

Case report

Occupational allergy caused by *Dianthus caryophyllus*, *Gypsophila paniculata*, and *Lilium longiflorum*

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Background Occupational allergy to ornamental flowers is seldom reported.

Methods We report a case of a 35-year-old male flower supplier who developed IgE-mediated allergic rhinoconjunctivitis with nasal polyps, and contact urticaria and dermatitis after handling *Gypsophila paniculata*, *Dianthus caryophyllus*, and *Lilium longiflorum*.

Results Studies revealed the presence of positive immediate skin prick tests. Rubbing tests were positive to *G. paniculata* and *D. caryophyllus*. Serum specific IgE was detected against *G. paniculata* and *L. longiflorum*. In IgE immunoblot, two IgE-binding bands (15 and 17 kDa) were obtained against *D. caryophyllus*, and two different bands (19 and 22 kDa) were visible against *L. longiflorum*.

Conclusions These findings suggest an IgE-mediated reaction as the mechanism responsible for the respiratory and cutaneous symptoms of the patient. No similar cases of allergy to *D. caryophyllus* and *L. longiflorum* have been previously reported.

Key words: allergy; contact urticaria; *Dianthus caryophyllus*; *Gypsophila paniculata*; *Lilium longiflorum*; occupational disease; rhinoconjunctivitis.

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Few examples of occupational respiratory allergy within the floral industry have been reported to date. Among the many etiologic agents in this work environment, the ornamental flower *Gypsophila paniculata*, commonly used in flower arrangements, is the most frequently reported (1–3). *Limonium tataricum* (4) and *Spathiphyllum* spp. (5) have been implicated in allergic respiratory diseases, as well. Allergens have been identified in *G. paniculata* (known as “baby’s breath” in the USA), *L. tataricum* (sea lavender), *Spathiphyllum* spp. (spathe flower), and *Tetranychus urticae*, a common parasite of vegetables and ornamental flowers (6).

The immunologic and clinical studies conducted on a worker at a flower supplier who presented immediate cutaneous and respiratory symptoms while working with *G. paniculata*, *Dianthus caryophyllus*, and *Lilium longiflorum* are described.

Case report

A 35-year-old man, a nonsmoker, with no family or personal history of atopy, was studied at our

allergy unit for hand dermatitis and respiratory disease. Ten years before, he had begun work as a flower supplier, selling and distributing fresh flowers. For the first 8 years, he showed no symptoms. For the last 2 years, however, he had noticed nasal congestion with itching, sneezing, water rhinorrhea, and conjunctival and palpebral edema. At the outset, nasal and eye symptoms occurred 15 min after he entered the greenhouse, but afterward, symptoms were continuous. One year later, he also developed pruritic wheals followed by papulae and vesicles on exposed hands and forearms. Symptoms appeared throughout the year and were clearly related to the work environment, since he noticed clinical improvement on weekends and holidays.

On physical examination, nasal congestion, water rhinorrhea, and hand dermatitis were present. Anterior rhinoscopy showed a pale mucosa, and one polyp was evident in the right middle meatus. The white blood cell count was 7300/mm³ with 212 eosinophils/mm³. Serum IgG, IgA, and IgM were within normal limits. Total serum IgE was 325 IU/ml.

Basal spirometry showed FEV₁ of 3.87 (107%) and FVC of 4.42 (103%).

Skin prick tests with a battery of commercially available allergens, including tree pollens, animal danders, molds, house-dust mites, and storage mites (ALK-Abelló, Madrid, Spain) were negative. Tests with grass-pollen species were positive.

Material and methods and Results

Extracts for in vivo skin tests

Twenty-five crude, unpurified extracts from the same number of different fresh flowers supplied by the patient were taken, PBS being used as diluent (1:10 w/v). Histamine phosphate 10 mg/ml and 0.9% saline solution were used as positive and negative controls, respectively. The presence of a wheal reaction 3 mm bigger than negative control was considered positive. Positive responses were obtained with *G. paniculata*, *D. caryophyllus*, and *L. longiflorum*. These three extracts were tested on five atopic, pollen-sensitive subjects and four normal persons who served as controls, with negative results. Skin test with *T. urticae* extract showed no reaction. Rubbing tests with the same three extracts were carried out on the patient's forearm. Twenty minutes later, strong positive responses of wheals and flare were observed on the contact area of *G. paniculata* and *D. caryophyllus* extracts. No reaction was detected to *L. longiflorum*. Patch tests were also performed on the patient's back and a positive response of erythema and papulae appeared at 48 h to *D. caryophyllus* and *L. longiflorum*. Ten control subjects were tested as controls with negative results.

A nonspecific bronchial provocation test with methacholine (Provocholine, Roche Laboratory, USA) was performed by the method of Cockcroft, but no significant differences in spirometric parameters were observed, even after the maximum dose of 25 mg/ml.

In vitro tests

In vitro tests were performed by ALK-Abelló, Madrid, Spain. Extracts from fresh *D. caryophyllus*, *G. paniculata*, and *L. longiflorum* were obtained. For that purpose, fresh flowers were frozen at -80°C, lyophilized, and then extracted in ammonium bicarbonate 50 mM, at pH 8.0, containing PMSF 1 mM, 1:20 w/v, for 1 h at room temperature. Afterward, the preparation was centrifuged at 20 000 g for 20 min at 4°C. After filtering, these solutions were dialyzed in distilled water for 24 h, and then passed through a 0.45-µm Sartorius filter. Extracts were lyophilized and stored at 4°C

until use. The total protein concentrations of the extracts, analyzed by the Smith et al. (7) method, were as follows: *D. caryophyllus*, 2.07 mg/ml; *G. paniculata*, 1.61 mg/ml; and *L. longiflorum*, 1.3 mg/ml. The presence of *T. urticae*, a common parasite in flowers, which can cause occupational respiratory allergy, had been ruled out by microscopic examination.

RAST

Newly prepared extracts were applied to paper disks activated with CNBr (8) (40 ml of 1 mg/ml solution of each extract per gram of paper disk). After an incubation period of 16 h at 4°C, the disks were washed with PBS, 40 ml of Tris-HCl 0.1 M, pH 8.0, was added, and the disks were then incubated for 1 h at room temperature. After being washed with PBS-Tween 20, 0.05%, the disks were stored in PBS at 4°C until required. RAST was conducted by the method of De Filippi et al. (9), and the results were reported in RAST classes. RAST values were class two for *D. caryophyllus* and *G. paniculata*, and negative for *L. longiflorum*.

SDS-PAGE and immunoblot

Proteins in the *D. caryophyllus*, *L. longiflorum*, and *G. paniculata* extracts were first dissociated with sodium dodecyl sulfate in 8–25% polyacrylamide gel (SDS-PAGE), by the method of Fling & Gregerson (10). Proteins in the gel were then transferred to a PVDF membrane (Immobilon-P, Millipore) by the method described by Towbin et al. (11). Immunodetection of IgE-binding components was conducted with the patient's serum and ¹²⁵I-labeled anti-IgE (12).

Extracts from *D. caryophyllus* and *G. paniculata* showed several different protein bands between 10 and 200 kDa. No protein bands were detected with the *L. longiflorum* extract (Fig. 1). However, two IgE-binding bands (15 and 17 kDa) were obtained against *D. caryophyllus*, and two IgE-binding bands (19 and 22 kDa) were visible against *L. longiflorum*. No IgE binding was detected against *G. paniculata* (Fig. 2).

Discussion

The patient described in this study experienced cutaneous and respiratory symptoms with occupational exposure to fresh *G. paniculata*, *D. caryophyllus*, and *L. longiflorum*, all of which are widely used in floral arrangements (Fig. 3). *G. paniculata*, *D. caryophyllus*, and *L. longiflorum* are perennial herbs which belong to the Caryo-

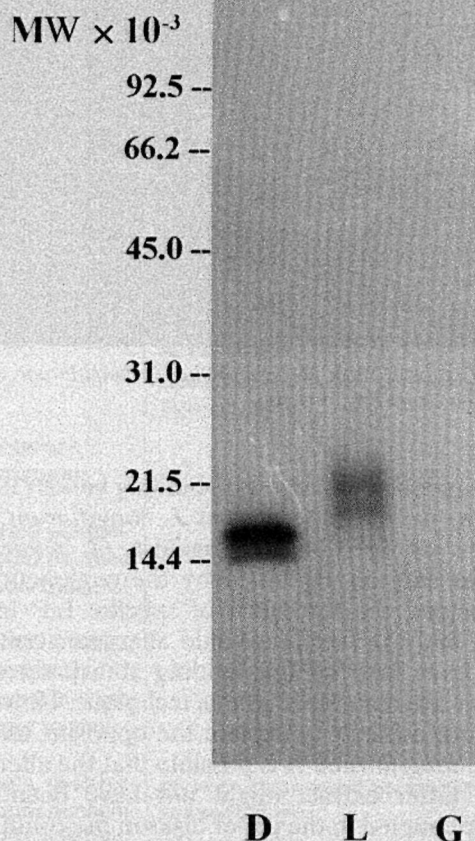
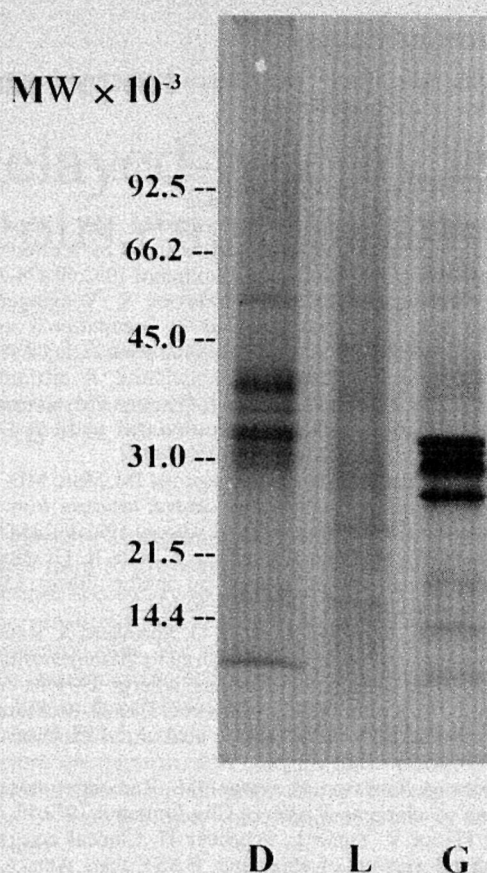


Fig. 1. Electrophoresis in polyacrylamide gel of *D. caryophyllus* (D), *L. longiflorum* (L), and *G. paniculata* (G) extracts dissociated with SDS-PAGE. Molecular weight (MW) expressed in kilodaltons.

Fig. 2. Autoradiograph of *D. caryophyllus* (D), *L. longiflorum* (L), and *G. paniculata* (G) extracts after immunoblotting with patient's serum. Molecular weight (MW) of IgE-binding antigens in kilodaltons.

phyllaceae family (*G. paniculata* and *D. caryophyllus*) and to the unrelated Liliaceae family (*L. longiflorum*).

The plants used by our patient were stored in greenhouses for commercial purposes. No preservatives were used. The presence of positive skin prick tests (*G. paniculata*, *D. caryophyllus*, and *L. longiflorum*), rub testing (*G. paniculata* and *D. caryophyllus*), and serum specific IgE (*G. paniculata* and *D. caryophyllus*), as well as the demonstration of IgE-binding bands on immunoblot (*D. caryophyllus* and *L. longiflorum*), strongly suggest type I hypersensitivity. As has been suggested, dried plants that crumble easily could facilitate the generation of airborne dust particles, leading to sensitization by inhalation of these particles (2, 4). However, the patient reported here suffered from symptoms when handling fresh flowers, and the sensitization mechanism is more difficult to explain. A similar case of sensitization to fresh *G. paniculata* has been reported by Antépara et al. (3). To the best of our knowledge, this is the first

report of allergy to *D. caryophyllus* and *L. longiflorum*.

The patient showed positive skin tests to grass pollen species, even though he presented no seasonal allergic symptoms; therefore, this sensitization seems irrelevant. Moreover, the lack of response to *G. paniculata*, *D. caryophyllus*, and *L. longiflorum* extracts in five pollen-sensitive patients, used as controls, does not indicate cross-reactions between grass pollen and allergens from *G. paniculata*, *D. caryophyllus*, and *L. longiflorum*.

The presence of *T. urticae* on the surface of the flowers were ruled out by microscopic examination. In any case, a skin test with *T. urticae* extract was negative, and allergy to this parasite may be ruled out.

The antigenic structure of the proteins in the extracts of *G. paniculata*, *D. caryophyllus*, and *L. longiflorum* was studied. Two IgE-binding bands (15 and 17 kDa) were obtained against



Fig. 3. Picture of fresh *D. caryophyllus*, *L. longiflorum*, and *G. paniculata* (from left to right).

D. caryophyllus, and two IgE-binding bands (19 and 22 kDa) were visible against *L. longiflorum*. The absence of IgE binding against *G. paniculata* proteins may be explained by the conjunction of two factors: the low level of specific IgE in the serum, and the fact that labile allergenic epitopes might have lost the IgE-binding ability, since the proteins are denatured in this technique. However, it is more difficult to explain the opposite, as seen with *L. longiflorum*. It is possible that the allergens in the latter extract might not have been adequately coupled to the paper disks in the conditions employed for the reaction. Certainly, the results of this study demonstrate that more than one *in vitro* technique may be needed for an accurate diagnosis of allergy in some cases.

In conclusion, the patient had developed severe rhinoconjunctivitis and contact urticaria and dermatitis from respiratory and skin exposure to *G. paniculata*, *D. caryophyllus*, and *L. longiflorum*.

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