

ATMOSPHERIC MOLD SPORES IN AND OUT OF DOORS

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SINCE the early days of studies in allergy to mold spores there has been among allergists considerable doubt concerning the relative importance of the indoor and outdoor air as a source of mold spores which can give rise to respiratory allergy. In several of the earliest published cases of mold allergy the source of the mold was attributed to the patient's home.¹⁻³ Such cases supported the then prevailing impression that allergy to mold spores was a rarity and was confined to individuals with a peculiar residential or occupational exposure. This view was not seriously contested until Feinberg⁴ pointed out that in his clinic allergy to mold spores was by no means rare and⁵ that the ordinary outside air was a widespread and potent source of exposure to mold spores.

As a result of Feinberg's work, studies on the incidence of mold spores in the air were begun in many places in the United States of America and in other countries. Usually investigations were made in the open air only. Several allergists, however, have emphasized the importance of making studies of the spore content of the air indoors, and more than a few such investigations have now been made. From purely botanical considerations Saito,⁶ Rostrup,⁷ and Peyronel⁸ had investigated the mold content of the air indoors before the question of mold allergy arose. Under the stimulus of mold allergy Prince and Morrow⁹; Rennerfelt¹⁰; Nilsby^{11, 12}; Flensburg and Samsøe-Jensen¹³; Alemany-Vall¹⁴; Wallace, Weaver, and Scherago¹⁵; Maunsell^{16, 17}; and Swaebly and Christensen¹⁸ have since carried out similar investigations.

Few of the previous investigations, however, took into account the possibility of seasonal variation in the mold spore content of indoor air, and rarely has an investigation of the air indoors been coupled with a similar investigation outdoors to allow a comparison of the two to be made.

The aim of the study which is described here was to obtain information on the relative importance as a source of exposure to mold spores of the air indoors and outside. The main thesis of this paper is concerned with the normal house, but it may be appropriate to include here a note on the abnormal damp house which itself is known to be a source of mold.

The Damp House.—There can be little doubt that in a house which is visibly moldy (i.e., where molds can be observed growing on, e.g., distempered walls, wallpaper, books, leather articles, or textiles) the residents are exposed to higher concentrations of mold spores than the residents of a normal, clean, dry house. Nilsby^{11, 12} exposed plates inside several hygienic and unhygienic homes and found that the average number of colonies which developed on a 9 cm. Petri dish exposed for fifteen minutes was 5 in the dry hygienic homes

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but was 55 in homes about which complaints had been made to the Bureau of Health. In several homes in Cardiff, I have examined the molds growing on damp wallpaper, distemper, linoleum, books, etc., by direct microscopic examination and by making from each area of moldy surface many isolations on culture plates. The platinum wire used for making isolations was flame sterilized between successive samplings. The molds most commonly found growing indoors in such situations were *Alternaria tenuis*, *Aspergillus niger*, *Aspergillus glaucus*, *Aspergillus fumigatus*, *Cephalosporium acremonium*, *Cladosporium herbarum*, several *Penicillium* species and *Pullularia pullulans*. It is difficult to say which among these species are commoner than the others. Several other species of the common saprophytes occur occasionally. Balfour-Browne¹⁹ and Smith^{20, 21} have both described new species of molds found growing on damp walls indoors. The molds growing in dark patches on walls and elsewhere indoors in Israel were found by Kessler²² to be identically the same as those which he caught most frequently from the air outdoors.

The predominance of a certain species or groups of molds at any particular indoor site would appear to be determined by properties of the immediate environment. Mold growths on damp walls in one house may be different in constitution from apparently similar growths in another house, and the variation can extend to two rooms in the same house. In one room with damp walls which I examined, many isolations from the wallpaper over an area of 1 to 2 square meters produced *Cladosporium* colonies only, while isolations from a similar area on an abutting wall produced mainly *Penicillium* together with some *Cladosporium* and *Aspergillus*. These moldy areas must be produced by the germination and growth of mold spores deposited on the surface from the air. The composition of any particular area of mold might be subsequently fixed by the initial fortuitous deposition alone, but it is also more than possible that within each mold-colonized area (or mold community) there may occur in a certain time a distinct ecological succession of mold species.

The Normal House.—A house which appeared to be a normal, dry, clean dwelling, and not itself a source of molds, was used for this investigation in which a pair of 9 cm. Petri dishes containing Sabouraud's (maltose) agar* were exposed in a bedroom on every weekday for twelve months. The house was adjacent to the hospital and on the same elevation (approximately 25 feet altitude). It was over 60 years old, of stone construction, in good repair and can be considered typical of a large number of houses in Britain. The bedroom where the Petri dishes were exposed was situated on the upper of the two floors. It connected with the remainder of the house through one door, and with the outside through a window (which was never opened during the twelve months of the experiment) and a chimney. No fires were lit in the bedroom during these twelve months. The occupant of the bedroom vacated this room each morning at about 8.30 A.M. and it was left undisturbed until the technician

*This medium was used for the sake of continuity and comparability of results with the previous investigations of Hyde and Williams.³² They had earlier, in a long series of Petri dish exposures, compared this medium with others such as potato-dextrose agar and glucose-peptone agar, and had found the mold catches on the several media to be closely similar.³² In view of these results Sabouraud's agar was chosen as the standard medium because of the simplicity of preparation of uniform successive batches of medium.

arrived bringing the Petri dishes. The dishes were taken into the bedroom, placed on the bed and at 10 A.M. uncovered with as little disturbance as possible. The technician then quietly left the room, later returning in time to recover the plates after an exposure of ten minutes by stop watch.

At the same time on every weekday a similar pair of plates were exposed for ten minutes in a Hyde and Williams²³ shelter situated on a lawn in the hospital grounds. This outdoor site was some 60 yards away in a straight line from the house.

To prevent the inclusion in the count of any laboratory contaminant, all plates were poured and sealed with adhesive paper at least five days before their exposure. After exposure the plates were again sealed and incubated at room temperature for five days after which the mold colonies which had grown were identified and recorded. Those colonies which bore no spores at the first observations were re-examined after further incubation. Where a mold colony proved difficult to identify accurately on the original plate, subcultures were made on slides and on plates of other media, e.g., malt agar, and Czapek-Dox + yeast agar.²⁴ Colonies which finally failed to produce spores were labeled *Mycelia sterilia*. For convenience this large and diverse group was subdivided by means of the color characteristics of mycelium into three sections—(a) entirely white mycelium, (b) white mycelium above, dark beneath or reverse, and (c) entirely dark-colored mycelium. In practice this classification, though arbitrary, brought together colonies of broadly similar morphologic and cultural characters. The group *Mycelia sterilia* (white) contained many *Oospora*-like colonies and several others resembling Basidiomycetes mycelia. The *Mycelia sterilia* (white and dark) included a large proportion of colonies closely resembling (in mycelial characteristics) nonsporing or poorly sporing colonies of *Alternaria* and several others resembling Basidiomycetes mycelia. The *Mycelia sterilia* (dark) were a small group without marked affinities.

RESULTS

During the whole year 626 Petri dishes were exposed as described at each site. The total mold catches in the bedroom and in the open air are shown separately in Table I. The main results were:

1. The outdoor catch was more than five times as large as the indoor catch.
2. The same molds were caught indoors as outside and they were caught in approximately similar proportions at both sites. (Fig. 1.)
3. Taking the year as a whole the mold caught in greatest numbers, both indoors and out, was *Cladosporium* followed by sterile mycelia and *Penicillium*.
4. Eight genera (*Cladosporium*, *Penicillium*, *Pullularia*, *Aspergillus*, *Phoma*, *Botrytis*, *Epicoccum* and *Oospora*) together with the sterile mycelia made up more than nine-tenths of the total catch at each site.
5. From June to October at both sites *Cladosporium* was by far the most prevalent mold, but during the rest of the year, when the numbers of *Cladosporium* were much lower, *Penicillium* predominated. *Penicillium* shows little variation in incidence throughout the year (Fig. 2).

TABLE I. ANALYSIS OF THE MOLD COLONIES RESULTING FROM THE DAILY EXPOSURE OF TWO CULTURE PLATES THROUGHOUT ONE YEAR

THE OUTDOOR CATCH			THE INDOOR CATCH		
GENUS OR GROUP	COLONIES CAUGHT ON 626 PLATES	PER CENT OF ALL COLONIES	GENUS OR GROUP	COLONIES CAUGHT ON 626 PLATES	PER CENT OF ALL COLONIES
Cladosporium	3,097	45.2	Cladosporium	463	35.9
Mycelia sterilia (white)	821	12.0	Mycelia sterilia (white)	293	22.7
Penicillium	668	9.7	Penicillium	194	15.1
Pullularia	588	8.6	Oospora	71	5.5
Mycelia sterilia (white & dark)	250	3.6	Aspergillus	53	4.1
Aspergillus	204	3.0	Mycelia sterilia (dark)	30	2.3
Phoma	199	2.9	Botrytis	29	2.2
Botrytis	160	2.3	Mycelia sterilia (white & dark)	26	2.0
Epicoccum	152	2.2	Pullularia	25	1.9
Oospora	144	2.1	Sporotrichum	24	1.9
Candida	113	1.6	Candida	14	1.1
Sporotrichum	105	1.5	Epicoccum	9	0.7
Mycelia sterilia (dark)	100	1.5	Monilia	9	0.7
Alternaria	44	0.6	Torula	9	0.7
Other genera	214	3.1	Other genera	40	3.1
	6,859			1,289	

THE OTHER GENERA CAUGHT, INDIVIDUALLY COMPOSING LESS THAN 0.5 PER CENT OF THE TOTAL CATCH

THE OUTDOOR CATCH		THE INDOOR CATCH	
	COLONIES CAUGHT ON 626 PLATES		COLONIES CAUGHT ON 626 PLATES
Cephalosporium	30	Scopulariopsis	2
Mucor	29	Trichosporium	2
Torula	25	Verticillium	2
Mycelia sterilia (dark)	25	Acrostalagmus	1
Monilia	21	Allantospora	1
Stemphylium	14	Botryotrichum	1
Fusarium	9	Chaetomium	1
Trichoderma	8	Diplodia	1
Trichothecium	8	Gliomastix	1
Rhizopus	6	Myxomycete	1
Heterobasidion	5	Spicaria	1
Diplococcium	4	Spondylocladium	1
Gliobotrys	4	Stysanus	1
Ciboria	3	Synsporium	1
Paecilomyces	2	Tilachlidium	1
Papularia	2	Trichocladium	1
		Phoma	6
		Fusarium	5
		Mucor	5
		Cephalosporium	4
		Rhizopus	4
		Trichothecium	3
		Heterobasidiom	2
		Trichosporium	2
		Acremonium	1
		Allantospora	1
		Diplodia	1
		Paecilomyces	1
		Papularia	1
		Stysanus	1
		Unidentified	3

6. No mold occurred more frequently on the indoor plates than it did on the outdoor plates.

The variation (among the predominant genera) of the ratio of the outdoor catch to the indoor catch is shown in Table II. This ratio varies from 2.3 (*Oospora*) to 33.2 (*Phoma*) and ∞ (*Alternaria*). Some explanation of this variation is attempted later.

The effect of season on the catches of the commoner molds is shown in Table III and Fig. 2. In the summer months *Cladosporium*, *Botrytis*, *Epicoccum*, and *Pullularia* occur on the outdoor plates in numbers which are strikingly

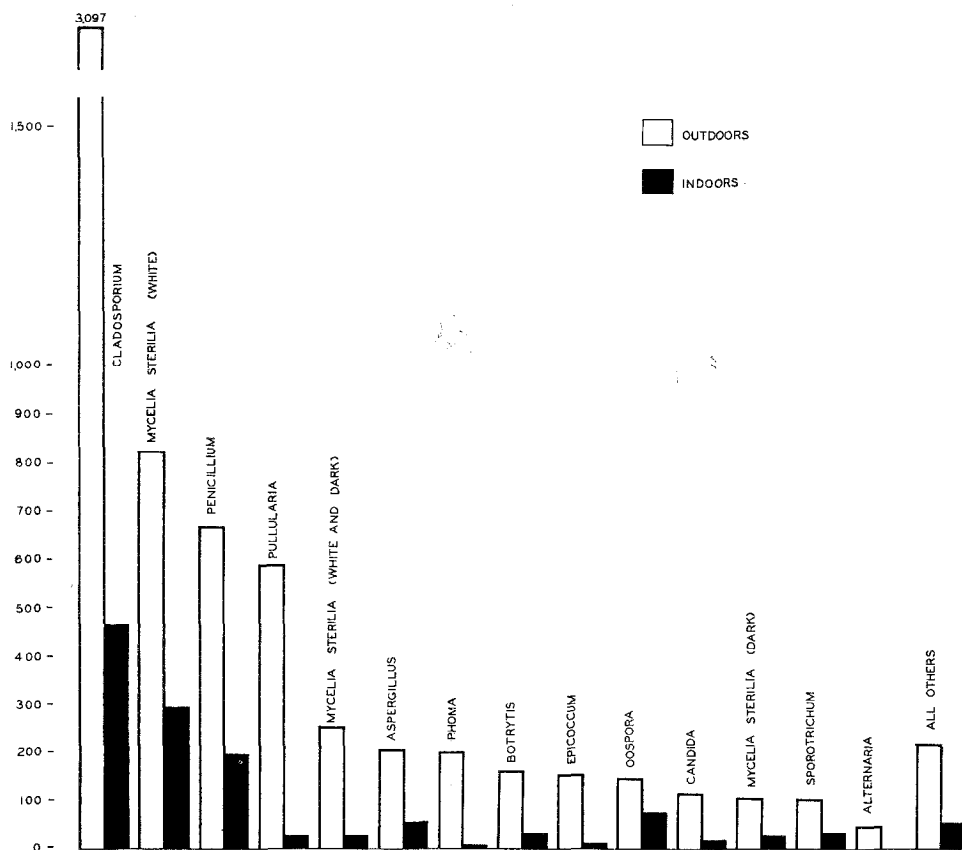


Fig 1.—The relative prevalence of the chief molds caught indoors and outside during the year April, 1951 to March, 1952.

TABLE II. THE RELATIONSHIP BETWEEN THE INDOOR AND OUTDOOR CATCHES

	OUTDOOR CATCH	INDOOR CATCH	OUTDOOR
			INDOOR
Cladosporium	3097	463	6.7
Mycelia sterilia (white)	821	293	2.8
Penicillium	668	194	3.4
Pullularia	588	25	23.5
Mycelia sterilia (white and dark)	250	26	9.6
Aspergillus	204	53	3.8
Phoma	199	6	33.2
Botrytis	160	29	5.5
Epicoccum	152	9	16.9
Oospora	144	71	2.3
Candida	113	14	8.1
Sporotrichum	105	24	4.2
Mycelia sterilia (dark)	100	30	3.3
Alternaria	44	—	∞
Total catches	6859	1289	5.5

higher than in the winter months. This seasonal incidence is equally well shown in the indoor catches. Similarly, the groups *Mycelia sterilia* (white) and (white and dark) also have a higher incidence in summer on the outdoor and indoor plates. *Aspergillus* and *Oospora* are the only two genera to show out of doors

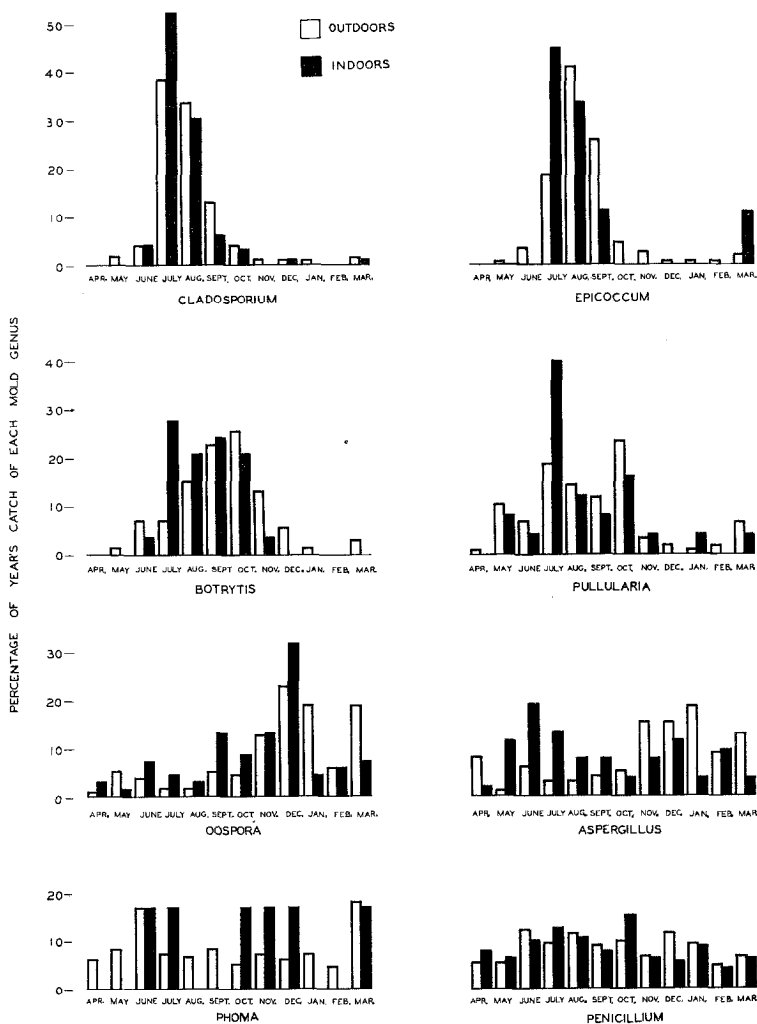


Fig. 2.—Seasonal variation in incidence of the commonest air-borne molds. The histograms show the percentage occurring in each month of the year's catch of each mold genus.

a higher incidence in the winter months. In the case of *Oospora* the higher winter incidence is reflected in the indoor catch, but that of *Aspergillus* is not. The reason for this difference is not clear.

The very large seasonal variation in numbers of *Cladosporium* colonies is shown in Fig. 3. The weekly catch (i.e., the total number of colonies on six successive daily pairs of plates) varies from 0 to 1 during February to April,

indoors and outside, to the highest catches of 440 colonies outdoors and 80 colonies indoors at the end of July. The correspondence between the outdoor and indoor catches is marked and the graph indicates that the indoor catch is merely a diminished reflection of the outdoor catch.

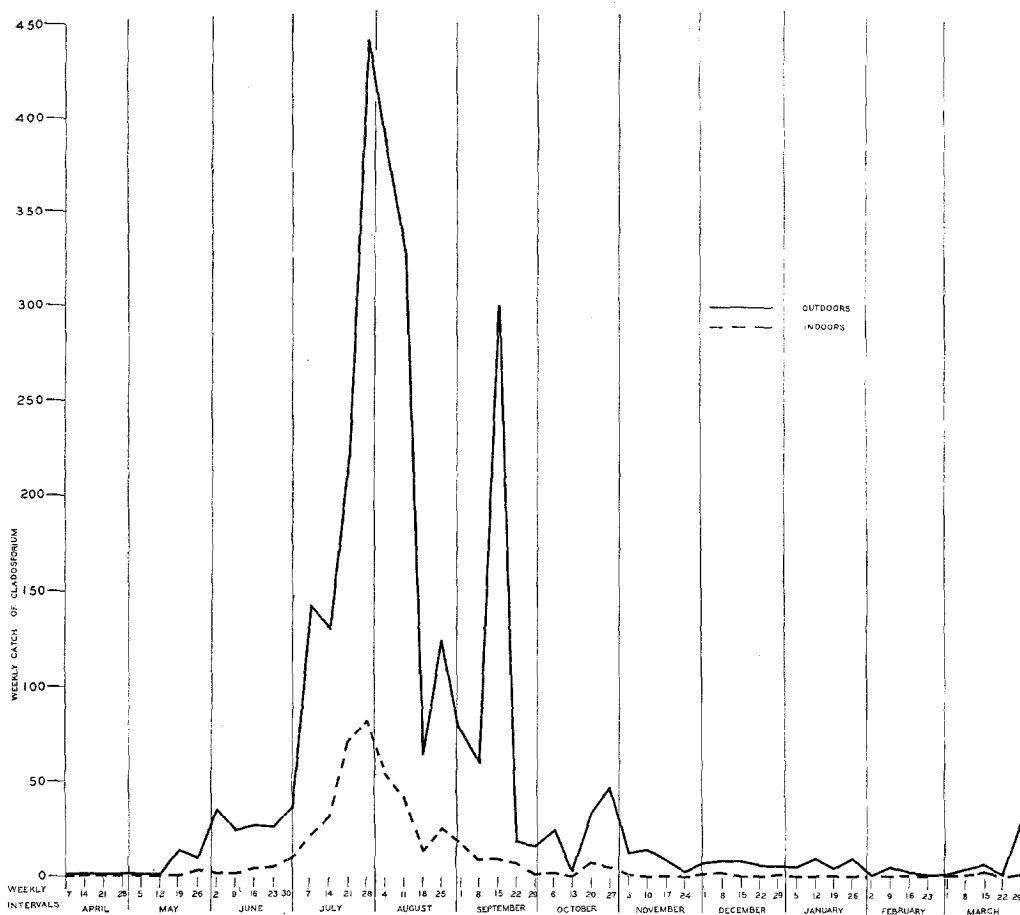


Fig. 3.—The seasonal variation in *Cladosporium* incidence. The graph is plotted at weekly intervals, each point representing the *Cladosporium* catch on six successive daily pairs of plates.

DISCUSSION

The variation from one genus to another in the ratio outdoor catch/indoor catch (Table II) indicates clearly that the factors governing the size of the mold catch are not the same both indoors and outside for all molds. The following remarks are offered as a possible partial explanation of the variation.

1. *Genera with large spores*, e.g., *Alternaria* and *Epicoccum* are known to sediment out of still air more swiftly than smaller spores. [Calculations based on Stokes's Law give the following (approximate) speeds of fall under gravity (terminal velocity) for smooth spherical bodies assuming unit density. A

TABLE III. THE EFFECT OF SEASON ON THE INDOOR AND OUTDOOR CATCHES OF THE COMMONER MOLDS

GENUS OR GROUP	OUTDOOR OR INDOOR	APRIL (25)*	MAY (27)	JUNE (26)	JULY (26)	AUG. (27)	SEPT. (25)	OCT. (27)	NOV. (26)	DEC. (26)	JAN. (27)	FEB. (25)	MAR. (26)
Cladosporium	Outdoor	2	54	152	1185	1037	400	120	38	28	27	10	44
	Indoor	1	4	23	242	140	28	15	0	4	1	0	5
Mycelia sterilia	Outdoor	24	66	72	56	142	140	41	115	43	46	20	56
(white)	Indoor	3	18	20	41	69	84	10	17	15	8	2	6
Penicillium	Outdoor	34	35	80	62	75	59	65	44	76	61	32	45
	Indoor	15	12	19	24	20	15	29	12	11	17	8	12
Pullularia	Outdoor	5	60	40	110	83	68	137	20	11	5	10	39
	Indoor	0	2	1	10	3	2	4	1	0	1	0	1
Mycelia sterilia	Outdoor	7	12	15	38	62	37	22	14	14	7	3	19
(white and dark)	Indoor	0	0	2	3	12	3	3	2	0	0	0	1
Aspergillus	Outdoor	16	2	12	6	6	8	10	31	31	38	18	26
	Indoor	1	6	10	7	4	4	2	4	6	2	5	2
Phoma	Outdoor	12	16	33	14	13	16	10	14	12	14	9	36
	Indoor	0	0	1	1	0	0	1	1	1	0	0	1
Botrytis	Outdoor	0	2	11	11	24	36	41	19	9	2	0	5
	Indoor	0	0	1	8	6	7	6	1	0	0	0	0
Epicoccum	Outdoor	0	1	5	28	62	39	7	4	1	1	1	3
	Indoor	0	0	0	4	3	1	0	0	0	0	0	1
Oospora	Outdoor	1	7	5	2	2	7	6	18	34	27	8	27
	Indoor	2	1	5	3	2	9	6	9	22	3	4	5
Total mold catch	Outdoor	117	309	496	1571	1562	846	497	349	313	284	150	365
	Indoor	22	48	93	360	271	171	87	57	75	40	27	38

*Number of daily pairs of Petri dishes contributing to the stated number of mold colonies.

sphere of 25 μ diameter (the order of size of the larger mold spores), terminal velocity—1.9 cm. per second, and a sphere of 5 μ diameter (the order of size of the smaller mold spores), terminal velocity—0.07 cm. per second]. Since the bedroom was undisturbed for about one and one-half hours before the Petri dishes were exposed, it is likely that most of the larger spores had settled out of the air. If, as is believed on morphologic grounds, a proportion of the *Mycelia sterilia* (white and dark) consists of nonsporing *Alternaria* colonies, then the same explanation fits the high outdoor/indoor ratio for this group.

2. *Genera with moist spores*, e.g., *Phoma*, *Pullularia*, and *Candida*, are frequently found in relatively higher numbers on our plates exposed outdoors in rainy weather. This probably increased the outdoor catch relative to the indoor catch of this type of mold spore.

3. *Genera with small spores*, e.g., *Penicillium*, *Aspergillus*, *Oospora*, and *Sporotrichum*. In air moving at low wind-speeds spores are caught on 'surface-traps' (e.g., Petri dishes) with a lower efficiency than in still air.²⁵ This effect becomes more pronounced the smaller the mass of the spore. During sampling, conditions in the bedroom were very still in contrast to the more or less constantly moving air outdoors, and it may be that the small spores were trapped relatively more efficiently indoors, resulting in a lower outdoor/indoor ratio.

PREVIOUS INVESTIGATIONS.—The first attempt at investigating the mold content of the air indoors appears to have been made by Saito⁶ in Japan. He exposed a total of 24 plates in an operating theater, a cloakroom, and a lecture room in conjunction with a longer series of outdoor exposures. Indoors he recorded mainly *Cladosporium* and *Penicillium*. Rostrup⁷ in Denmark exposed plates in

the same house at irregular intervals over a period of one year and found *Penicillium* commonest followed by *Cladosporium* (showing higher incidence in summer) and *Pullularia*. In Italy, Peyronel⁸ found *Penicillium* and *Mucor* the most frequent indoor molds. Prince and Morrow⁹ in Texas caught from the air indoors as many mold spores as they caught out of doors, and in spring the numbers caught indoors were even higher than those outside. Rennerfelt¹⁰ in Sweden found fewer molds in indoor air than outside. On the indoor plates *Penicillium* predominated followed by *Cladosporium*. In the same country Nilsby^{11, 12} found that *Penicillium*, *Cladosporium*, and *Aspergillus* predominated indoors, but this conclusion was based upon an aggregate of some exposures made in dry hygienic houses and others made in unhygienic moldy houses. Flensburg and Samsøe-Jensen¹³ in Copenhagen found *Penicillium*, *Aspergillus*, *Cladosporium*, and *Pullularia* predominating on their indoor plates, but 19 exposures only were made—none in the summer months. In Barcelona, Alemany-Vall¹⁴ found that the commonest mold in indoor air was *Penicillium* followed by *Cladosporium*, *Alternaria*, and *Aspergillus*. Wallace, Weaver, and Scherago¹⁵ reported that *Penicillium* and *Aspergillus* were the predominant molds in indoor air in Kentucky in the United States of America. They found no *Cladosporium* (either indoors or outside) which is surprising in view of the several reports²⁶⁻³¹ which indicate that high numbers of *Cladosporium* occur in the air perennially in the southern portion of the United States. In London, Maunsell¹⁶ using the slit sampler technique found fewer molds in the air in undisturbed rooms than outside. Later¹⁷ the same author showed how the catch of molds on agar plates was considerably increased during and after sweeping and dusting operations. Swaebly and Christensen¹⁸ exposed plates at various times of day in fifteen houses in Minnesota during early spring and summer, and also exposed plates out of doors at the same locations. They found that the indoor counts were lower than outdoors but generally, whereas *Penicillium* and *Aspergillus* predominated indoors, *Alternaria* and *Cladosporium* were predominant outside. One-quarter of their exposures, however, were made after sweeping had been carried on, and, as these exposures showed considerably higher mold catches than the others, they will have seriously influenced the indoor results as a whole. This may have been the cause of the difference between the indoor and outdoor results.

Among the aforementioned investigations, in one alone¹⁵ were regular (but not frequent) observations extended over as long as one year. The majority consisted of a relatively small number of exposures carried out at irregular intervals and it is probable that their results do not reflect the precise order of predominance of the molds in the indoor air throughout the year, nor in particular, during the summer months. Most authors, however, are in substantial agreement in finding lower concentrations of molds in the air indoors, and in naming as the four commonest genera in indoor air *Aspergillus*, *Cladosporium*, *Penicillium*, and *Pullularia*. These four genera are usually (except in the areas where *Alternaria* predominates) the commonest molds in the air outdoors.

SUMMARY AND CONCLUSIONS

In houses which are observably moldy, the mold spore content of the air may be different in constitution (as well as in quantity) from that of the outside air. Culture experiments show that the molds growing in such houses are usually the common atmospheric contaminants, though the predominant species can and does vary from one house to another.

Daily exposures of Petri dishes throughout one year showed that in a normal, dry, fairly old house, the air contains the same molds (though in lower concentrations) as the air outside. The indoor catch fluctuates seasonally in size and constitution parallel with the seasonal fluctuations in the outdoor catch.

The botanical findings indicate that in the normal house the most important source of air-borne mold spores is the outside air; that indoors relatively few spores are produced and released into the air. This suggests that in the investigation, diagnosis, and treatment of allergy to air-borne mold spores the most important prerequisite is a thorough understanding of the mold spore content of the air out of doors.

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