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A Three-Year-Survey of Microfungi in the Air of Copenhagen 1977–79

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The presence and prevalence of common saprophytic airborne microfungi has been studied. Since August 1977 culturable microfungi in the outdoor air of Copenhagen have been monitored. The method used is a volumetric analysis by means of a Biap Slit-Sampler. The microfungal flora was dominated by very few genera, thus *Cladosporium, Alternaria, Penicillium* and *Aspergillus* make up 86.8% of the organisms collected.

The mere number of microfungal spores might be misleading as a guide to the relative importance of atmospheric allergens. An alternative or a supplementary parameter could be the spore substance expressed as cubic spore substance/m^3 of air, as suggested by Hyde (20).

Most fungi show a seasonal dependence as well as fluctuations from year to year. The high season for living microfungi is found to be from June until October. The microfungi thus constitute a prolongation of the pollen season, a fact of importance in relation to patients suffering from inhalation-allergy.

Key words: aeroallergens; aerobiology; microfungi, volumetric sampling.

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During recent years aerobiological studies of atmospheric pollen have enabled clinicians to correlate the concentrations of pollen in the air with the symptoms of the patients sensitive to pollen. It appears, however, that patients with symptoms in prolongation of the pollen season might be sensitive to microfungal spores (8). The correlation between peak values in spore concentration and their ability to produce an attack of asthma is, however, less elucidated than in the case of pollen. The microfungal spores appear in the early summer, maximize in August and continue until October-November in rather a large amount (13, 16, 20, 22, 29).

Volumetric collectors with a fixed sampling efficiency are available today for studying the potentially allergenic airborne microfungi. Two methods: the "viable" and the "non-viable", are generally applied separately or simultaneously.

The slide method (Burkard Trap) is often used for pollen- and spore-counts today (4) with the drawback that only a selected group of moulds can be identified on the slides.

The viable technique requires that spores are able to germinate and grow (3, 22, 29).

Both modes of recovery have yielded valuable information on the prevalence of microfungi separately, but the limitations of either mode of sampling can be overcome by applying them together (9, 22).

In this research the "viable" (culture plate) method has been used with the aim of studying the fungus flora qualitatively as well as quantitatively in the outdoor air, and further, of elucidating the fluctuations in the aerospora during the year.

MATERIAL AND METHODS

During the period from August 1977 to December 1979 regular collections were made with a slit-sampler (7) in Copenhagen.

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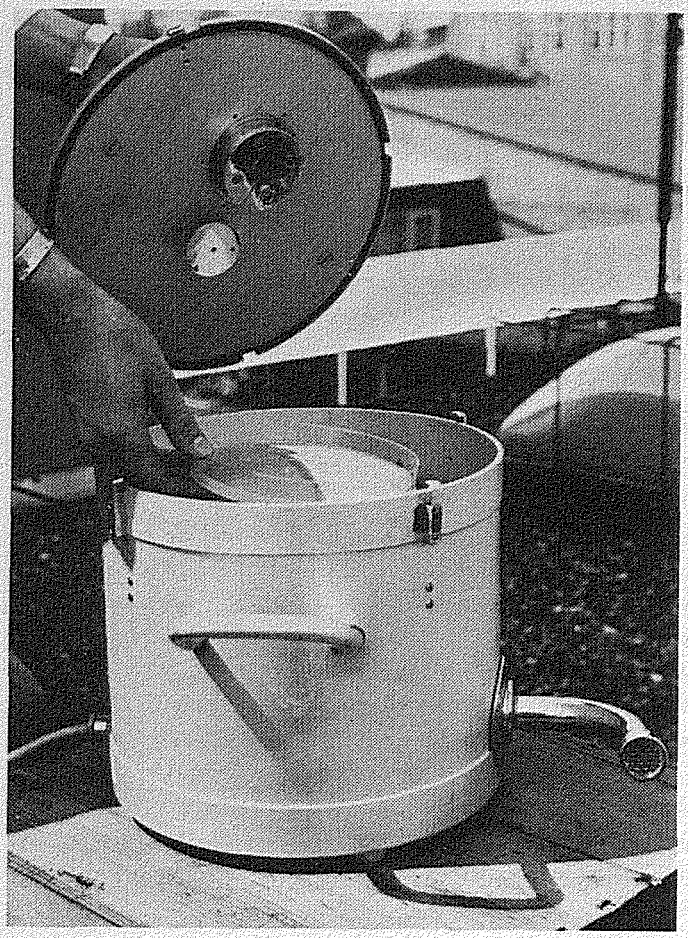


Fig. 1. The Biap slit-sampler.

This volumetric method is based on the principle that a well-defined volume of air is sucked through a slit (0.5 mm wide) and accelerated to a velocity leading the particles suspended in air to impinge on a surface of growth-medium in a slowly rotating petri dish. A built-in distance indicator ensures that the agar surface-slit distance is constantly 1 mm during sampling, so that an optimal retention efficiency is ensured through a linear airspeed of 20–25 m/sec. To prevent overloading of the agar surface, collections of 10 min duration were employed corresponding to 0.3 m^3 of sampled air.

The sampling took place on the roof of Allergologisk Laboratorium at Amager in the Copenhagen area. In 1977 and 1978 the analyses were run twice a week, but in 1979 daily measurements were done. All collections were performed between 1200 and 1300 hours on weekdays.

Recoveries were made on V-8 agar with antibiotics added to eliminate bacterial flora (13). This culture medium allows growth of most living saprophytic fungi, and favours their sporulation, which is a condition necessary for the identification of most of the microfungi.

Following the exposure, the culture plates were incubated at 26°C in high humidity.

After 4–6 days all the developed colonies were counted and identified on the basis of their macro- and microscopical characteristic features. (5, 11, 27, 33, 34). The microscopic slides of the fungi were made in water, or the fungi were stained in lactophenol added cotton blue.

The obtained number of colonies in a sample of 0.3 m^3 was converted to colonies/m^3 air. The culture plates were followed during 1 month, and colonies still without spores remained in the group of *Mycelia sterilia.*

RESULTS

The results of air samplings from August 1977 to December 1979 are given in Table 1. The total colony count is given per cubic meter of air ranked in descending order to the frequency of detection.

The flora is dominated by very few genera – *Cladosporium, Alternaria, Penicillium, Aspergillus* and *Botrytis* together contributing 87.6% of the microfungi collected.

The fungus most commonly met was *Cladosporium,* which alone contributes 68.9% of the total catch. *Cladosporium herbarum* was the most dominating, but *Cladosporium macrocarpum* as well as *Cladosporium cladosporioides* have also been identified. *Cladosporium macrocarpum* was rather frequent in June and July 1978.

The remaining genera were of infrequent occurrence – though non-sporulating fungi accounted for 6.3%, and yeasts together with "mirror yeasts" for 4.1%, represented mainly by *Rhodotorula, Sporobolomyces* and *Trichosporon.* Non-identified colonies account for less than 0.2%.

Fig. 2a. represents the occurrence of the total number of microfungi during the year. The counts are added for each month, here presented as the mean value of 3 years of investigation. Despite wide daily variations, a significant seasonal pattern is seen, with the highest numbers of colonies in summer and autumn and the low-

Table 1

Results of 313 Outdoor Analyses with the Slit-Sampler from August 1977 to December 1979 in Denmark (Percentages are from total number of colonies obtained).

Fungus	Total	%
Cladosporium	48671	68.9
Alternaria	7452	9.4
Penicillium	4682	6.0
Aspergillus	2049	2.5
Botrytis	577	0.8
Arthrinium	270	
Paecilomyses	178	
Stemphylium	126	
Ulocladium	105	
Aureobasidium	76	
Fusarium	72	
Epicoccum	63	
Chrysosporium	59	
Helminthosporium	57	
Acremonium	57	
Chaetomium	54	
Verticillium	42	
Phoma	39	12.4
Scopulariopsis	38	
Trichoderma	30	
Humicola	21	
Trichothecium	18	
Mucor	16	
Geotrichum	6	
Doratomyces	3	
Gliomastix	3	
Graphium	3	
Nigrospora	3	
Oedocephalum	3	
Pithomyces	3	
Rhizopus	3	
Stachybotrys	3	
Yeasts	2995	
Mycelia sterilia	4558	
Fungi imperfecti	131	
Total colony count / m³	72466	100 %

est numbers in winter. Maximum in August and minimum in February, but colonies were obtained even on the coldest days.

Figs. 2b, 2c, 2d and 2e show the monthly variation of *Cladosporium, Alternaria, Penicillium* and *Aspergillus* respectively.

Table 2 indicates the mean values of the microfungi count for each month (total number of microfungi and four genera respectively).

The highest number of total colonies is achieved in August (1086 col/m³), and the lowest number in February (20 col/m³).

From January to April the colony-count stays below 50 col/m³ air, starts to rise in May (80 col/m³) and declines in November.

In addition it is seen that *Cladosporium* dominates from May-November, the highest average (914 col/m³), occurred in August, the lowest (3 col/m³), in January. Contrary to this, *Penicillium* is rather frequent in the winter months, with the highest average (40 col/m³) in November, and the lowest in August (5 col/m³).

Alternaria is of little significance during 8 months of the year, with approx. 1–5 col/m³, but in August it shows a sudden increase to 125 col/m³, only dominated by *Cladosporium. Aspergillus* accounts for only about 3% of the total fungal flora on a yearly basis – and like *Penicillium* – is most frequent in November.

Fig. 3 indicates that *Cladosporium* contributed to 81% of the total colony count in 1977 (15,814 colonies distributed among 35 analyses), 79.7% in 1978 (29,239 col. distributed among 83 analyses), but only 46.2% in 1979 (27,421 col. distributed among 195 analyses) (mean value 68.9%).

The frequency of *Alternaria* shows an increase from 4–5% in 1977/78 to 18.7% in 1979 (mean value 9.4%).

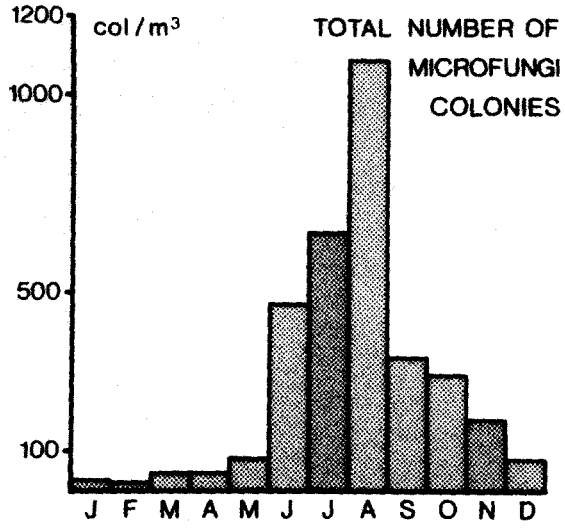
Concerning *Penicillium* the frequency varies from 3–4% in 1977/78 to 10.8% in 1979 (mean value 6%) and *Aspergillus* fluctuates from 1–2% in 1977/78 to 5.2% in 1979.

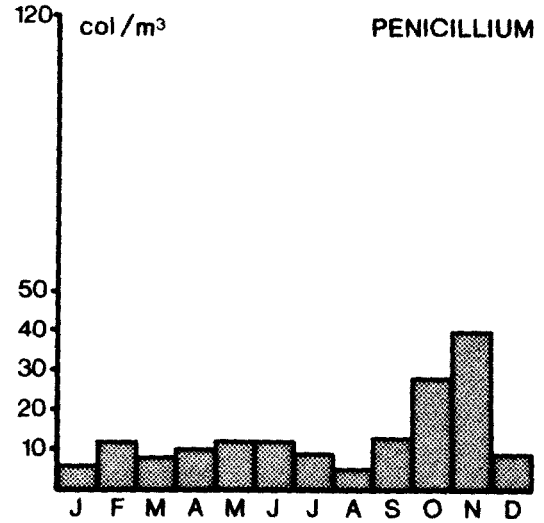
DISCUSSION

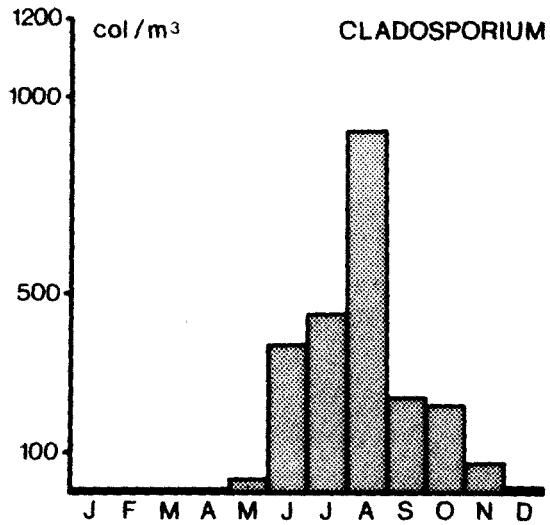
Prevalence

Cladosporium accounts for 68.9% of the total colony count (average 3 years) in this study. This dominance agrees well with previous Danish observations by Flensborg & Samsøe-Jensen (12). Other aerobiological studies have shown that *Cladosporium* is ubiquitous, living as a saprophyte on dead herbaceous plants, especially grasses. It dominates the outdoor microfungal flora in temperate regions and in the tropics (1, 16, 17, 19, 32).

Alternaria, fewer in number but not necessarily less in total volume, contributes with 9.4% of the total airspora in this period. This corresponds well with other aerobiological studies (1, 22, 28) although it may be found in large quanti-







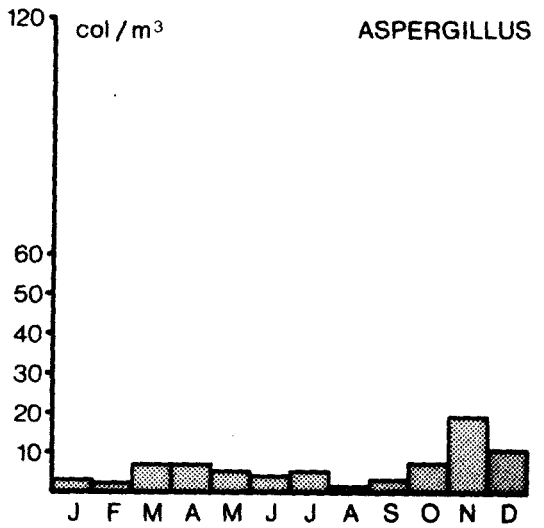
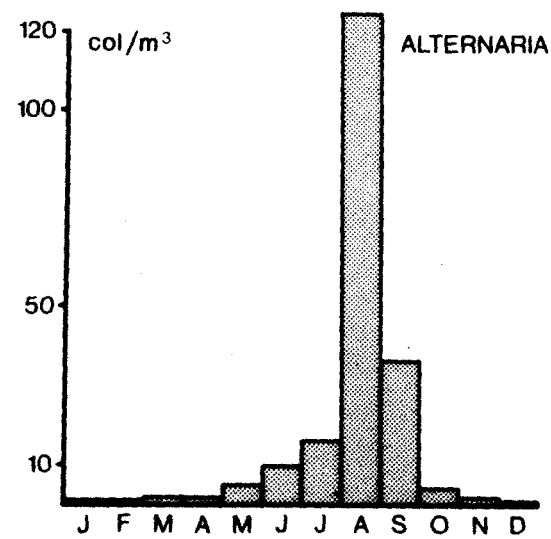


Fig. 2. Seasonal incidence of the overall fungal growth and the four most common outdoor moulds. Monthly average during 3 years, 1977–79.



ties in moister and warmer climates and even dominates the airspora of certain regions in the Unites States (25). In the Arctic and Alpine regions it is rare or even lacking (6).

Many *Penicillium* species are soilborne saprophytes, distributed all over the world, and frequencies from 2 to 15% have been reported (6). The frequency of *Penicillium* (6.0% and *Aspergillus* (2.5%) here in Copenhagen is in good agreement with these findings. Hamilton (17) found large quantities of *Penicillium* in the city of London compared with findings from the countryside.

Table 2

The monthly mean of the number of microfungi colonies per analysis from August 1977 to December 1979. Total colonies and four genera are listed

Month	Total colony count col/m³	Cladosporium col/m³	Alternaria col/m³	Penicillium col/m³	Aspergillus col/m³
January	24	3	1	6	4
February	20	4	1	12	2
March	42	8	2	8	8
April	47	9	2	10	5
May	80	27	5	12	5
June	470	367	10	12	4
July	652	449	16	9	6
August	1.086	914	125	5	2
September	333	237	36	13	3
October	291	214	4	28	6
November	177	71	2	40	19
December	67	8	1	9	11

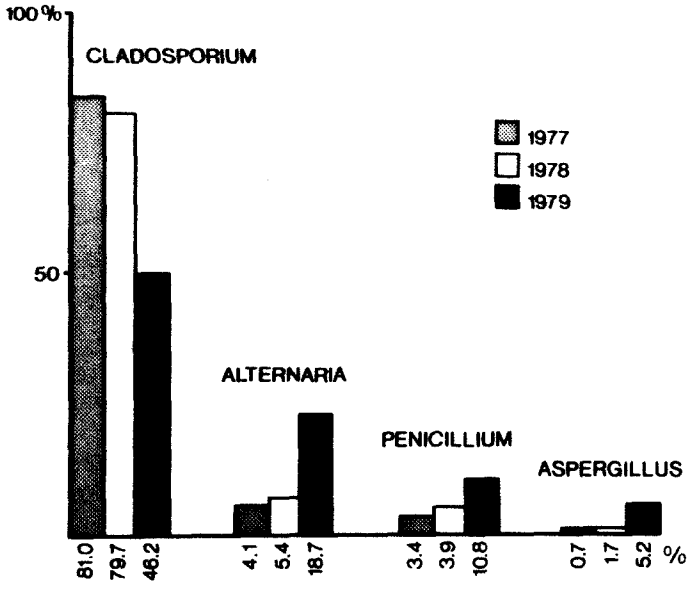


Fig. 3. The variation in frequency of the four dominating genera from 1977–1979. Percentages are from total number of colonies each year. The average total colony counts per analysis were: 1977: 452 col/m³, 1978: 352 col/m³, 1979: 141 col/m³.

Spores of *Aspergillus* are usually found in moderate counts in the atmosphere. The genus *Aspergillus* has mainly been represented by the species *Aspergillus fumigatus* here in Copenhagen (1977/79), but *Aspergillus candidus, ochraceus* and *niger* have also been identified. In the United States, however, Bassett (6) found *Aspergillus niger* the most frequent.

Continuous records, using the Hirst Automatic Volumetric spore trap, showed the "mirror-yeast" *(Sporobolomyces)* to predominate together with *Cladosporium* (16). Yeasts together with "mirror-yeasts" account for 4.1% in this survey.

Seasonal variations

In this study a seasonal variation has been demonstrated in spite of the fluctuations in the flora from day to day (Fig. 2). Seasonal development of the microfungi and maturation of the spores commonly determine the airflora at a given time, affected by external factors (e.g. substrate, temperature, humidity, precipitation) as well as internal biological factors. These fluctuations during the year have been observed in many surveys of microfungi (1, 16).

Summer peaks in spore concentrations might be obtained by favourable temperature and humidity. In late summer and autumn the peaks may be explained by the beginning process of seasonal decay of vegetable matter. The low spore frequency in the winter months is probably due to the snow cover, but the spore frequencies start decreasing already in November before snowfall, when the temperature falls below zero, preventing sporulation.

Cladosporium and *Alternaria,* obligate members of the phylloplane flora, i.e. leaf-surface flora, show pronounced seasonal periodicity. A close connection between the product of temperature and humidity and the number of *Cladosporium* colonies was discovered by Flensborg & Samsøe-Jensen (12) who found that when this product was below 1,000, the colony-count was low.

In contrast *Penicillium* and *Aspergillus* seem to be independent of seasonal changes, although the concentrations tend to increase in the winter months. In Finland, however, Ahlström & Kääärik found a maximum of *Penicillium* in July and a minimum in December (2).

Incidences of these four fungi in the indoor environment of Denmark seem to parallel the outdoor exposures in seasonal incidences (13). Indoor exposures, however, show a higher number of *Aspergillus* and *Penicillium* because they probably find a growth focus in the indoor environment. In the United States Lumpkins et al. and Solomon found corresponding seasonal relations between indoor and outdoor incidences of *Alternaria* and *Cladosporium* (23, 29).

The moulds, *Mucor* and *Rhizopus,* abundant in soils and commonly found in house-dust (14), are very seldom seen in outdoor analyses. Gravesen explains the overall representation of *Mucoraceae* in house-dust by the fact that the spores are very sticky because of their liberation from the sporangium in a mucilaginous droplet (21).

Average colony counts for each month might be useful botanical reference values to the actual colony count at a given time during the season (cf. Table 2). These values, too, may help the allergist in correlating periodical aggravations of symptoms in sensitized patients with fungal spores prevalent at the corresponding period.

Since the microfungal spores are arranged in order of numerical abundance, *Alternaria,* an important allergen, appears so far down the list that it must be evident that the number of spores, as a single parameter, is a misleading guide to the relative importance of atmospheric allergens. An alternative method has been suggested by Hyde, which involves reporting individual spores on the basis of cubic spore substance/m^3 air. He claims to achieve a closer correlation with clinical data by this method (20).

In contrast *Cladosporium* is by far the most frequent fungal spore in this study and other surveys. Yet, Street & Hamburger (31), by skin prick-testing patients sensitive to moulds, found only 60% of the patients with a positive reaction to *Cladosporium.* This has been investigated and confirmed by Gravesen (15), who found that among patients with positive skin prick test to fungi, only 60% reacted to *Cladosporium.*

Variation from year to year

The fungal flora from 1977 show a good correlation to that of 1978, qualitatively as well as quantitatively. In 1979 the total colony count was about 30% less than that of 1978, probably because of the cold summer in Denmark (Fig. 3).

According to Harvey (18) warm, dry summers favour high spore concentrations, whereas cool, wet summers are inhibitory, and Hyde claims that consistently cool summers relieve sufferers from *Cladosporium* asthma (20). This decline in *Cladosporium* colonies in 1979 might enhance the growth of other fungi on the culture plates, and the significant increase of *Alternaria* in 1979 could be explained by this fact.

It should be mentioned that the analyses from 1977 are only performed during 6 months (from August to December), and thus may not be representative of the whole year.

This study only describes the microfungal flora in a restricted area, and further study is required in order to evaluate the effects of local vegetation and climate on the fungal flora.

Materials and methods

Previously, the pollen and spore surveys were based entirely on the gravity slide and culture plate techniques. But when the volumetric apparatus appeared, it was preferred in outdoor studies due to its superiority to gravity technique in turbulent air.

Several surveys of the airborne microfungi have been performed by volumetric culture plate methods, "viable" methods (2, 3, 22). The advantages and disadvantages have been men-

tioned here, e.g. the selectivity of medium, temperature, and the competitive interactions between organisms present on the culture plate.

One of the advantages is the easiness with which the 10–15 μm small-spored genera can be identified, many of which are of allergological relevance.

The Hirst Trap (the "non-viable" method) has been applied for quite a number of years in outdoor surveys. The advantages are, for example, the statistically more reliable results due to the great number of spores caught by the trap (2). A diurnal periodicity can be demonstrated by the continuous recordings – but the hyaline, small-spored genera like for instance *Penicillium* and *Aspergillus,* are difficult to identify by this method. The superiority of applying the two traps at the same time has been demonstrated by several authors (1, 9, 22).

All the average concentrations mentioned in this work are low in comparison with the daily number of spores to which the patients may actually be exposed at the seasonal peaks. The concentrations of microfungal spores eliciting symptoms are, however, not quite known, but the results of indoor and outdoor studies of the fungal flora may be a helpful tool for the allergologist in the clinical work of elucidating fungal allergy.

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