

OCCUPATIONAL RESPIRATORY ALLERGY TO CYCLAMEN POLLEN: A CASE REPORT

R. Ariano, MD (1), G. Mistrello, MD (2), R.-C. Panzani, MD (3)

SUMMARY: A case of cyclamen (*Cyclamen persicum*) pollen allergy is described on a flower cultivator. The diagnosis was assessed by skin test, methacoline test, nasal provocation test and RAST. SDS-PAGE and immunoblotting have shown an intense response against a 18 kDa component and a weaker one at 100 Kda. A RAST inhibition against *Cupressus sempervirens* and *Acacia floribundia* showed no identity with cyclamen. This case confirm the cyclamen allergenity, in professional esposition.

The nasal challenge prove to be very useful to confirm allergic sensitizations an we emphasise the necessity of preventive measures of protection for those florists wich cultivate cyclamen plants.

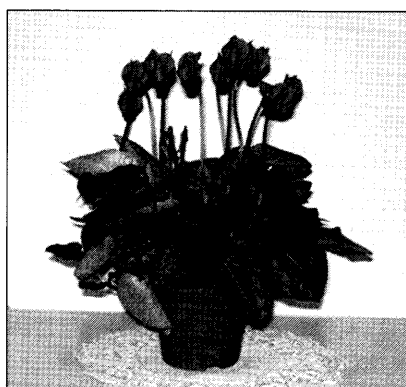


Fig. 1: *Cyclamen persicum*.

Flower cultivators and florists, more especially if they work in greenhouses, run a risk of developing both type I and/or type IV occupational allergy as it is the case with the now well-known *Ficus benjamina* (1) and some flowers such as Compositae

(2). Allergy to the pollen of cyclamen has already been described (3).

In this paper we describe the case of a 46 year old female flower cultivator who has cultivated cyclamens for seven years and who developed, after a 2 years exposure, an allergic rhinitis and asthma during the blooming season of the plant, i.e. between September and March with a peak in intensity of symptoms from December to February. This woman has never had allergic symptoms before and is otherwise in good shape (normal clinical exam, x-ray of thorax and sinus normal).

The patient suffered from mild persistent asthma with cough, wheeze and chest tightness every day, and night time symptoms, at least three times to week,

during the period of cyclamen blooming season. Lung function test FEV1 was equal 80 percent of normal value and methacoline test was positive to the dose of 600 mcg. The therapy assumed by the patient was inhaled steroids and albuterol intermittently as needed.

Cyclamen is an entomophilic plant with a heavy pollen, therefore sensitization occurs only by proximity. Cyclamen cultivars belong to the Primulaceae family and are originated from Iran and Greece. They are cultivated, but different species such as *Cyclamen hederifolium*, *Cyclamen repandum*, *Cyclamen purpurascens* are spontaneous in the Mediterranean area. The species found in trade is *Cyclamen persicum*.

It is known that Primula (Primrose) which belongs also to the Primulaceae family (Poison Ivy) contains an active substance (primine), responsible for an intense contact dermatitis (4). We have no other member of the primulaceae family in Italy such as Dodecatheon which is common in the U.S.A.

MATERIAL AND METHODS

Preparation of extracts (Lofarma laboratory Milano Italy)

The pollen of cyclamen (Gm) was defatted with ether before being extracted at 5% (w/v) in 0.15M phosphate-buffered saline, (PBS), pH 7.4 containing 0.5% Na₃N for 16 h at 4°C. After removing debris and insoluble material by centrifugation at 10000 x g for 20

(1) U.O.C. Medicina Interna. Ambulatorio di Allergologia ed Immunologia Clinica. A.S.L. n°1 Imperiese (Italy).

(2) Lofarma S.p.A Milano (Italy).

(3) Centre de recherche en allergologie, Marseille (France).

min, supernatants was dialyzed against PBS and filtered through 0.22 μ m membrane.

Protein content of extract was determined by BioRad method (Prodotti Gianni, Milan, Italy) with a result of 0.345 μ g/ml. The same extract was used for skin testing and nasal provocation test.

Patients

■ **Skin test:** Skin prick tests were performed with the Dome Hollister lancet. Extracts were used in 1/20 vol solution. We used a commercial extract (Lofarma laboratorio, Milan Italy). Glycerin with histamine 1:1000 was used as positive control and glycerin alone as negative control. Reactions were observed after 20 minutes. Wheals with a diameter of 3 mm or more than the negative control were considered positive. Skin prick tests were performed with a panel of the most common allergens on the patient and on two relatives. This panel included pollens (grasses mixt *Cupressus sempervirens*, *Olea europea*, *Parietaria judaica*, *Acacia floribundia*), molds (*Alternaria alternata*, *Aspergus mixt*), house dust mites (*Dermatophagoides - pteronyssinus*), household insects (cockroach, mosquito, horsefly), cat and dog danders.

The testing included patch tests with the leaves and petals of the flower. A written informed consent was obtained for the three subjects.

Nasal Provocation Test: NPT

We utilized the Youtlen peak inspiratory flow meter (PIFRn meter). It offers some advantage over the expiratory flow rate meter, especially when used in provocation tests, and is easier to handle than standard rhinomanometry (5). The results are expressed as the mean values of three consecutive registrations. A test was considered positive when we observed a drop in the basic value of 40% or more after instillation of at least 20 A.U. of a solution of the allergen. Nasal tests were carried out with the same extract used for Prick.

Preparation of solid phases and direct RAST

Polystyrene beads (6.4 mm diameter, Precision Plastic Balles, Chicago, Illinois) were coated with cyclamen extract according to the procedure described. The beads were activated with glutaraldehyde 0.2 M in PBS for 5 h at room temperature before being incubated with the extract (at 50 μ g/ in PBS, 150 μ l/bead)

overnight in end-over agitation. After several washings with PBS the beads were stabilized with NaHBO₃ 0.37% in PBS for 1 h and finally saturated with 5% in BSA in PBS for 24 h.

To perform direct RAST experiments 50 ml of each sera were added to a coated-bead and incubated overnight at room temperature. Bound specific IgE were detected incubating with a ¹²⁵I-labelled anti-human IgE for 8 hours, and after washing, measuring residual radioactivity in a gamma scintillation counter.

SDS-PAGE AND IMMUNOBLOTTING

Twenty micrograms of cyclamen pollen extract were electrophoresed in a 10% polyacrilamide precast Nupage Bis-Tris gel, according to manufacturer's instructions (Novex, Prodotti Gianni, Milan, Italy), at 180 mA for 1 h. The resolved proteins were stained with 0.1% Coomassie Brilliant Blue and their molecular weight was calculated using the software ImageMaster (Amersham Pharmacia-Biotech, Milan, Italy). For immunoblotting detection of allergenic components the resolved extracts were transferred onto a nitrocellulose membrane according to Towbin (6). The membrane was saturated in TBS buffer containing 2% defatted dry milk (saturating buffer) for 1 h, before to be incubated with patient's serum or with a pool of sera from non-allergic subjects, both diluted 1:10 in saturating buffer. Bound specific-IgE were detected by peroxidase-conjugated anti-human IgE serum, using an ECL western blotting kit (Amersham Pharmacia-Biotech, Milan, Italy) as substrate (5).

RAST INHIBITION

Rast inhibition was performed as described by Yman (7). Twenty ml of each allergic serum was incubated with 30 ml of inhibitor allergen and dilutions in PBS. The resulting solutions were incubated with the solid phase and then with I-anti-IgE tracer, following the common RAST procedure. A negative control was performed by mixing 30 ml of PBS and 20 ml of a normal (RAST-negative) serum pool. A positive control was performed by mixing 30 ml of PBS and 20 L of allergic serum pool. The bound radioactivity was measured by a (Packard) gamma counter and expressed in counts per minute (cpm). Inhibition rates, ranging from 0% to 100% were calculated according to the following formula:

$$\frac{(\text{cpm positive control}) - (\text{cpm inhibitor})}{(\text{cpm positive control}) - (\text{cpm negative control})} \times 100$$

RESULTS

■ Skin testing

We had the following results on our patient:

Histamine : 12 mm

Control : 2 mm

Cyclamen 12 mm

Dermatophagoides-pteronyssinus 10 mm

Cupressus-sempervirens 6 mm

Mimosa : Acacia floribundia 3 mm

All skin tests were negative on the two relatives, that didn't any other positivities to the different allergens.

The patch tests with the leaves and petals were also negative on the patient and the relatives.

■ **Nasal provocation tests:** NPT was strongly positive on the patient and negative on the two relatives, as appears in the following tables 1, 2):

N° administration	Patient PIFR (L/MIN)	1° relative PIFR (L/MIN)	2° relative PIFR (L/MIN)
1°	150	160	175
2°	140	150 (-6%)	180 (+ 3%)
3°	150	160	175
4°	150	160	175
5°	150	160	175
6°	150	160	175

Table 1: Nasal provocation test (control solution).

N° administration	Patient PIFR (L/MIN)	1° relative PIFR (L/MIN)	2° relative PIFR (L/MIN)
5 A.U.	150	160	175
10 A.U.	150	150 (-6%)	175
15 A.U.	130 (- 14%)	160	165 (- 6%)
20 A.U.	90 (- 40%)	160	170 (- 3%)
25 A.U.	STOP	160	175
30 A.U.	STOP	160	175

Table 2: Nasal provocation test (active extract).

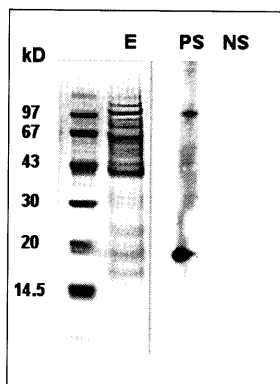


Fig. 1: SDS-PAGE and immunoblotting.

■ **SDS-PAGE and immunoblotting:** The protein profile of the cyclamen extract showed a great number of component with molecular weight ranging from 14 to over 100 kD (fig. 1, lane E).

In immunoblotting experiments patient's serum strongly reacted with two allergens at about 19 and 100 kDa (fig. 1 lane PS) while no reactivity was detected using a pool of sera from non-allergic people (fig.1, lane NS).

■ **RAST inhibition:** A RAST inhibition was carried out against *C.sempervirens* and *Mimosa* showing no identity between cyclamen and this two pollens.

We also performed patch tests with the leaves and petals of the plant which were negative.

DISCUSSION AND CONCLUSIONS

In this paper we signal a case of respiratory allergy towards the cyclamen, in a floriculturist, in Italy, to confirmation of to precedent report, in Holland.

In our case we have attempted to improve the search, in comparison to the paper above reported, using NPT and the RAST inhibition. Besides, we have also effected the immunoblotting foundig one allergenic component about 18 Kdas which represents the more relevant allergen.

We am able, therefore, to make some considerations. In first place is confirmed that the sensitization to the pollen of cyclamen happens under conditions heavy and prolonged exposure. In this case, nevertheless, the period of exposure is prolonged for two years, before the symptoms were manifested. Two subjects of the same family working under the same exposure are symptom free; this fact is further argument to sustain the specificity of the symptomatology of the patient. Moreover, close to the pricks tests and to the RAST, it appears very significant the positivities to the nasal challenge. This show an evident difference of behavior among the symptomatic subjects and the two relatives, that have act as control group.

It seems us opportune, besides, to sustain some practical suggestions. In first place, that the TPN is to methodic very useful to confirm allergic sensitizations. Besides, that the atopic subjects must be advise not to start activity with heavy allergenic esposition, same the floristic. In fact, this patient had some other preceding sensibilities.

Finally, we emphasise the necessity of preventive measures of protection (mask and gloves) for the those florists wich cultivate cyclamen plants.

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