

Occupational asthma and contact urticaria from dried flowers of *Limonium tataricum*

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Dried flowers of *Limonium tataricum* (LT) are widely used for semipermanent floral arrangements. We report a case of a floral industry worker in whom exposure to LT precipitated rhinoconjunctivitis, asthma, and contact urticaria. Studies revealed the presence of immediate skin test reactivity and positive leukocyte histamine release in response to LT extract. Specific anti-LT antibodies were detected in the patient's serum by direct RAST. Bronchial challenge with LT extract also resulted in an isolated immediate asthmatic response. Both the positive methacholine inhalation test and the significant changes observed in PEFR measurements when the patient handled LT supported the diagnosis of occupational asthma. These findings strongly suggest that an IgE-mediated immunologic mechanism is responsible for the patient's respiratory and cutaneous symptoms.

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It has long been known that exposure to vegetable-derived products may cause respiratory symptoms of rhinitis and asthma. In the workplace, organic dust from various sources, often plant-derived, is commonly identified as the etiologic agent of occupational asthma. This includes dust from grains, seeds, leaves, roots, gums, latex, woods, pollen, and tubers of various plants (3). However, in the floral industry itself, where there may, theoretically, be many potential allergens, several unique causes of work-related asthma have been reported. To the best of our knowledge, the only allergens identified in florists are those of baby's breath (*Gypsophila paniculata*) (11, 13) and weeping fig (*Ficus benjamina*) (1). In both cases, type I hypersensitivity to these plants has been demonstrated by *in vivo* and *in vitro* tests. Contact urticaria caused by plants or plant-derived products has also been reported to be due, in some instances, to an immunologic mechanism (1, 6). On the other hand, several plants and flowers, especially the family Compositae, have been shown to be contactant allergens in allergic contact dermatitis (10).

In this article we describe the immunologic and clinical studies conducted on a worker in a flower supplier who presented immediate respiratory and cutaneous symptoms after handling the dried plant *Limonium tataricum* (LT) (sea lavender) (Fig. 1).

Case report

A 27-year-old man, a smoker, with no family or personal history of atopy, was studied in our department. For the last 8 years, he had been working in a big flower supplier, which sold dried LT flowers. These flowers were purchased from Andalusia (Spain) and the Netherlands and were then unpacked, prepared in bunches, and distributed to local florists and customers. The fine dust of this plant was released during these processes. Three years after he had started this job, the patient began to notice nasal itching, sneezing, rhinorrhea, watery eyes, and pruritic wheals on exposed cutaneous areas a few minutes after handling LT. Several months later he developed dyspnea, wheezing, unproductive cough, and chest tightness on exposure to this plant at work. Although other plants were also sold in this store, he experienced no symptoms from them. When he avoided exposure to LT, his respiratory and cutaneous symptoms markedly decreased until he was asymptomatic. For this reason he was moved to the delivery section, but still had symptoms when handling LT. A therapeutic trial with disodium cromoglycate (DSCG), 20 mg q.i.d., was not very effective in preventing asthma attacks, but his condition markedly improved with inhaled betamimetics.

Clinical examination was normal. White blood cell count was 6700/mm³ with 268 eosinophils/mm³.

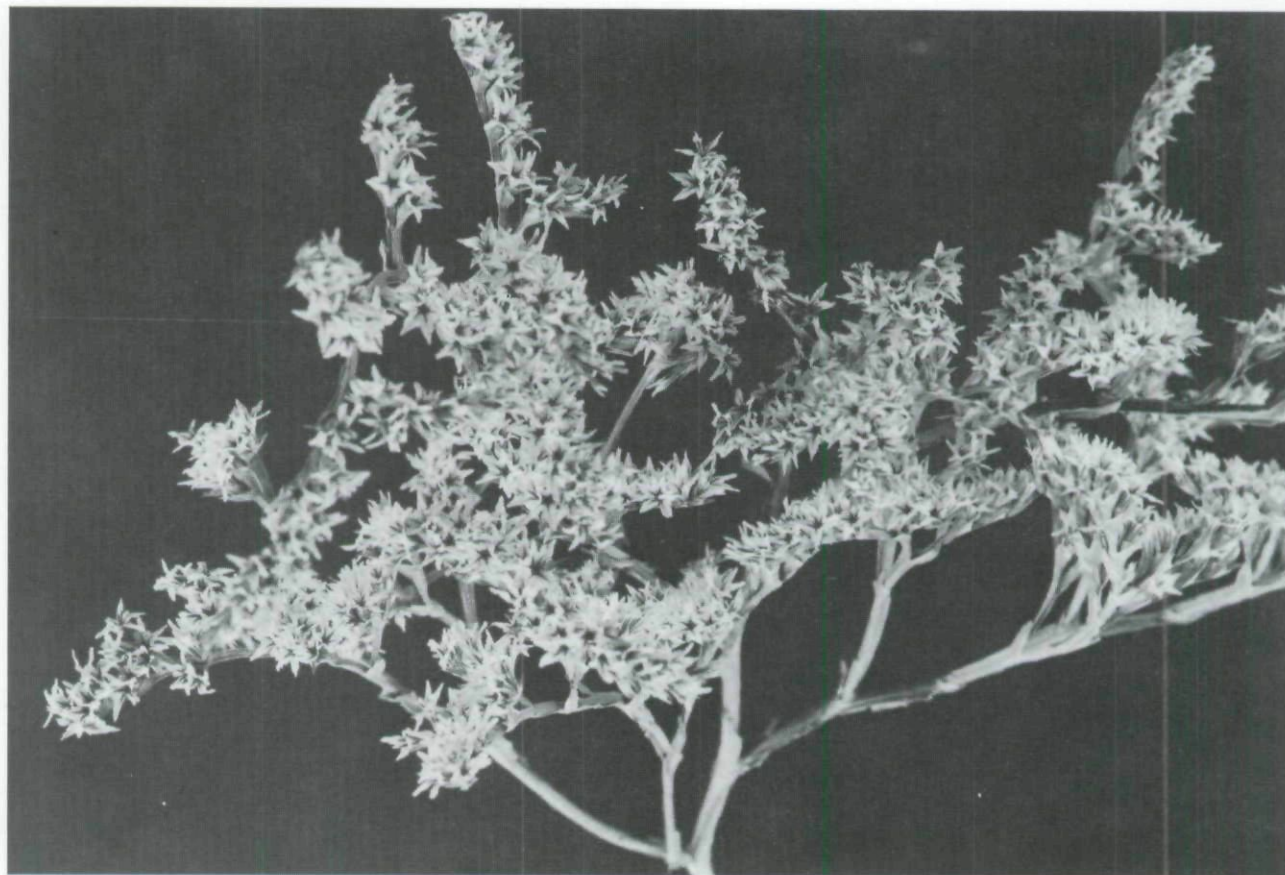


Fig. 1. Dried *Limonium tataricum* flowers as used in floral arrangements.

Total IgE was 378 kU/l. Chest and sinus radiographs were within normal limits. Spirometry revealed an FEV₁ of 93% and FVC 86% predicted.

Material and methods

Extract for in vivo and in vitro tests

Dried LT flowers were carefully separated by hand and then milled to a powder. Two grams of this material was dissolved in 20 ml of phosphate-buffered saline (PBS), pH 7.3, at laboratory temperature (24°C). After stirring for 1 h and passing through filter paper, we dialyzed the solution in PBS for 24 h, and then passed it through a 0.22- μ m Flow-pore filter (ICN Biomedicals, Irvine, UK) for sterilization. The protein concentration of this extract was determined by the method of Lowry et al. (8), with bovine serum albumin (BSA) as a standard. The protein concentration was 0.3 mg/ml. Tenfold dilutions were made for skin and inhalation tests.

Skin prick tests

Skin prick tests were done with LT extract (1:10 w/v) as well as with a battery of commercially avail-

able allergens, including environmentally important pollen species in our region (Alergia Abelló, Madrid, Spain). Histamine phosphate 10 mg/ml, and 0.9% normal saline were used as positive and negative skin test controls. Responses were examined after 15 min by measuring the two perpendicular diameters of the wheal. A positive response was defined as a wheal greater than 3 mm in the presence of a negative response to the saline control. The same tests were performed on five atopic and five normal, non-exposed persons who served as controls.

Rub testing

The patient's forearm was moistened with water and then rubbed with dried LT flowers for 30 s and examined 15 and 60 min later. Two atopic subjects were also tested as controls.

Histamine release test

This was performed by an automated fluorometric method according to Siraganian (12), with the modifications previously described (9). Four 10-fold dilutions of LT extract, the most concentrated being

equal to the 1:1 dilution used for skin tests, were used. *Phleum pratense* (timothy) pollen was also tested at a concentration of 10 and 1 BU/ml. Blood specimens from two grass-pollen-allergic patients were used as controls. Before allergen challenge, blood samples were tested with two dilutions of goat antihuman IgE. A result greater than 10% release was considered to be positive.

Specific IgE determination

To detect specific IgE antibodies against LT flower antigens, we treated nitrocellulose paper disks (Pharmacia, Uppsala, Sweden) with our own extract. The extract (1:10 w/v) was added to the disks, and, after an overnight incubation period at 24°C, the disks were washed three times in PBS 0.1% Tween 20 (PBS-TW). Then 1% BSA in PBS was added and the disks were incubated for 1 h at 24°C. After three more washes with PBS-TW, the disks were ready for use. They were stored in PBS at 4°C until required. RAST was conducted according to the standard technique, which uses ¹²⁵I-labeled rabbit antihuman IgE (Phadebas anti-IgE) and expresses the results in PRU/ml as derived from the Phadebas RAST reference system. Sera from 10 atopic patients allergic to grass or tree pollen were used as controls. Serum samples were analyzed in duplicate.

IgE antibodies specific to common environmental pollens were determined by RAST FEIA CAP System (Pharmacia Diagnostics AB, Uppsala, Sweden).

Total serum IgE

This was measured by Pharmacia CAP System IgE FEIA according to the manufacturer's instructions.

Ambulatory monitoring of peak expiratory flow rate (PEFR)

Serial determinations of PEFR with a Mini-Wright peak flow meter (Clement Clarke International, London, UK) were performed by the patient at 2-h intervals during waking hours at work and at home for 4 weeks. The maximum and minimum values were kept for analysis of daily variability. Records with a diurnal variation of more than 15–20% were considered to be significant for daily variability.

Bronchial provocation test (BPT)

Nonspecific BPT with methacholine inhalation was performed according to the method described by Chai et al. (4). Methacholine (Provocholine, Roche Lab., Nutley, NJ, USA) was administered with a nebulizer, model DeVilbiss 646 (DeVilbiss Co.,

Somerset, PA, USA) connected to a Rosenthal-French dosimeter calibrated to deliver 15 µl per breath. According to this method, PD₂₀ methacholine is expressed in cumulative inhalation units (IU) (an IU is arbitrarily defined as one inhalation of 1 mg/ml of methacholine in buffered diluent); 225 cumulative IU is considered to be the cutoff point to differentiate between responsive and nonresponsive patients (4).

Specific BPT was done with LT extract. The aerosolized particles were generated by a continuous pressurized DeVilbiss No. 646 nebulizer, with a fitted output of 0.28 ml/min. A control challenge with PBS was performed before antigen challenge. The patient was skin-tested with 10-fold dilutions of LT extract to determine a safe starting dose for the provocation, and this concentration was a 1:100 000 w/v extract. At daily intervals, the patient inhaled the aerosolized allergen in progressively increasing concentrations for 2 min at tidal volume. Base-line pulmonary function measures of FEV₁ and FVC were performed and repeated at 5, 10, 15, 20, 30, and 60 min after each challenge. A positive immediate response was defined as a greater than 20% fall from basal FEV₁. Then, hourly PEFR measurements were performed for 24 h (respecting sleeping periods) following each challenge in order to evaluate the late response. This was considered to be a greater than 25% fall in PEFR from base line. The inhibitory effect of DSCG on the bronchial reaction was studied. DSCG (40 mg) was given by inhalation with a spinhaler 30 min before the BPT.

Two nonexposed asthmatic patients with mild nonspecific reactivity were also challenged with 1:10 w/v LT extract after they had given informed consent.

Results

Fifteen minutes after skin testing with LT extract (1:10 w/v), the patient exhibited a 20 × 11 mm wheal and erythema response with pseudopods. A positive response was also observed to *Chenopodium album* (7 × 7 mm), *Olea europaea* (6 × 5 mm), *Platanus occidentalis* (8 × 7 mm), *Phleum pratense* (5 × 4 mm), and *Alnus glutinosa* (6 × 5 mm) pollens. The histamine phosphate-induced wheal was 6 × 6 mm, and no response was observed to the saline control.

Rub testing was strongly positive with LT flowers, producing confluent wheals, flare, and edema on the contact area. Both skin prick and rub tests with LT were negative in control subjects.

The leukocyte histamine release test was positive with all concentrations of LT extract tested, with a maximum release of 69%, and a plateau effect was observed (Fig. 2). The maximum release reached with *Phleum pratense* pollen (10 BU/ml) was 36%.

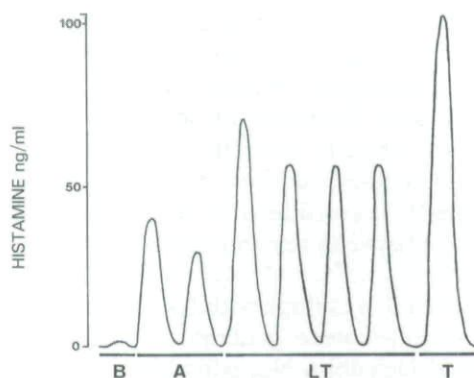


Fig. 2. Histamine release test with LT extract. (B) Basal release; (A) release with antihuman IgE; (LT) release obtained using progressive 10-fold dilutions (1:1, 1:10, 1:100, 1:1000) of LT extract; (T) total release (103.3 ng/ml).

In the two control atopic patients, no significant release was obtained with LT extract (<5%).

IgE antibodies specific to LT extract were found in the patient's serum. RAST value was 1546 cpm, which is equivalent to 2.6 arbitrary PRU/ml (class 2), and was negative (<0.35 PRU/ml) in 10 nonexposed atopic patients. The results of the RAST FEIA to common pollens in the patient's serum were as follows: *Chenopodium album* 0.69 kU/l, *Olea europaea* 4.81 kU/l, *Platanus acerifolia* 0.91 kU/l, *Phleum pratense* 4.47 kU/l, and *Alnus incana* 0.80 kU/l.

Monitoring of PEFR showed significant drops (>25%) from base-line values and a variability of 15–25% on the days the patient worked with LT (Fig. 3). The patient experienced bronchoconstriction within a few minutes of being exposed to this plant. Bronchial challenge with methacholine after a symptomatic period revealed mild bronchial hyperresponsiveness, with a PD₂₀ methacholine value of

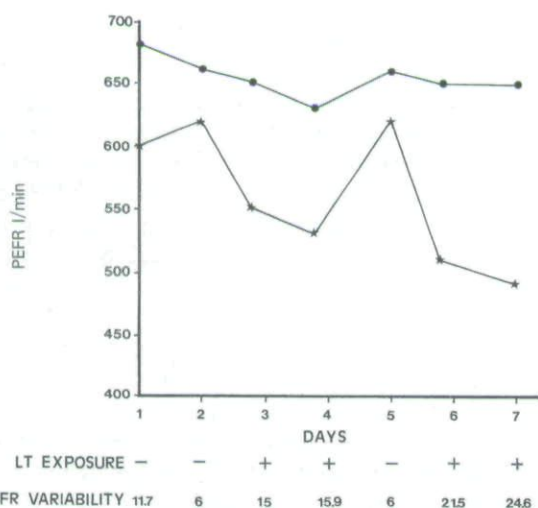


Fig. 3. Plot of daily maximum (●), minimum (★), and variability of peak expiratory flow rate (PEFR).

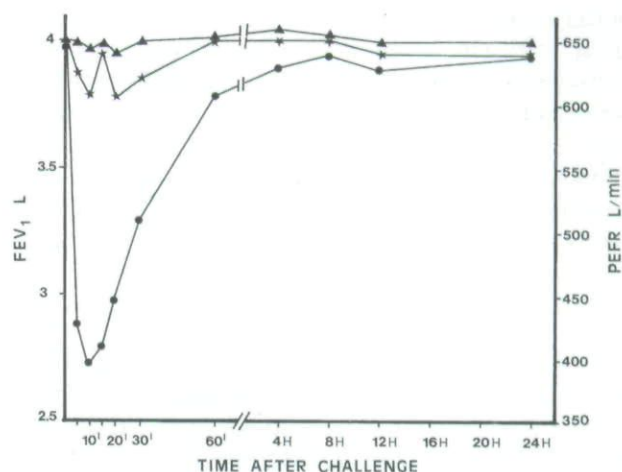


Fig. 4. Immediate asthmatic response after bronchial challenge with LT extract 1:1000 w/v (●), the same test performed after DSCG 40 mg inhalation (★), and control challenge with PBS (▲). Function measures refer to FEV₁ within the first hour and to PEFR in the following hours. Basal values: FEV₁ 3.99 l, PEFR 660 l/min.

225 IU (9.6 total μ mol of delivered methacholine). However, PD₂₀ methacholine was not obtained after inhaling 325 IU 1 month later when the patient was asymptomatic and not working with LT. After bronchial challenge with LT extract (1:1000 w/v), the patient had an immediate response, both clinically (cough, chest tightness, and wheezing) and spirometrically. FEV₁ decreased 32% at 10 min and returned to base line 1 h later. No late reaction was observed. Preinhalation of DSCG totally inhibited this immediate response (Fig. 4). Control subjects showed no response to bronchial challenge with LT extract.

Discussion

Ornamental dried plants are becoming very popular in many countries because, unlike green plants, they are long-lasting and need no attention. Many people have some kind of dried plant at home, and virtually anyone may be exposed to dust from these widespread ornamental plants.

LT is an annual plant, growing up to 300 mm high, that belongs to the Plumbaginaceae family; it is commonly known as "statice" or "sea lavender". The main *Limonium* spp. appreciated in the gardening world are *L. latifolium*, *L. sinuatum*, *L. tataricum*, *L. suworowii*, and *L. vulgare*. These spp. are endemic in salt marshes and shore areas because of their resistance to salt spray; they originated in the Mediterranean region and Asia. LT has rosettes of leaves at its base, and is topped by stems packed with masses of small pink or white flowers. Bunches may be gathered and dried for semipermanent bouquets

because of the plant's durability. During the processes of drying, storing, packing, and delivering, workers are exposed to a dust from this plant that can easily become airborne and thus be inhaled. The fact that these flowers are dry and papery may facilitate the generation of a dust aerosol.

In our patient it was demonstrated by provocative challenges that LT was responsible for the cutaneous and respiratory symptoms that he presented at work when exposed to this plant. The positivity of skin prick, leukocyte histamine release, and bronchial provocation tests, as well as the demonstration of LT-specific IgE antibodies, strongly suggests that the patient had developed type I hypersensitivity to LT flower antigen(s). The symptoms of contact urticaria were probably due to the same immunologic mechanism; thus, the skin could be not only a specific target organ of the IgE-mediated reaction, but also another contributory route for sensitization. In addition, the clinical characteristics indicate the possibility of air-transmitted occupational urticaria. It may be worth remarking that the plateau effect observed in the histamine release test, as well as the strong responses observed on skin tests with LT flowers, seems to indicate that the patient was highly sensitive to this material. Neither an irritating nor a toxic effect was suspected, as no response has been observed in control subjects.

Both the positive bronchial methacholine challenge and the significant changes on PEFR records when the patient was processing LT support the diagnosis of occupational asthma. The low degree of nonspecific bronchial hyperresponsiveness observed may have been due to intermittent exposure to LT because of its processing in batches. It has been shown in this patient that after a short, symptom-free period, bronchial hyperresponsiveness to methacholine was absent, indicating that nonspecific bronchial hyperresponsiveness could be detected only after some degree of allergen exposure, and, at this stage of the disease, it disappeared shortly after the cessation of exposure. In some cases it has been reported that occupational asthma may exist with negative methacholine challenges (5). It has been proposed that in these cases a sufficiently strong stimulus, or one to which the patient is particularly sensitive, might then be capable of provoking airflow obstruction even in the absence of measurable hyperreactivity (7). However, nonspecific reactivity to methacholine might be present only as a rapidly transient phenomenon, and, if so, testing should be performed when the patient is symptomatic (i.e. after a work shift).

Although this patient also showed positive skin prick tests to several taxonomically nonrelated pollens, he exhibited no seasonal allergic symptoms when away from work; therefore, these positive tests

should be considered to be latent sensitizations to common environmental allergens, indicating an atopic diathesis. This atopic status probably facilitated an IgE-mediated response to LT antigen(s). The possible existence of cross-reactivity between allergens from LT and these pollens could not be investigated because RAST scores were not considered to be suitable for the performance of inhibition studies. However, the lack of response to LT extract in pollen-sensitive patients, as well as the low RAST scores to these pollens in the patient's serum, does not seem to support that possibility.

It is not known whether individuals who have LT or other plants in their homes might be potentially exposed to antigens released from them in sufficient concentration to induce respiratory allergy, and so act as an unsuspected source of indoor allergens. In this connection, Axelsson et al. (2) have reported that nonoccupationally exposed persons may become sensitized to the indoor plant, weeping fig, with a risk for atopic patients of about 6%. However, we think that both people having floral hobbies and floral industry workers have high-grade exposure to many potential allergens that may lead, as in the case of LT, to allergic sensitization. To the best of our knowledge, this is the first report of allergy to *Limonium* spp.

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