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Common Airborne Allergens and their clinical relevance in the Caracas valley.

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Abstract. In order to determine the sequential prevalence of pollen grains and fungi spores in the city of Caracas by the volumetric method of collection and its effect on the population at risk, the first multidisciplinary team of aerobiological research was structured through a protocol designed for five experimental stages namely: a) Determination of the climatological parameters :temperature, relative humidity, speed and wind direction, precipitation and atmospheric stability, through simultaneous records of meteorological status located in the metropolitan area of Caracas; b) Collection, identification, classification and determination of the local distribution of the most important pollen grains existing in the zone under study; c) Collection, identification and classification of the most important fungi spores in the area; d) Preparation of the first pollinic calendar of Caracas and e) Evaluation of possible implication of the environment and the response through the IgE antibody in the selected patients in the area under study. Our results show: 1) The climatic conditions existing during the sampling period coincide with the analysis of the last 20 years in the Valley of Caracas. 2) The Venezuelan Central University (UCV) station was elected as the most representative point for permanent sampling. 3) An ideal statistical method is obtained in order to determine the spatial arrangement in the sampler rod of a dense type of fungi spores typical of the area under study. 4) The first pollinic calendar of Caracas was structured and 5) A seasonal tendency of the IgE response is shown. These results suggest an evident interrelation between the suggested concept of poliseasonality and antigenic polysensitization, and between individual seasonality with a specific reactivity and, finally, between tropical mixed seasonality with the expression of combined respiratory pathologies in our environment.

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INTRODUCTION

Severity of allergic symptoms induced by hypersensitivity reactions in individuals previously sensitized to environmental allergens is related to the exposure and concentration degree in a given geographic area.

In the last decades, the amount of aerobiological studies has increased worldwide. Valuable knowledge of season, environmental concentrations and their relationship with metereological parameters have been obtained (59). This has made possible that some countries or regions could report about the implementation of forecast methods against the adverse influence of environment and its contents of aeroallergens that can serve patients affected by these airborne particles (11).

Predictive information is based upon the correlations between the specific material, its interaction with climatological factors, and the severity of symptoms in patients at risk. However, such associations are not as simple as they seem, due to the influence of a great number of variables that must be taken into consideration. The application of this type of sequential and statistically significant observations enhances the use of even more rational and specific therapeutic measures in sensitized population.

In Venezuela, the presence and importance of aeroallergens in different regions of the country has been documented, trying to determine the concentration of aeroallergens in such regions. The importance of pollen in *Cecropia* by the gravimetric method was reported (24); pollen of *Melinis minutiflora* was obtained by means of an interesting volumetric collection work (Rotorod) carried out for five years consecutively (6). In Maracaibo, the presence of gramineous pollens in the air by the gravimetric method was reported (56). In such study, the predominance of *Cynodon dactylon* and *Dactylis glomerata* was perceived. Furthermore, pollen of Ambrosia, among others, is abundantly present in June, October and November. In a more recent study (23), through an aero sampling made by a gravimetric method of a suburban region of Caracas (Altos de Pipe), it was possible to determine that gramineous pollens show a seasonal peak between the last two months of the year.

In relation to mold spores collection in Caracas by means of exposure of Petri plates, the presence of different genus, being *Penicillium* the most prevalent, was reported (33). Besides, in Maracaibo a greater concentration of *Aspergillus* spores was found (32).

Allergic diseases are one of the most significant problems of public health that affect economic and social fields. In 1961, the prevalence of 34.6% in a school population in Caracas was reported (5), and in 1980 a value of 52.4% in a similar population was determined (2), finding a polysensitizing pattern of combined allergic diseases. Isturiz G., et al (personal communication) reported a 20.8% prevalence of allergic diseases in the population of the northern coast of Venezuela, and the result from the first multicentric study of prevalence of allergic diseases in different states of the country was 43.2% (31). In relation to specific sensitization towards different selected allergens in various socioeconomic strata of the tropical environment, an specific reactivity was demostrated (29), as well as a seasonal trend of IgE response through immunodiagnostic tests of a population at risk selected from the Caracas valley (63).

A valuable knowledge of aerobiology exists in the country, and at the same time we are aware of the research limitations of the aerobiological research in relation to the studied areas, collection time, equipment and methodology of analysis used, wich makes it difficult to correlate the results obtained from them.

In 1982, the Department of Allergic Diseases at the Venezuelan National Immunology Institute (SAS, UCV) started the first multidisciplinary research project on aerobiology of pollen grains and fungi spores existing in the Caracas atmosphere, and their relationship with allergic diseases. This project was carried out by a continuous counting within the urban area, inform correlation methods, estimation and prediction, wich allow to establish comparisons between the local records and their possible relationship with international parameters. Their results are the subject of this paper.

MATERIALS AND METHODS

1. Determination of meteorological parameters: temperature, relative humidity, speed and direction of the wind, rain, radiation media and atmospheric stability. These records were simultaneously gathered in equidistant stations distributed in the metropolitan area of Caracas, in order to determine the influence of these parameters over the degree of dispersion and transportation of aeroallergens in the area studied, as well as to select the most representative sampling station in the area.

2. Recovery, identification and classification of atmospheric pollen grains and determination of local distribution, as well as blooming time of the most important plants in the area. Relevant aspects included: sampling techniques, counting and identification of aeroallergens, production of an ilustrated atlas with the most important types of pollen and pictures (using a light and electronic microscope), creation of distribution maps of the most prevalent and important pollen grains, together with a collection of acetolized grains of pollen and died ones with special coloring (first aerobiologic palinoteca).

3. Preparation and structure of the first pollinic calendar of Caracas.

4. Identification and classification of sampling environmental fungi spores, using instruments selected and standardized, as well as the application of culture techniques in the laboratory.

5. Evaluation of the possible immunoclinic interrelationship between the environment (pollen grains and fungi spores) and population at risk, through immunodiagnostic evaluation in vivo and in vitro, with standardized and selected extracts for the study.

Meteorological parameters

In order to obtain a representative aerobiological sampling of Caracas, environmental factors that influence production, dispersion and transportation of aeroallergens, were taken into consideration. In this sense, the evaluation of topographic, climatologic and vegetation criteria, representative of the area under study, was carried out.

Topographic criteria: The area considered in this research was comprised betweeen coordinates 10°24'-10°33' N and meridians 66°44'-67°00' W with heights between 800 and 1000 above sea level. The city is located in the rear of the Guaire river, in an elongated form and aligning in direction WNW ESE, in the section corresponding to the urbanized area, wich is called the metropolitan Caracas area.

Climatologic criteria:
Climatologic data were obtained from the meteorologic stations network operated by the Venezuelan Air Force (FAV) and the Army of Venezuela. In those stations, experts make measurements of temperature by means of maximum, minimum and normal thermometers; relative humidity (thermohygrograph and psychrometer); wind (anemograph); rain (pluviograph); sun radiation (actinograph). The atmospheric stability was determined based upon isolation (heliophanograph) and cloudiness (visual observation) approximating Paskill curves.

Climatological values, considered to begin the aerobiological study, were based upon the analysis of the normalized average of measurements carried out in different meterological stations located in representative places of the metropolitan area and used in a climatologic study of the Caracas valley (14): Altos de Ñoleón, El Jarillo, Llano de Cura, Pozo de Rosas, Izcaragua, Macarao, Sabaneta, Montalbán, Caricuao, Altos de Pipe, La Mariposa, San Diego, Carrizal, Los Teques, El Hatillo, Los Palos Grandes, Chacaíto, Petare, Los Venados, Los Castillitos, La Carlota, Catia and Prados del Este.

The climatological study was updated in 1988, taking into consideration the following stations: La Carlota (Venezuelan Air Force FAV), Cagigal (Navy), the Department of Meteorology and Hydrology (Venezuelan Central University-UCV) and Los Castillitos (Navy), as well as the results of the climatological atlas of Venezuela (1).

Vegetation criteria: The types of vegetation were studied along the north, south and rear parts of the valley slopes. Besides, the scientists collected samples of different pollen grains and used them as reference patterns in the pallinologic study, and prepared slides wich were sent to the Aerobiology Laboratory at the UCV, laboratory of palleo-ecology (IVIC) and Venezuelan National-Herbarium (VNH). Evident characteristics of the pollen grains (form, number, type and position of the openings) were observed with an optical microscope and eyepiece 40X, whereas for the finest details (ornamentation, relative thickness of the exine layers), an eyepiece 100X was used.

Selection of the central sampling station: Due to orientation of the valley and wind prevalence in the area (SE, SSE, and ESE), three aerobiologic sampling places were chosen in order to determine a sampling central collection point of the particulate material, representative of the area under study. Therefore, pollen grains and fungi spores were collected every 6 minutes during 24 hours, by means of a Rotorod sampler placed in each area, as shown in Fig. 1. The Carlota (C) station at 8 5 meters (m) above sea level and at 4 m. over terrestrial roads; station UCV located in the internal campus of the Venezuelan Central University at 890 meters above sea level, situated at 20 m. of the street, and the station of the Cagigal Observatory (CO) at 835 m. above sea level located at 25 m. of the soil surface. From the first station C to the second UCV, there is a distance of 2.5Km. and from the second UCV to the third one CO, 2 Km. The criteria to select the sampling places included: good representation of the area, distribution of vegetation, accessibility, use of electric energy and closeness to a meteorological station (Class C1) wich provided very important climatological data for the aerobiological sampling.

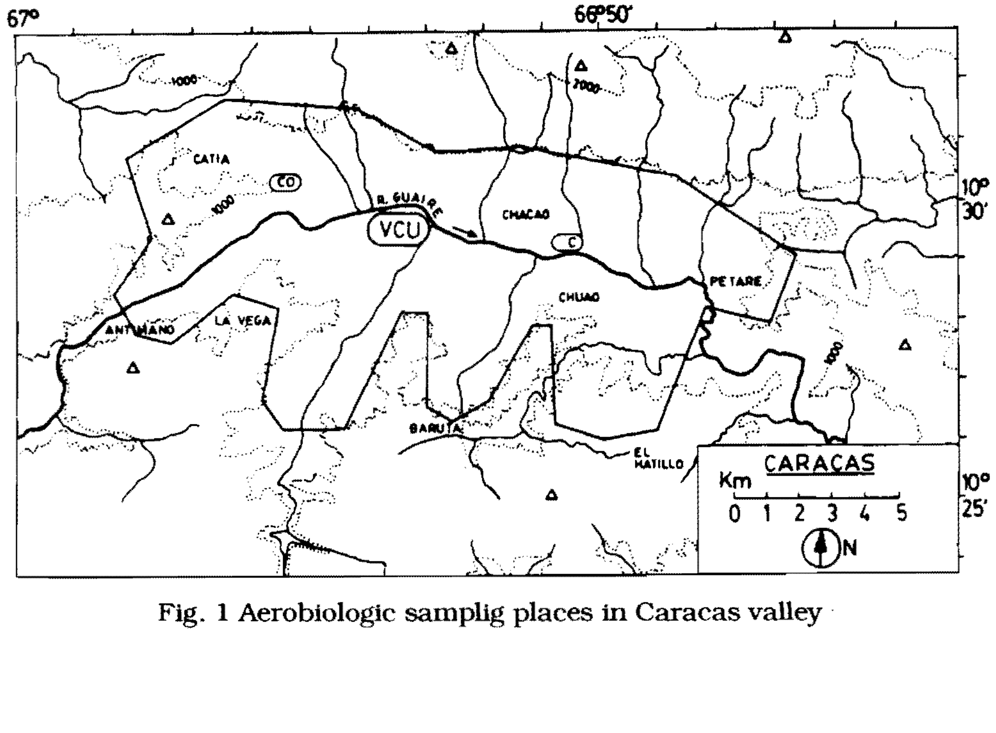


Fig. 1 Aerobiologic samplig places in Caracas valley

Sampling methods: Since the volumetric techniques are better indicators of particle prevalence due to satisfactory recoveries of pollen grains and larger spores (58), they were selected to determine the abundance of aeroallergens. The data obtained by the volumetric method was adapted to be compared with those obtained in other latitudes (18).

The effectiveness of the volumetric samplers obviates the influence of important deviations in the collection of particles of great size, such as speed effect, and wind direction and turbulence.

The collector was a Rotorod (Ted Brown Associates, Los Altos Hills, Calif., USA (39) used with intervals of 6 minutes for 24 hours per area during the preliminary selection study of the most representive stations. Pollen grains and fungi spores captured, identified and classified through reference samples, were prepared for each sampling station, according to conventional methods. This collector is an impactor of particles able to collect particulate pollens and fungi spores in small acrilic thin sticks which turn at 2400 rpm., using the intermittent form which guarantees the efficiency of both mechanical impactors and suction devices to recover aerosol of unequal sizes.

Sample counting: The two acrylic cylinders, which form the sampling gathering surface, are covered by a silicon fine layer to guarantee the retention of impacted particles. Once the sampling is concluded, the particles fixed in the adhesive- coated of the cylinders, its adherent particles are placed in a slide specially for this purpose (7), with 2 drops of Carberla solution, a slide cover, the sample is observed under an optical microscope (Zeiss WL) with an 8X ocular and 40X eyepiece.

The counting of pollen grains was carried out with a 40X optical microscope. Once the total number of collected particles was determined for each type of pollen, concentration by m^3 of sampled air (47) was determined and photomicrographies were taken with a Zeiss III photomicroscope and an Ilford FP4 black and white film type.

The space distribution pattern of particles collected by the Rotorod sampler was evaluated, based upon the establishment of the characteristics and number of subsamples which allowed an estimation of spatial or temporal comparisons, using standardized procedures and calculations with a high efficiency.

In a low density sampling collection by the Rotorod, the total particles were enumerated. Space arrangement observed in the different samples for pollens allowed to count all the grains adhered to the rods.

It is usual to extrapolate the counting of the total number of particles of one or more transverse transepts of the Rotorod cylinders, independent of the space location. However, at the observation of high density particle deposits in which the individual counting was impossible, it is necessary to perform a second estimation. For this reason, the way to determine efficiently the space disposition of such conglomerates was researched. Seventy samples (150 rods) were collected and analyzed at the sampling central station for deposit situation of the homogeneous particles and conglomerates (dense). Application of rigurous statistic methods allowed to carry out a particular analysis of this type of disposition for fungi spores at the Valley of Caracas, using the Rotorod sampler.

The grain shape was described using a modified (54) polar length index/equatorial length (P/E) (15). Terminology used to describe the countour with polar view (34), and for the remaining of the morphologic description (15). In cases in which pollen settled in an unfavorable position to visualize some outstandinng morphologic characteristics, they were mechanically rotated. Measurements were carried out using a Leitz ocular with a micrometric vernier. In population concentrations of less than 25 grains of pollen, an approximated numerical value was obtained and when the frequency of the polynic type allowed, measures were taken corresponding to 30 grains.

Identification of pollinic types and their corresponding taxonomic groups up to the minor possible category were carried out, using the collectionn of reference and specialized bibliography (14,26,52). Classification was confirmed through comparison with photographies, illustrations, descriptions, acetolized material, herborized field collections and consultations with palinologists.

By means of methods of multiple computarized correlations, possible interrelations were examined betweeen concentration of pollen grains and meteorologic parameters. A digital computer VAX-780, program PCA (multiple componentes) was used. Critical values to establish the statiscal study were taken from the Rohlf Sokal tables (49).

Immunologic study

This phase of the study was oriented to the evaluation of aeroallergens and sensitized patients to them by means of a daily record of symptoms and their reaction to immunodiagnostic in vivo and in vitro tests:

1. Clinical history to identify allergic diseases with more than 600 items. After an explanation of the project and awritten and voluntary authorization, 20 natural patients residing at the valley of Caracas were selected. Those patients presented some symptoms induced by hypersensitivity reactions at the high respiratory tract such as bronchial asthma (cough, dyspnea, sibilance and diminishing of the volume expired in the first second (FEV_1) equal or higher than 15%)and allergic rhinitis (nasal pruritus, sneezing, nasal obstruction and/or rhinhorrea), possitive skin Prick tests (≥ 3 mm average of the papule) (37) and possitive RAST tests ($\geq 2+$) for selected allergens, besides laboratory tests (complete haematology, fresh faeces and egg concentrations, and urine test), bilateral nasal cytology and pulmonary function determining FEV_1 and forced expiratory flow (FEF_{25-75}) using the criterium of significance previously stated. Patients were evaluated each trimester, obtaining four observations during the collected time.

2. Daily record of symptoms for rhinitics and asthmatics, besides measurements (three times daily) in variations of pulmonary functions with

the mini-peak-flow-meter using conventional bronchodilator drugs. A daily record form for symptoms severity was designed with a value scale of 0 to 4 for later statistical analysis.

3. Total IgE levels (PRIST-Pharmacia) and specific (Prick and RAST) test. Skin prick tests were carried out with conventional (Hollister-Stier mixture of trees: *Eucaliptus sp, Cupressus sp, Casuarina equisetifolia* and *Acacia sp.: Cynodon dactylon*, grass mixture (*Poa annua, D. glomerulata* and *Agrostis alba*) and corresponding individuals: weed mixture (*Ambrosia artemisifolia, Ambrosia psilostachya, Ambrosia trifida* and *Ambrosia acanthicarpa*) and their corresponding individuals: mold mixture (*Aspergillus fumigatus, Penicillium, Cladosporium* and *Alternaria*) and respective individuals and *Dermatophagoides pteronnysinus*.

The determination of specific IgE by means of radioimmunoessay technique (RAST-Pharmacia) was widely described (8,38) for a limited number of allergens: *Cynodon dactylon, Ambrosia artemisifolia, Aspergillus fumigatus* and *Dermatophagoides pteronnysinus*.

Statistical analysis

Climatologic data were determined by direct readings of the measurement instruments. The relationships among metereologic parameters were obtained by means of a Pearson's coefficient of lineal correlation and regression analysis. In the selection of the central sampling station, Kendall's concordance coefficient and correlation coefficients were used. Determining of spacial particle distribution of medium and high density was, according to Cochran's criterium (12), followed by a distribution of chi- square with a distribution of n-1 grade of freedom in case of distribution of homogeneous particles and an applied correction to the Cochran criterium when the optimum number of particles was higher than 10%.

In the analysis of immunologic study, chi-square tests were applied and the student t for normal distribution. Determining of average values and standard deviation of IgE for logarythmic transformation (base 10) and Mann-Whitney test for correlations were performed. Pattern curves for certain laboratory techniques were structured calculating correlations by means of the lineal regression coefficient.

RESULTS

Through the analysis of climate in Caracas, it was possible to determine that it depends on different atmospheric changes that cause rain in the valley, and at the same time rule the annual behavior of all meteorological parameters. Among those changes or disturbances we can mention: the invasion of cold northern air, tropical waves, intertropical convergence, local type of convergence and hurricane season. The effect of mountains causes conditional unstability or generates stable situations with a higher limit of a humid layer up to 1000 m., causing long lasting rains.

The dry season extent is between December and April with an annual 10% contribution of the total rain. The rainy season covers the months of May to November with a contribution of 77%. The total annual rain oscillates between 500 and 1300 mm.

Results may be seen in Table I related to the periodical determination of different climatic elements, and average values of the main metereological parameters of the last 20 years in Caracas. Generally, higest temperatures occur between April and May and the lowest in January. Average wind speed is 2.1 m. and the main directions are SE, SSE and ESE in 50%, in 20% NW, NNW and WNW, calm occupy 25% and other directions 5% (20).

In spite of the fluctuations which rule the dry and rainy seasons in the Caracas valley, an inter-relationship between meteorological parameters and prevalence cycle of the particulate material in the study has been determined.

TABLE I.
AVERAGE OF THE MAIN METEREOLOGIC PARAMETERS IN THE CARACAS CITY

	Sampling Period 1984 (DEC)	1985 (JAN-DEC)	Last 20 Years 1964-1985 (JAN-DEC)
R.H. (%)	78	76.1	77.0
Mean T. (^{oC})	21.6	22.1(15.1-30.5)*	22.5(12.3-32.6)*
Rain (mm)	5.0	956.2	800(500-1300)*
Wind Direction	SE/NW	SE/ESE	SE/SSE/ESE
Wind Velocity	1.9	2.1	2.3
Sun Radiation (Kcal/cm^2/day)	5.8	6.5	7.3

RH: Relative Humidity
* Range Values are in parenthesis

Selection of the sampling central station

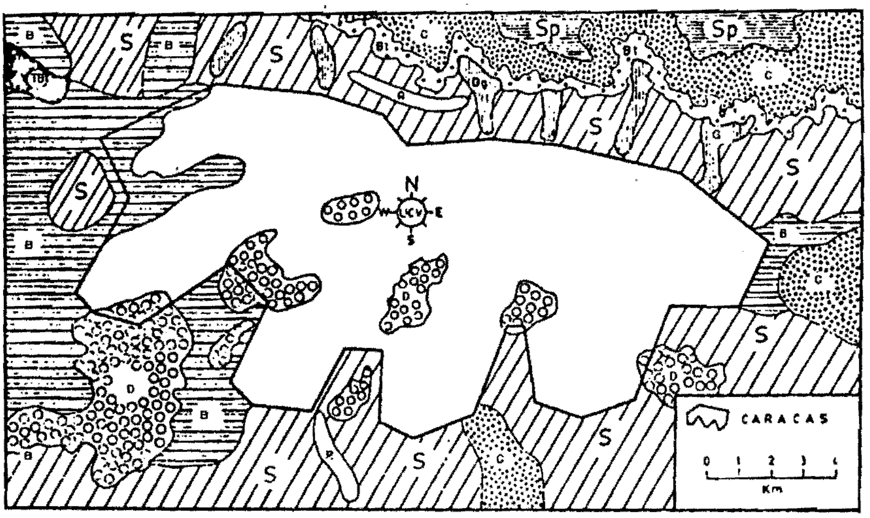
The number of pollen grains was similar in the three collection areas selected in the Valley of Caracas, but in a lower amount than the total fungi spores determined (43). *Cladosporium* spores and Gramineae pollens were the most abundant aeroallergens collected for the period of selection as the ideal point for permanent sampling station, representing 63% of the total of pollen grains collected. Kendall's coefficient for pollen grains was 0.834 with 8 degrees freedom and concordance probability $p > 0.95$. For the fungi spores it was 0.625 with 16 degrees freedom and $p > 0.98$. These results show that sampling and counting of particulate material in the three experimental stations were not done at random.

Other factors analyzed in relation to experimental points of air sampling show that station C, located at the local airport of the city, evidence a distorsion in the collections due to the turbulence of air induced by airplane traffic, besides the presence of a high degree of environmental pollution. Station CO, located at the astronomic observatory and with a greatest altitude, evidenced a high number of mold spores in relation to the pollen grains. The UCV station, which is in the center of the city, has all varieties of polen grains and fungi spores existing in other sampling stations. The influence of the Botanical Garden in the pollinic sampling was not significant, since the prevailing wind direction SE-SSE reaches the sampling station before getting to the mentioned area.

Pollen

In the north, south and rear end of the Valley of Caracas the folowing types of vegetation were observed: subparamo, tropical cloudy forest, transition forest, gallery forest, tropical decidual forest, savannah and bushy. In areas adjacent to the city dominate the savannah, decidual forest and bushy areas. In general, the east wing of the valley has a greater extension of cloudy forest while in the western area the dry and bushy forest dominate. Human intervention is noticed in the great extension of the savannah reforesting some slopes with exotic trees (*Pinus, Casuarina, Eucalyptus, Cupressus, Grevillea*). In the urban area there are native and exotic cultivated plants as well as Gramineae and ruderal herbs. Fig. 2.

After the standarization period of all the methodology to be used in this multidisciplinary research (1982-1984), the aerosampling period began in December 1984 and finished in November 1985, collecting 878.1 grains of pollen, which represents 87% of the total annual amount. Fig. 3 shows weekly averages of the concentration of pollen grains collected. Total concentration of pollens varies



S= Savannah B=Bushy D=Dry bushy T=Transient forest C=Cloudy forest
Sp = Subparamo Tb = Thorn bushes G = Gallery forest

Fig. 2.- Vegetation map of the Caracas Valley

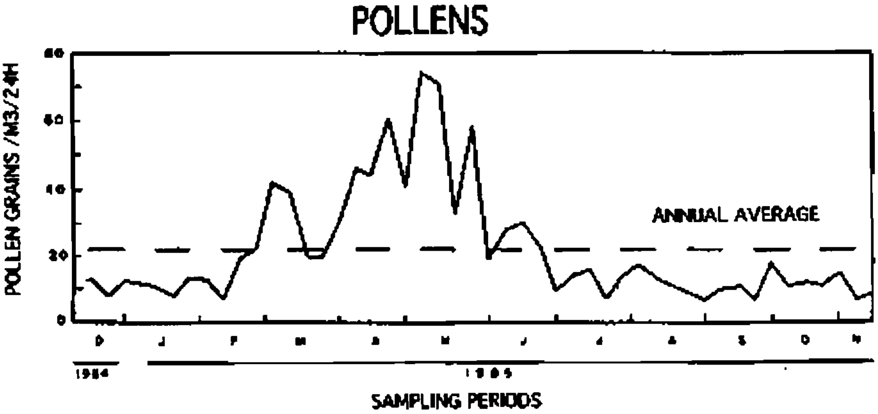


Fig. 3.- Pollen prevalence in the city of Caracas

between 1 to 118 grains/m^3/day with a mean value of 22.

In general, we may divide the results of the studied period in three periods (42). The first corresponds to December 1984 until February 1985, in which the pollen collected expressed low levels, with a progressive increase starting in March until reaching its highest value in the second period (March-June); the third, showed pollen concentration values similar to the one observed during the beginning period, extending the same from July until November of the same year.

Period I was characterized by the presence of pollen of *Mimosa, Casuarina, Cupressus*, Proteaceae and Gramineae in similar amounts and with sporadic appearances, and Cyperaceae, *Celtis* and *Cecropia* type. These two latter pollinic groups were responsible for the increase registered during February and March. Later on, Pollen of *Cecropia* became the dominating pollen grains until the final of the II period and beginning of the III period. The main accompanying pollens of *Cecropia* during the second period were mainly pollen of other Moraceas and Ulmaceas.

Greatest pollinic frequency is observed during the last week of April, with 68.9 grains/m^3, followed by the first week of May with 68.0 grains/m^3. Pollen of Ulmaceas and Moraceas represents 69% of the total aereal pollen gathered, being these families mainly responsible of the pollinic peaks from March to May. However, the secondary peak of June is mainly due to the contribution of Moraceae and Gramineae. Fig 4.

The third period was characterized by the dominance of Gramineae. All the pollinical types but *Cecropia* pollen, were present in a very low frequency or absent. Gramineae are relevant, however, during the sampling period exclusively during December 1984 and November 1985, this fact evidences an important contribution which surpassed the pollinic annual concentration media. The period which covers the present work does not truly reflect relevance of the Gramineae family, some of which

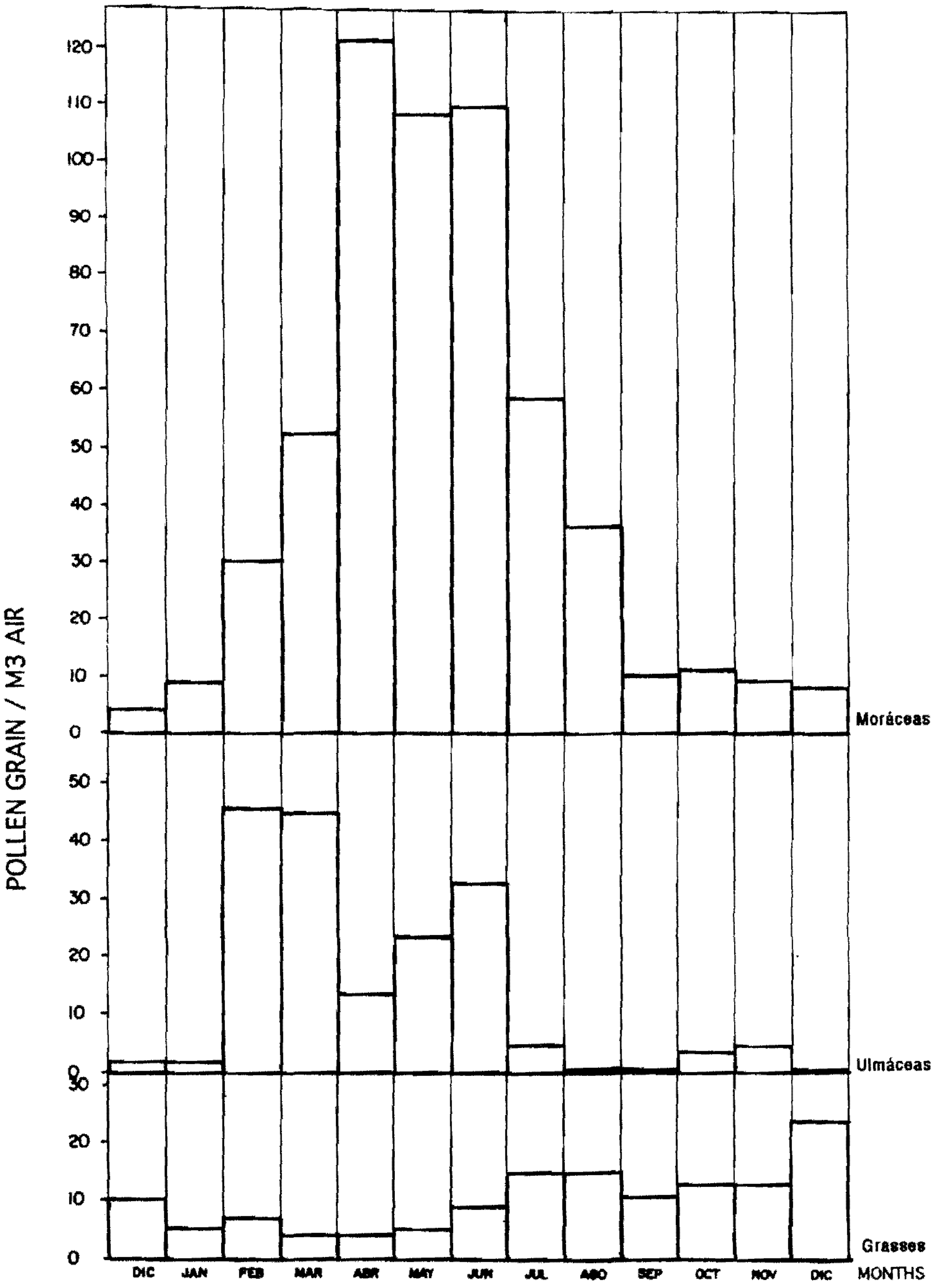


Fig. 4.- Pollinic frequency of mainly families

species bloomed in a notorious way in the landscape before the previously described months. It must be noticed that most of pollen grains belong to the Urticareae (*Cecropia, Celtis, Poulsenia, Trema, Sorocea, Chorophora*), which accounted for 61% of the total gathered pollen.

The three previously mentioned periods reflect a successive process of different types of pollen grains as expressed in Table II. The 50 morphologic types found were distributed in 16 families.

In relation to the morphologic characteristics of the pollen grains collected in the Valley of Caracas (9), morphologic description of the pollinic types comprised some plants such as: Amaranthaceae Family, type 1: *Amaranthus, Chenopodium, Ambrosioides*, type 2: *Alternanthera*, type 3: *Pfaffia*, Type 4: *Gomphrena*, Casuarinaceae family, *Casuarina* genus; Chenopodiaceae family, Compositae family, Cypressaceae family, *Cupressus* genus; Cyperaceae family, Euphorbiaceae family, species: *Ricinus communis*; Gramineae family; Leguminosae family, type: Acacia, Mimosaseae family; Moraceae family, species: *Cecropia palmatisecta*; species *Clorophora tinctoria*; species *Sorocea sprucei*; species *Poulsenia armata*; family Myrtaceae, Eucalyptus type; Palmae family; Proteaceae family; Sapindaceae family; type *Dodonaa viscosa*: type *Melicocca bijuga*; Ulmaceae family, type *Celtis iguanaea* type *Trema micrantha*; Urticaceae orders. Description of each pollinic types reported shall be matter of other publication due to the lengh of the subject. Observe the illustration in which the pollen of *Casuarina equisetifolia*, is presented MH, eyepiece 110/1.6 polar view (Fig. 5).

TABLE II

PERCENTAGES OF THE MOST IMPORTANT AIRBORNE POLLENS COLLECTED IN THE CARACAS CITY

Cecropia	33.1
T. Celtis	14.1
Graminae	13.9
T. Poulsenia	6.0
Leguminosae	4.4
T. Clorosphora	3.8
T. Sorocea	3.1
Cyperaceae	2.3
Myrtaceae	2.1
Cupressus	1.9
Casuarina	1.8
Mimosa	1.2
Melicocca	1.1
T. Trema	0.9
Proteaceae	0.7
Urticales	0.6
Others	9.0

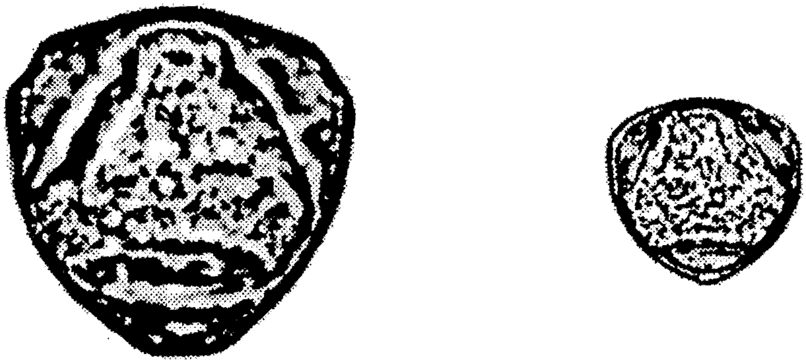


Fig. 5.- Pollen of Casuarina equisetifolia MH, eye piece 110/1.6 (left), and 40/2 (right) polar view

These results allowed to structure the bases of the first pollinic calendar of Caracas (50) (Fig. 6), as wellas classify the main plants (trees and weeds) whose pollinic frequencies were relevant during the air collecting periods (Table III).

Different meterorological facts affected the concentration of pollen grains in the air, observing a significant influence ($p < 0.05$) with sun radiation and temperature. However, an inverse relationship was determined with pluviousness. In Fig. 7 the levels of air

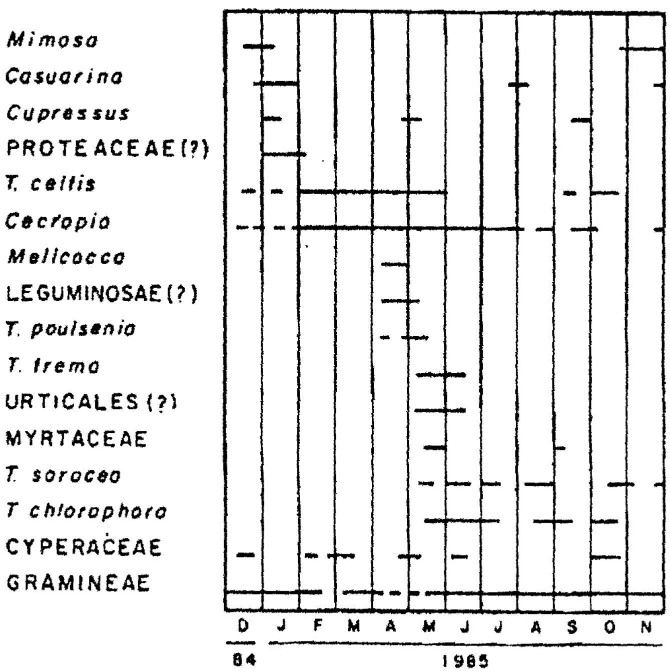


Fig. 6.- First airborne pollinic calendar of Caracas

TABLE III
CLASSIFICATON OF THE MAIN CARACAS ANEMOPHILOUS PLANTS

TREES		WEEDS	
Botanical name	Commom name	Botanical name	Common name
Cupressus sp	Ciprés	Compositae	
Cecropia palmatisecta	Yagrumo	Cyperaceae	Cyperaceae
Trema micrantha			
Poulsenia armata	Poulsenia armata		
Sorocea sprucel	Charo macho		
Casuarina equisetifolia	"Pino" Pino casuapira		
Melicocca bijuga	"Mamón" Macai macao		
Celtis iguanaeae	Guacharo		
Chiocrophera tinetoria	"Mora" Palo de Mora		
Mimosa sp	Dormidera		

pollen grains reaching a high concentration during the dry months (20 to 120 grains/m^3/air) and an important lowering during humid months are shown. The greater amount of pollen (maximum level) was determined in a transition time to rain (April to June), with a high pollen collection mean, continuing the lowering until the end of the sampling year (November 1985) from 50 up to 689 grains/m^3/air.

Fungi

Three types of space arrangement of fungi spores in the attraction rods of the Rotorod were determined, contrary to the conventional methods, where it is assumed that there is always a distribution of homogeneous particles in the attraction surface. The uniform or regular type, where a steady distance is observed between particles; the type at random, where a number of particles for sample follows a Poisson distribution with the media equal to the variance and finally the aggregate types where the presence of high density areas combined with others of low density is observed. In this case, the variance is greater than the media.

It is shown (51) that previous estimations had an error between 5 and 15% approximately, counting a high number of bands, while counts based upon a lower number of it are not statiscally trustworthy. Generally speaking, the *Cladosporium* spores and ascospores showed a dense pattern in the majority of the samples, worthy of a very high band count, in order to obtain a precise application of the conventional statistical methods. When spores are deposited in the attraction rod at random, the count are rigurous and practical.

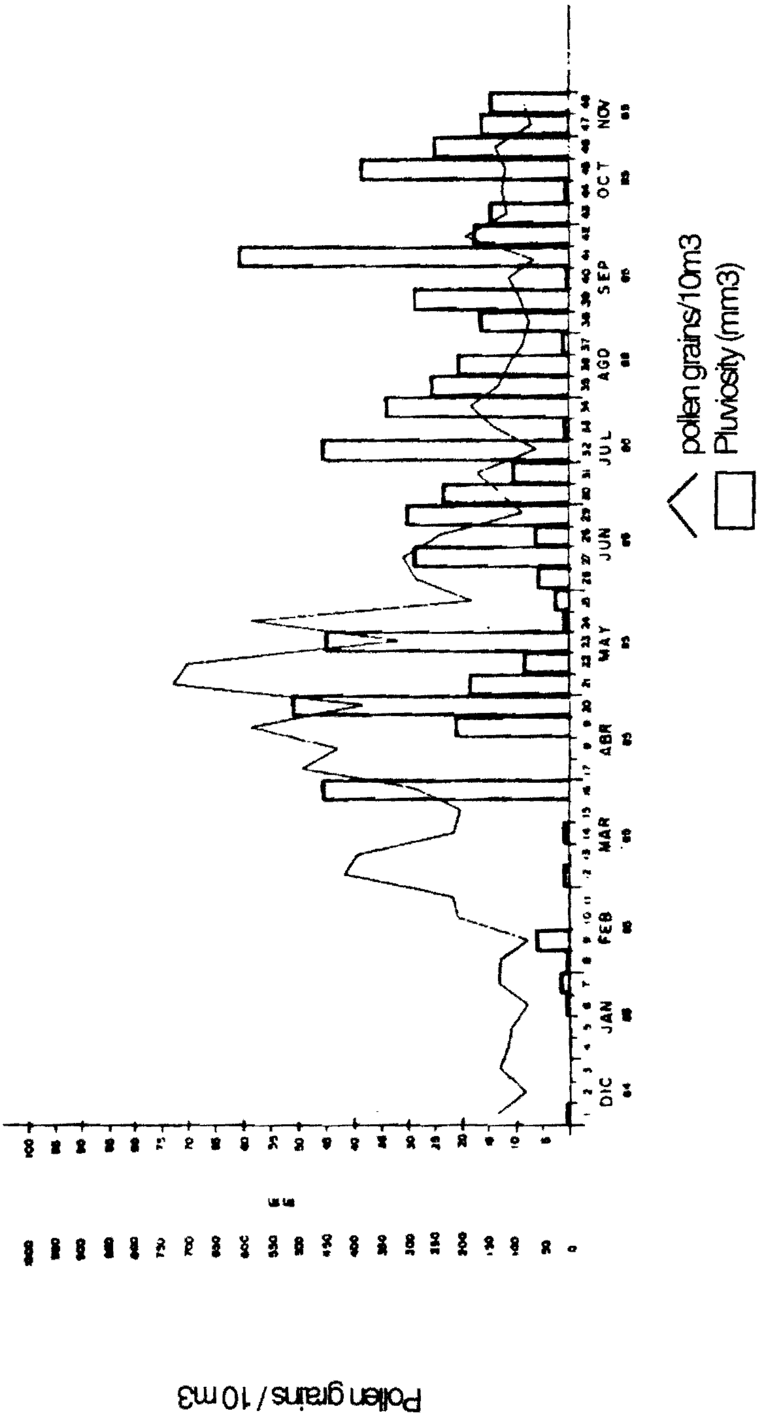


Fig. 7.- Air pollen concentration and season influences

This aerobiological study allowed to conclude, according to the application of distribution's criteria of Cochran and its correction, that for the reading of fungi spores deposited in a dense way it is important to take into consideration longitudinal bands with an equivalent surface to 15 transverse transects which include a longitudinal variability of the number of particles attracted.

Maximum concentration of fungi spores in the air of Caracas was determined in approximately 19.476/day and a minimum of 5.282 obtaining an average of $10.722/m^2$/day similar to the curve observed for the pollen grains. Mold spores begun the year with a concentration of 9.152 reaching progressively high levels starting in April and May. *Cladosporium* was the fungi which mainly contributed to determine the total concentration pattern in the air until June. *Ustilago* had irregular variations. Ascospores and basidiospores expressed a period of higher concentration from June to November (Fig. 8).

According to the environmental factors related to mold spores concentration in the Valley of Caracas, rain influenced significantly ($p< 0.05$), increasing its concentration during the rainy season in comparison with the dry season (Fig. 9).

Immunologic study

From 20 patients selected with respiratory allergic diseases, non helminth parasitized, 85% showed combined diseases such as bronchial asthma, and allergic rhinitis, divided in 17 asthmatics (6 extrinsic and 11 mixed types) and 3 perennial allergic rhinitis without other allergic diseases. From the total population selected 16 had allergic rhinitis (13 perennial and 3 seasonal types) and 4 patients with mixed bronchial asthma without other allergic diseases.

During the sequential evaluation (trimestral) the diagnosis changed from mixed to extrinsic type at the third term, as well as one patient showed a change.

A polisensitization was observed in a 75% of the patients studied by immunodiagnostic tests, to the allergens selected. From the total of individuals studied only three had no skin test reaction to *Dermatophagoides pteronnysinus*, two showed individual sensitization to Australian pine (*Casuarina equisetifolia*), and one patient to *Ambrosia artemisifolia* pollen grain.

The total sensitization prevalence from the fourth trimestral period showed: *Dermatophagoides pteronnysinus* (84%), mixed weed pollens (39%), mixed mold spores (35%), and mixed grass pollens (32%).

Specific sensitivities were high to Australian pine (*Casuarina equisetifolia*) 46%, *Acacia*, *Cladosporium*, and *Aspergillus fumigatus* 43% for each one.

Fig. 10 shows the results of the specfic IgE response in the selected population during the period studied. Significant results from a correlation scale symptoms and aeroallergens collected were obtained ($p < 0.05$). Fig 11 shows the correlation of pollen mean grain collection and allergic rhinitis symptoms scale and Fig. 12, the correlation between mold spores concentration and the severity scale of bronchial asthma symptoms.

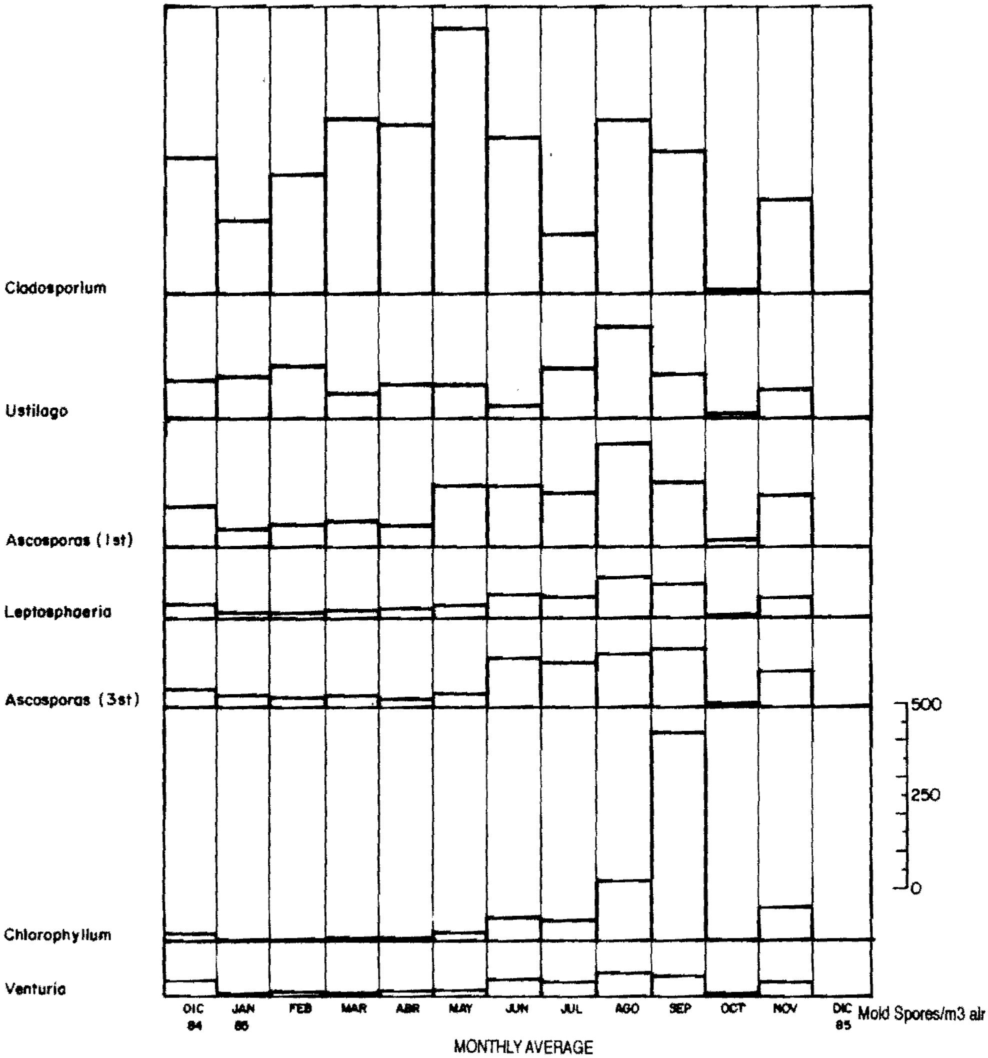


Fig. 8.- Prevalence of the main airborne mold spores of the Caracas area

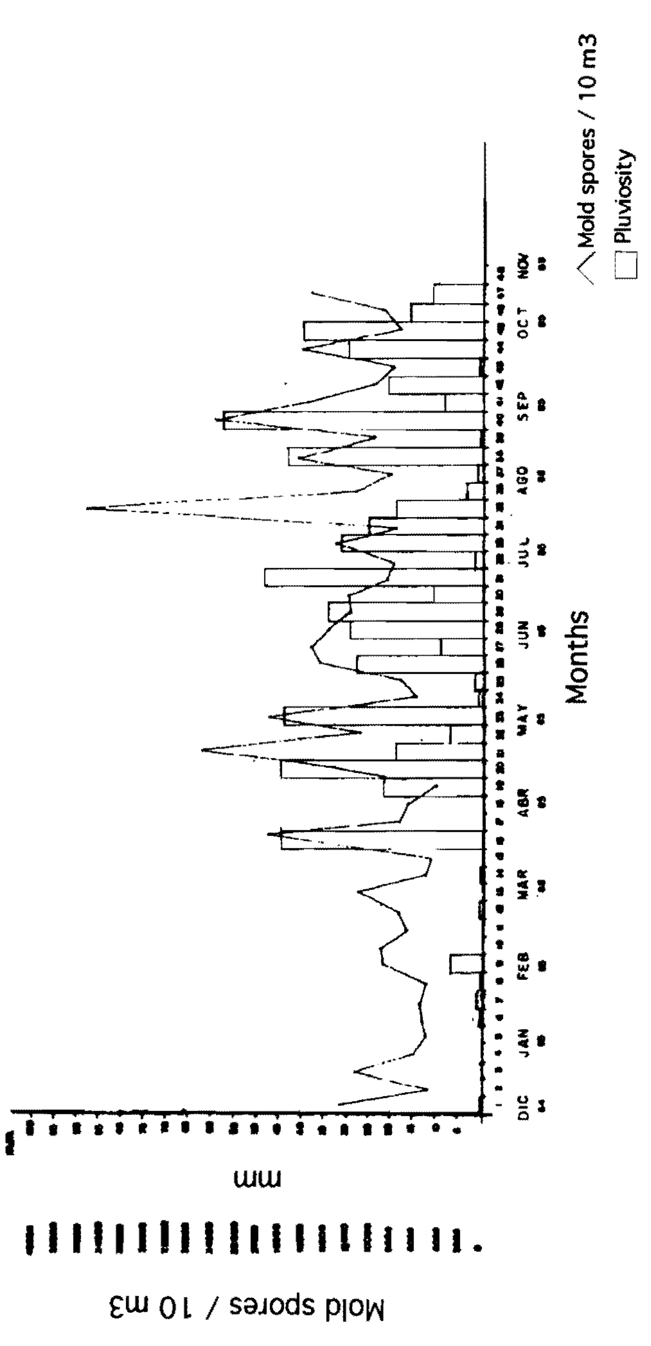


Fig. 9.- Environmental factors related to mold spores concentrations on the Caracas valley

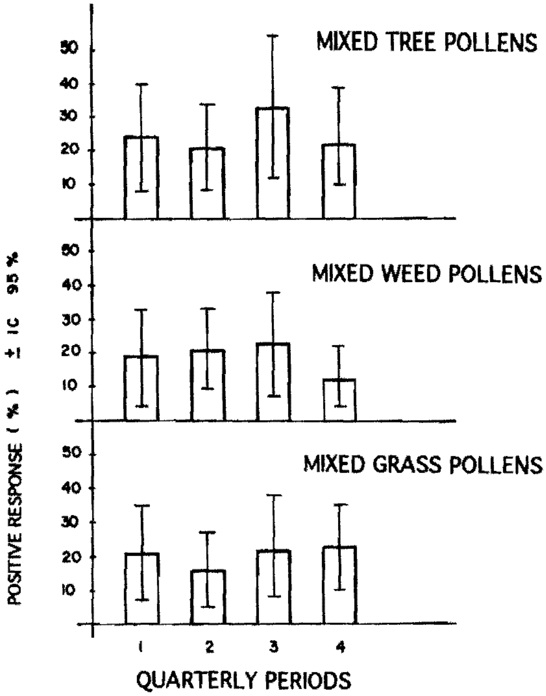


Fig. 10.- Population specific in vivo IgE response towards the selected allergens

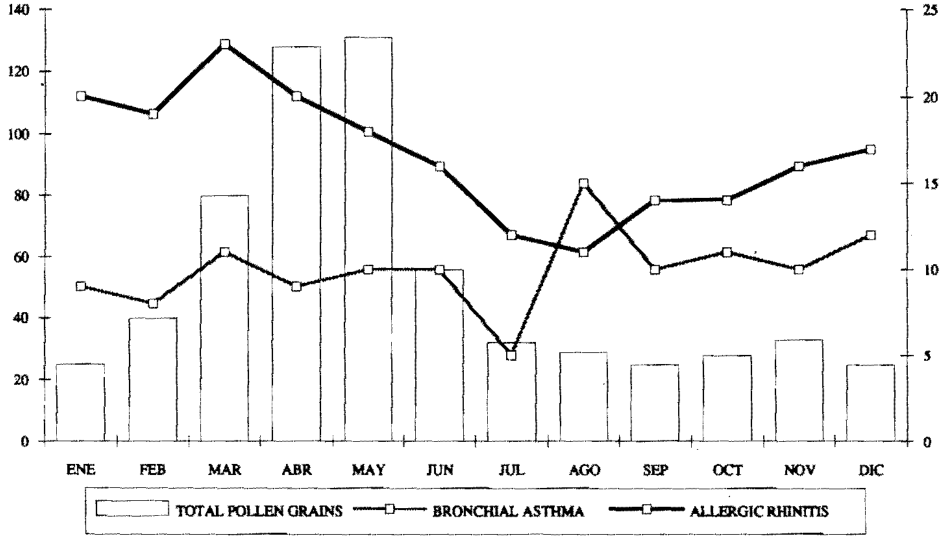


Fig. 11.- Correlation between respiratory allergic symptoms and monthly mean pollen grains count

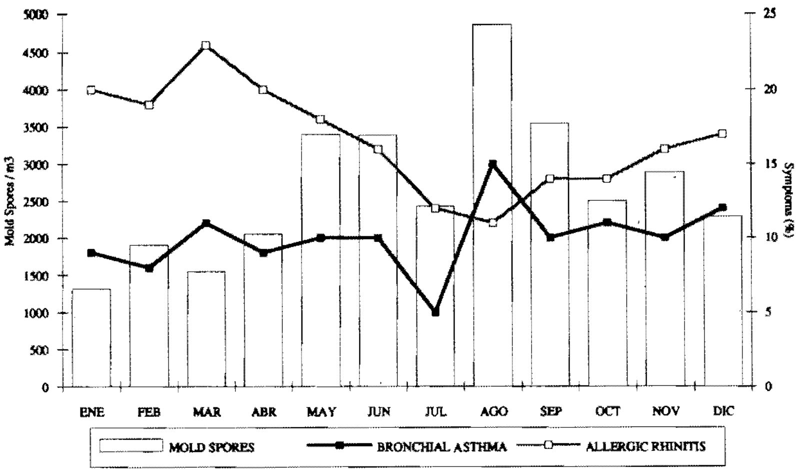


Fig. 12.- Correlation between respiratory allergic symptoms and monthly mean mold spores

DISCUSSION

Selection of the central sampling station

Although it was observed a significant statistical difference within the three eperimental stations during the 24 hours of aerobiological sampling, other considerations should be made, taking into account the number of collection equipments and the staff trained to analize, simultaneously, the study areas.

Results show that the three places are representative of the valley. However, the UCV station, due to its location and accessibility, was chosen as the ideal point for permanent sampling, that is related to the selection reports of central points of aerobiological sampling in the main cities of other latitudes (48).

Pollen grains

It was possible to identify 37 pollinic types from the 50 classes of morphologic pollen recognized in the atmosphere of the valley of Caracas. Those types provide a source of data for future studies. During this research, there was a lack of reports of detailed morphological identification of pollen of plants, pertaining to the zone under study, which serve as patterns of reference.

From the weekly report, it can be observed that *Cecropia* and *Celtis* genus are great producers of pollen, whereas there are other plants abundant in the area of collection that have a limited representativity due to their low production of pollen. Amaranthaceae, Myrtaceae and Compositae are in a low frecuency in the air, as well as Gramineae, although its representation in the studied area was proved. (Bolbochan D., In preparation).

Although a higher number of morphological types has been determined in relation to the number reported for the temperature zones (14), it does not reflect the diversity of plants in Caracas that characterize a model area of the tropic. This predominance has been evident thanks to the different previous works in which the sampling geographic area has been studied, the collection methodology of particles and the expression of results (grains/m^3 of air) in the country (6) and in other regions with similar characteristics were carried out (35). Besides, it was observed a predominance of anemophilous plants existing in the area under study, giving at the same time the higher concentration of pollen captured (76%).

In a research carried out in a region with a higher altitude (1500 - 1600 meters above the sea level) than Caracas and next to the Venezuelan capital (24), it was observed the presence in the air of pollinic types registered in the atmosphere of the metropolitan area. However, the abscence of other pollinic types (*Artocarpus*, *Proposia*, *Chrysanthemum*, *Miconia* and Polygalaceae), as well as the difference in the frequency values are mainly due to the involvement of different ecological habitats since these plants grow just in the mountains around the valley of Caracas (60).

Itis well known the great influence of temperature on the presence of pollen of seasonal plants in the air (11), similar to what it is observed in the stage of higher concentration of aerial pollen during 1985 and the athmospheric variables indicates the environmental conditions necessary to produce the opening of anthers of anemophilous plants, i.e. low rain and high average temperature (25), with the exception of gramineous plants which showed their highest concentration in conditions of high average humidity with a significance of 99% of reliance and a precipitation with a significance of a 95% of reliance (10).

These observations show the presence of seasonal changes related to the environmental temperature.

A significant relation between the prevailing wind direction (East) and the concentration of pollen existing in the vegetation of the easter area of Caracas was determined. Therefore, future research with models of dispersion are necessary.

The variability of different plants is related to the time of flowering and the pollination (53). Such factors are considered important and influential on their atmospheric concentration. Variability is also related to the floating phenomenon of the pollen grain and the future precipitation after the latency period towards inferior layers. Caracas has a significant annual bioseasonal variations with precipitations, which determines a division between the dry season (December-April) and the rainy season (May-November) (1). Some vegetal species flower at the beginning of the rainy season, other towards the middle of it, others during the transition period and other group at the beginning of the following season.

The values of pollen grains collected during the dry season, if compared to the rainy season, indicate that during the beginning of the latter season starts the flowering time of most of the plants in Caracas. In the records of the period beginning the last week of June up to November, it was observed a low concentration of total pollen due to the removal of particles produced by the rain (17,55).

In mild zones, flowering time are limited to specific seasons in contrast to the tropical areas, where there are plants with flowers and pollen during the whole year. It makes impossible to mark pollinic seasons without overlapping.

Species, in particular, have a characteristic period of flowering, mantaining levels of total pollen of the family while flowering season progress (25). This situation is reflected in the pollinic record of Maracaibo (56) and Caracas, where Graminae and Urticae are present during the whole year. There are two Graminae, *Axonopus pulcher* and *Melinis minutiflora* which differ from the rest of genus in the time and length of the period of flowering, and in the behavior related to environmental factors such as precipitation and day length (19).

The presence of a sole pollinic season, specially *Mellinis minutiflora*, signaled for other tropical cities since 1942 (40), stimulates the research of other possible season existing in this climatic zones of high controversy. The predominance of pollen of *Cecropia* (Yagrumo) from April to June coincides to the seasonal characteristic of Moraceae at the southeast of the United States and for Ulmaceae from February to May (57) represented in this study by pollen of the *Celtis* tree (Marinisa, Uña de Gavilán). These evidences allow us to determine the presence of a record of high pollinic frequency which delimits the concept of a specific season for these kinds of pollen and shows the presence of the tropical polyseason variations, characterized by the overlapping of individual pollinic season during a particular period.

The statistical correlation existing between the concentration of aerial pollen and the atmospheric parameters during the period of serial collection, show the presence of a mixed seasonal component for Caracas. This component is formed by the presence of a defined period of an isolated, high and significant pollinic prevalence (individual season) and by periods combined and constituted by the overlapping of individual curves of pollinic prevalences (Polyseasonal variation).

Mold spores

It was possible to observe characteristics different to those of pollen, because of the higher absolute abundance and the variability between their maximum and minimum concentrations of short duration. The quantitative analysis of the total spores collected is higher than the total of serial pollen (46). This ratio differs from the record of Altos de Pipe (22) where the total number (6.311/6.25 cm) of spores is lower than the concentration of aerial pollen collected (11.093). This fact is explained because of the difference existing in methodology and sampling ecosystem.

The spores prevailing in this study are Cladosporium, ascosporae, *Ustilago* (?) and basidiosporae, besides 35 additional types of low concentration observed in the samples.

As well as the studies reported in most of the European countries (4) and America (27), *Cladosporium* is relevant because of its high concentration that reaches a maximum level of 81%. Therefore, spores of this genus are considered the main responsible factor of the oscillations in the total concentration of fungi collected in Caracas; this differs from the previous researches (33) carried out in 1962 to determine possible environmental differences related to the sampling period and coinciding to the higher abundance of this fungus in the report of the mountain zones at the south of Caracas (23).

Different authors have pointed out the climatic influences on the recovery of spores in the air (21,61), observing an important correlation between the number of spores and the meteorologic variables such as high temperature and low relative humidity, a finding observed in Caracas for *Cladosporium*, prevalent fungi during the dry season. The humidity factor correlated with the presence of basidiospores. In comparison to the reports of other latitudes, the total average of spores present in the air was related to the general meterological situation.

Immunodiagnostic tests

In most of the allergens inhaled, an association between positive dermic tests and suggestive symptoms of sensitization toward antigens investigated as possible causals of allergic diseases was observed. Negative prick test and clinical history favor the absence of allergic pathology (36). There are confirmed evidences of sensitization in population at risk of our tropical environment for universal allergens, such as mites, fungi spores and domestic animals epithelium (44, 28). However, it is posible to observe controversies in relation to the importance of pollinic sensitization, seasonal variations and clinical repercussion in sensitized patients.

The immunoclinical stimulus - response interrelationship for pollen is still under study. Levels of 20 grains/m^3 of air containing Graminae pollen have been associated with the symptoms of individuals specially sensitive from urban areas in England. Similar studies for *Ambrosia* pollen have estimated 9 grains/m^3 of air, as a critical value of response, which support in our study the importance of the pollinic cocentration detected during the sampling period, and their possible implications in hipersensivity reactions observed in the population under study.

The importance of pollen to each species of plants is evaluated by the local counting of pollen grains compared with the allergic symptoms in patients of the geographic area under study (13). This is similar to the phenomenon observed in Caracas between the relationship of allergic rhinitis symptoms with the prevalence of pollen grain collected and the relationship of bronchial asthma symptoms and fungi spores collected in the air. This coincides with the particular affinity between the fungi spores and bronchial mucosa (3).

Previous results indicated that polen grains and fungi spores play a vary important role in the development of respiratory symptoms in patients from the metropolitan area of Caracas (5, 30, 41, 45). Different studies show such observations through immunodiagnostic tests even in population of different socioeconomic levels, patients with or without parasites living in rural and urban zones of Venezuela.

The correlation existing between percentage and response intensity towards the immunodiagnostic tests and the local aerobiologic countings have made it possible to identify the most important allergens of a particular place. Time of pollination is influenced by the environmental conditions and the tendency to be reasonably constant during each period of collection (46). This observation has an evident relation with the climatological analysis of the Caracas valley during the last 20 years (1) where it is observed the sequential repetitiveness of environmental conditions of the area under study with an acceptable range of variability reflected in the collection period used during this research.

The results show the importanceof pollen of *Casuarina* (Australian pine), Graminae, spores of *Cladosporium* and *Aspergillus fumigatus* as allergens of higher reactivity in the population studied, as well as a pattern of polysensitization previously reported (2). Besides, a great number of cases sensitized to the *Ambrosia artemisifolia* (imported extract) was identified. This pollen was not found during the sampling period. Different types of pollen pertaining to the Compositae family were observed, showing a higher pollinic frequency during the second and third collection term. This fact underlines the importance of carrying out future immunoclinic researches with standardized extracts, according to the list of pollen grains of the most important plants in Caracas. Furthermore, it determines the possible crossed reactivities among different types of autochthonus pollen pertaining to families growing in other latitudes.

Tendencies of higher response of hypersensitivity reactions observed towards the selected allergens for the immunodiagnostic evaluation are related to the sesonal variations reported for the most important groups of plant and fungi spores existing in Caracas. The similar tendencies of specific reactivity (62) in 211 rhinitics and asthmatics patients from the zone under study are determined, duringa pe iod of five consecutive years of evaluation through immunodiagnostic and immunoclinical observations. This proves the possible existence of a seasonal component of the response mediated by IgE.

Our results suggest that the pattern of polysensitization showed in the allergic population hides the specific seasonal reactivity, as it was determined in patients suffering from seasonal allergic rhinitis patients which are individually sensitive to differente pollen grains. That particular perennial symptoms comes from the sensitivity towards *Dermatophagoides pteronnysinus* and or environmental fungi spores, associated to the pollinical sensitivities expressed in a seasonal way. This suggests a posible interrelationship between the concept of pollinic polyseasonal variations of sampling of the tropical environment and the antigenic polysensitivity of the patient comming from the same region, the interrelationship between the pollinic seasonal variation with the individual specific reactivity and the mixed pollinic seasonal variations (conjugation varieties of identified pollinic season) with the immunoclinic expression of combined pathologies with a high prevalence in our environment.

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RESUMEN

Aeroalergenos mas comunes del valle de Caracas y su relevancia clínica. Perdomo-Ponce, D. (Centro Nacional de Inmunología Clínica y Hospital de Clínicas Caracas. Av. Panteon con Alameda, Consultorio 203, San Bernardino, Caracas, Venezuela), Salgado-Labouriau M., Hernández A., Alvarez F., Rull V., Guariglia M., Bolbochán D., Suárez V. *Invest Clín 32(4): 157 - 186, 1991.*

Con el fin de determinar la prevalencia secuencial de granos de polen y esporas de hongos de la ciudad de Caracas, utilizando el método volumétrico de captación y su efecto en la población a riesgo, se estructuró el primer equipo multidisciplinario de investigación aerobiológica a través de un protocolo diseñado para cinco fases experimentales, siendo las siguientes: a) Determinación de los parámetros climatológicos (temperatura, humedad relativa, velocidad y dirección del viento, precipitación y estabilidad atmosférica), por registros simultáneos de estaciones metereológicas distribuidas en el área metropolitana de Caracas; b) Captación, identificación y clasificación y determinación local de los granos de polen más importantes de la zona de estudio; c) Captación, identificación y clasificación de las esporas de hongos más importantes del área; d) Elaboración del primer calendario polínico de la ciudad de Caracas y e) Evaluación de las posibles implicaciones del medio ambiente y la respuesta mediada por el anticuerpo IgE en los pacientes seleccionados, del área en estudio. Nuestros resultados expresan: 1. Las condiciones climáticas durante el período de muestreo coinciden con el análisis de los últimos 20 años del valle de Caracas; 2. La estación Universidad Central de Venezuela (UCV) se eligió como el punto más representativo para muestreo permanente; 3. Se obtiene un método

estadístico ideal para determinar la disposición espacial en la varilla del muestreador, de un tipo denso de esporas de hongos provenientes del área estudiada; 4. Se estructura el primer calendario polínico de la ciudad de Caracas, y 5. Se demuestra una tendencia estacional de respuesta IgE. Estos resultados sugieren una evidente interrelación entre el concepto sugerido de poliestacionalidad y polisensibilización antigénica, estacionalidad individual con reactividad específica y estacionalidad mixta tropical con la expresión de patologías respiratorias combinadas en nuestro medio.

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