	12	FS	-	adrenaline
	39	FS	-	-
Mi	1	FS	SR (I)	clemastine
Wu	36	FS	LR	adrenaline
Bo	9	FS	LR	-
	26	FS	LR	-
Ke	8	FS	LR	-
	15	FS	SR(II)	-
	30	FS	SR(I/II)	-
Kjr		-		
Bu	< 12	4xFS	-	-
Vi		-		
SC	10	2xFS	LR	-

HC: in-hospital challenge
FS: field sting
LR: local reaction
SR: systemic reaction (grade according to Mueller (2)).

Concerning side effects it can be compared to bee and wasp venom immunotherapy. No unacceptable high incidence of side effects was seen. Large local reactions were seen in 6,2 % during induction phase and 2,8 % during maintenance phase. During induction phase 2 systemic reactions occurred in a patient with instable asthma. It must be stressed that more side effects always occur with faster schedules (11). That is why we used a semi-rush procedure to lower the risk of reactions.
As efficacy is concerned we have 18 field stings and two hospital challenges to evaluate. A provocation test with a sting from the responsible insect is without any doubt the most reliable control of the effectiveness of immunotherapy but as some authors mentioned (12), the low grade of a sting reaction, does not assure the patient to remain free of having a systemic reaction on any next sting. Nevertheless we will emphasise that our patients are still working among bumblebees and underwent several field stings, which were tolerated without complications. As misidentification by the patient at work of the insect is nearly impossible, more provocation tests seemed not to be necessary. The only three systemic reactions, which occurred in 2 patients, were less serious in relation to the anaphylaxis prior to immunotherapy. In 1 patient the SR took place before reaching the maximum dose. This confirms the effectiveness of BBV immunotherapy (85 %) comparable with other venom immunotherapy. Earlier studies in which patients on maintenance VIT were exposed to a provocation test showed that more than 90% of yellow jacket venom allergic and 75-80% of honeybee venom allergic patients were fully protected (5,13).
In summary we conclude that immunotherapy with bumblebee venom is safe and effective for patients with occupational bumblebee venom anaphylaxis.

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Chapter 9

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Part seven

Chapter 10

General discussion and summary

General discussion

The prevalence of food and occupational allergies is increasing, and the variety of allergens is expanding as well. For instance, occupational allergy among greenhouse employees in the Netherlands is a growing problem. This is probably related to the growing export and annual turnover of flowers e.g. *chrysanthemum*. Not only greenhouse employees, but florists and gardeners also suffer from occupational allergic symptoms. Furthermore, the incidence of immediate type reactions to natural rubber products (latex) is rising. This is a consequence of the increased use of latex gloves among health care personnel.
Besides occupational allergies, the number of food allergic patients is increasing extensively as well. Most frequent sensitisation to food is observed in pollen related foods. The diagnosis of uncommon allergies caused by relatively new allergens is difficult. Apart from the exposure to new and therefore unknown occupational and food allergens the observed increased prevalence may fit in the general increase in allergy and allergic disorders.
The aim of this thesis is, first, the diagnostic work-up of occupational and food allergies in the absence of well-validated commercially available standardised extracts for Skin Prick Test. Second, to investigate cross-reactivity in occupational and food allergic patients. Third, the treatment of, employees with an occupational allergy in order to enable the continuation of work.

1: Sensitisation and diagnosis of allergy.

1.1 Occupational allergy

1.1.1 Pollen, mites

The prevalence of work related allergic symptoms, found among employees in greenhouses is high. The reported symptoms among employees e.g. in *Chrysanthemum* and Bell pepper pollen (Groenewoud et al. 2002) greenhouses were 57% and 54% respectively, the main symptoms being rhinitis and conjunctivitis. Symptoms of the lower airways, which are considered to be the most serious manifestations of an occupational allergy, were found in 9% and 13.3% respectively. Besides employees in greenhouses, 14 flower growers with allergic rhino conjunctivitis symptoms due to the handling of flowers visited our hospital in a period of two years. In these patients asthma was reported in 10/14.
To analyse these occupational allergic symptoms, SPT with in house manufactured extracts of the pollen were performed. The concordance between SPT and symptoms in most cases was satisfying, e.g. 74% in the gardeners and florists (*Chapter* 3); however, not all work- related symptoms could be confirmed by a positive SPT. In only 29% of greenhouse workers who presented work related symptoms after exposure to *Chrysanthemums* (*Chapter 2*) the SPT was positive; 55% of symptomatic bell pepper pollen employees had a positive SPT after testing with a bell pepper pollen extract. Apparently, the greenhouse environment elicits more symptomatology, which cannot be explained only by IgE mediated allergy to the most likely responsible allergens. It seems quite probable that there are more airborne sensitising agents from unknown origin.
At a site visit of one of the greenhouses we found mites in the bell pepper flowers which appeared to be the *Amblyseius cucumeris* (a biological control agent against thrips). SPT with extracts of these mites were positive in 23% of 472 employees (Groenewoud et al. 2002). Another frequently found biological control agent in greenhouses is the Orius (*Orius laevigatus*) (van Stelt et al. 1999) We have not tested this biological agent in skin prick tests yet. Furthermore irritating agents and non-specific stimuli can provoke a non-immunologic response in employees with hyperreactivity of upper and lower airways. The observation that the most predominant and obvious allergens may not be the cause of all presented symptoms emphasises the need for visits at the workplace to establish a proper diagnosis.

Discrepancies, between symptoms and SPT results might be due to several other reasons:

1.The quality of the extract
2.Less reliable case history, e.g. interference of unidentified allergens or stimuli
3.Cross-reactivity
4.Sensitisation without symptoms

1. The quality of the extract.
First of all, a possible explanation might be that the extracts have failed to detect sensitisation. The extraction procedures used were the same as used earlier by Goldberg et al.1998 and Waisel et al.1996. Goldberg found 52% positive SPTs with an ornamental flower extract among 75 flower growers. He made pollen extracts of the *Asteraceae* (Compositeae) family. Most positive SPTs were induced by these pollen extracts. The potency of an extract is dependent on the possibility of gathering pollen, as extracts of pollen yield better SPT results (Gerth van Wijk et al. 1989). Sometimes, in our studies (especially in *chrysanthemums*) the flowers were very small and/ or did not produce enough pollen. In such cases we took a small piece of the heart of the flower. A low potency causes false negative SPT reactions. The differences in allergenicity in *chrysanthemum* pollen extracts are explained by differences in protein concentrations (0.25 g/L - 1.16 g/L.)
For the diagnosis of occupational *A.cucumeris* allergy we prepared an extract of these mites. A positive SPT with this extract was found in 109/ 472 (23%) employees. Additionally, we carried out nasal challenge tests on 23 sensitised employees (Groenewoud et al. 2002) to examine the biological activity of Ac extract on human mucous membranes. The consequent clinical-allergological relevance of sensitisation could be confirmed by a nasal challenge test.

2. A less reliable case history.
It could be envisaged that other flowers or plants not used in the study were responsible for the symptoms. An incorrect identification of flowers is imaginable when employees work with different flowers. Furthermore, we used for the preparation of SPTs only seven of the most frequently cultivated *chrysanthemum* varieties in the Netherlands. It is likely that employees are sensitised to other chrysanthemum pollen than used in SPT. To obtain a reliable flower specific case history of symptoms after occupational exposure, an extensive questionnaire was used. Exacerbations during workweek and remissions on weekends and holidays were considered to be work related. However, in the bell pepper and *chrysanthemum* study employees were positive for common inhalant allergens in 37.5% and 35% respectively. These symptoms to common allergens could also be present during work and therefore bias the questionnaire results. This may be also the case with employees sensitised to *A.cucumeris.*

3. Cross reactivity.
Cross-reactivity can give positive SPT reactions, which are not reflected by clinical reactivity to the allergen. Taking into account that cross-reactivity exists between different members of flower families (Sriramarao et al.1996, Fernandez et al.1993) sensitisation to ornamental plants may also indicate cross sensitisation between different flowers of one family. This is certainly conceivable in occupational flower allergic employees. The results in *Chapter 2 and 3* suggest strong cross-reactivity between flowers of the same family. Maybe SPT reactions may not have clinical relevance in all cases. This could also explain in part the SPT results with *A.cucumeris* in *Chapter 7*. Cross-reactive antigens were found between this mite and the house dust mite *D.pteronyssinus*.

4. Sensitisation without symptoms
Sensitisation to food or inhalant allergens can occur without symptoms. Epidemiological studies have shown that a substantial number of subjects may be sensitised to inhalant allergens without experiencing symptoms of asthma or allergic rhinitis (Baldacci et al. 1996). Recently, Kerkhof et al. (2000) found in a study among 1904 Dutch subjects, that forty-three percent with specific IgE to inhalant allergens was asymptomatic.

1.1.2 Latex

Allergy to natural rubber latex is a major occupational problem in health care settings and in other occupations where protective gloves are used. (Condemi et al.2002) Latex hypersensitivity can induce local symptoms (Type IV) as well as the more serious Type I allergic reactions. Prevalence studies focussing on type I and IV is scarce. In 1997 a prevalence study among a population at risk was carried out. (*Chapter 4*) Glove related urticaria, rhinoconjunctivitis and/ or asthma was reported in 24.6% and glove related local dermatitis in 36.4% of all cases. SPT was done with extracts from four different gloves and patch tests were done with 1 cm^2 of each glove. Five employees (8.3%) appeared to have a positive SPT with one or more glove extracts; a positive patch test was found in 3 of them. There appeared to be two distinct disease groups. A type I group, all with atopy and a high prevalence of coexisting tropical fruit allergy; and a type IV allergic population without evidence of type I allergic reactions to inhalant allergens, fruit or NRL.
In the absence of standardised commercial latex extracts, in house manufactured extracts were made (Turjanmaa et al.1996). Although the protein concentration of the extracts did not vary widely, the results between the SPTs did. Nevertheless, all employees with a positive SPT with one or more latex extracts had symptoms of occupational latex allergy. In one employee only one extract was positive. The same was found in a later study among 226 surgery employees (Bijl et al.1999). In this study we found 14.1% latex sensitisation on latex extracts from gloves used in operation rooms. Again there was some difference in allergenicity between the different glove extracts. In recent years much research has been done on latex allergy, and commercial standardised extracts are available now. Nevertheless, in some patients only in house manufactured extracts can confirm an IgE mediated latex allergy.
Supported by the results of both studies changes in latex policy in our hospital were carried out. All gloves were replaced by powder-free or low-allergen latex gloves. Nowadays, the use of low-allergen latex or non-latex gloves is recommended for health care workers who have natural rubber latex allergy. This is the conclusion of Turjanmaa et al. (2002), who performed a long-term follow-up study of 160 adult patients with natural latex allergy.

1.2 Food

There is evidence that the prevalence of known food allergies such as peanut allergy is rapidly increasing (Kagan et al.2003) and that new food allergies are emerging, e.g. to sesame seed and kiwi fruit. This has serious consequences for allergic subjects, their quality of life and for public confidence in the safety of food. Extensive research concerning food allergy is going on in large projects in the EU. For example, the S.A.F.E. project brings together many scientists from all over Europe (www.akh-wien.ac.at/safe/). The European Commission funds this scientific project. The project focuses on apple allergy: its aim is to characterise allergens and their relationship with symptoms in fruit-allergic patients from across Europe (Asero et al.2004). It is the policy of the EU to actively encourage the consumption of fruit and vegetables for the promotion of health. Hence the necessity of research concerns the increasing number of plant-food allergic patients.
However, the diagnosis of food specific allergy is complex. Standardised commercially extracts are not available or of insufficient quality (Akkerdaas et al. 2003). Moreover, it is well known that skin prick tests with fresh foods and vegetables have a higher sensitivity than commercial extracts. (Ortolani et al. 1989, Dreborg et al.1983, Rance et al. 1997.) But this prick-to-prick method can be very impracticable. In the absence of standardised extracts in house manufactured extracts were made. In *Chapter 5,* we examined four in house manufactured food extracts: coriander, hazelnut, peach and sesame seed on stability, reproducibility and dose response. We performed SPTs in 21 food-sensitised patients with a positive history for the four mentioned allergens. Overall, pair wise comparisons of dose responses were significant; no significant differences were seen between fresh extracts, 3 months and 6 months old extracts. The mean concordance between SPT and CH was 72%. Furthermore, we found neither significant differences in varieties of fruits, nuts or seeds, nor significant seasonal variation, in the SPT results, as described previously by Skampstrup et al. (2001)
Extensive sensitivity and specificity research on in house manufactured allergen extracts as described by Niemeyer et al. (1996) has not yet been done. He evaluated the concentration and cut-off levels of

SPT and ICT in standardised inhalation extracts. Extensive further investigations are necessary to examine, sensitivity, specificity, cut-off values and optimal concentrations on in house manufactured food extracts. As described previously, in house manufactured food extracts are of most importance for research projects, as demonstrated in a study on cross reactivity between birch pollen and apple, peach and hazelnut (*Chapter 6*). Concordance between SPT and a positive history was found in 77%, 52% and 54% for apple, peach and hazelnut, respectively. The explanations for the discrepancies between SPT and case history are already given in the previous section of this discussion: the quality of the extract, a less reliable case history and/or cross-reactivity. Concerning extraction methods, we compared three apple extraction procedures in *Chapter 6.* The juice appeared to give the best results. When we compare the concordances between both studies it is obvious that the differences are due to the selection criteria of the patients. In the birch-pollen study the mean concordance between SPT and case history for peach and hazelnut is considerable lower (53%) then in the 21 food sensitised patients in *Chapter 5* (72%) The last group was selected on food allergy symptoms, and the first group on birch-pollen allergy. The lower concordance in the latter group fits in the well-known phenomenon that cross-reactivity may lead to SPT reactions not correlating with clinical symptoms. (Wensing et al. 2002, Ferrera et al 2004)

Finally, the use of these extracts for a proper diagnosis can be discussed. In case of a convincing case history concerning specific food allergy symptoms e.g. anaphylaxis or oral allergy symptoms, which can be confirmed by positive SPT with an in house manufactured extract a double blind placebo controlled food challenge is unnecessary. Dietary advice is the next step. Only in case, SPT and or RAST cannot identify the sensitising allergens responsible for symptoms, a DBPCFC should be performed.

2. Cross-reactivity

2.1 Between inhalation allergens

Cross-reactivity is a well-known problem in the diagnosis of IgE mediated allergy. Many researchers have studied cross- reactive patterns and its clinical relevance. Insights in cross- reactivity patterns can help elucidate to what extent exposure to allergens is an independent risk factor for the development of allergic disorders. Of particular interest are those studies showing the structural relationships between allergens. In our occupational prevalence studies cross-reactivity is most likely. Most of the sensitised employees are atopic and an association was found between the occupational allergen and one or more common pollen allergens like birch, grass and mugwort (*Artemisia vulgaris*). Focussing on *Chapter 2 and 3,* there seems to be a striking correlation between sensitisation to flowers and mugwort pollen. We concluded that SPT with mugwort could be used as screening test for a possible flower allergy. Furthermore, sensitisation patterns suggest strong cross sensitisation between several members of flower families. Fernandez et al (1993) found cross-reactivity between sunflower pollen and other pollen of the *Compositae* group. In *Chapter 2*, all employees with a positive SPT for *chrysanthemum* were also sensitised to mugwort. We do not know whether sensitisation to chrysanthemum is a consequence of primary sensitisation to mugwort. Further research should be done on this subject to examine whether the sensitisation to mugwort has predictive value developing a *chrysanthemum* allergy. In some cases cross- reactivity can be a helpful tool in diagnostic methods. For instance, as *chrysanthemum* is not easy to use for extraction, *Solidago* appeared to be very useful in SPT when flowers of the *Compositae* group are involved.
Recently, Vermeulen et al. (2003) found that most IgE binding structures in sweet bell pepper (Groenewoud et al. 2002) pollen did not cross-react with allergens in grass, birch or mugwort pollen. In this study, co-sensitisation was expected but not proven.
Cross- reactivity between mites was reported before by van der Heide et al. (1998) In *Chapter 7*, we examined cross- reactivity between *Amblyseius cucumeris* (Ac) and *Dermatophagoides pteronyssinus* (Dp). We found two different patterns: one with common protein bands between both mites and one showing no shared protein bands. Our findings can be explained by the fact that Ac is only distantly related to Dp. The results are of importance, as in some cases employees appear to be primarily

sensitised to this biological agent. Furthermore, a strong association between sensitisation to Ac and *Tyrophagus putrescentiae* (48/ 109) employees suggests cross- reactivity between these mites (Groenewoud et al. 2002). Future experiments are planned on this issue.

2.2 Between inhalation and food allergens

Four out of five employees who were sensitised to natural rubber latex had a positive SPT on exposure to avocado, banana and kiwi. (*Chapter 4*) These results are in line with the first reports of the latex fruit allergy syndrome demonstrating that the most common associations exist between latex and banana, avocado and kiwi (Hjorth et al. 1976). Nowadays, 25 years later, in latex sensitised patients clinical or serological sensitivity to almost 30 different foods e.g. beets, buckwheat, figs, spinach, watermelon, passion fruit have been found (Condemi et al. 2002).
Vandenplas et al. (1996) reported that the enzyme papain and bromelin present in papaya and pineapple also have epitopes that are present in NRL. Bijl et al. (1999) examined 163 surgery personnel. Sensitisation to natural rubber latex was found in 23 (14.1%) employees. In only 10 (6.1%) they reported symptoms due to kiwi, banana and/ or avocado. In 43 cases (26.4%) a positive SPT was found with extracts of these fruits. This difference emphasises that a positive SPT, due to cross-reactivity is not always clinically relevant. Nevertheless, employees who are sensitised to latex should be aware of the fact that a large variety of fruits, nuts and other food substances can give allergic symptoms.
The cross-reactivity between birch-pollen and food has been known for several years. In *Chapter 6*, we found SPT positive for apple, peach and hazelnut in 64.5%, 77.2% and 89.9% respectively, in 79 consecutive patients suffering from tree pollinosis. Case histories for apple, peach and hazelnut were positive in 44.3%, 29% and 44.3% respectively. The discrepancies between SPT and symptoms can be due to cross-reactivity. New insights into the nature and biological function of cross- reactive food allergens have become available (Wensing et al.2002). Ferreira et al. (2004) mentioned 28 major groups of cross-reactive proteins from various sources. Allergens of six of these groups belong to some families of pathogenesis related (PR) proteins from plants. They are induced in response to infections by pathogens (fungi, bacteria and viruses), by wounding or other stress including drought, flooding, freezing temperature, ozone and ultraviolet B light. With regard to the allergens involved in birch pollen allergy two groups could be identified. Firstly, the fagales pollen group – group 1 (PR-10, plant steroid hormone transporter), e.g. Bet v *bettula verrucosa* (Birch) 1, Cor a, *Corylus avellana* (Hazel) 1 and secondly the actin binding protein, profilin. Both groups are responsible for strong cross-sensitisation, but not in all cases clinically relevant. Besides the three food sources examined in our study, the list of cross-reactive foods is expanding. Consequently, taking into account that food allergy is increasing, the role of cross-reactivity becomes more and more important. Recently, Bolhaar et al. (2004) examined the effect of birch-pollen immunotherapy on cross-reactive foods. They found that birch-pollen IT decreases allergy to foods containing Bet v 1-homologous allergens.

In summary, the impact of the huge number of studies on allergenic molecules and extracts and their relationships is highly relevant for diagnostic procedures. Further research is necessary to understand the sensitising process, which often begins with exposure to a single source.

3. Treatment

3.1 Avoidance

Occupational rhinitis has a profound effect on the of quality of life and raises substantial costs due to loss of productivity. Avoidance of exposure and protective measures at the workplace can prevent progression of the disease from rhinitis to asthma. (Hellgren et al. 2003). Thus far, no prospective intervention studies have been performed concerning decreasing pollen exposure in greenhouses.
In *Chapter 8*, we examined whether the reduction of pollen output from the bell pepper plant could protect the employees. To accomplish a lower pollen exposure we used honeybees, as they can be used to pollinate flowers. The results of the pollen counts after 3 and 6 months of high and low dose of bees

are very promising. In the group greenhouses with high numbers of bees the percentage of pollen was reduced to 12-18%. In the allergic greenhouse workers we found a significant decrease of the VAS (visual analogue scale) in four items. Furthermore, after feed-back with the employees this year, two years after the study, the greenhouses reported the application of honeybees on a large scale, not for research, but to reduce allergic symptoms among workers. As we mentioned before the bell pepper pollen are probably not the only cause of allergic symptoms. Obviously, a combination of several interventions e.g. using less *A. cucumeris* will improve the results.
It is a challenge to create a balance between plants, animals and employees in these complex biological and environmental conditions. We are satisfied with the promising findings of our intervention study; further research has to be done with the aim to let the employees work free of symptoms.

3.2 Immunotherapy

Specific immunotherapy with Hymenoptera venom (VIT) has been successful with wasp (*Vespula germanica*) and honeybee (*Apis mellifera*) (Muller et al. 1993). With the increase in commercial vegetable production in greenhouses, occupational sensitisation to bumblebee (*Bombus terrestris*) venom is becoming more common. This is the consequence of a newly developed Dutch bio industry, using bumblebees (BB) in greenhouses, for the pollination of vegetable flowers such as those of tomatoes. Employees working in a BB farm are most likely to get stung and in some cases serious anaphylactic reactions occur. Immunotherapy is the treatment of choice in these patients. As honeybees and bumblebees are closely related species the question arises if the already available honeybee venom (HBV) could be used for this purpose. Kochuyt et al. (1992) performed VIT with HBV successfully in patients with bumblebee venom (BBV) allergy. Nevertheless, Stapel et al. (1998) concluded after RAST-inhibition and immunoblotting experiments that honeybee venom will not be effective as immunotherapy in all bumblebee venom allergic patients. Hoffman et al. (2001) came to a similar conclusion after founding significant species group-specific epitopes on bumblebee venom proteins. In house made extracts cannot be applied in immunotherapy. Therefore, ALK Abello was asked to produce bumblebee venom extract for immunotherapy. In a first study de Groot et al (1995) evaluated this extract and described three patients treated with bumblebee venom immunotherapy. It was our objective to investigate the safety and efficacy of VIT with bumblebee venom (BBV) (*Chapter 9*) Eleven patients with severe occupational anaphylaxis caused by stings of bumblebees underwent immunotherapy with BBV. All patients reached a maintenance dose in 6 weeks without severe side-effects. During a follow-up period of 1.5-5 years, three systemic reactions were seen in 20 bumblebee stings, milder compared to the index sting. This confirms that the effectiveness of BBV immunotherapy (85%) is comparable with that of other VIT. We can therefore conclude that BBV immunotherapy is safe and effective in the treatment of BBV occupational allergic patients.

4. Conclusions and directions for future research

It is obvious that the environmental situation in greenhouses is responsible for a growing number of occupational allergic employees. Our results give evidence that the ubiquitous presence of bell pepper pollen and chrysanthemum pollen in greenhouses generate a constant high exposure in employees. We speculate that other not yet elucidated environmental factors play also a role in the development of allergic symptoms in these employees. For instance, the finding that *A. cucumeris* can induce rhinitis and asthma, emphasise the importance to set up new clinical trials. We concluded that for the diagnosis of relatively new occupational allergies in house manufactured extracts are safe, reliable and more or less indispensable.
Further research should be focussed on the presence of other biological and chemical agents, the airborne levels of these sensitising agents and the primary or secondary – due to cross reactivity - sensitisation of the employees.
Since we found significant results using honeybees in bell pepper greenhouses, the next step is to investigate whether the long term use of bees can prevent the development of allergic symptoms in new employees. And, second, will the presence of bees lower the exposure in already sensitised

employees and eventually decrease symptomatology. Another occupational allergy is latex allergy. To diagnose latex allergy in house manufactured latex extracts have proven to give reliable results. In recent years latex exposure is lowered due to the use of low-allergen powder-free latex and non-latex gloves. Follow up studies should be done to confirm a decrease in the prevalence of latex allergy in employees.

In the second part of this thesis we investigated a new method to diagnose food allergy. This is a very complex and difficult area of research. The main reasons are the absence of well validated and standardised commercial food extracts and cross- reactivity between inhalation and food allergens.

When - in case of a convincing history - specific food allergic symptoms can be confirmed by a positive SPT with the responsible food allergen (and a positive RAST) an IgE mediated food allergy is most likely. This approach can be discussed and criticised, but in the absence of good diagnostic tools e.g. well-accepted standard procedures for DBPCFC and the impracticability to test series of allergens in multi-sensitised patients with DBPCFC, a diagnosis of allergy based on skin tests with in house manufactured extracts combined with a reliable and convincing history, may be appropriate in daily clinical practice.
Recently, it has been suggested that low-dose challenges may be safe to use in highly sensitised patients (Taylor 2004). Therefore, it is to be expected that the determination of threshold doses (Taylor 2004) and the development of standardised challenge materials that can be properly masked (Vlieg-Boerstra 2004) will lead to a more prominent position of the DBPCFC in daily practice.

Finally, the quality of the extract. The definition of a ‘good extract’ is widely discussed. A good extract is safe, reliable, can confirm specific allergic symptoms, contains the allergen in its native form, contains the major allergen, do not give irritative, non-specific reactions, has an optimal concentration, is reproducible, is stable, is standardised.
Standardisation of allergen products is extraordinary complex even for the common inhalant allergens (Vieths et al. EAACI 2004). Licensing procedures differ, standardisation procedures by manufacturers are based on in house references (IHR), and there is no common system for labelling activity units for allergen extracts. Furthermore standardisation methods are considerably different between the US method and the Nordic method (Turkeltaub PC. 1988, Nordic Counsel on Medicines 1989). Recently, van Ree et al. (2003) started an EU-funded project (CREATE) aiming to develop certified reference materials based on purified natural or recombinant major allergens. Twenty-nine partners from industry, research labs, regulatory bodies and clinical centres work together on 8 allergens from birch, grass and olive pollen and house dust mite. They compare ~1000 serum samples from 8 different EU member states. This large study illustrates the complexity of standardisation of extracts.
The in house manufactured extracts used in the occupational and food allergic patients described in this thesis are so called “named patient products“. They are prepared in accordance with a medical prescription for an individual patient or a group of occupational allergic employees. These products are excluded *per se* from licensing procedures.
It can be concluded that, although the extracts that have been used in the studies mentioned in this thesis, they did not meet all criteria of a ‘good extract’, the greater part of the allergic symptoms could be confirmed by SPT. The extracts were certainly safe and reliable, contained the allergen in its native form, and contained the major allergen. They did not give non-specific reactions. Stability and reproducibility were only examined for 4 food extracts from different food allergen groups: nuts, seeds, fruits and spices. The results are promising and give sufficient support for larger projects. More in house manufactured allergen extracts should be evaluated and more patients should be tested in comparison with clinical history and oral challenge to provide insights in sensitivity, specificity, cut-off values and optimal concentration. Furthermore, our occupational in house manufactured extracts proved to be safe and reliable as well. They were not examined on quality, but they could confirm presence of occupational allergy and they were indispensable in performing the first extensive prevalence studies in occupational allergic employees in horticulture in the Netherlands.
In summary, we conclude that the use of in house manufactured extracts is a reliable tool to diagnose IgE mediated allergy to food - and occupational allergens in the absence of standardised commercially available extracts.

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Summary

The aim of this thesis is, first, the diagnostic work-up of occupational and food allergies in the absence of well-validated commercially available standardised extracts for Skin Prick Test. Second, to investigate cross-reactivity in occupational and food allergic patients. Third, the treatment of, employees with an occupational allergy in order to enable the continuation of work. The number of work-related symptoms among greenhouse workers is increasing and the prevalence of food allergies is rising. The sequence of diagnostic procedures is the history first, followed by skin tests and RAST tests, and challenge tests as final instruments. Unfortunately, commercial standardised extracts to diagnose the more uncommon allergies caused by relatively unknown allergens are generally not available. Therefore, we made new in house manufactured extracts, for the diagnosis of occupational and food allergies e.g. several flower pollen, *A. cucumeris*, latex, fruits (peach, kiwi, banana, avocado), and hazelnut.

In *Chapter 2* we found that the prevalence of work related symptoms and sensitisation among greenhouse workers appeared to be very high, e.g. 57 % in *Chrysanthemum* growers. Main symptoms in these flower growers were rhinitis and conjunctivitis. Symptoms of the lower airways were found in 9%. Furthermore, in *Chapter 3*, we examined 14 florists and gardeners with severe allergic symptoms due to the handling of flowers. Sensitisation in both studies was confirmed by SPT with in house manufactured extracts. The concordance between SPT and symptoms was satisfying: 74%. In greenhouse workers the discrepancies were considerably larger than in florists. Apparently the greenhouse environment elicits more symptomatology, which cannot be explained only by IgE mediated allergy to the most likely responsible allergens. Furthermore, discrepancies can be due to quality of the extract, less reliable case history, cross-reactivity and sensitisation without symptoms.
Allergy to natural rubber latex is a major occupational problem in health care settings and in other occupations where protective gloves are used. Latex hypersensitivity can induce local symptoms (Type IV) as well as the more serious Type I allergic reactions. In *Chapter 4*, the prevalence among a laboratory population at risk was studied and 24,6% reported urticaria, rhino conjunctivitis and/ or asthma. Five employees (8.3%) appeared to have a positive SPT with one or more glove extracts; a positive patch test was found in 3 of them. All patients with positive SPT had symptoms of occupational latex allergy. There appeared to be two distinct disease groups. A type I group, all with atopy and a high prevalence of coexisting tropical fruit allergy; and a type IV allergic population without evidence of type I allergic reactions to inhalant allergens, fruit or NRL. This study and a study among surgery personnel legitimised changes in latex policy in our hospital. All gloves were changed for powder-free and or low-allergen latex.
There is evidence that the prevalence of known food allergies such as peanut allergy is rapidly increasing and that new food allergies are emerging, e.g. to sesame seed and kiwi fruit. However, the diagnosis of food specific allergy is complex. Standardised commercially food extracts are not available or of insufficient quality. In *Chapter 5,* we examined four in house manufactured food extracts: coriander, hazelnut, peach and sesame seed on stability, reproducibility and dose response. We performed SPTs in 21 food-sensitised patients with a positive history for the four mentioned allergens. Overall, pair wise comparisons of dose responses were significant; no significant differences were seen between fresh extracts, 3 months and 6 months old extracts. The mean concordance between SPT and CH was 72%. Furthermore, we found neither significant difference in varieties of fruits, nuts or seeds, nor significant seasonal variation, in the SPT results.
In *Chapter 6*, we studied sensitivity to apple, peach and hazelnut in 79 birch- pollen allergic patients. Again in house manufactured extracts were used. In this study concordance was 77%, 52% and 54% for apple, peach and hazelnut respectively which, is most likely caused by cross- reactivity as we selected our patients on birch pollen allergy. It is a well-known phenomenon that cross-reactivity may lead to false positive SPT reactions.
In case of a convincing case history concerning specific food allergy symptoms e.g. anaphylaxis or oral allergy symptoms, which can be confirmed by positive SPT with an in house manufactured extract a double blind placebo controlled food challenge is unnecessary. Dietary advice is the next step. Only in case, SPT and or RAST cannot identify the sensitising allergens responsible for

symptoms, a DBPCFC should be performed. Further investigations, examining cut-off values and optimal concentrations will be accomplished in a large study.
Throughout the thesis, cross-reactivity is involved in the sensitisation and diagnosis. In C*hapter 2 and 3,* there seems to be a striking correlation between sensitisation to flowers and mugwort pollen. We concluded that SPT with mugwort could be used as screening test for a possible flower allergy. Furthermore cross-reactivity is examined between *A.cucumeris* and *D. pteronyssinus* in *chapter 7*. In the last case two different patterns were found, one with common protein bands between both mites and one showing no shared protein bands, illustrating that allergy to *A.cucumeris* is not in all cased caused by primary sensitisation. Between inhalation and food allergens cross-reactivity is studied in *Chapter 4* (latex-fruit) and *Chapter 6* (birch pollen–food). In *Chapter 4*, four out of five employees who were sensitised to natural rubber latex had a positive SPT on exposure to avocado, banana and kiwi. Not all these patients had food specific allergic symptoms. In *Chapter 6*, extensive cross-reactivity is shown among 79 consecutive birch pollen allergic patients. In this group, SPT was positive for apple, peach and hazelnut in 64,5%, 77,2% and 89,9% respectively. We described new insights into nature and biological function of major groups of cross-reactive proteins from various sources e.g. pathogenesis related (PR) proteins.
Finally, in *Chapter 8 and 9* intervention and treatment is described. In *chapter 8*, we examined whether the reduction of pollen output from the bell pepper plant could protect the employees. To accomplish a lower pollen exposure we used honeybees, as they can be used to pollinate flowers. In the group greenhouses with high numbers of bees the percentage of pollen was reduced to 12-18%. We found a dose dependent significant decrease of VAS (visual analogue scale) in itching, sneezing droplet and total nose scores in allergic greenhouse employees. Our results are quite promising and further research is to be done. Furthermore, in *Chapter 9,* we examined whether VIT (venom immunotherapy) with BBV (bumblebee venom) is safe and effective. Eleven patients with occupational anaphylaxis were examined during a follow-up (1,5-5 years) period. Our results confirmed that the effectiveness of BBV immunotherapy (85%) is comparable with that of other VIT.

In summary, we conclude that the use of in house manufactured extracts is a safe and reliable tool to diagnose IgE mediated allergy to food - and occupational allergens in the absence of standardised commercially available extracts.

Samenvatting

In deze thesis wordt voornamelijk ingegaan op de diagnose van beroeps – en voedsel allergieën. Het aantal werknemers met beroepsgebonden klachten in de glastuinbouw neemt de laatste jaren fors toe. Daarnaast is er een stijgend aantal patiënten met een voedselallergie. Voor het vaststellen van een diagnose wordt eerst een zorgvuldige anamnese afgenomen, gevolgd door huidtesten en radioallergosorbent test met als laatste instrument een provocatie. Helaas zijn gestandaardiseerde extracten voor de diagnose van deze (minder voorkomende) beroeps - en voedsel allergieën nog niet commercieel verkrijgbaar. Daarom zijn er nieuwe 'in huis geprepareerde' extracten ontwikkeld van diverse beroeps – en voedsel allergenen zoals: paprikapollen, bloempollen, roofmijt, hazelnoot, perzik, kiwi, appel en banaan.

In *Hoofdstuk 2* is onderzoek gedaan naar de prevalentie en werk gerelateerde klachten onder chrysant kwekers: 57% bleek klachten te ondervinden tijdens het werken met deze bloemen. De voornaamste klachten waren rhinitis en conjunctivitis. Negen procent had klachten van de lagere luchtwegen. Daarnaast bezochten 14 bloemisten (*Hoofdstuk 3*) onze afdeling met beroepsgebonden klachten door het werken met diverse bloemen. Bij deze bloemisten zijn huidtesten gedaan met 17 verschillende bloempollen extracten. Overeenkomst tussen huidtest en klachten kon gevonden worden in 74% van de bloemisten. Dit getal lag beduidend lager bij de werknemers in de glastuinbouw. Van de werknemers met beroepsgerelateerde klachten had 29% een positieve huidtest met chrysantpollen extract. De atmosfeer in een kas bevat blijkbaar meer stoffen die een allergische reactie kunnen veroorzaken dan alleen de (meest voor de hand liggende) pollen. Verder kunnen er diverse oorzaken zijn voor verschillen tussen huidtest en klachten. Ten eerste kan een mindere kwaliteit van het extract de oorzaak zijn, daarnaast kan het verhaal van de patiënt onduidelijk zijn en verder kan kruis reactiviteit met andere allergenen een goede reden zijn.

Latex allergieën zijn inmiddels een bekend probleem. In *Hoofdstuk 4* hebben we bij een groep laboratorium werknemers die regelmatig met latex werkt (werknemers immunologie Erasmus MC) onderzocht hoeveel procent gesensibiliseerd is voor dit allergeen. Er bleek 24,6 % klachten van urticaria, rhinoconjunctivitis en/ of astma te hebben gedurende het werken met handschoenen. Er werden huidtesten gedaan met 5 verschillende handschoen extracten. De huidtest was positief bij 8,3 % van de werknemers. Deze werknemers hadden allen klachten. Door deze studie en een studie onder OK personeel is besloten de latex richtlijnen van het ziekenhuis te veranderen. Er worden alleen nog latex arme en poedervrije handschoenen gebruikt.

Het diagnosticeren van voedsel allergieen is bijzonder gecompliceerd niet in de laatste plaats door het feit dat er geen goede gestandaardiseerde extracten voor huidtesten verkrijgbaar zijn. Daarnaast is de dubbel blinde placebo gecontroleerde voedsel provocatie (DBPCFC) de gouden standaard voor de diagnose van een voedselallergie. Helaas is deze methode arbeidsintensief en nog niet voor alle voedingsproducten bruikbaar. Algemeen geaccepteerde standaard procedures zijn niet voorhanden. Er wordt veel onderzoek gedaan naar de juiste receptuur en protocollen.
Bij gebrek aan gestandaardiseerde extracten en procedures worden er op de afdeling Allergologie van het ErasmusMC 'in huis geprepareerde' extracten vervaardigd voor de diagnose van IgE gemedieerde voedselallergie.
In *Hoofdstuk 5*, zijn vier van deze extracten (koriander, hazelnoot, perzik en sesam zaad) onderzocht op stabiliteit, houdbaarheid en dosis relatie. Er zijn huidtesten gedaan met deze extracten bij 21 patiënten met klachten na het eten van deze voedingsmiddelen. De gemiddelde concordantie tussen huidtest en klachten was 72%. Gepaarde vergelijkingen en dosis response waren significant. Er waren geen significante verschillen tussen verse extracten, en 3 maanden en 6 maanden oude extracten. In *Hoofdstuk 6*, wordt een onderzoek beschreven waarbij 79 berkenpollen allergische patiënten getest zijn op sensibilisatie voor appel, perzik en hazelnoot. In dit onderzoek werden ook 'in huis geprepareerde extracten' gebruikt. De concordantie tussen klachten en huidtest lag in dit onderzoek lager: 77%, 52% en 54% voor appel, perzik en hazelnoot respectievelijk. De reden hiervoor kan gevonden worden in het feit dat we berkenpollen allergische patiënten hebben geselecteerd. Kruis

sensibilisatie is een bekend fenomeen in boompollen allergische patiënten. Dit kan een positieve huidtest tot gevolg hebben zonder dat de patiënt klachten heeft bij het eten van het voedingsmiddel. Aangezien er nog geen goede gestandaardiseerde extracten verkrijgbaar zijn en DBPCFC vooralsnog onvoldoende geprotocolleerd en niet makkelijk implementeerbaar zijn, vormen huidtesten met deze 'in huis geprepareerde' extracten een acceptabel diagnosticum om een zorgvuldige anamnese te ondersteunen. Verder onderzoek dient te worden gedaan naar de optimale concentratie en cut-off waarden van meerdere verschillende extracten.

In de diverse beschreven beroeps – en voedsel allergie onderzoeken in deze thesis speelt kruissensibilisatie tussen allergenen een rol. In *Hoofdstuk 2* is een sterke kruisreactiviteit gevonden tussen bloemen van de *Compositae* familie en Bijvoet. Een huidtest met Bijvoet kan gebruikt worden als screening voor een mogelijke bloemenallergie. Daarnaast is in *Hoofdstuk 7* kruisreactiviteit onderzocht tussen *A.cucumeris* en *D.pteronyssinus.* Daarbij werden twee duidelijk verschillende patronen gevonden. In sommige gevallen werden dezelfde eiwit banden gevonden in *A.cucumeris* en *D.pteronyssinus* en in andere gevallen was er juist geen overeenkomst te vinden. Hieruit blijkt dat het bij een allergie voor *A.cucumeris* niet altijd gaat om een primaire sensibilisatie. Kruissensibilisatie tussen inhalatie – en voedsel allergenen is onderzocht in *Hoofdstuk 4* (latex en fruit) en *Hoofdstuk 6* (berken pollen en voedsel) In de latex studie hadden 4/5 werknemers met een latex sensibilisatie tevens een positieve huidtest met avocado, banaan en kiwi. Deze werknemers hadden niet altijd klachten van deze voedingsmiddelen. Verder is intensieve kruissensibilisatie is beschreven in 79 berkenpollen allergische patiënten in *Hoofdstuk 6.* Er werden positieve huidtesten met appel, perzik en hazelnoot gevonden in 64.5 %, 77.2% en 89.9% respectievelijk. Niet al deze patiënten hadden klachten bij het eten van deze voedingsmiddelen. Recent onderzoek heeft uitgewezen dat deze allergenen tot bepaalde kruisreaktieve eiwit groepen behoren, zoals bijvoorbeeld pathogenese gerelateerde eiwitten.

Als laatste is in *Hoofdstuk 8 en 9* interventie en behandeling beschreven. Interventieonderzoek (*Hoofdstuk 8*) had tot doel de pollen expositie in de paprikakassen te verlagen zodat de allergische werknemers hun baan kunnen behouden. Dit is gebeurd door bijen in te zetten die het stuifmeel uit de bloemen halen. Er is een significantie daling van de allergische neusklachten gevonden in deze paprikapollen allergische werknemers. Deze resultaten zijn hoopgevend en er zal meer onderzoek gedaan worden om de werkomstandigheden van deze werknemers te verbeteren.
Daarnaast is er in *Hoofdstuk 9*, onderzocht of immunotherapie (IT) met hommelgif veilig en efficiënt is voor werknemers met een hommel allergie. Gedurende een studie van 5 jaar werden elf hommel allergische patiënten vervolgd, die IT met hommelgif ondergingen. De resultaten bevestigden dat IT met hommelgif net zo veilig en efficiënt is (85%) als IT met andere insectengiffen (wespen – en bijengif)

Dankwoord

Voorafgaand aan elk hoofdstuk of artikel van dit proefschrift dacht ik: “dit lukt me nooit”. Gelukkig dachten mijn familieleden, collega’s vrienden en kennissen daar anders over. Zonder hun steun, vertrouwen en liefde was het me niet gelukt dit proefschrift te schrijven. Ik wil hierbij een aantal mensen daarvoor persoonlijk bedanken.
Allereerst Dr. R. Gerth van Wijk, co-promotor. Roy, hartelijk bedankt voor je vertrouwen in mij, je was zo overtuigd van mijn kansen om te gaan promoveren, dat ik er in ging geloven. Je hebt me enorm veel vrijheid gegeven om mijn ideeën uit te voeren en je hebt me de kans gegeven om opleidingen te volgen. Vooral ben ik je dankbaar voor je geduld en je begeleiding de afgelopen maanden, die absoluut de zwaarste waren in mijn carrière.
Zonder Dr. H. de Groot had ik nooit al dit onderzoek kunnen doen. Hans, jaren van keihard werken, creativiteit, tegenslagen en successen. We konden goed samenwerken en organiseren. Samen begonnen we aan het boompollen onderzoek. Wie had kunnen weten dat het de start was van 8 jaar onderzoek dat nu geleid heeft tot mijn promotie. Je hebt me enorm veel geleerd en gestimuleerd, daar ben ik je erg dankbaar voor. Maar ook voor al het plezier en de lol. Je zorgde voor de krenten in de pap. Congressen bezoeken, presentaties, posters, het was altijd leuk. Ik hoop dat we nog jaren samen onderzoek doen.
Prof.dr. H.A.P. Pols, promotor. Huib, ik ben je dankbaar voor het vertrouwen en je enthousiaste reacties op mijn werk. Ik hoop op een goede samenwerking in de toekomst.
De leescommissie, bestaande uit Prof.dr. H.F.J. Savelkoul, Prof.dr. J.M.W. Hazes en Dr.ir. A.Burdorf dank ik voor het beoordelen van dit proefschrift.
Dr. P.G.M. Mulder. Beste Paul, wat ben ik blij dat jij voor mij de statistiek wilde doen. We hebben wat uurtjes in jou kamer doorgebracht. Ik begreep er vaak niks van en je probeerde met alle geduld uit te leggen hoe je aan die significante getallen kwam. Zonder jou was het niks geworden. Nogmaals mijn hartelijke dank.
Dr. A.W. van Toorenenbergen. Albert, mijn interesse in de allergologie is bij jou op de afdeling ontstaan. Vaak hebben we samen langdurig zitten filosoferen over nieuwe allergenen, bepalingstechnieken en onderzoeksvragen. Je gaf me de vrijheid zelf met ideeën te komen en uit te voeren. Toch kon je mijn vertrek naar de afdeling Allergologie goed begrijpen, ik was razend nieuwsgierig naar de klinische achtergronden achter de serumwaarden. Ik ben je dankbaar voor alles wat je me geleerd hebt en hoop op een langdurige samenwerking.
Dr. P.H. Dieges. Paul, je hebt me deze baan aangeboden in 1994. Ik heb geen moment getwijfeld en ik ben je enorm dankbaar voor het vertrouwen en de kans die je me gegeven hebt om mezelf op deze afdeling te bewijzen.
Ab Vermeulen. Ab, zeker 25 jaar was je een kanjer van een collega met veel humor. We zijn vrienden geworden en dat blijft zo. Je rust, je geduld, je probeerde me alles te leren. Met die rust is het niks geworden en je werd vaak moe van mijn (ADHD) gedrag. Toch konden we goed samenwerken en uiteindelijk zijn we allebei docent. Door de jaren heen heb je mij vreselijk veel geleerd op allerlei terreinen. Daar ben ik je dankbaar voor en wens je alle succes in het onderwijs.
Dr. G.C.M. Groenewoud. Giséle, samen hebben we moeten zwoegen en ploeteren in de paprika- en chrysantkassen. Vroeg op weg met een auto vol en laat weer terug op de afdeling. Bloedprikken (niet mijn hobby), kwaliteit van leven lijsten, huidtesten, allemaal in de kas. Het was zwaar, maar enorm gezellig. In die tijd at iedereen paprika’s en namen we vaker een bloemetje mee. Bedankt voor de leuke tijd en de geweldige samenwerking. Ik wens je alle succes in je verdere carrière.
Dr. A. Berghout. Arie, ik wil je bedanken voor het kritisch lezen van de introductie en discussie en je adviezen wat betreft het Engels. De tips waren onmisbaar en hebben het echt af gemaakt.

Ik wil alle personeelsleden van de poli Allergologie heel hartelijk bedanken: Irene, Ingrid, Jiska, Ellen, Marina, Rinia, Linda, Wilma en Conny. Ik was de afgelopen maanden niet altijd even gezellig en werd alleen maar drukker. Als het allemaal niet lukte of tegenzat kwam er altijd wel iemand me moed inpraten. Ik ben jullie enorm dankbaar voor de verdraagzaamheid en hulp. Ik hoop dat we nog jaren met elkaar op deze afdeling blijven werken en veel gezellige koffie uurtjes doorbrengen. Irene en

Ingrid wens ik heel veel succes in hun nieuwe baan. Annemarie Bijl, met jou heb ik het latex onderzoek op de OK gedaan. Het was een leuke tijd en daar wil ik je graag voor bedanken.

De personeelsleden van lab Allergologie wil ik ook graag bedanken: Paul, Ingrid en Joep. Paul, ik ben je dankbaar voor je hulp bij het doen van de RAST-en. Je hielp me met de apparatuur en was altijd bereid me van alles uit te leggen. Wat ik wilde kon soms eigenlijk niet of mocht eigenlijk niet. Je bent (bijna) nooit boos geworden op mijn dwingende karakter en hielp me om het toch voor elkaar te krijgen. Het moest ook altijd gisteren klaar. We hebben een hoop lol gehad, ik ben je voor alles enorm dankbaar. Kees van Leeuwen van de Afdeling Klinische Chemie (AKC) bedank ik hartelijk voor de vele eiwit bepalingen die hij voor mij gedaan heeft.
Tijdens het onderzoek heb ik gebruik gemaakt van apparatuur van andere afdelingen. Ik wil de medewerkers hartelijk bedanken voor het gebruik van hun apparatuur, hun hulp en gezelligheid. Ten eerste, het personeel van de afdeling Metabole ziekten van Inwendige Geneeskunde. Trinet, en alle andere medewerkers, bedankt. Daarnaast het personeel van het Hypertensie lab van Inwendige Geneeskunde. René, Jeanette en Usha, bedankt.
De groenten – en fruithandel M.Vreugdenhil wil ik bedanken voor alle moeite die zij hebben gedaan om buiten het seizoen perziken en andere vruchten te leveren.

Ria Bikker, paranimfe. Lieve Ria, je bent mijn beste vriendin. Al jaren bespreken we van alles en nog wat tijdens het lopen op zondagmorgen. Je hebt de promotie aan zien komen en nu is het zover. Ik wil je bedanken voor alle adviezen, je hulp en je aandacht. Je hebt me erdoorheen gesleurd. Ik heb een enorm veel respect voor je. Ik dank je voor alles.
Marianne de Vries, zusje, paranimfe. Lieve Marianne, ik ben je dankbaar dat je mijn paranimfe wilt zijn. Je hebt de afgelopen jaren vaak mijn verhalen over de promotie met geduld aangehoord. Je hebt me enorm blij gemaakt met de verrassingsparty vorig jaar toen ik mijn 2^{e} graad Biologie haalde. Dat was geweldig.
Alfred de Jong, broertje. Lieve Alfred, je gaat tijdens de promotie filmen en foto's maken. Telkens als ik iets spannends meemaak sta jij daar met je camera's. Het is altijd perfect, je bent een kanjer. Ik heb je al zo vaak beloofd mee te gaan zeilen of surfen. Ik hoop dat ik die belofte eindelijk waar ga maken na mijn promotie.
Verder dank ik familie, schoonfamilie, vrienden en kennissen die ik niet bij naam genoemd heb voor alle steun, liefde en begrip.

Loes en Ilse. Lieve meiden, het valt niet mee met een moeder die twee banen heeft, afstudeert en vervolgens promoveert. Toch was het een leuke drukke tijd en sinds ik voor de klas sta begrijp ik veel beter waar jullie mee bezig zijn. De mailtjes waren leuk: "eejj mams hoezzie, 't is egt fa2 zo'n promo fiksuh, maar ff w88 want ik ga loesoe, BRB CU lateRRrr…:-))))))))-xXx". In die drukke tijd gingen jullie gewoon door met keihard leren en hockeyen. Ik ben enorm trots op jullie en dankbaar dat ik dit allemaal heb mogen doen.
Mijn ouders wil ik bedanken voor hun eindeloze steun en vertrouwen. Dit boekje is niet voor niks aan jullie opgedragen. Jullie waren altijd bereid te helpen, hebben altijd en overal voor me klaargestaan. Zonder jullie hulp en liefde was het absoluut niet gelukt. Ik ben jullie enorm dankbaar.
Lieve Jan, het is af. Terwijl ik avonden, zaterdagen en zondagen achter mijn computer in het ziekenhuis zat te werken, heb jij thuis de kar alleen getrokken. Ik ben je daar dankbaar voor. Ik hoop dat er nu een rustiger tijd aankomt.

Curriculum Vitae

Nicolette Wilma de Jong is geboren op 9 september 1957 te Rotterdam. Het MAVO diploma is behaald in 1972 in Ridderkerk. Direct daarna is zij gestart met de in-service opleiding MBO (klinische chemie) in het toen geheten "Dijkzigt" ziekenhuis in Rotterdam. Het diploma werd behaald in 1975. Vervolgens in 1977 begonnen aan HBO (klinische chemie) op het van 't Hoff instituut in Rotterdam. In 1987 heeft zij de opleiding post - HBO immunologie aan het van 't Hoff instituut met positief resultaat gevolgd. HBO 2^{e} graad Biologie (*B Ed*) heeft zij afgerond in 2003 aan de Hogeschool Rotterdam. Zij heeft in 1999 en 2003 een posterprijs ontvangen van de Nederlandse Vereniging voor Allergologie.
Als analiste was zij werkzaam vanaf 1978 op de afdeling algemene klinische chemie (AKC) van het Erasmus MC. Vervolgens was zij werkzaam als research analiste van 1991- 1994 op het laboratorium Allergologie van het AKC onder supervisie van Dr. A.W. van Toorenenbergen. Vanaf 1994 werd zij aangesteld als research analiste op de afdeling Allergologie van het Erasmus MC onder supervisie van Dr. P.H. Dieges. Vanaf 1997 tot heden verricht zij, onder supervisie van Dr. R. Gerth van Wijk en Dr. H. de Groot, naast routine werkzaamheden, onderzoek naar beroeps – en voedsel allergieën, hetgeen geresulteerd heeft in dit proefschrift. Inmiddels is zij aangesteld als wetenschappelijk medewerker op deze afdeling. Sedert 2003 is zij tevens werkzaam als docente Biologie en Scheikunde op een middelbare school.

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