

AIR-BORNE FUNGI IN BARCELONA CITY (SPAIN). I. A TWO-YEAR STUDY (1976-1978)

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Quantitative and qualitative studies were made of the fungi of the air in Barcelona City. The genera *Cladosporium* and *Alternaria* formed two of the most important components of the fungus population studied during a two-year period running from January 1976 through January 1978. *Penicillium* species formed the 16.3 % of the total colonies while *Aspergillus* species represented only the 2.8 %. The occurrence of these genera was greatly affected by climatic conditions. An attempt was made to summarize the data of various kinds of fungi on a volumetric basis. Most of the fungi reported here have been identified as far as genera.

Une étude qualitative ainsi que quantitative a été réalisée sur la population fongique présente dans l'atmosphère de la ville de Barcelone. Les genres *Cladosporium* et *Alternaria* constituent les moisissures les plus importantes quant à leur présence parmi la population fongique étudiée durant la période de deux ans comprise entre le mois de Janvier 1976 et le mois de Janvier 1978. Le genre *Penicillium* représentait le 16.3% de la totalité des colonies isolées, tandis que le genre *Aspergillus* ne représentait que le 2.8%. La présence d'espèces de ces genres était largement influencée par les conditions climatologiques. Les données sur la présence des différents genres de moisissures ont été établies sur une base volumétrique. La plupart des moisissures identifiables l'ont été jusqu'au genre.

Introduction

Aerobiological investigations of pathogenic fungi, particularly of dissemination and concentration of fungus spores, are valuable in understanding the epidemiology of allergies and pulmonary diseases. Previous workers have reported studies on the fungus population of the air in Barcelona (Alemany-Vall, 1 and Frouchtman, 9), in

Madrid (Canto et al., 6), in Léon (Aller et al., 2), in Cádiz (Díaz-Rubio et al., 7 and 8), and in Alcázar de San Juan (Morales et al., 12). However, it seemed desirable to undertake quantitative and qualitative studies in a more exhaustive way in order to obtain a large body of data with the purpose of establishing a mycological calendar in the various parts of Spain. This calendar could be very useful to medical doctors in their diagnostics on allergies and pulmonary diseases.

The purpose of the present work has been to correlate the fungus population of the air in Barcelona with the high degree of incidence of allergies and pulmonary diseases the year around, and to provide additional information on the fungi obtained in the two-year sampling period (1976-1978). An attempt has been made to summarize the data of the various kinds of fungi on a volumetric basis. Owing to the large number of samples that were taken and to the problems involved in culturing and subculturing, particularly of those colonies which were nonsporulating, it was not possible to identify all fungi, and in most of the work reported here they have been identified as far as genera.

Material and methods

The present work was carried out over a 24-month period in Barcelona City which is located at the NW of Spain, enjoying a typically mild mediterranean climate. The number of spores of the air was estimated by the volumetric spore trap method (Bourdillon et al., 4), using a modification of the Bourdillon's apparatus in which spores entering a narrow orifice, directed into the wind, were impacted on a sterile Petri dish containing a proper sterile culture medium. This device permitted to know the volume of air passing per minute into the apparatus. The culture medium em-

Table 1. List of fungus genera most frequently found.

Genus	% of total colony counts
<i>Cladosporium</i>	34.6
<i>Penicillium</i>	16.3
<i>Alternaria</i>	7.1
<i>Aureobasidium</i>	6.6
<i>Aspergillus</i>	2.8
<i>Phoma</i>	1.0
<i>Mucor</i>	0.7
<i>Arthrinium</i>	0.4
<i>Botrytis</i>	0.2
<i>Rhizopus</i>	0.1
<i>Fusarium</i>	0.1
<i>Trichoderma</i>	0.1

ployed was 2 % malt extract agar, which proved to be quite efficient in previous experiments (Calvo et al., 5).

Three plates were exposed daily for 4 minutes each at four different sampling areas of the city: at the outskirts (A.Z.), at the University (B.Z.), at the downtown zone (C.Z.) and finally at the maritime zone (D.Z.). The flow rate was of 20 liter per minute. The plates were incubated during seven days at 27 °C. Counting and pure culture

Table 2. Monthly average concentration of spores per cubic meter at the four sampling zones.

Month	A zone	B zone	C zone	D zone
February	77.9	115.6		92.5
March	102.4	59.6	37.5	83.7
April	155.4	107.5	54.6	71.2
May	198.3	148.7	63.5	136.2
June	198.0	172.5	287.5	126.0
July	190.8	132.9	270.0	185.0
August	174.9	261.2	287.5	135.0
September	237.4	104.9	120.0	103.7
October	339.9	231.8	198.5	161.2
November	295.4	286.2	314.2	319.4
December	315.8	296.2	355.6	260.0
January	346.2	373.1	284.0	290.0
February	381.6	281.2	512.5	306.2
March	313.3	276.1	191.2	275.6
April	265.7	275.0	381.5	337.5
May	340.4	247.7	142.5	294.0
June	245.4	211.8	258.7	200.0
July	234.9	212.4	25.0	156.9
August	220.0	300.3	60.7	140.3
September	320.2	150.1	50.3	110.3
October	350.4	320.2	70.3	120.3
November	310.3	321.4	150.2	161.2
December	315.4	320.2	170.4	240.5
January	340.3	334.2	180.3	280.9

isolation of fungi were performed after a period of incubation for further identification. Those colonies which were nonsporulating after 30-day incubation period were considered as 'mycelia sterilia'.

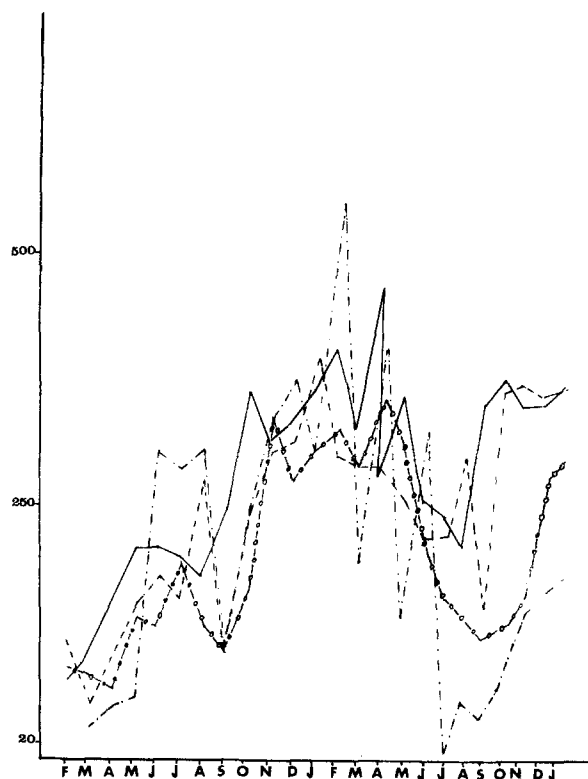


Fig. 1. Monthly average values of spore numbers per cubic meter of air at the four sampling zones: — = A zone; - - - = B zone; - . - . - = C zone; - ○ - ○ - = D zone. Abscissa: Months of the year.

Results

A total of 10,438 plates were exposed during the two-year sampling period and 202, 241 colonies were identified as far as genera. The average viable spore concentration of the air in Barcelona City was about 236.2 spores per cubic meter.

Table 1 shows the list of genera most frequently encountered at the four sampling zones with indication of the percentage of the total colony counts.

Table 2 shows the monthly average concentration of spores per cubic meter in the four sampling zones.

Figures 1 and 2 show the spore number values for each zone and the total values respectively.

Figures 3 and 4 show the monthly average concentration

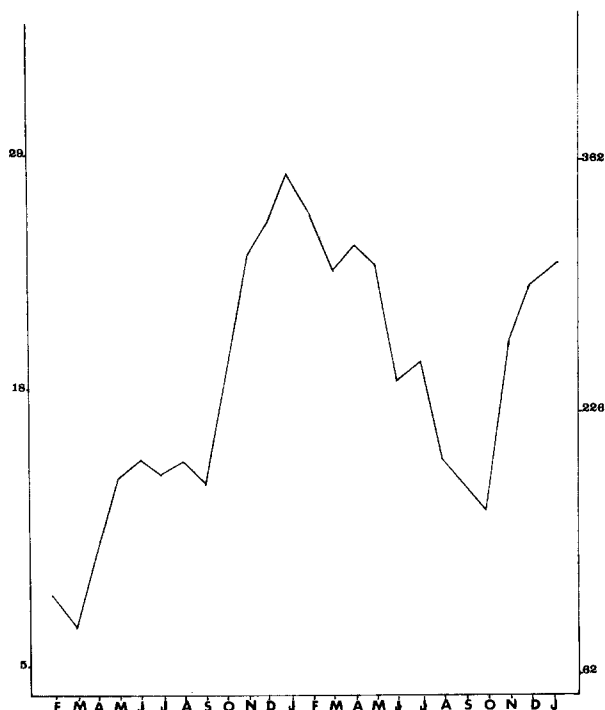


Fig. 2. Monthly average values of spore numbers per plate and per cubic meter of air.
Right ordinate: average of spore numbers per plate.
Left ordinate: average of spore numbers per cubic meter of air.
Abscissa: Months of the year.

of *Cladosporium* and *Alternaria* spores respectively, which were the most frequently found fungi in the air of Barcelona, indicating the hour of the day and the sampling zone.

The statistical analysis of the viable spore number per cubic meter of air at each sampling area is presented in table 3. From the data presented in it there were no significant differences above 5% between the number of viable spores per cubic meter and the different hour of the day and the sampling zones with the exception of sampling hours between 1.30 p.m. to 2.30 p.m. and 10.30 p.m and 11.30 at the outskirt zone. The statistical treatment of data was performed following Schwartz statistical methods (Schwartz, 15).

Table 4 presents the relationship between monthly data corresponding to the climatic factors considered and the logarithm of the viable number of spores per cubic meter.

Discussion

The spore concentration of the air in Barcelona City which is about 236.2 viable spores per cubic meter, was inferior

to that reported by previous workers, (Kramer et al., 10, 11), (Ogunlana, 13), and (Barkai-Golan et al., 3) of the air in Kansas, Nigeria and Israel respectively.

The studies on this two-year sampling period suggested that important variations in the spore concentration may be found concerning the genera *Cladosporium*, *Penicillium*, *Alternaria*, *Aureobasidium*, and *Aspergillus*, but otherwise they all kept the first ranks in order of their frequency regardless of the sampling zone.

Our observations in the present work agreed quite well with most studies carried out by other workers in various cities of the world (Barkai-Golan, et al., 3, Kramer, 10 and 11, Ogunlana, 13, and Ripe, 14). However, since some authors used in their studies gravimetric sampling methods, they were not quite comparable with our own. Nevertheless, their data permitted to determine the relative frequency of the identified genera.

Table 2 clearly shows that the average spore concentra-

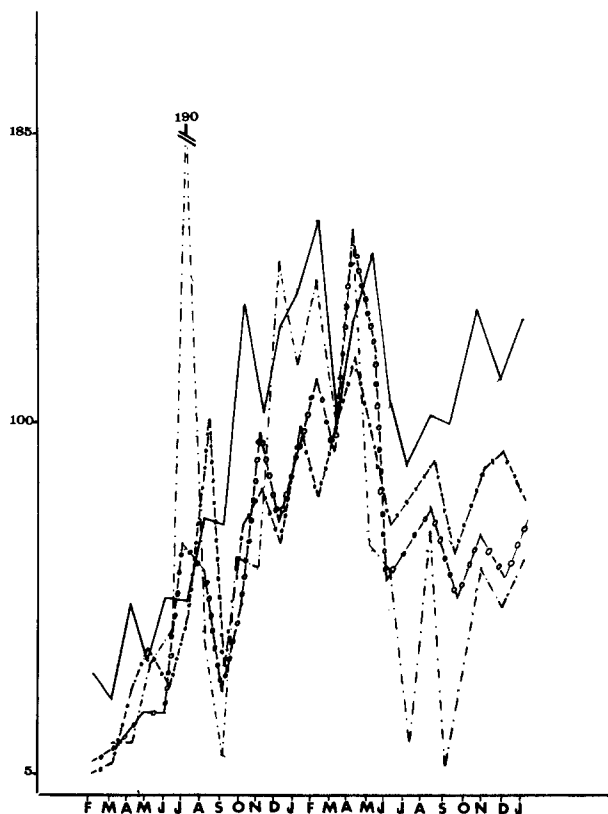


Fig. 3. Monthly average values of spore numbers per cubic meter of the genus *Cladosporium* at the four sampling zones: — = A zone; - - - = B zone; - . - . - = C zone; - ○ - ○ - = D zone.

Table 3. Average values, variance, standard deviation and standard error of data obtained at the four sampling zones.

Zone	Average values	Variance	Standard deviation	Standard error
A zone	245.5	7 130.0	84.4	4.7
B zone	210.7	6 647.7	81.5	4.5
C zone	222.8	17 432.2	132.0	7.7
D zone	191.1	7 543.3	86.8	4.8

Table 4. Monthly values of climatic factors and logarithm of the viable number of spores per cubic meter.

Month	Temperature (°C)	% R.H. ¹	W.S. ² (Km/h.)	R.D. ³	W.D. ⁴	mm.R. ⁵ (sqr.m.)	Log. (col./c.m.)
February	11.0	71.0	16.0	10	S.E.	32.5	2.0
March	12.3	63.0	3.6	5	N.E.	4.0	1.9
April	13.4	66.0	20.0	11	N.	15.8	2.1
May	17.4	72.0	16.0	8	N.E.	20.3	2.2
June	22.9	63.0	14.3	7	S.W.	41.4	2.2
July	24.2	73.0	15.0	10	W.	54.0	2.2
August	23.3	69.0	2.0	17	N.W.	154.1	2.2
September	20.6	70.0	15.0	9	W.	38.4	2.2
October	16.6	67.0	14.0	11	S.W.	53.0	2.3
November	12.3	63.0	13.0	5	S.E.	9.8	2.4
December	12.2	72.0	13.0	14	N.W.	41.2	2.5
January	11.0	72.0	14.0	7	N.E.	33.1	2.5
February	13.7	63.0	15.0	5	S.W.	6.1	2.5
March	13.5	69.0	15.0	11	N.W.	48.1	2.4
April	14.2	67.0	15.0	9	S.E.	37.5	2.5
May	15.8	77.0	16.0	17	N.E.	199.7	2.4
June	14.7	72.0	17.2	13	S.S.E.	68.4	2.4
July	22.1	70.0	18.0	11	N.E.	66.7	2.3
August	22.0	70.6	9.8	17	N.W.	10.5	2.2
September	21.0	74.0	8.5	9	W.	20.6	2.2
October	18.8	79.0	33.0	11	S.W.	15.0	2.3
November	13.5	67.0	15.0	5	S.E.	12.3	2.4
December	11.4	77.0	17.8	14	N.W.	23.0	2.5
January	8.4	63.5	8.0	7	N.E.	10.0	2.5

1: Relative humidity (%); 2: Wind speed; 3: Rainy days; 4: Wind direction; 5: mm of rain per square meter.

tion per cubic meter was higher during the months of February 1977 and October 1978 at the A zone, of January 1977 and 1978 at the B zone, and of February and April 1977 at the C zone and B zone. The highest values were obtained in February 1977 at the B zone. A coincidence could be observed between the drawings representing the four sampling zones when the average values were considered as shown by figure 1. A dramatical encrease of the viable number of spores was observed starting at the month of October 1976, correlated with climatic changes. The genera *Cladosporium* and *Alternaria* followed the same pattern with respect to the total number of viable spores per

cubic meter. This encrease became apparent for the former genus from July 1976, followed by further oscillations which were replaced by a marked decrease at the C zone. The commonest fungus recovered the year around was *Cladosporium*, but the peak of its frequency was attained in summer.

Ripe (14) studying the fungus population of the air in Stockholm reported the highest values during the months of June, July and August for this genus, with a decrease starting in November. These findings were in agreement in a general basis with the result presented here.

The genus *Alternaria* on the other hand, presented the

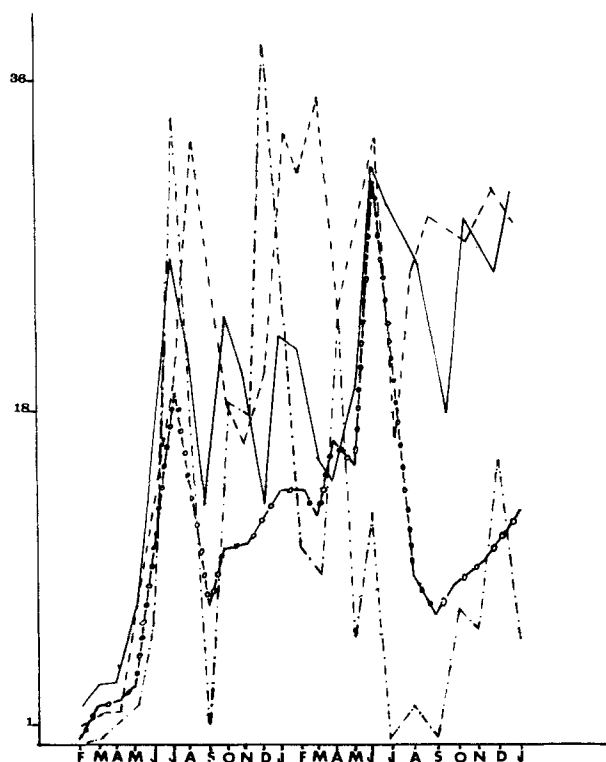


Fig. 4. Monthly average values of spore numbers per cubic meter of the genus *Alternaria* at the four sampling zones: — = A zone; — — = B zone; — . — . — = C zone; —○—○— = D zone.

same wide variations as those presented by *Cladosporium* at all tested areas. The data concerning the frequency and intensity of this genus as reported here were in a total agreement with those presented by Kramer, Pady and Rogerson (11) in their study of the fungus population of air in Kansas.

Studies are now in progress to determine whether there is a correlation between air-borne fungi and the incidence of allergies and pulmonary diseases in Barcelona City.

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