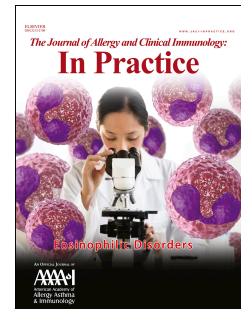


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High prevalence of sensitization to mites and insects in greenhouses using biological pest control

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Title

High prevalence of sensitization to mites and insects in greenhouses using biological pest control

Running title

Greenhouse workers' mite and insect sensitization

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The authors declare no conflicts of interests

Abstract**Background**

Mites and insects are widely used as biological pest control in greenhouses. A few studies have reported sensitization to mites among greenhouse workers, but the prevalence of sensitization to pest control insects is not known.

Objective

We aimed to determine the prevalence of IgE-mediated sensitization to pests and their control organisms in the population of exposed greenhouse workers and the relationship between sensitization and allergic symptoms.

Methods

In a cross-sectional study, we interviewed 117 tomato and cucumber greenhouse workers from eight companies that use biological pest control. Sensitization to nine organisms was assessed by serum specific IgE measurement, fractional exhaled nitric oxide (FeNO) was also measured.

Results

The prevalence of specific sensitization to pests and pest control organisms was 50%, to mites 29% and to insects 46%. Of the individual species, *Macrolophus pygmaeus* insect sensitization had the highest prevalence (46%). Asthma symptoms were significantly associated with sensitization to pest and pest control organisms (OR 3.9, 95% CI 1.2-12.5) and with a FeNO level of ≥ 25 ppb (OR 4.8, 95% CI 1.7-13.8), indicating eosinophilic airway inflammation. South-East Asian origin was significantly associated with sensitization (OR 5.1, 95% CI 2.1-12.1) and rhinitis (OR 2.8, 95% CI 1.2-6.3).

Conclusion

Tomato and cucumber greenhouse workers were commonly sensitized to predatory insect *Macrolophus pygmaeus* and pest control mites. Our findings stress the importance of the surveillance and prevention of work-related allergic diseases among greenhouse workers.

Highlight box

What is already known about this topic? Mites and insects are widely used as biological pest control in greenhouses. A few studies have reported sensitization to mites among greenhouse workers, but sensitization rates to insects is are not known.

What does this article add to our knowledge? Half of the greenhouse workers were sensitized to at least one of the nine tested organisms, asthma symptoms were significantly associated with sensitization. *Macrolophus pygmaeus* insect sensitization had the highest prevalence.

How does this study impact current management guidelines? Our findings stress the importance of the surveillance and prevention of work-related allergic diseases among greenhouse workers. Regular health surveillance of exposed workers including testing for specific sensitizations should be considered.

Keywords

Biological pest control, *Macrolophus pygmaeus*, Occupational allergy, Predatory insects, Predatory mites

Abbreviations

BSA (bovine serum albumin), CI (Confidence interval), FeNO (Fractional exhaled nitric oxide), IQR (Interquartile range), PPB (Potassium phosphate buffer), OR odds ratio, SPT (Skin prick test)

Introduction

In recent decades, concerns over environmental pollution, occupational safety and food quality related to chemical pesticides has led to widespread use of biological pest control in greenhouses. Typical pests in vegetable greenhouses are plant-feeding mites and insects such as thrips, whiteflies and aphids. To reduce these, various types of beneficial organisms including mites, insects, worms, bacteria and moulds are used. Mites (*Acari*) are typically predatory species that feed on pests. Insects are mostly either parasitic wasps (*Hymenoptera* family) that infect the target pest with their eggs, or predatory bugs (*Hemiptera* family) that feed on pests (Figure 1). These may be applied to growth either regularly, for example, at a few weeks' intervals, or on demand, to halt already-observed excessive growth see Table E1 in the Online Repository. They can be delivered in small sachets, by hanging tapes on the plants or sprinkling on the growing bed and leaves. Currently, vegetables are produced in greenhouses throughout the year in Nordic countries, which means that workers encounter these biological materials indoors all year round.

Pests and control organisms' allergenic protein structures, such as dried exuviae, scales, hair, fragmented dead remains and faeces, may end up in the air in dust particles or aerosols (1). A few reports have described cases of allergic diseases caused by biological pest control organisms (2-6). Moreover, previous larger studies have shown an 18%–25% prevalence of sensitization to pest or pest control mites among greenhouse workers (2, 7-9). Although insects are known sensitizers in workplaces (10), little is known about sensitization to pests and pest control insects among greenhouse workers.

The aim of this study was to evaluate the prevalence of sensitization to common pests and pest control organisms (mites and insects) among Finnish tomato and cucumber greenhouse workers. Furthermore, we examined the relationship between this sensitization and asthma, rhinitis and urticaria symptoms, and assessed eosinophilic airway inflammation.

Methods

Allergen extracts

We selected seven pest control organisms and three other organisms present commonly in Finnish tomato and cucumber greenhouses. The predatory mites (*Neoseiulus cucumeris* and *Amblyseius swirskii*, both in aqua), parasitic insects (*Encarsia formosa* and *Eretmocerus eremicus* eggs in whitefly pupae, *Encarsia formosa* adults as such, *Aphidius colemani* in aqua) and predatory bugs (*Macrolophus pygmaeus* as such) were supplied by Biotus Oy (Forssa, Finland). We also acquired pest mites (*Tetranychus urticae* as such), nutritive mites (*Suidasia medanensis*) and pest flies (*Trialeurodes vaporariorum*, whitefly, as such) from Biobest Group NV (Vesterlo, Belgium).

We stored the organisms (0.2–5 grams/species) supplied as such in a freezer at arrival and diluted them later in 1.75–10 ml sterile potassium phosphate buffer (PPB). The extraction method followed that previously reported by Carnes et al., with some modification (11). We extracted the solutions either by ultrasonication for 10–15 minutes or by shaking at 4 °C overnight. Those supplied as aqueous solutions were shaken as such at 4 °C overnight. We centrifuged the extracts for 10 minutes at 20 °C and 5000 g. The supernatants were concentrated and purified from small molecules with Amicon Ultra-15, 10 kD centrifugal filters at 5 °C. We determined their protein contents using a NanoDrop® spectrophotometer with the sample mode ‘Other protein (E1%)’ and a dilution series of 0.06–2 mg/ml bovine serum albumin (BSA) for calibration. Depending on the concentration, each extract was either diluted with potassium phosphate puffer (PPB) or concentrated by centrifugation under vacuum (500 mmHg) to 2 mg protein/ml. In the case of *Encarsia formosa* adults, only 1 mg/ml protein content was achieved. The extracts were filtered through Millex-GV 33 mm, PVDF 0.22 µm sterile filter (Merck GmbH, Germany) directly into 5 ml drop bottles and stored at -20 °C until investigations.

Study population

The study was conducted in eight greenhouse companies in Western and Southern Finland that grow tomatoes and/or cucumbers and use biological pest control. Six of the companies were recruited via a local producer association and two were found via the internet and contacted directly. All the employees and entrepreneurs who were present during the visiting day were invited to participate. Five people were unwilling to participate, and we were unable to obtain blood samples from four people. The final number enrolled in this study was 117.

Ethical considerations

The study was approved by the Helsinki University Hospital Ethical Committee (HUS/3372/2017). Each participant gave their written informed consent.

Interview

The occupational physician (PH) performed structural face-to-face interviews to all participants at their workplace assisted by an online interpreter and translated written questions when needed. The questions covered demographic information, countries participants parents are from (ethnicity), work history, smoking habits, and airway and skin symptoms. We adapted asthma and rhinitis questions from Tuohilampi questionnaire validated in Finnish language (12). Rhinitis symptoms were defined as the presence of at least two of four nasal symptoms (rhinorrhoea, nasal obstruction, nasal itching, sneezing) over the last 12 months excluding symptoms during respiratory infection. Asthma symptoms were defined as the presence of at least two of four asthma symptoms (coughing,

dyspnoea, wheezing, chest tightness) over the last 12 months. Urticaria was defined as the presence of self-reported contact urticaria on the hands and/or forearms. For clarification, the workers were shown a picture of typical contact urticaria on the skin. The questions related to skin symptoms followed the Nordic Occupational Skin Questionnaire (NOSQ-2002) for surveys on work-related skin disease and exposures to environmental factors (13).

Skin prick tests

The skin prick test (SPT) panel included a negative control, a positive control (histamine). The common environmental allergens panel included six standardized environmental antigens (birch, timothy grass and mugwort pollen and dog, cat and house-dust mite *Dermatophagoides pteronyssinus*) (ALK-Abello, Hørsholm, Denmark). A wheal at least 3 mm larger than the negative control was considered positive (14). Atopy was defined as at least one positive SPT to common environmental allergens.

Specific IgE assessment

Specific IgE to the pests and pest control organisms were measured using the in-house allergen extracts described above and a previously reported immunospot method (15). Briefly, three microlitres of the allergen extracts were applied to nitrocellulose membranes (0.2 µm) (Biorad Laboratories, Germany). Free binding sites were blocked with 0.5% Tween 20 in PPB, pH 7.4, for one hour at room temperature. After washing, the membrane was incubated with serum overnight at room temperature, followed by iodine 125-labelled anti-IgE (Immuno-Biological Laboratories, Germany). The membranes were incubated in cassette for ten days. The image plates were scanned with FLA-300 (Fujifilm). The results were analysed using AIDA Image Analyser Software (Elysia-Raytest, Germany). Human IgE full-length protein (Abcam, UK) was used as a standard to measure pest- and pest control organism-specific IgE in the serum (ng/ml). The values are presented as ISRU (immunospot relative unit) which is equivalent to kU/l. Values of >0.4 kU/l (ISRU) were considered positive.

Fractional exhaled nitric oxide (FeNO)

FeNO was measured at the workplace during the workday using an online chemiluminescence analyser (NIOX mino; Aeroctine AB, Solna, Sweden) in compliance with ATS/ERS recommendations (16).

Statistical analysis

We used SPSS version 27.0 (IBM Corporation, Armonk, NY) for our analyses. Continuous measures were summarized by medians, and interquartile range (IQR) and categorical variables by their frequencies and proportions. We analysed the differences between the groups using the Mann–Whitney U test for continuous variables and chi-squared tests for categorical variables. Logistic regression analysis was used for calculating odds ratios (OR). The variables were tested in a multivariate logistic regression model and adjusted for sex, age, smoking, atopy and ethnicity.

Results

Demographic and clinical characteristics

The study population included 117 greenhouse workers (107 employees and 10 entrepreneurs). They were mainly male, their median age was 39 (IQR 31–50 years) and 40 (34%) were current smokers (Table 1). Most of them were migrant workers from Eastern Europe and South-East Asia (Vietnam, Thailand). The median duration of total greenhouse work was 5.8 (2.8–12.1) years and time with current employer 4.8 (1.6–10.2) years. Working in both types of greenhouses was common, 102 (87%) worked in tomato and 73 (62%) in cucumber greenhouses.

Atopy was detected in 30 (25%) of the cases, and 24 (21%) reported asthma, 49 (42%) rhinitis and 14 (12%) urticaria symptoms (Table 1). Eosinophilic airway inflammation ($\text{FeNO} \geq 25 \text{ ppb}$) was detected in 25 (22%) of the workers.

Sensitization to pest control organisms and pests

Of the workers, 58 (50%) were sensitized to one or more of the nine tested pest or pest control organisms (Table 1). The sensitized workers were more commonly of South-East Asian origin ($p < 0.001$) and reported more asthma ($p = 0.019$) and rhinitis symptoms ($p = 0.012$) than the others. In addition, an increase in eosinophilic airway inflammation marker ($\text{FeNO} \geq 25 \text{ ppb}$) was more common among the sensitized workers ($p = 0.015$).

We detected sensitization to one or more tested mites in 34 (29%) workers (Table 2). Sensitization was most common to *Amblyseius swirskii* 29 (25%) and *Suidasia medanensis* 29 (25%). Only one worker was sensitized to *Neoseiulus cucumeris*.

Altogether 54 (46%) cases were sensitized to one or more of the five tested insects, 25 (21%) to Hymenoptera and 54 (46%) to Hemiptera (Table 2). We did not find any cases of sensitization to adult stage *Encarsia formosa*, whereas 23 (19%) cases were sensitized to *Encarsia formosa* in *Trialeurodes vaporariorum* pupae, of whom 11 (48%) were also sensitized to *Trialeurodes vaporariorum*. The most common sensitization to insects was that to *Macrolophus pygmaeus*, at 54 (46%), and the median IgE of positive cases was 1.04 (IQR 0.62–3.23) (Table 2).

Venn comparisons indicated a considerable overlap in mite sensitization (Figure 2A). Of the 34 sensitized cases, 12 (35%) were sensitized to three mites and 13 (38%) to two mites. Of the Hymenoptera insects, 13 (52%) people were sensitized to only *Encarsia formosa* in pupae, 10 (40%) to two or three species, and only one (4%) person was sensitized to only *Eretmocerus eremicus* or *Aphidius colemani* (Figure 2B). All those sensitized to Hemiptera were sensitized to *Macrolophus pygmaeus*, and 13 (24%) were also sensitized to *Trialeurodes vaporariorum* (Figure 2C). A comparison of sensitization to mites (Acari), Hymenoptera and Hemiptera (Figure 1D), revealed that 19 (33%) people were sensitized to organisms of all the three classes: all those sensitized to Hymenoptera were also sensitized to Hemiptera, but 18 (31%) cases were sensitized only to Hemiptera.

House dust mite *Dermatophagoides pteronyssimus* sensitization was detected in 12 (10%) cases, 7 of them were sensitized to tested mites and 9 to insects, a total of 9 cases were sensitized to mites or insects. *D. pteronyssimus* sensitization was significantly associated with sensitization to the studied mites ($p = 0.016$) and insects ($p = 0.029$) (Table E2), whereas sensitization to any of the tested pollen or domestic animals was not significantly associated with sensitization to pests and pest control organisms.

In a logistic regression model, sensitization to the pests and pest control organisms was a significant risk factor for asthma symptoms (Table 3). The association remained significant after adjusting for sex, age, smoking, ethnicity and atopy (OR 3.9, 95% confidence interval (CI) 1.2-12.5). We found a trend towards an association between mite and insect sensitization and rhinitis. No association was found between sensitization and self-reported urticaria (OR 1.2, 95% CI 0.3-4.2). We also detected a significant association between asthma and the eosinophilic airway inflammation marker, FeNO of ≥ 25 ppb (OR 4.8, 95% CI 1.7-13.8). FeNO of ≥ 25 ppb was also significantly associated with rhinitis symptoms (OR 3.5, 95% CI 1.3-9.3). South-East Asian origin was a risk factor for rhinitis (OR 2.8, 95% CI 1.2-6.3) and for sensitization to pests and control organisms (adjusted OR 5.1, 95% CI 2.1-12.1).

Discussion

Workers are exposed to various species of mites and insects in greenhouses that use biological pest control. We found a 50% prevalence of sensitization to one or more pest or control organism. Sensitization was most common to the pest control insect *Macrolophus pygmaeus*, and the mites *Amblyseius swirskii* and *Suidasia medanensis*. Of the workers, 21% reported asthma symptoms, 42% rhinitis and 12% contact urticaria.

We selected the pest control organisms for the allergen extracts based on information from the suppliers, including local market shares and typical target pests, and aimed to include the most common ones. We also selected different taxonomical classes to represent the variety of possible allergens. Many pest control products are used on a needs basis; *Macrolophus pygmaeus*, *Amblyseius swirskii* and *Encarsia formosa* and/or *Eretmoceris eremicus* were used in several greenhouses at the time of the investigations, but we were unable to confirm that all the tested products were used recently in the studied greenhouses. Moreover, we were unable to test all the pest control organisms used in the greenhouses. Thus, we may have underestimated the total sensitization rate to pest control organisms and pests among the greenhouse workers.

We used the previous method of Carnes et al. (11) with some modifications related to the form of the raw material we received. In nearly all the raw materials, the amount of biomass was very limited, which hampered obtaining enough proteins in the extracts. This was particularly the case with mites whose masses are very low, and with *Encarsia formosa* adults, which are usually supplied as infected whitefly pupae rather than as the insect itself. For the same reason, we were unable to obtain *Eretmoceris eremicus* adults. The protein concentration was set to a similar level in all except one extract. Due to the limited raw materials, allergen concentration may have been low in some extracts. Our study stresses the need for commercial allergen extracts in related health surveillance of greenhouse workers.

The study population comprised mainly migrant workers from Eastern Europe and South-East Asia. Interestingly, the South-East Asian workers had a higher prevalence of rhinitis and sensitization to mites and insects. In general, migrants moving from low- to middle-income countries to a high-income country have a lower asthma and similar allergic disease prevalence than the host population (17), although a few studies have shown conflicting results (18, 19). Migrant workers are at a considerable risk of occupational morbidity (20), but data on their occupational allergies are lacking. As the natural environment of many pest control organisms used in greenhouses is (sub)tropical, it is possible that South-East Asian workers have been exposed (and sensitized) to the same or similar organisms in their home country before migration. Work duration did not have a significant effect on sensitization to mites and insects. This could be partly explained by the 'healthy worker effect', i.e. unhealthy workers being more likely to leave employment (21). Because of limited language skills, changing jobs may be more difficult for South-East Asian workers than others, which may partly explain their higher sensitization rate.

The prevalence of atopy (25%) was similar to that previously reported among Swedish greenhouse workers (31%) (9). Previous studies have identified atopy as a risk factor for predatory mite sensitization (2, 9), and we found a similar trend. We detected more asthma (21%) and rhinitis symptoms (42%) than the Swedish study (14% and 33% respectively) which may be due to their more stringent definition criteria (9). The prevalence of contact urticaria was 12%. In a Danish cross-sectional study of bell pepper greenhouse workers, the prevalence of urticaria or angioedema was 9% (22). Due to limited language skills of many participants, we screened their symptoms with a physician's structured interview. Thus, the prevalence of symptoms cannot be directly compared

with studies using validated questionnaires. For the same reason we did not evaluate the order in which airway symptoms appeared.

In our population, 29% of workers were sensitized to mites. Former studies of greenhouse workers have reported a similar mite sensitization prevalences. A Spanish study of 246 greenhouse workers reported a 25% sensitization rate to the pest mite *Tetranychus urticae* (7). A Swedish study of 31 greenhouse workers reported a 32% prevalence of sensitization to *Phytoseiulus persimilis*, 52% to *Hypoaspis miles* and 26% to *Tetranychus urticae* (23). A more recent study of 96 Swedish greenhouse workers showed an 18% sensitization rate to the predatory mites *Phytoseiulus persimilis* and *Hypoaspis miles* (9). A Danish study with 579 participants reported a 25% sensitization rate to the predatory mite *Neoseiulus cucumeris* (also named *Amblyseius cucumeris*) and *Phytoseiulus persimilis* (8) and in a Dutch study of 472 greenhouse workers, 23% were sensitized to *Neoseiulus cucumeris* (2). Contrary to previous studies, we found only 1% sensitization rate to *Neoseiulus cucumeris*. This could be due to the more frequent use of *Amblyseius swirskii* than *Neoseiulus cucumeris* in Finnish tomato and cucumber greenhouses. Nevertheless, we cannot exclude too low concentration of allergenic proteins in that extract.

We found a high sensitization prevalence (46%) of the predatory insect *Macrolophus pygmaeus*, a 3 mm-long slender pale-green plat insect belonging to the Hemiptera insect family Miridae, which is widely distributed in Europe, North Africa, Asia Minor and Central Asia (24). The species is commonly used in greenhouses to prevent whiteflies. A previous report described occupational asthma, rhinitis and contact urticaria in two greenhouse workers sensitized to *Macrolophus caliginosus*, belonging to same *Macrolophus* genus, and other pest control organisms (5). We found no reports of sensitization to or allergic diseases due to *Macrolophus pygmaeus*. We detected a 23% sensitization rate to *Encarsia formosa* in whitefly pupae, but no sensitization to the adult insect. Half of the people sensitized to the pupae extract were also sensitized to whitefly, suggesting that the positive tests could partly be explained by whitefly sensitization. The protein concentration in the adult extract (1 g/ml) was lower than that in the other test extracts (2 g/ml), proposing that low allergen content could partly explain the low sensitization rate.

The level of IgE-mediated sensitization to mites and insects was generally low (median IgE values in sensitized persons 0.76-1.96 ISRU corresponding to kU/l), which may reflect either low allergen concentrations in the extracts or low-level sensitization. We used the immunospot method to detect specific IgE to pests and pest control organisms. Previous studies using EIA (8), immunoCAP (9) and RAST (2) methods to assess IgE binding have found similar rates of sensitization to predatory mites in greenhouses, suggesting that the assessment method has little impact on sensitization rates. Venn diagrams showed a considerable overlap in sensitization to individual species, proposing cross-reactivity. Seven cases were sensitized to *Dermatophagoides pteronyssinus* and studied mites and insects. Previous studies have shown cross-reactivity between house dust mites and other mites including *Tetranychus urticae* (25) and some insects i.e. cockroaches. Tropomyosins have been assumed to be a major cause of cross-reactivity (26). Cross-reactivity related to tropomyosins may partly explain sensitization to mites and insects in our study. Lack of non-exposed reference group was a weakness of this study. Without reference group we do not know the sensitization rate of studied organisms in general population or the proportion of detected sensitization caused by cross-reactivity with allergens that are not related to greenhouse work.

Logistic regression analysis showed that sensitization to pest control organisms and pests was a significant risk factor for asthma symptoms and a similar trend was seen for rhinitis suggesting that sensitization has clinical relevance. Our findings are in line with previous studies of mite-sensitized

workers. Astarita et al. found that seasonal occupational rhinitis symptoms were significantly associated with *Tetranychus urticae* sensitization among farmers in southern Italy (27). Kronqvist et al. found that sensitization to predatory mites was significantly associated with asthma and rhinoconjunctivitis (9). A prospective three-year study of greenhouse workers revealed skin rash, nose and eye symptoms in those who developed sensitization to predatory mites (8). We found that elevated FeNO was more common among workers sensitized to mites and insects and was significantly associated with asthma and rhinitis symptoms, which indicates eosinophilic airway inflammation and type 2 inflammatory processes typical of allergic asthma (28). Recent studies have shown promising FeNO measurement results in occupational asthma surveillance among bakers (29). Our findings suggest that FeNO measurement might also be useful in the surveillance of greenhouse workers.

In conclusion, we report a 50% prevalence of sensitization to pest and pest control mites and insects among greenhouse workers. The prevalence of mite sensitization (29%) was similar to previous reports. We also found that 46% of the workers were sensitized to the predatory insect *Macrolophus pygmaeus*. Workers sensitized to tested species had more asthma symptoms. The results propose that sensitization to *Macrolophus pygmaeus* may be common and clinically relevant among greenhouse workers. Our findings stress the importance of preventing allergic diseases from these organisms among greenhouse workers and suggest that South-East Asian workers may be at an increased risk of these diseases. Regular health surveillance and education of exposed workers, work modifications and the use of protective equipment should be considered. Employees who report work-related allergic symptoms should be invited to undergo testing for specific sensitization. Nowadays, the lack of allergen extracts restricts testing for specific sensitization. There is a need for commercially available allergy tests and allergen extracts of pests and pest control organisms.

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Table 1. Demographic and clinical characteristics of 117 greenhouse workers.

	All	Workers sensitized to pest control organisms or pests, n=58	Workers not sensitized to pest control organisms or pests, n=59	P-value
Male, n (%)	72 (62)	42 (72)	16 (28)	0.017
Age, median (IQR)	39 (31–50)	35 (30–44)	44 (33–53)	0.019
Ethnicity, n (%)				<0.001
Finnish	24 (21)	8 (14)	16 (27)	
Other European	36 (31)	10 (17)	26 (44)	
South-East Asian	54 (46)	38 (66)	16 (27)	
Other [†]	3 (3)	2 (3)	1 (2)	
Smoking, n (%)	40 (34)	19 (33)	21 (36)	0.747
Duration of greenhouse work with current employer, years, median (IQR)	4.8 (1.6–10.0)	4.8 (2.0–10.0)	4.6 (1.4–10.3)	0.781
Duration greenhouse work, median (IQR)	5.8 (2.8–11.9)	4.8 (2.6–10.2)	7.9 (2.8–13.6)	0.193
Working in tomato greenhouse, n (%)	102 (87)	50 (86)	52 (88)	0.755
Working in cucumber greenhouse, n (%)	73 (62)	40 (69)	33 (56)	0.146
Atopy [‡] , n (%)	30 (25)	19 (34)	11 (19)	0.062
Asthma symptoms, n (%)	24 (21)	17 (29)	7 (12)	0.019
Rhinitis, n (%)	49 (42)	31 (53)	18 (31)	0.012
Contact urticaria, n (%), n=116	14 (12)	7 (12)	7 (12)	0.945
FeNO ≥25ppb, n (%), (n=115)	25 (22)	18 (31)	7 (12)	0.015

[†]Armenia, Georgia, Nigeria; [‡]At least one positive SPT to common environmental allergens; IQR= interquartile range

Table 2. IgE-mediated sensitization to pest control organisms and pests among 117 greenhouse workers.

Pest control organisms and pests	Sensitized workers n (%)	Median (IQR) of specific IgE values [#]	Median (IQR) of positive specific IgE values
Mites (Acari)			
<i>Amblyseius swirskii</i>	29 (25)	0.19 (0.05-0.46)	1.65 (0.81-6.29)
<i>Neoseiulus cucumeris</i>	1 (1)	0.09 (0.00-0.17)	0.88 (0.88-0.88)
<i>Suidasia medanensis</i> [†]	29 (25)	0.17 (0.07-0.40)	1.82 (0.69-6.82)
<i>Tetranychus urticae</i> [‡]	16 (13)	0.13 (0.02-0.25)	0.95 (0.55-2.06)
Sensitization to least one mite	34 (29)		
Insects			
Hymenoptera			
<i>Encarsia formosa</i> [§]	23 (19)	0.19 (0.08-0.36)	0.98 (0.73-2.88)
<i>Encarsia formosa</i>	0 (0)	0.06 (-0.01-0.16)	NA
<i>Eretmocerus eremicus</i>	9 (7)	0.09 (0.01-0.20)	0.81 (0.45-1.46)
<i>Aphidius colemani</i>	8 (7)	0.08 (0.00-0.20)	0.76 (0.41-2.42)
Sensitization to at least one Hymenoptera	25 (21)		
Hemiptera			
<i>Macrolophus pygmaeus</i>	54 (46)	0.29 (0.09-0.97)	1.04 (0.62-3.23)
<i>Trialeurodes vaporariorum</i> [¶]	13 (11)	0.11 (0.00-0.24)	0.68 (0.54-2.87)
Sensitization to least one Hemiptera	54 (46)		
Sensitization to at least one insect	54 (46)		
Sensitization to least one pest control organism	58 (50)		
Sensitization to least one pest	18 (15)		
Sensitization to least one pest or control organism	58 (50)		

[†]Nutritive mite in cultivation of control mites; [‡]Pest, vegetable mite; [§]in *Trialeurodes vaporariorum* pupa; [¶]Pest, Whitefly; [#]Presented as immunospot relative unit (ISRU) which is equivalent to kU/l. Values of >0.4 were considered positive, IQR= interquartile range

Table 3. Odds ratio for having asthma and rhinitis symptoms in relation to sensitization to pest control organisms or pests and eosinophilic airway inflammation (FeNO $\geq$ 25 ppb) calculated using logistic regression model.

	Asthma symptoms OR (95% CI)	Rhinitis symptoms OR (95% CI)
Sensitization to pest control organisms and pests		
Model 1 [†]	3.1 (1.2-8.1)	2.6 (1.2-5.6)
Model 2 [‡]	4.0 (1.4-11.5)	2.1 (1.0-4.7)
Model 3 [§]	3.9 (1.2-12.5)	1.7 (0.7-4.1)
FeNO $\geq$ 25 ppb		
Model 1 [†]	5.1 (1.9-13.9)	4.0 (1.6-10.4)
Model 2 [‡]	5.5 (1.9-15.3)	4.0 (1.5-10.6)
Model 3 [§]	4.8 (1.7-13.8)	3.5 (1.3-9.7)
South-East Asian origin		
Model 1 [†]	2.3 (0.9-5.8)	2.9 (1.4-6.2)
Model 2 [‡]	2.3 (0.9-6.1)	2.7 (1.2-6.0)
Model 3 [¶]	2.5 (0.9-6.8)	2.8 (1.2-6.3)

[†]crude, [‡]adjusted for sex, age, smoking, [§]adjusted for sex, age, smoking, ethnicity and atopy,

[¶]adjusted for sex, age, smoking and atopy; FeNO=fractional exhaled nitric oxide

Figure legends

Figure 1. Taxonomical classification of organisms covered in this study.

Figure 2. Venn diagrams showing cases of sensitization to various organisms in the A) Mite (Acari) class, B) Hymenoptera insect class, C) Hemiptera insect class, and D) Acari, Hemiptera and Hymenoptera classes.

Figure 1

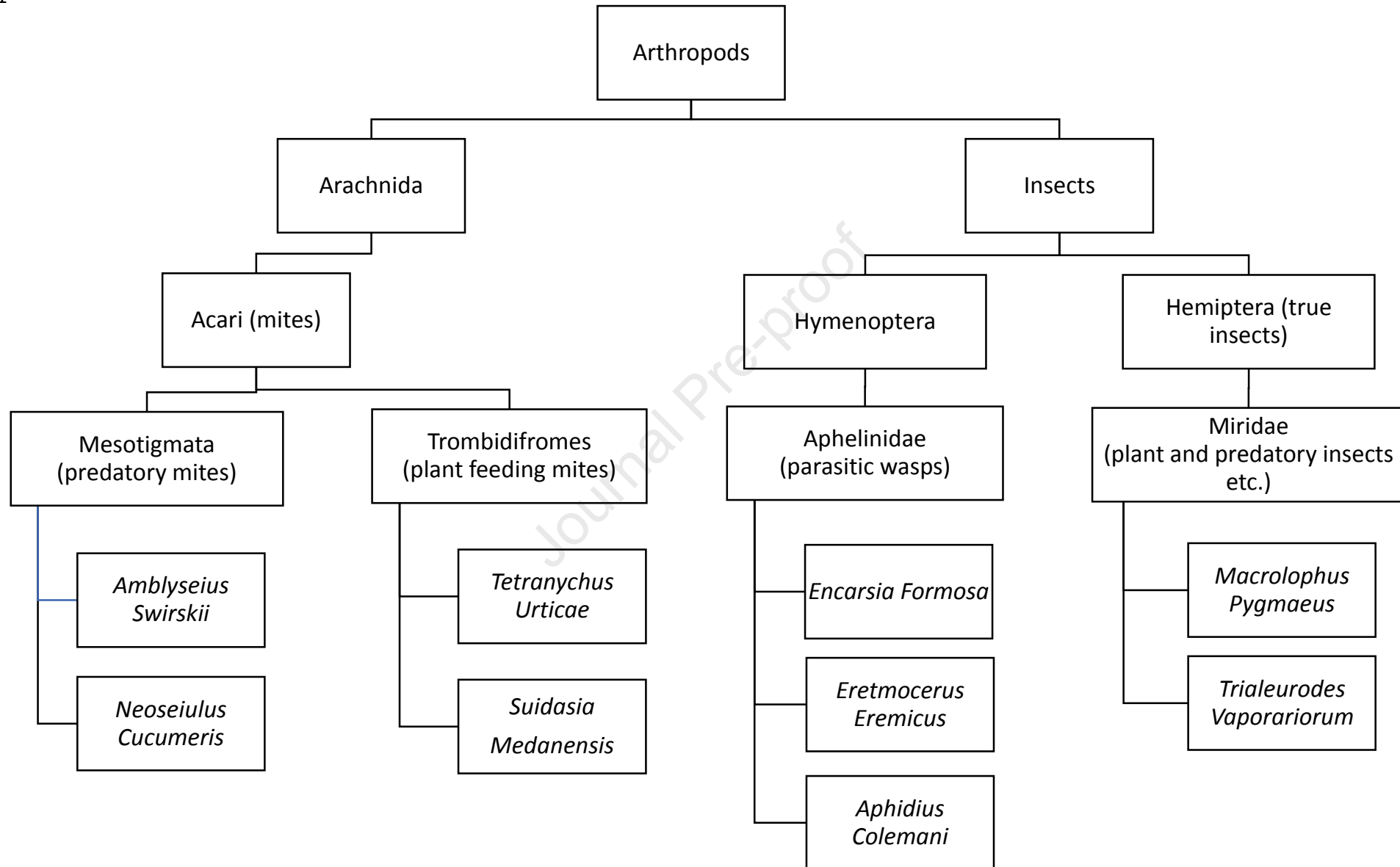
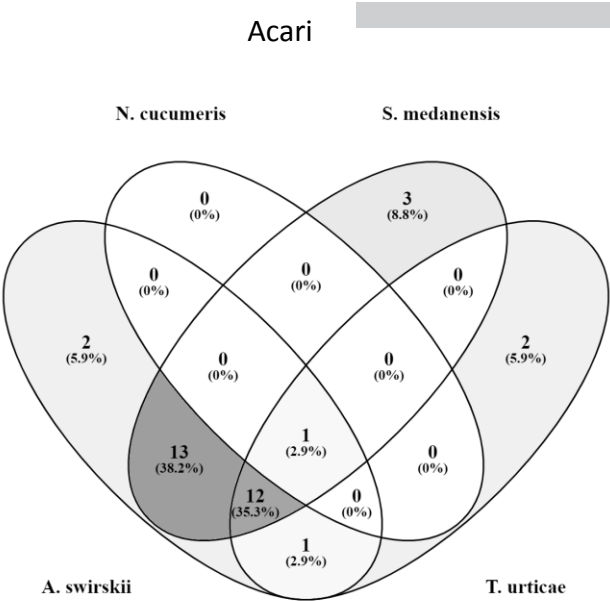
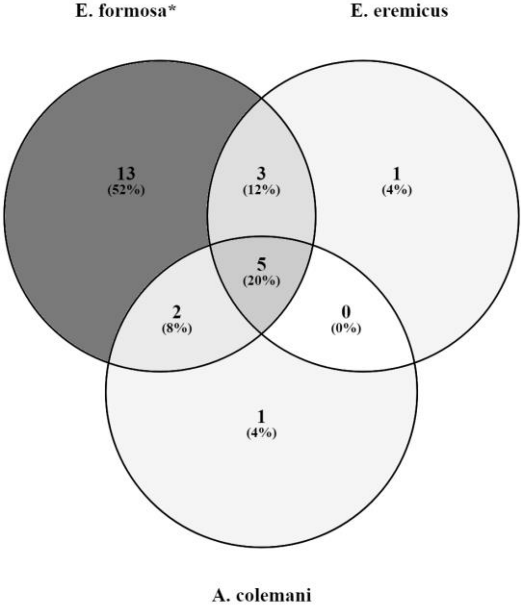


Figure 2

A

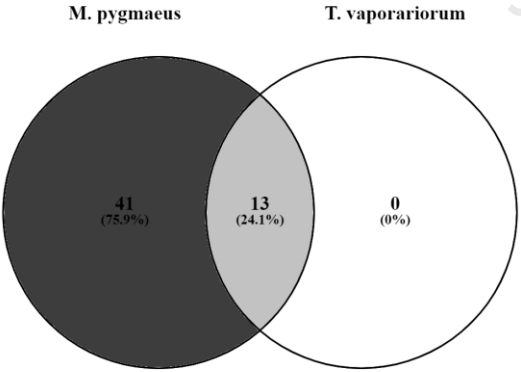


Hymenoptera



C

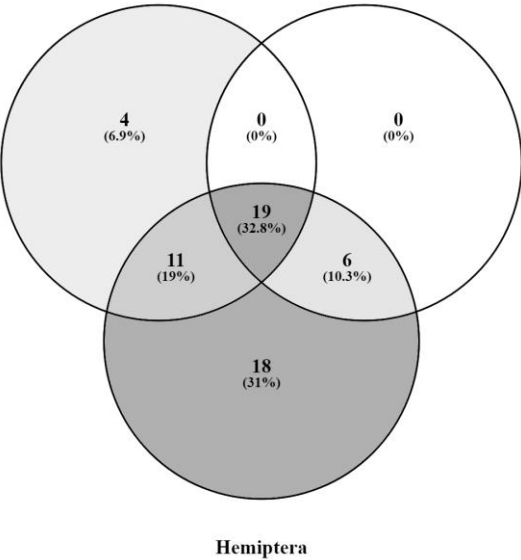
Hemiptera



D

Acari

Hymenoptera



* In *T. vaporariorum* pupa

Table E1. Micro-organisms used for allergen extracts: species, target species and mode of usage.

Species	Target species	Mode of usage and remarks
<i>Amblyseius swirskii</i> (predatory mite)	Thrip (insect)	- Mites mixed with carrier substance such as vermiculite or brans - Placed in growth as such or in small sachets - Varying amounts in repeated additions, usually 150–250 pieces/m²
<i>Neoseiulus cucumeris</i> (predatory mite)	Thrip (insect)	- Mites mixed with carrier substance such as vermiculite or brans - Placed in growth as such or in small sachets - Varying amounts in repeated additions, usually 250–500 pieces/m² - Also called <i>Amblyseius cucumeris</i>
<i>Suidasia medanensis</i> (nutritive mite)	None	- Used as nutritive for <i>Amblyseius swirskii</i> and other mites during cultivation, storage and delivery; may be contained in packages of predatory mites - Domestic mite in tropical climate
<i>Tetranychus urticae</i> (vegetable mite)	None	- Is a pest itself
<i>Encarsia formosa</i> (parasitic insect)	Whitefly (<i>Trialeurodes vaporariorum</i> , insect)	- Card slips with whitefly pupae infected by <i>Encarsia formosa</i> eggs are hung in growth or infected pupae are spread on growth as such - Amount varies according to contamination, 2–20 pieces/m² - Extract contains proteins from both control organism (<i>Encarsia formosa</i>) and pest (whitefly)
<i>Eretmocerus eremicus</i> (parasitic insect)	Whitefly (<i>Trialeurodes vaporariorum</i> , insect)	- Card slips with whitefly pupae infected by <i>Eretmocerus eremicus</i> eggs are hung in growth or infected pupae are spread on growth as such - Amount varies according to contamination, 2–20 pieces / m² - Extract contains proteins from both control organism (<i>Eretmocerus eremicus</i>) and pest (whitefly)
<i>Encarsia formosa</i> (adult insect)	Whitefly (<i>Trialeurodes vaporariorum</i> , insect)	- Not available as such for pest control
<i>Aphidius colemani</i> (parasitic insect)	Aphids	- Insect pupae mixed with carrier substance such as vermiculite or brans - Spread on growth as such - Amount varies according to contamination, 0.5–10/m²
<i>Macrolophus pygmaeus</i> (predatory insect)	Whitefly (<i>Trialeurodes vaporariorum</i> , insect)	- Spread on growth as such

		- 1–2 additions/growth - Amount varies according to contamination, 2–10/m ²
<i>Trialeurodes vaporariorum</i> (white fly, insect)	None	- Is a pest itself

Table E2. Association between sensitization to house dust mite *Dertmatophagoides pteronyssimus* and sensitization to studied mites and insects.

Pest control organisms and pests	P-value
Mites	
<i>Amblyseius swirskii</i>	0.004
<i>Suidasia medanensis</i>	0.004
<i>Tetranychus urticae</i>	0.002
Insects	
<i>Encarsia formosa</i> in <i>Trialeurodes vaporariorum</i> pupae	<0.001
<i>Eretmocerus eremicus</i>	0.009
<i>Aphidius colemani</i>	0.105
<i>Macrolophus pygmaeus</i>	0.029
<i>Trialeurodes vaporariorum</i>	<0.001