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Immunologic and cross-reactivity studies of Cupressaceae antigens in the rabbit, and skin test results from suspected Juniper Cypress pollinosis patients, indicate that a large number of species of the Cupressaceae family are of possible allergenic importance in our mobile population in many areas of the United States and the world.

CONIFER POLLEN ALLERGY: STUDIES OF IMMUNOGENICITY AND CROSS ANTIGENICITY OF CONIFER POLLENS IN RABBIT AND MAN

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MOUNTAIN CEDAR (*Juniperus Sabinoides*) is a recognized cause of inhalant allergic disorders in certain areas of the west, especially Texas, and the cross reactivity of this pollen with Port Orford Cypress (*Chamaecyparis lawsoniana*) of California and Oregon was suggested by skin tests and results of hyposensitization.¹

The cross reactivity of Mountain Cedar with the winter pollinating Eastern Red Cedar was demonstrated by similar techniques.² The Cupressaceae family contains a large number of species of potential interest to allergists. These include, in addition to the above, Monterey Cypress (*Cupressus macrocarpa*) of the Fort

Ord, California, area, the Mediterranean Cypress (*Cupressus sempervirens*) of Israel, Weeping Cypress of North China (*C. Funnebris*), Arizona Cypress of Fort Huachuca, Arizona area, and Alaska Cedar (*C. Nootkaensis*). In Japan Sugi Cedar is the most prevalent cause of pollinosis.³ In San Francisco the *Cupressus*/*Juniperus* type is the most prevalent airborne pollen and a wide variety of Junipers are used as ground cover. The pollen is present perennially, with a sharp midwinter increase in January, and reaches a peak in April.⁴ It has not been regarded as being highly sensitizing, despite experience with the highly allergenic Mountain Cedar and Incense Cedars.⁵

This report is part of a study to determine the relative importance of *Juniperus*/*Cupressus* in causing allergic disease in the San Francisco area. We now report the immunogenicity and cross-reactivity of several Cupressaceae antigens in the rabbit and skin test results from sus-

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pected Juniper Cypress pollinosis patients.

Methods and Materials

1. Antigens

Allergenic extracts were prepared by Berkeley Biologicals and were 1:20 dilutions. The following antigens were used:

1. Red Cedar (*Juniperus virginiana*)
2. Western Red Cedar (*Thuja plicata*)
3. American Arborvitae (*Thuja occidentalis*)
4. Western Juniper (*Juniperus occidentalis*)
5. Redwood (*Sequoia sempervirens*)
6. Australian Pine (*Casuarina equisetifolia*)
7. Port Orford Cypress (*Chamaecyparis lawsoniana*)
8. Monterey Cypress (*Cupressus macrocarpa*)
9. Arizona Cypress (*Cupressus arizonica*)
10. Mountain Cedar (*Juniperus sabinoides*)
11. Pfitzer Juniper (*Juniperus chinensis*)
12. Incense Cedar (*Libocedrus decurrens*)
13. Japanese Sugi (*Cryptomeria japonica*)

The protein concentration of the antigens was determined by Lowry's method at the Letterman Army Institute of Research. Japanese Cedar pollen was provided by Dr. Setsuo Kobayashi of Gumma University, Japan. These concentrations vary between 50.3 mg/ml and 61.5 mg/ml.

2. Immunization

Random bred, young adult rabbits were used for the immunization with pollen antigen. One ml. of each concentrated pollen antigen (1:20 dilution) was mixed with one ml. of complete Freund's adjuvant and the emulsion was injected in the four foot pads.

Three weeks later the same amount

of antigen with equal quantities of Freund's adjuvant was given intramuscularly for the booster dose. For Sugi immunization the rabbits were injected with 3.5 cc. of a 10% suspension of pollen in normal saline, mixed with complete Freund's adjuvant.

3. Immunodiffusion

Immunodiffusion was performed in one percent agarose in a Petri dish or a slide by placing the antigen in the center well (same as immunizing antigens) and antiserum in the outer well or vice versa. The dish was kept at room temperature and observed 24 and 48 hours later and the immunoprecipitin line was read after standard methods of washing and staining plates. When there was no precipitation line with immunodiffusion, the rabbit was re-immunized with antigen I.M. or S.Q.

4. Human Direct Skin Tests

All of the patients in this study were followed at the Allergy Clinic of Letterman Army Medical Center, San Francisco.

We have followed a small group of patients whose history suggested possible conifer sensitivity because of respiratory symptoms while working in the garden in the vicinity of their juniper plants or because of repeated late winter symptoms. We have now tested about 30 of these individuals with extracts of the local evergreen plants or those to which their travels might expose them. The extracts were used at 1:20 weight to volume dilution for scratch testing and 1:500 for intradermal testing.

Results

Skin Test

Table I shows the skin test results. The number of positive skin reactions obtained with each antigen is indicated in the first column ("pos"). Only 16

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out of 30 gave positive reactions to these antigens. Testing individuals with positive reactions to the antigen listed in the left-hand column, the antigens in the column headings were found to produce positive reactions in the number shown. For example, patients found to have a positive skin test (at least two plus more than control on a scale of 0-4) to Mountain Cedar would in 15 of 16 instances also react to Monterey Cypress. It is evident that there is a high probability that patients with a positive skin test to Pfitzer Juniper also will have a positive reaction to Monterey Cypress. This was true in 15 of 16 instances. Cross reactivity appears to center in that group of conifers which are members of the Cupressaceae family. Not included on the chart are positive reactions to Japanese Cedar in four of seven patients reactive to Mountain Cedar.

The statistical analysis was prepared by Dr. Judy Bean of the Department of Biostatistics of the University of Iowa from skin test results weighted for positivity. Using Kendall's rank order coefficients, a relationship between two events is statistically more significant the closer the coefficient approaches one, and the less likely it is to be of significance the closer it approaches minus one. The conifer Redwood of a different family, and especially the non-conifer Australian pine, are noted to have very low rank order coefficients in comparison to most of the others.

Immunodiffusion of Rabbit Antibodies

Most of the antigens were immunogenic and were able to produce antibodies. For reasons we do not understand, Monterey Cypress and Port Orford Cypress were not effective immunogens in this system. Table II de-

TABLE I. PATIENT'S SKIN TEST (SCRATCH AND INTRADERMAL) WITH CONIFER ANTIGEN.

PLANT ANTIGEN	POS	CROSS REACTIONS														
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
I Red Cedar	16	...	14	14	15	6	8	15	15	15	16	13	15	11	8	8
II W. Red Cedar	15	14	...	14	14	5	7	14	14	14	14	13	14	9	7	6
III Am. Arborvitae	14	14	14	...	14	5	7	14	14	14	14	13	14	9	7	6
IV W. Juniper	16	15	14	14	...	5	7	15	14	16	16	14	15	9	7	6
V Redwood	6	6	5	5	5	...	5	6	6	5	5	5	5	4	4	5
VI Australian Pine	12	8	7	7	7	5	...	7	7	7	8	6	7	10	9	10
VII Port Orford Cypress	16	15	14	14	15	6	7	...	15	15	15	14	15	11	7	7
VIII Monterey Cypress	16	15	14	14	15	6	7	15	...	15	15	13	14	9	7	7
IX Arizona Cypress	16	15	14	14	16	5	7	15	15	...	16	14	15	9	7	6
X Mountain Cedar	16	16	14	14	16	5	8	15	15	16	...	14	16	10	8	7
XI Incense Cedar	14	13	13	13	14	5	7	14	13	14	14	...	13	9	6	5
XII Pfitzer Juniper	16	15	14	14	15	5	7	15	14	15	16	13	...	10	8	7

TABLE II. CROSS REACTIVITY OF 13 ANTIGENS

Antigen	Antisera to Antigen												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Red Cedar	+	+	+	+	...	...	...	...	+	+	+	+	...
2 Western Red Cedar	+	+	+	+	...	...	...	...	+	+	...	+	...
3 American Arborvitae	...	+	+	...	...	...	...	...	+	+	...	+	...
4 Western Junipers	+	...	...	+	...	...	...	...	+	+	+	+	...
5 Redwood	...	...	...	...	+	...	...	...	...	...	...	...	...
6 Australian Pine	...	...	...	...	...	+	...	...	...	...	...	...	...
7 Port Orford Cypress	...	...	...	...	...	...	0/5	...	...	...	...	...	...
8 Monterey Cypress	+	+	+	+	...	...	...	0/8	...	+	...	+	...
9 Arizona Cypress	+	+	...	+	...	...	...	...	+	...	...	+	...
10 Mountain Cedars	+	+	+	+	...	...	...	...	+	+	+	+	...
11 Incense Cedar	+	...	...	+	...	...	...	...	+	+	+	+	...
12 Pfitzer Juniper	+	+	+	+	...	...	...	...	+	+	+	+	...
13 Sugi Cedar	...	...	...	...	...	...	...	...	+	...	+	...	+

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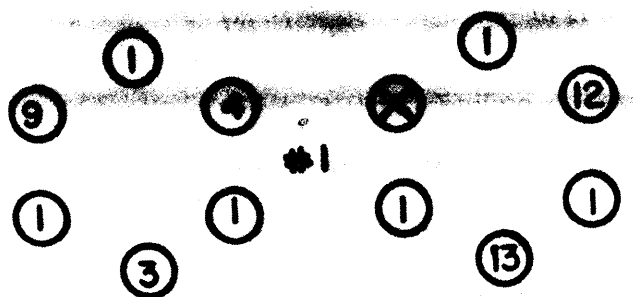
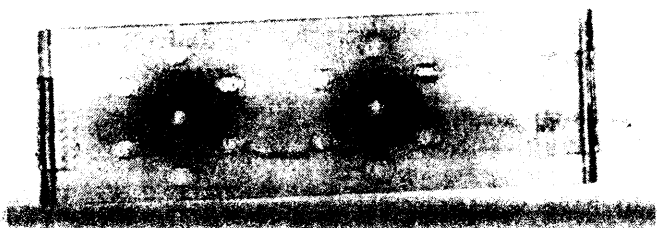


Figure 1. Immunodiffusion plate. Antiserum #1 in the center well was made against Red Cedar antigen. Numbers in the outer well indicate the antigen number: #1 Red Cedar, #3 American Arborvitae, #4 Western Juniper, #9 Arizona Cypress, #12 Pfitzer Juniper and #13 Sugi Cedar.

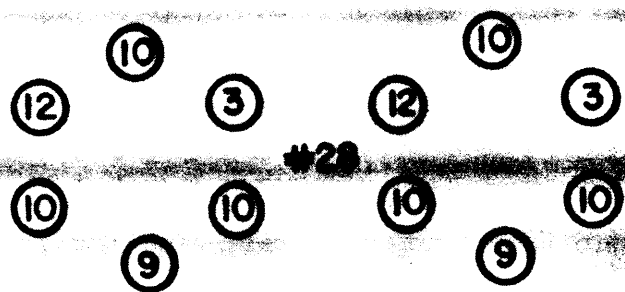
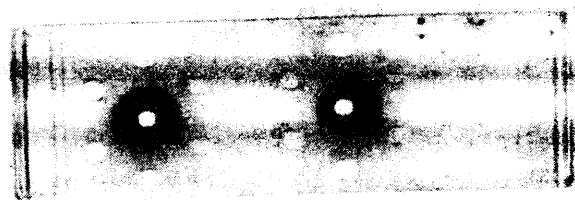


Figure 2. Immunodiffusion plate. Antiserum #28 in the center well was made against Mountain Cedar antigen. Numbers in the outer well indicate antigen number: #3 American Arborvitae, #9 Arizona Cypress and #12 Pfitzer Juniper.

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monstrates the cross reactivity as indicated by immunodiffusion studies (Figures 1-3) with the rabbit antisera to the individual pollens. Antisera against Redwood and Australian Pine did not cross-react with any Cupressaceae antigen. Port Orford Cypress failed to induce any precipitating antibody in five rabbits by immunodiffusion test. Monterey Cypress also failed to produce any precipitating antibody in eight rabbits used but this antigen cross-reacted

to the antisera against Red Cedar, Western Red Cedar, American Arborvitae, Western Juniper, Mountain Cedar and Pfitzer Juniper. The rest of the juniper antisera reacted with more than two of the juniper antigens. Antisera against Arizona Cypress and Incense Cedar reacted with Sugi Cedar antigen.

Pfitzer Juniper produced an antiserum cross reactive with all the other Cupressaceae members except

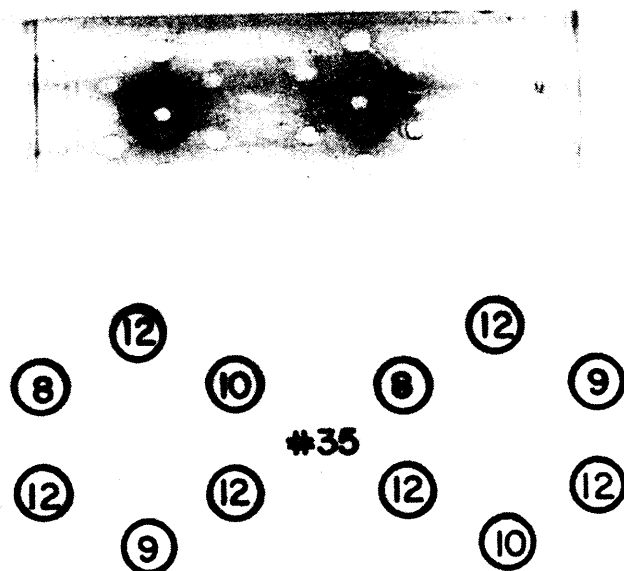


Figure 3. Immunodiffusion plate. Antiserum #35 in the center well was made against Pfitzer Juniper antigen. Numbers in the outer well indicate antigen number: #8 Monterey Cypress, #9 Arizona Cypress, #10 Mountain Cedar and #12 Pfitzer Juniper.

TABLE III. KENDALL'S RANK-ORDER CORRELATION COEFFICIENTS OF PATIENT'S SKIN TESTS

Antigen #		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12
Antigen #													
1	Red Cedar		.40	.53	.71	.32	−0.35	.49	.39	.52	.59	.40	.65
2	Western Red Cedar			.59	.40	.28	−0.18	.35	.61	.44	.52	.58	.61
3	American Arborvitae				.60	.40	0.03	.55	.64	.36	.40	.34	.50
4	Western Junipers					.23	−0.15	.72	.45	.52	.58	.32	.62
5	Redwood						0.13	.17	.33	.09	.26	.10	.29
6	Australian Pine							−0.19	−0.04	−0.20	−0.05	−0.15	−0.35
7	Port Orford Cypress								.48	.30	.32	.43	.51
8	Monterey Cypress									.47	.51	.56	.47
9	Arizona Cypress										.79	.52	.62
10	Mountain Cedars											.49	.63
11	Incense Cedar												.38
12	Pfitzer Juniper												

Port Orford Cypress. This antigen also reacted with the antisera produced by the other family members. Mountain Cedar antigen reacted with available antisera of the family. The immunodiffusion plates are shown in Figures 1-3.

Discussion

Cedar pollinosis has been recognized throughout the world. J.H. Black in 1929 appears to have been the first to speak and write about Mountain Cedar sensitivity.¹ A few months later French reported from the same area of central Texas that approximately 4% of his hay fever patients had sensitivity to this pollen in addition to their other sensitivity.⁶ Shortly thereafter, based on results of skin testing and hyposensitization, Kahn reported² that there appeared to be cross antigenicity between the pollen of Mountain Cedar and Red Cedar found over much of the southeast United States. Our results confirmed these observations (Tables I and II). Juniper is the most frequent woody plant used for landscape planting in the western part of the United States. The liberal use of species such as Pfitzer Juniper suggests its possible allergenic importance in the sprawling suburban areas.

Black demonstrated the skin sensitivity of his Mountain Cedar patients to injections of extract of Port Orford Cedar pollen. They had no known exposure to this pollen.

Our results with human skin tests parallel those of Black. However, Port Orford Cypress antigen did not cross react with rabbit antisera directed against Mountain Cedar. Black's study of cross antigenicity of these two pollens may have been prompted by confusion arising from the application of the common name of cedar. This confusion has obscured understanding of the relationship in allergenic diseases of many members of the conifer

family. Australian Pine, which is not a conifer, did not cross-react with the other antisera. Redwood and Sugi behave similarly. This is in accord with the phylogenetic relationship of these trees.

The true cypress of *Cupressus* genus was first implicated in the production of hay fever by Ordman in South Africa in 1945.⁸ Two decades later sensitivity to Mediterranean or Italian Cypress was reported from Israel.⁷

Monterey Cypress was found originally in the Monterey area of California and is now cultivated widely throughout the world. The prevalence of this pollen in San Francisco prompted this study. Previously it has not been considered a significant cause of allergic disease. Our results indicate this was a poor immunogen in the rabbit, but the human skin test indicated the patients had positive skin tests and therefore could develop symptoms to this allergen.

Japanese Cedar pollen also is the predominant pollen causing asthma and hay fever in Japan.^{4,7}

The *Arborvitae* is an American native that is used widely as a landscaping and foundation plant. Western Red Cedar is an important timber material of the northwestern United States.

The Kendall coefficient analysis revealed a general pattern of skin reactions which correlate reasonably well with the rabbit precipitin data. Australian Pine and Redwood skin test correlation coefficient is a very low number, while the correlation coefficient of Mountain Cedar and Pfitzer Juniper is a relatively higher value. The clinical inference is that a patient who develops clinical symptoms on exposure to Mountain Cedar in San Antonio, Texas might, on transfer to San Francisco, continue to have symptoms because of similar antigens

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in Monterey Cypress. On the other hand, his probability of being sensitive to Redwood or Australian Pine is the same or less than his probability of sensitivity to grasses or weeds. It is noted that the so-called Australian Pine is an evergreen of an entirely different botanical order. Japanese Cedar and the coast Redwood of northern California were the two members of the Taxodiaceae family considered during this work.

The Redwood and Incense Cedar are December-January pollinators. The mid-winter pollination of some of these species suggests that the usual pollen collection months from Elm through the ragweed season may not be sufficient for the determination of the role of the conifers in disease.

Summary

Conifers, with few exceptions (Mountain Cedar, Eastern Red Cedar and Incense Cedar), have not been considered significant allergen producers in this country. Previous reports have suggested cross allergenicity of pollen from several species of the order Coniferales. The Cupressaceae family is one of the major airborne pollen producers of San Francisco, but it has been considered to have minor clinical importance. Our studies indicate that the pollen extracts of a large number of species of the Cupressaceae family are immunogenic and cross reactive in man and rabbits, which suggests their possible allergenic importance to our mobile population in many areas of this country and the world.

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