

A STUDY OF THE ANTIGENIC COMPOSITION OF POLLEN OF THE MARSH ELDER (*Iva frutescens*)

N. G. Astaf'eva, N. S. Gurina,
V. A. Ado, and L. A. Goryachkina

UDC 616-056.43-022.854.
2-097.2:582.998

KEY WORDS: marsh elder pollen; pollinoses.

Recent investigations have established the well-marked allergenic properties of pollen of the marsh elder (*Iva frutescens* L.) and have established its important role in the etiology of pollinoses in the southeastern part of the RSFSR [2, 8]. Since the marsh elder belongs to the Compositae family, the morphological similarity of its pollens to that of ragweed, the almost equal strength of its anaphylactogenic action, and cases of polyvalent sensitization of patients to pollen of marsh elder and other weeds (ragweed, wormwood, orache, hemp), the degree of antigenic kinship between the above-mentioned species of plants was investigated.

EXPERIMENTAL METHOD

To determine the antigenic composition of marsh elder pollen Ouchterlony's gel-diffusion test in Petri dishes was used in accordance with the usual recommendations [1]. Antiserum against pollen was obtained by immunizing rabbits with a 5% suspension of pollen in Freund's complete adjuvant, optimal to obtain highly active sera in the diffusion test [6]. The precipitating properties of the serum also were determined by the turbidity spectrum method, based experimentally on measurement of turbidity using several light filters [3, 4].

By spectroturbidimetric titration (STT) it is possible to determine the mean size of the particles ($r\lambda$), and their concentration by weight (M) and number (N) in the course of the test.

An ordinary production model of the FET photoelectric turbidimeter was used for the investigations which were carried out in the Laboratory of Polymers, Research Institute of Chemistry, Chernyshevskii Saratov University, by Scientific Officer L. G. Lebedeva. The working cuvette filled with antiserum against marsh elder pollen, diluted in the ratio of 1:5 with phosphate-salt buffer, pH 7.2. The syringes of the doser contained 3% saline extract of marsh elder, wormwood, orache, ragweed, and hemp pollen. The diffusion test was carried out by adding the solution of the allergen to the measuring cuvette of the instrument. The components were mixed by adding the allergen solution in small portions to the cuvette by means of an automatic doser. While the doser was in action, the shaker was switched off. The rate of addition varied from 1.15 to 0.2 ml/min. The order of determination of optical density, changes in turbidity (r), the wave exponent (n) of the mean particle size ($r\lambda$), and the weight of precipitate (M) were the same as described previously [5].

To confirm the results of the precipitation test, crossed anaphylactic shock was produced. Batches of ten guinea pigs were sensitized respectively with wormwood, orache, hemp, and ragweed pollen by the scheme described above. On the 21st day after the beginning of sensitization, as reacting injection the animals were given an intravenous injection of 1 ml of a 3% saline extract of marsh elder pollen. The animals developed anaphylactic shock of a varied degree of severity, assessed by an anaphylactic index, calculated by the equation in [7].

EXPERIMENTAL RESULTS

In Ouchterlony's gel-diffusion test rabbit antiserum against marsh elder pollen gave two precipitation lines with homologous antigen only (3% saline extract of marsh elder pollen), containing 15,000 PNU. No cross precipitation lines were found with allergens of wormwood,

Allergologic Center, Saratov Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR M. Ya. Studenikin.) Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 91, No. 2, pp. 200-201, February, 1981. Original article submitted May 27, 1980.

TABLE 1. Degree of Anaphylactic Reaction in Guinea Pigs Sensitized with Wormwood, Orache, Ragweed, and Hemp Pollen in Response to Injection of Reacting Dose of Marsh Elder Pollen Extract

Animals	Number of animals	Degree of anaphylactic reaction to injection of marsh elder pollen					Anaphylactic index
		-	+	++	+++	++++	
Sensitized with ragweed pollen	10	—	1	3	4	2	2,7
Sensitized with wormwood pollen	10	—	4	3	2	1	2,0
Sensitized with orache pollen	10	—	2	4	2	2	2,5
Sensitized with hemp pollen	10	10					
Control (not sensitized)	10	10					

orache, hemp, and ragweed (standard allergens prepared at the Stavropol' Institute of Vaccines and Sera, containing 10,000, 15,000, and 20,000 PNU/ml respectively). The absence of common antigenic determinants in the gel diffusion test can be explained by the low sensitivity of the method [7]. Accordingly, to continue the study of common antigen properties in the allergens tested the more sensitive STT method was used.

In the STT test particles of precipitate were seen to be formed in a solution of serum from a guinea pig sensitized with marsh elder on the addition of the homologous antigen (marsh elder). However, in experiments in which standard solutions of ragweed were used as the added allergen, complex formation also was found. The phenomena described above were observed, to a lesser degree, on the addition of wormwood and orache allergens. On the addition of hemp allergen to the serum of a sensitized guinea pig in the STT test the optical system of dispersion of the serum was stable and no precipitates were formed.

As an example, the results of titration of antiserum with solutions of marsh elder, ragweed, wormwood, and hemp allergens are compared in Fig. 1. As the critical concentration of marsh elder allergen was reached the turbidity of the system increased, due to the formation of particles of precipitate, the mean size of which varied in this experiment from 0.05 to 0.3 μ , and the weight of precipitate increased from 0.5 to 1.5 mg. These phenomena could be observed to a lesser degree when ragweed allergen was added. During titration of the antiserum with hemp allergen, no change in turbidity was observed with time.

The presence of antigenic similarity between marsh elder and ragweed pollen was thus demonstrated by means of a sensitive method capable of revealing the formation of precipitate (allergen-antibody complexes) in the STT test. Wormwood and orache possessed a lower degree of antigen kinship with marsh elder. The laboratory tests used failed to reveal any common determinant of marsh elder and hemp pollen.

To confirm the results of laboratory tests revealing antigenic kinship between marsh elder, ragweed, wormwood, and orache experiments were carried out to produce cross anaphylactic shock. In guinea pigs sensitized with ragweed, wormwood, and orache allergens, anaphylactic shock of varied degrees of severity was observed in response to intravenous injection of a reacting dose of marsh elder pollen extract. The results of these experiments are given in Table 1. They show that guinea pigs, sensitized with ragweed pollen, reacted to injection of marsh elder pollen extract more strongly than guinea pigs of the other group. Their anaphylactic index was 2.7. The anaphylactic index in the group of animals sensitized with orache pollen was a little lower than in the previous group (2.5) and crossed reactions also were observed with wormwood pollen. The anaphylactic index for this group was 2.0. No cross reactions were observed with hemp pollen.

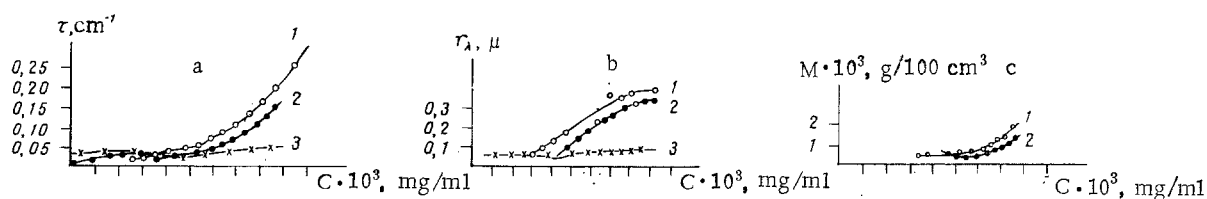


Fig. 1. Changes in turbidity of system (a), mean particle size (b), and weight of precipitate (c) during STT of marsh elder antiserum with solutions of marsh elder (1), ragweed (2), and hemp (3) allergens.

The formation of a precipitate during titration of marsh elder antiserum with ragweed, wormwood, and orache allergen in the STT test and ability to produce crossed general anaphylaxis in guinea pigs points to the possibility of cross reactions between substances in marsh elder and ragweed and, to a lesser degree, between marsh elder, wormwood, and hemp; this is a fact of great importance to the interpretation of clinical and immunologic data when persons with allergy to pollen of the above-mentioned plants are investigated.

LITERATURE CITED

1. A. D. Ado and A. A. Pol'ner (editors), Modern Practical Allergology [in Russian], Moscow (1963).
2. N. S. Gurina, "Aeropalynologic investigations and regional variations in pollinoses," Author's Abstract of Candidate's Dissertation, Saratov (1979).
3. V. I. Klenin, N. G. Astaf'eva, and N. V. Uzun, in: Allergy as a Problem in Social Hygiene [in Russian], Saratov (1974), pp. 329-330.
4. V. I. Klenin and S. Yu. Shchegolev, Dokl. Akad. Nauk SSSR, 204, No. 5, 1158 (1972).
5. F. F. Lukmanova, "Antigenic properties of plant pollen," Author's Abstract of Candidate's Dissertation, Moscow (1967).
6. A. I. Ostroumova, "Ragweed pollinosis," Author's Abstract of Doctoral Dissertation, Moscow (1972).
7. A. I. Polyak and K. A. Rakova, in: Pollinoses [in Russian], Krasnodar (1973), pp. 33-39.