

## Immediate type of allergy in stasis growers

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*Three stasis growers complaining of immediate allergic symptoms induced by harvesting stasis in a plastic greenhouse were examined to clarify their allergic conditions. Distinct positive reactions in the intradermal test and nasal and eye provocation test to the stasis extracts were shown in all three cases. High score of radioallergosorbent test (RAST) IgE and remarkable RAST inhibitory effects to the stasis extracts were seen in these cases. The immunologic cross-reactivity between stasis and chrysanthemum was absent in the RAST inhibition in the patient labeled case C who had complained of the same allergic symptoms when handling chrysanthemums in the off-season for harvesting stasis. Those results indicated that the allergic conditions of the present cases were from an immediate type of allergy mediated by a specific IgE antibody to the stasis extract. (J ALLERGY CLIN IMMUNOL 1992;90:742-8.)*

**Key words:** Immediate allergy, stasis grower, occupational allergy, flower, case study, intradermal test, provocation test, RAST IgE, RAST inhibition, cross-reaction

Flower growers are working under conditions of continuous exposure to flower components generally recognized as sources of sensitizing substances. From the authors' previous investigations higher prevalences of allergic symptoms and findings have been observed in flower growers together with the workers engaged in tobacco growing and poultry than in other agricultural workers.<sup>1,2</sup> Previously, some allergic cases such as asthma,<sup>3,4</sup> rhinitis,<sup>5</sup> and contact dermatitis<sup>6-9</sup> induced by flower growing have been reported sporadically.

The stasis, *Limonium Sinuatum* Miller (*Statis Sinuata* L.), is an important flower in the cut flower market, and recently the production of that flower has shown a rapid increase. Although there have been no reports of a confirmed allergic case induced by growing stasis to date, the authors have assessed several persons complaining of immediate allergic symptoms induced by harvesting stasis in a plastic greenhouse.

### Abbreviation used

RAST: Radioallergosorbent test

The present paper is to clarify the allergic conditions of those stasis growers and to study the causative antigens.

## METHODS

### Cases

Three flower growers, a 49-year-old woman (case A), and two men, 22 (case B) and 33 (case C) years old, who complained of allergic symptoms occurring immediately after they entered a plastic greenhouse to harvest stasis flowers were examined. The husband of the woman in case A, who had no complaint of allergic symptoms (case D), and a healthy woman engaged in raising cattle in the same areas (case E) received some of the same tests as controls. Each subject was examined at his or her home on 10 to 12 July, soon after the end of the harvesting season.

### Examinations

The examination was performed as follows: a detailed history of the allergic onset and its relation to the working process was taken, blood and nasal smear analysis and skin tests were performed, serum immunoglobulin and RAST-IgE were determined, and nasal and eye provocation tests were done. The RAST inhibition test to stasis extracts and the cross-reaction between stasis and chrysanthemum were also studied.

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TABLE IA. Results of examinations in three cases

|                                | Case A                                                             | Case B                                                                            | Case C                                                 |
|--------------------------------|--------------------------------------------------------------------|-----------------------------------------------------------------------------------|--------------------------------------------------------|
| Sex: age (yr)                  | F: 49                                                              | M: 22                                                                             | M: 33                                                  |
| Duration (yr): Flowers         | 21                                                                 | 7                                                                                 | 15                                                     |
| Statis                         | 10                                                                 | 2                                                                                 | 6                                                      |
| Chief complaints               | Nasal obstruction<br>Nasal discharge<br>Eye irritation<br>Sneezing | Nasal obstruction<br>Nasal discharge<br>Eye irritation<br>Sneezing<br>Sore throat | Nasal obstruction<br>Nasal discharge<br>Eye irritation |
| Past histories                 | Conjunctivitis<br>Urticaria                                        | Rhinitis<br>Urticaria                                                             | Rhinitis                                               |
| Family histories               | Son (rhinitis)                                                     | Mother (urticaria & conjunctivitis)                                               | Sister (rhinitis)                                      |
| Habit: Smoking                 | None                                                               | ½ pack/day                                                                        | None                                                   |
| Alcohol intake                 | None                                                               | 5 days/week                                                                       | 3 days/week                                            |
| Blood: WBC (/mm <sup>3</sup> ) | 5200                                                               | 5800                                                                              | 6000                                                   |
| % of eosinophils               | 8                                                                  | 11                                                                                | 8                                                      |
| Serum: IgE (U/ml)              | 100                                                                | 1170                                                                              | 270                                                    |
| IgA (mg/ml)                    | 238                                                                | 293                                                                               | 259                                                    |
| IgG (mg/ml)                    | 1110                                                               | 1160                                                                              | 1160                                                   |
| IgM (mg/ml)                    | 46                                                                 | 94                                                                                | 130                                                    |
| Nasal smear: Eosinophils       | +++                                                                | —                                                                                 | +                                                      |
| Neutrophils                    | +                                                                  | +                                                                                 | +                                                      |
| Basophils                      | —                                                                  | —                                                                                 | —                                                      |

Self-made antigens

*Statis.* The flower portion (statis[F]) or leaf (statis[L]) were cut to appropriate lengths and extracted with use of Coca's solution.<sup>10</sup> The extract was freeze-dried after dialysis in distilled water. The dried powder was dissolved in physiologic saline solution from 10<sup>-2</sup> wt/vol to 10<sup>-10</sup> wt/vol and sterilized by Millipore (Millipore Corp., Bedford, Mass.) filter (0.22 µm) for preparing test antigens. One kind of chrysanthemum (brand name Kanzanyo) and several other flowers cultivated in the same areas as statis were also prepared in the same way for control antigens.

*Commercial extracts.* 1:1000 wt/vol in 50% glycerin of house dust, pollen of Japanese cedar, short ragweed, and Japanese hop by Torii & Co., Ltd. (Tokyo, Japan) were used for intradermal test. Saline solutions including 0.5% phenol and/or 50% glycerin were used for the control solution.

*Pesticides.* The routine concentration in appropriate medium of benomyl, chlorothalonil, triforine, and other 11 pesticides were used for the patch test.<sup>11</sup>

*Leaves.* The leaves of chrysanthemum, statis, and other flowers (snapdragon, gypsophia) were extracted by the method of Bleumink et al.<sup>6</sup> for the patch test.

Intradermal test

Each concentration of 0.02 ml diluted solution of the antigens was injected in the forearm of the patient, and the diameter of the wheal and flare were measured after 15 minutes.

Provocation test

*Intranasal test.* A small filter disk permeated with 10<sup>-2</sup> solution of statis[F] and control solution (saline solution including 0.5% phenol) were placed on the lower turbinate mucosa of the patient on separate days in a single-blind manner, and the reaction of the topical mucosa was observed 15 minutes after the treatment.

*Eye provocation test.* 10<sup>-4</sup> solutions of statis[F] and control solution (saline solution including 0.5% phenol) were dropped into the patient's eyes on separate days in a single-blind manner, and the reaction was observed 15 minutes after the treatment.

Serum immunoglobulin

Total IgE was determined by enzyme immunoassay method by enzyme immunoassay IgE test kit (Medical and Biological Laboratories Co., Ltd., Nagoya, Japan), and IgA, G, and M were determined by single radial immunodiffusion method by N-immunoring kit (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan).

The RAST-IgE to the statis extracts and other antigens was determined by referring to the method described by Ceska et al.<sup>13</sup>

RAST inhibition

Fifty microliters of sera of patients in cases A and C were incubated with various doses of the statis[F] and [L] at room temperature for 30 minutes, and each value of the RAST to the statis disks was determined by the same method as

TABLE IB. Allergic findings in the three cases

|                             | Case A            | Case B            | Case C            |
|-----------------------------|-------------------|-------------------|-------------------|
| Intradermal test            |                   |                   |                   |
| Statis (F)*10 <sup>-2</sup> | 75 × 72 (27 × 20) | 50 × 50 (22 × 21) | NT                |
| 10 <sup>-4</sup>            | 45 × 42 (21 × 13) | 40 × 35 (13 × 13) | 45 × 47 (27 × 16) |
| 10 <sup>-6</sup>            | 40 × 30 (18 × 16) | 28 × 28 (9 × 9)   | 41 × 36 (15 × 12) |
| Statis (L)†10 <sup>-2</sup> | 28 × 20 (13 × 11) | 48 × 45 (22 × 15) | NT                |
| 10 <sup>-4</sup>            | 18 × 14 (8 × 5)   | 34 × 26 (10 × 10) | 30 × 23 (12 × 11) |
| 10 <sup>-6</sup>            | 34 × 24 (12 × 10) | 21 × 20 (9 × 8)   | 26 × 23 (9 × 9)   |
| Chrysanthemum (L)‡          | NT                | 42 × 34 (13 × 13) | 25 × 17 (10 × 9)  |
| House dust§                 | 5 × 5 (3 × 3)     | 30 × 30 (12 × 12) | 11 × 11 (7 × 7)   |
| Japanese cedar§             | 6 × 5 (4 × 4)     | 25 × 24 (8 × 7)   | 3 × 3 (0 × 0)     |
| Short ragweed§              | 6 × 6 (4 × 4)     | NT                | 21 × 21 (9 × 8)   |
| Japanese hop§               | 6 × 5 (3 × 3)     | NT                | 5 × 5 (0 × 0)     |
| RAST-IgE (score)            |                   |                   |                   |
| Statis (F)                  | 3                 | 3                 | 4                 |
| Statis (L)                  | 3                 | 3                 | 4                 |
| Chrysanthemum (L)           | 0                 | 3                 | 3                 |
| House dust§                 | 0                 | NT                | 0                 |
| Mite§                       | 0                 | NT                | 0                 |
| Provocative test            |                   |                   |                   |
| Intranasal                  | +                 | +                 | +                 |
| Eye conjunctiva             | +                 | +                 | +                 |
| Patch test                  |                   |                   |                   |
| Statis¶                     | —                 | —                 | +                 |
| Chrysanthemum¶              | —                 | —                 | —                 |
| Other flowers#              | —                 | —                 | —                 |
| Pesticide (chlorothalonil)  | +                 | —                 | —                 |
| (triforine)                 | +                 | —                 | —                 |
| (others)**                  | —                 | —                 | —                 |

Diameter of flare (wheal) in millimeters NT, Not tested.

\*F: From flower portion extracted by Coca's solution.

†L: From leaf portion extracted by Coca's solution.

‡10<sup>-4</sup> of the chrysanthemum extract.

§Commercial antigens by Torii & Co., Ltd., Japan.

||10<sup>-2</sup> of the statis (F) for intranasal and 10<sup>-4</sup> for eye provocation.

¶Leaves of statis and chrysanthemums extracted by Bleumink's method.

#Leaves of snapdragon and gypsophila extracted by Bleumink's method.

\*\*Other pesticides: benomyl, captafol, captan, cyhexatin, dichlorvos, iprodione, mancozeb, maneb, methidathion, methomyl, quino-methionate and thiophanate methyl.

described herein. The cross-reactivity between statis and chrysanthemum was evaluated by determining the RAST inhibitory effect of different doses of the chrysanthemum extract to the statis disks in the patient in case C.

### Patch test

Test samples in Finn chambers (Epitest Ltd., Oy, Finland) were attached to the forearms of the patient. After 48 hours, the patch was detached and the reaction was evaluated by the criterion of the International Contact Dermatitis Research Group.<sup>12</sup>

The study was carried out after completing informed consent procedures with all of the subjects in accordance with the Guide for Human Examination, Faculty of Medicine, Kagoshima University.

## RESULTS

The results of allergic examinations of the three cases are summarized in Tables I, A, and B.

### Case A

As shown in Table I, A, she was a full-time agricultural worker who had been engaged in flower growing with her family (cases B and D) for 21 years, and she had been growing statis for 10 years. She was growing several kinds of chrysanthemums and other flowers along with statis. For the previous 6 years she had complained of sneezing, nasal discharge, and eye irritation immediately after entering a plastic greenhouse to cut the statis, especially in May and June.

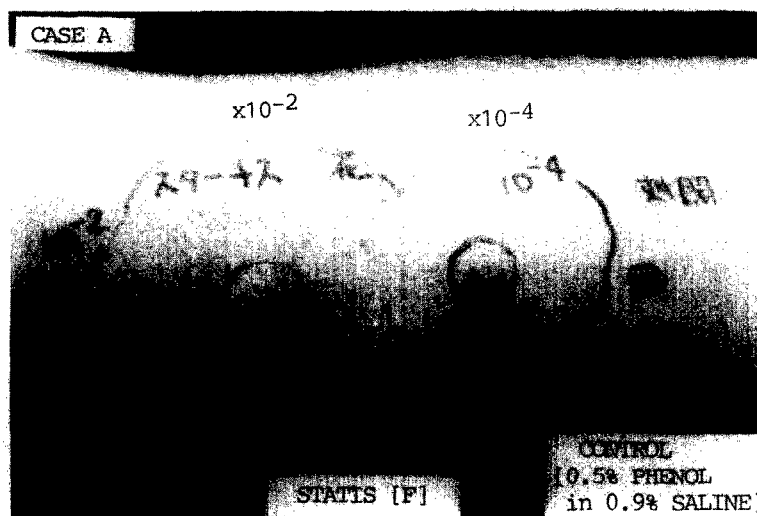


FIG. 1. Intradermal test of patient in case A.

the peak season of harvesting stasis. Those symptoms had subsided shortly after leaving the work place. She was free of delayed skin disorders and never complained of allergic symptoms in handling other flowers.

As shown in Table II, the intradermal test reactions to the four commercial antigens were negative, but distinct positive reactions were seen to both stasis[F] (Fig. 1) and [L]. The intensity of reaction was stronger to stasis[F] than [L]. The threshold reaction was  $10^{-10}$  for stasis[F] and  $10^{-8}$  for stasis[L].

The RAST scores to stasis[F] and [L] were determined to be 3 (7.2 Phadebas RAST unit (PRU)/ml for stasis[F] and 6.3 PRU/ml for [L]). On the other hand, RAST scores to the chrysanthemum extract, house dust, and mite were 0.

By the nasal provocation test, nasal itching, increase of nasal hydnorrhoea, and sneezing appeared 3 minutes after the treatment. By the eye provocation test, a positive reaction appeared 5 minutes after the treatment. The patch test reactions to eight kinds of flowers and 14 kinds of pesticides were negative except to chlorothalonil and trifonine.

As shown in Table I, A, a slightly elevated value was observed in the proportion of peripheral blood eosinophils, and plenty of eosinophils were also seen in the nasal smear. Values of total serum immunoglobulins were within normal range.

### Case B

He was a son of the woman in case A, with 2 years of experience at stasis growing. He also complained of eye and nasal symptoms after entering the plastic greenhouse to cut the stasis (Table I, A). He complained also of the same immediate allergic symptoms

in handling chrysanthemums (especially a particular kind of chrysanthemum called Kanzanyo).

As shown in Table I, B, a distinct reaction of intradermal test not only to stasis[F] and [L] but also to house dust, Japanese cedar, and the chrysanthemum extract were seen. Nasal and eye provocation tests were also positive not only to the stasis but also to the chrysanthemum. Remarkable elevation of total IgE and high RAST score to the stasis extract (9.6 PRU/ml) were also seen. He refused to receive further examinations, so the cross-reactivity between stasis and chrysanthemum could not be proved in the RAST inhibition.

### Case C

He was a full-time agricultural worker, with 6 years experience of growing stasis. The scale of flower cultivation was about the same as that of the patient in case A. He was growing seven kinds of chrysanthemums, gypsophila, liatris, dracaena, and stasis with his parents.

He complained of nasal and eye symptoms accompanied by a low-grade fever 1 hour after entering the plastic greenhouse where stasis was growing, and these symptoms lasted through the daytime even outside of the greenhouse (Table I, A). He had had these complaints for 3 years throughout the harvesting season, from December to May. He also complained of the similar symptoms in handling chrysanthemums in off-season of growing stasis and was free of delayed skin disorders.

As shown in Table I, B, the intradermal test reaction was positive to both stasis[F] and [L], with mild positive reaction to the chrysanthemum, house dust, and short ragweed. The RAST scores to stasis[F] (more

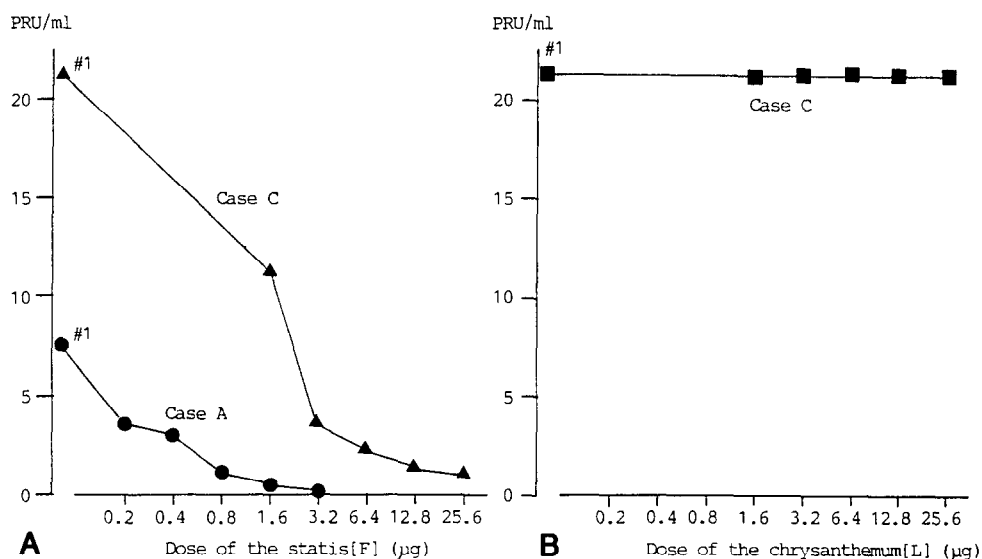


**FIG. 2.** RAST inhibition. **A**, Inhibitory effects of statis[F] extract. After preincubation of sera of the patients with various doses of statis[F] extract at room temperature for 30 minutes, each value of  $^{131}\text{I}$  on statis[F] disks was determined. No. 1 represents initial value of RAST (0 μg dose of statis[F] extract). Sera of patients in cases A and C were used in the test. **B**, An absent inhibitory effect of chrysanthemum[L] extract to statis[F] disks in the RAST. No. 1 represents initial value of the RAST (0 μg dose of chrysanthemum[L] extract). Serum of patient in case C was used in the test.

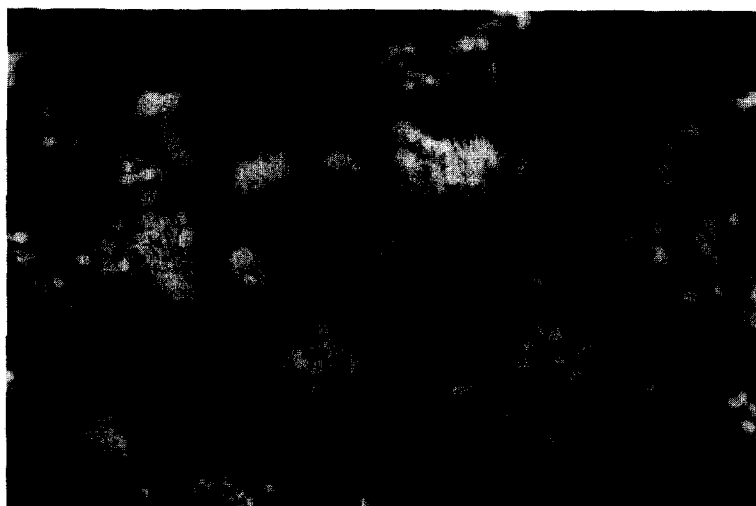


**FIG. 3.** Statis in a plastic greenhouse.

than 17.5 PRU/ml), [L] (14.6 PRU/ml), and chrysanthemum[L] (more than 17.5 PRU/ml) were determined to be 4. On the other hand, the scores to house dust and mites were 0. The provocation tests were also positive. No positive reaction was seen in the patch test, except to statis.

#### Cases D and E

The control subjects showed no abnormal reaction in the intradermal and scratch test to the statis and

other extracts, determination of total IgE, or patch test, except for a weak reaction in the intradermal test to statis[F] ( $15 \times 15$  [ $10 \times 12$ ]) for the patient in case D. The RAST scores to statis[F] and [L] were determined to be 0 (less than 0.34 PRU/ml) in both controls.

**RAST inhibition test.** As shown in Fig. 2, in the sera of patients in both cases A and C remarkable inhibitory reactions to the statis[F] were shown. In the serum of the patient in case C, the inhibitory effect

of chrysanthemum[L] was also shown. However, the cross-reaction between stasis and chrysanthemum was absent in the RAST inhibition in the patient in case C.

## DISCUSSION

Stasis is a popular cut flower grown mainly in the southeast part of Japan. The nursed seedlings of the flowers are set in a plastic greenhouse from September to October and harvested in the intervals between the busy harvesting seasons of summer and winter chrysanthemums. The stasis plant is shown in Fig. 3. The workers are exposed to not particularly dense concentration in plastic greenhouses. The diameter of the pollen was measured to be 30  $\mu\text{m}$ , with a rounded shape (Fig. 4), and it swelled to bursting immediately in water.

No reports exist on allergic cases confirmed by allergologic consideration among stasis growers to date. As a result, the present cases were the first to receive systemic allergic examinations for immediate allergic symptoms induced by stasis growing.

The onset of symptoms of the three cases and relatively high proportions of eosinophils in the blood and nasal smear of the cases suggested that the symptoms of the present cases might be from an immediate type of allergy induced by growing stasis.

The intradermal tests in the present three cases showed distinct positive reactions to the stasis extracts both from the flowers and leaves. On the other hand, negative or mild reactions were seen to some commercial antigens commonly recognized as strong sensitizers.

The provocation tests to the stasis extract were also positive, and the RAST-IgE score to stasis[F] and [L] were determined to be 3 for the patient in case A and 4 for the patients in cases B and C. However, the scores to other well-known commercial antigens were 0. Moreover, remarkable inhibitory effects of the stasis extracts were shown in both case A and C. On the other hand, the control cases showed no specific reactions in those immunologic tests except weak intradermal reaction to stasis[F] in the patient in case D, who worked together with the patients in cases A and B. These results indicated that the allergic conditions of the three cases were an immediate type of allergy mediated by a specific IgE antibody of the extracts from the stasis.

A high score of the RAST to the chrysanthemum extract was seen in cases B and C, suggesting a possibility of cross-reaction between stasis and chrysanthemum. However, the cross-reactivity between stasis and chrysanthemum was absent by RAST inhibition in case C. These results indicated that the stasis and

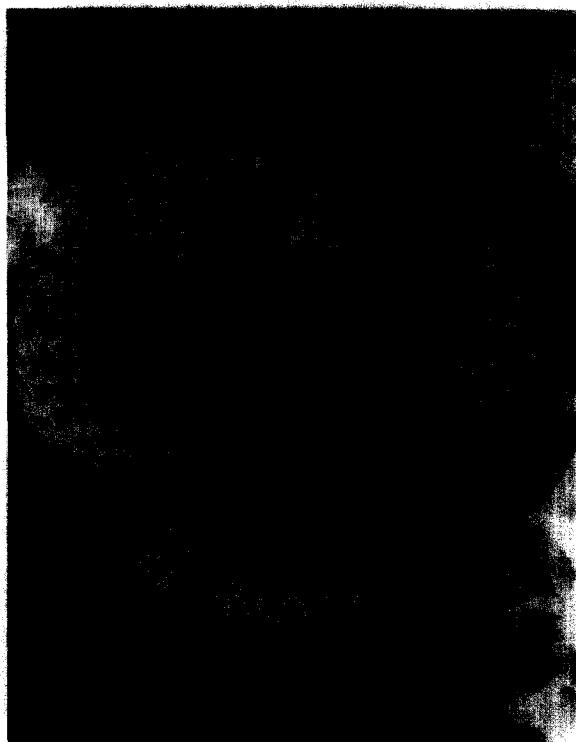


FIG. 4. The pollen of stasis (Original magnification  $\times 2000$  by electron microscope).

chrysanthemum extracts might cause separate immunologic reactions as specific antigens.

The patient in case B also showed positive reactions in the intradermal test to other well-known antigens as well as elevation of IgE value, suggesting he had inherited a tendency to have allergies from his mother (case A). These results emphasized that further examination is required to clarify the question of mixed sensitization to multiple allergens among flower growers.

Positive patch test reactions to two kinds of pesticides and to the stasis extract in cases A and C suggested that a delayed allergic reaction must be counted as an allergic condition in flower growers. However, these findings might not be of etiologic importance for the patients in the present cases who had no serious complaint of skin disorders.

The results indicated that the extract from flowers was a stronger causative antigen than that from leaves. This seems reasonable, because the workers are more likely to be exposed to pollen than to other components of stasis. However the RAST scores were determined to be equally high to both antigens, indicating that these two solutions have a common antigen.

Although various cases of patients with occupational pollinosis have been reported,<sup>14-19</sup> not many re-

ports exists on flower growers among those cases. In fact, the cases of immediate allergy in chrysanthemum growers reported to date seem to have mainly developed by inhalation of cilia of the flower,<sup>4</sup> except in a few reports.<sup>3</sup>

The present patients were advised to apply disodium cromoglycate to their nasal passages for 1 month before the beginning of harvest and/or on every day before work and to take antiallergic drugs, and they were also advised to wear a mask while working and to set up ventilation equipment in the plastic greenhouse. These measures were shown to be effective, even though not complete, in the present cases, supporting the idea that the allergic conditions of the present cases developed by the patient's being sensitized to the statis.

The present case studies indicate that the pollen of flowers may also be an important antigen to flower growers, and further study is necessary to find and clarify other unknown allergic cases among flower growers and to develop preventive measures for those allergies induced by pollen.

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## REFERENCES

1. Ueda A, Ueda T, Matsushita T, Ueno T, Nomura S. Prevalence rates and risk factors for allergic symptoms among inhabitants in rural district. *Jpn J Ind Health* 1987;29:3-16.
2. Ueda A, Aoyama K, Fujita Y, et al. Survey on allergic symptoms and their pathogenesis in chrysanthemum growers. *J Jpn Assoc Rural Med* 1986;35:55-66.
3. Jyo T, Kohmoto K, Katsutani T, Otsuka T, Tsuji S. A case of occupational asthma in a chrysanthemum grower. *Jpn J Allergol* 1979;28:619-23.
4. Ebihara I. A study on the inhalative allergy of cilia of leaves (Rep. 2). *J Sci Labour* 1976;52:407-18.
5. Nakagawa S, Katsuda M. On the pollinosis caused by dalmation chrysanthemum. *Jpn J Allergol* 1975;24:535-42.
6. Bleumink E, Mitschell JC, Nater JC. Contact dermatitis to chrysanthemums. *Arch Dermatol* 1973;108:220-2.
7. Tanaka T, Moriwaki S, Morio T. Occupational dermatitis with simultaneous immediate and delayed allergy to chrysanthemum. *Contact Dermatitis* 1987;16:152-4.
8. Cairns R. Plant dermatoses. Some chemical aspects and results of patch testing with extracts of *Primula obconica*. *Trans St John's Hosp Derm Soc* 1964;261:311-6.
9. Hjorth DS, Wilkinson DS. Contact dermatitis IV. Tulip fingers, hyacinth itch and lily rash. *Br J Dermatol* 1968;80:696-8.
10. Coca AF. Studies in specific hypersensitivity V. The preparation of fluid extracts and solutions for use in the diagnosis and treatment of atopic conditions. *J Immunol* 1922;7:163-78.
11. Nomura S, Matsushita T, Kanbe Y, Horiuchi N, Sugaya T, Kimura M. Studies on diagnostics of dermatitis due to agricultural chemicals. *J Jpn Assoc Rural Med* 1978;27:734-41.
12. Fregert S, Hjorth N, Magnusson B, et al. Epidemiology of contact dermatitis. *Trans St John's Hosp Derm Soc* 1967;55:17-35.
13. Ceska M, Wriksson R, Varga JM. Radioimmunosorbent assay of allergens. *J ALLERGY CLIN IMMUNOL* 1972;49:1-9.
14. Ursing B. Sugar beet pollen allergy as an occupational disease. *Acta Allergologica* 1968;23:396-9.
15. Matsuyama R, Sato M, Miyata R, Azuma Y, Ozaki H, Ito K. Sugar beet pollinosis as an occupational disease. *Jpn J Allergol* 1972;21:235-43.
16. Nakazawa T, Kobayashi S, Hichijo K, Yamaji T. A case of bronchial asthma caused by orchard pollen in Gumma. *J Jpn Int Med* 1971;60:43-6.
17. Kobayashi T, Ozeki H, Inazawa S, et al. Bronchial asthma induced by strawberry pollen and some epidemiological study of the pollinosis. *Jpn J Allergol* 1973;22:699-705.
18. Teranishi H, Kasuya M, Aoshima K, Kato T, Ikarashi T. Pollinosis due to artificial pollination of Japanese pears. *Jpn J Ind Health* 1981;23:166-7.
19. Sawada Y. Epidemiological study of apple pollinosis among apple farmers. *Jpn J Allergol* 1980;29:293-305.