

FUNGUS COLONY COUNTS FROM AIR-BORNE SPORES IN BIRMINGHAM, ALABAMA

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THE air-borne spores of *Alternaria* and *Cladosporium* have received considerable attention as inhalant allergens. The allergist should be informed as to the variety of spores in a local area, since many fungus spores have been incriminated as allergy incitants. The purpose of this investigation was to determine the kinds and prevalence of air-borne fungus spores in Birmingham, Alabama, by the culture plate method. A yearly count was undertaken in an effort to obtain information on the seasonal fluctuation of the fungi encountered and to attempt an interpretation of the data obtained in relation to precipitation, relative humidity, and wind direction.

The advantages of plate cultures over direct mold spore slide counts are many. Colony counts and concurrent slide spore counts are seldom in agreement.¹ Only those molds possessing relatively large spores or spores with marked characteristics can be recognized by the slide method. *Alternaria* spores, which are large and typical, and *Cladosporium* spores, which usually occur in clumps, can be identified readily. *Aspergillus*, *Penicillium*, and others, the spores of which are relatively small and indeterminant, are more difficult, if not impossible, to identify. By the use of an artificial medium supporting growth of the majority of air-borne fungi, positive identification following incubation of the exposed plates is usually possible. Although culturing with consequent identification of genera is more time-consuming, it is generally recognized as a more accurate method than that of slide spore counting.

The survey covered the year from June 1, 1953, to May 31, 1954. Three exposures a week of three plates each for a five-minute period were made. Following development of colonies, usually in five to seven days, results were tabulated, unidentifiable genera were isolated for further study, and the monthly total of each recognizable genus was compiled. Species identification was not undertaken.

Birmingham, situated at the foothills of the Great Smoky Mountains, has an elevation of between 500 and 1,000 feet. The average monthly rainfall during the survey was 3.3 inches. The average relative humidity was 66.6 per cent, and the average temperature was 63.2 degrees. Since the rainfall and humidity were not excessive, abundance of the *Phycomycetes* was not anticipated.

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PROCEDURE

Exposure of Sabouraud's plates to the air was made after trial exposures of malt agar, potato-dextrose agar, and Sabouraud's dextrose agar. Sabouraud's agar was found to satisfy the criterion of a favorable medium, since it was easy to prepare, supported the growth of the common air-borne fungi, limited bacterial growth, and was a medium on which the common mold genera were easy to recognize. Pigmentation of certain colonies and reverse coloration were readily apparent.

COMPARISON OF TOTAL MOLD COLONY COUNTS TO COMBINED ALTERNARIA AND CLADOSPORIUM COUNTS

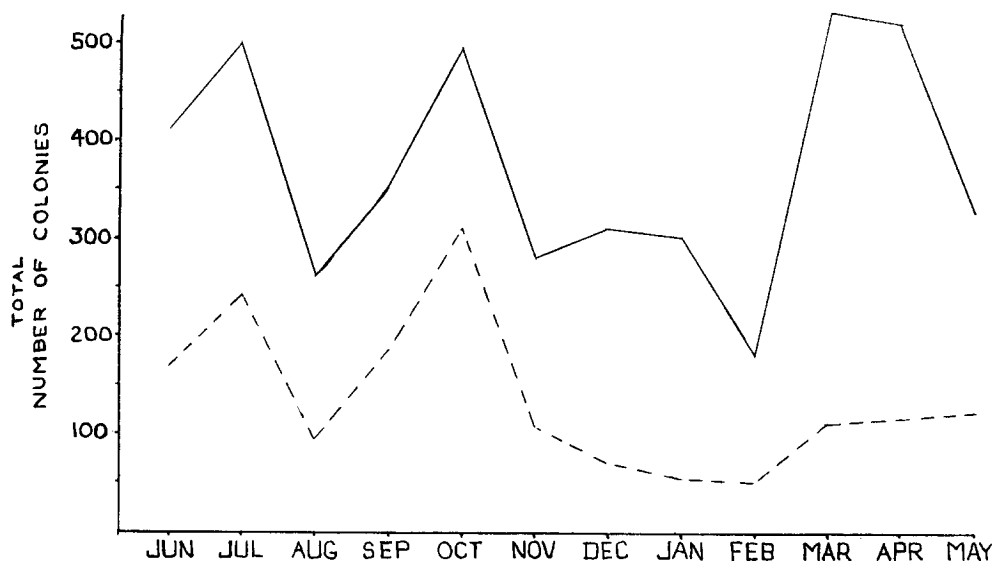


Fig. 1.

Numerous types of media have been employed by other investigators.²⁻⁴ Since there seemed little likelihood, from a cursory observation of trial exposures and general climatic conditions, that many spreading *Oospora*, *Aspergillus*, and *Rhizopus* or other *Phycomycete* colonies would be found which might overgrow on the plates, the choice of Sabouraud's medium was made.

Each Monday, Wednesday, and Friday midmornings three plates were exposed for five minutes on a brick ledge on the roof of the Medical Center Building. Previous test exposures indicated that five minutes was the maximum exposure time for eventual maturation of discrete colonies. There were 156 exposures of three plates each, or the equivalent of 468 separate exposures.

Plates were incubated at room temperature, undisturbed, for five to seven days, after which time colonies were identified macroscopically, and microscopically by use of cotton blue moist preparations. Unrecognizable colonies were isolated and further identification attempted.

DISCUSSION

Fig. 1 shows the total mold colony counts by months, compared to the combined total *Alternaria* and *Cladosporium* spore counts. Those two mold colonies were found throughout the entire year, comprising, roughly, one-third of all colonies counted. The summer season and on through October showed the heaviest fall of both of these spores. The greatest number of these colonies was recorded in October. From November through March, the *Alternaria* and *Cladosporium* counts remained consistently lower, and a slow rise occurred in April and May approaching the summer peak season.

The total number of colonies counted during August was low, as were the counts of both *Alternaria* and *Cladosporium*. The prevailing wind that month was from the southwest, a dry arid region where normal counts of these two molds are low.⁵ Eleven days of rain may have been instrumental in washing spores from the air.

Table I shows the distribution of the nine most frequently encountered molds, the miscellaneous mold distribution, the total mold colony count, the unidentified molds, and the meteorologic data for the year. The genera, *Alternaria*, *Cladosporium*, *Stemphylium*, and *Acrothecium*, belong to the *Dematiaceae* and *Botrytis*, *Penicillium*, and *Aspergillus* are *Moniliaceae*, whereas *Phoma* is classed in the family *Sphaerioidaceae*, and *Epicoccum* in the family *Tuberculariaceae*. Of the total of 4,488 colonies counted, 174 were not identified. No marked seasonal variation was observed for *Penicillium*, *Aspergillus*, *Acrothecium*, or *Epicoccum*. During June a high *Stemphylium* count was recorded, and a shower of *Phoma* with a record of 195 colonies on April 30 may have occurred as a result of a strong wind from the south on that day. Heavy falls of *Botrytis* during December, January, and March occurred on rainy days when the wind was from the south.

Combinations of climatic factors undoubtedly influenced daily spore dissipation. In general, *Alternaria* and *Cladosporium* were more prevalent when the prevailing wind was from the west. Rain temporarily washed spores from the higher atmosphere, and counts were lower following precipitation. Plates exposed during the rain often developed increasing numbers of *Pullularia* and *Cryptococcus* colonies.

Alternaria and *Cladosporium* spores, although more prevalent in the summer, were found during the entire year. Winds from the north and midwest may well have brought some of these spores from the wheat-growing areas during the height of the wheat season. Since these two molds were found consistently during the year, they must have a natural habitat and maturation in this area.

The remaining seven mold genera, while demonstrating no seasonal fluctuation, were recovered during each month and may be considered as potential allergens. However, the quantity of air-borne spores of any genus does not in itself incriminate that fungus as an allergy incitant.

TABLE I. MONTHLY MOLD COLONY COUNTS AND LOCAL CLIMATOLOGICAL DATA, JUNE 1953 TO MAY 1954

GENUS	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	JAN.	FEB.	MARCH	APRIL	MAY	TOTAL	PER CENT OF TOTAL	YEARLY AVERAGE
Cladosporium	96	120	40	94	209	57	48	32	31	81	89	67	964	21.5	
Alternaria	65	117	51	91	101	50	25	22	20	32	30	57	661	14.7	
Botrytis	8	95	17	24	11	19	101	106	21	281	55	25	763	17.0	
Penicillium	25	25	9	36	25	18	33	60	30	17	18	13	309	6.9	
Phoma	5	8	4	5	2	2	24	10	9	6	204	14	293	6.5	
Stemphylium	125	22	23	19	18	10	8	10	4	9	19	12	199	6.2	
Acrothecium	9	17	23	23	24	13	4	5	2	6	9	15	150	3.3	
Aspergillus	4	2	8	6	8	24	30	8	5	2	5	20	122	2.7	
Epicoecum	6	7	6	4	9	7	4	14	7	20	17	24	125	2.8	
Total miscellaneous colonies	65	76	72	52	75	65	31	36	43	68	70	75	728	16.3	
Unidentified	5	11	6	9	11	15	6	3	6	11	6	5	174	2.1	
Total count	413	500	259	363	493	280	314	306	178	533	522	327	4488	100	63.2
Average temperature (degrees)	81.4	79.7	79.5	74.0	65.2	51.6	43.4	46.2	51.2	53.4	67.2	65.1			
Total precipitation	1.22	4.71	2.80	2.98	0.21	1.62	9.04	6.35	3.37	3.51	3.20	3.23			3.33
Number of rainy days	7	11	11	4	2	5	17	10	6	9	11	8	101		
Average relative humidity	65.8	73.0	66.3	61.1	61.0	59.5	71.9	72.1	60.1	72.7	68.9	66.7			66.6
Wind direction	NE	N	SW	NE	W	SE	NW	SW	SW	SW	NW	NW			

TABLE II. INCIDENCE OF AIR-BORNE FUNGI

I. FUNGI IMPERFECTI			
A. <i>Dematiaceae</i> (Dark to black hyphae and/or spores)		B. <i>Moniliaceae</i> (<i>Conidia</i> hyaline or bright-colored, usually on conidiophores)	
	(PER CENT)		(PER CENT)
Cladosporium	21.5	Botrytis	17.0
Alternaria	14.7	Penicillium	6.9
Stemphylium	6.2	Aspergillus	2.7
Acrothecium	3.3	Monilia	1.5
Pullularia	1.3	Trichoderma	1.4
Helminthosporium	0.5	Oospora	1.2
		Paecilomyces	0.6
Miscellaneous—less than 0.5 per cent each		Miscellaneous—less than 0.5 per cent each	
Curvularia		Cephalosporium	
Montospora		Scopulariopsis	
Nigrospora		Fusidium	
Mycogone	0.8	Trichothecium	
Synsporium		Verticillium	1.2
Stachybotrys		Geotrichum	
Mesobotrys		Gliocladium	
Spondylium		Botrytrichum	
Per cent of total	48.3	Cylindrocarpon	
		Per cent of total	32.5
C. <i>Stilbaceae</i> (<i>Conidiophores</i> bound into coremia)		D. <i>Tuberculariaceae</i> (Fruiting layer of hyphae and conidiophores colored, intercalary chlamydospores)	
Stysanus	0.05	Fusarium	1.7
		Epicoccum	2.8
Per cent of total	0.05	Per cent of total	4.5
E. <i>Sphaerioidaceae</i> (<i>Pycnidia</i> dark, membranous)		F. <i>Melanconiaceae</i> (<i>Conidiophores</i> in superficial or erumpent stratum, conidia may have appendages)	
Phoma	6.5	Pestalozzia	0.1
Chaetomella	0.4	Per cent of total	0.1
Per cent of total	6.9		
II. PHYCOMYCETES			
A. <i>Mucoraceae</i> (<i>Columellate</i> sporangia, nonseptate hyphae, zygospores)			
	Rhizopus		0.8
	Per cent of total		0.8
III. MISCELLANEOUS			
A. <i>Streptomycetaceae</i> (Hyphae usually not more than 1.5 μ in diameter, white or colored, conidia in chains)		B. <i>Saccharomycetaceae</i> (No mycelium, asci, loosely attached spores)	
Streptomyces	1.3	Sacchromyces	0.5
		C. <i>Cryptococcaceae</i> (No mycelium, after budding spores)	
Per cent of total	1.3	<i>Cryptococcus</i> (red-orange)	2.3
		Per cent of total	2.8
D. <i>Mycelia-Sterilia</i> (indefinite or globose sclerotium)		E. Unidentified	
	0.6		2.1
Per cent of total	0.6	Per cent of total	2.1

Table II is a composite tabulation of the total percentage of each mold genus found during the year. The Phycomycetes account for only 0.8 per cent of all the colonies counted. The Fungi Imperfecti comprised 92.4 per cent of the total; 80.8 per cent of these belonged to the families Dematiaceae (48.3 per cent) and Moniliaceae (32.5 per cent). The remaining 6.8 per cent

were miscellaneous or nonsporulating, unrecognizable genera. Cryptococci, Streptomyces, and Saccharomyces comprised only a small percentage of the recorded colonies.

SUMMARY

A total of 4,488 colonies of air-borne fungi was recorded in a one-year period from 468 plate exposures.

The Phycomycetes (0.8 per cent) and the Fungi Imperfecti (92.4 per cent) composed 93.2 per cent of all the identified colonies counted.

Cryptococci, Saccharomyces, Mycelia-Sterilia, and unidentifiable fungi made up the remaining 7.6 per cent of the colonies counted.

Alternaria and Cladosporium accounted for 36.2 per cent of the total colonies, showing the heaviest production during June, July, September, and October.

No marked seasonal variations were found among the remaining genera.

Showers of Botrytis and Phoma occurred during a number of months of the year.

At least nine mold genera occurred in quantities sufficient to be incriminated as possible allergens.

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