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Health effects of predatory beneficial mites and wasps in greenhouses

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The reports are, however, published because the Danish EPA finds that the studies represent a valuable contribution to the debate on environmental policy in Denmark.

Contents

Preface

The present report is the final report for the study, started in December 2003 based on material from a 3-year follow-up study of greenhouse workers carried out in the period 1997-2001 based on grants from the Danish Environment Protection Agency's Pesticide Fund and the Danish Environmental Research Program. The present study has been carried out based on grant no. 7041-0244 and from the Danish Environmental Protection Agency's Pesticide Research Fund.

The aim of the present study is the health effects of exposure to beneficial arthropods in the form of parasitoids and mites. In the study the individual exposure to the arthropods has been assessed and methods to measure the specific IgE antibodies and in vivo test of the allergic response against the animals have been developed.

Annie Enkegaard, Senior Scientist, M.Sc., Ph.D, Danish Institute of Agricultural Sciences, Flakkebjerg, Denmark has authored the chapter on biology of predators. Per Stahl Skov, Research director, M.D., Dr. Med. Sc., RefLab A/S, Copenhagen has planned and carried out analysis of histamine release. Gert Doekes, Associate professor, M.Sc., Ph.D, Institute of Risk Assessment Sciences, University of Utrecht, the Netherlands has made extracts for the testing of IgE and HR as well as the analyses of specific IgE. Research Assistant M. Sc., Mia Birkhøj Kjærstad, has made the exposure assessments, Professor M.D., Ph.D. Torben Sigsgaard, Department of Environmental and Occupational Medicine, Institute of Public Health; Aarhus University has helped with planning of the study and revision of the report.

Besides the authors a large group of persons have contributed to the project: Preben Larsen, M.D., Ph.D-student, Department of Occupational and Environmental Medicine, Odense University Hospital was the main researcher on BIOGART study, the basis of the present study. Laboratory assistant Kirsten Østergaard, Department of Environmental and Occupational Medicine, Institute of Public Health, Aarhus University has made preparation and weighing of filters and assisted in collecting airborne samples of the predators. Research Secretary, Rikke Lørup Larsen and Research Assistant Heidi Pöckel Department of Occupational and Environmental Medicine, Odense University Hospital have helped with the administration and writing of the report. Professor, Ph.D Michael Væth, Institute of Public Health, Aarhus University has given advise about the statistical analyses.

We thank the workers and employers at the participating greenhouse firms for their help in the different phases of the project.

On behalf of the project group

Jesper Bælum
Project Manager

Odense, February 2007

Sammenfatning

Formålet med projektet er at vurdere, om nyttedyr udgør en risiko for allergi, astma og andre luftvejssygdomme hos de ansatte i væksthusgartnerier.

Nyttedyr har vundet stor udbredelse i danske væksthuse. Det drejer sig især om fire typer: snyltehvepse, rovmider, rovtæger og nematoder. Udenlandske undersøgelser har vist, at trips-rovmiden *Amblyseius cucumeris* og spinde-rovmiden *Phytoseiulus persimilis* giver anledning til dannelse af IgE antistoffer hos 20-25 % af de ansatte. Snyltehvepse *(Aphidius colemani* og *Encarsia formosa)* kan give astma og allergi hos personer, som opformerer snyltehvepsene. Forekomsten af astma og høfeber i gartnerierhvervet har i europæiske undersøgelser været næsten lige så høj som hos personer i landbruget.

Undersøgelsen er baseret på data og materiale fra en stor epidemiologisk 3-års follow-up undersøgelse af ansatte på 31 væksthusgartnerier gennemført i perioden 1997-2001 (BIOGART studiet). Formålet med dette studie var at undersøge den sundhedsmæssige effekt af de tre hyppigst forekommende typer af mikrobiologiske bekæmpelsesmidler med vægten lagt på udvikling af allergi og inflammatoriske luftvejssygdomme.

I undersøgelsen deltog 579 personer. Heraf blev 256 personer fulgt gennem tre år og ca. 400 fulgt i mindst et år. I hver undersøgelsesrunde blev der foretaget interview vedrørende eksponering for mikrobiologiske bekæmpelsesmidler, kemiske bekæmpelsesmidler og nyttedyr, håndtering af planter samt en række baggrundsoplysninger. Der var desuden detaljerede oplysninger om luftvejs- og hudsymptomer, målinger af lungernes funktion og følsomhed for histamin samt priktest over for standardallergener. Desuden var der blodprøvemateriale (serum) fra personerne.

I studiet af nyttedyr blev der gennemført en vurdering af personernes eksponering for rovmider og snyltehvepse. Vurderingerne er baseret på deltagernes opgivelser i løbet af undersøgelsen suppleret med resultater af en rundspørge til gartnerierne i 2004. Resultaterne viste, at 80 % af de ansatte arbejdede i væksthuse, hvor nyttedyr blev anvendt og 4-10 % håndterede selv dyrene ved udspredning. Eksponeringen for seks nyttedyr, snyltehvepsene *Aphidius colemani* og *Encarsia formosa samt* rovmiderne *Phytoseiulus persimilis, Hypoaspis miles, Amblyseius cucumeris* og *Amblyseius californicus* blev vurderet.

Der blev foretaget undersøgelse for IgE antistoffer mod *A. colemani, A. cucumeris, P. persimilis, H. miles* og spindemiden *Tetranychus urticae* i prøverne fra de sidste to undersøgelsesrunder på alle deltagere, i alt 662 analyser af hvert dyr. Der anvendtes en metode udviklet i forbindelse med projektet.

Ved brug af de samme ekstrakter af tre af de inkluderede nyttedyr *A. colemani, A. cucumeris* og *P. persimilis* er der testet for en allergisk reaktion (histamin release test) i blodet hos personerne. Princippet ved metoden er, at personens hvide blodlegemer (de basofile leukocytter) kan være sensibiliseret til at reagere mod det udefrakommende allergen, her ekstraktet fra nyttedyrene. Hvis de reagerer, frigiver de histamin, som kan måles. I dette studie anvendes

en indirekte metode, hvor celler fra bloddonorer behandles med den undersøgte persons serum og herefter testes med allergenet. Histaminfrigørelsestesten blev udført på alle de personer, som viste reaktion i IgE (70 personer) samt en udvalgt kontrolgruppe uden IgE reaktion (30 personer). Tre eller fire prøver blev analyseret for hver af disse personer, i alt 380 analyser. Desuden blev 138 personer uden kontakt til gartnerier undersøgt. Disse personer havde deltaget i en befolkningsundersøgelse af astma.

Resultaterne viste, at der var målbare IgE antistoffer mod alle dyrene. *A. colemani* viste positive værdier hos ca. 4 %, men der var ikke nogen sammenhæng med den opgivne eksponering. Miden *A. cucumeris* viste positive værdier hos 8 % med en vis overhyppighed af høje værdier blandt dem, der arbejdede i væksthuse, hvor miden blev brugt. *P. persimilis* og *H. miles* viste hyppigheder på ca. 3 % uden relation til eksponering. *T. urticae* havde en hyppighed på ca. 6 %. I modsætning til hos nyttedyrene var der en øget hyppighed af posititv IgE over for *T. urticae* hos personer med kendt allergi over for de almindelige allergener (pollen, dyr, husstøv).

Histamin-frigørelsestesten viste betydeligt større følsomhed end IgE. Blandt personer som arbejdede i gartnerier, hvor *A. cucumeris* blev anvendt reagerede 13 % i starten af studiet stigende til 22 % i slutningen, mens 5 % af personerne uden udsættelse for *A. cucumeris* viste reaktion. Otte personer, heraf syv eksponerede, blev sensibiliseret over for *A. cucumeris* i løbet af studiet, målt ved mindst to-trins stigning i histaminreaktionen.

P. persimilis viste et lignende billede, men syntes at være lidt mindre specifik, idet andelen af eksponerede personer, som reagerede, steg fra 30 % til 35 %, De ikke eksponerede varierede mellem 15 % og 32 %, men viste svagere reaktioner end de eksponerede. Syv personer, heraf fire eksponerede blev sensibiliseret i løbet af undersøgelsesperioden.

A. colemani viste positive værdier hos ca. 30 % men uden forskel mellem eksponerede eller ikke eksponerede. Fem personer viste tegn til sensibilisering i løbet af studiet, fire eksponerede og en ikke-eksponeret.

På basis af resultaterne af histaminfrigørelsestesten blev der gennemført et feltforsøg med måling af allergen fra *A. cucumeris* i luften på et gartneri. Over tre arbejdsdage blev der foretaget personbårne opsamlinger af støv. Opsamlingerne blev foretaget i tre afdelinger, hvor *A. cucumeris* blev anvendt i de to. Miderne var placeret på bordene og blev udskiftet på undersøgelsens anden dag. I alt 25 støvprøver fra luften og fra det anvendte mideprodukt blev testet med reaktion i serum fra fem personer, som havde vist kraftig reaktion på *A. cucumeris.*

Der kunne måles mideallergen i fire af luftprøverne, alle fra områder, hvor *A. cucumeris* blev anvendt. Koncentrationen varierede stærkt, og metodens følsomhed var ikke optimal, antageligt fordi en stor del af allergenet hang fast på filtrene.

Der er foretaget en statistisk analyse af sammenhængen mellem eksponering, sensibilisering og de sundhedsmæssige oplysninger fra den epidemiologiske follow-up. Det drejer sig om symptomer fra lunger, næse, øjne og hud samt ændringer i lungefunktionen og lungernes reaktivitet over for histamin. Der er analyseret dels for de første målinger for alle 579 personer, som deltog i

undersøgelsen, og dels den sidste måling for de 338 personer som havde deltaget i de sidste to undersøgelsesrunder og stadig var ansat i et af gartnerierne. Endelig blev der lavet en analyse af effekten af eksponeringen på antallet af nyopdukkede (incidente) symptomer og ændringer i lungefunktionen.

Der var en vis sammenhæng mellem målene for eksponering og forekomsten af de mange forskellige symptomer, men ikke et helt systematisk billede. Der var en vis sammenhæng mellem forekomsten af IgE over for *A. cucumeris* og *P. persimilis* og forskellige symptomer fra lungerne, mens der var en sammenhæng mellem IgE over for snyltehvepsen *A. colemani* og symptomer fra næse og øjne. Der var ingen effekt af eksponering eller sensibilisering på symptomscore for astma eller på lungefunktionen.

Et paradoks var, at de personer, som selv håndterede nyttedyrene og derfor var mest eksponerede, generelt havde lavere symptomhyppighed end de øvrige grupper. Dette kan enten skyldes, at der er sket en udvælgelse, idet personer med luftvejsgener holder op med denne aktivitet ("healthy worker effect"), eller at den væsentligste eksponering sker ved håndtering af planterne senere hen.

Fem personer viste tegn til at udvikle allergiske reaktioner over for et eller flere af nyttedyrene i løbet af undersøgelsesperioden. Det så ud til, at symptomer udviklede sig samme år som sensibiliseringen. De fem personer udviklede gener fra næse og øjne, mens astma ikke blev registreret. De fleste havde samtidig allergi over for et eller flere almindelig forekommende allergener.

Projektet viste, at arbejde med rovmiderne *A. cucumeris* og *P. persimilis* giver risiko for udvikling af allergi og symptomerne overvejende af høfeberlignende karakter med gener i øjne og næse og svælg. Snyltehvepsen *A. colemani's* sundhedsmæssige betydning er mere usikker. Den sundhedsmæssige betydning af nyttedyrene er antageligt større end for de mikrobiologiske midler, som blev undersøgt med samme metoder.

Undersøgelsen viser, at der bør udarbejdes retningslinjer for brugen af nyttedyr. Undersøgelsen giver dog ikke mulighed for at foreslå omfang og karakter af disse retningslinjer. Nyttedyrene er kun en mindre del af det miljø i gartnerierne, som kan give anledning til allergi hos de ansatte, idet planterne og den mikrobielle vækst i gartnerierne også har væsentlig betydning.

Yderligere forskning må vise, hvor stor udsættelsen er for allergener fra nyttedyrene, dels ved håndtering, men også fra planterne gennem deres vækst og ophold hos konsumenterne. Desuden ønskes en bedre vurdering af hvilke personer, som er i risikogruppen for allergisk sygdom, og hvor meget, der skal til for at udløse sensibilisering eller symptomer. Endeligt er det væsentligt at vide, om nyttedyrene kan udløse en mere alvorlig sygdom som astma.

Summary

The aim of the project was to describe to which extend beneficial predatory arthropods are risk factors for development of allergy, asthma, and other airway diseases among employees in greenhouses culturing ornamental plants.

Beneficial predatory arthropods are widely used in Danish greenhouses. The most frequently used are parasitoids, predatory bugs, mites, and nematodes. The mite *Amblyseius cucumeris* used for the control of thrips and *Phytoseiulus persimilis* used for the control of spider mites have in studies caused sensitization in about 25 % of the persons exposed. No direct information of the predatory wasps in study is available but some other wasps have caused asthma in the persons breeding them. European studies have shown a high prevalence of asthma among greenhouse workers, almost on level with that in farmers.

The investigation has been based on data and biologic material from an epidemiological follow-up study of employees at 31 different greenhouse firms (the BIOGART study). The primary goal of this study was to investigate the health effects of microbiological pest management on allergic and inflammatory airway diseases.

In the study 579 persons participated of who 256 were followed during three years with annual examinations while 400 were followed for at least a year. At each examination an interview was done including the use of predators, microbiological pest management, and pesticides. Besides, the persons were asked about symptoms from the lungs, nose, eyes, and skin. Finally, lung function tests, a test of the bronchial reactivity, prick tests for common allergens were made and blood samples were taken.

The actual project included an evaluation of the exposure to predatory mites and wasps. This evaluation was based on the information from the participants in the annual examinations combined with the results of a survey conducted in the participating greenhouses in 2004.

Eighty percent of the participants worked in greenhouses where the beneficial arthropods were applied while 4-10 % handled the products themselves during the observation period. Two predatory insects, *Aphidius colemani* and *Encarsia formosa* and three mites *Amblyseius cucumeris*, *Phytoseiulus persimilis*, and *Hypoaspis miles* were included as well as the spider mite *Tetranychus urticae*, which is naturally occurring in the greenhouses and used as prey for the beneficial species.

Screening for IgE antibodies against *A. colemani*, *A. cucumeris*, *P. persimilis*, and *H. miles* in the last two blood samples of the persons detected antibodies against all the species. For *A. colemani* 4 % showed positive values while *A. cucumeris*, *P. persimilis*, and *H. miles* showed sensitization rates of 8, 3, and 3 %, respectively. Only antibodies against *A. cucumeris* were to some extend, related to the measures of exposure while no relation was seen in the other species. Particularly the persons with the highest exposure, those who had

handled the animals themselves, had the lowest rate of sensitization to all the animals.

A histamine liberation test against the two mites *A. cucumeris* and *P. persimilis* and the wasp *A. colemani* was carried out on a sample of the persons. The principle in this is that basophile leucocytes from the persons blood reacts with the antigen in the extract by liberating histamine depending on the degree of sensitization. In the actual setup an indirect method was used as donor blood cells were incubated with the person's serum and then reacted with the antigen. The 70 persons tested were those who had reacted with a positive IgE to one of the arthropods and 30 persons without a positive IgE. For each person all samples, three or four were analyzed. Besides, test was carried out on 138 persons without relation to agriculture or the greenhouse trade. These persons were participants in a population study of asthma.

The histamine reaction was considerably more sensitive than the IgE. Among the persons exposed to *A. cucumeris* the rate of positive reactions during the study increased from 13 % to 22 % during the observation period while the unexposed showed a rate of 5 %. Eight persons showed signs of sensitization during the study defined by two levels increase in the reaction. Seven of these persons were exposed in the greenhouse.

The rate of sensitization against *P. persimilis* was somewhat higher than for *A. cucumeris* from 30 % to 35 % among the exposed compared to 15 % to 32 % among the unexposed. Seven persons showed signs of sensitization during the study, four of these were exposed to *P. persimilis.*

For *A. colemani* the sensitization rate was 30 % without relation to the exposure. Five persons were sensitized, four exposed and one non-exposed.

Based on the histamine reaction a pilot study of exposure measurements was conducted in a large greenhouse firm using *A. cucumeris.* During three workdays airborne antigen was sampled in dust filters of personal samplers in three different departments, *A. cucumeris* being used in two. Samples were taken before, during, and the day after the application of mites. Dust samples from 25 filters were eluated and tested against five different sera from persons with a strong reaction against *A. cucumeris.*

In four of the 25 filters antigen could be quantified. They were sampled in the departments where the mites were used and the concentration varied from 0.2 to 70 µg/m^3. The method, however, is not optimized and needs improvements to achieve a satisfactory recovery of antigen from the filters.

The statistical association between the prevalence of symptoms on one side and the estimates of exposure and sensitization on the other side was analyzed. The prevalence of symptoms at the first examination of the 579 persons was tested in a logistic regression with sex, status of atopy, and smoking habits as additional variables. Similar analyses were made on the last samples on the persons participating in the two last examination rounds (338 persons). In this group the relation between symptoms and the sensitization to each of the four beneficial species and *T. urticae* was tested, too. Besides, the incidence of symptoms, the decline in lung function, and the variation in bronchial reactivity were tested against the exposure variables.

There was some correlation between exposure variables and some of the vast number of prevalent symptoms, although not systematically. There were some significant relations between sensitization to the mites and lung symptoms while sensitization to the wasp *A. colemani* was correlated with nasal and eye symptoms. There was no effect of exposure on either asthma score or the different measures of lung function.

Consistently, the persons actually handling the animals generally had fewer symptoms than the rest. This may either be attributed to "healthy worker selection" or the exposure may not be specially related to the actual handling of the predators.

Five persons showed both signs of developing sensitization to one of the mites and a change in symptoms during the observation period. Symptoms from eyes and nose seemed to develop concomitantly with sensitization while no sign of asthma was seen. Most of the persons also had or developed other common allergies.

The study showed that occupational exposure to the two mites *A. cucumeris* and *P. persimilis* give rise to allergic sensitization and possibly eye and upper airway symptoms. The picture of the insect *A. colemani* with no sign of sensitization but some exposure related symptoms is more unclear. The group of predators are then one among a number of allergens in the greenhouses i.e. certain plants and naturally occurring fungi.

As the study shows possible health effects of the predatory animals guidelines and preventive measures when handling at least the mites are suggested. More information about the exposure is needed before these measures can be planned in more details.

Further studies are needed to evaluate the exposure to antigen both directly during handling of the products and by indirect exposure when handling the plants. The dose needed to sensitize or cause symptoms has to be characterized as well as the persons at risk. Finally, the possibility of the beneficial arthropods to cause the more serious disease asthma will have a great impact on the need for regulation of the use of the beneficial arthropods.

1 Introduction

The increasing awareness of the problems with pesticides used in greenhouses has led to a widespread use of biological control of pests both in the form of microbiological pest management and the use of beneficial arthropods. Beneficial arthropods as mites, parasitoids, ladybirds and nematodes have been introduced and used extensively in the production of vegetables and ornamental plants in Danish greenhouses during the last 30 years.

In parallel microbiological pesticides have been introduced in form of bacteria (i.e. *Bacillus thuringiensis*) for the control of larvae of butterflies and moths, ascarids etc., and fungi (i.e. *Verticillium lecanií)* for the control of different pests, aphids and whiteflies in environments with a high humidity. *Trichoderma harzianum* has been used as an antagonist for control of the pathogenic fungus *Botrytis cinerea* (grey mould). The health effects of these microbiological products have been investigated in the study which is the base of the actual project (Larsen & Bælum, 2002).

Introducing a new technology always gives rise to questions about the possible harmful effects on the health of the workers and consumers who may get in contact with the organisms or their waste products.

Toxicity, sensitization, and inflammatory airway diseases are the most likely adverse health effects of the arthropods. This is due to the protein content of these highly complicated animals, supported by the well known cases of allergy to closely related species as house dust mites, storage mites, and insects as cockroaches.

The possible health problems of exposure to beneficial arthropods (macrobiological pest controls) were anticipated in the planning of the study, and questions about handling of the animals have been included in the questionnaires, filled out at the annual examinations.

The present study is based on the material sampled during the follow-up study from 1997 to 2001 and supplemented with an inquiry in 2004 and field studies carried out in selected greenhouses in 2006.

1.1 Health of greenhouse workers

Greenhouse workers are a group of unskilled and skilled workers. In Denmark mostly women work in this trade.

According to mortality and general morbidity this group does not differ from other comparative working groups. They might actually be healthier when living in rural areas with lower morbidity than seen in the more densely populated urban areas. In the Danish register of hospital admissions in different jobs and trades 1995-99 (Hernandez *et al.*, 2005) the greenhouse workers were included in the group of “aids in agriculture, forestry, and fishing”. Morbidity rates were compared with all actively working persons stratified according to gender. A large difference between males and females

was seen for asthma with an index of 172 (CI95%: 105-266) for males while the females had a below average index of 53 (CI95%: 32-82). The males may primarily be farmer's hands, a group with a well known high prevalence of asthma. In a Finnish study of occupationally related incidence of asthma, gardeners had an incidence ratio of 1.62 (CI95%: 1.19-2.19), higher than the average of the working population but low in relation to other agricultural workers (Karjalainen *et al.*, 2002).

Occupationally related allergy and respiratory diseases have been studied in the large European study of respiratory diseases (ECRHS) covering 34 centers in 15 different European and other industrialized countries. In the study, greenhouse workers were included among other agricultural workers having a prevalence rate ratio (PRR) for asthma of 1.79 (95%CI 1.02-3.16), the reference group being office workers. (Kogevinas *et al.*, 1999). Comparable jobs were farmers (PRR =2.62), painters (PRR = 2.34), and cleaners (PRR=1.97).

Among growers in four European countries (Denmark, Germany, Sweden, and Spain) the prevalence of asthma was higher among those cultivating flowers (5.4 %) than among those cultivating other crops (grain, vegetables etc.) (3.1 %). In the crops which both were cultivated indoors and outdoors work in greenhouses was a risk factor for asthma (odds ratio 2.1 95%CI 0.9 – 4.5) but not for respiratory symptoms per se. (Monso *et al.*, 2000). The greenhouse workers were mainly from Spain as the Danish group only included 20 persons corresponding to 1.1 %.

In a study of Spanish greenhouse workers included in the above mentioned study, 40 randomly selected workers were examined in details (Monso *et al.*, 2002). Five of these (13 %) had asthma and three of these had impairment of the lung function during work indicating occupational asthma giving a prevalence of 7.6 %. Exposure measurements revealed high concentrations of different moulds, both the widespread types *Cladosporium, Aspergillus,* and *Penicillium* species to which the persons with occupational asthma were sensitized as well as *Botrytis cinerea.* Totally, 13 persons (33 %) were sensitized to work place allergens, 7 to moulds and 8 to flowers (Gladiolus, Narcissus, Solidago, Helianthus, and Chrysanthemum).

1.2 Beneficial arthropods

1.2.1 Production and use in the greenhouses

1.2.1.1 Suppliers of beneficial arthropods

The greenhouses included in this study used predatory animals supplied by three major suppliers of predatory animals. Most products were imported from two large Dutch and Belgian suppliers, Koppert and Biobest (Information from importers (GARTA and Borregaard)), while two major suppliers have production of *Amblyseius cucumeris* in Denmark. Some of the larger enterprises had in some periods cultured their own *Hypoaspis miles.*

1.2.2 Health effects of predatory animals

The hypothesis is that the greenhouse workers are exposed to the animals or their debris by inhalation either during mixing and applying the products (direct exposure) or when handling the plants or plant products to which predatory animals have been applied (indirect or reentry exposure).

The development of a disease is normally a complicated interaction between one or more external factors and the person's individual capacity to react. Different pathogenic mechanisms may be involved at the same time.

Figure 1.1 shows a model of the possible respiratory effects of predatory animals. The different factors are included in the figure. For each factor the methods of estimation in the actual study are included.

The arrows show the relations between the different factors. The exposure may give rise to allergy and the allergic response may elicit symptoms or physiological changes. On the other hand exposure may give rise to symptoms or psysiological changes via another pathway not involving the mechanisms of allergy.

Personal factors, such as sex, age, and tendency to react against normally occurring allergens may have an influence on all the relations in the figure.

Exposure is ideally estimated by individual measurements during the whole period. However, normally indirect information about time and method of use, annual consumption etc. has to substitute. This estimation is normally the most difficult and critical factor in revealing a cause effect relationship. The exposure assessment is described in chapter 4.

In the present study *allergy* was defined as a biochemical estimation of a sensitization to one of the actual beneficial species. It was estimated by two different methods. One was the identification and measurement of the concentration of a specific antibody within the type IgE (typical the allergy antibodies) against an extract of the animals.

The other type of method (Platzer *et al.*, 2005) was a functional test of basophile leucocytes response to an antigen, in this case extract from the animals. In the assay the liberation of histamine from the basophiles were visualized as a result of the allergic response. In the original form the test has to be made on fresh whole blood. However, in a new method, the serum of the person was incubated with leucocytes from donor blood. This modification makes it possible to make the analyses on frozen plasma or serum as in the present study.

An allergic response is in itself not a disease, but in many respects a pathogenic pathway to development of *health effects*: *symptoms,* measurable physiological responses, or manifest diseases. These diseases may be asthma, other lung diseases, or upper airway diseases as rhinitis. These conditions were detected (diagnosed) by a combination of symptoms and functional tests.

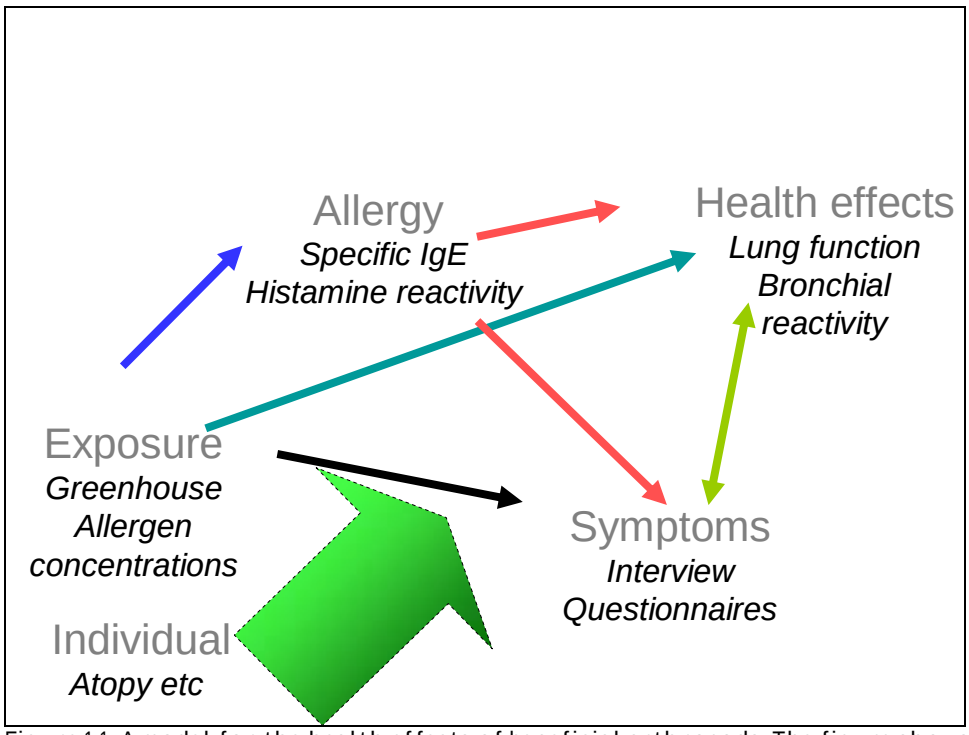

Figure 1.1. A model for the health effects of beneficial arthropods. The figure shows the different factors of interest in the study (*Exposure, allergy, health effects, symptoms,* and *individual* factors) and their interrelationship. Below each factor the type of measurements used in the study are shown. The arrows show the different relations which will be tested in the study. The individual factors will be modifiers in all the possible relations.

1.2.2.1 Amblyseius cucumeris and *Amblyseius californicus*

Only a single Dutch study has addressed the health effects of *A. cucumeris* (Groenewoud *et al.*, 2002a) in relation to their study of allergy to bell pepper. No information about *A. californicus* could be found in the literature.

In the Dutch cross sectional study 472 greenhouse workers, all potentially exposed to *A. cucumeris*, were tested with skin prick test with an extract of the mite (Groenewoud *et al.*, 2002a).

Of the 472 persons in the study, 109 (23 %) were prick positive, defined as a veal of 3 mm or more. Additionally an allergen-specificIgE (RAST) against *A. cucumeris* was made, which were only reported as positive or negative. Of the 100 prick positive persons where samples were available, 54 (54 %) had a positive specific IgE while among the about 350 persons with negative prick test for *A. cucumeris*, only 10 (ca. 3 %) had a positive IgE.

Additionally a nasal challenge test was performed on 23 persons with positive prick test to only *A. cucumeris*, to *A. cucumeris* and *Tyrophagus putrescentiae*, or a strong reaction in prick test to *A. cucumeris*. The results showed that persons with reported rhinitis had a much stronger response to the extract than persons, although sensitized, but without reported rhinitis symptoms.

The sensitization to *A. cucumeris* was followed by a high number of rhinitis and asthma symptoms, and there were a high proportion of persons with positive prick test to common allergen (defined as atopics) and sensitization to bell pepper as well as *T. putrescentiae*.

The study shows a high prevalence of sensitization to *A. cucumeris*, very often in relation to other allergies to other greenhouse related allergens. According to the effect of exposure to *A. cucumeris* on respiratory symptoms, the study only gives limited information as all persons were exposed to bell pepper which had a substantial effect (Groenewoud *et al.*, 2002b), and there was no control group without exposure to the mites in the study.

1.2.2.2 Phytoseiulus persimilis

Two recent articles from a Swedish group is the only available information of health effect of this predator in the medical scientific literature (Johansson *et al.*, 2003; Kronqvist *et al.*, 2005).

The first article reports on a pilot study of 31 greenhouse workers working in houses where *Phytoseiulus persimilis* and *Hypoaspis miles* were used (Johansson *et al.*, 2003). A method of SDS-PAGE and immunoblotting was used and 10 persons (31 %) had IgE binding in immunoblotting to *P. persimilis* but no relation was seen between years working in greenhouse and sensitization. Of the 31 workers, 6 (19 %) were atopics defined as a positive reaction to a screening test for common allergens (Phadiatop®, Pharmacia). Five of these were sensitized to mites.

The aim of the study was mainly to describe the possibility of detecting sensitization to the mites and no health effects were recorded.

In the following study 96 greenhouse workers were investigated (Kronqvist *et al.*, 2005). All the persons worked in greenhouses where mites were used for pest control but neither the method of recruiting, the individual level of exposure to the mites nor the working conditions were described in the article.

For measurements of specific IgE a UNICAP® (Pharmacia Diagnostics, Sweden) method was used and targets were *P. persimilis*, *H. miles* as well as the spider mite *Tetranychus urticae*, the storage mite *Tyrophagus putrescentiae*, and the two house dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*.

Reports of respiratory work related symptoms as well as lung function were included. Sensitization to mites was related to the occurrence of asthma as well as rhino-conjunctivitis. In the crude analyses, sensitization to predatory mites was related to asthma. However, in the multiple regression analysis where several factors were included, only sensitization to mites in general was related to asthma and to rhino-conjunctivitis.

In the group, 76 persons (79 %) handled mites themselves and among the 15 persons sensitized to mites, 13 (88 %) handled predatory mites. There was no information about the methods of application, the frequency of the use of mites, or the actual culture. Handling of mites was neither related to asthma nor to rhino-conjunctivitis.

The study demonstrated a higher frequency of symptomatic persons among those sensitized to mites in general. This is not surprising as sensitization to one biological agent, naturally occurring or occupational, increase the probability of sensitization to more agents. The relation between exposure to *P. persimilis* or *H. miles* and health effects can not be revealed from the article.

1.2.2.3 Hypoaspis miles

Only the above mentioned Swedish articles report about *H. miles* (Kronqvist *et al.*, 2005). Sensitization was seen in 14 of the 96 workers. However these persons were also sensitized to *P. persimilis*.
Therefore no specific effects of this widespread mite can be revealed.

1.2.3 Health effects of *Tetranychus urticae* and *Tyrophagus putrescentiae*

In contrast to the scarce information about the beneficial species more information is available about these two mites known as spider mites and mould mites, respectively. They are naturally occurring in greenhouses as well as used as prey fodder for the beneficial mites.

In an Italian prevalence study of *Tetranychus urticae* including 960 farmers and greenhouse workers, 58 (6 %) were sensitized to *T. urticae* measured by skin prick test (Astarita *et al.*, 2001). Of these persons, 38 had positive tests to a mixture of standard and food allergens. In 20 persons (26 %) an isolated positive test to *T. urticae* was seen.

Sensitization was correlated with respiratory symptoms at work with a mean time from start of farm work to onset of symptoms of 3 years. Greenhouse workers had a higher prevalence of sensitization to *T. urticae* than open field farmers and their time to onset of symptoms was shorter.

A Spanish study of 246 greenhouse workers and 227 controls showed a positive skin prick test to *T. urticae* in 25 % of the greenhouse workers compared to 8 % in the controls (Navarro *et al.*, 2000). In the greenhouse workers, 16 (26 %) of the positive values were solitary while in the rest and in all controls, positive values were seen in persons who responded to other common allergens. In 48 % of the workers, but none of the controls with positive skin prick test, specific IgE antibody (RAST) against *T. urticae* were detected. There was a five fold relative risk of respiratory symptoms in greenhouse workers with a positive prick test to *T. urticae*. However the high prevalence of multiple allergies in this group makes it difficult to conclude.

In the above mentioned Swedish study sensitization to *T. urticae* was seen in 23 persons (24 %), the highest sensitization rate in the study (Kronqvist *et al.*, 2005). This mite might then be of importance in the development of respiratory symptoms, but the study does not permit any firm conclusions.

A Korean study of 725 apple-cultivating farmers found a prevalence of sensitization to *T. urticae* in 26 % and there was some relation to occupationally related symptoms (Kim *et al.*, 1999). In the study, however, the prevalence of sensitization to an European red mite (*Panonychus ulmi)* was 40 % and there was a considerable correlation between the sensitizations to the two mites. Older case studies have demonstrated both allergy and exposure related symptoms from *T. urticae* (Reunala *et al.*, 1983; Delgado *et al.*, 1997) and a study has shown that the allergenicity of *T. urticae* from different sources in greenhouses can not be distinguished (Orta *et al.*, 1998).

Regarding *Tyrophagus putrescentiae* a number of studies have addressed the prevalence in farmers (Hage-Hamsten *et al.*, 1985), bakers (Revsbech & Dueholm, 1990), grain elevator workers (Revsbech & Andersen, 1987) as well as in a general population in a humid area in Northern Spain (Vidal *et al.*, 2004). In the general population a very high rate of sensitization (28 %) was

seen. In comparison the rate for house dust mites was 14 %. A considerably higher prevalence of sensitization was seen among the younger persons while it was lower in farming occupation than in others. In Swedish farmers a prevalence of sensitization was 6,8 %, often in combination with other storage mites. In the Dutch study of *A. cucumeris* mentioned above, 44 % of the persons sensitized to *A. cucumeris* had a positive prick test to *T. putrescentiae* (Groenewoud *et al.*, 2002a).

1.2.4 Conclusions from the studies of predatory mites

From the literature it can be concluded that in greenhouse workers sensitization to *A. cucumeris, P. persimilis,* and *H. miles* was seen. Sensitization was seen in atopic persons as part of multiple allergies, but also as solitary allergies. Respiratory symptoms are less well documented, but still a probable effect in analogue with allergies to other biological agents.

A considerable problem is the cross reactions between the different mites and the predators. The naturally occurring mites *T. urticae* and *T. putrescentiae* present a special problem as they show high sensitization rates in greenhouses, they are both naturally occurring pests, and they are used in the production of predators as prey.

The studies have serious limitations as they are cross sectional and only describe exposed groups. Therefore the prevalence of sensitization of the normal population without occupational exposure to mites and the time course of the sensitization and development of related symptoms are not known.

1.2.5 Health effects of the beneficial arthropods

According to the general literature, no information on the human health effects of the beneficial insects *Aphidius colemani, Aphidius ervi,* or *Encarsia formosa* is available. The literature mainly concerns the biology, control efficiency and the effects of different pesticides on the insects.

On the other hand health effects of the insects by occupational exposure are possible, if not probable. Allergy to cockroaches is one of the most prevalent allergies in several countries (Arlian *et al.*, 1997) and occupational allergies in persons working with breeding of insects (*Ephestia kuehniella* and *Orius laevigatus)* are well known (Belisario *et al.*, 2001; Cipolla *et al.*, 1997), also in Scandinavia (Nielsen, 2000).

In greenhouses the exposure is probably lower but the number of sensitive persons much higher, why a sensitization may be seen.

2 Biology of the selected species of arthropods

Below is given a brief biological character of relevance of the 6 beneficial species *Amblyseius cucumeris, Amblyseius californicus, Phytoseiulus persimilis, Hypoaspis miles, Aphidius colemani* and *Encarsia formosa*, as well as of the 2 pest species, the two-spotted spider mite *Tetranychus urticae* and the mould mite *Tyrophagus putrescentiae*.

The descriptions encompass methods for release, frequently treated cultures, biological parameters (survival, reproductive capacity, and population growth rate), possible remains from the species and the possibility to survive and reproduce at the consumers place.

In the descriptions, information on survival and reproduction pertains to normal glasshouse conditions, here defined as 20-25°C, 70-80 % rh. Only crops relevant for Denmark are mentioned. When referring to the possibility for the species to survive at the consumers place, the following conditions are assumed: in hobby glasshouses: as in the industry; in windowsills, patios etc.: 25-40°C, 20-50 % rh. It should be noted that glasshouse products prior to their arrival at retailers and consumers are subjected to transport conditions different from those during production. These transport conditions vary depending on producer, product and destination. In addition, season will influence the actual conditions in the refrigerated vans used for transport – during summer it will be more difficult to maintain a specific low temperature than during winter. Generalising, however, the conditions during transport are 1-4 days at 10-17°C for pot plants (Lene Petersen, DEG; Niels Peter Bach, GASA Group) and ½-1 day at 10-15°C for tomatoes and cucumbers (GASA Odense Frugt & Grønt). These conditions may affect the ability of pests and beneficials on the products to survive during transport hereby influencing their potential establishment at the consumers place.

An overview of the collected information is found in table 2-1.

2.1 The predatory mite *Amblyseius cucumeris*

(Synonym *Neoseiulus cucumeris*)

Photo: H.F. Brødsgaard, DJF

2.1.1.1 Overview

Predatory mite primarily used for control of thrips. Also eats various mites (Malais & Ravensberg, 2003) and may survive on pollen (Matsuo *et al.*, 2003).

2.1.1.2 Cultures

The mite is used in a number of cultures, including cucumber and various pot plants (e.g. Chrysanthemum, Gerbera, and Cyclamen).

2.1.1.3 Systems for release

Several systems for release. Direct releases are often used preventively (i.e. before the appearance of the target pest, thrips). This means that distribution of the mites often are repeated at short intervals (2-3 weeks) in case no other food sources are available.When breeding units are used, these may be replaced continuously throughout the cultural period with 4-5 week intervals.

Direct release 1: sprinkler bottles with several thousand adults or adults and nymphs (numbers varies from 10,000 up to 500,000), mixed with bran and mould mites (*T. putrescentiae)* as prey during transport (ratio between predatory mites and mould mites is approximately 1: 2-5). The content of the bottle is distributed evenly by hand in the crop, on leaves or in small piles on rock wool[1].

Direct release 2: as above but vermiculite[2] is added which makes the product suited for distribution with mechanical blowers. *A. cucumeris* is the only beneficial mite for which distribution may take place with blowers.

Breeding units: sachets with different stages of the mite (numbers 200-1,000), as well as bran and mould mites (ratio between predatory mites and mould mites is approximately 1: 15:30). The sachets are placed evenly in the crop by hanging them from the plants. The mites reproduce within the sachets and migrate slowly out in the culture over a period of up to 5-8 weeks.

[1] (Biobest, http://www.biobest.be/; EWH BioProduction http://www.bioproduction.dk/; Koppert http://www.koppert.nl/e002.shtml)

[2] Vermiculite may cause sensitisation by inhalation at mechanical application – dust masks are recommended.

2.1.1.4 Release rates
Direct releases: preventively: 50-100 mites /m² every 2 weeks; curatively: 100-250 mites /m² every week. Breeding units: 1 sachet /2-3m² in ornamentals (to be replaced every 4-5 week); 1 sachet /3. cucumber plant (to be used twice per cucumber planting)[3].

2.1.1.5 Survival and reproduction
The population of released mites will survive for up to 3-4 weeks (Zhang *et al.*, 2003) and will reproduce in the culture (both provided that suitable prey is available). Reproduction characteristics: 30-35 eggs/female (Castagnoli & Simoni, 1990; Zhang *et al.*, 2003); sex-ratio ca. 65 % females (Castagnoli & Simoni, 1990; Zhang *et al.*, 2003) population growth rate (rm) 0.15-0.18 day-1 [i.e. doubling time 3.8-4.6 days; finite rate of increase 1.2 (for every individual in one generation there will be 1.2 present the next)] (Castagnoli & Simoni, 1990; Cloutier *et al.*, 1995; Li *et al.*, 2003). Development from egg to adult last ca. 10 days (Castagnoli *et al.*, 1990).

Minimum temperature for development is around 8°C and maximum temperature approx. 35°C (Castagnoli *et al.*, 1990; Malais & Ravensberg, 2003). Optimum temperature for development and reproduction is 25-30°C (Castagnoli *et al.*, 1990; Cloutier *et al.*, 1995; Li *et al.*, 2003; Malais & Ravensberg, 2003). Optimum humidity for survival of adults and juveniles is approx. 75-95 % rh (Shipp & van Houten, 1997). Survival of juveniles and adults decreases with temperature above 25°C and with humidity below ca. 55 % (Shipp & van Houten, 1997).

The commercially available strain of *A. cucumeris* does not enter diapause (i.e. hibernation).

For survival and reproduction of mould mites, see the section on *T. putrescentiae*.

2.1.1.6 Possible remains
A. cucumeris passes through 4 stages (egg, larva, protonymph, deutonymph) in its development to adults. Between each stage the old cuticle is shed and will remain in the crop on leaves and in flowers until it deteriorates. Dead individuals will shrink and shrivel but the outer cuticle being hardier will remain in the crop until deterioration. No information is available on the durability of these remains. The feeding stages of the mites produce faeces that deposit on plants and the surroundings. No information is available on the durability of this material.

For remains of mould mites, see the section on *T. putrescentiae*.

2.1.1.7 Survival at consumers
In the absence of food *A. cucumeris* may survive for some time (Malais & Ravensberg, 2003); (no further specifications found).

The survival with access to prey or pollen/nectar is stated above. *A. cucumeris* is likely to thrive in hobby glasshouses but may find it difficult to sustain population development in more interior places due to a generally high temperature and low humidity.

[3] (Biobest, http://www.biobest.be/; EWH BioProduction http://www.bioproduction.dk/; Koppert http://www.koppert.nl/e002.shtml)

For survival and reproduction of mould mites see the section on *T. putrescentiae*.

2.2 The predatory mite *Amblyseius californicus*

(Synonym *Neoseiulus californicus*)

Photo: BioBest, www.biobest.be

2.2.1.1 Overview

Predatory mite used primarily for control of spider mites (Malais & Ravensberg, 2003). In the absence of spider mites the predatory mite may survive on thrips (Malais & Ravensberg, 2003), other mites (Castagnoli & Falchini, 1993) and pollen (Dindo, 1995).

2.2.1.2 Cultures

The mite is used in a number of different glasshouse cultures, both vegetables and ornamentals.

2.2.1.3 Systems for release

The mite is marketed in bottles with 2,000 individuals (nymphs and adults) mixed with vermiculite[4]. The content of the bottle is distributed evenly by hand in the crop on leaves[5].

2.2.1.4 Release rates

Preventively: 1-2 mites /m² every 2-3 weeks; curatively: 6 mites /m² one time[6]. Some recommend the simultaneous use of both *A. californicus* and *P. persimilis* when spider mites are present in the culture[7].

2.2.1.5 Survival and reproduction

The population of released mites will survive for up to 4-8 weeks (de Courcy Williams & Kravar-Garde, 2002; El-Laithy & El-Sawi, 1998) and will reproduce in the culture (provided that suitable prey is available). Reproduction characteristics: 60-65 eggs/female (Castagnoli & Simoni, 1991; El-Laithy & El-Sawi, 1998); sex-ratio 50-65 % females (Castagnoli & Simoni, 1991; Castagnoli & Falchini, 1993); population growth rate (rm) 0.12-0.26 day-¹ [i.e. doubling time 2.7-5.8 days; finite rate of increase 1.1-1.3 (for every individual in one generation there will be 1.1-1.3 present the next)] (Castagnoli & Simoni, 1991; Rencken & Pringle, 1998). Development from egg to adult last ca. 6-7 days (Castagnoli & Simoni, 1991).

Lower temperature threshold for development, survival and reproduction is around 8-9°C (Castagnoli & Simoni, 1991; Rencken & Pringle, 1998) and populations can still develop at 33°C (Malais & Ravensberg, 2003). No

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[4] Vermiculite may cause sensitization by inhalation at mechanical application – dust masks are recommended.

[5] (Biobest, http://www.biobest.be; Koppert http://www.koppert.nl/e002.shtml)

[6] (Biobest, http://www.biobest.be; Koppert http://www.koppert.nl/e002.shtml)

[7] (Biobest, http://www.biobest.be)

information can be found on the upper temperature threshold. Optimum temperature for development and reproduction seems according to (Castagnoli & Simoni, 1991) to be around 28-32°C. Relative humidity below 60 % has a negative effect on population growth (Malais & Ravensberg, 2003).

The commercially available strain of *A. californicus* does not enter diapause (i.e. hibernation).

2.2.1.6 Possible remains

A. californicus passes through 4 stages (egg, larva, protonymph, deutonymph) in its development to adults. Between each stage the old cuticle is shed and will remain in the crop on leaves or in flowers until it deteriorates. Dead individuals will shrink and shrivel but the outer cuticle being hardier will remain in the crop until deterioration. No information is available on the durability of these remains. The feeding stages of the mites produce faeces that deposit on plants and the surroundings. No information is available on the durability of this material.

2.2.1.7 Survival at consumers

In the absence of food *A. californicus* may survive for some time ((Malais & Ravensberg, 2003); no further specifications found).

The survival with access to prey or pollen/nectar is stated above. *A. californicus* is likely to thrive in hobby glasshouses and perhaps also in more interior places, provided that temperature and humidity conditions are not too server.

2.3 The predatory mite *Phytoseiulus persimilis*

Photo: F. Lind, DJF

2.3.1.1 Overview

Predatory mite used for control of spider mites (Malais & Ravensberg, 2003).

2.3.1.2 Cultures

The mite is used in a number of different glasshouse cultures, including cucumbers and ornamentals (e.g. Gerbera). On tomato a special strain adapted to tomato is marketed[8].

2.3.1.3 Systems for release

Several systems for release exist.

Release only of predatory mites:

Mixed with carrier material. Here the mite is delivered in bottles with 1,000-2,000 individuals (nymphs and adults) mixed with vermiculite[9] or wood chips. The content of the bottle is distributed by hand in the crop on leaves[10]. The strain used for bio control of spider mites on tomato is formulated in this way.

Release of both spider mites and predatory mites[11]:

1) On leaves with spider mites. Here the mite is delivered in packages with 2,000 individuals (adults and nymphs) on bean leaves, with spider mites serving as food during transport and establishment (ratio between predatory mites and spider mites is approximately 1: 5). The leaves are distributed in the crop.

2) Bottles with predatory mites and with spider mites, respectively. Here both spider mites and predatory mites are delivered. Spider mites are released and for every second dose of spider mites, predatory mites are added.

2.3.1.4 Release rates

(valid for both the usual strain of *P. persimilis* and for the tomato-adapted strain)

Preventively: 1-2 mites /m² every 2-3 weeks[12]; curatively for light infestations: 3-10 mites /m² once a week for 2 or more weeks; curatively for heavy

[8] (Biobest, http://www.biobest.be; EWH BioProduction http://www.bioproduction.dk/)

[9] Vermiculite may cause sensitisation by inhalation at mechanical application – dust masks are recommended.

[10] (Biobest, http://www.biobest.be; EWH BioProduction http://www.bioproduction.dk/; Koppert http://www.koppert.nl/e002.shtml)

[11] (Biobest, http://www.biobest.be; EWH BioProduction http://www.bioproduction.dk/)

infestations: 20-50 mites /m² once a week for 2 or more weeks[13]. When using the mites curatively for light infestations they are placed on plants with symptoms, as well as on plants without[14]. When used curatively for heavy infestations they are introduced only in the infested areas[15].

2.3.1.5 Survival and reproduction

The population of released mites will survive for up to 3-4 weeks (Cho *et al.*, 1995; Galazzi & Nicoli, 1996; Laing, 1968) and will reproduce in the culture (both provided that spider mites are available). Reproduction characteristics: 40-75 eggs/female (Gillespie & Quiring, 1994; McClanahan, 1968; Schulten *et al.*, 1978); sex-ratio 60–80 % females (Kilincher *et al.*, 1996; Toyoshima & Amano, 1998); population growth rate (rm) 0.2-0.4 day-¹ [i.e. doubling time 1.7-3.5 days; finite rate of increase 1.2-1.5 (for every individual in one generation there will be 1.2-1.5 present the next)] (Hance, 1988; Kilincher *et al.*, 1996; Laing, 1968; Mesa *et al.*, 1988). Development from egg to adult last ca. 5-7 days (Mesa *et al.*, 1988; Sabelis, 1981).

Minimum temperature for development is around 11°C (Morewood, 1992) and maximum temperature ca. 30-35°C (Ashihara *et al.*, 1976; Hamamura *et al.*, 1976; Malais & Ravensberg, 2003). Optimum temperature for development and reproduction is around 25-27°C (Santi & Maccagnani, 2000; Stenseth, 1979). Lower humidity threshold is around 65 % rh. (Perring & Lackey, 1989).

P. persimilis does not enter diapause (i.e. hibernation).

For survival and reproduction of spider mites either present in the culture, where *P. persimilis* is released, or released together with the predatory mites, see the section on *T. urticae.*

2.3.1.6 Possible remains

P. persimilis passes through 4 stages (egg, larva, protonymph, deutonymph) in its development to adult. Between each stage the old cuticle is shed and will remain in the crop on leaves or in flowers until it deteriorates. Dead individuals will shrink and shrivel but the outer cuticle being hardier will remain in the crop until deterioration. No information is available on the durability of these remains. The feeding stages of the mites produce faeces that deposit on plants and the surroundings. No information is available on the durability of this material.

2.3.1.7 Survival at consumers

In the absence of spider mites, *P. persimilis* will first act cannibalistic and subsequently starve and die after a short time (2-4 days or 1-2 weeks without and with water, respectively (de Courcy Williams & Kravar-Garde, 2002; Mori & Chant, 1966; Sabelis, 1981)). In the absence of food, egg laying ceases (Sabelis, 1981).

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[12] (EWH BioProduction http://www.bioproduction.dk/; Koppert http://www.koppert.nl/e002.shtml)
[13] (Biobest, http://www.biobest.be; EWH BioProduction http://www.bioproduction.dk/; Koppert http://www.koppert.nl/e002.shtml)
[14] (EWH BioProduction http://www.bioproduction.dk/; Koppert http://www.koppert.nl/e002.shtml)
[15] (Koppert http://www.koppert.nl/e002.shtml)

The survival with access to prey is stated above. Spider mites will thrive in consumers' hobby glasshouses and are, in addition, able to survive on plants in windowsills, patios and similar interior parts of the homes of consumers. *P. persimilis* will likewise thrive in hobby glasshouses but normally finds it difficult to sustain population development in more interior places, due to a generally high temperature and low humidity.

2.4 The predatory mite *Hypoaspis miles*

Photo: S. Ydergaard, DJF

2.4.1.1 Overview

Soil-dwelling predatory mite used for control of arthropods with soil-dwelling stages, e.g. sciarids, fly larvae, spring tails, mites and thrips (Malais & Ravensberg, 2003).

2.4.1.2 Cultures

The mite is used in a variety of glasshouse cultures, especially ornamentals (e.g. Poinsettia, Cyclamen).

2.4.1.3 Systems for release

The mite is marketed in bottles with 10,000-50,000 individuals (nymphs and adults) mixed with peat and vermiculite[16]. Some products also contain the mould mite, *T. putrescentiae* (ratio between predatory mites and mould mites is approx. 1: 1-2). The content of the bottle is distributed evenly by hand in the crop on the compost or on rock wool cubes[17]. The mite may be introduced below the tables to help control of sciarids[18].

H. miles is completely soil-dwelling in all stages inhabiting the top layer of the soil (at a depth of 1-4 cm). The mite seldom occurs on the above-ground plant parts.

2.4.1.4 Release rates

Preventively: 100 mites /m² once or twice[19]; curatively: 200-1000 mites /m² applied at least once[20].

2.4.1.5 Survival and reproduction

The population of released mites will survive for up to 9-16 weeks (Enkegaard *et al.*, 1997; Ydergaard *et al.*, 1997) and will reproduce in the culture (both provided that suitable prey is available). Reproduction characteristics: 45-55 eggs/female (Enkegaard *et al.*, 1997; Ydergaard *et al.*, 1997); sex-ratio 60-90 % females (Enkegaard *et al.*, 1997; Ydergaard *et al.*, 1997); population growth rate (rm) 0.07-0.13 day-¹ [i.e. doubling time 5.3-9.9 days; finite rate of increase 1.1 (for every individual in one generation there will be 1.1 present

[16] ¹Vermiculite may cause sensitisation by inhalation at mechanical application – dust masks are recommended.
[17] (Biobest, http://www.biobest.be; Koppert http://www.koppert.nl/e002.shtml)
[18] (EWH BioProduction http://www.bioproduction.dk/)
[19] (EWH BioProduction http://www.bioproduction.dk/; Koppert http://www.koppert.nl/e002.shtml)
[20] (Biobest, http://www.biobest.be; EWH BioProduction http://www.bioproduction.dk/; Koppert http://www.koppert.nl/e002.shtml)

the next)] (Enkegaard *et al.*, 1997; Ydergaard *et al.*, 1997). Development from egg to adult last ca. 13-20 days (Enkegaard *et al.*, 1997; Ydergaard *et al.*, 1997).

Minimum temperature for population development is around 10-12°C (Ydergaard *et al.*, 1997) and maximum temperature approx. 33°C (Wright & Chambers, 1994). Optimum temperature is around 22-25°C (Ydergaard *et al.*, 1997). Population development requires that the soil medium is moist but not too wet[21].

H. miles does not enter diapause (i.e. hibernation).

For survival and reproduction of mould mites, see the section on *T. putrescentiae*.

2.4.1.6 Possible remains

H. miles passes through 4 stages (egg, larva, protonymph, deutonymph) in its development to adults. Between each stage the old cuticle is shed and will remain in or on the soil until it deteriorates. Dead individuals will shrink and shrivel but the outer cuticle being hardier will remain in or on the soil until deterioration. No information is available on the durability of these remains. The feeding stages of the mites produce faeces that deposit in or on the soil. No information is available on the durability of this material.

For remains of mould mites, see the section on *T. putrescentiae*.

2.4.1.7 Survival at consumers

H. miles brought to the home of consumers (hobby glasshouses, windowsills, patios and similar interior parts) in the soil of potted plants are likely to be able to survive and reproduce provided that the above mentioned conditions are met and provided that the soil contains prey items suited for the mite. In the absence of food, the mite may survive for 3-4 weeks. In the absence of food, egg laying ceases (Malais & Ravensberg, 2003).

For survival and reproduction of mould mites, see the section on *T. putrescentiae*.

[21] (Koppert http://www.koppert.nl/e002.shtml)

2.5 The parasitoid *Aphidius colemani*

Photo: Jack Kelly Clark, University of California,
http://www.ipm.ucdavis.edu/IPMPROJECT/ADS/manual_naturalenemies.html

2.5.1.1 Overview

Parasitoid used for control of aphids (Malais & Ravensberg, 2003). Parasitoids are obligatory parasites that for reproduction require hosts in which the eggs are laid and in which development to adults takes place. Adult *A. colemani* feeds on honeydew[22].

2.5.1.2 Cultures

The parasitoid is used in a number of cultures where suitable host aphids (e.g. cotton aphids, *Aphis gossypii* and peach-potato aphids, *Myzus persicae*) occur, including tomato and cucumber and various pot plants (e.g. Chrysanthemum, Gerbera).

2.5.1.3 Systems for release

Two systems for release.

Direct release: sprinkler bottles with 500-5,000 parasitoids (a few adults, the rest in the mummy stage (parasitized aphids in the last stage of parasitisation)) mixed with sawdust or wood chips. The content of the bottle is distributed evenly by hand in the crop on leaves, growing media or rock wool[23].

Banker plants: an open rearing system created by placing wheat plants infested with approx. 500 cereal aphids, *Rhopalosiphum padi* in the glasshouse and introducing *A. colemani* onto these. The parasitoids will reproduce on the cereal aphids and disperse to the glasshouse crop[24].

2.5.1.4 Release rates

Direct releases: preventively: 0.15 parasitoids /m² every week [25] or ½-1 parasitoids /m² every 2 weeks[26]; curatively: ½-1 parasitoids /m² every ½ week or every week for 3-6 weeks[27].

Banker plants: min. 5 banker plants /1,000 m² [28] which are substituted every 2-3 weeks. *A. colemani* is introduced at 0.1-0.15 parasitoids /m² each time.

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[22] (Biobest, http://www.biobest.be/)
[23] (Biobest, http://www.biobest.be/; EWH BioProduction http://www.bioproduction.dk/; Koppert http://www.koppert.nl/e002.shtml)
[24] (Biobest, http://www.biobest.be/; EWH BioProduction http://www.bioproduction.dk/; Koppert http://www.koppert.nl/e002.shtml)
[25] (Biobest, http://www.biobest.be/; Koppert http://www.koppert.nl/e002.shtml)
[26] (EWH BioProduction http://www.bioproduction.dk/)
[27] (Biobest, http://www.biobest.be/; Koppert http://www.koppert.nl/e002.shtml)

2.5.1.5 Survival and reproduction

The population of released parasitoids will survive for up to 1 week (Hofsvang & Hagvar, 1975; van Steenis, 1995) and will reproduce in the culture provided that suitable aphid species are present. Reproduction characteristics: 300-390 eggs/female (Hofsvang & Hagvar, 1975; van Steenis, 1993; van Steenis, 1995); sex-ratio ca. 66 % females (Gucuk & Yoldas, 2000); population growth rate (rm) 0.3-0.35 day-¹ [i.e. doubling time 2-2.3 days; finite rate of increase 1.35-1.4 (for every individual in one generation there will be 1.35-1.4 present the next)] (Ahmad & Hodgson, 1997; van Tol & van Steenis, 1994). Development from egg to adult last ca. 10-13 days (van Steenis, 1993).

Minimum temperature for population development is around 3-5°C (Elliott *et al.*, 1995; Prinsloo *et al.*, 1993; Sampaio *et al.*, 2003) and maximum temperature 25-30°C (Ahmad & Hodgson, 1997; Guenaoui, 1991; Malais & Ravensberg, 2003). Optimum temperature for development and reproduction is around 22-25°C (Ahmad & Hodgson, 1997; Sampaio *et al.*, 2003).

A. colemani does not enter diapause (i.e. hibernation).

2.5.1.6 Possible remains

The development of an individual *A. colemani* takes place within an aphid. In the process, the entire aphid is consumed and all that remains after the parasitoid has emerged is the outer cuticle of the parasitized aphid. No information is available on the durability of these remains. The adult parasitoids produce faeces that deposit on plants and the surroundings. No information is available on the durability of this material.

2.5.1.7 Survival at consumers

Without access to hosts or honeydew the lifespan of *A. colemani* is reduced to 1-2 days at temperatures around 25°C (Gucuk & Yoldas, 2000). Supplement of sugar solutions prolongs longevity to about 2-4 days (Gucuk & Yoldas, 2000).

The survival with access to hosts is stated above. Aphids will thrive in consumers' hobby glasshouses and are, in addition, able to survive on plants in windowsills, patios and similar interior parts of the homes of consumers. *A. colemani* will likewise thrive in hobby glasshouses but normally finds it more difficult to sustain population development in more interior places, due to a generally high temperature and low humidity.

[28] (Biobest, http://www.biobest.be/; EWH BioProduction http://www.bioproduction.dk/) or 5 banker plants /10,000 m² (Koppert http://www.koppert.nl/e002.shtml)

2.6 The parasitoid *Encarsia Formosa*

Photo: F. Lind, DJF

2.6.1.1 Overview

Parasitoid used for control of whiteflies (Malais & Ravensberg, 2003). Parasitoids are obligatory parasites that for reproduction require hosts in which the eggs are laid and in which development to adults takes place. Adult *E. formosa* feeds on honeydew, as well as on host body fluid[29].

2.6.1.2 Cultures

The parasitoid is used in a number of glasshouse vegetables (tomato, cucumber) and various pot plants (e.g. Poinsettia, Gerbera).

2.6.1.3 Systems for release

The parasitoids are supplied as parasitized whitefly pupae glued to cards (100-500 pupae per card) that are hung from the plants in an even distribution[30].

2.6.1.4 Release rates

Preventively: 1½ parasitoids /m² every week or every second week[31]; curatively: 3-10 parasitoids /m² every week until sufficient parasitisation is achieved (minimum 5 weeks)[32]. For treatment of "hot-spots" (areas with high infestation of whiteflies) more than 10 parasitoids /m² may be needed[33].

2.6.1.5 Survival and reproduction

The population of released parasitoids will survive for up to 2-3 weeks (Burnett, 1949; Dindo, 1995; Enkegaard, 1993; Yoldas, 2001; Di Pietro, 1977) and will reproduce in the culture provided that suitable whitefly species are present. Reproduction characteristics: 100-350 eggs/female (Gast & Kortenhoff, 1983; Kajita, 1979; Madueke, 1979; Parr *et al.*, 1976; van Lenteren *et al.*, 1987; Yoldas, 2001); sex-ratio ~100 % females (Ghahhari & Hatami, 2001; Heimpel & Lundgren, 2000; van Roermund & van Lenteren, 1992); population growth rate (rm) 0.13-0.28 day-¹ [i.e. doubling time 2.5-5.3 days; finite rate of increase 1.14-1.32 (for every individual in one generation there will be 1.14-1.32 present the next)] (Arakawa, 1982; Enkegaard, 1993; Lopez & Botto, 1995; van Roermund, 1995). Development

[29] (Biobest, http://www.biobest.be/)
[30] (Biobest, http://www.biobest.be/; EWH BioProduction http://www.bioproduction.dk/; Koppert http://www.koppert.nl/e002.shtml)
[31] (Koppert http://www.koppert.nl/e002.shtml)
[32] (Biobest, http://www.biobest.be/; EWH BioProduction http://www.bioproduction.dk/; Koppert http://www.koppert.nl/e002.shtml)
[33] (EWH BioProduction http://www.bioproduction.dk/)

from egg to adult last ca. 15-25 days (Arakawa, 1982; Di Pietro, 1977; Jansen, 1974; Madueke, 1979).

Minimum temperature for population development is around 10-11°C and maximum temperature approx. 38°C (van Roermund & van Lenteren, 1992). Optimum temperature for development and reproduction is around 25°C (van Roermund & van Lenteren, 1992; van Roermund, 1995), and optimum humidity is 50-85 % rh (Malais & Ravensberg, 2003).

E. formosa does not enter diapause (i.e. hibernation).

2.6.1.6 Possible remains

The development of an individual *E. formosa* takes place within a whitefly. In the process the entire whitefly is consumed and all that remains after the parasitoid has emerged is the outer cuticle of the parasitized whitefly. No information is available on the durability of these remains. The adult parasitoids produce faeces that deposit on plants and the surroundings. No information is available on the durability of this material.

2.6.1.7 Survival at consumers

Without access to hosts or honeydew *E. formosa* only survives for few days (ca. 3) at temperatures around 20°C (Gast & Kortenhoff, 1983; van Lenteren *et al.*, 1987). In the absence of hosts, but supplied with honeydew or other sugar solutions, the parasitoids can sustain life 4 weeks or more (Gast & Kortenhoff, 1983; van Lenteren *et al.*, 1987; Vet & van Lenteren, 1981).

The survival with access to hosts is stated above. Whiteflies will thrive in consumers' hobby glasshouses and are, in addition, able to survive on plants in windowsills, patios and similar interior parts of the homes of consumers. *E. formosa* will likewise thrive in hobby glasshouses, but normally finds it more difficult to sustain population development in more interior places, due to a generally high temperature and low humidity.

2.7 The two-spotted spider mite *Tetranychus urticae*

Photo: Jack Kelly Clark, University of California,
http://www.ipm.ucdavis.edu/IPMPROJECT/ADS/manual_naturalenemies.html

2.7.1.1 Cultures

Spider mites occur as pests in a number of different glasshouse cultures, including tomato, cucumber and ornamentals (e.g. Hedera, Aster, Chrysanthemum). In connection with the use of the predatory mite, *P. persimilis*, spider mites may be part of the product or recommended to be released simultaneously to serve as prey during establishment and/or transport. The mite feed on the underside of the leaves and produce webbing that at high infestation levels may cover the plants. The mites spread through the culture by migration between plants or along wires or by use of silk threads that are carried by air currents. Also mechanical spread, via movement of infested plant material or on clothes or other objects, occurs.

2.7.1.2 Survival and reproduction

Spider mites survive and reproduce where sufficient plant material is available. Uncontrolled infestations of spider mites will lead to the collapse of the culture. Adult spider mites live for 12-20 days (Ahn *et al.*, 1997; Rao *et al.*, 1996) and the reproduction characteristics are: 60-130 eggs/female (Ahn *et al.*, 1997; Rao *et al.*, 1996; Sabelis, 1981); sex-ratio 70-80 % females (Ho & Lo, 1979; Kim *et al.*, 2001; Margolies & Wrensch, 1996); population growth rate (rm) 0.12-0.4 day^{-1} [i.e. doubling time 1.7-5.8 days; finite rate of increase 1.1-1.5 (for every individual in one generation there will be 1.1-1.5 present the next)] (Ahn *et al.*, 1997; Ho & Lo, 1979; Rao *et al.*, 1996; van Impe & Hance, 1991). Development from egg to adult last ca. 10-22 days (Ahn *et al.*, 1997; Rao *et al.*, 1996; Sabelis, 1981).

Minimum temperature for development is around 11-12°C (Kim *et al.*, 2001; Lu *et al.*, 2002; Malais & Ravensberg, 2003) and maximum temperature ca. 35-40°C (Candolfi *et al.*, 1991; Malais & Ravensberg, 2003). Optimum temperature for development and reproduction is around 24-30°C (Chen, 2000; Malais & Ravensberg, 2003; Sabelis, 1981). Optimal humidity is around 40-80 % rh (Ferro & Chapman, 1979).

When environmental conditions change adversely (decreased daylenght, reduced temperatures, decline or deterioration of food supply), female spider mites enter diapause and remain hidden in the glasshouse structures (Malais & Ravensberg, 2003). When conditions become more favourable, diapause is broken and feeding and egg laying resumes.

2.7.1.3 Possible remains

T. urticae passes through 4 stages (egg, larva, protonymph, deutonymph) in its development to adults. Between each stage the old cuticle is shed and will

remain in the crop on leaves or in flowers until it deteriorates. Dead individuals will shrink and shrivel but the outer cuticle being hardier will remain in the crop until deterioration. No information is available on the durability of these remains. The feeding stages of the mites produce faeces that deposit on plants and the surroundings. No information is available on the durability of this material.

2.7.1.4 Survival at consumers

Spider mites will thrive in consumers' hobby glasshouses and are, in addition, able to survive on plants in windowsills, patios and similar interior parts of the homes of consumers.

2.8 The mould mite *Tyrophagus putrescentiae*


Photo: USDA

Mould mites occur in various environments e.g. in grain stores, in soil or litter feeding on organic material and on mould. The mites often occur in the soil of potted plants in glasshouses and are, in addition, added to some products of beneficial organisms (e.g. *A. cucumeris* and *H. miles*) serving as prey during transport and first establishment. In glasshouses they may establish in e.g. shoots and flowers of some crops (e.g. cucumber, Begonia, Gerbera) and cause damage (Malais & Ravensberg, 2003).

2.8.1.1 Survival and reproduction

Adult mould mites live for about 30-50 days (Eraky, 1992; Li *et al.*, 1998; Malais & Ravensberg, 2003) and the reproduction characteristics are: 300-500 eggs/female (Boczek, 1974; Eraky, 1992; Li *et al.*, 1998); sex-ratio ca. 50 % females (Eraky, 1992; Ignatowicz, 1986). No information has been found regarding population growth rate (rm). Development from egg to adult last ca. 13-21 days (Li *et al.*, 1998).

Minimum temperature for development is around 6-10°C (Sanchez-Ramos & Castanera, 2001) and maximum temperature ca. 35-37°C (Sanchez-Ramos & Castanera, 2001). Optimum conditions for development and reproduction are around 30°C and 85-95 % rh (Sanchez-Ramos & Castanera, 2001). Lower humidity threshold for survival is around 65 % rh (Ree & Lee, 1997). The conditions of the substrate in which the mites live are also of importance for survival and reproduction (Malais & Ravensberg, 2003).

2.8.1.2 Possible remains

T. putrescentiae passes through 4 stages (egg, larva, protonymph, deutonymph) in its development to adults. Between each stage the old cuticle is shed and will remain in the crop on leaves or in flowers until it deteriorates. Dead individuals will shrink and shrivel but the outer cuticle being hardier will remain in the crop until deterioration. No information is available on the durability of these remains. The feeding stages of the mites produce faeces that deposit on plants and the surroundings. No information is available on the durability of this material.

T. putrescentiae does not enter diapause (i.e. hibernation).

2.8.1.3 Survival at consumers

Mould mites can under high humidity conditions be found in house dust and food items such as flour, oats and grain[34]. Mould mites brought to the consumers place in the soil of potted plants will be able to survive and reproduce here, provided that conditions are suitable.

[34] (Danish Pest Infestation Laboratory http://www.dpil.dk/frames/spom_frm.htm)

Species	Release methods	Mixed with	Release rates	Survival of adults[1]	Development time for immatures[1]	Reproduction[1]	Population growth rate[1]	Conditions	Remains	Survival at consumer
Amblyseius cucumeris	Direct releases, incl. with blowers Breeding units	*T. putrescentiae* *T. putrescentiae*	50-100/week or 100-250/2 weeks /m² see text	3-4 weeks	10 days	30-35 eggs/♀	0.15-0.18 day^{-1}	Min. 8°C; 55 % rh Max. 35° Opt. 25-30°C; 75-95 % rh	Cast cuticles, faeces	Hobby glasshouses
Amblyseius californicus	Direct releases	-	1-2/m²/2-3 weeks or 6/m² once	4-8 weeks	6-7 days	60-65 eggs/♀	0.12-0.26 day^{-1}	Min. 8-9°C; 60 % rh Max. >33°C Opt. 28-32°C	Cast cuticles, faeces	Hobby glasshouses, perhaps interior places
Phytoseiulus persimilis	Direct releases	Sometimes *T. urticae*	1-2/m²/2-3 weeks to 3-10 or 20-50/ m²/ week	3-4 weeks	5-7 days	40-75 eggs/♀	0.2-0.4 day^{-1}	Min. 11°C; 65 % rh Max. 30-35°C Opt. 25-27°C	Cast cuticles, faeces	Hobby glasshouses
Hypoaspis miles	Direct releases	Sometimes *T. putrescentiae*	100/m² 1-2 times or 200-1000/m² once or more	9-16 weeks	13-20 days	45-55 eggs/♀	0.07-0.13 day^{-1}	Min. 10-12°C Max. 33°C Opt. 22-25°C	Cast cuticles, faeces	Hobby glasshouses, perhaps interior places
Aphidius colemani	Direct releases Banker plants	- Cereal aphids	0.15/m²/week or ½-1/m²/2 weeks or ½-1/m²/½-1 week see text	1 week	10-13 days	300-390 eggs/♀	0.3-0.35 day^{-1}	Min. 3-5°C Max. 30°C Opt. 22-25°C	Remaining cuticles from hosts, faeces	Hobby glasshouses
Encarsia formosa	Direct releases	-	1½/m²/1-2 weeks or 3-10/m²/week	2-3 weeks	15-25 days	100-350 eggs/♀	0.13-0.28 day^{-1}	Min. 10-11°C Max. 38°C Opt. 25°C; 50-85 % rh	Remaining cuticles from hosts, faeces	Hobby glasshouses
Tetranychus urticae	Direct releases	*P. persimilis*	See text for *P. persimilis*	1½-3 weeks	10-22 days	60-130 eggs/♀	0.12-0.4 day^{-1}	Min. 11-12°C Max. 35-40°C Opt. 24-30°C; 40-80 % rh	Cast cuticles, faeces	Hobby glasshouses, interior places
Tyrophagus putrescentiae	Direct releases	*A. cucumeris, H. miles*	See text for *A. cucumeris* / *H. miles*	4-7 weeks	13-21 days	300-500 eggs/♀	?	Min. 6-10°C; 65 % rh Max. 35-37°C Opt. 30°C; 85-95 % rh	Cast cuticles, faeces	Hobby glasshouses, interior places

[1] at temperatures around 20-25°C, rh around 70-80 %.

Table 2-1 Overview of the 8 different glasshouse arthropods.

3 The epidemiological study

3.1 Aims and general study design

The study was a three-year follow up study. A group of greenhouse workers were studied including annual examinations.

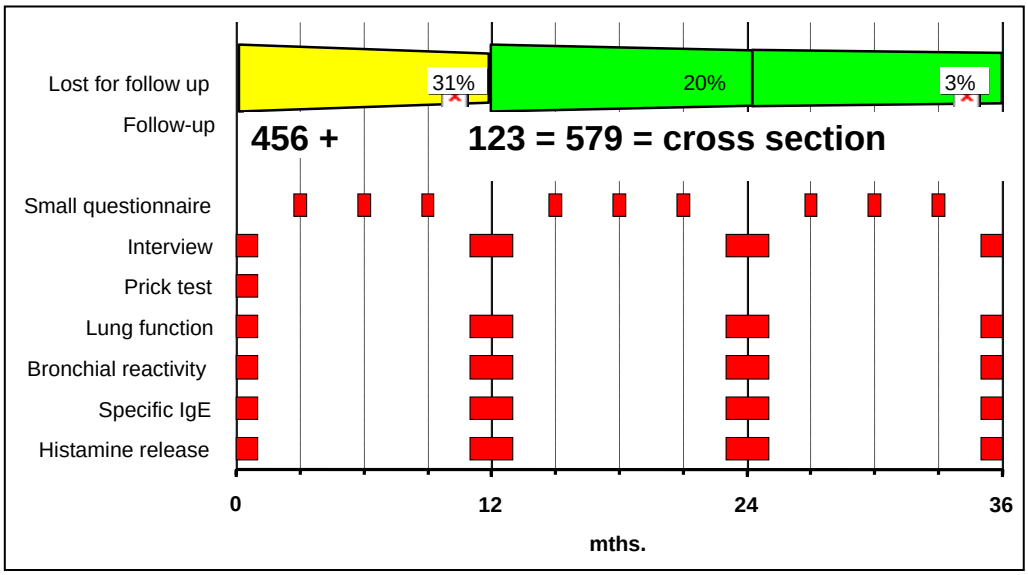

Figure 3.1 The general design of the follow-up study as a time line with the annual examination from 0 to 36 mths. (run 0 to run 3, respectively). The top row shows the flow of persons in the study while the lower shows the investigations made within and between the annual examinations.

3.2 The greenhouses

The greenhouse firms were selected from the responders in a survey made in 1996 on the 205 firms culturing ornamental flowers in Funen's county in order to get a group of greenhouses using microbiological pesticides and a group not using these products. The use of beneficial arthropods had no influence on the selection process.

The greenhouses all produced ornamental flowers covering more than a hundred different cultures. The most frequently produced culture was Christmas tree Stars which were cultured in the fall and sold up to Christmas. Other produced products of high frequency were Marguerites, Chrysanthemums, and roses (Larsen & Bælum, 2002).

3.3 The persons

A detailed description of the persons included is given in the former report of the study (Larsen & Bælum, 2002).

The persons were recruited at the 31 greenhouses which in the beginning of the study employed 773 persons of whom 456 (59 %) were willing to participate. The cause of non response was either leave from the work place the actual day or unwillingness of having taken a blood sample.

In run 1 (1998) the persons were reexamined and those employees who were present in the greenhouse and not previously included were offered participation. These were persons hired in the previous year and those who otherwise were not available at the first examinations. This resulted in an additional inclusion of 123 persons while 316 persons from run 0 were examined adding up to 436 persons.

In run 2 and run 3 no additional persons were included but all the persons who had participated in any of the previous runs were invited.

Persons who did not show up or did not want to participate were mailed a questionnaire about the reason for leaving the study.

In all, 579 persons participated in the study. Of these 262 were followed for three years, 237 participated in all the runs while 97 were followed for two years, and 74 persons were examined in two consecutive years. 146 persons were examined only once in run 0 or run 1.

The study was thereby based on 1592 single observations and 1056 person years for follow up.

		Run0	Run1	Run2	Run3
	Males	148	148	123	116
		(32 %)	(34 %)	(35 %)	(34 %)
	Females	308	291	227	224
		(68 %)	(66 %)	(65 %)	(66 %)
	Total	456	439	350	340
Age (years)	Males	34.8	34.7	35.8	36.3
		(17 - 60)	(17 - 67)	(19 - 67)	(20 - 67)
	Females	35.7	35.9	36.8	36.9
		(16 - 59)	(19 - 61)	(19 - 59)	(19 - 59)
Seniority	Males	14.9	14.5	16.0	16.8
in trade		(0 - 45)	(0 - 53)	(0 - 53)	(0 - 53)
	Females	8.5	8.7	9.4	9.5
		(0 - 38)	(0 - 38)	(0 - 38)	(0 - 38)
Seniority	Males	8.4	8.0	8.8	9.3
at the actual		(0-39)	(0-39)	(0-39)	(0-39)
workplace	Females	5.7	5.5	6.3	6.5
		(0-33)	(0-33)	(0-32)	(0-33)
Positive	Males	34	38	29	28
pricktest		(23 %)	(26 %)	(24 %)	(24 %)
	Females	49	50	43	42
		(16 %)	(17 %)	(19 %)	(19 %)
Atopy	Males	47	42	32	32
in the family		(32 %)	(28 %)	(26 %)	(28 %)
	Females	97	96	74	73
		(31 %)	(33 %)	(33 %)	(33 %)

Table 3-1. Key variables for the persons in the study. Age and seniority were at the inclusion into the study.

3.4 Unexposed persons without contact to greenhouses

As the measures of sensitization and health effects may not be specific for the exposure to the beneficial species and there may be difficulties in evaluating the individual exposure status, a group of 149 persons not related to the greenhouse trade, were included.

The samples were taken from subjects participating in a large population study, Risk Factors for the development of Asthma in Adults (Hansen *et al.*, 2005), based on the protocol of the European multi center study ECRHS (European Community Respiratory Health Study{Kogevinas, 1999 2622 /id}).

The persons were selected among participants in the clinical examinations in Funen's County carried out between September 2003 and February 2004. The persons were responders to a questionnaire sent to a random sample of 10.000 persons in five Danish counties. Among the 73 % responders, persons with symptoms of asthma and a healthy control group were invited to an examination including a detailed interview, standard skin prick test, lung function, bronchial provocation test, and blood samples. A protocol closely related to that used in the BIOGART study (Larsen & Bælum, 2002), although the instruments used for measuring lung function and bronchial responsiveness were different.

For each person 2 aliquots of plasma were sampled and kept frozen at -80°C until analysis.

Of the persons 71 were males and 78 females, the mean age being 34 years (ranging between 20 and 44 years). 55 persons (38 %) had self reported asthma while 68 (46 %) reported nasal allergies.

The persons were employed in different trades. In about 10 persons the information indicated employment in relation to agriculture or gardening. These persons will be excluded from the analyses.

4 Exposure assessment

The purpose with this part of the project has been to get estimates of the person and time specific exposure to each of the relevant beneficial animals.

Due to lack of direct measurements of exposure to airborne antigens from the predatory animals and the non-beneficial mites, this exposure assessment has to be based on information about the use in the relevant time period 1997-2001.

4.1 Sources of information

There have been three sources to this information:

1. Data from the initial inquiry in 1996 before the start of the study.
2. Questionnaire reports from the workers at the annual examinations.
3. An inquiry conducted in 2004 at the participating greenhouse firms.

4.1.1 Inquiry in 1996

As part of the planning of the BIOGART study in 1996 a survey was carried out among the 205 registered greenhouse enterprises that had employees. The greenhouses were selected from the employers' organization and the owners of the enterprises were mailed a questionnaire followed by a telephone interview.

The interview mainly concerned microbiological pesticides, but there was a final, open question about beneficial species.

Interviews were obtained from 193 (94 %) responders and among these the actual group of participating greenhouses was chosen.

4.1.2 Information from questionnaires 1997-2001

The annual examinations included a questionnaire on the working conditions including handling of beneficial species.

The questionnaire comprised a section about present and former employments, skills (skilled or unskilled worker), time of employment at the actual greenhouse and in the trade as a whole.

Sections about working function, cultures, handling of microbiological pesticides, beneficial species as well as chemical pesticides during the past year, were included.

The work tasks carried out in the previous year were scored by ranking a preset series of working procedures according to the part of the working time spent (most of the time, ¾ of the working time, ½ of the time, ¼ of the time or more rare).

The time spent at each of the different cultures was scored in the same way.

Regarding beneficial species only the persons own handling of the products were registered. It was possible to register up to seven different beneficial species and for each of these the type, mode and frequency of application, the treated culture, the area applied and the use of personal protection.

Similar groups of questions were made for the microbiological and the chemical pesticides.

4.1.3 Horticulture inquiry 2004

In 2004 an inquiry was conducted with reference to the use of beneficial species in greenhouse firms in Funen in the period of 1997-2001.

The purpose of this part of the investigation was to verify the workers reports about the use of beneficial species in the greenhouse firms and hereby helping to assess the extent of exposure, both directly when applying the species and indirectly by handling the plants.

4.1.3.1 Method

The study group consisted of employees at the 31 greenhouse firms in Funen who participated in the BIOGART study.

Data collection was conducted by telephone interviews between the 8th and the 28th of June 2004. All the greenhouse firms received 1-2 weeks prior to the interview, written information about the investigation and an outline with a table of the information we would like to obtain. Receiving the form before hand gave the greenhouse firms the possibility of finding the information that would be 3-7 years old at the time of contact.

Some of the greenhouse firms were in the meanwhile closed down and others had changed ownership. If possible, the previous owner or contact person was contacted and interviewed. Contact attempts were made up to four times and were successful with 30 out of 32 greenhouse firms (93 %).

The interviews lasted from a few to 45 minutes and were based on the form received. The interviews were focused on the use of six selected beneficial species and one pest species in the greenhouse firms in the period of 1997-2001. For each of the species was asked: when it was used, how and in which cultures it was released, what was the size of the treated area, what about the frequency and the amount at each release, and who the supplier was.

Data were keyed in using Epidata, version 3.02, and analyzed in the statistical package STATA, version 8.2.

4.1.3.2 Results

Valuable information was obtained from 15 of the 31 greenhouse firms. These 15 firms had employed 404 of 579 or 70 % of the participants in the follow up. Eleven of the firms had been using beneficial species in the period whereas four had not.

The sixteengreenhouse firms did not participate, due to various reasons (lack of time or lack of interest, change of ownership since last approach three years ago, approaching close down, two firms stated that they were fed up with inquiries and others simply could not or did not have the capacity to find data from the time period).

4.2 Individual exposure assessment

As the first step in the assessment of the individual exposure, each person in each run was assigned a level of exposure to each of the six beneficial species. If the person had reported use of the species in the questionnaire in a run, exposure was assigned “Applying” in the specific period.

If at least one person in a greenhouse in a run had reported “Applying”, it was compared with the results of the 2004 inquiry. If there was agreement, the exposure was assigned “Exposed in the greenhouse” for the rest of the participants in the greenhouse in the particular run and the level of confidence was set to “high”.

If there was no agreement between the information from the greenhouses and the questionnaires from the employees (mostly if one of the persons had registered ”Applying” and the greenhouse had stated that it was not used in the particular year), an individual evaluation was made, based on the data in the questionnaire and the use the year before or after. The level of confidence of the greenhouse was then assigned to “low”.

For the greenhouses where information only was available from the questionnaires the level of confidence was set to “intermediary”.

The distribution of the exposure assignments for the three levels of confidence is shown in table 4-1.

In the analyses of the effects of exposure on the different effect measurements, separate analyses were made of the whole material and for the subgroup, with high level of confidence which amounted to about 75 % of the observations.

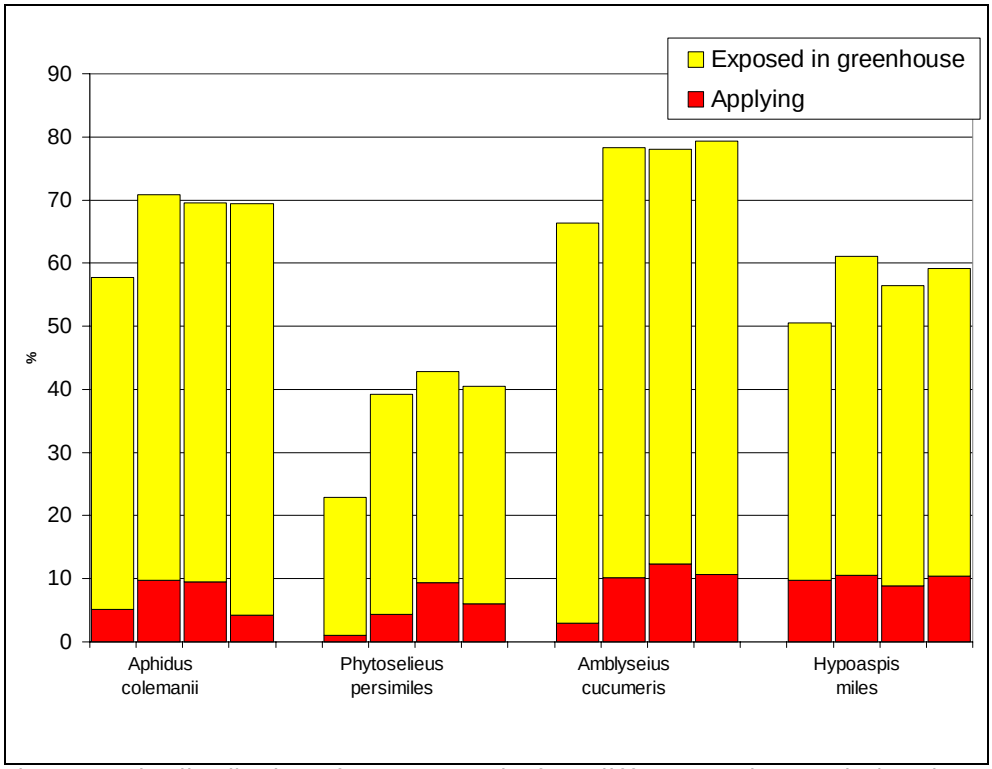

Figure 4.1. The distribution of exposure to the four different predators. The bars in each group show the frequency in run 0 through run 3.

	Level of confidence					
	Low. Disagreement between employees and inquiry 2005		Intermediary. Only information from employees		High. Agreement between employees and inquiry 2005	
Aphidius colemani	n	%	n	%	n	%
No exposure	0		299	(59 %)	264	(25 %)
Exposed in greenhouse	0		192	(37 %)	729	(70 %)
Applying	0		22	(4 %)	51	(5 %)
Encarsia formosa						
No exposure	45	(57 %)	349	(68 %)	305	(32 %)
Exposed in greenhouse	34	(43 %)	148	(29 %)	633	(65 %)
Applying	0	(0 %)	17	(3 %)	27	(3 %)
Amblyseius cucumeris						
No exposure	13	(45 %)	238	(46 %)	173	(17 %)
Exposed in greenhouse	15	(52 %)	247	(48 %)	771	(76 %)
Applying	1	(3 %)	29	(6 %)	71	(7 %)
Amblyseius californicus						
No exposure	0		507	(99 %)	694	(66 %)
Exposed in greenhouse	0		6	(1 %)	342	(33 %)
Applying	0		1	(0 %)	8	(1 %)
Phytoseiulus persimilis						
No exposure	88	(25 %)	513	(100 %)	495	(71 %)
Exposed in greenhouse	258	(74 %)	1	(0 %)	176	(26 %)
Applying	4	(1 %)	0	(0 %)	23	(3 %)
Hypoaspis miles						
No exposure	51	(21 %)	388	(75 %)	312	(39 %)
Exposed in greenhouse	180	(75 %)	108	(21 %)	439	(56 %)
Applying	9	(4 %)	18	(4 %)	53	(5 %)

Table 4-1. Exposure of the individuals in run 2 and run 3 to the different beneficials. Based on the level of confidence of the information from the annual questionnaires from 1997 to 2001 and the inquiry to the greenhouse owners in 2005.

5 Analyses of specific IgE

5.1 Screening for IgE

In order to test whether an IgE response to the different species could be detected, a screening was done on the samples from run 2 and run 3.

5.2 Material and methods

Allergen preparations of the following species were made;
Tetranychus urticae, Phytoseiulus persimilis, Hypoaspis miles, Amblyseius cucumeris, and *Aphidius colemani.* The invertebrates used for the analyses were purchased from Koppert BV, The Netherlands.

The detailed description of the methods of producing extracts and analysis of the specific IgE can be read in Appendix 1.

The results of the IgEs were presented in units of OD (Opticl densities) as there was no possibility for standardizing the values according to the normally used IUs (International units).

5.3 Reanalyses of the positive screening values for all the measurements

For all persons with positive IgE in either run 2 or run 3 all the samples from the person were analysed including reanalysis of the previously analysed. For datails see Appendix 1.

5.4 Statistical analysis

For each of the beneficial species (*A. colemani, A. cucumeris, P. persimilis,* and *H. miles*) the distributions of IgE values were graphically inspected. They were separated in groups according to the individual exposure in the actual run (no exposure, exposure in the greenhouse and handling predators themselves ("Applying")) (see chapter 4). Differences were tested by a Kruskal-Wallis non-parametric analysis of variance.

Additionally a logistic regression analysis was made on IgE values dichotomized at the chosen detection value, 0.05 OD including the personal characteristics sex, seniority, and atopy, based on either information about atopic disease in the family or one or more positive reactions to the standard prick test at the entrance into the study. Besides, the total IgE measured in run 0 and run 1 was included (logarithmically transformed).

For the reanalyses a cross sectional time series analysis on negative binomial distribution was carried out.

5.5 Results

The values of IgE in response to the five species are shown in the figures 5.1 to 5.5. Each figure shows the distribution of the 680 values from run 2 and run 3. Each curve shows the distribution of subjects not exposed to the animal within the last year, exposed in greenhouse within the last year, and applying the beneficials more than once within the last year, respectively.

For *T. urticae* no exposure estimates were available. Therefore the curves were separated by the personal value atopy defined as one or more positive prick tests.

Table 5-1 shows the number of persons with positive IgE defined by a cut-off of 0.05 OD units (see Appendix 1) in run 2 and run 3. The numbers of positive values for *A. cucumeris* were 8.7 % and 4.3 %, for *P. persimilis* 2.6 % and 3.0 % and for *H. miles* 2.9 % and 2.6 %. The detailed values are described for each specimen in the following chapters 5.3.1 through 5.3.5.

Table 5-2 shows the odds ratios and confidence intervals of the logistic regression analysis for the five species. In this analysis the IgE values were dichotomized with a threshold value of 0.05 OD as proposed in Appendix A.

	Run 2			Run 3		
	No exposure	Exposed in greenhouse	Applying	No exposure	Exposed in greenhouse	Applying
Aphidius colemani						
< 0.05 OD	112	205	20	98	197	9
≥ 0.05 OD	6 (5.4 %)	7 (3.4 %)	1 (5 %)	3 (3.1 %)	7 (3.6 %)	0 (0 %)
Amblyseius cucumeris						
< 0.05 OD	81	213	29	70	208	23
≥ 0.05 OD	7 (8.6 %)	19 (8.9 %)	2 (6.9 %)	3 (4.3 %)	8 (3.8 %)	2 (8.7 %)
Phytoseiulus persimilis						
< 0.05 OD	214	116	12	195	103	7
≥ 0.05 OD	6 (2.8 %)	3 (2.6 %)	0 (0 %)	4 (2.1 %)	5 (4.9 %)	0 (0 %)
Hypoaspis miles						
< 0.05 OD	161	164	16	138	150	18
≥ 0.05 OD	4 (2.5 %)	6 (3.7 %)	0 (0 %)	5 (3.6 %)	3 (2 %)	0 (0 %)

Table 5-1. The distribution of the IgEs against the four predatory species in the exposure groups in run 2 and run 3, respectively.

	Odds ratio	CI95%	Odds ratio	CI95%
Aphidius colemani				
In greenhouse	1.38	(0.30-6.32)	1.25	(0.31-4.98)
Applying	*)			
Positive prick test	0.92	(0.22-3.97)	**4.40**	**(1.23-15.68)**
Total IgE	**3.35**	**(1.87-6.01)**		
Amblyseius cucumeris				
In greenhouse	0.59	(0.17-2.05)	0.62	(0.18-2.12)
Applying	1.40	(0.23-8.33)	1.38	(0.24-8.1)
Positive prick test	1.27	(0.35-4.63)	2.46	(0.79-7.62)
Total IgE	**1.54**	**(1.02-2.31)**		
Phytoseiulus persimilis				
In greenhouse	2.19	(0.57-8.49)	2.36	(0.62-8.99)
Applying	*)			
Positive prick test	0.24	(0.03-2.24)	0.54	(0.07-4.38)
Total IgE	**1.65**	**(1.03-2.64)**		
Hypoaspis miles				
In greenhouse	0.53	(0.12-2.26)	0.53	(0.12-2.27)
Applying	*)			
Positive prick test	1.71	(0.28-10.5)	1.57	(0.31-8.01)
Total IgE	0.94	(0.54-1.63)		
Tetranychus urticae				
Sex (male>female)	**0.23**	**(0.06-0.94)**	0.32	(0.09-1.18)
Positive prick test	0.36	(0.07-1.82)	1.58	(0.39-6.34)
Total IgE	**2.70**	**(1.59-4.58)**		

Table 5-2. Logistic regression of the IgE of the five different specimens. The threshold of positive values is 0.05 OD. (run 2 and run 3 together n=629) The left column shows the odds ratios including total IgE in the model, the right column without total IgE. * means that the model predicts perfect failure (no difference from non-exposed) and the observations are not included.
Significant effects ($p<0.05$) are shown in **bold.**

5.5.1 *Aphidius colemani*

The rate of sensitization of *A. colemani* was 4.2 % and 3.3 % in run 2 and run 3, respectively. Figure 5.1 shows the distribution of the actual values. There was no difference between the different exposure schemes, but those applying tended to have lower values than the others.

The IgEs were correlated with atopy (one or more positive prick tests) and total IgE. Significant correlations between IgE of *A. colemani* and prick test responses to birch, the two house dust mites, and *Alternaria* were shown. On the other hand there was a relatively low correlation with the IgEs against the mites. This was in agreement with the lack of inhibition by the mite antigens (see Appendix 1).

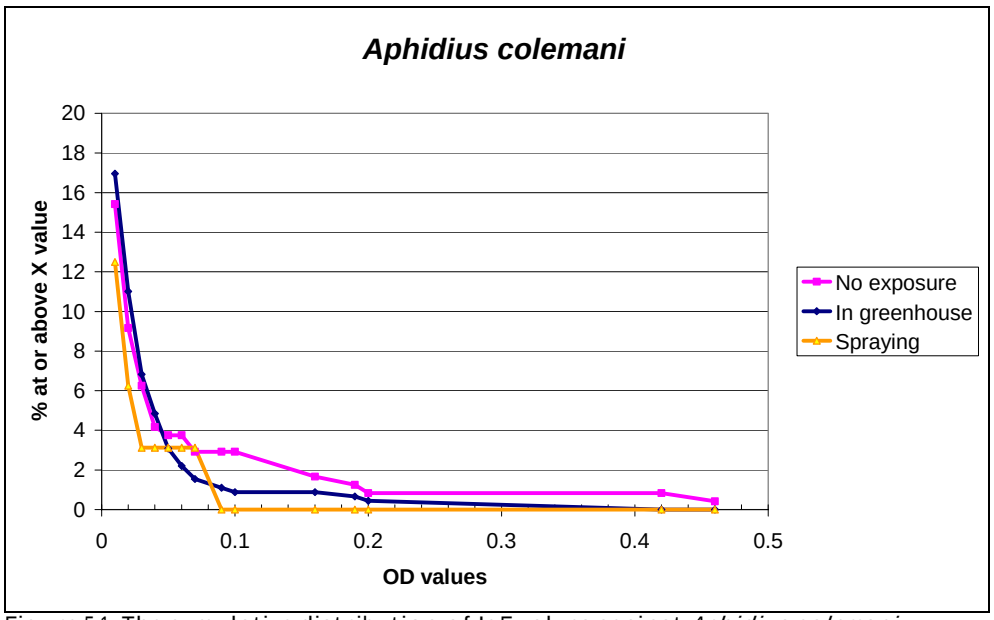

Figure 5.1. The cumulative distribution of IgE values against *Aphidius colemani* according to the exposure groups.

5.5.2 *Amblyseius cucumeris*

The rate of sensitization was 8.6 % and 4.3 % in run 2 and run 3, respectively. There was a tendency to a higher rate in the two groups of exposure (Mann-Whitney p= 0.07) although those with the possibly highest exposure had lower values than the others. The difference was mainly seen in a limited number of high levels, which might be indicative of a higher rate of sensitization. In the logistic regression no effect was seen on the dichotomized data.

The IgE of *A. cucumeris* correlated with the other mites and the prick tests for both house dust mites, while the cross inhibition analyses (Appendix 1) showed only partial cross inhibition from house dust mites, but not from any of the other mites.

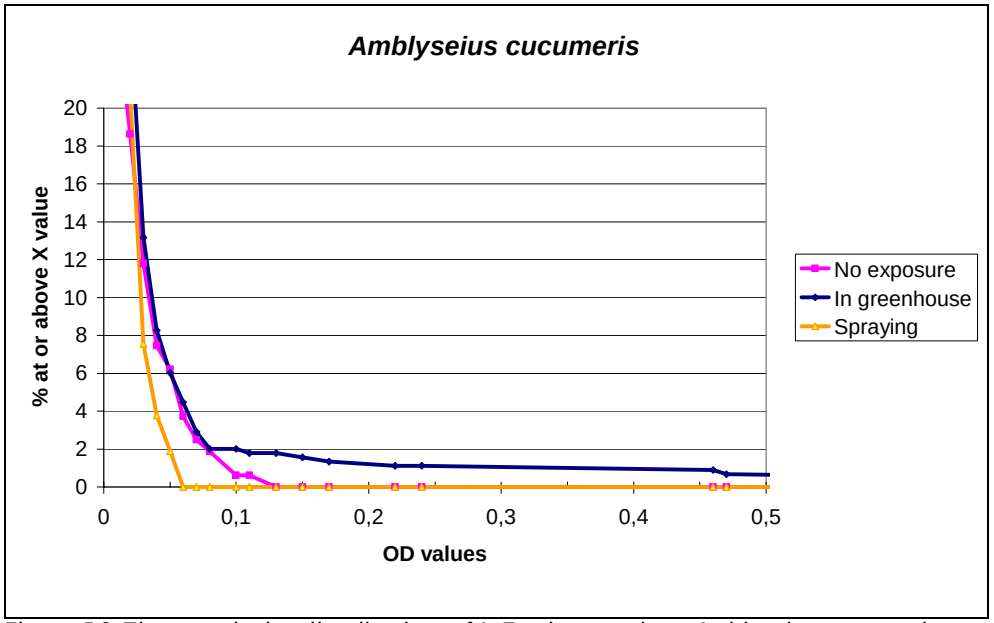

Figure 5.2. The cumulative distribution of IgE values against *Amblyseius cucumeris* according to the exposure groups.

5.5.3 *Phytoseiulus persimilis*

The prevalence rate of IgE values over the chosen limit (0.05 OD) was 2.5 % and 2.9 % in run 2 and run 3, while no relation to the exposure was seen. Generally the IgE values were lower than those of *A. cucumeris*. *P. persimilis* and *T. urticae* showed a high correlation and the cross inhibition experiments showed a considerable mutual cross inhibition while the inhibition from *A. cucumeris* was moderate.

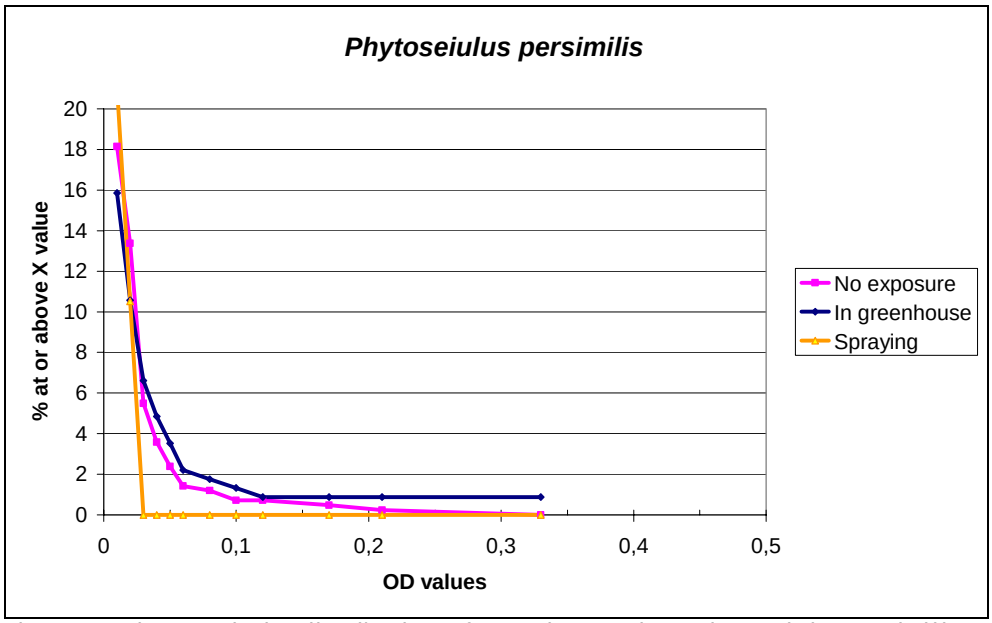

Figure 5.3. The cumulative distribution of IgE values against *Phytoseiulus persimilis* according to the exposure groups.

5.5.4 *Hypoaspis miles*

The IgEs of *H. miles* showed low values with 2.8 % and 2.5 % above 0.05 OD, only a single value was above 0.1 OD. In the cross inhibition study this did - not show any inhibition indicating this being caused by a non specific reaction.

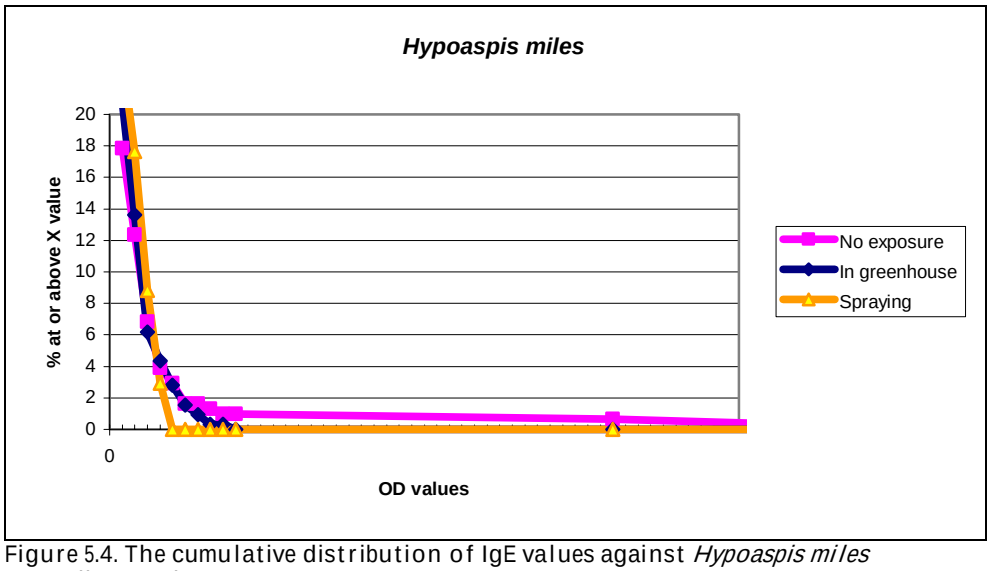

Figure 5.4. The cumulative distribution of IgE values against *Hypoaspis miles* according to the exposure groups.

5.5.5 *Tetranychus urticae*

This naturally occurring mite showed higher values than the beneficial mites and there was a clear difference related to atopy, probably primarily correlated with allergy to house dust mites (see fig 5-6). The inhibition experiments showed a clear inhibition from the mite itself and a less abundant inhibition from *P. persimilis*. Curiously the relation with atopy was only seen in run 2 while only 3 out of 68 atopics had positive values compared with 10 out of 74 in run 2.

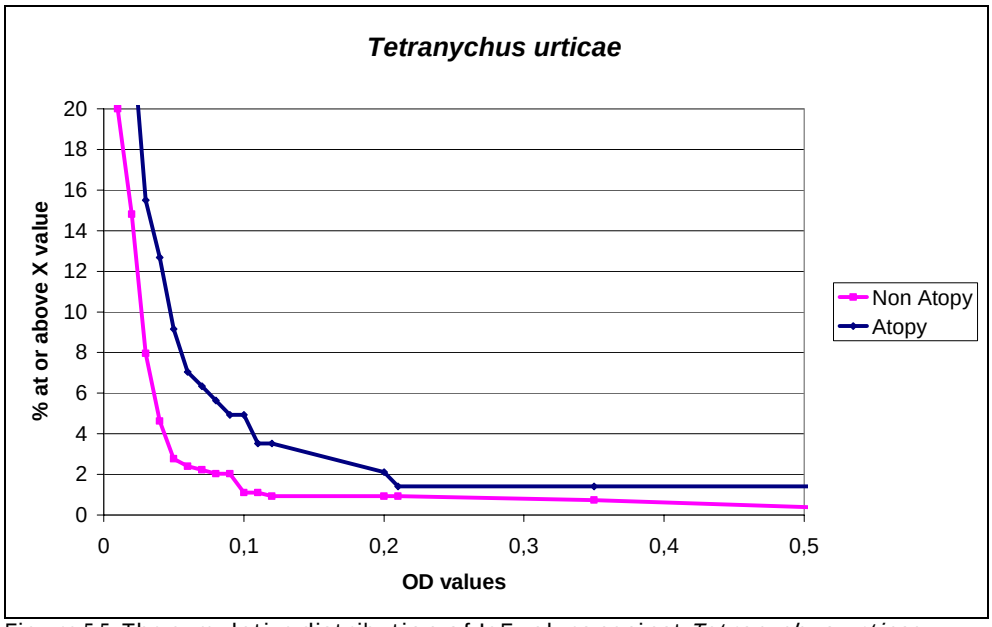

Figure 5.5. The cumulative distribution of IgE values against *Tetranychus urticae* according to status of the prick test (Atopy ~ 1+ positive test).

5.5.6 Correlation between the different IgEs

	ige_*A. colemani*	ige_*A. cucumeris*	ige_*P. persimilis*	ige_*H. miles*
ige_*A. cucumeris*	0.2373			
ige_*P. persimilis*	0.1913	0.3265		
ige_*H. miles*	0.2084	0.2181	0.1633	
ige_*T. urticae*	0.1843	0.1968	0.3749	0.1020

Table 5-3. The Spearman non-parametric correlation between the values of the five different IgEs in the last sample of each person (n=369). All correlations are significantly different from 0 ($p<0.001$).

Previous studies have indicated cross allergy between the different mites and house dust mites (*Dermatophagoides*).Therefore a correlation analysis was made between the results of the prick tests made at the persons first examination 1 to 3 years before the measurements of the IgEs.

Figure 5.6 shows the correlation coefficients between the five IgEs and the prick tests against the two house dust mites, birch and *Alternaria*. There were no coefficients above 0.10 with any of the other 7 prick tests (grass, mug worth, horse, dog, cat, *Cladosporium*, and *Trichoderma*).

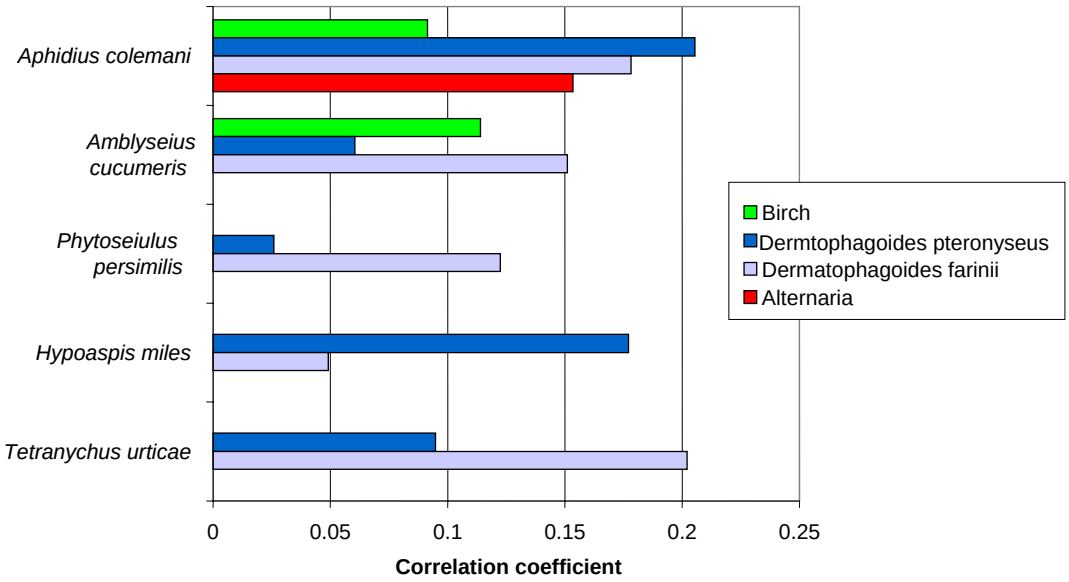

Figure 5.6. The Pearson correlation coefficients between selected prick tests made at the start of the study and the IgE dichotomized at 0.05 OD. n=363 and correlation coefficients above 0.10 are significant (p<0.01).

5.5.7 Correlation between the IgEs in the follow-up

The results showed a close correlation between the consecutive samples and there were only slight signs of change in sensitization following exposure. The change in the indivual measurements is shown in Appendix 1, chapter 1.

5.6 Discussion

The IgEs were analysed using extracts made from the whole animal as there was no previous standard of extracts and the antigens are not well defined. This may lead to a lot of unspecific effects and a considerable effort was needed to get a stable and low background. This may in part be the cause of the relatively low values measured. On the other hand the sensitization rate did not differ from those measured against *A. cucumeris* in the Dutch study (Groenewoud *et al.*, 2002a). A strong correlation between the individual values in the two runs may support that the measured values represent a true biological sensitization.

The very limited correlation with the indicators of exposure may have different causes. Firstly, the exposure estimates were crude based on information on the use anywhere in the greenhouse. More refined information would be preferable but due to the use of historic data this could not be revealed. The greenhouse workers may previously have worked with beneficial species which had been introduced in larger scale from the late 1970´s. There may be an exposure in the other greenhouses or cross reaction with other unknown allergens. A study of persons without relation to greenhouse work can give a background level of the sensitization.

On the other hand none of those having reported to be in direct contact with the beneficial arthropods in the previous year and thereby the highest and best documented exposure, had high values of IgE. This low sensitization rate in handlers of beneficials might be caused by selection as those who has been sensitized stop applying or leave the job (healthy worker selection). The

atopics being more sensitive and having more work related respiratory symptoms would then be a risk group. However, there was no indication of fewer atopics among handlers than among non-handlers.

Comparing the IgEs against the four different beneficial species showed some differences. The IgE against the parasitoid *A. colemani* seemed to be clearly different from the others; the levels were comparable with those of the mites. No previous information on the sensitization is available, why a further study of this specie is warranted.

A. cucumeris had the highest frequency of use; about 75 % of the persons were exposed. Some indications of an exposure relationship were seen although weak. In the Dutch study both sensitization and nasal reactions were seen, although the exposure estimates were very crude and all persons were exposed to bell pepper pollen as well (Groenewoud *et al.*, 2002b; Groenewoud *et al.*, 2002a). We have in our clinic seen a patient with a clear reaction to *A. cucumeris* followed by a very strong prick test reaction and histamine release. This mite therefore needs a further investigation of its health effects.

P. persimilis has in the Swedish study shown health effects comparable with those of *A. cucumeris*. In our study the IgEs against *P. persimilis* were clearly lower than that of *A. cucumeris* and there was a considerable influence of *T. urticae*. This may be due to a cross sensitization but *T. urticae* is used for prey in the production of *P. persimilis*, and there may therefore be a contamination of *P. persimilis* with *T. urticae* protein in our preparations. To avoid this *P. persimilis* fed on another diet is needed for the analyses.

The IgE against *H. miles* seems to be less stable than the others. Only little information of the health effects of this mite is available as the persons showing sensitization in the Swedish study were also sensitized to *P. persimilis* (Kronqvist *et al.*, 2005).

Looking at the time course the positive values were stable over time, and there was a satisfactory analytical stability when the concentration was high enough. On the other hand the IgEs did only show limited information about the development of sensitization.

5.7 Conclusions

The IgEs against the beneficials all showed some positive values although with relatively weak relations to relevant exposure or personal characteristics. The parasitoid, *Aphidius colemani* and the mite *Amblyseius cucumeris* seem to be the best candidates for a further analysis, but the differences between the four species were limited and can not exclude any of the others.

As expected the naturally occurring mite *Tetranychus urticae* had an influence and needs to be taken into account. It is a well known allergen, but the actual study does not permit any estimate of individual exposure.

6 Histamine release tests

6.1 Background

As an alternative to IgE against the predators it is possible to measure histamine release from IgE-sensitized basophiles circulating in the patient's blood. Two types of histamine release tests can be applied: 1) direct histamine release using fresh peripheral blood from the study subjects or 2) by passive sensitization of stripped basophiles using serum from the study subjects.

For practical and logistic reasons it was not possible to obtain fresh anticoagulated blood from the persons included in the study, and it was therefore decided to use the second type of histamine release (serum sensitization of stripped basophiles). In contrast to specific IgE determinations, histamine release from sensitized basophiles indicates the biological significance of the allergen specific reaction.

6.2 Material and methods

From the results in chapter 5, screening of IgE, a number of species were selected for further analysis.

Due to the very weak correlation with the measures of exposure on one hand and spurious correlations with the concomitant occurrence of symptoms there was no obvious candidate for the selection of persons for the second analysis. On the other hand there was a significant correlation between the two samples from the individual person and some cross reactivity between the mites, but not with the parasitoid *Aphidius colemani.* From the inhibition experiments IgE of *A. colemani*, *Phytoseiulus persimilis*, and *Amblyseius cucumeris* seem to be the most specific, while *Hypoaspis miles* seems to be more unspecific.

In the following investigations *A. colemani*, *A. cucumeris* and *P. persimilis* were chosen for investigation.

6.2.1 Selection of persons for analysis

Persons with at least 1 positive IgE (>=0.05 OD) towards *A. colemani*, *A. cucumeris*, *P. persimilis*, or *H. miles* were chosen (71 persons).

In order to get a contrast, controls were chosen as those having values below 0.025 OD in any of the IgE in any of the samples.

A match on atopy status (positive prick test), sex, smoking habits (smoker/non smoker), and age was made. Matching was carried out by sorting all the eligible persons according to the above mentioned criteria. The controls were then those just after the persons with positive values (n=37).

Hereafter only persons with three or four samples were chosen (70 positive and 30 controls) providing 261 and 119 samples, respectively, in total 380 samples.

The other group was 138 valid samples from the 149 participants from Funen in the population based RAV-study in the period September 2003 to February 2004 (see paragraph 3.4).

The persons in this group with job title or trade related to agriculture or horticulture (6 persons) or who in the questionnaire had mentioned that they had left any of these trades due to respiratory complaints (4 persons) were included, but marked in order to be excluded from some of the analyses.

Group	No. persons	No. samples
1+positive IgE	70	261
Controls	30	119
Population sample	138	138

Table 6-1. The number of samples selected for further analysis of Histamine release and specific IgE.

6.2.2 Analytical methods

The passive sensitization method is based on the use of buffy coat from the blood bank (donor cells) where stripped basophiles in the cell suspension are passively sensitized with sera from study subjects. The passively sensitized cells are then incubated with allergen (i.e. extracts of predators) in different concentrations. To determine the presence of biologically active IgE in the serum samples, histamine release is measured.

In brief, buffy coat blood samples used for the sensitization were screened and selected for the capability to elicit an anti-IgE response (histamine release (HR) > 30 %) and with no histamine release reactivity towards 10 common inhalant allergens, 10 food allergens and the allergen with the unknown biological activity.

Sensitization of stripped basophiles is performed as described earlier (Dirks *et al.*, 2005). In brief, peripheral blood monocytes (PBMC's) from the selected buffy coat were isolated by Lymphoprep gradient centrifugation and contained 1-2 % basophiles. Cell bound IgE was removed by washing the PBMC's in a phosphate buffer (pH 3.55). Stripped basophiles were then sensitized with sera from allergen sensitized patients and serum from a healthy non-allergic control, respectively.

The passively sensitized cells were then incubated with extracts of *A. cucumeris*, *P. persimilis*, *A. colemani*, and a mixture of the growth substrates for the predators. Extracts were tested in six concentrations (from 1:100 to 1:50.000, dilution factor 3.5) and histamine release was determined by the glass fibre method (HR-Test, RefLab, Denmark) according to the manufacturer's standard procedure, and described in (Untersmayr *et al.*, 2005). Results are expressed in percentage of total cellular histamine content, and a HR > 10 % is considered a positive response.

The histamine release responses were classified according to the highest sample dilution inducing > 10 % histamine release. This classification implies that cells responding to the lowest concentration will be a class 6 reaction and

cells only responding to the highest concentration will be a class 1 reaction. No reaction to any of the dilutions is a class 0 reaction. It should be noted that extracts of predators in concentrations above 1:100 induced unspecific histamine release in healthy controls and these concentrations were therefore omitted.

6.2.3 Statistical methods

Due to the skew distribution and a variance larger than the mean a negative binomial distribution is suggested. The values were then tested with a regression analysis (prevalence data) and a cross sectional time series analysis (change in HR titres). As independent variables, besides the exposure status, sex, actual smoking habit and atopy (positive standard prick test) were chosen. The correlations between IgE and HR were tested using a non-parametric correlation analysis (Spearman).

6.3 Results

6.3.1 Persons without exposure to greenhouses

The histamine release test was obtained from 138 persons, 72 females and 66 males aged between 20 and 44 (average 34.3) years. The prick test results are shown in figure 6.1. 44 persons (32 %) had at least one positive prick test. Of the 12 persons with work in agriculture or gardening one person had one positive prick test while one had three.

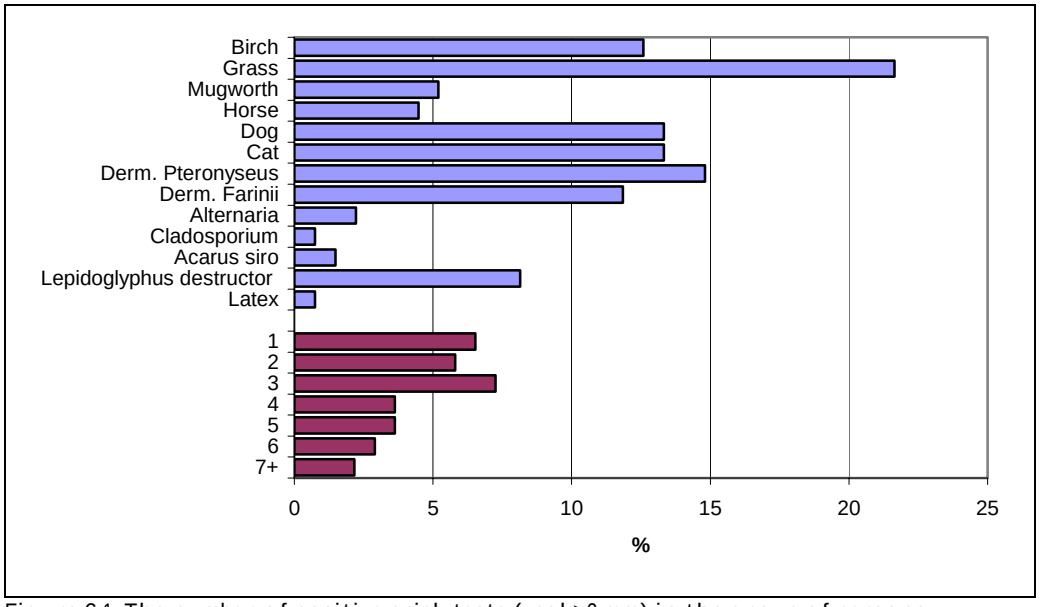

Figure 6.1. The number of positive prick tests (veal ≥3 mm) in the group of persons from the population sample (n=138). The upper part shows the frequency of sensitization against the different tests, while the lower part shows the distribution of number of positive tests.

The frequency of the positive titres of *A. cucumeris, P. persimilis,* and *A. colemani* according to positive prick tests (atopic), is shown in figure 6.2.

The figure shows a relatively high frequency of positive titres, for *A. cucumeris* higher in atopics than in non-atopics, a difference not seen in the other species. The correlations between the titres against any of the beneficial arthropods on one hand and positive reactions against storage mites *Acarus*

siro and *Lepidoglyphus destructor* or against other prick test reactions were all negative.

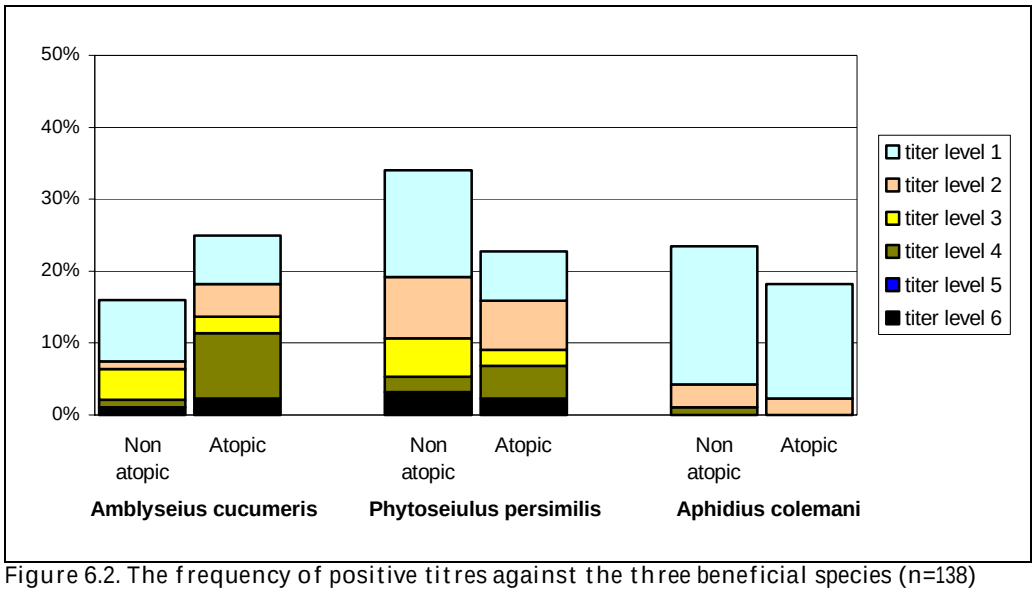

Figure 6.2. The frequency of positive titres against the three beneficial species (n=138) in persons with and without at least one positive prick test.

Among the twelve persons with relation to agriculture or gardening two non-atopics showed reaction to all three beneficial species.

6.3.2 Greenhouse workers

6.3.2.1 Amblyseius cucumeris

The frequency of titres of the HR measurements in each run for those not exposed the previous year and those exposed in greenhouse or applying themselves, are shown in figure 6.3.

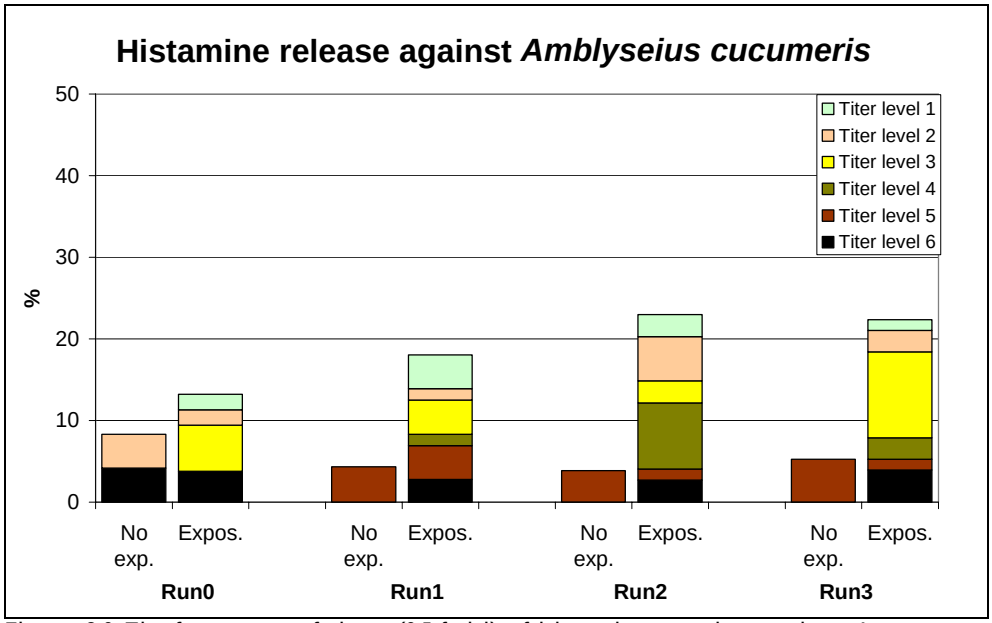

Figure 6.3. The frequency of titres (3.5 fold) of histamine reaction against *A. cucumeris*. No exp. = not using *A. cucumeris* in the previous year. Expos. = *A. cucumeris* used in the greenhouse or applied by the person.

There was a clear significant (p=0.002) difference between exposed and non-exposed persons in the titre levels of *A. cucumeris*. However, those having applied *A. cucumeris* during the previous year did not differ from the rest of the exposed persons.

The increase in HR titres was related to the exposure in greenhouse, although not significant (p=0.20). Restricting the analyses to those with a high validity of exposure characterization did not alter the results.

The positive titres were characteristically concentrated on relatively few persons (22 %) but they had in average relative high titres and titres within each person were closely related. As seen in Table 6-2 a set of significant increases in titres (increase >= 2 levels) were seen in 8 persons.

Persons with at least one positive standard prick test had higher titres than those with no positive prick tests, while the 8 episodes with significant increase in titres were seen in mostly non atopics.

The prevalence rates shown are biased estimates as the persons were selected by having a positive IgE. Minimal rates could be estimated by proposing that those not tested were negative thereby giving rates of *A. cucumeris* of 1.4 % and 6.0 %, *P. persimilis* of 9.5 % and 10.1 %, and *A. colemani* of 8.9 % and 7.7 %, for non-exposed and exposed persons in the last run, respectively. In comparison with the only other studies the rates are somewhat lower.

The time course of the sensitization to *A. cucumeris* was clear. The rates increased and a number of individuals showed rise in titre of more than two steps. Eight persons showed this by giving a sensitization rate of 8/1050 person years = $7.6 * 10^{-3}$ (CI95%: $2.3 - 12.9 * 10^{-3}$).

Subject	sex	Positive prick test	Exposure to *A. cucumeris*				Histamine release titre			
			Run0	Run1	Run2	Run3	Run0	Run1	Run2	Run3
1	f	yes	2	1	1	1	2	3	2	2
2	m	no	1	1	1	1	0	0	**4**	4
3	f	no	1	1	1	1	3	**5**	4	4
4	f	yes	1	1	1	1	3	3	2	2
5	f	yes	0	0	0	0	6	5	5	5
6	m	yes	1	1	1	1	0	1	**3**	3
7	m	no	1	1	1	1	3	**6**	4	3
8	m	no	0	2	2	2	2	3	2	3
9	f	yes	1	1	1	1	6	6	6	6
10	f	no	1	2	2	2	1	1	2	3
11	f	yes	1	1	1	1	0	1	0	.
12	f	no	1	1	1	1	6	5	6	6
13	f	yes	.	1	1	1	.	4	3	3
14	m	yes	.	1	1	1	.	5	4	3
15	f	yes	.	1	1	1	.	0	4	.
16	m	yes	.	1	1	1	.	2	1	**3**
17	m	no	.	1	1	1	.	0	1	**3**
18	f	no	.	1	0	.	.	0	0	1
19	f	yes	.	1	1	1	.	.	5	5
20	m	yes	.	1	1	.	.	.	4	**6**

Table 6-2. Individual histamine release results of persons with at least 1 positive titer (20 persons out of 95 included). **Bold** numbers denote an at least two-step increase in titre from one observation to the next. Exposure - non-exposed, 1 - exposed in greenhouse, 2 - applying.

6.3.2.2 Phytoseiulus persimilis

The titre of HR tests against *P. persimilis* is shown in Figure 6.4. In comparison with *A. cucumeris* the frequency of positive titres was higher but the average titre lower and less consistent for the individual person. The relation to exposure was significant ($p=0.001$). Besides, the rate of sensitization was related to exposure ($p=0.045$). Very few persons were applying and this group did not differ from the rest.

The titres were slightly, but significantly higher in atopics, while no other significant relation with personal factors was seen.

Presuming that those not tested were negative, the minimal prevalence rates were *P. persimilis* of 9.5 % and 10.1 % for non-exposed and exposed persons in the last run, respectively.

Seven persons had a more than 2 step increase in *P. persimilis* giving a rate of sensitization of $6.7 * 10^{-3}$ (CI95%: $1.7 - 11.6 * 10^{-3}$).

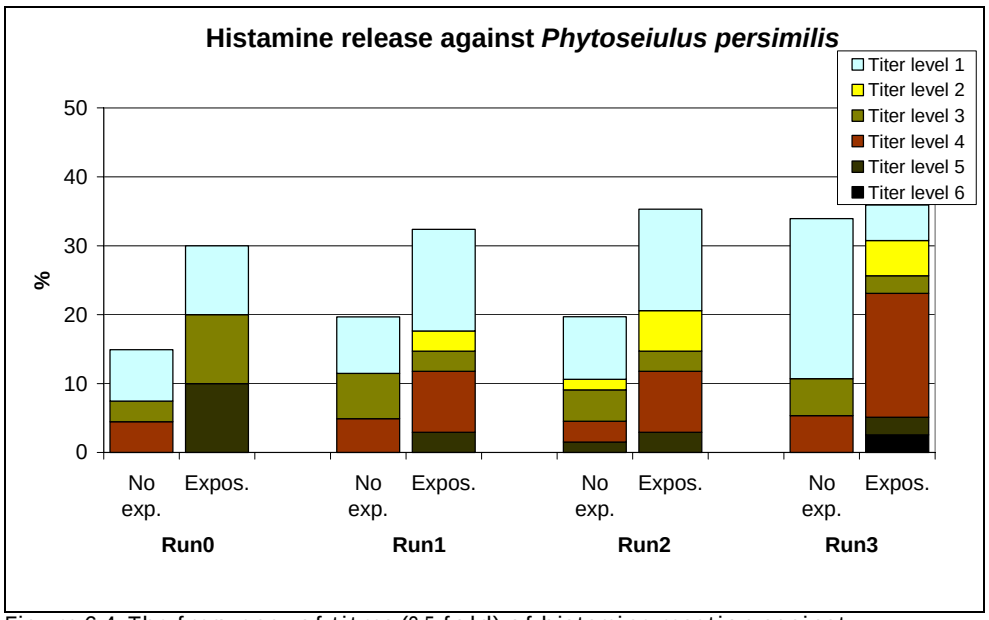

Figure 6.4. The frequency of titres (3.5 fold) of histamine reaction against *Phytoseiulus persimilis*. No exp. = not using *P. persimilis* in the previous year. Expos. = *Amblyseius cucumeris* used in the greenhouse or applied by the person.

6.3.2.3 Aphidius colemani

The distribution of the positive HR tests is shown in Figure 6.5. In comparison with the two mites, *A. cucumeris* and *P. persimilis* the titres were lower and no relation to the exposure variables was seen neither in the level (p=0.36) nor the rate of sensitization (p=0.54). Atopics had lower HR titres against *A. colemani* than non-atopics (p=0.002).

The prevalence rates were 8.9 % and 7.7 %, for non-exposed and exposed persons in the last run, while the incidence rate was $4.8*10^{-3}$ (CI95%: 0.6 – $8.9*10^{-3}$).

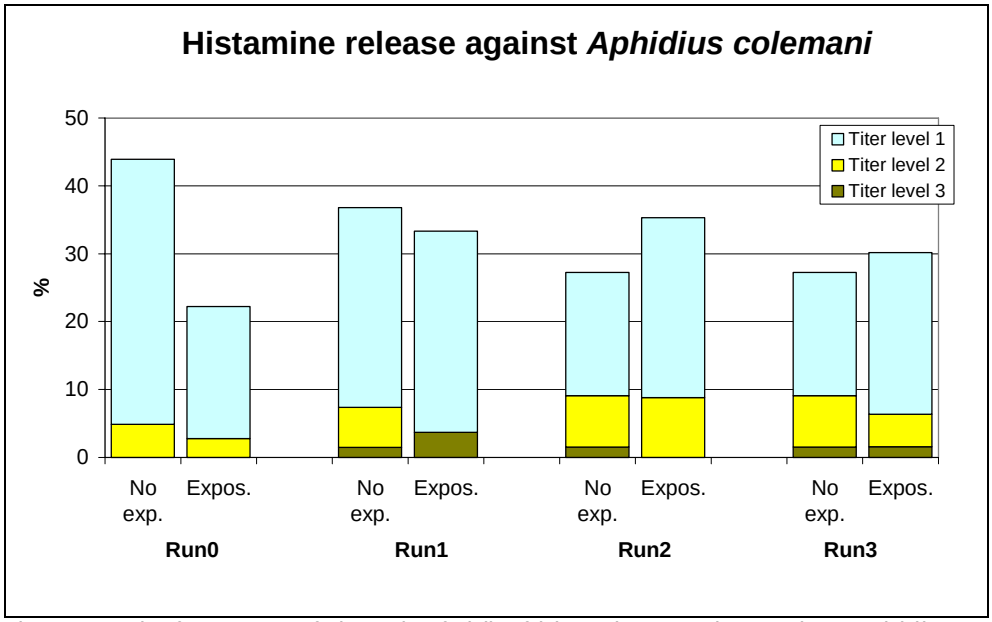

Figure 6.5. The frequency of titres (3.5 fold) of histamine reaction against *Aphidius colemani*. No exp. = not using *Phytoseiulus persimilis* in the previous year. Expos. = *A. colemani* used in the greenhouse or applied by the person.

6.3.3 Correlation between histamine release and specific IgE

Figure 6.6 shows the relation between Histamine release and IgE against *A. cucumeris* in the 218 measurements where both were analysed. As also shown by the statistical analysis a significant increase in IgE was only seen in those with the high titre in HR.

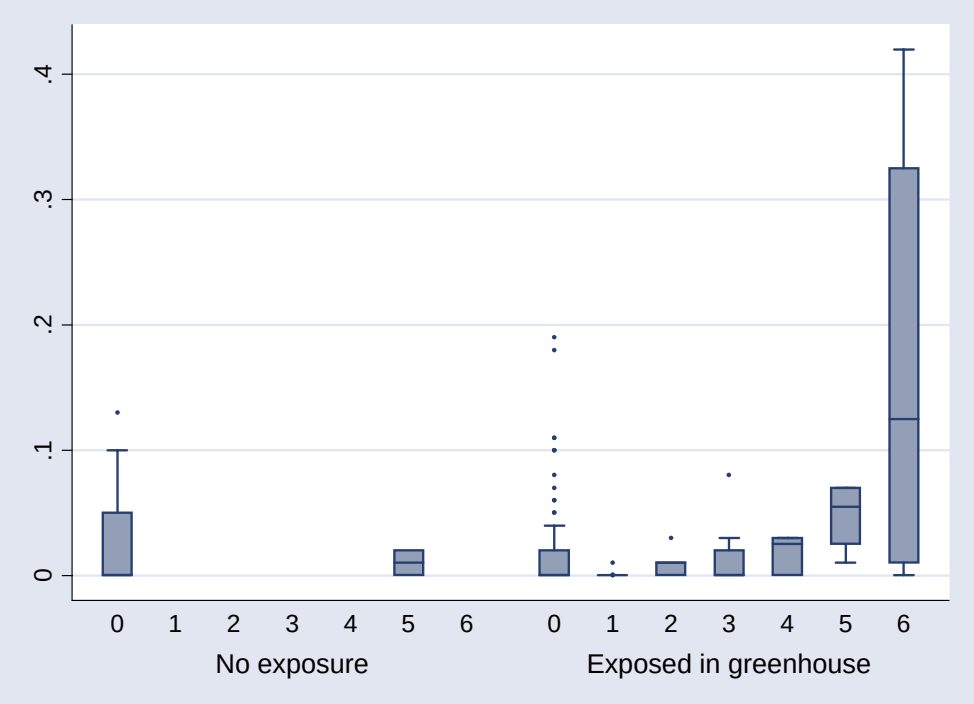

Figure 6.6. The IgE against *Amblyseius cucumeris* in relation to the HR titres. The median, interquartile range (box), 1.5 time the interquartil range (whiskers), and outliers (points).

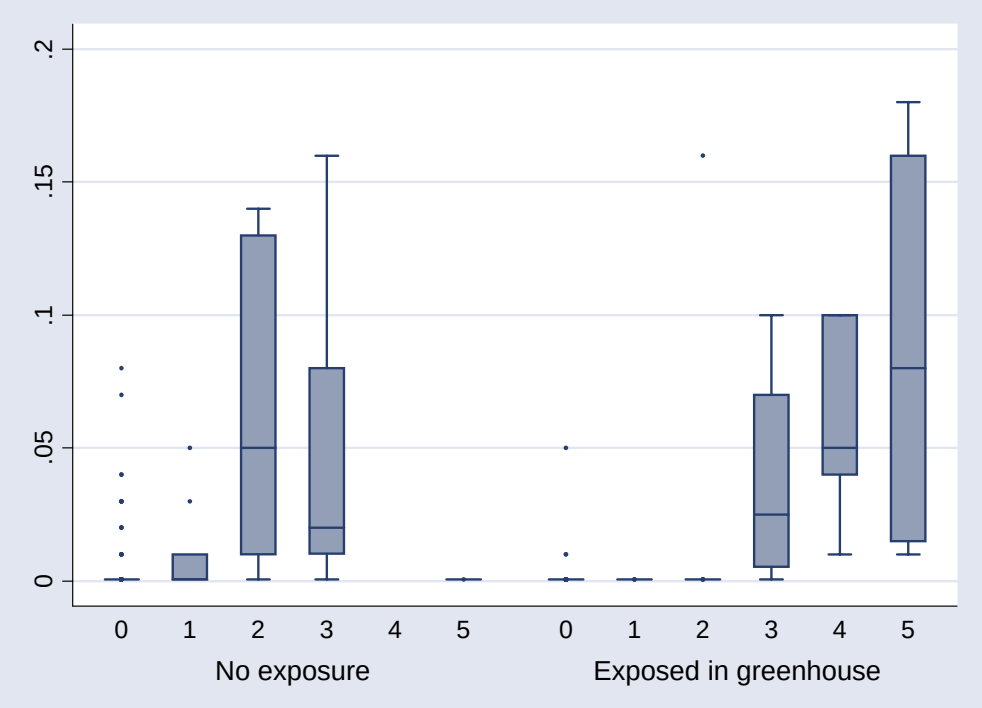

Figure 6.7. The IgE against *Phytoseiulus persimilis* in relation to the HR titres. The median, interquartile range (box), 1.5 time the interquartile range (whiskers), and outliers (points).

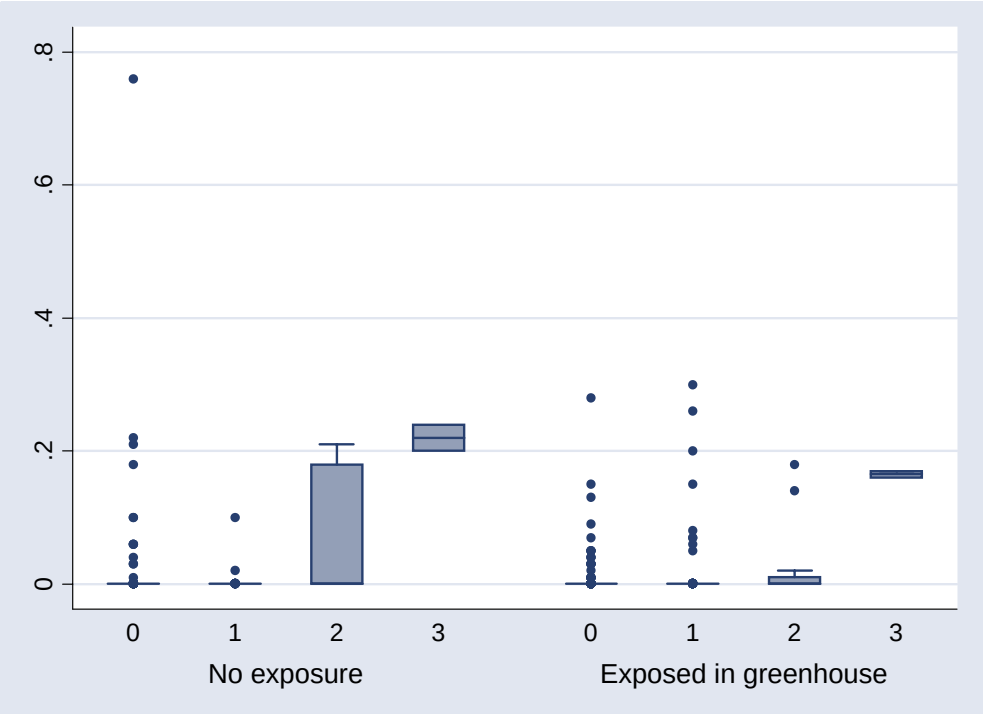

Figure 6.8. The IgE against *Aphidius colemani* in relation to the HR titres. The median, interquartile range (box), 1.5 time the interquartil range (whiskers), and outliers (points).

6.4 Discussion

Despite using the same extracts for analysis, the HR tests seem to be more discriminating between exposed and non-exposed persons than specific IgE. At least the reactions against the mites *A. cucumeris* and *P. persimilis* showed a significant relation to the exposure estimates, both in the cross section and the time course.

The prevalence rates shown are high estimates as the persons were selected by having a positive IgE. Minimal rates could be estimated by proposing that those not tested were negative thereby giving rates of *A. cucumeris* of 1.4 % and 6.0 %, *P. persimilis* of 9.5 % and 10.1 %, and *A. colemani* of 8.9 % and 7.7 %, for non-exposed and exposed persons in the last run. In comparison with other studies the rates are somewhat lower. This may depend on the actual exposure and the fact that sensitization in the Dutch study was based on prick tests (Groenewoud et al., 2002a).

There was a considerable difference between the distributions of sensitization against the two mites. The positive HR against *A. cucumeris* was restricted to relatively few persons who almost exclusively had been exposed while *P. persimilis* showed higher rates, still with a clear exposure relation, but distributed with more varied levels. This may imply a difference in specificity between the two measurements. On the other hand, in the population sample relatively high sensitization rates were seen for both mites, higher than in the non-exposed greenhouse workers. The reason for this was unclear, a relation to atopy was as would be expected, but this could not explain the difference. The only difference in preparation was that the population sample was based on plasma while the other was serum.

Sensitization against *A. colemani* seems to be less pronounced as the levels were lower and no relation to exposure was seen. This can be due to a number of reasons, the most probable is that the sensitization is unspecific against various insects and the actual exposure to this group was insignificant. A testing of persons with a higher exposure, i.e. in the production of the species may reveal a possible effect. In the actual study the sensitization seems to be a minor problem.

The time course of the sensitization to both *A. cucumeris* and *P. persimilis* was clear, the rates increased and a number of individuals showed a rise in titre of more than two steps. Eight persons showed this giving a sensitization rate of about 0.7 % for both *A. cucumeris* and *P. persimilis.* These rates are surprisingly high, because the persons at the start of the study had been exposed for several years. They may therefore have been sensitized already or been selected due to healthy worker effect as their sensitized colleagues have left the trade.

In comparison with the measurements of IgE the histamine release seem to be more sensitive as the exposure relationship was much clearer and the correlation between IgE and HR showed that IgEs were only increased at the two highest levels of HR against *A. cucumeris* and *P. persimilis*. Besides, it was shown that the IgEs did not show any significant changes in the persons over time.

The extracts used in the analyses of HR and IgE were the same, so the mechanism in the sensitization may imply more significant factors than the actual level of specific IgE.

6.5 Conclusions

In conclusion histamine release reaction against the mites *A. cucumeris* and *P. persimilis* seem to be valid measures of sensitization against the mites. An improvement of the technology, mainly by a better characterization of the antigens in the extracts, may increase the sensitivity and specificity.

7 Relation between exposure and health effects

7.1 Introduction

A considerable part of the study was measurements of health effects of the exposure.

In the BIOGART study the persons were, at the annual examinations asked about a series of symptoms mainly from the respiratory tract. For every group of symptoms the first question was whether the person had experienced the symptom in question the previous year. The next questions were about the frequency, type, and relation to factors and activities, both at work and outside work (Larsen & Bælum, 2002).

The analyses follow the model of disease shown in figure 1.1, which shows the relation between exposure, allergy and symptoms.

During the four annual investigations the persons registered a series of symptoms, mainly from eyes, nose, and lungs (Larsen & Bælum, 2002). The symptoms were grouped in categories. Three categories from the lower airways; cough, chest tightness, and wheeze. From the upper airways; symptoms of always stuffed nose, itching nose or pharynx, and running nose. Questions about itching eyes and skin rash were also included. For each category there was a general question about symptoms at work, symptoms after a holiday, and specific additional symptoms to the main symptom.

Finally the persons were asked about asthma within the last three months.

In a follow-up study two principally different methods of estimating health effects are possible. As in a cross sectional study the prevalence of symptoms in relation to the concomitant measures of exposure can be estimated at each session. The other possibility is to estimate incident symptoms, i.e. newly developed symptoms between the examinations. Both methods have advantages and disadvantages. Therefore both types of analyses have been done.

7.2 Analysis of the symptom score

7.2.1 Analytical methods

7.2.1.1 Analysis of prevalences

Analyses were made on two subsets of the observations, the first sample for each person (n=579) and the last sample restricted to either run 2 or run 3 (n=365). In the latter group those who had left the trade but anyhow were examined (n=27) were excluded, as no recent exposure estimates could be obtained.

The latter sample corresponded with those who had measures of specific IgE against the four beneficial animals and *Tetranychus urticae*. Therefore, a third group of analyses were made, the relation between the prevalence of symptoms and sensitization against the four beneficial animals and *T. urticae*.

In order to study the occurrence of asthma more specifically, an index adopted from the European study of asthma (ECRHS) was applied (Pekkanen *et al.*, 2005). This index consists of eight symptoms of which seven were included in the present interview.

For each set of data a logistic regression was made. Firstly, analyses were made only with the exposure variable (0= not exposed, 1= exposed in greenhouse, 2= applied within the last year). Secondly, the additional factors, sex, atopy (one or more positive standard skin prick tests at inclusion into the study) and smoking habits (been smoking within the last 1 year) were included. The results are presented as odds ratios with 95% confidence intervals.

Due to the type of distribution of the asthma index a negative binomial regression analysis was made.

7.2.1.2 Analysis of incidence

The incident cases for five main symptoms: cough, chest tightness, wheeze, asthma, and running nose were calculated. An incident case was defined, if a symptom occurred which had not been reported in the previous run. The time of occurrence was set to the day of examination in the actual run. However, if the symptom was reported in any of the questionnaires returned every three months between the examinations, the date of occurrence was set to the day of the first report in these questionnaires.

The incidences were then analysed by a Cox-regression proportional hazard analysis including the exposure variables as well as sex, atopy, and actual smoking habit. The asthmascore variable was analysed by a cross sectional time series analysis based on the negative binomial distribution.

7.2.1.3 Lung function

The values of FEV_1, FVC, and FEV_1/FVC in the first and the last measurement for each person were selected and the decline per year was calculated. These declines were anlysed by a linear regression including exposure variables as the averages over the period, height, smoking habits, and atopy as independent variables.

According to bronchial hyperreactivity very few observations showed a positive value of PD_{20} (Larsen & Bælum, 2002). Therefore a slope value for the change in FEV_1 in response to the increasing doses of histamine was calculated by the formula

$$\text{slope} = \log((x0-x)/(x0*\text{dose})+1)$$

adopted by (Miller *et al.*, 2002). In order to normalize the distributions the values were log transformed before the linear regression analysis.

7.3 Results

The tables 7-1 to 7-5 and the figures 7.1 to 7.5 containing the results are situated at the end of this chapter.

Aphidius colemani
Figure 7.1 shows the prevalences in the first sample in relation to exposure to *Aphidius colemani*. In table 7-1 odds-ratios between the exposure estimates and the prevalence of the symptoms corrected for sex, status of atopy, and smoking habits are shown. Odds-ratios significantly different from 1 are shown in bold. If no odds-ratio is shown, one of the groups had no observations.

In the initial samples a lower frequency of cough at work, chest tightness at work and itching eyes at work was seen in those exposed to *A. colemani* in the greenhouses, while skin rash was more frequent in those directly applying the wasp.

In the last sample no significant differences between groups were seen, but characteristically no lung or nose symptoms were seen in those directly applying the species.

A more consistent pattern was seen in correlation with IgE, where increased chest tightness at work, stuffed nose, itching nose or pharynx and skin rash was seen in those sensitized to *A. colemani*.

Those exposed to *A. colemani* showed a higher incidence of chest tightness (IRR = 1.82 (1.07-3.10)) and running nose (IRR=2.10 (1.03-4.23)).

Amblyseius cucumeris
Figure 7.2 shows the prevalences in the first sample. In table 7-2 odds-ratios between the exposure estimates and the prevalence of the symptoms corrected for sex, status of atopy, and smoking habits are shown. Odds-ratios significantly different from 1, are shown in bold. If no odds-ratio is shown, one of the groups had no observations.

No significant differences between the exposed groups were seen in the first sample, except a lower prevalence of skin rash at work in the exposed groups. In the last sample the same pattern was seen, especially no wheeze or asthma was seen in those applying *Amblyseius cucumeris.*

Coughing at work and chest tightness at work were marginally more frequent in those sensitized to *A. cucumeris.* Wheeze at work was significantly increased while the general question “Wheeze” tended to be higher in the sensitized group.

Phytoseiulus persimilis
Figure 7.3 shows the prevalences in the first sample acoording to exposure to *Phytoseiulus persimilis.* In table 7-3 the odds-ratios between the exposure estimates and the prevalence of the symptoms corrected for sex, status of atopy, and smoking habits are shown. Odds-ratios significantly different from 1 are shown in bold.

In the first measurement only one person applied *P. persimilis* himself, why figure 7.3 only has two columns. No relation between exposure and symptoms was seen. On the other hand, those sensitized to *P. persimilis* had

significantly more chest tightness at start of work and wheeze than those not sensitized.

Exposure to *P. persimilis* showed no effect on the incidence of symptoms.

Hypoaspis miles
Figure 7.4 shows the prevalences in the first sample in relation to exposure to *Hypoaspis miles.* In table 7-4 odds-ratios between the exposure estimates and the prevalence of the symptoms corrected for sex, status of atopy, and smoking habits are shown. Odds-ratios significantly different from 1 are shown in bold.

No relation between the estimates of exposure in either the first or the last sample was seen, except a lower prevalence of wheeze in those exposed in the first sample.

Those sensitized to *H. miles* had more cough and chest tightness at work than those not sensitized, while a number of other lower airways symptoms showed the same pattern all though not reaching significance.

Exposure to *H. miles* showed no effect on the incidence of symptoms.

Tetranychus urticae
Figure 7.5 shows the relation between sensitization to *Tetranychus urticae* and symptoms. The odds-ratios corrected for sex, atopy, and smoking habits are shown in table 7-5.

Significantly more chest tightness and wheeze at start at work, was seen in those sensitized to *T. urticae.* No specific pattern in the other symptoms was seen.

7.4 Asthma score, lung function, and bronchial reactivity

The asthma score (sum of symptoms from 0 to 7) was highly skewed, 5.2 % of the persons had more than one symptom, more among females than males and this prevalence decreased from run 0 to run 3 (7.5 % to 2.5 %).

Table 7-6 shows the analyses of the change in asthma score in relation to the different exposures. This composite score did not show any relation with the exposure estimates, if any tendency, this was negative. A clear relation with atopy and smoking habits shows that relevant factors influence on the score.

In the lung function values only one significant effect and no general tendencies were seen. Again smoking habit and atopy showed the expected effect, an indication of the validity of the measurements.

7.5 Cases with possible symptoms related to sensitization

To look for the relation between sensitization and development of symptoms the persons developing sensitization during the study were scrutinized.

Five persons with suspected symptoms related to sensitization are mentioned here:

Female working with poinsetta exposed to *A. colemani*, *A. cucumeris*, and *P. persimilis*. She develops positive HR against *A. colemani* and concomitantly report itching nose and eyes as well as skin rash. IgE against *A. colemani* increased gradually from 0.08 to 0.17 OD.
She has a positive prick test for birch, grass, dust mites and *Alternaria*.

Male (no. 6 in table 6-2) exposed to *A. cucumeris* develops positive HR against *A. cucumeris* and transient weak reactions to *P. persimilis* and *A. colemani*. IgEs were, except against *A. colemani* in a single measurement, very low. He had a single nose symptom before sensitization but develops more as well as skin rash and eye symptoms. He had a positive prick test for birch, grass, mug worth, and dog.

Female working with Campanula exposed to *A. cucumeris*, and *H. miles*, and from run 1 *A. colemani*. She develops in run 2 HR titre level 3 against *P. persimilis* and *A. colemani* disappearing in run 3. IgEs were negative. She develops cough, chest tightness, wheeze and nose symptoms in relation to work and general annoyance. In run 3 both sensitization and symptoms disappear.

Female (no. 10 in table 6-2) working with Campanula applying *A. cucumeris* and *H. miles*. She develops a gradual increase in HR against *A. cucumeris* and *P. persimilis* although not exposed to the latter. IgEs against *A. cucumeris* were below 0.01 OD and the others negative. She develops nose and eye symptoms in run 2 but no report in run 3.
In the study she had negative prick tests but was later in 2003 tested having several positive tests (cat, the two house dust mites, and *Lepitoglyphus destructor*) and prick test using a live *A. cucumeris* gave a very strong allergic reaction. The eye and nose symptoms were clearly related to the handling of *A. cucumeris*.

Male (no. 16 in table 6-2) working in a large firm with several plants. They were using both *A. colemani*, *A. cucumeris*, *P. persimilis*, and *H. miles*. He had in all three runs positive HR against *A. cucumeris*, developed a titre of 2 against *P. persimilis*, and a titre of 1 against *A. colemani* in the first two measurements but not in the last measurement. IgEs were negative. He had eye and nose symptoms in all the runs. He had a well known seasonal rhinitis and positive prick test against birch.

7.6 Discussion

A large number of symptoms correlated with the five different exposures in three different settings gives a very large number of possible statistical tests and thereby a considerable probability of mass significance. Therefore the interpretation of the relation between symptoms and exposure rely on the pattern and directions of the significances or tendencies to effect.

In the present chapter it is seen, that only spurious effects are seen in relation to the exposure estimates, especially a very low prevalence of symptoms in those actually applying the beneficial animals.

On the other hand, a number of significant relations between sensitization and especially lower airway symptoms as chest tightness and wheeze were seen for all the three mites, while the wasp, *A. colemani* showed effects on the nose and

skin rash. This wasp also showed, as the only one of the tested animals, exposure relation with the incidence data.

The pattern was the same for *T. urticae,* a well known allergen.

In the statistical analysis no relation was seen between sensitization to mites and symptoms from the upper air ways, a finding seen in both the Dutch study of *Amblyseius* species and the Swedish study of *P. persimilis* (Kronqvist *et al.*, 2005; Groenewoud *et al.*, 2002a).

In the cases suspected of developing reactions against the mites a parallel development of sensitization and symptoms seem to be the case. It was also seen that the persons suspected of developing a clinical allergic disease were atopics with mostly several allergies to the most frequently occurring allergens. Besides, when reacting to one of the beneficial species there was a tendency to reaction to one of the others, too.

The expected relations between both upper and lower air way symptoms, especially, chest tightness, wheeze, and the composite asthma score were seen with status of atopy, but correcting for this did not change the effects of beneficial mites.

When looking at the composite score of asthma and lung function parameters no effect was seen in any of the exposure variables. A two or three years observation period for measuring a decline in FEV_1 or FVC is very short although the large number of observations gives a high precision. On the other hand bronchial hyperreactivity is generally a sensitive measure of an asthmatic reaction. Therefore the possible effect of the predatory animals on inflammatory lung diseases, if apparent, is limited.

The use of general epidemiological methods as used in the present study for allergic effects give some problems, as only a fraction of the population is in risk of developing the diseases. This risk group can only to some extend be identified and the studies will always be hampered by a lack of power, unless very large populations are used. Using a logistic regression in this setting may be a problem, when adjusting for a strong factor such as atopy. Therefore a control analysis using stratification and a Mantel-Hentzell test was done. This did not change the results.

Therefore case findings can give additional information although the etiology often is hard to prove. In this present study a number of suspected cases were found. Characteristics were that only upper airway symptoms were seen, and the sensitization to the predator might be part of a general development of allergy to several factors. Whether asthma will develop at a later stage is uncertain. A very low frequency of bronchial reactivity can be a sign of a selection in the material, as persons with reactive airways leave the trade because of work related symptoms.

7.7 Conclusions

In case reports a development of concomitant sensitization and development of upper airway and eye symptoms were seen.

A number of relations between exposure estimates and symptoms were seen, both upper and lower airways symptoms as well as skin rash.

On the other hand no indication between exposure to any of the predators and asthma was seen, neither in the composite asthma score nor in the lung function tests.

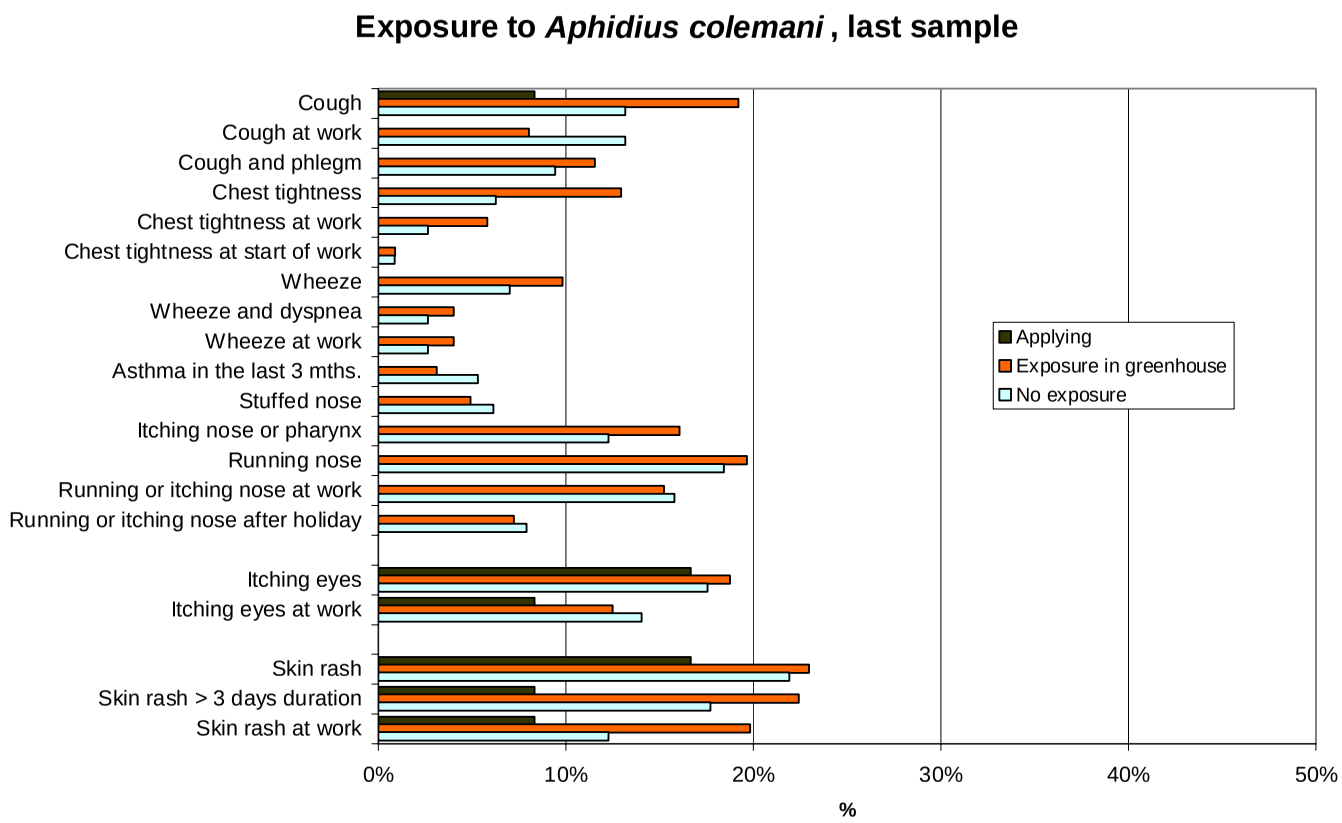

Figure 7.1. Prevalence of the symptoms for each of the exposure groups of *Aphidius colemani* in the last sample of persons participating in run 2 or run 3 and still employed in the greenhouses (n=338).

Aphidius colemani	Initial samples		Last samples		IgE in the last sample
	Exposure in greenhouse	Applying	Exposure in greenhouse	Applying	≥0.05 OD
Cough	0.80 (0.54 - 1.18)	0.27 (0.06 - 1.23)	0.81 (0.52 - 1.26)	0.56 (0.12 - 2.62)	1.67 (0.41 - 6.87)
Cough at work	0.61 (0.36 - 1.03)	0.35 (0.04 - 2.75)	0.61 (0.35 - 1.08)	0.54 (0.07 - 4.34)	0.83 (0.10 - 6.98)
Cough and phlegm	1.08 (0.58 - 2.03)	0.39 (0.04 - 3.52)	0.80 (0.45 - 1.45)	0.63 (0.07 - 5.42)	0.60 (0.07 - 5.04)
Chest tightness	0.83 (0.53 - 1.29)	0.81 (0.22 - 2.93)	1.06 (0.62 - 1.82)		1.03 (0.12 - 8.64)
Chest tightness at work	**0.45 (0.23 - 0.90)**	0.68 (0.08 - 5.56)	1.42 (0.54 - 3.71)		1.87 (0.21 - 16.82)
Chest tightness at start of work	0.94 (0.15 - 5.75)		0.97 (0.17 - 5.45)		**16.24 (1.02 - 259.24)**
Wheeze	1.23 (0.73 - 2.07)	0.81 (0.17 - 3.81)	1.19 (0.66 - 2.14)		1.50 (0.30 - 7.63)
Wheeze and dyspnea	1.04 (0.49 - 2.19)		1.33 (0.53 - 3.37)		1.45 (0.15 - 14.27)
Wheeze at work	1.03 (0.38 - 2.79)		1.00 (0.41 - 2.44)		1.63 (0.17 - 15.27)
Asthma in the last 3 months	1.11 (0.54 - 2.25)	0.93 (0.11 - 7.92)	0.55 (0.17 - 1.71)		
Stuffed nose	0.68 (0.37 - 1.24)		0.53 (0.26 - 1.06)		4.88 (0.88 - 27.24)
Itching nose or pharynx	0.89 (0.58 - 1.36)	0.22 (0.03 - 1.74)	1.07 (0.65 - 1.74)		**3.98 (1.11 - 14.25)**
Running nose	0.90 (0.58 - 1.39)	0.23 (0.03 - 1.76)	0.99 (0.62 - 1.58)	0.33 (0.04 - 2.64)	1.43 (0.36 - 5.73)
Running or itching nose at work	**0.61 (0.39 - 0.93)**	0.41 (0.09 - 1.86)	0.96 (0.50 - 1.83)		1.08 (0.22 - 5.44)
Running or itching nose after holiday	0.55 (0.28 - 1.05)		0.89 (0.36 - 2.21)		
Itching eyes	1.01 (0.69 - 1.48)	0.84 (0.29 - 2.49)	1.05 (0.67 - 1.64)	1.75 (0.57 - 5.36)	0.73 (0.15 - 3.56)
Itching eyes at work	**0.55 (0.35 - 0.89)**	1.22 (0.38 - 3.93)	0.80 (0.48 - 1.33)	1.93 (0.57 - 6.48)	1.09 (0.49 - 2.42)
Skin rash	0.96 (0.65 - 1.44)	2.41 (0.92 - 6.31)	0.95 (0.62 - 1.45)	1.75 (0.57 - 5.40)	**4.51 (1.33 - 15.26)**
Skin rash > 3 days duration	0.80 (0.54 - 1.18)	1.62 (0.60 - 4.37)	0.90 (0.59 - 1.39)	0.84 (0.23 - 3.11)	1.15 (0.29 - 4.48)
Skin rash at work	**0.54 (0.31 - 0.92)**	1.03 (0.32 - 3.27)	1.27 (0.77 - 2.09)	1.34 (0.35 - 5.18)	0.93 (0.19 - 4.55)

Table 7-1. The odds-ratios corrected for sex, atopy, and smoking habits for the symptoms in the first sample (n_579), the last sample (n=338), and in relation to the specific IgE (n=365).

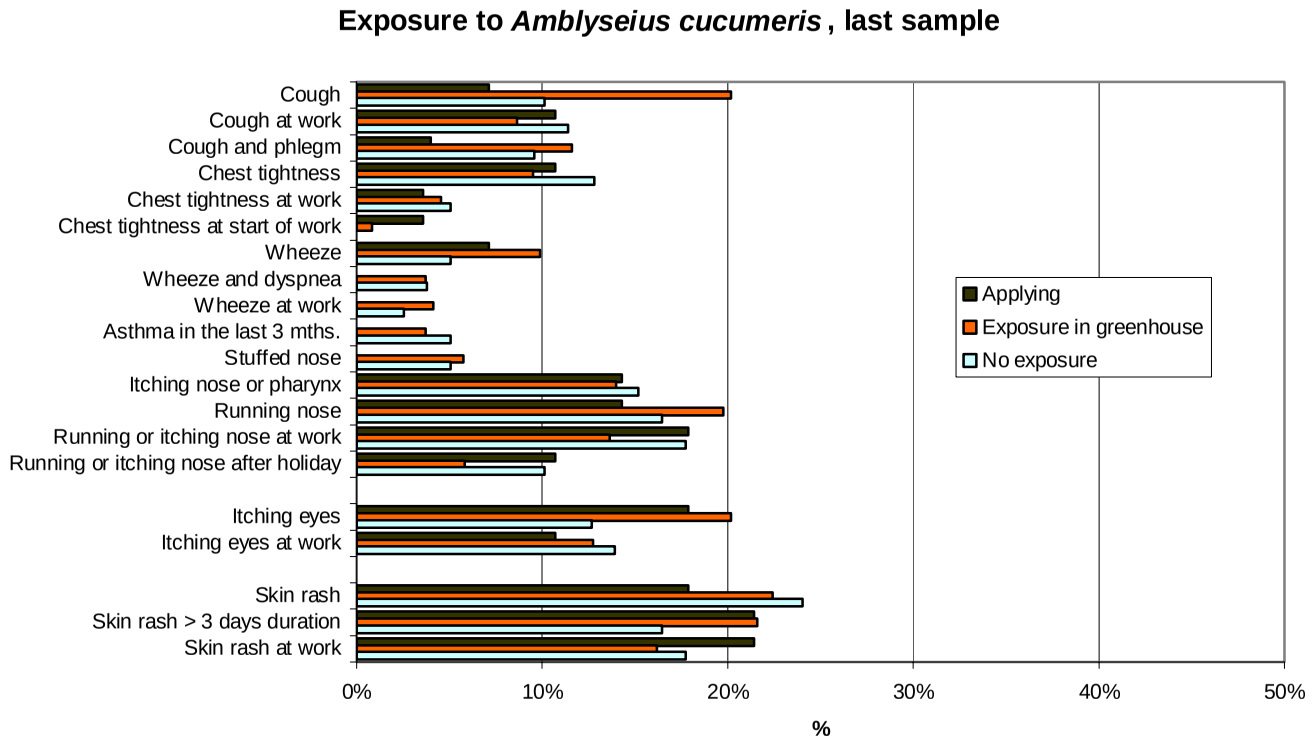

Figure 7.2. Prevalence of the symptoms for persons with and without positive IgE (>0.05 OD) to *Amblyseius cucumeris* in the last sample of the persons (n=365).

Amblyseius cucumeris	Initial samples		Last samples		IgE in the last sample
	Exposure in greenhouse	Applying	Exposure in greenhouse	Applying	≥0.05 OD
Cough		0.76 (0.24 - 2.47)	1.05 (0.65 - 1.70)	0.48 (0.13 - 1.72)	0.96 (0.20 - 4.54)
Cough at work	1.16 (0.65 - 2.06)	0.46 (0.06 - 3.72)	0.72 (0.40 - 1.32)	0.94 (0.25 - 3.51)	3.33 (0.84 - 13.26)
Cough and phlegm	0.69 (0.34 - 1.38)		1.02 (0.53 - 1.96)	0.24 (0.03 - 1.94)	0.63 (0.08 - 5.11)
Chest tightness	1.24 (0.77 - 2.01)	0.63 (0.14 - 2.89)	0.92 (0.52 - 1.62)	0.81 (0.22 - 2.99)	1.89 (0.38 - 9.32)
Chest tightness at work	1.13 (0.54 - 2.35)	1.05 (0.12 - 8.95)	0.81 (0.32 - 2.07)	0.92 (0.10 - 8.04)	4.28 (0.81 - 22.72)
Chest tightness at start of work	0.65 (0.11 - 4.02)		1.51 (0.16 - 13.78)	7.37 (0.39 – 140)	12.96 (0.97 - 173)
Wheeze	0.76 (0.45 - 1.29)	0.26 (0.03 - 2.06)	1.51 (0.76 - 2.98)	0.67 (0.14 - 3.29)	2.91 (0.73 - 11.59)
Wheeze and dyspnea	0.59 (0.28 - 1.26)		0.94 (0.37 - 2.38)	**4.63 (1.94 - 11.07)**	1.75 (0.18 - 16.83)
Wheeze at work	1.56 (0.49 - 4.92)		1.51 (0.54 - 4.20)	**2.84 (1.14 - 7.09)**	**9.06 (1.90 - 43.22)**
Asthma in the last 3 months					
Stuffed nose	1.03 (0.53 - 1.99)	1.35 (0.28 - 6.61)	0.73 (0.35 - 1.52)	0.47 (0.06 - 3.87)	4.12 (0.78 - 21.78)
Itching nose or pharynx	1.23 (0.76 - 1.97)	0.28 (0.04 - 2.19)	1.08 (0.64 - 1.85)	1.15 (0.39 - 3.43)	0.81 (0.16 - 4.04)
Running nose	0.93 (0.59 - 1.48)	0.51 (0.11 - 2.35)	0.95 (0.58 - 1.55)	0.69 (0.22 - 2.19)	0.69 (0.14 - 3.34)
Running or itching nose at work					1.00 (0.20 - 4.95)
Running or itching nose after holiday					3.50 (0.60 - 20.37)
Itching eyes	1.31 (0.87 - 1.97)		1.25 (0.77 - 2.04)	0.95 (0.35 - 2.58)	1.17 (0.31 - 4.42)
Itching eyes at work	1.08 (0.66 - 1.78)		0.79 (0.47 - 1.35)	0.59 (0.16 - 2.14)	1.38 (0.29 - 6.59)
Skin rash	0.95 (0.62 - 1.46)	2.39 (0.90 - 6.35)	0.84 (0.53 - 1.31)	1.16 (0.47 - 2.87)	0.50 (0.11 - 2.37)
Skin rash > 3 days duration	0.96 (0.63 - 1.45)	2.25 (0.84 - 6.04)	0.91 (0.57 - 1.45)	1.58 (0.65 - 3.85)	0.60 (0.13 - 2.78)
Skin rash at work	**0.47 (0.26 - 0.84)**	**0.17 (0.03 - 0.87)**	**0.56 (0.34 - 0.92)**	0.78 (0.28 - 2.15)	0.83 (0.18 - 3.97)

Table 7-2. The odds-ratios corrected for sex, atopy, and smoking habits for the symptoms in the first sample (n_579), the last sample (n=338), and in relation to the specific IgE (n=365). **Bold** numbers denote odds-ratios significantly different from 1.

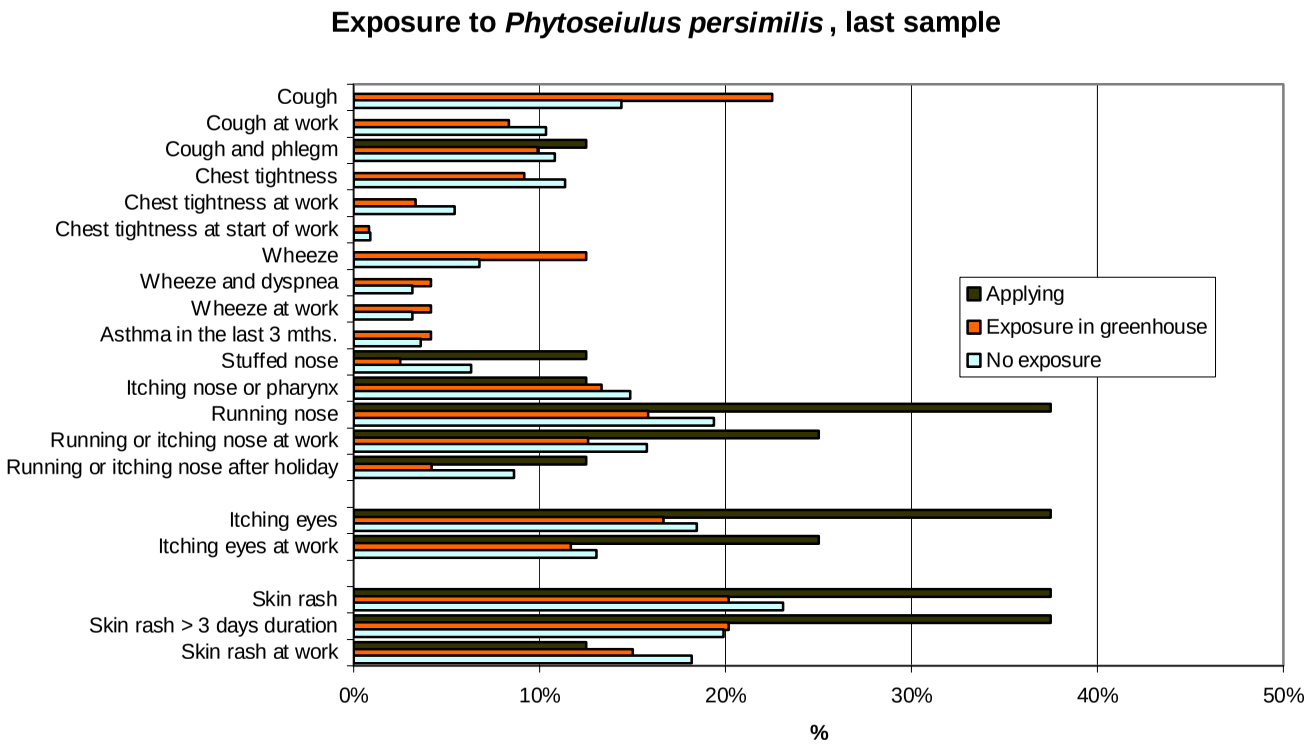

Figure 7.3. Prevalence of the symptoms for persons with and without positive IgE (>0.05 OD) to *Phytoseiulus persimilis* in the last sample of the persons (n=365).

Phytoseiulus persimilis	Initial samples		Last samples		IgE in the last sample
	Exposure in greenhouse	Applying	Exposure in greenhouse	Applying	≥0.05 OD
Cough	0.76 (0.48 - 1.18)		0.98 (0.62 - 1.53)		1.36 (0.26 - 6.98)
Cough at work	1.13 (0.64 - 2.00)		0.69 (0.37 - 1.29)		1.38 (0.16 - 11.66)
Cough and phlegm	0.73 (0.37 - 1.42)		0.83 (0.45 - 1.53)	0.67 (0.08 - 5.76)	1.05 (0.12 - 8.93)
Chest tightness	0.84 (0.51 - 1.38)		0.77 (0.44 - 1.35)		1.47 (0.17 - 12.64)
Chest tightness at work	0.57 (0.24 - 1.32)		0.56 (0.20 - 1.57)		3.67 (0.40 - 33.84)
Chest tightness at start of work	0.60 (0.07 - 5.43)		0.89 (0.16 - 5.03)		**26.5 (1.57 - 446)**
Wheeze	1.00 (0.57 - 1.76)		1.35 (0.76 - 2.40)		**5.02 (1.15 - 21.86)**
Wheeze and dyspnea	1.22 (0.54 - 2.78)		1.43 (0.59 - 3.46)		**7.24 (2.11 - 24.81)**
Wheeze at work	1.28 (0.46 - 3.58)		0.86 (0.34 - 2.17)		5.30 (0.51 - 55.08)
Asthma in the last 3 months	0.89 (0.39 - 2.05)		1.35 (0.41 - 4.39)		
Stuffed nose	1.06 (0.54 - 2.07)		**0.32 (0.12 - 0.84)**	1.60 (0.19 - 13.72)	3.56 (0.39 - 32.72)
Itching nose or pharynx	0.93 (0.57 - 1.51)		0.85 (0.51 - 1.41)	0.82 (0.10 - 6.85)	0.87 (0.10 - 7.39)
Running nose	0.79 (0.48 - 1.32)		0.79 (0.48 - 1.30)	3.06 (0.69 - 13.55)	1.53 (0.30 - 7.73)
Running or itching nose at work	0.71 (0.43 - 1.18)		0.80 (0.40 - 1.58)	2.26 (0.42 - 12.22)	
Running or itching nose after holiday	0.65 (0.29 - 1.46)		0.40 (0.13 - 1.23)	1.97 (0.21 - 18.36)	
Itching eyes	0.99 (0.65 - 1.50)		0.98 (0.62 - 1.55)	2.44 (0.56 - 10.66)	0.53 (0.06 - 4.37)
Itching eyes at work	0.65 (0.37 - 1.13)		0.85 (0.50 - 1.45)	1.91 (0.37 - 9.89)	
Skin rash	1.09 (0.71 - 1.69)		0.80 (0.51 - 1.25)	1.99 (0.46 - 8.64)	1.71 (0.42 - 6.99)
Skin rash > 3 days duration	0.97 (0.63 - 1.50)		0.73 (0.46 - 1.15)	1.98 (0.46 - 8.59)	0.98 (0.20 - 4.78)
Skin rash at work	0.72 (0.41 - 1.25)		0.61 (0.36 - 1.02)	0.52 (0.06 - 4.40)	

Table 7-3. The odds-ratios corrected for sex, atopy, and smoking habits for the symptoms in the first sample (n_579), the last sample (n=338), and in relation to The specific IgE (n=365). **Bold** numbers denote odds-ratios significantly different from 1.

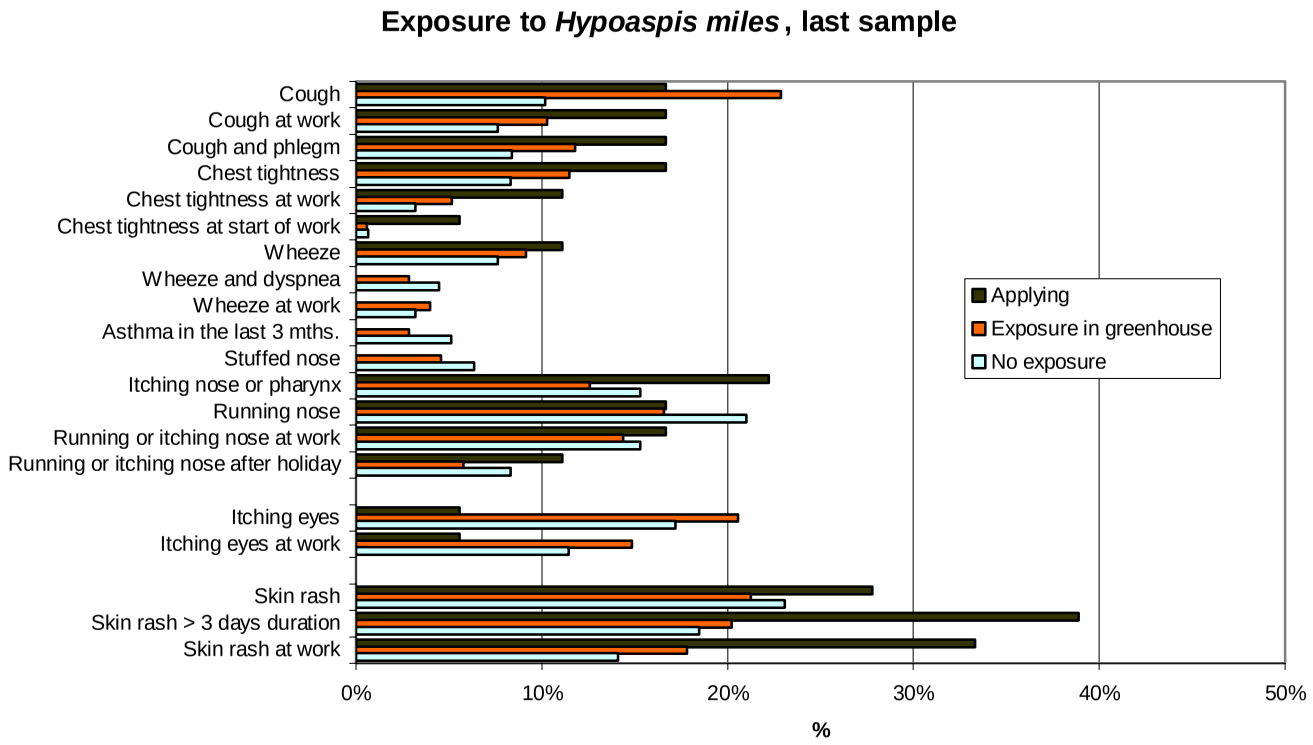

Figure 7.4. Prevalence of the symptoms for persons with and without positive IgE (>0.05 OD) to *Hypoaspis miles* in the last sample of the persons (n=365).

Hypoaspis miles	Initial samples		Last samples		IgE in the last sample
	Exposure in greenhouse	Applying	Exposure in greenhouse	Applying	≥0.05 OD
Cough	0.80 (0.54 - 1.18)	0.27 (0.06 - 1.23)	0.81 (0.52 - 1.26)	1.17 (0.41 - 3.34)	1.76 (0.34 - 9.05)
Cough at work	0.61 (0.36 - 1.03)	0.35 (0.04 - 2.75)	0.61 (0.35 - 1.08)	2.08 (0.65 - 6.68)	**5.71 (1.31 - 24.86)**
Cough and phlegm	1.08 (0.58 - 2.03)	0.39 (0.04 - 3.52)	0.80 (0.45 - 1.45)	2.59 (0.84 - 8.01)	1.11 (0.13 - 9.34)
Chest tightness	0.83 (0.53 - 1.29)	0.81 (0.22 - 2.93)	1.06 (0.62 - 1.82)	1.87 (0.65 - 5.40)	2.45 (0.47 - 12.68)
Chest tightness at work	**0.45 (0.23 - 0.90)**	0.68 (0.08 - 5.56)	1.42 (0.54 - 3.71)	**4.93 (1.17 - 20.7)**	2.54 (0.29 - 22.50)
Chest tightness at start of work	0.94 (0.15 - 5.75)		0.97 (0.17 - 5.45)	6.10 (0.52 - 72.0)	**22.76 (1.64 – 315)**
Wheeze	1.23 (0.73 - 2.07)	0.81 (0.17 - 3.81)	1.19 (0.66 - 2.14)	0.67 (0.15 - 3.08)	3.93 (0.74 - 20.86)
Wheeze and dyspnea	1.04 (0.49 - 2.19)		1.33 (0.53 - 3.37)		
Wheeze at work	1.03 (0.38 - 2.79)		1.00 (0.41 - 2.44)		3.01 (0.31 - 29.21)
Asthma in the last 3 months	1.11 (0.54 - 2.25)	0.93 (0.11 - 7.92)	0.55 (0.17 - 1.71)		
Stuffed nose	0.68 (0.37 - 1.24)		0.53 (0.26 - 1.06)	0.45 (0.06 - 3.50)	2.36 (0.27 - 20.73)
Itching nose or pharynx	0.89 (0.58 - 1.36)	0.22 (0.03 - 1.74)	1.07 (0.65 - 1.74)	1.28 (0.48 - 3.44)	0.52 (0.06 - 4.51)
Running nose	0.90 (0.58 - 1.39)	0.23 (0.03 - 1.76)	0.99 (0.62 - 1.58)	0.72 (0.23 - 2.21)	0.44 (0.05 - 3.65)
Running or itching nose at work	0.61 (0.39 - 0.93)	0.41 (0.09 - 1.86)	0.96 (0.50 - 1.83)	1.11 (0.30 - 4.12)	1.53 (0.30 - 7.97)
Running or itching nose after holiday	0.55 (0.28 - 1.05)		0.89 (0.36 - 2.21)	1.38 (0.28 - 6.65)	
Itching eyes	1.01 (0.69 - 1.48)	0.84 (0.29 - 2.49)	1.05 (0.67 - 1.64)	0.44 (0.12 - 1.54)	0.49 (0.06 - 4.07)
Itching eyes at work	**0.55 (0.35 - 0.89)**	1.22 (0.38 - 3.93)	0.80 (0.48 - 1.33)	0.55 (0.12 - 2.44)	0.88 (0.10 - 7.31)
Skin rash	0.96 (0.65 - 1.44)	2.41 (0.92 - 6.31)	0.95 (0.62 - 1.45)	1.06 (0.42 - 2.66)	1.53 (0.36 - 6.43)
Skin rash > 3 days duration	0.80 (0.54 - 1.18)	1.62 (0.60 - 4.37)	0.90 (0.59 - 1.39)	1.76 (0.74 - 4.18)	1.86 (0.45 - 7.72)
Skin rash at work	**0.54 (0.31 - 0.92)**	1.03 (0.32 - 3.27)	1.27 (0.77 - 2.09)	1.34 (0.49 - 3.70)	2.34 (0.55 - 9.88)

Table 7-4. The odds-ratios corrected for sex, atopy, and smoking habits for the symptoms in the first sample (n_579), the last sample (n=338), and in relation to specific IgE (n=365). **Bold** numbers denote odds-ratios significantly different from 1.

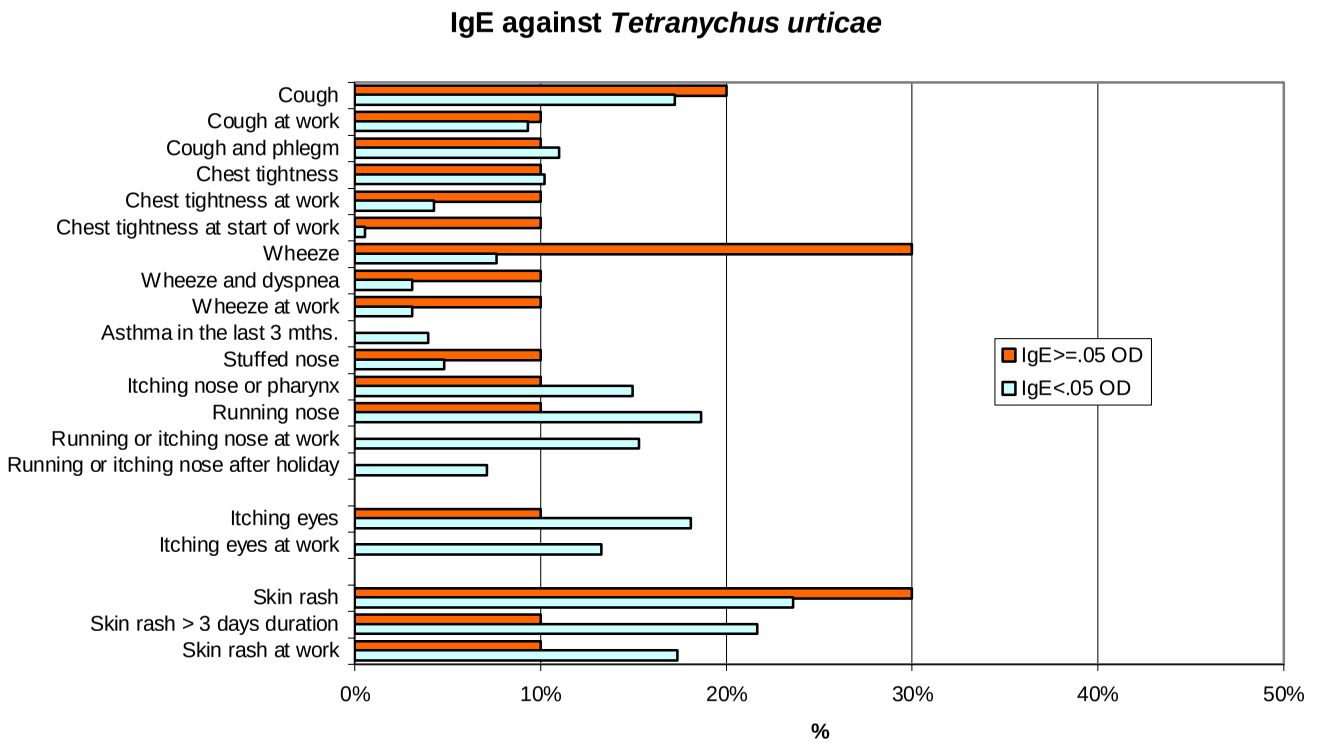

Figure 7.5. Prevalence of the symptoms for persons with and without positive IgE (>0.05 OD) to *Tetranychus urticae* in the last sample of the persons (n=365).

Tetranychus urticae	IgE in the last sample
	≥0.05 OD
Cough	1.40 (0.27 - 7.28)
Cough at work	1.33 (0.16 - 11.23)
Cough and phlegm	0.97 (0.12 - 8.16)
Chest tightness	1.44 (0.17 - 12.35)
Chest tightness at work	3.15 (0.35 - 28.29)
Chest tightness at start of work	**22.09 (1.61 – 303)**
Wheeze	**5.04 (1.16 - 21.95)**
Wheeze and dyspnea	4.33 (0.44 - 42.56)
Wheeze at work	3.63 (0.37 - 35.8)
Asthma in the last 3 months	0.00 (0.00 - 0.00)
Stuffed nose	3.07 (0.34 - 27.5)
Itching nose or pharynx	0.70 (0.08 - 6.15)
Running nose	0.58 (0.07 - 4.95)
Running or itching nose at work	0.00 (0.00 - 0.00)
Running or itching nose after holiday	0.00 (0.00 - 0.00)
Itching eyes	0.53 (0.06 - 4.41)
Itching eyes at work	1.00 (0.45 - 2.22)
Skin rash	1.68 (0.40 - 7.05)
Skin rash > 3 days duration	0.46 (0.06 - 3.73)
Skin rash at work	0.68 (0.08 - 5.66)

Table 7-5. The odds-ratios corrected for sex, atopy, and smoking habits for the symptoms in relation to the specific IgE (n=365). **Bold** numbers denote odds-ratios significantly different from 1.

		Amblyseius cucumeris	*Phytoseiulus persimilis*	*Aphidius colemani*	*Hypoaspis miles*
Cough	Exposed in greenhouse	1.14 (0.66-1.99)	1.03 (0.64-1.68)	0.88 (0.53-1.46)	1.45 (0.9-2.34)
	Applying	1.20 (0.46-3.12)	0.44 (0.06-3.24)	1.58 (0.64-3.91)	0.61 (0.14-2.58)
Chest tightness	Exposed in greenhouse	1.27 (0.63-2.53)	1.57 (0.89-2.77)	**2.10 (1.04-4.24)**	1.26 (0.71-2.23)
	Applying	2.20 (0.78-6.22)	0.89 (0.12-6.63)	2.10 (0.57-7.79)	0.45 (0.06-3.35)
Wheeze	Exposed in greenhouse	1.22 (0.57-2.61)	1.40 (0.75-2.63)	1.41 (0.68-2.94)	1.21 (0.62-2.34)
	Applying	1.43 (0.42-4.9)		1.77 (0.47-6.65)	2.58 (0.86-7.77)
Asthma	Exposed in greenhouse	0.75 (0.14-3.96)	0.32 (0.04-2.65)	0.50 (0.11-2.32)	0.35 (0.07-1.82)
	Applying				
Running or itching nose	Exposed in greenhouse	1.08 (0.65-1.81)	0.96 (0.6-1.54)	**1.82 (1.07-3.1)**	1.30 (0.82-2.06)
	Applying	0.95 (0.38-2.42)	1.59 (0.49-5.16)	2.21 (0.87-5.66)	1.51 (0.63-3.65)

Table 7-6. The incidence rate ratios and their 95% confidence intervals for the five main symptoms in relation to exposure to the four species. The results are corrected for, sex, smoking habits, and atopy. **Bold** numbers denote values significantly different from 1.

		Change (IRR)	Initial samles (RD)	Last sample (RD)	IgE in the last sample
Aphidius colemani	Exposed in greenhouse	0.85 (0.64 - 1.12)	-0.04 (-0.41 - 0.32)	0.50 (-0.11 - 1.12)	0.91 (-0.43 - 2.26)
	Applying	0.76 (0.44 - 1.30)	-0.33 (-1.45 - 0.79)	*)	
Amblyseius cucumeris	Exposed in greenhouse	0.83 (0.60 - 1.16)	0.14 (-0.53 - 0.24)	0.19 (-0.48 - 0.86)	0.14 (-1.68 - 1.96)
	Applying	0.58 (0.33 - 1.02)	-0.97 (-2.21 - 0.27)	-0.13 (-1.39 - 1.14)	
Phytoseiulus persimilis	Exposed in greenhouse	0.79 (0.59 - 1.05)	-0.15 (-0.61 - 0.31)	0.21 (-0.36 - 0.78)	0.50 (-0.79 - 1.79)
	Applying	0.44 (0.12 - 1.71)	*)	*)	
Hypoaspis miles	Exposed in greenhouse	**0.73 (0.54 - 0.99)**	-0.14 (-0.51 - 0.23)	0.07 (-0.50 - 0.63)	0.80 (-0.81 - 2.40)
	Applying	0.81 (0.49 - 1.34)	0.28 (-0.59 - 1.14)	0.19 (-1.13 - 1.51)	

Table 7-7. The relation between asthmascore and the exposure variables. The values are the estimates of the negative binomial regression on the score (0-7) corrected for sex, smoking habits, and atopy. *) denotes no or only single observations. IRR Incidence rate ratio, RD risk difference.

	Decline in lung function /year			Slope, bronchial hyperreactivity (log transformed values)		
	FEV_1 (ml)	FVC (ml)	FEV_1/FVC (%)	Change over time	First sample	Relation to HR
Aphidius colemani	-14.15 (-28.65 - 0.36)	-5.86 (-26.03 - 14.31)	-0.25 (-0.59 - 0.09)	-0.09 (-0.22 - 0.04)	-0.14 (-0.32 - 0.04)	-0.09 (-0.27 - 0.10)
Amblyseius cucumeris	**-17.16 (-33.45 - -0.87)**	-19.43 (-42.04 - 3.17)	-0.10 (-0.48 - 0.28)	0.00 (-0.13 - 0.14)	-0.01 (-0.21 - 0.19)	0.05 (-0.05 - 0.14)
Phytoseiulus persimilis	3.26 (-10.84 - 17.36)	13.91 (-5.59 - 33.41)	-0.25 (-0.58 - 0.08)	-0.02 (-0.15 - 0.11)	0.04 (-0.16 - 0.25)	-0.04 (-0.15 - 0.08)
Hypoaspis miles	5.32 (-8.45 - 19.09)	-2.35 (-21.43 - 16.73)	0.09 (-0.24 - 0.41)	0.03 (-0.09 - 0.16)	0.04 (-0.15 - 0.22)	

Table 7-8. The values of lung function and bronchial hyperreactivity in relation to the exposure values. The estimates corrected for sex, height, atopy status, and smoking habits are shown. The persons applying the products are included in the exposed group. **Bold** values are signifant effects ($p<0.05$)

8 Exposure estimation and individual sensitivity

8.1 Background

In the test of HR (see chapter 6), the predator which showed the strongest exposure response relationship was *Amblyseius cucumeris*. An exposure study was therefore set up for this.

Several methods can be used to determine the allergenic content in sample material (foods & dust). Usually the determination is based on RAST (Radio Allergo Sorbent Test) or EAST (Enzyme Allergo Sorbent Test) – inhibition. These methods are based on the fact that IgE-allergen binding in a solid phase system is inhibited by addition of free allergen. Based on the standard inhibition curve using known amount of allergen the inhibitory capacity in an unknown sample can be detected. In some cases it is time-consuming and laborious to develop a reliable inhibition assay due to a less reliable IgE determination for the allergen in question or interfering compounds in the sample material. Another problem may be lack of sufficient sample material or high tittered IgE sera.

An alternative to the above mentioned assays is to perform a biological determination of the allergen in a sample. This can be done *in vitro* by measuring allergen induced histamine release from human basophiles sensitized with a high tittered IgE serum. These passively sensitized basophiles will release histamine by incubation with different concentrations of the allergen and a dose-response curve can be established. A sample containing unknown amount of the allergen in question will induce histamine release and the biological concentration can be established from the standard curve. This method is very sensitive, provided the access to high tittered sera, and it is relatively unaffected by contaminating material.
In the present study we used allergen induced histamine release from passively sensitized basophiles.

The finding of several high tittered sera specifically directed against *A. cucumeris* in the previous histamine release screening (see chapter 6) made the investigation possible.

8.2 Material and methods

In a large greenhouse firm *A. cucumeris* has been used routinely for several years and is actually applied in their R&D department and in their production of mother plants of Campanula.

The mites, product name *Amblyline cu CRS* were supplied from GARTA A/S, produced by Syngenta Bioline, Little Clacton, Essex, UK and delivered the day before appliance.

A. cucumeris was applied in small letters (sachets) with an average of 1.000 mites in each including some fodder. The letters are then placed with interval among the plants.

In the R&D department the mite letters are put with a high density, about one letter per 50 cm on normal growth tables. They are renewed about every thirty days. The R&D department is situated in a series of small older greenhouses. Three to four persons are continuously working in the area with a high plant contact, handling and controlling the plants. Two persons handled the sachets. On the same day the bug Orius was applied.

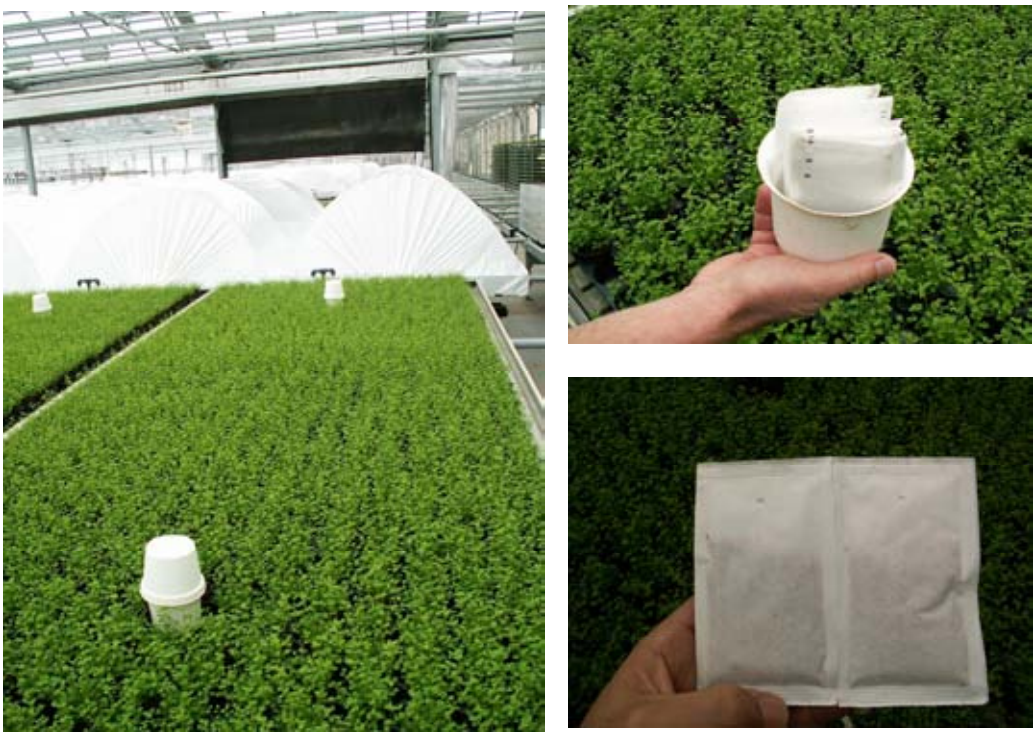

Figure 8.1. The sachets with *Amblyseius cucumeris* were placed in dishes and placed on each table. (Photo J.Bælum)

The production of mother plants is situated in two glasshouses of about 10,000 m^2 on mobile tables. Tasks are sorting, irrigating, and controlling.

The production of sales plants where no mites are applied takes place in a large greenhouse. Plants on mobile tables are automatically moved around in the house. A number of persons work here packing and controlling the plants.

8.2.1 Sampling strategy

Three areas have been taken out:

1. the R&D glasshouses,
2. the production of mother plants,
3. a control area, the packing area for the normal production of Campanula, where *A. cucumeris* is not used.

The study was carried out from May 31 to June 2. In the production of mother plants and in the R&D department *A. cucumeris* was applied on June1 thereby giving possibility for a pre day and a post day.

From the logger of the climate computer the average outdoor temperature on the three days was 12.5° 11.5°, and 11.7°C, respectively while the temperature during the workday (07:00-16:00) was 15.5° 13.2°, and 14.6°C. 5 % of the time it was sunny and there was no rain.

In the greenhouses temperatures ranged between 19 and 21°C. The relative humidity was 57-67 % in the packing area and 62-78 % in the other areas.

Area	31.5 (day 1)	1.6 (day 2)	2.6 (day 3)
R&D	2 persons	2 persons	2 persons
Mother plants	3 persons	1 person handling *A. cucumeris* 4 other persons (bystanders)	3 personal samples
Packing of plants for sale (control area)	3 persons	1 person	1 person
Controls (blind filters)	1 filter	1 filter	1 filter

Table 8-1. The program for the sampling of antigen against *Amblyseius cucumeris.*

8.2.2 Sampling methods

Sampling of the inhalable dust fraction was done with IOM Personal samplers and SKC portable pumps type 224-5 (SKC, Dorset, UK, www.skcinc.com) equipped with 25 mm Teflon-filters with an average pore size of 1,0 µm (MFS, Frisinette, Ebeltoft). The flow was 2,0 l/min and sample time 3-4 hours excluding pauses or other stays outside the production area.

8.2.3 Analytical methods

In the study we determined allergenic activity in 25 filters, three different batches of *A. cucumeris* (in the original bags), labelled: 22 14 6; 17 106 17 & 17 106 (1-6-06) and one sample from a dish. Unexposed filters were included as blanks.

Standard *A. cucumeris*-extract was a mixture of crude *A. cucumeris* (21 µg/ml) and pure *A. colemani* extract (5 µg/ml). Filters and cassettes were weighed after acclimatization, before and after sampling at Department of Environmental and Occupational Medicine, Aarhus University. Filters were sent to RefLab, Copenhagen. Samples were then extracted in pipes buffer and tested against sera from: 1) two persons with a clear reaction to *A. cucumeris* and 2) two persons with no reaction to *A. cucumeris* but reaction to *P. persimilis*, which has not been in use at the actual greenhouse.
Five high titter sera (HR-Test ≥ class 5) against *A. colemani* from five different persons were included.

8.2.3.1 Extraction of material from filters
Before and after extraction each filter was examined by eye and visible colour of the filter was noted as 0; (+); + and ++. There was no change in the colour after 3 hours extraction and the extraction was therefore continued for another 69 hours, but no change in colour was observed. This crude inspection indicates that the extraction is far from complete.

Each of the 25 filters and one unexposed were cut into four pieces and extracted 3 hours in 3 ml Pipes buffer at room temperature. After the three hour extraction period a sample of the extract (500 µl) was removed. The extraction was then continued for 72 hours at 4º C.

8.2.3.2 Extraction of A. cucumeris from original bags
Samples from each of the three different bag-batches were extracted in pipes buffer (100 mg/mL) for one hour at 4º C. Then the samples were centrifuged and the supernatants used in the experiments.

8.2.3.3 Histamine release method
Blood bank buffy coat basophiles were used in the study. The basophiles were screened for activity against 10 inhalation and 10 food allergens and Anti-IgE. Only cells showing no histamine release response to the 20 allergens and a good release (> 30 %) to Anti-IgE were included in the study. Before sensitizing the cells with the above mentioned sera, IgE from the basophile surface was removed by a short exposure to low pH.

The IgE deprived cells were passively sensitized with the above mentioned sera for 60 min. at 37º C. The passively sensitized cells were incubated with extracts from the 25 filters, one unexposed filter, and the three samples as well as the standard *A. cucumeris* sample. All extracts and samples were tested in 6 concentrations by 3.5 fold dilutions (the standard *A. cucumeris* sample was used in concentrations from 26 to 0.26 µg/mL). Control experiments using cells sensitized to healthy non allergic sera were included.

8.3 Results

The amount of dust on filters varied considerably in a non-predictable manner.
No significant reactions were found when sensitized basophiles were incubated with the 3 hour extracts. The standard *A. cucumeris* sample showed a class 6 reaction (release already at 0.26 µg/mL) corresponding to and hereby confirming common allergenicity with the samples from the large screening study.

Some of the 72 hour filter extracts showed however reactions. As can be seen from table 8-1 there is some concordance to positive reactions in the histamine release and the amount of dust determined by weighing the filters.

The three crude samples were potent inducers of histamine release from the passively sensitized cells. By extrapolation from the release curve induced by the standard extract the allergenic material constituted about 5-10 % of the samples. In the three original batch bags the content was 8-10 % allergen content (w/w) while extraction from the dish indicated about 15 % allergen content (w/w).

Date	Department	Person	Duration (min.)	mg dust/m^3	Handling (min.)	Allergen µg /ml	Allergen µg /m_3
05-31	Packing	01	95	0.070		0	n.d.
05-31	Packing	02	325	0.051		0	n.d.
06-01	Packing	03	335	0.205		0	n.d.
06-02	Packing	03	234	0.093		0	n.d.
05-31	Mother plants	11	250	1.198		26	21.7
05-31	Mother plants	12	390	below l.d.		0	n.d.
05-31	Mother plants	13	340	1.503		0	n.d.
06-01	Mother plants	14	365	0.774		0.85	1.1
06-01	Mother plants	15	345	0.321		0	n.d.
06-01	Mother plants	11	340	0.740		52	70.3
06-01	Mother plants	12	370	below l.d.	185	0.3	n.d.
06-01	Mother plants	13	370	0.391		0	n.d.
06-02	Mother plants	11	292	0.479		0	n.d.
06-02	Mother plants	12	294	0.652		0	n.d.
06-02	Mother plants	13	111	1.170		0	n.d.
05-31	R&D	31	250	below l.d.		0	n.d.
05-31	R&D	32	410	0.211		0	n.d.
06-01	R&D	31	405	below l.d.	60	0	n.d.
06-01	R&D	32	300	0.249	250	0	n.d.
06-02	R&D	31	330	1.569		96	61.2
06-02	R&D	32	317	0.324		0	n.d.
05-31, 06-01, 06-02			Blanks			0	n.d.

Table 8-2. The results of the measurements of antigen against *Amblyseius cucumeris* in air.

8.3.1 Individual sensitivity

There were only 4 filters with quantifiable antigen concentrations so there was no possibility of testing individual sensitivity to these.

8.4 Discussion and Conclusions

Our results indicate that it is possible to determine allergenic material from *A. cucumeris* in the filters. The extraction efficacy from the filters seem however to be low. This is indicated by the visual inspection and the relatively low histamine release responses observed in the filter extracts where the allergenic material constitutes varying amounts from 0.1 to about 10 % of the actual material deposited on the filters.

On the other hand, the results show that high concentrations of antigen were found in the samples of material, in both fresh and old sachets as well as dust from the dishes. This implies that an airborne exposure is relevant.

Further studies are needed to establish a more efficient extraction method to enhance the sensitivity. It is also possible that the use of less hydrophobic filters will allow a more efficient extraction. It should be noted that allergens eliciting an IgE mediated reaction are almost exclusively water soluble. This is indeed demonstrated in this study where crude material extracted with buffer releases up to 15 % allergenic material on a weight to weight basis.

9 Discussion and conclusions

The present study has been an attempt to describe the possible health effects of introducing a new technology with the goal to replace chemical pesticides with more ecologically friendly agents. The question is whether the introduction of beneficial animals poses a risk to the persons employed in the greenhouse or to the consumers who are exposed to the plants after sale. Originally, the beneficial animals have been introduced without any risk assessment and it is thus not known if there is any measurable risk to be considered and need for regulation of the use of the technology.

The beneficial animals have been introduced in an environment with a considerable number of allergens and the problem is firstly, whether there is a measurable risk of the animals and secondly to compare it with those of competing technologies.

Table 9-1 shows an overview of the different exposure related effects found throughout Chapter 4 to 7.

	Aphidius colemani	*Amblyseius cucumeris*	*Phytoseiulus persimilis*	*Hypoaspis miles*
Exposed in greenhouse (range over the 4 runs)	53-65%	63-69%	22-35%	41-49%
Applying (range over the 4 runs)	4-10%	3-12%	1-9%	9-11%
IgE in relation to exposure	0	0	0	0
HR in relation to exposure	0	+	+	-
Symptoms in relation to exposure				
Prevalent symptoms, first run	0	0	0	0
Prevalent symptoms, last run	0	- Wheeze and dyspnea (A) - Wheeze at work (A)	0	- Chest tightness at work
Incident symptoms	- Chest tightness - Running or itching nose	0	0	0
Decline in lung function	0	0	0	0
Bronchial reactivity	0	0	0	0
Symptoms in relation to sensitization				
Prevalent symptoms, last run	- Chest tightness at start of work - - Itching nose or pharynx - Skin rash	- Wheeze at work	- Chest tightness at start of work-Wheeze - Wheeze and dyspnea	-Cough at work - Chest tightness at start of work
Incident symptoms	0	0	0	0
Decline in lung function	0	0	0	0
Bronchial reactivity	0	0	0	0

Table 9-1. Overview of the effects of exposure to the four different predators. 0 denotes no significant effects, - denotes not measured. (A) the group of applying persons.

Estimating the allergic effects of beneficial animals in a complex environment poses many problems. The assessment of health effects caused by the house dust mite is an example of the work done to solve these problems. Standardised extracts of specific components have been developed as well as measures of exposure in the environment (beds, mattresses, carpets) both by measuring the number of vital animals and the amount of antigen. Finally standardized solutions of antigen for prick tests have been developed for these mites as well as some of the storage mites.

For the beneficial animals in the present studies no such extracts were available and therefore the first step was to produce these in sufficient amount and purity. It was a problem that animals were fed on a mixture of wheat bran and mites used for fodder (*Tetranychus* and *Tyrophagus* species). This has inevitably decreased the specificity of the extracts and thereby decreased any dose-response relations between exposure estimates and sensitization. On the other hand the rate of sensitization did not differ from the two other studies of beneficial animals and sensitization (Groenewoud *et al.*, 2002a; Kronqvist *et al.*, 2005).

Another problem in the study was the characterization of exposure. This study was made on observations and sampling of blood done several years before and the study was not originally set up to study beneficial animals, but microbiological pest management. Therefore we had to make retrospective investigation with interview of the greenhouse owners about the use of the individual beneficial animals during the observation period some years before. The information in the questionnaires from the participants in the study was obtained at the actual time and probably more reliable but only for those who had been applying the animals themselves. However, a good agreement between the two different measures was seen for about 70 % of the material.

The pilot study on *Amblyseius cucumeris* described in chapter 8 did partly show measureable airborne antigen. A series of technical problems decreased the sensitivity of the assay. A development of the method may increase the sensitivity, but as it relies on serum from persons with proved sensitization a method using other techniques may be preferred if used in a larger scale. This may be combined with methods for counting viable mites in the environment.

The employees who actually handled the animals were probably the most exposed and therefore at the largest risk of being sensitized or developing symptoms. However, no indication of either increased sensitization or respiratory symptoms was seen in this group. On the contrary this group typically had the lowest prevalence of the exposure groups. One of the cases, however, clearly reported symptoms in relation to the handling of *Amblyseius cucumeris.* Generally, however, the present study was not aimed at the detailed description of symptoms in relation to specific processes.

The reason for low prevalence of symptoms may be “healthy workers selection” where only the most healthy and fit persons continue being exposed while persons with symptoms formally or informally are transferred to other jobs or out of the work place. Persons with atopic disposition, shown by sensitization to one or more of the common allergens, are in elevated risk of developing allergy. However there were no indications of decreased number of atopics in the group who were applying any of the beneficial animals. An alternative hypothesis may be that the amount of time the individual person

was exposed during handling was too short to increase the risk of sensitization and symptoms.

The cross sectional analyses have limitations with reporting bias and selection bias. At the present stage this probably has a minor importance as the beneficial animals were not stated as the primary goal of the study. On the other hand the three follow-up years were only a short period of a working life. Therefore a selection might already have taken place and therefore a healthy group has been investigated. The rate of self reported asthma of 7 %, however, is at the same level or higher than that of a referent population in five Danish counties including Funen County (Skadhauge *et al.*, 2005). On the other hand the frequency of bronchial hyperreaction was very low, so persons with active asthma might not be able to work in the greenhouse athmosphere.

Among the specific animals the wasp *Aphidius colemani* showed a sensitization on the same level as the mites; however, in contrast to the mites no exposure relation was seen. On the other hand there was a correlation between exposure to *Aphidius colemani* and the incidence of upper air way symptoms which was in contrast to the mites which showed some relation to lower airway symptoms.

Among the mites *Amblyseius cucumeris* was the most frequently used and showed a clear relation between exposure estimates and sensitization by the histamine release reaction. The relation between sensitization and symptoms was seen in wheeze and chest tightness at work, symptoms most related to inflammatory diseases, while cough, normally related to smoking or dust exposure, also was seen in the present study. In the Dutch study, relation was mainly seen to upper air way symptoms and nasal allergies(Groenewoud *et al.*, 2002a). Why the two studies are different may be explained by the upcoming measures in the present study.

Exposure to *Phytoseiulus persimilis* caused a clear sensitization, although in comparison with *Amblyseius cucumeris* the level in the unexposed group was higher. This pattern of sensitization is in agreement with the Swedish study (Kronqvist *et al.*, 2005).

According to *Hypoaspis miles* the level of sensitization was lower and only a single person had a high IgE and the inhibition studies indicated that this IgE probably was the least reliable of the four measures. On the other hand a relation of the same size between symptoms and sensitization was seen in this mite although it was mostly seen in the symptom “Cough at work”.

Tetranychus urticae showed a relation between chest tightness at the start of work in combination with wheeze. It was not different from the pattern of the other mites.

The relation between symptoms and sensitization in the different species were very much alike. This could suggest that the relation is caused by a common background factor. However exposures in the population were different and there were only a partial overlap between those having IgE to the different species. This supports partially the hypothesis that the mites, both the beneficial and *Tetranychus urticae* have at least some specific biological effects.

10 Conclusions

The use of beneficial arthropods is a well consolidated technology in Danish greenhouses. In contrast to the chemical pesticides and to some extend the microbiological control agents there are no regulations of the use.

The present study showed development of sensitization to the predatory mites and description of cases with exposure related symptoms.

Amblyseius cucumeris caused sensitization in about 7% of the exposed persons and development of new sensitization was seeen in several persons during the observation period. A number of persons developed allergic symptoms in the eyes, nose and throat in parallel with the sensitization.

Phytoseiulus persimilis gave rise to a higher sensitization rate than *A. cucumeris*. The sensitization seems to be less specific due to a relatively high sensitization rate in the unexposed group. Development of sensitization and symptoms were seen in a small number of persons during the observation period.

There were signs of sensitization against the wasp *Aphidius colemani* but both in those exposed and those not exposed. A number of symptoms, mainly from the upper airways, may be related to exposure to *A. colemani*.

Two different measures of sensitization were used. We were able to show reliable antibody response to the above mentioned arthropods, while IgE antibodies against the mite *Hypoaspis miles* were not reproducible. On the other hand the specific IgE levels were low and no relation to exposure was seen. In contrast, histamine release test seems to be a more sensitive and reliable measure of sensitization, although considerably more expensive.

Exposure to the predators seems to give rise to some symptoms from eyes, nose and throat. On the other hand there was no indication of that any of the beneficial animals may cause asthma or other imflammatory lung diseases.

The study has been limited by the possibility of good individual exposure estimates, but a limited pilot study has shown that antigen from *A. cucumeris* can be traced in the work room air. Better individual exposure estimates may improve the results but they will still be influenced by the selection of workers ("healthy worker effect").

The study shows that in workers exposed to the beneficial mites and possibly wasps give rise to health effects. The effects are clearly more pronounced than those of the microbiological control agents *Bacillus thuringiensis, Trichoderma harzianum,* and *Vertilcillium lecanii,* but several other factors in the greenhouses, i.e. plants and naturally occurring microbial fauna have comparable effects.

11 Suggestions for regulation, preventive measures, and further research

As both well documented cases of allergic rhinitis caused by *Amblyseius cucumeris* and *Phytoseiulus persimilis* and clear signs of sensitization in a broader group are shown, some regulation of the use of beneficial animals should be introduced. The data for regulation is limited and detailed guidelines will require more data, especially about the exposure levels during the different work processes.

Guidance for the application of the mites may be the first priority, especially to limit the airborne antigen level or protect the persons working in the area when the products are applied.

Regulation on the predatory wasp *Aphidius colemani* will have a lower priority based on the very limited data.

11.1 Suggestions for further research

The beneficial animals have some potential health effects and in order to document the benefit of regulatory measures more information of the exposure to the relevant allergens in different work processes is needed.

A better characterization of the relevant allergens of the different mites and testing on the blood of sensitized persons will give a more precise description of the relevant exposure and give the possibility for better analytical methods for the beneficial mites. Besides, good methods for counting viable animals in the greenhouses will give a better description of the possible exposure to allergens from the beneficials species, also during the plants life at the consumers.

An investigation of the relation between exposure and symptoms in closer follow-up studies will reveal a possible dose-response relation. The importance of the other, naturally occurring mites and insects will be of importance as well as characterization of persons in risk of developing symptoms or diseases.

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1 Methods for analysis of specific IgE

Sera from the 2nd and 3rd year follow-up phase of the BIOGART cohort study, 344 and 338 samples, respectively. Additionally, 59 sera from previous runs have been tested for comparison.

1.1.1 Preparation of allergen extracts

Allergen preparations made by extraction of the following species, purchased from Koppert BV, The Netherlands:

Tetranychus urticae
Phytoseilus persimilis
Hypoaspis miles
Amblyseius cucumeris
Aphidius colemani

The first two were obtained as live animals (n = 25,000 – 200,000; varying per species) largely free of substrate material, while one (*) was supplied with as carrier material corn grid, the second (**) a mixture of sieved wood particles, wheat bran, yeast lyofilisate flakes, grinded buckwheat shells, and vermiculite while the third (***) needs to be kept in enriched fertilized garden soil. In an attempt to separate the carrier material from the animal bodies, these preparations were sieved through a 425 m sieve, and the crude (>0.425 mm) and fine (<0.425 mm) fractions were extracted separately.

Extraction was performed with 5 % (w/v) dry material suspended in PBS pH 7.00, shaken overnight at room temperature then incubated in an ultrasonic bath for 15 minutes, and vortexed for 30 minutes. The extracts were centrifuged at 25,000 g and the supernatants dialysed against PBS pH 7,40. The dialysed extract was filtered through a 0.45 m filter and stored in aliquots at –20°C.

Protein yields varied largely, from 1.11 (*Hypoaspis miles* > 0.425 mm) to 31.6 (*Tetranychus urticae*) mg protein per g starting material.

Of the pure carrier materials 25 grams were suspended in 400 ml PBS pH 7.00 and extracted as described above. Here protein content varied from 0 mg (Vermiculite) to 38.1 mg (Wheat bran) protein per gram raw material

SDS-PAGE was performed to assess the protein composition: a neat band pattern with many (>20) proteins in the MW range from 10 to 100 kD was found for the *Tetranychus urticae* and *Phytoseiulus persimilis* extracts; *Amblyseius cucumeris* ‘crude’ extract showed a range of ~10-15 proteins at 5-30 kD, while in the other predator extracts hardly any clear protein bands

could be distinguished. From the carrier materials Yeast and Wheat showed band patterns in the 4-38 kD and 6-70 kD range respectively.

1.1.2 Screening for IgE

Specific IgE sensitization was tested with an EIA method in which sera are tested in microwells coated overnight with 0.1 mL diluted allergen extract at 20 µg protein per mL, in PBS, as described by Doekes et al (1996), with some modifications:

- Sera incubated for 2 hrs in allergen-coated microwells; all sera tested at 1/10 dilution; dilution medium: PBTG with a 10% diluted pool of negative control plasma (*see below).
- Detection of IgE binding with mouse monoclonal anti-IgE, followed by incubations with biotinylated rabbit anti-mouse Ig, avidin-peroxidase – all diluted in PBTG (PBS-0.05% Tween-20 with 0.2% gelatin -; detection of avidin-peroxidase-binding with the peroxidase-substrate o-phenylenediamine (OPD) in phosphate-citrate buffer with 0.015% H_2O_2
- Peroxidase reactions stopped after 30 min with 1 M HCl, and optical density (OD) read at 492 nm.
- All sera tested in rows of six micro-wells: five wells coated with the various allergen preparations and one non-coated control well (control 1).
- In all plates 'no serum/buffer only' controls for each coated allergen (control 2).

Results expressed as OD values of test sera in coated wells corrected for both OD (control 1) and OD (control 2; reagent blank):

$OD_c(i, x) = [\text{Crude OD } (i, x) - \text{OD } (\text{PBTG}, x)] - [\text{OD}(i, \text{coating} = 0) - \text{OD}(\text{PBTG}, 0)]$

After the first complete test series, all sera giving an OD_c value >0.025 on at least one of the five test allergens, and a random sample of completely negative sera were retested on all five allergens. In a few cases where a marked discrepancy was noted between the first and the second test results a third test was performed and the average of the two most nearest OD values was used. For all other serum/allergen test combinations, the OD of the first test was used.

A serum was considered definitely positive for IgE against a specific allergen if the OD_c of the final test result was >0.050, a threshold level at which the chance of false-positive reactions is much lower than at lower cut-off values like 0.025.

All serologic data were provided as data-files with thus corrected OD values (OD_c) for each serum on each allergen, and with a 0/1 code indicating specific IgE positivity as defined above.

1.1.3 Modification of IgE test method

In the first IgE test series, serious technical problems were encountered: with several allergen preparations, high background values were observed in the

‘no serum’ control wells, and these high OD values showed high inter-plate and inter-day variation, which further hampered the production of reliable, reproducible test results.
Since it was noted that OD values were often lower in wells in which – apparently negative - serum or plasma had been present, compared to the control wells with coated allergen but PBTG instead of serum (control 2), a panel of plasma samples from a presumably negative population (rubber industry workers) was screened, and a pool of >10 plasma samples with no evidence of any IgE reaction to the five allergen extracts was prepared. Since addition of this pooled plasma (at 10% v/v) to the dilution medium PBTG resulted in much better and more constant background OD values, all subsequent tests were performed with sera diluted 1/10 in PBTG containing 10% of the negative plasma pool. Only IgE serology results obtained in this test system are included in the final data files.

Serum series	N	No (%) of sera positive on:					
		≥1 allergen	*Tetranychus urticae*	*Phytoseiulus persimilis*	*Hypoaspis miles*	*Amblyseius cucumeris*	*Aphidius colemani*
1-9999	59	6 *(10.1)*	2 *(3.3)*	1 *(1.7)*	1 *(1.7)*	2 *(3.3)*	3 *(5.1)*
20000 (run 2)	344	37 *(10.8)*	14 *(4.1)*	9 *(2.6)*	8 *(2.3)*	23 *(6.7)*	13 *(3.8)*
30000 (run 3)	338	35 *(10.4)*	10 *(3.0)*	8 *(2.4)*	7 *(2.1)*	15 *(4.4)*	10 *(3.0)*

Table 1-1 Test sera: numbers and %'s of positive IgE reactions (ODc >0.05):

1.1.3.1 Numbers and associations of positive IgE reactions

Approximately 10 % of all sera showed a positive reaction to at least one allergen. The positive reactions to different allergens showed significant associations:

Thus, of the 6 sera in the 1-9999 series with at least one positive IgE reaction, 1 serum was positive for three allergens (*T. urticae*, *P. persimilis* and *A. colemani*), and one for two allergens (*T. urticae* and *A. colemani*). Associations for the 20000 and 30000 series are summarized in the following two-by-two tables.

Associations were most pronounced for IgE to *T. urticae* and *P. persimilis* (χ^2 = 80 to >100), for the combination of IgE to *T. urticae* and *A. colemani* (χ^2 = 63-73), while IgE to *H. miles* showed least associations with other positive reactions.

Run 2	*Phytoseiulus persimilis* +	*Phytoseiulus persimilis* -		Run 3	*Phytoseiulus persimilis* +	*Phytoseiulus persimilis* -
Tetranychus urticae +	6	8		*Tetranychus urticae* +	5	5
Tetranychus urticae -	3	327		*Tetranychus urticae* -	3	325
χ^2 >100	p < 0.001			χ^2 = 81	p < 0.001	
Run 2	*Hypoaspis miles* +	*Hypoaspis miles* -		Run 3	*Hypoaspis miles* +	*Hypoaspis miles* -
Tetranychus urticae +	2	6		*Tetranychus urticae* +	1	9
Tetranychus urticae -	6	330		*Tetranychus urticae* -	6	322
χ^2 = 21.8	p < 0.001			χ^2 = 0.44	p = NS	
Run 2	*Amblyseius cucumeris* +	*Amblyseius cucumeris* -		Run 3	*Amblyseius cucumeris* +	*Amblyseius cucumeris* -
Tetranychus urticae +	9	5		*Tetranychus urticae* +	4	6
Tetranychus urticae -	14	316		*Tetranychus urticae* -	11	317
χ^2 = 62.3	p < 0.001			χ^2 = 22.7	p < 0.001	
Run 2	*Aphidius colemani* +	*Aphidius colemani* -		Run 3	*Aphidius colemani* +	*Aphidius colemani* -
Tetranychus urticae +	7	7		*Tetranychus urticae* +	5	5
Tetranychus urticae -	6	324		*Tetranychus urticae* -	5	323
χ^2 = 73.0	p < 0.001			χ^2 = 63.4	p < 0.001	
Run 2	*Hypoaspis miles* +	*Hypoaspis miles* -		Run 3	*Hypoaspis miles* +	*Hypoaspis miles* -
Phytoseiulus persimilis +	2	7		*Phytoseiulus Persimilis* +	1	7
Phytoseiulus persimilis -	6	329		*Phytoseiulus persimilis* -	6	324
χ^2 = 8.37	p = 0.004			χ^2 = 0.71	p = NS	

Run 2	*Amblyseius cucumeris* +	*Amblyseius cucumeris* -		Run 3	*Amblyseius cucumeris* +	*Amblyseius cucumeris* -
Phytoseiulus persimilis +	6	3		*Phytoseiulus persimilis* +	3	5
Phytoseiulus persimilis -	17	318		*Phytoseiulus persimilis* -	12	318
χ^2 = 43.9	p < 0.001			χ^2 = 13.9	p < 0.001	
Run 2	*Aphidius colemani* +	*Aphidius colemani* -		Run 3	*Aphidius colemani* +	*Aphidius colemani* -
Phytoseiulus persimilis +	6	3		*Phytoseiulus persimilis* +	4	4
Phytoseiulus persimilis -	7	328		*Phytoseiulus persimilis* -	6	324
χ^2 = 83.5	p < 0.001			χ^2 = 47.5	p < 0.001	
Run 2	*Amblyseius cucumeris* +	*Amblyseius cucumeris* -		Run 3	*Amblyseius cucumeris* +	*Amblyseius cucumeris* -
Hypoaspis miles +	4	4		*Hypoaspis miles* +	1	6
Hypoaspis miles -	19	317		*Hypoaspis miles* -	14	317
χ^2 = 18.0	p < 0.001			χ^2 = 0.12	p = NS	
Run 2	*Aphidius colemani* +	*Aphidius colemani* -		Run 3	*Aphidius colemani* +	*Aphidius colemani* -
Hypoaspis miles +	3	5		*Hypoaspis miles* +	1	6
Hypoaspis miles -	10	326		*Hypoaspis miles* -	9	322
χ^2 = 17.0	p < 0.001			χ^2 = 0.44	p = NS	
Run 2	*Aphidius colemani* +	*Aphidius colemani* -		Run 3	*Aphidius colemani* +	*Aphidius colemani* -
Amblyseius cucumeris +	8	15		*Amblyseius cucumeris* +	2	13
Amblyseius cucumeris -	5	316		*Amblyseius cucumeris* -	8	315
χ^2 = 56.3	p < 0.001			χ^2 = 2.71	p = 0.10	

Table 1-2 The association of positive IgE reactions to different allergens.

1.1.3.2 Correlation between year 2 and year 3 results

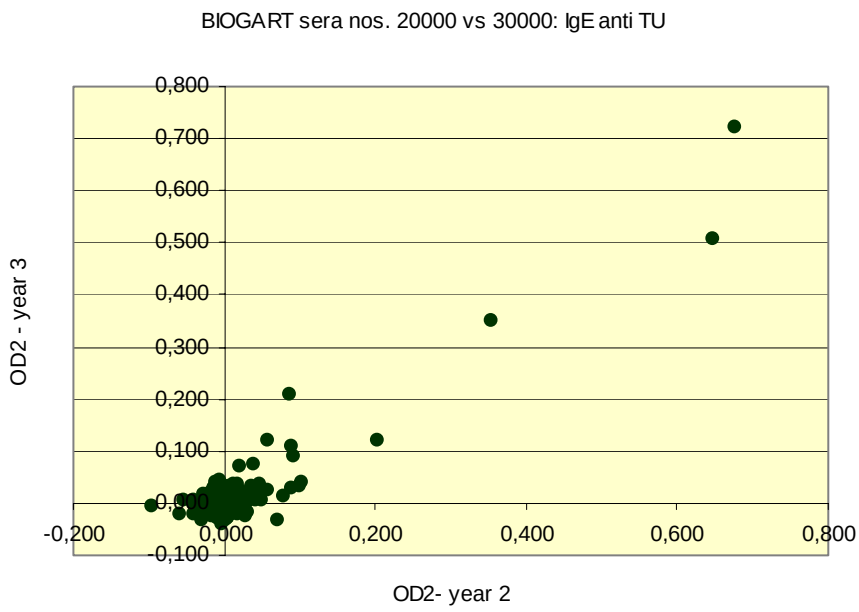
For 310 subjects sera from both year 2 and year 3 were tested, and the results showed a strong correlation. Table …gives the associations as two-by-two tables for each of the five allergens:

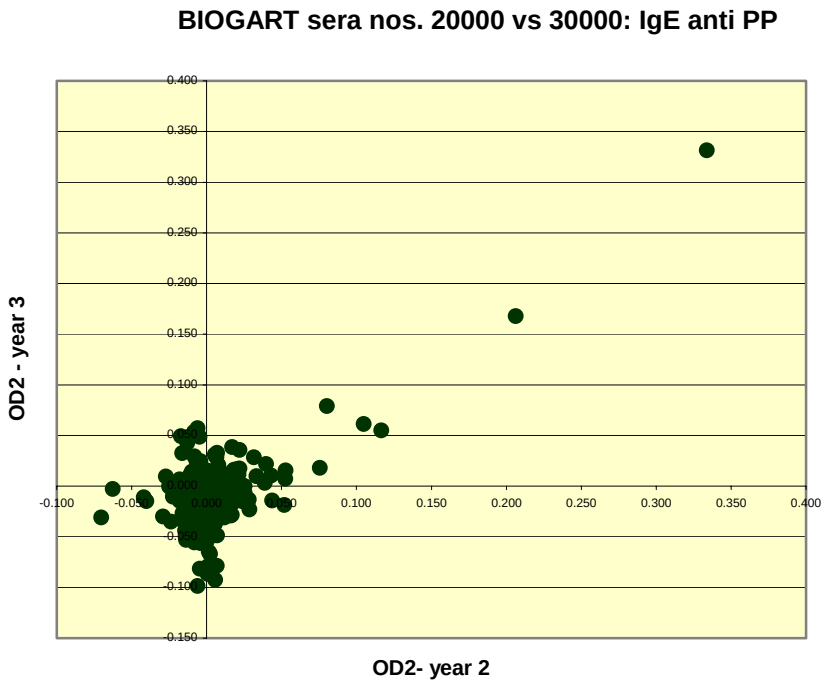
Tetranychus urticae	Year 3+	Year 3 -		*Phytoseiulus persimilis*	Year 3+	Year 3 -
Year 2 +	8	6		Year 2 +	5	4
Year 2 -	2	294		Year 2 -	3	298
χ^2 > 100	p < 0.001			χ^2 = 82.9	p < 0.001	
Hypoaspis miles	Year 3+	Year 3 -		*Amblyseius cucumeris*	Year 3+	Year 3 -
Year 2 +	2	6		Year 2 +	7	16
Year 2 -	4	298		Year 2 -	7	280
χ^2 = 12.2	p < 0.001			χ^2 = 32.5	p < 0.001	
Aphidius colemani	Year 3+	Year 3 -				
Year 2 +	8	5				
Year 2 -	2	295				
χ^2 > 100	p < 0.001					

Table 1-3 The correlation between the IgE in run 2 and in run 3.

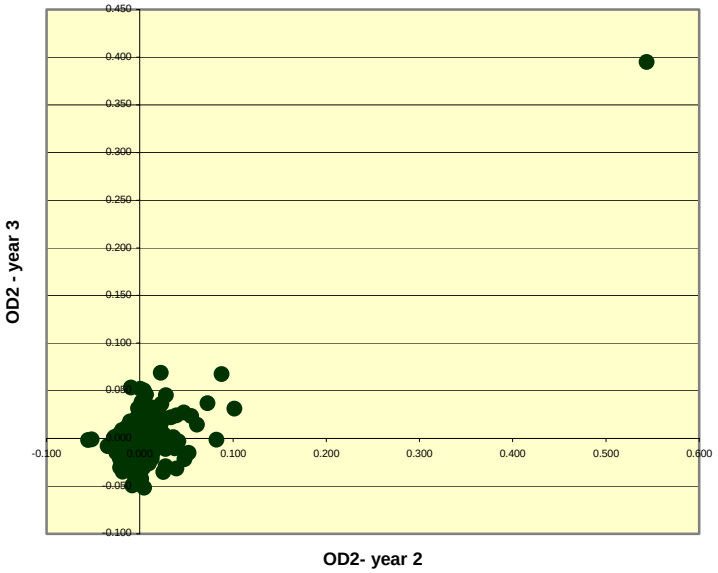
Quantitative relations between levels of IgE to each allergen as measured in the two consecutive years were assessed as the (Pearson) correlations for the OD_c values; thus calculated correlation coefficients (for non-transformed values) were 0.915, 0.666, 0.784, 0.906 and 0.781 for IgE to *T. urticae*, *P. persimilis*, *H. miles*, *A. cucumeris* and *A. colemani*, respectively.

These relations are further illustrated in the following graphs, which clearly show that the stronger reactions (OD's >0.1-0.2) in year 2 were practically always reproduced in year 3; that there were apparently no examples of a markedly present 'new' incident sensitization during follow-up; and that the large majority of 'discordant' results was found among sera with relatively weak to borderline reactions.

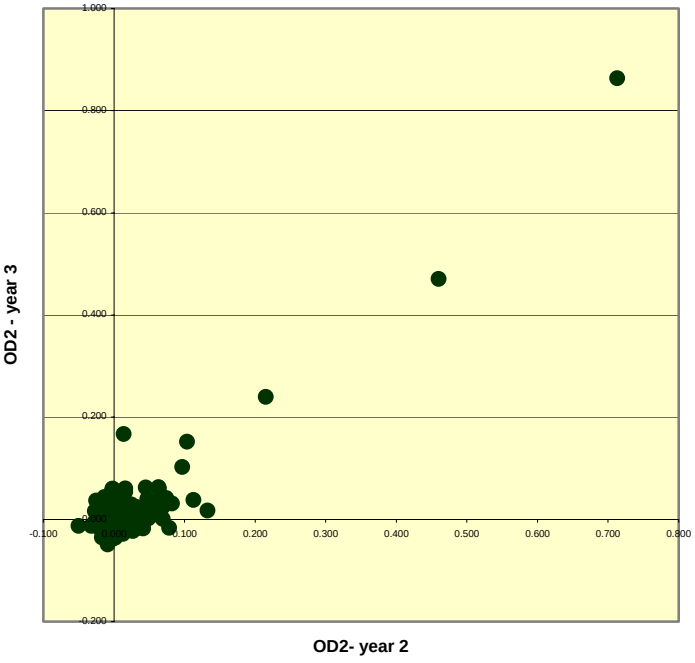

BIOGART sera nos. 20000 vs 30000: IgE anti TU
0,800
0,700
0,600
0,500
0,400
0,300
0,200
0,100
0,000
-0,100
-0,200
0,000
0,200
0,400
0,600
0,800
OD2 - year 3
OD2- year 2

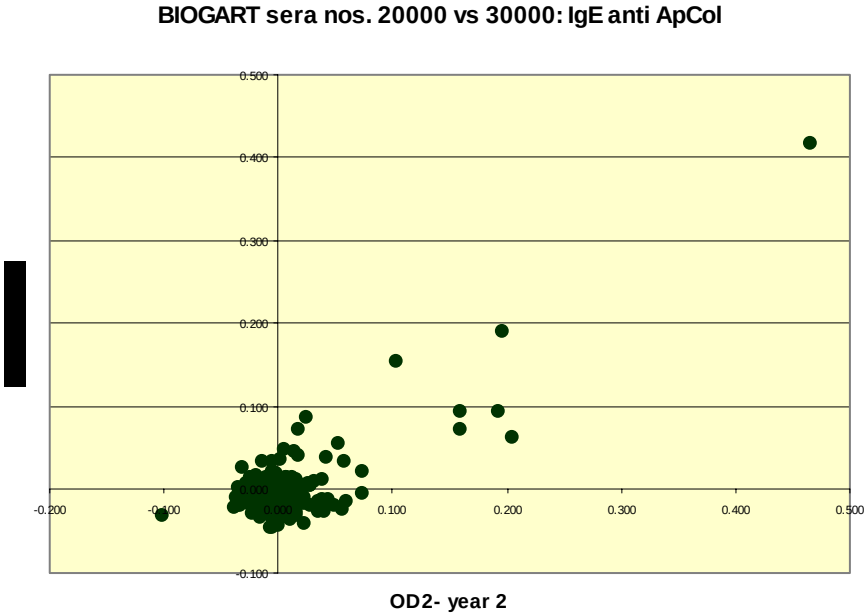

BIOGART sera nos. 20000 vs 30000: IgE anti PP
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.000
-0.100
-0.150
0.100
0.150
0.200
0.250
0.300
0.350
0.400
OD2 - year 3
OD2- year 2

BIOGART sera nos. 20000 vs 30000: IgE anti HM



BIOGART sera nos. 20000 vs 30000: IgE anti AmCu





1.1.4 IgE inhibition experiments and immunoblotting

The strong or at least significant correlations between reactions to IgE different allergens suggest that the *T. urticae* and *P. persimilis* preparations might be partially cross-reactive. Therefore IgE inhibition EIAs were performed in which the reactions of moderately to strongly positive sera with a coating of *T. urticae* or *P. persimilis* allergens respectively were blocked by adding the same or a possibly cross-reacting allergen extract to the fluid phase.

The results indicated that

- reactions of anti-*T. urticae* positive sera with *T. urticae* allergens could be dose-dependently inhibited with *T. urticae* extract, such that 50% inhibition was achieved at approximately 4 µg/mL *T. urticae* extract (protein concentration);
- IgE anti-*T. urticae* could also be inhibited with the *P. persimilis* extract, with 50% inhibition reached at approx. 45 µg/mL;
- partial inhibition was seen after incubation with *A. cucumeris* extract: approx. 30% at the highest concentrations (45 µg/mL).
- other extracts: *H. miles, A. colemani*, and a house dust mite extract (HDM) did not show any significant inhibition.

The results of the plate with *P. persimilis* coating in which anti-*P. persimilis* positive serum was tested confirmed the cross-reactivity between *T. urticae* and *P. persimilis* extracts:

- *P. persimilis, T. urticae* and *A. cucumeris* showed dose-dependent inhibition, with C (inh, 50%) values of approx. 10, 5, and 20 µg/mL, respectively. Thus, *T. urticae* was a more effective inhibitor of IgE anti-*P. persimilis* than *P. persimilis* itself, suggesting that the observed IgE anti-*P. persimilis* reactions – at least of the sera used for these

inhibition experiments - may be largely or completely due to an initial sensitization to *Tetranychus* allergens and cross-reactivity with *P. persimilis*.
- The other extracts, *H. miles*, *A. colemani* and HDM did not inhibit the IgE anti-*P. persimilis* binding.

For the *H. miles* inhibition experiment only serum from one subject with sufficiently high titre was available. Curiously enough, the reaction could not be inhibited with *H. miles* extract (and neither with the other extracts), which suggest that even this relatively strong reaction might in fact be non-specific.

In the IgE anti-*A. cucumeris* inhibition EIA only *A. cucumeris* itself showed sufficient potency to reach close to 50% inhibition – at ~60 µg/mL. None of the other extracts showed inhibition except HDM, which showed a dose-response inhibition curve levelling off at ~30% inhibition – which suggested partial cross-reactivity by allergens shared by *A. cucumeris* and HDM.

The IgE anti-*A. colemani* reaction was only inhibited by *A. colemani* itself, with a C(inh, 50%) of approximately 1 µg/mL , whereas none of the other preparations showed any inhibition.

SDS-PAGE and immunoblotting were performed with the same sera. Results confirmed
- the similarity of *T. urticae* and *P. persimilis* preparations, with similar staining patterns for proteins after SDS-PAGE, and after incubation of blot strips;
- the relatively weak, but clearly demonstrable reactions of IgE with one or several proteins in each of tested allergen preparations.

1.2 Analysis of follow-up

1.2.1 Selection of material

A remarkable finding of the IgE serology in the material from run 2 and 3 was the strong correlation between the levels of IgE sensitization for each specific allergen – expressed as the blank-adjusted OD values in EIAs with 1/10 diluted sera - in the two consecutive follow-up years (see Appendix 1, paragraph 1.2.3, Table 3, and the graphs). This strongly suggested that the incidence of new sensitization during the study would have been low – at least during the later follow-up years. To assess whether this was indeed the case, and also true (or not) for the earlier years in the study, all still available sera from workers with one or more positive reactions in run 2 and /or 3 in the thus far reported test series were simultaneously retested on the same allergen(s). Thus, from each greenhouse worker in this group the corresponding serum samples from run 0 and run 1 were recovered and tested in allergen-coated EIA plates, together with his/her serum samples from run 2 and/or 3 of which at least one had been found positive.

1.2.2 Analytical methods

The test method for specific IgE was essentially the same as described in Appendix I for the primary test series, with sera tested at a 1/10 dilution in PBTG plus negative control serum in microwells coated with the various allergen preparations, and with appropriate control microwells in each plate:

- for each allergen controls without serum (reagent blank or no serum control);
- for each serum a non-coated control well to correct for non-specific sticking of IgE to the plate (Appendix 1.1.2).

The general design per test plate was however different, and always such that for each subject all available serum samples (from 2 to 4) were tested on a specific allergen in adjacent wells of the same plate, to allow an optimal comparison of longitudinal changes in allergen-specific IgE reactivity for each individual worker.

For practical reasons these sera were clustered in a number of groups:

a) sera tested (runs 0 and 1) or retested (run 2 and 3) on four allergens: *T. urticae*, *P. persimilis*, *A. cucumeris* and *A. colemani*; n = 9
b) sera (re)tested on *T. urticae* and *P. persimilis*; n = 12
c) sera (re)tested on *A. cucumeris* and *A. colemani*; n = 35

Most of the run 2 and run 3 series included in set a) had been positive on only 2 or 3 of the four test allergens, and similarly many of the run 2 and/or run 3 sera of groups b) and c) had been positive on only one of the allergens. Therefore this design also implicated retesting of a substantial number of previously negative IgE tests.

Given the low number of clearly positive IgE anti-*H. miles* reactions, and their apparent lack of specificity shown by the EIA inhibition experiments (Appendix 1.2.4), no longitudinal analyses of IgE anti-*H. miles* reactions were performed.

1.2.3 Results

A relatively high proportion of the positive IgE reactions found in the previous test series were very weak – just above the pre-set cut-off OD values of 0.05. As a consequence, retest results for several of these previously positively tested samples were in a number of cases now just below the limit of detection, and positivity could thus not be confirmed. This was particularly the case for sera from workers who had shown a positive reaction in only one of the two test runs.

In the following tables the results are summarized semi-quantitatively for all tested workers involved in the follow-up serology, with the strength of the IgE reaction (adjusted OD value) given in categories as follows:

below 0.05:	- / neg.
0.05 – 0.10	+/-
0.10 - 0.20	+
0.20 – 0.50	++
>0.50	+++

Not done (serum not available): ND.

Amblyseius cucumeris		IgE in follow-up study			IgE response vs. time
participant	year 0	year 1	year 2	year 3	
20x (3-4 years)*	neg	neg	neg	neg	
2x (2 years)*	neg	neg	neg	neg	
A	neg	+/-	+	+	new sensitization in year 1
B	+/-	+/-	+	+	slight increase year 1 -> 2
C	neg	neg	+/-	+/-	new sensitization in year 2
D	+/-	+/-	+/-	+/-	constant, low
E	neg	+	neg	neg	transient
F	++	++	++	++	constant positive
G	neg	neg	+/-	nd	new, low in year 2
H	+/-	+/-	+/-	nd	constant, low
I	+	+/-	+/-	nd	constant, low
J	+	neg	neg	nd	transient
K	+	+	+	nd	constant positive
L	+	+/-	+/-	nd	constant, low
M	+/-	+/-	+/-	nd	constant, low

*) For 20 subjects, sera from all 3 or 4 years were negative; for 2 workers, sera from 2 years were available and both negative.

Table 1.3.1: IgE response to *Amblyseius cucumeris* – development in time

Thus, 7 subjects showed a constant (weakly to moderately) positive IgE response to *A. cucumeris*, 3 showed newly developed sensitization, in one the data suggested a slight increase in an already positive response, and in two there had been an early response that seemed to decrease in later years.

Aphidius colemani		IgE in follow-up study			IgE response vs. time
participant	year 0	year 1	year 2	year 3	
22x (3-4 years)*	neg	neg	neg	neg	
1x (2 years)*	neg	neg	neg	neg	
N	+/-	+	+	+	slight increase year 0 -> 1
O	neg	+	neg	neg	transient
C	neg	neg	++	+/-	new sensitization in year 2, thereafter decrease
P	neg	neg	+/-	+/-	new, low in year 2
Q	+/-	++	neg	neg	transient
R	++	++	+	++	constant positive
F	neg	neg	+	neg	transient
S	+/-	+/-	+/-	+/-	constant, low
H	++	+	+/-	nd	constant, gradual decline
I	++	++	+	nd	constant, gradual decline
T	+/-	nd	+	nd	constant, (low) positive
L	++	++	++	nd	constant positive

* For 22 subjects, sera from all 3 or 4 years were negative; for 2 workers, sera from 2 years were available and both negative.

Table 1.3.2: IgE response to *Aphidius colemani* – development in time.

Over-all, 4 subjects showed a constant (weakly to moderately) positive IgE response to *A colemani*, and two a positive response that seemed to become gradually weaker in time. In 2 workers there appeared to be new sensitization during the study, and in one the data suggested a slight increase in an already positive response. In three workers a transient response was observed.

Phytoseiulus persimilis		IgE in follow-up study			IgE response vs. time
participant	year 0	year 1	year 2	year 3	
15x (3-4 years)*	neg	neg	neg	neg	
U	+/-	+	+	+	increase year 0 -> 1
P	neg	neg	+	neg	transient
R	+	+	+	+	constant positive
V	neg	neg	neg	+	new in year 3
H	+	+/-	+/-	nd	constant, with decline
I	+	+	+/-	nd	constant, with decline

* For 15 subjects, sera from all 3 or 4 years were negative; for 2 workers, sera from 2 years were available and both negative.

Table 1.3.3: IgE response to *Phytoseiulus persimilis* – development in time.

Over-all, 1 worker showed a constant positive IgE response to *P. persimilis*, and two a positive response that seemed to become gradually weaker in time. In 1 worker there appeared to be new sensitization during the study, and in one the data suggested a slight increase in an already positive response. In one worker a transient response was observed.

Tetranychus urticae		IgE in follow-up study			IgE response vs. time
participant	year 0	year 1	year 2	year 3	
9x (3-4 years)*	neg	neg	neg	neg	
X	+/-	+/-	neg	neg	transient (weak)
Y	+/-	neg	neg	neg	transient (weak)
U	++	+++	++	+++	constant positive, with increase year 0 -> 1
Z	++	+	+	+	constant positive
aa	neg	neg	neg	+	new in year 3
R	++	+	+	++	constant positive
ab	+/-	+/-	+/-	neg	weak, gradual decline
ac	neg	nd	neg	+/-	new (weak) sensitization in yr 3
V	+/-	+/-	+/-	+/-	constant, weak
S	+/-	+/-	+/-	+/-	constant, weak
I	+	+	+/-	nd	constant positive, with decline (?)
ad	nd	++	+++	nd	strong, increase in time

* For 15 subjects, sera from all 3 or 4 years were negative; for 2 workers, sera from 2 years were available and both negative.

Table 1.3.4: IgE response to *Tetranychus urticae* – development in time

Over-all, 7 workers showed a constantly positive IgE response to *T. urticae*, and one a positive response that seemed to become gradually weaker in time. In 2 workers there appeared to be new sensitization during the study, and in 2 the data suggested a slight increase in an already positive response. In 2 workers a transient response was observed.

1.3 Discussion

No data are available in the literature on the development in time of these IgE responses, thus no comparison with previously published data is possible. The results however further underline that the IgE responses detected with these EIA methods were very weak and therefore in many cases difficult to reproduce upon re-testing. Nevertheless, definitely positive IgE reactions (eg. OD values >0.15-0.20) could in general be easily reproduced, and for a number of the workers clearly positive IgE responses could be noted over all the years. On the other hand, sera from these subjects did - with a few exceptions - not show an obvious increase in IgE levels in time. Instead it appeared that the most pronounced IgE responses had been present from the start of the study. This may be in line with the fact that the study is no real cohort study but essentially a large cross-sectional survey with an extensive follow-up component. Thus the relatively strong IgE reactions of some sera from the first study periods (runs 0 and 1) could be due to work-related sensitization in the years of employment preceding the study. Alternatively, the reactions may reflect cross-reactivity with other insects or mites encountered in teh general environment, in which case the IgE responses might have been much less relevant.

1.4 Conclusions

The rather low frequency of specific IgE responses to the beneficial predatory mites and insects, and to the pest mite *Tetranychus urticae* was confirmed in this follow-up serology study. In fact, where positive, the reactions of most sera were rather weak. There was very little evidence of incident sensitization during the follow-up; in those few individuals where serology data suggested an increase in the response, or newly emerging sensitization, the levels remained usually relatively low, at least much lower than those found in sera from subjects with more or less constant positive responses.