

Honeybee Interference as a Novel Aid to Reduce Pollen Exposure and Nasal Symptoms among Greenhouse Workers Allergic to Sweet Bell Pepper (*Capsicum annuum*) Pollen

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Key Words

Honeybees · Occupational allergy · Bell pepper pollen · Skin prick test

Abstract

Background: In 1999, an extensive study among bell pepper growers showed a prevalence of 53.8% work-related symptoms and 35.4% sensitisation to bell pepper pollen. Experiments with bees to remove pollen from bell pepper flowers have been done for a few years. **Objectives:** It was the aim of this study to investigate whether bees can reduce the pollen exposure in bell pepper greenhouses and whether this reduction results in a decrease in allergic complaints in the greenhouse workers. **Methods:** Eighteen greenhouses were selected for the study. In each greenhouse, pollen exposure was estimated. In 6 and 3 of the greenhouses, high, respectively low, numbers of honeybees were placed throughout the pollen season of the sweet bell pepper plant. Nine greenhouses without honeybees were used as control. At baseline, as well as after 4 and 8 months, nasal symptoms assessed with a visual analogue score and lung function parameters were used as outcome measure. **Results:** Forty-four of the 133 employees invited reported work-related symptoms. High numbers of bees reduced the pollen amount in a dose-dependent way to 18% of the baseline ex-

posure. A significant trend relationship between the visual analogue scale in nasal symptoms and the number of colonies of bees was seen. **Conclusions:** The interference of bees in bell pepper greenhouses significantly reduces the pollen amount. This reduction is associated with less work-related rhinitis symptoms in allergic greenhouse workers. This intervention study supports the hypothesis that allergic work-related complaints of greenhouse workers, sensitised to bell pepper pollen, are caused by occupational exposure to this pollen in the greenhouse.

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Introduction

Occupational respiratory complaints are well-recognised problems [1–6]. In the surveillance of the work-related and occupational respiratory disease (SWORD) project [1, 2], the causal agents were classified into 44 categories, and a third of the suspected causes of asthma were organic. Studies addressing risk factors for occupational allergy among greenhouse workers are rarely found in the literature [3–5].

In 1999, an extensive study in bell pepper culture in the Netherlands among 472 employees showed that 53.8% had work-related respiratory symptoms. Sensitisation to

bell pepper pollen was found in 35.4%. Association between positive skin prick test (SPT) reactions with the pollen and complaints appeared to be 90% [7].

Due to the close contact between employees and plants, inhalation of pollen is difficult to avoid, whereas personal protection is considered as inconvenient and impractical. Reduction in pollen exposure would be the best solution to protect the employees from sensitisation and symptoms. Honeybees can be used to pollinate flowers [8]. They collect pollen to feed their brood. Therefore, honeybees may be effective in gathering pollen and may be capable to survive under these artificial conditions. The sweet bell pepper plant produces pollen during the whole production process. The actual pollen output does not change, but by removing pollen from the flower, bees prevent pollen from becoming airborne. However, application of bees is not common practice, since the bell pepper plant is a rather good self-pollinator under normal conditions. Furthermore, it is unknown how effective the use of bees is in reducing respiratory symptoms. Since a few years, the Applied Plant Research, Bee Research Unit, has been experimenting with bees to remove pollen from the flowers. If honeybees are helpful in reducing the pollen load in the greenhouse air, this might lead to a reduction in allergy symptoms.

The aim of this prospective intervention study was to investigate whether bees can reduce the amount of pollen in sweet bell pepper greenhouses. Secondly, we aimed to investigate whether this reduction has a notable effect on the work-related complaints caused by bell pepper pollen in allergic employees.

Material and Methods

Bell pepper greenhouses in the western part of the Netherlands were approached at random by telephone and asked to participate in the study. Eighteen greenhouses were recruited. Initially, they had no bee populations. The greenhouses were arbitrarily allocated into three groups with distinct numbers of honeybees throughout the flower season of the sweet bell pepper plant. Group I comprised a control group of 9 greenhouses without bees (total area 308,500 m², total number of employees 72, number of employees with work-related symptoms 22). Group II consisted of 3 greenhouses with a low number of bees (total area 61,000 m², total number of employees 17, number of employees with work-related symptoms 8). Group III consisted of 6 greenhouses with a high number of bees (total area 126,500 m², total number of employees 44, number of employees with work-related symptoms 14). There was no significant difference in baseline characteristics between the three groups.

Employees were considered eligible for the follow-up study if they had at least one of the following symptoms at work: rhinitis,

conjunctivitis, or asthmatic complaints. The symptoms were defined as work related if the employees notified a substantial improvement or complete regression of their complaints during weekends and holidays. The investigators paid three visits to each greenhouse. The first visit took place in January/February at baseline (measurement 1) before introduction of bees, the second in May/June (measurement 2), and the last in September/October (measurement 3). During the first visit, the volunteers gave informed consent and were asked questions about their age, gender, use of medication, symptoms at work, and allergic symptoms. The symptoms comprised five categories: redness, itching and/or eczema of the skin, urticaria/angioedema, rhinitis, conjunctivitis, and asthmatic symptoms (asthma was defined as shortness of breath and/or coughing and/or wheezing). Sensitisation was determined by means of a SPT performed according to international guidelines with homemade extracts of the bell pepper pollen and common inhalant allergens [9]. During the first, second and last visit, symptom severity was assessed by the visual analogue scale (VAS). The use of medication during the period between the actual visit and the prior visit was asked and noted by the investigator. The Hospital Medical Ethical Committee approved the study. Confidentiality was maintained.

Prick Tests

SPTs were done. Reactions were expressed as the mean wheal diameter in millimetres. A diameter of 3 mm or more was considered positive [9]. Dilution buffer was used as negative control, histamine chloride 10 mg/ml as positive control. The same investigator performed all skin tests.

Allergens

Pollen from flowers of the bell pepper plants was collected in a greenhouse. The flowers were in full bloom. A 25% (w/v) extract was prepared in PBS, pH 7.4, containing 0.03% human serum albumin and 0.5% phenol. The extracts were centrifuged for 10 min at 2,000 g, and supernatants were passed through a 0.22-µm Millex GS filter (Millipore, the Netherlands). All extracts were stored in appropriate aliquots at -20°C until use in skin tests. Before use, extracts were defrosted for 1 h before skin test and mixed. In addition, SPTs were performed with 6 common inhalant allergens from ALK Abelló (Nieuwegein, the Netherlands): *Dermatophagoides pteronyssinus* (SQ 503), tree mix (SQ 108), grass mix (SQ 293), mugwort (SQ 312), dog dander (SQ 553) and cat dander (SQ 555).

Honeybees

In January 2002, all greenhouses were visited. After the first visit, honeybees were placed in the selected greenhouses. All colonies originated from the experimental bee stand of Applied Plant Research, Bee Research Unit (Hilvarenbeek, the Netherlands). The colonies were healthy and had not been used recently for experiments that might influence their development. During the experiments, all colonies were fed with Apifonda sugar.

Approximately 1 colony (10,000) of bees per 10,000 m² (low number of bees) was used in group II, whereas approximately 2 colonies of bees per 10,000 m² (high number of bees) were used in group III. The Bee Research Unit paid visits every 3 weeks to inspect whether the size of the colonies was large enough to warrant good foraging and to check whether the colonies were healthy.

In every greenhouse with bees, an epinephrine-containing syringe was present, to use in case of bee sting anaphylaxis. Instructions how to use the syringe were given to each employee.

Visual Analogue Scale

To score the severity of the rhinitis complaints, as experienced by the employees, we used a VAS. The VAS is a continuous horizontal line of 100 mm, with 0 mm indicating no symptoms and 100 mm representing severe complaints (totally hampered by nose complaints throughout the week). Thus, the VAS score ranges from 0 to 100 [10, 11]. The employee is asked to indicate the severity of symptoms of itching of the nose, sneezing, running nose, nasal congestion and total nose complaints experienced over the past 2 weeks during work in the greenhouse on this horizontal line.

Pollen Measurements

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

Statistical Analysis

The coefficient of variation across the six petri dishes was estimated using a variance-components analysis after natural logarithmic transformation of the pollen counts.

The non-parametric Jonckheere-Terpstra test was used for testing differences in the ordinally scaled VAS between the three groups (I–III) at 4 and 8 months as from the start of the trial [11]. Data are summarised as geometric median and interquartile ranges. Significance is supposed to be reached if the *p* value is smaller than 0.05.

Results

Employees

Of the 35 greenhouses approached by telephone, 18 participated in the study. The total area of the greenhouses was 496,000 m². Reasons for refusal to participate were lack of time and/or lack of interest. Another reason was the current use of honeybees or bumblebees for pollination. The invited group comprised 133 employees. Of this total group, 44 employees appeared to have work-related symptoms, and therefore were considered to be eligible for the follow-up study according to the predetermined criteria. SPTs were positive in 39 of these 44 employees. Baseline characteristics of these 44 selected employees are shown in table 1. Twenty-four employees used anti-

Table 1. Characteristics of the employees

Employees (n = 44)	
Age, years	
Mean	36
Range	21–50
Males	35 (80)
Medication use	24 (55)
Smoking	9 (20)
Symptoms at work (n = 44)	
Skin (itching/redness/eczema)	17 (39)
Rhinitis	44 (100)
Conjunctivitis	33 (75)
Asthma	20 (45)
SPT positive for bell pepper pollen extract (n = 43)	
Bell pepper pollen extract	39 (91)
SPT positive for inhalant allergens (n = 39)	
<i>Dermatophagoides pteronyssinus</i>	15 (38)
Tree pollen	10 (26)
Grass pollen	14 (36)
Mugwort pollen	3 (8)
Cat dander	5 (13)
Dog dander	9 (23)
Any inhalant allergen	25 (64)

Figures in parentheses are percentages.

allergic medication. Twenty-two of the 44 employees used medication for their rhinitis, and 11 workers used medication for asthma. During the whole study, there was no substantial change in use of medication by the workers, with the exception of a few employees who temporarily used an antihistamine.

Honey Bees

During the first 2 weeks of placement in the greenhouses, the colony strength (number of bees) generally decreased, probably because they got lost in the greenhouse or outside (open vents). Also, the brood area decreased during the first weeks, but then remained constant. In most cases, after 10 weeks, the number of bees decreased, due to a lowered brood production under greenhouse circumstances. Subsequently, the colonies had to be replaced. In all colonies, sweet bell pepper pollen was found in the cells of the beehive. The pollen-collecting behaviour of the bees was generally higher during the morning than during the afternoon. An interference of crop protection measures with bee activity was observed: after application of Admire®, an insecticide toxic to honeybees, the bees remained inactive for at least a week. During applications of crop protection substances, the closed bee colonies were placed in a cool place outside

Table 2. Pollen counts (mean of six petri dishes)

Greenhouse No.	Measurement			% of baseline	
	1	2	3	2	3
<i>Without bees (group I)</i>					
5	420	347	238	83	75
10	516	728	354	141	69
11	502	525	947	105	189
13	1,127	758	533	67	47
14	263	340	221	129	84
15	222	225	n.d.	101	n.d.
16	935	328	591	35	63
17	391	437	439	112	112
18	814	507	637	62	78
Median	502	437	486	101	69
<i>With low numbers of bees (group II)</i>					
2	715	233	202	33	28
6	701	181	201	26	29
7	467	202	150	43	32
Median	701	202	201	33	29
<i>With high numbers of bees (group III)</i>					
1	619	217	128	35	21
3	436	34	83	8	19
4	393	26	n.d.	7	n.d.
8	811	112	75	14	9
9	748	192	136	26	18
12	959	91	79	9	8
Median	684	102	83	12	18

n.d. = Not done.

the greenhouse. After re-entering the greenhouse, the activity resumed only slowly. In September and October, the bees were remarkably less active in all greenhouses. A possible cause of this phenomenon may be that the flowers produce less pollen in the late season. The plants produce less fruit and November is the end of the season for this cultivation.

The workers were not hampered by the presence of the bees; only a few were stung, resulting in only local reactions. Anaphylactic reactions were not observed. Therefore, epinephrine was not used throughout the study.

Pollen Counts

Pollen counts ranged from 25 to 1,127 across the 18 greenhouses and 3 measurement times, with a mean of 414 and a standard deviation of 277. Because the distribution of pollen counts was skewed to the right, the median of this distribution was taken as the appropriate location measure. The median percentage of pollen, expressed as

percentage of the baseline (measurement 1), in the group without bees was 101% for measurement 2 and 69% for measurement 3. The median percentages of pollen in the group with low numbers of bees in measurements 2 and 3 were 33 and 29%, respectively. The median percentages of pollen in the group with high numbers of bees in measurements 2 and 3 were 12 and 18% of the baseline, respectively. All results are shown in table 2.

Visual Analogue Scale

Median VAS and interquartile ranges are summarised in table 3. No significant differences were seen in VAS in measurement 1. At 3 months, a significant trend relationship between itching ($p = 0.034$), sneezing ($p = 0.038$), running nose ($p = 0.030$) and bee numbers was seen. Nasal congestion and total nose symptoms yielded no significant results. At 6 months, a significant trend relationship between itching ($p = 0.038$), running nose ($p = 0.045$) and bee numbers was seen. This relationship tended to reach significance for total nose score ($p = 0.094$). Sneezing and nasal congestion scores did not reach significance.

Discussion

The high percentage of bell pepper pollen-sensitised workers (35.4%) in bell pepper culture forced us to consider possible solutions. The most obvious solution is to reduce the pollen output. However, until now, attempts to decrease pollen exposure have never been made. Since honeybees pollinate flowers [8], we decided to investigate whether bees can be used to reduce symptoms caused by bell pepper pollen in these sensitised employees. The results of pollen counts proved that the bees are certainly capable of removing pollen from the flowers. The third measurement in the group with high numbers of bees even showed a decrease of 82% in pollen. Importantly, we established a dose-response relationship between the number of bees and pollen counts in greenhouses. The more bees were used, the less pollen was found. This relationship was seen in spite of a decrease in pollen in the group without bees at the third measurement. We assume that the bell pepper plant is not that active in the late season, therefore producing less pollen.

The interference with bees was associated with less severe nasal symptoms.

Before accepting these results, several potential flaws of this study have to be addressed. First, this study was designed as an exploratory investigation to a novel inter-

Table 3. Visual analogue scale

	Measurement 1	Measurement 2	Measurement 3
Itching			
No bees	4.0 (2.0–5.7)	4.5 (2.6–6.7)	3.4 (0.8–6.6)
Bees (low numbers)	3.4 (0.9–6.2)	3.2 (0.4–5.5)	3.5 (1.1–5.4)
Bees (high numbers)	2.1 (0.0–5.5)	1.6 (0.5–5.6)	0.4 (0–2.6)
p value		0.034	0.038
Sneezing			
No bees	4.7 (2.4–7.3)	4.2 (3.0–7.3)	4.1 (1.7–6.6)
Bees (low numbers)	3.2 (1.9–5.8)	3.0 (1.8–5.9)	4.5 (0.8–7.8)
Bees (high numbers)	5.3 (1.3–7.9)	2.4 (0.8–4.3)	2.2 (0–4.9)
p value		0.031	0.156
Running nose			
No bees	6.2 (1.0–7.3)	3.5 (1.6–6.9)	3.5 (0.9–5.5)
Bees (low numbers)	2.3 (1.3–3.8)	2.8 (0.5–6.6)	2.0 (0.3–6.5)
Bees (high numbers)	3.3 (0.0–8.8)	0.6 (0.2–4.3)	0.4 (0.1–3.9)
p value		0.030	0.045
Nasal congestion			
No bees	5.0 (1.4–7.6)	3.3 (1.7–5.1)	2.0 (0.4–5.4)
Bees (low numbers)	2.0 (0.7–7.4)	2.9 (0.4–6.6)	1.2 (0.2–7.5)
Bees (high numbers)	4.8 (1.8–9.4)	3.7 (0.4–7.4)	1.4 (0.3–6.4)
p value		0.973	0.615
Total nose score			
No bees	5.5 (2.8–7.8)	5.0 (3.4–7.2)	4.4 (2.8–6.7)
Bees (low numbers)	4.8 (2.0–7.6)	4.4 (1.4–6.2)	1.7 (0.5–8.4)
Bees (high numbers)	5.0 (4.0–9.3)	4.0 (1.4–6.6)	1.0 (0.6–4.7)
p value		0.390	0.094

Figures indicate median values, with interquartile ranges in parentheses. p values refer to the significance of a linear trend of bee concentrations per VAS per measurement time; $p \leq 0.05$ is significant.

vention. As private enterprises were involved, it appeared to be difficult to design the study as a strictly randomised controlled trial. Greenhouses were selected and arbitrarily allocated to the different groups I–III. Neither formal randomisation procedure nor power calculation was carried out. The absence of randomisation might have led to differences in outcome measures at baseline. However, VAS scores did not differ between the groups at baseline, suggesting that the results are not affected by the absence of randomisation. Secondly, a control group without sensitisation to bell pepper pollen was not included. Theoretically, the presence of allergy to common inhalant allergies or to predatory mites or other unknown allergies to occupational agents could have influenced the results. However, in a recent study, multiple regression modelling showed that in greenhouse workers, nasal disease expressed in terms of impaired rhinitis-related quality of life could be exclusively attributed to bell pepper sensitisation in spite of concomitant sensitisation to common inhalant allergens or predatory mites [12]. The study

demonstrated that the effect of bell pepper pollen sensitisation overshadows all other concomitant allergies. This study supports the hypothesis that work-related symptoms in these 44 subjects are predominantly caused by bell pepper pollen allergy, thereby providing an explanation for our findings that interference of bees leads to pollen reduction, and intervention is associated with fewer symptoms. Thirdly, not all outcome measures were affected by the intervention (i.e. nasal congestion). An explanation could be that changes in chronic nasal blockage are less well perceived by patients than changes in symptoms such as sneezing and rhinorrhoea. It is possible that the study was underpowered in this respect.

The results of this study suggest that in concordance with common inhalant allergy to birch or grass pollen, allergy to bell pepper pollen is mainly characterised by nasal disease.

Pollen exposure was estimated using a very simple method. We are aware that measurement of airborne pollen – even with nasal samplers [13] – might have led to

more accurate results. However, even with this crude but practical methodology, we were able to detect substantial changes in the amounts of pollen.

In conclusion, the interference of bees in bell pepper greenhouses is a novel way to reduce the pollen output. This reduction is accompanied by a reduction in work-related rhinitis symptoms in allergic greenhouse workers. Furthermore, we found a dose-response relation in the number of bees and a decrease in rhinitis symptoms of the workers. Therefore, this intervention study sup-

ports our hypothesis that allergic work-related complaints of greenhouse workers, sensitised to bell pepper pollen, are mainly caused by occupational exposure to this pollen in the greenhouse.

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