New disease

Type 1 sensitisation against a *Steinernema feltiae* productTrine Thilsing,¹ David Sherson,² Anne Mette Madsen,³ Jesper Baelum²¹Research Unit for Occupational Medicine, University of Southern Denmark, Odense C, Denmark²Department of Occupational Medicine, Odense University Hospital, Odense C, Denmark³The National Research Centre for the Working Environment, Copenhagen, Denmark

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Summary

The use of biological control measures (biopesticides) is a widespread and fundamental technique for crop protection in greenhouses. Previous reports have documented allergic sensitisation against predatory mites, bacteria and fungi. Till now no cases of sensitisation against nematode products have been described. Two subjects working at a flower greenhouse were examined at the Department of Occupational and Environmental Health at Odense University Hospital. A histamine release test revealed positive reaction against a nematode product containing *Steinernema feltiae*, but negative reaction when testing formulation ingredients separately. Skin prick testing with samples containing *S feltiae* or mixed carrier medium, separately, revealed positive reactions in both subjects against *S feltiae* samples only. Skin prick testing among seven control subjects revealed no positive reactions. This is the first report indicating type 1 sensitivity against a *S feltiae* product. These findings point to the need for guidelines and preventive measures when handling biopesticide products and biopesticide-treated plants.

BACKGROUND

Over the past two decades, the use of biological control measures (biopesticides) has become a widespread and fundamental technique for crop protection in greenhouses. In particular, predatory beneficial mites, nematodes and insects are widely used, along with bacteria and fungi. Recent studies have shown allergic sensitisation against certain predatory mites,^{1–3} bacteria and fungi⁴ among greenhouse workers. Till now no cases of sensitisation against biopesticides containing the nematode *Steinernema feltiae* have been described.

CASE PRESENTATION

Two employees (subject A and B) from a flower greenhouse producing campanula, lavender (*lavendula*), Christmas rose (*helleborus*), spring cactus (*rhipsalidopsis*) and autumn cactus (*schlumbergera*) were referred to the Department of Occupational and Environmental Health at Odense University Hospital owing to work-related respiratory symptoms.

Subject A (a 57-year-old woman) and subject B (a 32-year-old man) had been working in the greenhouse for 23 and 5.5 years, respectively. Biopesticides used routinely in the greenhouse included *S feltiae*, *Amblyseius cucumeris*, *Amblyseius swirskii*, *Hypoaspis miles*, *Orius laevigatus*, *Encarsia formosa* and *Verticillium lecanii*. Subject A was spreading out biopesticides every week. *S feltiae* was dispersed by sprinkling. Subject A mixed the *S feltiae* product with water before pouring it in to the automatic sprinkling system. Subject B did not spread out biopesticides.

Subject A reported work-related rhinitis and conjunctivitis when working with biopesticides, in particular *A cucumeris*. Both symptoms resolved in weekends and on holidays.

Subject B reported work-related rhinitis when working with campanula. The symptom resolved in weekends and

on holidays. During the past 12 months he had experienced two episodes of wheezing and whistling in the chest and he had been woken at night by attacks of shortness of breath. Both subjects were non-smokers.

INVESTIGATIONS

A clinical examination was performed including lung function testing according to ERS guidelines,⁵ test for bronchial responsiveness (a modification of the method described by Yan *et al*⁶), exhaled nitrogen oxide (FeNO) using the handheld device NIOX MINO and skin prick testing using 10 common inhalation allergens (birch, grass, mugwort, horse, dog, cat, *Dermatophagoides farinae*, *D. pteronyssinus*, *Alternaria alternata* and *Cladosporium herbarum*), along with the storage mites *Acarus siro*, *Tyrophagus putrescentiae* and *Lepidoglyphus destructor* (Soluprick ALK-Abello, Hørsholm, Denmark).

In addition, the prick-prick technique was performed with plant material from three campanula types, one *schlumbergera* and one *helleborus*.

A blood sample was drawn for histamine release (HR) testing⁷ against three biopesticides (*A cucumeris*, *S feltiae* and *Orius majusculus*) and plant material with positive prick-prick test results.

OUTCOME AND FOLLOW-UP

Initial results

Subject A revealed positive HR reaction against the *S feltiae* and *A cucumeris* products, campanula and *schlumbergera*, and positive skin prick test against birch, timothy, cat, *T. putrescentiae*, campanula and *schlumbergera*. Lung function and FeNO were normal, and there were no signs of bronchial hyperreactivity.

Subject B also revealed positive HR reaction against the *S feltiae* product, along with campanula, and *helleborus*. Skin prick test revealed hypersensitivity against birch, timothy,

mugwort, dog, campanula, schlumbergera and helleborus. Lung function was normal, but there were signs of bronchial hyperreactivity (FEV1 dropped 23.4% following an accumulated metacholine dose of 1.166 mg). Exhaled NO showed signs of airway inflammation (96 ppb).

Further testing

As hypersensitivity against *S feltiae* products has never previously been described further examinations were conducted. According to the material safety data sheet the product contained *S feltiae* paste and an inert carrier. As the product is not considered to be hazardous no detailed information on ingredients was listed. However, the producer agreed to supply samples of product formulation ingredients separately (including *S feltiae* nematodes without additives).

In addition, a sample from the batch of *S feltiae* products used in the greenhouse was processed to separate nematodes from other ingredients: A sample was placed on MF filters (inert mixtures of cellulose acetate and cellulose nitrate filters, pore size 8 µm; Millipore, Bedford, Massachusetts) and sterile water was repeatedly sucked through the product. A total 0.17 g of the product was used and 44 ml water. The *S feltiae* larvae were re-suspended in 4 ml sterile water by shaking it for 3 min (300 rpm). After cleaning, the concentration of the nematode larvae was 10⁶/ml, the concentration of fungi was below 5 cfu/ml (the detection limit) and the concentration of bacteria was above 10⁵ cfu/ml.

Half the *S feltiae* samples (from producer and from greenhouse) were frozen at –20°C for 24 h before skin prick testing.

Skin prick testing was performed using (1a) producer-supplied *S feltiae* larvae without additives, frozen, (1b) not frozen; (2a) purified *S feltiae* product from the greenhouse, frozen, and (2b) not frozen; (3) mixed-carrier medium (four additives) from the producer (no nematodes), (4) positive control (histamine) and (5) negative control (ALK-Abello, Hørsholm, Denmark).

In addition to subject A and B, a control group comprising seven subjects never employed in a greenhouse was skin prick tested with the above-mentioned samples.

HR testing was performed in subject A and B with sample 1a, 1b, 2a, 2b and with the four additives, separately.

Skin prick testing showed positive reaction (wheel diameter >3 mm) against all samples containing *S feltiae* (sample 1a, 1b, 2a and 2b) in subject A and B, but no reaction against the mixed-carrier medium.

None of the seven control subjects showed positive reactions against any of the samples tested.

HR testing revealed no positive reactions.

DISCUSSION

This is the first case report indicating type 1 sensitisation against an *S feltiae* product. As positive skin prick tests were seen in the two greenhouse employees only, occupa-

tional exposure may be a prerequisite for sensitisation, but re-entry exposure through handling treated plant seems to be sufficient. The incongruent HR test results may be due to a generally low level of IgE during the second testing (weak reaction on the positive control solution applied—anti-IgE). It is unknown whether the work-related symptoms are caused by *S feltiae* sensitisation, as other occupational hypersensitivities were detected. However, these and previous findings on biopesticide hypersensitivity^{1–3} emphasise the need for regulation, guidelines and preventive measures when handling biopesticide products and biopesticide-treated plants.

Learning points

- The use of biological control measures (biopesticides) is a widespread and fundamental technique for crop protection in greenhouses.
- The use of biopesticides is only poorly regulated, and no specific guidelines exist to protect greenhouse workers from exposure.
- Previous studies have shown allergic sensitisation against biopesticide products based on predatory mites, bacteria and fungi among greenhouse workers.
- This report is the first to identify type 1 hypersensitivity against a commonly used nematode product containing *Steinernema feltiae*.
- These and previous findings on hypersensitivity against biopesticides emphasise the need for regulation, guidelines and preventive measures when handling biopesticide products.

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Competing interests None.

Patient consent Obtained.

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