

A ONE-YEAR AEROALLERGEN SURVEY OF PUERTO RICO

Correlation of Results With Clinical Findings in 300 Patients

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THE INCREASE in business and pleasure travel to Puerto Rico, as well as the large migration of Puerto Ricans to the United States, has aroused interest among practicing allergists on the mainland in the specific regional factors that may affect allergic patients in this island. With the exception of isolated air-borne fungus cultures,¹ of mention of possible fungus and bacterial allergens in Puerto Rico,² and of listings of potential hay fever plants,³ no studies of the air-borne allergens of the island have been performed. This communication will present the results of the first one-year fungus and pollen survey of Puerto Rico; a partial report of this study has already been published.⁴ The findings will be correlated with the clinical features of 300 patients with respiratory allergy studied in the island.

Puerto Rico is located in the West Indies at latitude 18° 30' N, longitude 65° 35' W. It is densely populated by 2,210,703 inhabitants (1950 census) in an area of only 3,421 square miles. The topography is varied, with a central axis of mountains surrounded by coastal plains. The tropical-marine climate is pleasantly influenced by trade winds; the yearly extremes of temperature in San Juan range between 62° to 94°. Rainfall is 61.19 inches yearly and it is fairly well distributed throughout the year, although much the greater amount occurs from May to December, inclusive; the sun shines on an average of 360 days a year. Sugar cane has traditionally been the main agricultural crop but, in the last decade, industrial and touristic development has caused a distinct rise in living standards.

MATERIAL AND METHODS

The survey was carried out from Feb. 1, 1956, to Jan. 31, 1957, according to the standards of the American Academy of Allergy for air-borne allergen surveys in the United States.⁵ Slides coated with petroleum jelly were exposed daily for twenty-four hours in a Durham air sampling apparatus located on the roof of a one-story building at San Patricio Hospital in suburban San Juan. Pollen grains and fungus spores were counted on the slides over an area

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of 4.48 sq. cm. and the counts were reduced to an area of 1 sq. cm. Individual *Hormodendrum* spores were counted as recommended by Durham.⁶ Simultaneous twelve-minute weekly exposures of Petri plates containing modified Mehrlich's medium were performed in three different locations in San Juan, namely, a third-floor balcony overlooking the ocean in a residential section, a third-story roof in the business district, and at San Patricio Hospital. The plates were kept at room temperature for five days, at which time the fungus colonies were counted. The colonies from the plates exposed at the Hospital were subcultured on Sabouraud's, Czapek, corn meal, and V-8 juice media, and morphological identification of the genera of fungi was carried out. The nomenclature of genera as established by Clements and Shear⁷ was followed throughout.

The case records of 300 patients with bronchial asthma, allergic rhinitis, or both, were studied in regard to occurrence of seasonal symptoms, variation of symptoms during residence in Puerto Rico and in temperate zones, and skin test reactions by the scratch and intracutaneous methods to approximately 80 food and inhalant allergens. All the patients were studied by the senior author between 1952 and 1956. The selection of cases was limited to patients who had had symptoms of at least one year's duration and who showed definite clinical and skin test evidence of allergy to environmental factors. Patients with bronchial asthma who showed predominantly intrinsic factors were excluded from the study, as well as patients who showed inconclusive skin test reactions. The series is believed to be representative of cases seen in the practice of clinical allergy in Puerto Rico.

SURVEY RESULTS

The daily slide counts showed a marked predominance of fungus spores over pollen grains (Table I and Fig. 1). The range of the daily spore count was 2 to 2,193 per square centimeter, and the daily pollen counts varied from 0 to 15 per square centimeter. The average daily spore count for the entire year studied was 41.2 spores per square centimeter; that of pollen was only 2.2 grains per square centimeter. Although there were marked day-to-day variations in both fungus and pollen counts, no significant seasonal variations occurred. The high fungus count for December reflects one daily count of 2,193 spores per square centimeter, while the next highest spore count in the entire year was 163 per square centimeter.

TABLE I. AVERAGE DAILY COUNT OF FUNGI AND POLLEN (1956-1957) PER SQUARE CENTIMETER

	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	JAN.
Fungi	25.9	33.9	28.7	38.8	36.9	34.3	36.7	38.1	42.3	37.2	114.8	32.6
Pollen	1.6	2.6	3.3	2.9	3.1	1.9	2.9	1.9	1.9	1.3	1.3	1.3

Upon comparing the monthly total of fungus spore counts per square centimeter with the *Alternaria* spore counts included in the Statistical Report of the Mold and Pollen Committee of the American Academy of Allergy for

1956,⁷ we find that our counts are much higher than those reported for twenty-two states and Canada. There are only two exceptions: San Antonio (Texas) reported a total count of 1,294 *Alternaria* spores per square centimeter for the month of June, and Lexington (Kentucky) reported a count of 1,281 per square centimeter for the month of July. Both of these counts are higher than ours for the corresponding months, even though they include only *Alternaria* spores.

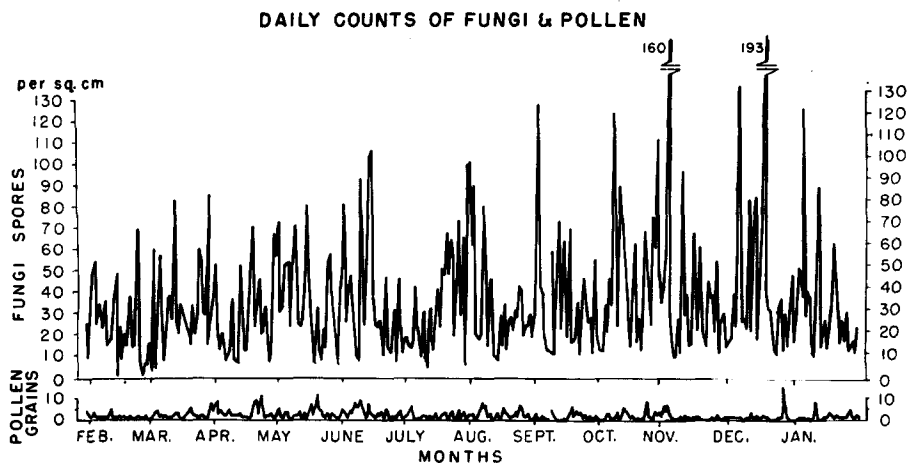


Fig. 1.

The total daily, weekly, and monthly fungus spore counts were analyzed in relation to corresponding climatological data of rainfall, temperature, dew point, wind velocity, and wind direction, as obtained from monthly reports of the U. S. Department of Commerce Weather Bureau. No obvious relationships were apparent. Simple correlation analyses were carried out with the daily spore counts throughout the year and relative humidity, and, independently, with the spore counts and dew point. No correlation was found to exist. It must be pointed out that the climatological data was obtained from readings taken at the Isla Verde Airport, at a distance of 5.6 miles from the slide exposure site.

TABLE II. WEEKLY AVERAGE OF PLATE FUNGUS COUNTS PER MONTH (1956-1957)

	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	JAN.
Suburban area	48.5	41.4	58.2	22	39	31.7	117.6	27.5	122	12	49	64
Business area	12.2	10	14	20	8.2	9	9	13.2	46.5	14	13.5	8.7
Seashore	5.2	12.2	6.3	13	3.2	4	16.4	9.2	4.7	17	2.5	1.5

Fungus counts by the plate method ranged from 0 to 473 colonies per plate. The average count for the year (50 exposures) was 36 colonies per plate, for a 12 min. exposure. Table II summarizes these results in terms of weekly averages. As in slide counts, there were marked variations in the number of colonies obtained on different exposures, but no seasonal trends were noted.

The lowest counts were obtained almost invariably from the plates exposed at the seashore, and the highest occurred in the suburban area, the results in the business section being intermediate (Table II).

A total of 1,824 fungus colonies were counted in the plates exposed in the suburban area. Of these 71 per cent were identified morphologically, 17 per cent could not be identified, and 12 per cent were overgrown or could not be subcultured. Forty-three different fungus genera were identified. Among the identified *so-called* genera we have listed three groups which are not true genera (Table III). *Yeast-like* includes *Candida*s, other white yeast-like forms which did not produce hyphae, and colored yeast-like organisms. The fungi in group 5 A 35 show very similar macroscopic and microscopic characters among themselves. A similar resemblance can be noted among the fungi which constitute group 5A 60. Both produce large dark pycnidia- or perithecia-like bodies which resisted all attempts to make them produce spores, even to cultivation on bits of sterilized leaves, stems, etc. The most numerous genera of fungi in order of appearance were: 5 A 35, *Hormodendrum*, 5A 60, *Basisporium*, *Penicillium*, *Phomopsis*, yeast-like, *Phoma*, *Aspergillus*, *Fusarium*, *Pullularia*, *Helminthosporium*, *Stemphylium*, and *Pestalozzia* (see Table III). These genera make up 1,184 colonies, or 65 per cent of the total fungi identified. Other genera which appeared less frequently were *Ustilago*, *Monilia sitophila*, *Stachybotrys*, *Diplodia*, *Heterosporium*, *Zygosporium*, *Spondylocadium*, *Trichoderma*, *Spicaria*, *Rhizopus*, *Pringsheimia*, *Alternaria*, *Verticillium*, *Periconia*, *Fusidium*, *Cladosporium*, *Clasterosporium*, *Cephalosporium*, *Trichosporum*, *Sporotrichum*, *Scopulariopsis*, *Tetraploa*, *Pyrenochaeta*, *Mucor*, *Mycogone*, *Mycosphaerella*, *Graphium*, *Beltrania*, *Acrotheca*, and *Trichosporon*.

TABLE III. MOST COMMON GENERA OF FUNGI IN FIFTY WEEKS

GENERA	NO. OF COLONIES	NO. OF APPEARANCES
1. 5A 35	298	26
2. <i>Hormodendrum</i>	219	37
3. 5 A 60	141	12
4. <i>Basisporium</i> *	97	28
5. <i>Penicillium</i>	96	12
6. <i>Phomopsis</i>	72	15
7. Yeast-like†	50	22
8. <i>Phoma</i>	41	11
9. <i>Aspergillus</i>	38	16
10. <i>Fusarium</i>	34	11
11. <i>Pullularia</i>	30	9
12. <i>Helminthosporium</i> ‡	24	11
13. <i>Stemphylium</i>	23	11
14. <i>Pestalozzia</i>	21	9
Total	1,184	

These fourteen main genera make up 64.9 per cent of the total number of isolated colonies (1,823), or 91 per cent of the total number of identified colonies.

**Basisporium* includes *Nigrospora*.

†Includes pigmented and nonpigmented yeasts and *Candida*.

‡Includes the genus known as *Curvularia*.

There were no particular seasonal relationships in the appearance of individual fungus genera, with the sole exception of fungus 5A 60 which appeared only from December to June. The genus *Alternaria* was twenty-eighth

in the order of number of appearances and accounted only for 0.2 per cent of the colonies that were identified. Table III shows that the fungus groups dominant in total number of colonies are also dominant in number of appearances. This had been previously noted by Collier and Ferguson⁸ in Brunswick, Ga.

The pollen counts were uniformly low throughout the year. Pollen counts of over 6 grains per square centimeter were obtained in only twenty-seven days; these counts occurred on four consecutive days in June and on three days in August, while the other "high" counts occurred on isolated days throughout the year. Because of the low pollen counts, no attempt was made at a botanical classification of the pollen grains. A set of slides exposed in October, 1956, was examined by Mr. Oren C. Durham. Grass pollens were most frequently found, and a few tree pollens were noted.⁹

The slides were studied daily for the presence of recognizable insect particles from Sept. 15, 1956, to Jan. 31, 1957. A total of 439 particles were identified in 133 days, rendering an average daily count of 3.3 particles.

CLINICAL RESULTS

There were 223 cases of bronchial asthma and 77 of allergic rhinitis in the 300 patients studied. The composition of the series by age, sex, and duration of illness is summarized in Table IV. The predominance of children and young adults reflects the selection of cases showing clear-cut extrinsic allergy. The high incidence of reactivity to skin test allergens is shown by the reaction of the group to house dust (Endo's dust): 17 per cent showed positive scratch tests; 16 per cent gave 4+ intradermal, 57 per cent, 3+; 8.67 per cent, 2+; and only 0.67 per cent (two cases gave 1+ or negative reactions to this potent antigen).

TABLE IV. COMPOSITION OF CLINICAL SERIES OF 300 CASES (AVERAGE AGE—20.7 YR.; AVERAGE DURATION OF ILLNESS—7.1 YR.)

	BRONCHIAL ASTHMA—223 CASES AVERAGE AGE—18.2 YR. AVERAGE DURATION OF ILLNESS— 7.9 YR.		ALLERGIC RHINITIS—77 CASES AVERAGE AGE—23.2 YR. AVERAGE DURATION OF ILLNESS— 6.3 YR.	
	MALES (100 CASES)	FEMALES (123 CASES)	MALES (34 CASES)	FEMALES (43 CASES)
Age range	3-58 yr.	3-65 yr.	5-46 yr.	4-47 yr.
Average age	13.7 yr.	22.8 yr.	22.0 yr.	24.4 yr.
Duration of illness	1-39 yr.	1-40 yr.	—	1-20 yr.
Average duration	6.1 yr.	9.7 yr.	5.7 yr.	7.0 yr.

Seasonal Variations of Allergic Symptoms.—Practically all the patients had year-round allergic symptoms. Sixty per cent observed no seasonal differences at all; 5.7 per cent had year-round symptoms but claimed to be worse in the summer; 26 per cent had symptoms in any season but had exacerbations in the winter (many of the latter were related to respiratory infections). Only one patient in the 300 claimed to have symptoms in one particular month of the year, the reason for which was not explained.

Fungus Allergy in Individual Patients.—A history of exacerbation of allergic symptoms on entering damp places, such as old homes and closets, or handling objects, such as moldy clothes or mildewed foods, was considered suggestive of fungus allergy.¹⁰ All the patients were tested intracutaneously with extracts from cultures of eight genera of fungi, which include about 60 per cent of the genera identified in Puerto Rico, and reactions were graded as strongly positive (3 to 4+), moderately positive (2+), weakly positive (1+), and negative. On this basis, the following criteria for the presence and importance of fungus allergy in individual patients were established:

1. Fungus allergy important:

Positive history and strongly positive skin tests.

2. Fungus allergy contributory:

Positive history and moderately positive skin tests.

3. Fungus allergy questionable:

Positive history and weakly positive skin tests, or negative or doubtful history and strongly positive skin tests.

4. Fungus allergy absent:

Positive history with negative skin tests, or negative history with moderate or weak positive skin tests, or negative history with negative skin tests.

Fungus allergy was considered an important factor in 6.3 per cent of the entire group (asthma and allergic rhinitis), and a contributory factor in 19.7 per cent; thus, fungus allergy appeared to be significant in 26 per cent or about one fourth of all cases. Twenty per cent had questionable fungus allergy. In 54 per cent no fungus allergy was found but there was clinical and skin test evidence of allergy to universally occurring allergens such as dust, other inhalants, and foods.

Comparison of Allergic Symptoms in the Same Patients Living In and Out of Puerto Rico.—Sixty-five of the 300 patients had traveled to the temperate zones (United States or Europe) after the onset of their allergic symptoms (Table V). Forty-three patients claimed that there was no change in symptoms in or out of Puerto Rico, and showed almost identical evidence of fungus allergy (25.6 per cent) as that of the entire group of 300 patients. Of the 22 patients who improved while away from Puerto Rico, 50 per cent appeared to show important or contributory fungus allergy.

TABLE V. INCIDENCE OF FUNGUS ALLERGY

	SYMPTOMS IN AND OUT OF PUERTO RICO (43 CASES)	SYMPTOMS ONLY IN PUERTO RICO (22 CASES)	ENTIRE SERIES (300 CASES)
Important	4.6% }	9.1% }	6.3% }
Contributory	21.0% }	40.9% }	19.7% }
Questionable	23.2%	13.6%	20.3%
None	51.2%	36.4%	53.7%

DISCUSSION

The survey here reported demonstrates that fungus spores are the most common air-borne allergens of Puerto Rico. The fungus spore count, both by the slide and colony-plate methods, show day-to-day variations but there are no demonstrable seasonal differences. In the absence of any relationship to corresponding meteorological data, the high fungus counts in the suburban region could be related to the rich vegetation in the area involved, which would favor fungus growth. Similar results were obtained by Collier and Ferguson⁸ in the Brunswick, Ga., area and by Wallace, Weaver and Scherago¹¹ in the Lexington, Ky., area.

We found an average of 36.5 colonies per plate throughout the year, while Myers,¹² in Honolulu, reported a total of 1,803 colonies in 248 plates exposed over a period of sixteen months, giving a lower average of 13.6 colonies per plate. Since both these surveys were done according to the standard procedure recommended by the Sub-Committee on Mold Survey of the American Academy of Allergy, the results are comparable.

It is interesting to note that the genus *Alternaria* appeared in Puerto Rico only five times in fifty exposures, and that it produced less than 1 per cent of the total number of colonies isolated. This is in striking contrast to the situation in Canada,¹³ the United States,¹⁴ and Mexico and Cuba,¹⁵ where *Alternaria* is one of the genera appearing most frequently. However, the situation in other tropical countries, such as Costa Rica,¹⁶ Brazil (Rio de Janeiro),¹⁷ and Honolulu,¹² where *Alternaria* made up less than 1 per cent of the total fungi isolated, is similar to that of Puerto Rico. We isolated Actinomycetes. Targow and Plunkett¹⁸ found them to make up 6 to 7 per cent of the total count in their study carried out in 1940-1945 in the Los Angeles, Calif., area. They remark that, in other current reports, Actinomyces make up 1 per cent or less of the total fungi isolated. They consider this fact difficult to understand in view of the widespread distribution of these organisms in the soil. It must be noted that they employed Sabouraud's medium and a thirty minute exposure period. Gordon¹⁹ in his 1953-1954 survey of the Atlanta, Ga., area found that Streptomyces ranked with *Alternaria* and Hormodendrum as the three consistent dominants as to total number of isolates, frequency of appearance, and frequency of dominance on any one day. He used twenty minute exposure periods and Czapek-Dox or potato dextrose agar. However, Myers¹² in Honolulu and Quintero-Fossas in Cuba¹⁵ do not list Actinomycetes at all, and Salazar Mallen in Mexico¹⁵ and Evans and Ruiz¹⁶ in Costa Rica report them as comprising only 2.1 and 1.5 per cent of the total fungi isolated. As suggested by Targow and Plunkett,¹⁸ this discrepancy may be due to the fact that slow-growing Actinomycetes may be masked by more luxuriant growers and thus pass unnoticed.

Hormodendrum, *Penicillium*, and *Aspergillus* seem to be ubiquitous. Assuming that pollen counts of 6 to 11 grains per square centimeter are necessary to cause symptoms in allergic patients,²⁰ the pollen counts obtained in this study appear to be too low to have clinical significance.

Plans capable of producing allergenic pollen do occur on the island. Durham has identified some ragweed plants on the north shore about sixty miles from San Juan. In view of the low pollen counts, residence in the immediate vicinity of any such plants would be necessary for the development of symptoms in sensitive persons. We know of no instance of clinical ragweed sensitivity in patients who never left the island.

The lack of definite seasonal exacerbations in the patients studied is in agreement with the similar absence of differences found in the air-borne allergen survey. This is in sharp contrast with the experiences with seasonal asthma and hay fever in the temperate zones. Fungus allergy appears to be a significant factor in the over-all problem of the patients studied. The doubled incidence of fungus allergy in those patients whose symptoms were improved when they were in the temperate zones suggests the importance of fungi as regional allergens in Puerto Rico. In over half of the series, the problems encountered were similar to those found in allergy practice in any part of the world. Rarely have we seen isolated instances of sensitivity to unusual allergens, such as bat excrement, pollen from the mango tree, or foods, such as the mango and guava. These patients constitute an insignificant portion of the allergic population of Puerto Rico. The findings of insect particles on the slides suggests the possibility that there may be cases of hypersensitivity to insect allergens on the island.

Allergic patients coming to Puerto Rico from the United States should generally expect relief of symptoms due to pollen sensitivity. Patients with fungus allergy may have persistence of their symptoms; for those living on the island, residence in the immediate vicinity of the ocean may be beneficial. The year-round pleasant, warm weather would make the island a satisfactory place for patients with chronic asthma and respiratory infection who develop exacerbations of their disease in cold and inclement weather. The findings of this study could well be applicable to other tropical islands geographically similar to Puerto Rico.

SUMMARY

1. A one-year aeroallergen survey of San Juan, Puerto Rico, is presented.
 - a. Fungus spores are the most commonly found aeroallergens in Puerto Rico.
 - b. The pollen counts are too low to be clinically significant.
 - c. There are marked day-to-day variations in the fungus and pollen count, but there are no seasonal differences.
 - d. The most commonly encountered fungi are: *Hormodendrum*, *Basisporium*, *Penicillium*, and two unidentifiable groups.
2. Clinical findings of 300 patients with respiratory allergy living in Puerto Rico are evaluated.
 - a. There is no seasonal variation of allergic symptoms.
 - b. Fungus allergy appears to be a significant factor in one fourth of all patients and in half of those patients who traveled to the temperate zones and obtained relief of symptoms.

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