

Respiratory allergy to the pollen of *Mercurialis annua* (Euphorbiaceae)

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We have described ten cases of respiratory allergy to the pollen of *Mercurialis annua* in the area of Bordighera (Riviera dei Fiori) on the Italian Mediterranean coast. The incidence of sensitivity is only 0.9% in a population of 1081 patients with pollinosis. Since the Euphorbiaceae family (spurges) contains strongly sensitizing allergens (eg, seeds of *Ricinus communis* and latex of *Hevea brasiliensis*) our findings raise the possibility that respiratory allergy to the pollens of *Mercurialis annua* and other spurges has been underestimated throughout the world.

INTRODUCTION

The discovery a few years ago of a typical case of respiratory allergy to the pollen of *Mercurialis annua*^{1,2} prompted us to investigate more closely this aspect of pollen allergy which has been, until now, poorly described and without convincing evidence.³⁻⁶ *Mercurialis annua* belongs to the Euphorbiaceae (spurge) family which includes strongly sensitizing allergens (eg, seeds of castor bean: *Ricinus communis* and latex of *Hevea brasiliensis*). The pollens of the Euphorbiaceae (although in some cases anemophilous) are not known for their allergenicity. On the other hand, the irritant or toxic properties of spurges are well known.⁷ In Israel, Lindenbaum reported in 1966 a case of pollinosis to *Ricinus communis*⁸ and Shivpuri et al in India found positive skin tests to *Ricinus communis* pollen in 40% of 100 cases of patients presenting with a respiratory allergy.⁹ We have also described in many cases of respiratory allergy to the seed of *Ricinus communis* a cross reactivity between the seed and the

pollen¹⁰ and between the seeds of different spurges.¹¹

We present here the results of an investigation carried out in and around Bordighera (Riviera dei Fiori) a town in Liguria, Italy.

PATIENTS AND METHODS

In making a diagnosis of clinical sensitivity to *Mercurialis annua* we considered as relevant the following points:

(1) The pollen season, which generally starts in late winter (February–March) (together with *Cupressus sempervirens*) and ends in September.

(2) The proximity of *Mercurialis annua* plants to the patients' environment at a distance not exceeding 200 m.

(3) A positive skin test (prick test) and a positive RAST; prick testing was performed with the Dome Hollister lancet. The extracts were kindly provided by Lofarma Laboratory (Milan, Italy) and were used in a 1/20 solution (wt/vol). We adopted the following scores: a positive reaction of 3+ corresponds to the wheal and flare of the histamine 10 mg/mL prick test. A positive reaction of 4+ corresponds to a greater reaction than the one of histamine. A positive reaction of 2+ is 75% and a positive reaction of 1+ is 50% of the histamine reaction. The tests were performed on the forearm and were read after 20 minutes. The saline controls were negative.

RAST

The RAST was performed as described by Wide et al.¹² Commercial reagents were purchased from Lofarma, Milan (SferikitR) and consisted of a solid phase, which were commercial polystyrene balls with a diameter of 6.4 μ covalently coated with allergens; a reference system, *Poa pratensis* solid phase and human grass-positive serum pool; and a tracer, ¹²⁵I anti-human antibody raised in goats. The bound radioactivity was measured by a (Packard 800) γ counter and the percentage of bound radioactivity calculated.

The same extracts were used for RAST as those used for prick tests.

RAST Inhibition

RAST inhibition was performed as described by Yman L.¹³ Twenty microliters of each allergic serum was incubated with 30 μ L of inhibitor allergen and dilutions in PBS. The resulting solutions were incubated with the solid phase and then with ¹²⁵I-anti-IgE as tracer, following the common RAST procedure. A negative control was performed by mixing 30 μ L of PBS and 20 μ L of a normal (RAST-negative) serum pool. A positive control was performed by mixing 30 μ L of PBS and 20 μ L of allergic serum pool. The bound radioactivity was measured by a (Packard 800) γ counter and expressed in counts per minute (cpm). Inhibition rates, ranging from 0% to 100% were calculated

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according to the following formula:

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

Nasal Provocation Test

We utilized the Youlten peak inspiratory flow meter (PIFRn meter) which has some advantages as compared with the expiratory flow rate meter especially when used in provocation tests. Plus, it is easier to handle as compared with standard rhinomanometry.¹⁴ The results are expressed as the mean values of three consecutive measurements. A test was considered positive when we observed a 20% drop or more in the basic value after instillation of at least 20 allergenic units (A.I.) of a solution of the allergen. Allergenic units are based on the RAST inhibition technique in comparison to an internal standard, the potency of which was tested by skin tests on

allergic volunteers (Lofarma Laboratories).

We also performed skin and RAST tests on the ten *Mercurialis annua*-sensitive patients with the following extracts: *Ricinus communis* pollen and seed, and *Hevea brasiliensis* latex. Furthermore, skin tests only were performed with *Dermatophagoides pteronyssinus*, *Alternaria tenuis*, and pollens (*Parietaria officinalis*, *Olea europea*, grasses, and Betulaceae).

Pollen Count

Aerobiologic sampling was done with a continuous volumetric apparatus (spore trap, Burkard-Hirst) located 20 m above ground level on the roof of a building in the center of the city of San Remo, far from sources of pollution and exposed to all winds.

We took weekly samplings with the possibility of having a daily count according to the usual technique. The air is sucked by a pump permanently directed toward the wind by a rotating air vane. The

particle content of the sucked air is deposited on a sticky tape moving 2 mm/h. The sticky tape is a cellulose acetate ribbon coated with a solution of 2% silicon fluid in carbon tetrachloride. The adhesive tape, mounted on the drum (rotating in the trap at 2 mm/h) was changed every week. It was divided into seven segments 48 mm long, each corresponding to one day of sampling, and mounted on slides. The slides, with adhesive segments, were covered with fuchsin-stained glycerol jelly and with cover glasses. The daily mean concentration of the number of pollen particles was determined by use of an optical microscope for measuring tangential microscopic fields on the slide. An algorithm was used to calculate a conversion factor that considers the area of microscopic field, the adopted magnification and the number of tested strips. This value multiplied by the number of particles gives the average concentration for 1 m³ of air examined.¹⁵

Table 1. Clinical and Immunologic Data

N	Age	Sex	Clinical History	<i>Mercurialis annua</i>		<i>Ricinus communis</i> Pollen		<i>Ricinus communis</i> Seed		<i>Hevea brasiliensis</i>		Nasal Provocation Test	Other Allergies	
				Prick	RAST	Prick	RAST	Prick	RAST	Prick	RAST		Prick	RAST
1	58	M	Asthma	3+	2	3+	0	0	0	0	0	+	PA 3+	3
2	38	M	Asthma	3+	3	0	0	0	0	0	0	+	GR 3+	4
													BE 2+	3
													PA 4+	4
													OL 3+	4
3	40	M	Allergic rhinitis	1+	1	0	0	0	0	2+	1	+	—	—
4	24	M	Asthma	4+	3	0	0	0	0	2+	1	+	DP 3+	3
													GR 3+	3
													OL 1+	2
													BE 2+	2
5	55	M	Allergic rhinitis	3+	2	0	0	0	0	0	0	+	GR 3+	4
													PA 3+	3
													OL 4+	4
6	38	F	Allergic rhinitis	4+	3	0	0	0	0	0	0	+	—	—
7	41	M	Asthma	2+	2	0	0	0	0	2+	2	+	DP 4+	3
													PA 4+	4
													GR 3+	2
8	37	F	Asthma	3+	3	3+	0	0	0	0	0	+	—	—
9	52	M	Allergic rhinitis	3+	3	0	0	0	0	0	0	+	PA 2+	1
10	32	M	Allergic rhinitis	3+	3	0	0	0	0	1+	1	+	GR 3+	3
													PA 2+	2
													DP 2+	2

* GR: grasses, BE: Betulaceae, PA: *Parietaria officinalis*, OL: *Olea Europea*, and DP: *Dermatophagoides pteronyssinus*.

RESULTS

During the period 1980–1991 we found a total of ten patients with allergy to *Mercurialis annua*. This represents an incidence of *Mercurialis annua* allergy among patients with pollinosis of ten out of 1081 (0.9%).

Seven of the ten *Mercurialis annua*-sensitive patients were skin reactive to other pollens: grasses, *Parietaria officinalis*, *Olea europea*, and Betulaceae, as well as to *Dermatophagoides pteronyssinus* (Table 1). We found no case of skin reactivity to *Cupressus sempervirens* and *Acacia floribunda*. On the ten patients with positive skin tests to *Mercurialis annua*, RASTs specific to *Mercurialis annua* were also positive with a good correlation between skin tests and RASTs (RAST scores ranging from 1 to 4). Also in all ten cases, a nasal provocation test was

positive. As a control we tested five patients presenting with a respiratory allergy to the pollens of *Parietaria officinalis* with the same technique for nasal provocation. Although all gave positive responses to *Parietaria officinalis* the response to *Mercurialis annua* was always negative.

In two cases the prick test was positive to the pollen of *Ricinus communis* (RAST always negative); RASTs and pricks with seed allergens of *Ricinus communis* were negative. Conversely, with the latex of *Hevea brasiliensis*, we found four cases of positivity by prick test and by RAST with a good correlation between the in vivo and in vitro tests (Table 1).

Finally a RAST inhibition (*Mercurialis annua* solid phase) was performed with sera from the seven cases that were polysensitized using

pollens of grasses (mixture), *Parietaria officinalis*, *Olea europea*, Betulaceae, and the mite *Dermatophagoides pteronyssinus*. The results showed an important cross-reactivity between the pollen of *Mercurialis annua* and the pollen of grasses (five cases) and *Olea europea* (three cases). The cross-reactivity that occurs also in six cases with *Parietaria officinalis* and in two cases with *Betula alba* is less important. Less frequently in three cases with *Dermatophagoides pteronyssinus* and only one case with *Dermatophagoides farinae* we found a cross-reactivity that was even lower. The RAST inhibition was of course 100% with *Mercurialis annua* itself (Fig 1).

DISCUSSION

The Euphorbiaceae is an important family of angiosperm dicotyledon plants, which belongs to the order of Euphorbiales, Euphorbiaceae genera and species.¹⁶

The spurge family includes 8,800 species classified roughly into 275 genera, and many of these are of considerable economic importance, such as castor bean (*Ricinus communis* Linn.); rubber tree (*Hevea brasiliensis* Muell.); tung (*Aleurites cordata* R. Br.); cassava (*Manihot ulitissima* Pohl.), the source of tapioca; and more recently, a potential new oilseed crop (*Euphorbia lagascae* Spreng.).

Many species of the Euphorbia genus are used as ornamental plants in homes and gardens. These include poinsettia (*Poinsettia pulcherrima* R. Grah.), (*E. poinsettia* Willd.) also called "Christmas flower," and a multitude of cactiforms often misidentified as "dwarf cacti." In the Exotic Gardens of the Principality de Monaco, there is a vast collection of ornamental succulents which includes more than 100 species of giant cactiforms and blossoming Euphorbiaceae.

Members of Euphorbiaceae are cosmopolitan but with most species occurring in warm parts of the world. They range from herbs to

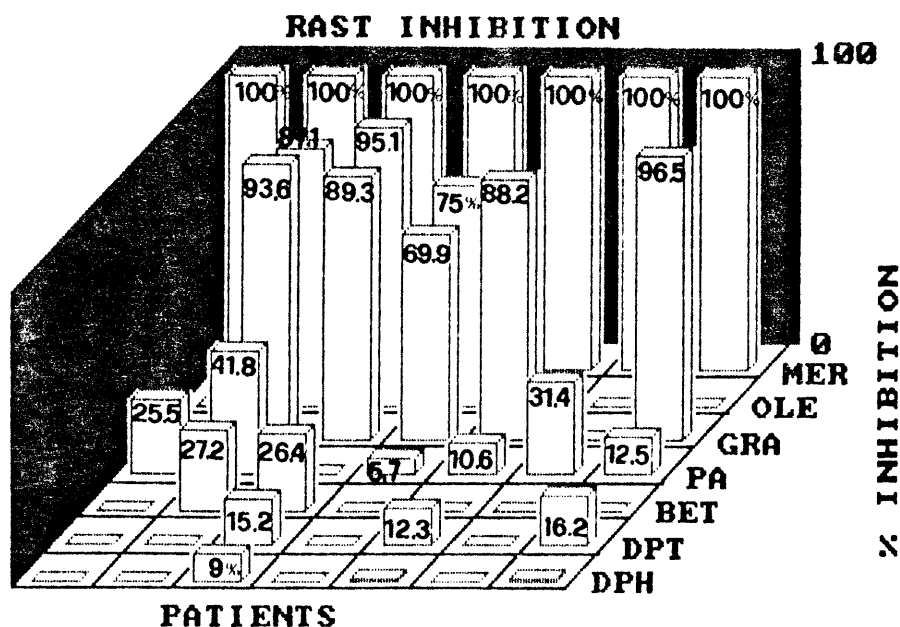


Figure 1. RAST inhibition is very high with grasses (from 96.5% to 69.9%) and *Olea europea* pollen (from 95.1% to 87.1%). Conversely, it is lower with *Parietaria officinalis* (from 41.8% to 5.7%) and *Betula alba* (26.4% to 27.2%). With *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* the RAST inhibition is even lower between 16.2% and 0.6%.

MER = *Mercurialis annua*

OLE = *Olea europea*

GRA = Grasses mixture

PA = *Parietaria officinalis*

BET = *Betula alba*

DPT = *Dermatophagoides pteronyssinus*

DPH = *Dermatophagoides farinae*

shrubs and trees. Most of them have laticiferous (latex bearing) channels, which is one of the most important characteristics of spurge.

In Italy and the South of France there are approximately 50 native species, the most common being: *Euphorbia helioscopia*, *Euphorbia cyparissias*, *Euphorbia peplus*, *Euphorbia dulcis*, *Euphorbia lathyris*, and of course *Mercurialis annua* (Fig 2) which is the most abundant. There are also *Mercurialis perennis* and *Mercurialis corsica*.

Mercurialis annua is a smooth and ramified plant with greenish unisexual flowers disposed in separate individuals. All the flowers (male or female) are joined together in long spikes. This morphology makes the dispersion of the pollens by the wind easier. It is the reason

why the pollination in this species is mostly anemophilous.

Ricinus communis is a hermaphrodite plant with female flowers in the upper part and male flowers in the lower part. The flowering starts from the lower part. The pollinization is anemophilous or entomophilous.

Other Euphorbiaceae (*Euphorbia peplus*, *Euphorbia resinifera*, *Euphorbia cyparissias*, *Euphorbia lathyris*, and *Euphorbia helioscopia*) have unisexual flowers with a special morphology: at the center there is one female flower surrounded by several male flowers. The pollination is only entomophilous.

These different characteristics of the dispersion of pollens explain, in our opinion, the greater presence of

Mercurialis annua pollens in our pollen counts in comparison to the pollens of *Ricinus communis* and other Euphorbiaceae as well as the greater frequency of clinical allergic symptoms related to *Mercurialis annua* pollen.

The pollen of *Mercurialis annua* (Fig 3)^{17,18} can be characterized in the following manner. The grains are tricolporate, prolate, isopolar, spheroidal to spheroidal, and small or rather small, $21 \times 25 \mu$. The pollen exine is rather thick between furrows; ectexine slightly thicker than endexine, thinning toward the furrows, and containing numerous rods which form the islets of a fine inverse reticulum. There are three furrows, $20.6 \times 6.2 \mu$, abruptly narrowing outside the pore to 4.0μ , acutely; membrane closely beset with fine granules. The polar field is small; index I: 6. There are three pores, one in the middle of each furrow, circular, 6.2μ diameter; membrane protruding up to about 2.5μ beyond the general surface of the grain and beset with large warts, which are more numerous around the margin. The intine is thin, thickening around and over the pores to form a membrane 1.5μ thick.

There are two small differences between the pollen of *Mercurialis annua* and the pollen of *Ricinus communis*: (1) the pollen of *Mercurialis annua* is smaller than the pollen of *Ricinus communis* ($28-31 \mu$) and (2) the exine of *Mercurialis annua* is thicker.

The pollen of other Euphorbiaceae has a diameter of 22 to 28μ . It is also tricolporate with a thin exine which has less asperities than the pollen of *Ricinus communis* and much less than the pollen of *Mercurialis annua*.

Mercurialis annua is anemophilous, the pollination starts in February and ends in September in Italy. According to pollen counts carried out from 1980 to 1991 the Euphorbiaceae show a very low incidence compared to some other pollens (Table 2).



Figure 2. Plants of *Mercurialis annua*.

Table 2. Pollen Count of Euphorbiaceae Compared with Other Common Pollens

Months	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.
<i>Mercurialis annua</i>	0	2	5	7	15	30	26	24	10	3	1
<i>Ricinus communis</i>	0	0	0	5	7	9	6	5	3	0	0
<i>Parietaria officinalis</i>	84	87	261	610	1082	1451	1204	1045	720	473	150
<i>Betula alba</i>	82	324	462	380	223	0	0	0	0	0	0
Grasses	0	0	0	52	291	450	675	520	103	0	0



Figure 3. *Mercurialis annua* pollen.

We present here clinical and immunologic evidence that *Mercurialis annua* pollen can cause respiratory allergy in patients living close to the plants. In all cases skin tests, RASTs and nasal provocation tests were positive. There was, in general, a good correlation between skin tests and RAST scores.

Only three patients were monosensitized. The others were allergic to pollens such as grasses, *Parietaria officinalis*, and *Olea europea*. As far as the diagnosis is concerned, since skin tests to *Cupressus sempervirens* and *Acacia floribunda* were in all cases negative and since their blooming season partly coincides with the season of *Mercurialis annua*, it is easy to eliminate the possibility of a sensitization to both pollens. The existence of positive skin tests to Betulaceae, the blooming season of which starts in February

and ends in April, could partly explain the clinical symptoms. However, only two *Mercurialis annua*-sensitive patients were allergic to these pollens.

After March, with the beginning of the blooming season of *Parietaria officinalis* and after May with the blooming season of grasses, it is more difficult to separate sensitivity related to *Mercurialis annua* from that related to *Parietaria officinalis* or grasses.

In view of the allergenicity of nonpollen parts of several members of the Euphorbiaceae, it is not surprising to find respiratory allergy to the pollen of a member of this family which is rather common in some parts of the world. It is possible that allergy to this plant has been underestimated. Furthermore, the fact that there is a cross-reactivity with other spurges, and especially with

Hevea brasiliensis, might explain the appearance of allergic reactions to the latex of *Hevea brasiliensis* in cases where no previous contact with this allergen has been noticed.

The RAST cross-reactivity with grasses and *Olea europea* and the lack of cross-reactivity with *Parietaria* and Betulaceae are more difficult to explain. Hemmens et al have shown cross-reactivity between birch and alder pollen allergens.¹⁹ In a recent study on olive and privet pollen allergy, we pointed out that "blotting, adsorption and elution, and inhibition studies clearly demonstrated allergenic cross reactivity between olive, privet, *Lolium perenne* and *Cynodon dactylon* pollen components. The presence of pollen-reactive IgE antibodies may not necessarily be a true reflection of the sensitizing pollen species."²⁰

It is likely that this statement could be applied to the cases in which we found strong RAST inhibition with the pollen of grasses and *Olea europea*. The three cases of monosensitization, however, enable us to describe *Mercurialis annua* pollen allergy as a clinical entity. The next step will be the identification and purification of *Mercurialis annua* antigens.

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DOSE-DEPENDENT SUPPRESSION OF GROWTH BY BUDESONIDE

The authors studied 38 mildly asthmatic children, 6 to 14 years of age, to evaluate effects of an inhaled adrenal corticosteroid on linear growth. Each child was assigned randomly to one of three dose groups to receive total daily doses of 200, 400, or 800 μ g budesonide by inhalation, divided into two doses each day for 8 weeks in the double-blind, parallel group study. Each dose group also received placebo for an additional 4 weeks either before or after treatment with budesonide. Weekly knemometry disclosed no significant difference in mean growth velocity among the three dose groups during treatment with placebo and no significant differences from placebo during treatment with 200 or 400 μ g daily. During treatment with 800 μ g daily mean lower leg growth velocity was decreased significantly to .13 mm/wk (SD = .19) from .39 mm/wk (S.D. .15) during treatment with placebo.

These data suggest doses of inhaled budesonide of up to 400 μ g daily probably do not significantly affect linear growth in asthmatic children 6 to 14 years of age. Larger doses may suppress growth but much less than oral prednisolone, 2.5 mg daily, as found previously by these authors.

—RMS

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