THE AIR-BORNE FUNGI IN JOHANNESBURG

A FIVE-YEAR SURVEY AS A BASIS FOR THE STUDY OF FUNGUS ALLERGY IN SOUTH AFRICA

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It is well known that the inhalation of pollen may give rise to hay-fever in sensitive persons. The possibility that air-borne saprophytic fungi have a similar allergenic effect has long been suspected and numerous investigations have amply confirmed the fact that hypersensitivity to fungi not infrequently occurs, giving rise to bronchial asthma or other allergic respiratory conditions.

This survey was carried out with the object of obtaining qualitative and quantitative information about the fungi present in the atmosphere as a basis for the study of fungus allergy in South Africa. With such knowledge the physician is better equipped to assess the etiological factors in the allergic complaints of patients.

Fungi are universal in distribution and they appear in the atmosphere from soil and from living plants and dead vegetation. Air-borne fungi are also found in the dust, furniture, upholstery and bedding materials in the home and may be responsible for respiratory allergy in the persons living there.

Fungi grow in masses made up of mycelia or threads and also of spores, which are constant in the different fungi and characterize the varieties. Fungus spores are very small and light, the average diameter being 3-5 μ and are much smaller than pollen grains, which have a diameter of 15-40 μ.

The true fungi are divided into the following Classes: Phycomycetes (non-septate mycelium and asexual spores in a sporangium), Ascomycetes (septate mycelium, ascospores in an ascus), Basidiomycetes (septate mycelium, basidiospores on short sterigmata on the outer surface of a basidium) and Fungi imperfecti (septate mycelium, and include conidial and asexual reproduction stages of other Classes). It is interesting to observe that the great majority of the air-borne fungi fall into the Class of Fungi imperfecti, the commonest being *Acromoniella*, *Alternaria*, *Aspergillus*, *Botrytis*, *Cephalosporium*, *Cephalothecium*, *Cladosporium* (*Hormodendrum*), *Epicoccum*, *Fusarium*, *Helminthosporium*, *Paecalomyces*, *Penicillium*, *Pestalotiopsis*, *Phoma*, *Pullularia*, *Scropulariopsis*, *Sporotrichum* and *Trichoderma*. It is also in the Order Moniliales of Fungi imperfecti that the yeast-like fungi, *Monilia* and *Torula* belong. The *Ascomycetes* are represented by *Chaetomium* and some species of *Penicillium* and *Aspergillus* as well as the true yeasts. The plant parasites *Ustilago* (smuts) and *Puccinia* (rusts) belong to the Basidiomycetes, and *Mucor* and *Rhizopus* to the Phycomycetes.

Sometimes non-fruiting mycelia are obtained from the atmosphere and their identification is not possible in the absence of the characteristic spores. Such unidentified fungi are referred to as *Mycelia sterilia*.

SURVEYS IN OTHER COUNTRIES

Some of the atmospheric fungus-surveys reported from Europe and the Americas are summarized below to indicate the fungus varieties generally encountered:

Bernstein and Feinberg[1] exposed 2 plates of potato dextrose agar consecutively for 15 minutes each day in Chicago over a period of 5 years. They found that *Alternaria* contributed 30% and *Hormodendrum* (*Cladosporium*) 42% of all the spores grown. *Penicillium* and *Aspergillus* came next with 11 and 4% respectively. A large number of other fungi were found but each contributed only 3% or less of the total.

Morrow *et al.*,[2] studying the atmospheric fungus content in 9 States of the USA from the Great Lakes to the Gulf Coast, also found that *Alternaria* and *Hormodendrum* (*Cladosporium*) were encountered more frequently and occurred in higher numbers than any other mould. *Aspergillus* and *Penicillium* counts were, however, low. In a later study Morrow[3] confirmed that the commonest genera found at all stations were *Alternaria*, *Hormodendrum* (*Cladosporium*), *Penicillium*, *Aspergillus*, *Pullularia*, sterile pale species, sterile dark species, *Torula*, *Fusarium* and *Trichoderma*.

Nilsby,[4] working in Sweden, found *Hormodendrum* (*Cladosporium*), *Penicillium*, *Pullularia*, and yeast-like fungi to be the commonest air-borne fungi.

Passarelli *et al.*[5] carried out a 2-year study of the incidence of air-borne fungi in Rio de Janeiro, Brazil. Plates were exposed at weekly, and later at fortnightly, intervals for 15 minutes each time. The commonest fungi found were yeasts (30·2%), *Hormodendrum* (*Cladosporium*) (16·5%), *Rhodotorula* (16·4%), *Penicillium* (14·3%), *Aspergillus* (8·0%) and *Fusarium* (3·5%). Numerous other fungi were recovered in lesser quantities—principally *Phoma*, *Trichoderma*, *Stemphyllium* and *Alternaria*.

In a 6-months survey in Mexico, the observations being made at fortnightly intervals, Blackaller[6] found *Hormodendrum* (*Cladosporium*) to be most numerous, with lesser numbers of *Alternaria*, *Penicillium*, and *Aspergillus*.

Targow and Plunkett[7] found the following fungi in the atmosphere over a 4-year period in the Los Angeles area: *Hormodendrum* (*Cladosporium*) (53·8%), *Alternaria* (14·8%), *Actinomyces* (7·2%), *Pullularia* (3·8%), *Epicoccum* (3·6%), *Penicillium* (3·4%), *Yeasts* (3·2%), with other genera each contributing 2% or less to the total catch.

Bruskin[8] found 52 genera of air-borne fungi in the New Brunswick and New Jersey areas. The following 12 genera made up 92% of the total count in a 21-month sampling period: *Hormodendrum* (*Cladosporium*) contributed half of the total and the following genera an additional 42%, viz. *Penicillium*, *Epicoccum*, *Alternaria*, *Pullularia*, *Aspergillus*, *Stemphyllium Botrytis*, *Cylindrocarpon*, *Fusarium*, *Helminthosporium* and *Phoma*. The remaining 8% consisted of 40 additional genera.

Hyde, Richards and Williams[9] have recently summarized the findings in a 3-year survey of atmospheric mould spores in Great Britain. Nearly 100 genera were represented but 96% of the total catch, other than sterile colonies belonged to the following 11 genera only: *Cladosporium* (37·8%), *Pullularia* (10·4%), *Penicillium* (9·1%), *Epicoccum* (3·4%), *Phoma* (3·0%), *Aspergillus* (2·9%), *Botrytis* (2·7%), *Oospora* (2·6%), *Sporotrichum* (2·1%), *Alternaria* (1·0%), and *Candida* (1·6%).

It will be observed that there is a considerable uniformity in the predominant air-borne fungi, regardless of where the observations have been made.

INVESTIGATION OF AIR-BORNE FUNGI IN JOHANNESBURG

The studies of the air-borne fungi in Johannesburg were commenced in 1948 but systematic work was delayed until preliminary experiments to determine the basis of future work on the subject had been completed. The actual investigation was begun in 1950 and records have been kept since that time. This report refers to the findings in the 5-year period 1950-1954.

Johannesburg (Lat. 26° 1′ S; Long. 28° 0′ E) is an inland city of the Highveld of South Africa, 5,600 ft. above sea-level, with cold dry winters and summer

TABLE I. CLIMATE DATA OF JOHANNESBURG (1950)

	June		*December*		*Year*	
	8 *a.m.*	2 *p.m.*	8 *a.m.*	2 *p.m.*	8 *a.m.*	2 *p.m.*
Mean Barometric Pressure, m.m.	846		841		843	
Rainfall, inches	0·8		7·5		31·5	
Mean Relative Humidity, per cent.	83	37	74	52	75	43
Mean Air Temperature, °F	42·1	64·0	65·6	75·2	56·3	71·6

rainfall. Although there is no agricultural activity in a major sense there are nevertheless small farm-holdings in the periphery of Johannesburg and in many of the suburbs nearly every home possesses a large or small garden. Certain climate data pertaining to Johannesburg which may be relevant to atmospheric fungus occurrence are shown in Table I.

Methods and Materials

Four-inch diameter plates of suitable culture media were exposed to the atmosphere at 11 o'clock each morning at the top of a 3-storey building in Hospital Hill, a Northern suburb on one of the ridges overlooking the city. As far as possible plate exposures were made each working day except during rain. Experimental trials revealed that 3 minutes was the optimum exposure time. Longer exposures resulted in plates unduly overgrown. After exposure the plates were covered and left undisturbed at room temperature in the laboratory and examined after 4 or 5 days both qualitatively and quantitatively for fungus colonies. They were then observed daily in order to identify the fungi as their characteristic spores appeared. Unidentified colonies were subcultured and re-examined about 10 days later and if necessary grown on various media to encourage sporulation.

At first dextrose agar medium plates alone were used. It was soon found, however, that on this medium the colonies increased rapidly in size with a tendency to spread over large areas. There was thus the possibility that other colonies coming up subsequently might be obscured. For this reason it was decided to use in addition Littman's ox-gall agar plates, because on this medium colonies were much smaller in size, development was slower and, on account of the streptomycin in the medium, bacterial growth was inhibited. With these two media used simultaneously it was felt that a truer estimate of the atmospheric fungus content was possible. Shortly thereafter it was decided to expose 2 plates each of dextrose and Littman's medium, the catch being calculated on the average of each pair of plates.

During 1953 a plate of tomato agar medium was exposed along with the other two media. It was found that *Cladosporium*, *Alternaria*, *Rhizopus* and *Trichoderma* grew equally well on the three media. Dextrose agar medium was best for *Epicoccum*, *Phoma*, *Nigrospora*, *Stemphyllium*, *Fusarium* and *Helminthosporium*. Littman's medium was best for *Penicillium*, *Aspergillus* and *Cephalosporium*. The use of tomato agar medium was discarded as it did not reveal the presence of fungus varieties additional to those recovered on the other media employed.

During the whole period of the investigation and for many years before its commencement slides had been exposed at weekly intervals to study the pollen content of the atmosphere. There was thus also available during the period of the atmospheric fungus survey a record of the fungus elements caught on the pollen slides. These included rusts and smuts in relatively small numbers, which of course do not necessarily reflect their quantitative occurrence in the atmosphere. The slide-exposure method is in general not adequate for fungus counts because few fungus spores are identifiable by direct microscopic examination of the slides and in any event the nature of fungus elements in a non-sporing phase cannot be determined.

The culture-plate exposure method is not entirely satisfactory for atmospheric fungus studies. Any artificial medium used must of necessity be selective in that it permits the growth of some but not other varieties of fungi. Further, not every air-borne fungus grows on artificial medium and some will thus be lost in colony counts made. There are in addition, certain difficulties in regard to colony counts which must be considered: (*a*) Some fungi (e.g. *Penicillium*) rapidly produce 'daughter colonies' which must be watched for lest they be included in the count of colonies derived from the actual exposure, (*b*) some fungus genera (e.g. *Rhizopus*) overgrow other fungi, which may still be in the early stages of their development and so be overlooked in

the counts; and (*c*) some colonies remain unidentified in spite of various techniques and media used to induce sporulation.

In spite of the difficulties and inadequacies mentioned above, plate exposures at the same place and at the same time each day over a period of years provide satisfactory information for practical purposes in a comparative sense even if precise and absolute knowledge of the atmospheric fungus content is not obtained.

No attempt was made to identify air-borne fungi as to species. This may become necessary in the future if species differences in fungus allergens are proved to be important.

Atmospheric Fungus Findings in Johannesburg

The quantitative estimation of the atmospheric fungi in the survey in Johannesburg was recorded in two ways: (1) *Abundance*—the total number of colonies of each fungus appearing on the plates during the exposure days per annum, and (2) *Frequency*—the number of times each fungus appeared, irrespective of quantities, during the exposure days per annum.

The Abundance and Frequency of the specific air-borne fungi found in Johannesburg in the 5-year survey in an average of 282 exposure days *per annum* is shown

TABLE II. ANNUAL ABUNDANCE OF AIR-BORNE FUNGI IN JOHANNESBURG IN THE 5-YEAR PERIOD, 1950-1954

(Average Annual Exposure Days=282)

Fungus	*Average Number of Colonies per Year*		
	Number	*Percent of Total*	
Cladosporium	1,058	32·5	
Alternaria	402	12·3	
Penicillium	330	10·1	
Epicoccum	329	10·1	84·3
Phoma	271	8·3	
Monilia *	202	6·2	
Torula *	152	4·7	
Rhizopus	55	1·7	
Yeasts *	55	1·7	
Nigrospora	54	1·6	
Stemphyllium	50	1·5	
Trichoderma	49	1·5	
Acrospeira	45	1·4	
Pleospora	37	1·1	
Fusarium	26	0·8	
Helminthosporium	23	0·7	
Aspergillus	23	0·7	
Mucor	13	0·4	
Cephalosporium	8	0·2	
Chaetomium	5	0·2	
Amblyospora, *Acromoniella*, *Botrytis*, *Cephalothecium*, *Cystophora*, *Diplodina*, *Macrosporangium*, *Paecalomyces*, *Pestalotiopsis*, *Periconia*, *Scropulariopsis*	26	2·1	15·7
Unidentified	41		

* Average for 4-year Period.

TABLE III. ANNUAL FREQUENCY OF AIR-BORNE FUNGI IN JOHANNESBURG IN THE 5-YEAR PERIOD, 1950-1954

Fungus	*Average Number of Days per Year on which the Fungus Appeared*	
	Number	*Percent of Exposure Days* (282)
Cladosporium	235	83·3
Alternaria	188	66·6
Penicillium	169	59·3
Phoma	139	49·3
Monilia	139	49·3
Epicoccum	105	37·2
Torula	86	30·5
Rhizopus	54	19·0
Yeasts	53	18·7
Nigrospora	50	17·7
Stemphyllium	43	15·3
Acrospeira	37	13·2
Trichoderma	32	11·4
Pleospora	25	9·9
Fusarium	23	8·1
Helminthosporium	22	7·8
Aspergillus	20	7·1
Mucor	12	4·2
Cephalosporium	7	2·5
Chaetomium	4	1·4
Other fungi (including 41 unidentified)	52	Each less than 1·0

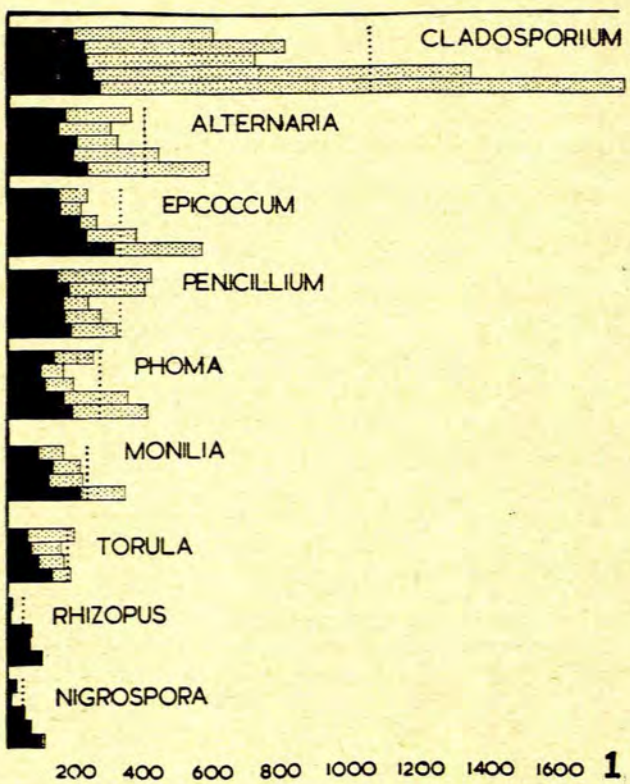

Fig. 1. The commoner air-borne fungi in Johannesburg, 1950-1954. Whole Column=*Abundance:* The total number of colonies of the specific fungus recovered in the exposure days each year. The annual average for the 5 years is shown by a dotted line. Black Column=*Frequency:* The number of days on which the specific fungus was recovered in the exposure days each year. The ratio of the whole column to the black column each year indicates the average number of colonies of the specific fungus recovered per exposure day.

in Tables II and III. It will be observed that *Cladosporium* was the most abundant fungus deposited on the plates, contributing nearly a third of all the fungi collected. The next commonest fungi were *Alternaria*, *Penicillium*, *Epicoccum* and *Phoma*, each contributing 8-13% of the total. The above 5 fungus genera together with *Monilia* and *Torula* accounted for as much as 84·3% and the remaining 15·7% was made up of 26 additional identified genera and 41 unidentified *Mycelia sterilia*. In Fig. 1 both the Abundance and Frequency of the 9 predominant air-borne fungi of Johannesburg are shown for each of the 5 years 1950-1954, the average Abundance for this period being indicated by a dotted line. The relation of the Abundance to Frequency reflects the average number of colonies of each variety of fungus found per exposure day.

It will be observed from Fig. 1 that both in Abundance and Frequency *Cladosporium*, *Alternaria*, *Penicillium*, *Phoma*, *Monilia*, *Torula* and *Rhizopus* constitute the commonest fungi in the air.

SEASONAL INCIDENCE

In many parts of the world it has been found that some of the atmospheric fungi have a seasonal incidence. Thus Hyde, Williams and Richards[9] reported that *Cladosporium*, *Pullularia*, *Epicoccum*, *Botrytis*, *Alternaria* and *Candida* had a summer predominance while *Aspergillus* and *Oospora* occurred mainly in the winter. *Phoma*, *Sporotrichosis* and *Penicillium* showed no special seasonal prevalence. In Rio de Janeiro, Passarelli, de Maranda and de Castro[5] found *Rhodotorulae* predominant in the winter and *Hormodendrum* (*Cladosporium*) commonest from the end of autumn to the beginning of spring, while the *Penicillium* incidence was lowest in the summer. No seasonal incidence was observed with *Aspergillus* and *Fusarium*. The more commonly occurring air-borne fungi in Johannesburg were similarly submitted to analysis from the point of view of seasonal occurrence. The findings are graphically shown in Fig. 2 and may be summarized as follows:

Cladosporium: Peaks of incidence have occurred in various years both in winter and in summer but there is no well-defined seasonal similarity in the 5-year period. There is however evidence of a consistent moderate rise in incidence in early summer (November).

Alternaria: No obvious seasonal incidence.

Penicillium: In some years there was a slight rise in incidence in August and November, but in general there was no significant seasonal incidence.

Epicoccum: There was generally a small rise in incidence in the Autumn (March to June) but this rise was quantitatively significant in only one of the 5 years.

SUMMARY

A survey was carried out of the air-borne fungi in Johannesburg over the 5-year period, 1950-1954.

The object of this survey was to establish a basis for the study of atmospheric fungus allergy in South Africa.

The principal genera of fungi found in the atmosphere of Johannesburg were: *Cladosporium* (32·5%), *Alternaria* (12·3%), *Penicillium* (10·1%), *Epicoccum* (10·1%), *Phoma* (8·3%), *Monilia* (6·2%), *Torula* (4·7%) and *Rhizopus*, yeasts, *Nigrospora*, *Stemphyllium*, *Trichoderma*, and *Acrospeira* (each about 1·5%).

The remaining 6·4% were constituted by 18 other fungus genera and 41 unidentified varieties.

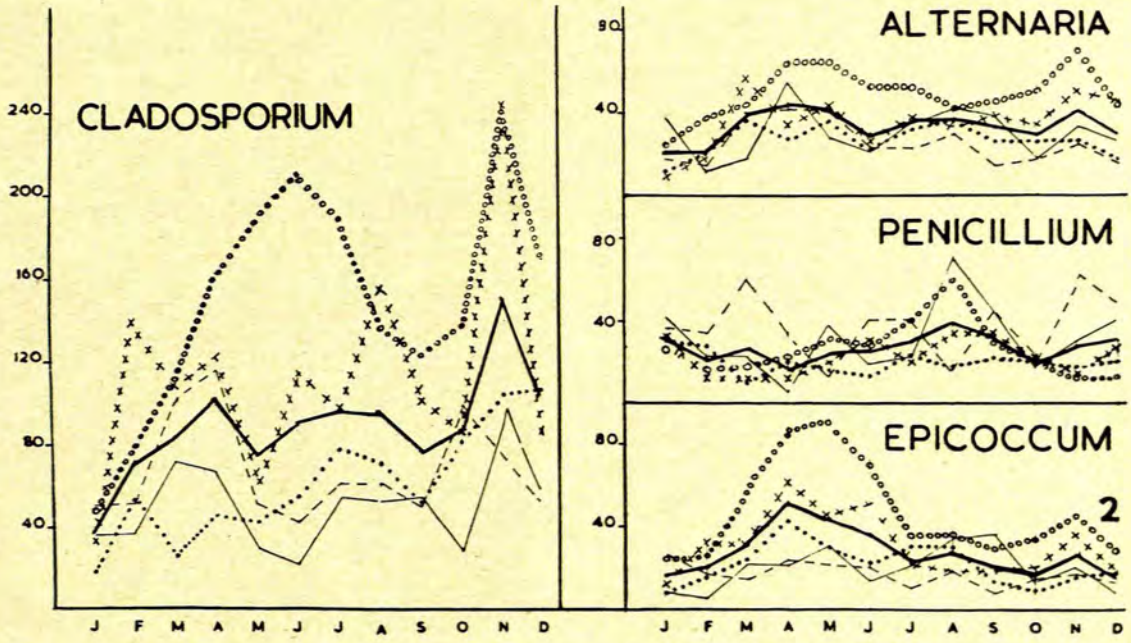

Fig. 2. Analysis of seasonal incidence of the commoner air-borne fungi of Johannesburg, 1950-1954. The total number of colonies (Abundance) of the specific fungus recovered monthly during each of the 5 successive years. The average for the 5-year period is shown by a heavy black line.

No significant seasonal incidence was noted in any of the commoner air-borne fungi.

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