

THE ANTIGENIC RELATIONSHIP OF THE POLLEN, SEED, AND LEAVES OF GIANT RAGWEED

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THE antigenic relationship between ragweed pollen, seed, and leaves is of practical and theoretical interest. It is known that wind-borne particles of plants, other than pollen, can induce coryza, conjunctivitis, and even asthma in susceptible persons. Often patients suffering from ragweed-pollen allergy complain of symptoms on windy autumn days, long after pollen has disappeared from the air. The minute particles of the ragweed leaves and stems, dried by the sun and wind and replacing the pollen, might account for this.

Another practical point of interest with respect to relationship of the antigens of pollen, seed, and leaves is the possible use of the latter two, which are more easily available, as sources of extracts for diagnostic and desensitizing material used in the management of allergic conditions. This study was undertaken not only to elucidate the practical aspect of the problem but also to obtain basic information on the immunologic relationship of the seeds and leaves to pollen.

EXPERIMENTAL PROCEDURES

1. *Materials*.—Giant ragweed pollen was exhaustively defatted with petroleum ether in a Soxhlet apparatus. A 3 per cent weight per volume extract of the pollen in Coca's fluid (1:33) was sterilized by filtration through a Seitz pad and stored at 4° C. All subsequent sterilizations were accomplished in the same manner. Fresh giant ragweed plants were gathered and stored at room temperature until they were completely dry. The seeds were removed, the dried stems and leaves cut into small pieces and ground as finely as possible by passage through a meat grinder. This material was defatted and extracted as described above for pollen. The seeds were similarly ground, defatted, and extracted.

Tests on eight subjects with a history of ragweed pollen hypersensitivity indicate that specific allergens were present in the seeds and stems and leaves.

2. *Fractionation Methods*.—Four fractionation procedures were carried out individually on pollen, seed, and leaves.

a. *Acetone Fractionation*: A 10 per cent extract of pollen in 0.1N NaOH was precipitated by the addition of seven volumes of cold acetone. The supernatant fluid was decanted into an evaporating dish and the acetone allowed

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Presented at the Tenth Annual Meeting of the American Academy of Allergy, Houston, Texas, Feb. 1-3, 1954.

This investigation was supported, in part, by a research grant from the Upjohn Company, Kalamazoo, Mich., and the Asthmatic Children's Aid, Chicago, Ill.

Received for publication, Feb. 1, 1954.

*This work was done as partial fulfillment of the requirements for the M. S. degree.

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TABLE I. RESULTS OF INTRADERMAL TESTS WITH ACETONE PRECIPITATE FROM SEED AND LEAVES

SUBJECT NO.	POLLEN EXTRACT CONTROL DILUTION*						SEED EXTRACT CONTROL DILUTION						SEED ACETONE PRECIPITATE DILUTION						LEAVES EXTRACT CONTROL DILUTION						LEAVES ACETONE PRECIPITATE DILUTION														
	1	2	3	4	5	6	11	12	13	14	15	16	17	18	11	12	13	14	15	16	17	18	11	12	13	14	15	16	17	18	11	12	13	14	15	16	17	18	
	2+	2+	2+	2+	2+	-	2+	2+	2+	2+	2+	2+	2+	2+	-	2+	2+	2+	2+	2+	2+	2+	-	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	
1	2+	2+	2+	2+	2+	-	2+	2+	2+	2+	2+	2+	2+	2+	-	2+	2+	2+	2+	2+	2+	2+	-	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	
2	4+	4+	3+	3+	3+	3+	4+	3+	3+	3+	2+	3+	2+	2+	-	3+	3+	3+	3+	3+	3+	2+	-	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	
3	+	+	-	-	-	-	3+	3+	2+	+	-	-	-	-	-	3+	2+	2+	-	-	-	-	-	-	3+	2+	2+	+	+	+	+	+	+	+	+	+	+	+	+
4	2+	+	-	-	-	-	2+	2+	+	+	+	+	+	+	+	2+	2+	2+	2+	2+	2+	+	-	2+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	4+	4+	3+	2+	2+	2+	+	+	+	+	-	-	-	-	-	2+	+	+	-	-	-	-	-	2+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	-	-	-	-	3+	3+	2+	-	-	-	-	-	-	3+	2+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
7	2+	+	+	-	-	-	4+	3+	3+	2+	+	+	+	+	-	4+	3+	3+	2+	2+	2+	+	-	2+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
8	3+	3+	2+	2+	2+	+	4+	3+	3+	2+	+	+	+	+	-	2+	+	+	+	+	+	-	-	3+	2+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	3+	3+	2+	+	+	-	2+	2+	+	-	-	-	-	-	-	2+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	
*Dilutions in thousands:						No. 1 = 1:33	No. 6 = 1:7,290	No. 11 = 1:0.33	No. 15 = 1:27							No. 15 = 1:27																							
						No. 2 = 1:90	No. 11 = 1:0.33	No. 16 = 1:81	No. 16 = 1:81							No. 16 = 1:81																							
						No. 3 = 1:270	No. 12 = 1:1	No. 17 = 1:243	No. 17 = 1:243							No. 17 = 1:243																							
						No. 4 = 1:810	No. 13 = 1:3	No. 18 = 1:729	No. 18 = 1:729							No. 18 = 1:729																							
						No. 5 = 1:2,430	No. 14 = 1:9																																

to evaporate as completely as possible in the air. The residue was dissolved in one-tenth of the original volume of 0.1N NaOH, diluted to 30 per cent with Coca's fluid, sterilized and labeled "pollen supernatant." The precipitate was drained well of acetone and dissolved in one volume of 0.1N NaOH. A portion of this solution, diluted to 3 per cent with Coca's fluid, was sterilized and labeled "pollen acetone precipitate." The remaining portion was allowed to evaporate to dryness in the air, ground finely, dissolved in one volume of 0.1N NaOH, diluted to 3 per cent with Coca's fluid, and sterilized. This was labeled "dry acetone precipitate." It is to be noted that "pollen supernatant" was made up ten times as concentrated as the other fractions.

Essentially the same fractionation procedures were carried out on 10 per cent extracts of seeds ("seed extract control") and leaves and stems ("leaves and stem control") except that both "leaves acetone precipitate" and "seed acetone precipitate" were made up as a 10 per cent solution in Coca's.

The above extracts were tested by the scratch procedure in 1:33, 100, 300, 900, 2,700 dilutions on the volar aspects of the arms and forearms or the backs of subjects clinically sensitive to ragweed pollen. The dilutions used for intradermal testing ranged from 1:33,000 to 1:7,290,000 for the pollen extracts and 1:330 to 1:729,000 for the leaves and seeds extracts. The results of the intradermal titrations are shown in Table I. In this and all subsequent titrations control tests done simultaneously on nonallergic subjects gave negative results.

b. *Dioxane and ammonium sulfate fractionations*: This technique was essentially the same as described by Boatner and Efron¹ except that dioxane was added to the extracts gradually, with continuous shaking. Fractions isolated by this procedure were a "25 per cent dioxane fraction," a "75 per cent dioxane fraction" and an "ammonium sulfate fraction."

The "pollen extract control" was tested simultaneously with the above fractions using the same dilutions for the intradermal tests as in the previous experiments. For the scratch tests, the dilutions were 1:10, 30, 100, 300, 1,000, 3,000, and 10,000. The sites chosen were the same as before. The results of the scratch test are presented in Table II.

TABLE II. RESULTS OF SCRATCH TESTS WITH CONTROL EXTRACTS OF POLLEN, SEED AND LEAVES

SUBJECT NO.	SCRATCH TEST WITH 1:33 EXTRACT		
	POLLEN	SEED	LEAVES
1	2+	-	-
2	4+	2+	2+
3	4+	2+	2+
4	±	±	-
5	+	-	+
6	±	-	-
7	±	-	-
8	2+	-	-
9	-	-	-

c. *Acetone and dioxane fractionation*: To the cold solution of "pollen acetone precipitate" sufficient cold dioxane was slowly added to make a 25 per cent concentration. The precipitate was collected by centrifugation and made up

TABLE 11A
Results of Scratch Tests With Dioxane and Ammonium Sulfate Precipitate From Pollen Extract

Results of Scratch Tests With Dioxane and Ammonium Sulfate Precipitate From Pollen Extract																									
SUBJECT NO.	POLLEN EXTRACT CONTROL						POLLEN 25% DIOXANE FRACTION						POLLEN 75% DIOXANE FRACTION						POLLEN AMMONIUM SULFATE						
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	
1	3+	+	±	-	-	-	+	+	+	±	-	-	+	+	-	-	-	-	-	+	-	-	-	-	
2	3+	3+	+	+	±	-	+	+	±	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	
3	4+	3+	2+	-	-	-	2+	+	+	±	-	-	2+	+	±	-	-	-	2+	2+	±	+	+	+	
4	4+	4+	4+	3+	2+	+	2+	2+	+	-	-	-	3+	+	+	2+	±	-	3+	3+	3+	+	+	+	
5	4+	2+	+	+	+	+	+	+	+	-	-	-	2+	±	±	±	±	±	2+	2+	+	-	-	-	

Results of Scratch Tests With Dioxane and Ammonium Sulfate Precipitate From Seed and Leaves Extract																																										
SUBJECT NO.	POLLEN EXTRACT CONTROL						POLLEN 75% DIOXANE FRACTION						POLLEN AMMONIUM SULFATE FRACTION						SEED EXTRACT CONTROL						SEED 75% DIOXANE FRACTION						LEAVES EXTRACT CONTROL						LEAVES 75% DIOXANE FRACTION					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6						
1	4+	4+	2+	+	-	-	+	+	+	2+	2+	±	4+	3+	2+	2+	±	-	2+	2+	±	-	-	-	+	±	-	-	-	+	-	-	-	-	-	-	-					
2	2+	+	-	-	-	-	2+	±	-	-	-	-	2+	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
3	2+	+	±	-	-	-	4+	2+	+	-	-	-	4+	2+	+	±	-	-	±	±	-	-	-	-	±	±	-	-	-	±	-	-	-	-	-	-	-	-				

*Dilution No. 1 = 1:10, 2 = 1:30, 3 = 1:100, 4 = 1:300, 5 = 1:1,000, 6 = 1:3,000.

to the original volume with 0.1N NaOH and sterilized. This was labeled "acetone-25 per cent dioxane fraction." Additional dioxane was added to the supernatant to make a 75 per cent concentration. The precipitate was redissolved in one volume 0.1N NaOH and divided into two portions. One portion was sterilized and will be referred to as "acetone-75 per cent dioxane fraction." The second portion was fractionated by ammonium sulfate as described in section 2b; the preparation will be referred to as "acetone-dioxane-ammonium sulfate fraction." Tests were made by the scratch method.

d. *Sodium Benzoate and Acetone Fractionation*: This method was carried out essentially according to McIntire's modification⁶ of that of Rimington and associates.⁷ To one volume of 10 per cent extract control was added sodium benzoate, 4 Gm. per 100 ml. Concentrated hydrochloric acid was added dropwise with continuous stirring of the solution until a pH of at least 3.5 was reached. After chilling for 10 to 15 hours in the refrigerator, the precipitated benzoic acid was collected by suction filtration on a Büchner funnel, without washing, and the suction was continued until the filter cake was nearly dry. The filter cake was treated with 9 times its weight of acetone. The suspension was centrifuged, the supernatant discarded, and the precipitate dissolved in one-half volume of Coca's extracting fluid. This "sodium benzoate fraction" was made up in the same dilutions as in the previous experiment and tested by the scratch technique.

3. *Comparative Clinical Studies*.—In order to evaluate these fractions with respect to their reactivity titrations were carried out simultaneously on the same subject in three ragweed-sensitive patients. Table III indicates the representative results obtained by intradermal testing in one of these subjects (K. S.).

RESULTS

1. *Purified Extracts*.—a. In skin reactivity "pollen acetone precipitate" was about one-third as strong as the "pollen extract control." When compared by cutaneous response in different subjects "dry acetone precipitate" ranged from one-tenth to one-third of the strength of the "pollen acetone precipitate" and one-thirtieth to one-ninth of the strength of the "pollen extract control." The "pollen supernatant," although used for skin testing in tenfold concentration, gave only mild reactions in two patients.

The control extracts and the acetone precipitated preparations of seed and leaves were tested simultaneously with "pollen extract control" but in a tenfold stronger concentration.

b. During the process of purification by dioxane precipitation there was a successive loss of pigment. Much of the plant pigment was lost also by absorption on the Seitz pad during filtration. The greater portion of dioxane insoluble material precipitated out at 25 per cent concentration. The precipitates were gummy and difficult to redissolve. The antigenic potency of the 25 per cent and 75 per cent dioxane fractions as measured by cutaneous response was weaker than that of each of the control extracts. The ammonium sulfate fractions generally gave a stronger response than did either of the two

dioxane fractions. The ammonium sulfate fraction of leaves, however, gave a weaker reaction than did the 75 per cent dioxane fraction and the control leaf extract.

c. The cutaneous response to the acetone-25 per cent dioxane fraction of pollen was about one-tenth as strong as the acetone-75 per cent dioxane fraction. The acetone-dioxane-ammonium sulfate fraction was from 3 to 100 times as potent as the acetone-75 per cent dioxane fraction but only about one-tenth to one-third the potency of the 10 per cent pollen control extract, even though it was made up twice as concentrated as the original.

d. The sodium benzoate fraction of pollen in a 20 per cent concentration gave about the same average result as did a 10 per cent concentration of the ammonium sulfate fraction.

TABLE III. RESULTS OF INTRADERMAL TESTS WITH FRACTIONS OF POLLEN, SEED, AND LEAVES

SUBJECT	PREPARATION	DILUTIONS*														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
K. S.	Pollen extract control						4+	3+	2+	2+	2+	2+	2+	2+	+	-
	Pollen acetone precipitate				4+	3+	3+	2+	+	+	+	+	-			
	Pollen ammonium sulfate fraction				4+	3+	3+	2+	2+	2+	2+	2+	+	±		
	Pollen sodium benzoate fraction					3+	2+	+	+	±	-					
	Seed extract control	2+	2+	+	+	-										
	Seed acetone precipitate	4+	3+	2+	±											
	Seed ammonium sulfate fraction	4+	3+	2+	±	-										
	Seed sodium benzoate fraction	4+	3+	+	±	-										
	Leaves extract control	4+	4+	3+	3+	2+	+	±	-							
	Leaves acetone precipitate	3+	3+	2+	2+	±	-	-								
	Leaves ammonium sulfate fraction	+	-	-												
	Leaves sodium benzoate fraction	2+	+	-												
*Dilution No.		1 = 1:10					9 = 1:2,430,000									
		2 = 1:100					10 = 1:7,290,000									
		3 = 1:1,000					11 = 1:21,870,000									
		4 = 1:10,000					12 = 1:65,610,000									
		5 = 1:30,000					13 = 1:196,830,000									
		6 = 1:90,000					14 = 1:590,490,000									
		7 = 1:270,000					15 = 1:1,771,470,000									
		8 = 1:810,000														

2. *Skin Tests in Allergic Subjects.*—The results of simultaneous and comparative titrations done with all preparations indicate that fractionation with acetone produces a preparation which is somewhat less potent than the control extract. Subsequent fractionations with ammonium sulfate and benzoic acid result in progressively less potent preparations. This pattern of reactivity is reflected for pollen, leaves, and seeds in that order, indicating that the same reagin-reacting antigen is separated from each substrate in approximately the same ratio by parallel fractionation. Although there is a certain amount of variation for each individual subject, an over-all pattern does seem to exist.

3. *Passive Transfer and Reagin Exhaustion Tests.*—Passive transfer sites with human ragweed reagin were prepared on the backs of nonallergic human subjects. After 48 hours the control extracts of pollen, seeds, and leaves were

titrated by the intradermal technique. That dilution of each extract which gave a 2+ reaction was used to exhaust a row of passively sensitized sites by repeated daily or twice daily injections of 0.2 ml. of extract until no further local reaction (erythema or wheal formation) occurred. Cross reactivity to each of the other two antigens was determined the day following complete exhaustion. The results which are given in Table IV indicate that pollen antigen completely exhausted the test sites so that no cutaneous response was elicited by either leaf or seed antigen.

The sites exhausted by seed antigen always gave positive and significant reactions on challenge with the pollen extract. Similar sites challenged with the leaf antigen resulted in positive reactions in three subjects, a doubtful reaction in one, and negative reaction in two.

Sites exhausted by the leaf extract gave inconsistent results when challenged with the pollen extract. Two subjects showed a strong response, one, a suggestive, and three, none. When challenged with seed antigen there was no reaction at the sites.

4. *Anaphylaxis in the Guinea Pig.*—Each of the 44 guinea pigs weighing 300 to 500 grams was sensitized by injecting 3 ml. of a mixture of 10 per cent giant ragweed pollen extract in Freund's adjuvant. Eighteen days after sensitization the animals were challenged by intravenous administration of one of the fractionated preparations. The volume of the challenge dose was adjusted so that each animal received 0.2 mg. total nitrogen. After fifteen days the same animals were challenged with 0.2 mg. protein N. The results are submitted in Table V.

The strongest anaphylactic reactions were shown by the guinea pigs challenged with pollen extract control based on either total or protein nitrogen. On the basis of the total nitrogen content the next potent preparation was the ammonium sulfate fraction of pollen. When tested on the protein nitrogen basis the sodium benzoate and the ammonium sulfate fractions of pollen were next and were of comparable potency.

5. *Precipitin Test.*—Two-tenths milliliter of rabbit antiragweed pollen serum diluted 1:3 was placed in each of several precipitin tubes. A similar volume of antigen solution containing 0.024 mg. total nitrogen and in multiple dilutions of 1:5 was layered over the serum and the tubes incubated for 1 hour at 37° C. After the interfacial zone was examined for a precipitin ring, the contents of the tubes were well mixed and stored at 4° C. for 24 hours. The degree of precipitation indicating a reaction was then recorded. A similar comparative titration was carried out with the various preparations based on a protein nitrogen content of 0.007 mg. A similar precipitin test was carried out using that concentration of antigen which gave a 2+ cutaneous response in subject K. S.

The highest titers were obtained with pollen extract control and ammonium sulfate fractions of pollen and leaves on the basis of either the total nitrogen or protein nitrogen content.

DISCUSSION

As a result of the many investigations on its antigenicity and chemistry, the tissue of the giant ragweed which has been best characterized is the pollen. Present knowledge concerning the antigens of ragweed pollen is far from complete, however, and most investigators agree that the antigenic makeup of the extract is quite complex. For these reasons, the results of experiments with all dilutions of the extracts of the leaves and seeds were compared with those obtained with the same dilutions of pollen control extracts as the standard.

The results obtained with skin testing emphasize the individual differences in sensitivity that subjects will exhibit to a given extract. In spite of a different threshold of reactivity to the same dilutions of pollen fractions, however, there was, in general, a similar pattern of responsiveness to the antigens of the three tissues of the giant ragweed. The reagin exhaustion studies suggest that in addition to unrelated antigens there exists a common antigen.

The possibility that some denaturation may have occurred during the fractionation procedures with modification in antigenic behavior is not to be overlooked. Thus, the response in the allergic subject to the fractions of the same tissue was not in proportion to the total nitrogen or to the protein nitrogen content per unit of volume. Also, the ability of each of the extracts of the three plant tissues to sensitize guinea pigs was not proportional to the protein or total nitrogen content. Of the fractionation procedures studied, the ammonium sulfate fractionation of pollen and leaf extract seemed to produce the most potent preparations, even though they contained the least amount of total nitrogen. Paradoxically, ammonium sulfate fractionation of the acetone-precipitable material did not result in a more potent fraction.

The cross reactivity which demonstrates the antigenic relationship between the fractions is best evident from the results of anaphylaxis in the guinea pig (Table V) and the precipitin test. Thus, we disagree with the findings of Thommen,⁸ who did not obtain cross reactivity between tissues of the same plant using extracts made from the leaf, stem, roots, and seed of giant ragweed and of timothy. The results of Duke² also refute the concept of the antigenic relationship of different parts of the plants. Wartenberg¹² observed a difference in the serologic behavior of different parts of plants. Dunbar³ noted that extracts of the pollen, grain, and leaf of rye showed a high degree of specific reactivity based on the results of the complement fixation test. Wodehouse¹³ was able to differentiate between pollen and seeds by their anaphylactic properties.

The results of Vallery-Radot and associates,⁹ however, based on their studies on the pollen, stem, and roots of the plant, *Dactylis glomerata* (orchard grass), support the likelihood of antigenic relationship of the different tissues of the plant. Comparable skin reactions were observed by testing with the pollen and stem extracts. The extract of the root failed to show any reaction. Waring and Pope¹¹ have reported that patients who are sensitive to the pollen of the cottonwood trees are also sensitive to the hair tufts of the seed.

TABLE IV. PASSIVE TRANSFER AND REAGIN EXHAUSTION

SUBJECT NO.	SITES EXHAUSTED WITH POLLEN EXTRACT CONTROL		SITES EXHAUSTED WITH SEED EXTRACT CONTROL		SITES EXHAUSTED WITH LEAVES EXTRACT CONTROL	
	CROSS TESTED WITH EXTRACT CONTROL OF SEED LEAVES		CROSS TESTED WITH EXTRACT CONTROL OF POLLEN LEAVES		CROSS TESTED WITH EXTRACT CONTROL OF POLLEN SEED	
1	-	-	2+	-	-	-
2	-	-	±	2+	-	-
3	-	-	2+	2+	-	-
4	-	-	3+	3+	±	-
5	-	-	3+	-	2+	-
6	-	-	3+	-	3+	-

Dutton,⁴ found that beet pollen and beet seed dust caused hay fever and asthma in patients sensitive to beets. Grubb and Vaughan,^{5, 10} studying group and species specific allergens of pollen and foods by clinical, chemical, and immunologic procedures, came to the conclusion that about one-half of those patients allergic to the pollen of wheat, corn, and beet were also allergic to the corresponding food.

TABLE V. ANAPHYLACTIC SHOCK IN GUINEA PIGS SENSITIZED WITH POLLEN EXTRACT CONTROL AND CHALLENGED WITH POLLEN, SEED, AND LEAF FRACTIONS

TISSUE OF THE PLANT	GUINEA PIG NO.	EXTRACT CONTROL	GUINEA PIG NO.	ACETONE PRECIPITATE FRACTION	GUINEA PIG NO.	AMMONIUM SULFATE FRACTION	GUINEA PIG NO.	SODIUM BENZOATE AND ACETONE FRACTION
<i>Total Nitrogen Basis (0.2 mg. Total N./Dose)</i>								
Pollen	1	4+	10	-	19	2+	28	±
	2	3+	11	-	20	4+	29	+
	3	+	12	±	21	4+	30	-
Seed	4	-	13	-	22	-	31	-
	5	-	14	-	23	-	32	-
	6	-	15	-	24	-	33	-
Leaves	7	-	16	-	25	-	34	-
	8	±	17	-	26	±	35	-
	9	-	18	+	27	±	36	-
<i>Protein Nitrogen Basis (0.2 mg. Protein N./Dose)</i>								
Pollen	1	4+	10	±	19	+	27	3+
	2	4+	11	-	20	2+	28	2+
	3	3+	12	-	21	3+	29	±
Seed	4	-	13	2+	22	-	30	-
	5	-	14	+	23	-	31	-
	6	-	15	±	24	-	32	±
Leaves	7	±	16	-	25	4+	33	-
	8	-	17	-	26	4+	34	-
	9	-	18	±			35	±

SUMMARY

Fractions of the giant ragweed pollen, seed, and leaves were prepared in a purified form. The antigenic relationships of these preparations were compared by direct skin tests in ragweed-sensitive patients, by cross testing of reagin sites exhausted with a related antigen, by anaphylaxis and by precipitin tests. The results showed that:

1. There exists an antigenic relationship of the three investigated giant ragweed tissues.

2. Comparative titrations suggest a difference in potency decreasing from pollen through leaves to seed.

3. There seems to be a very close relationship between the leaf and pollen antigen. The seed does not contain an antigen present in the pollen in that it is not capable of exhausting sites inoculated with human reagin. The restricted number of tested subjects, however, does not allow final conclusions to be drawn on this point.

4. Quantitative titrations and other investigations showed that ammonium sulfate fractionation results in the most potent preparations for leaves and pollen, but acetone fractionation is apparently required to produce potent seed preparations, especially as observed by its anaphylactic properties in sensitized guinea pigs.

5. The relatively small amount of pollen antigen in the leaves and seed would speak against the use of these plant tissues as a practical source of the antigen for diagnostic and therapeutic purposes.

6. The results suggest that the plant debris may serve as a natural source of allergy to the pollen-sensitive patient. Further work would have to be done before this can be accepted as a final opinion.

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