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IDENTIFICATION AND QUANTITATION OF INDOOR AIRBORNE MICRO-FUNGI DURING 12 MONTHS FROM 44 DANISH HOMES

By

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It is well known that airborne spores and conidia from micro-fungi may cause inhalation-allergy of various forms (3, 11, 15, 22, 32, 35, 36).

Aas (1) and *Liebeskind* (16) suggest a certain relation between the intensity of a given influence and the inhalation-allergy. As this question still needs discussion and perhaps a better understanding a qualitative and a quantitative investigation of the indoor flora of fungi, to which these patients are largely exposed, seems to be of importance. In Scandinavia previous works have especially dealt with outdoor microflora (6, 11, 12, 21, 26), and outside Scandinavia investigations of the same kind have been made (2, 10, 12, 13, 31, 33, 35).

The purpose of the present study is to detect the types of micro-fungi that can be found in Danish homes in the towns and in the country and to investigate if seasonal variations can be demonstrated in the indoor microflora. For practical reasons the investigation was performed in homes of children supposed to be hypersensitive to fungi, in order to obtain results that could be correlated to the clinical studies. It was planned later to relate these results to the effect of hypersensitization with the strain of a given species from the home of the patient with the effect of an analogous standard-strain.

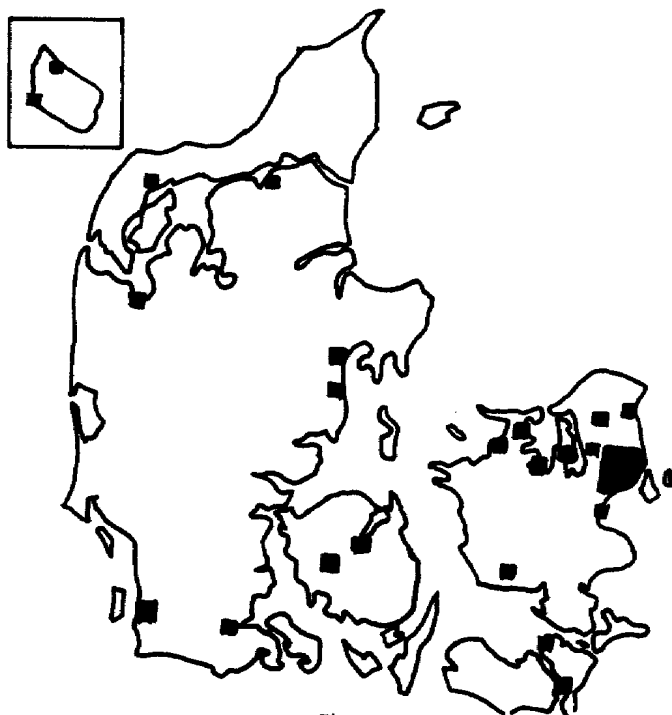


Fig. 1.

A map of Denmark showing the geographical distribution of the 44 examined homes.

MATERIAL

50 children referred in 1970 to the Pediatric Allergy Department of the University Hospital of Copenhagen were chosen as their anamnesis and the clinical examinations (scratch-tests and provocation-tests by sniffing) revealed hypersensitivity to fungi. During one year three agar-plates were sent monthly from Allergologisk Laboratorium with information to the childrens' homes to have the Petri-dishes exposed in the childrens' room, the sitting-room and the kitchen. In 44 homes the investigation was followed up, so that the material includes 1584 agar-plates.

The investigation ran from February 1970 to September 1971 as the homes were successively included in the investigation and recieved agar-plates in the

following 12 months. The geographical distribution of the homes is shown on the map Fig. 1.

METHODS

The agar-plate method. 9 cm ethylene-oxide-sterilized plastic-Petri-dishes were used; largely they can be mailed all over the country without damages. V-8-agar was used as culture medium. V-8 is a vegetable-juice consisting of juice from tomato, carrot, celery, beet root, parsley, lettuce, water cress and spinarch, canned by Campbell's Soup Ltd. The medium is sugar-deficient, but rich in mineral salts and vitamins, and most culturable micro-fungi will sporulate well on it (7). Furthermore the development of mycelium will be suppressed for the benefit of generation of conidia or spores, which is an advantage for the identification of the fungi. To eliminate growth of bacteria Penicillin and Streptomycin were added. The medium has the following composition:

V-8 medium	200 ml
CaCO ₃	3 g
Agar "New Zealand"	20 g
Aqua destillata	ad 1000 ml
Penicillin	100,000 IU/l
Streptomycin	1g/l

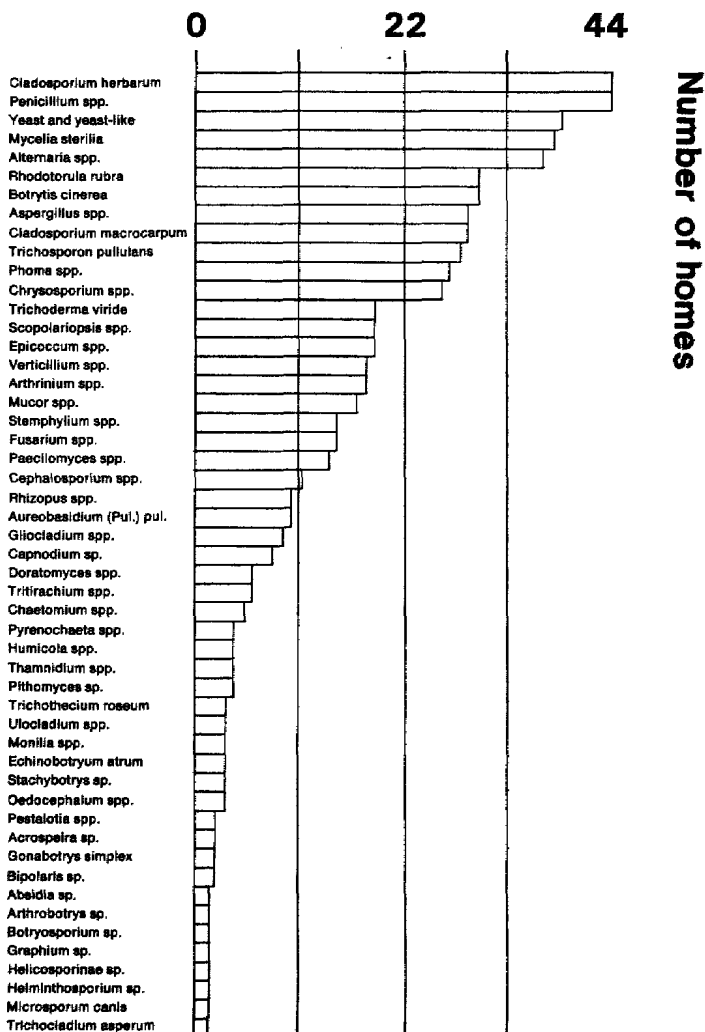
After sterilization (120° C, 20 min.) the antibiotics were added, and about 28 ml of the medium poured into each Petri-dish.

Exposition of the plates. The sterile agar-plates were sent to the patients with instructions to expose one plate during 20 minutes in sitting-room, childrens' room and kitchen respectively. Immediately after exposure they were re-sealed and by return of mail sent to the laboratory for incubation at 25° Celcius for about one week.

Identification of the fungi. After one week of incubation the colonies were counted and identified to genus and if possible to species by their macro- and microscopical character (4, 5, 9, 17, 24, 25, 34, 37).

Slides of the fungi were made either in water, or the fungi were stained in lactophenol with methylene blue (7). When identification was problematic, subcultures were made according to the cover-slip-method: The fungus is cultivated on an 5 mm agar-cube cut out of an agar-plate under sterile circumstances. A sterile cover-slip is placed on top of the cube, and the fungus will germinate on the surface of the cube on the bottom side of the cover-slip. When the cover-slip is subsequently placed directly on the slide with the staining medium, an undisturbed microscopical examination of the fungus is possible, as the conidia by this method will remain on the conidiophores.

Quantitation of the colonies. The number of colonies on the agar-plates were counted one to two weeks after the incubation of the plates. As a control the plates were re-examined after incubation during one month.

*Fig. 2.*

The occurrence of micro-fungi in the 44 homes examined.

RESULTS

In Fig. 2 the occurrence of micro-fungi in the 44 homes is demonstrated. It is emphasized that the results illustrated in the different diagrammes will not always be comparable as both a whole genus and singular species are quoted.

Table 1 shows the occurrence of micro-fungi in all plates examined ($3 \text{ plates} \times 12 \text{ months} \times 44 \text{ patients} = 1584 \text{ plates}$). *Cladosporium herbarum* e.g. was observed in 718 agar-plates out of 1584 (45 per cent). 4253 colonies of *Cladosporium herbarum* were counted 3398 of which are undoubtful primary colonies, while the rest may be secondary colonies developed in plates due to prolonged expedition.

Several problems are attached to the quantitative estimation of the distribution of the fungi (colony counting). The question will be further treated in the discussion.

Fig. 3 shows the monthly occurrence during one year of the most prominent fungi of the rooms examined in the 44 homes.

Fig. 4 shows the seasonal variations of the material based upon the total amount of colonies per month during one year.

Fig. 5 shows the qualitative and the quantitative occurrence of the fungi of each home during one year. The ordinate shows the total amount of fungi observed and the total amount of colonies of the 36 plates investigated from each home. The grouping of the homes in four rough sections reflects a certain supposed uniformity of outer environment.

DISCUSSION

The procedure employed was preferred in spite of its limitations, because it reasonably depicts the composition and variation of the indoor microflora, eliminates problems of expedition by mail and admits a simultaneous investigation of about 50 homes during a certain period. Other procedures and their applicability have been previously discussed (2, 20, 27, 28).

For practical reasons the homes investigated were chosen on account of their co-operability with clinical studies. It was realized that these homes might be particularly infected by

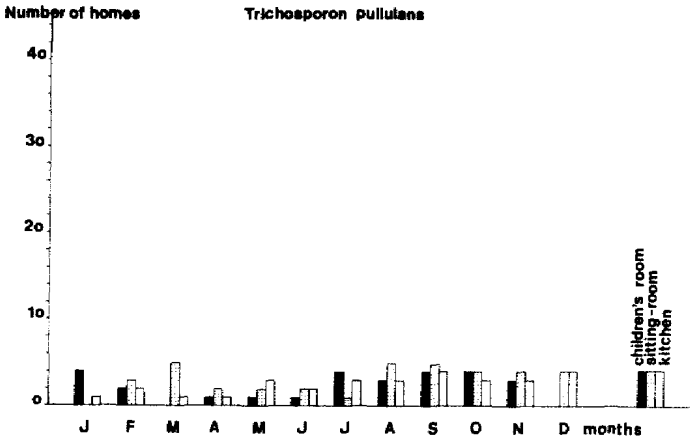


Fig. 3 a.

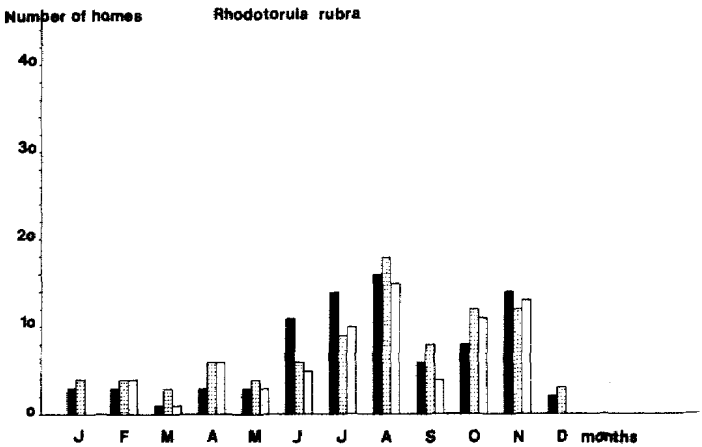


Fig. 3 b.

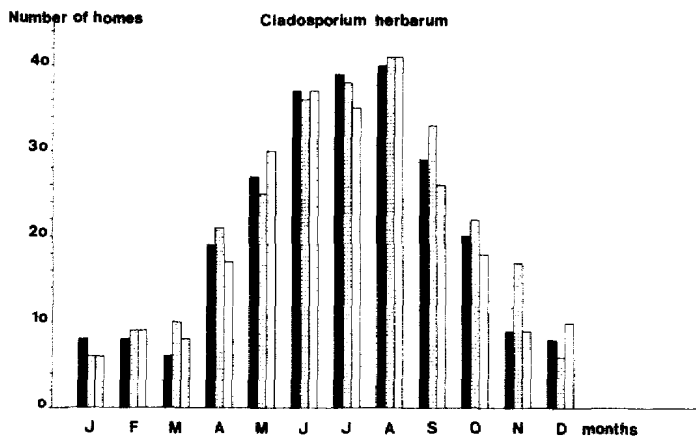


Fig. 3 c.

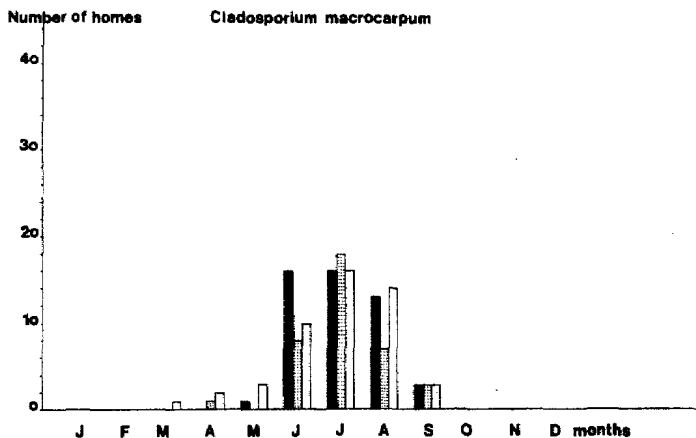


Fig. 3 d.

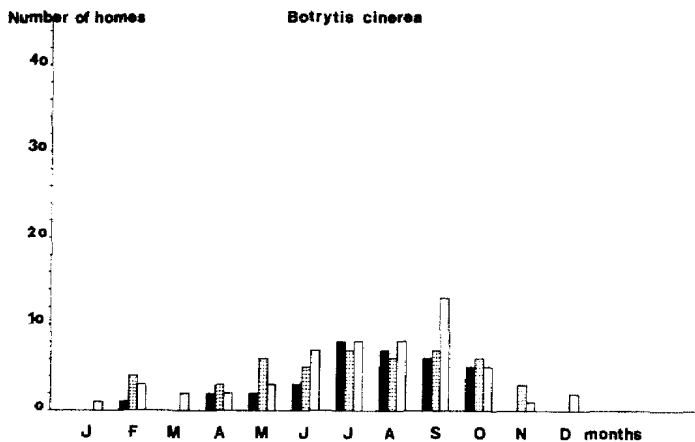


Fig. 3 e.

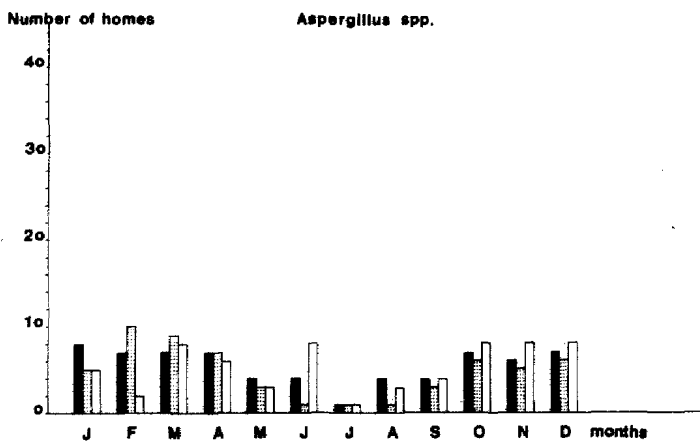


Fig. 3 f.

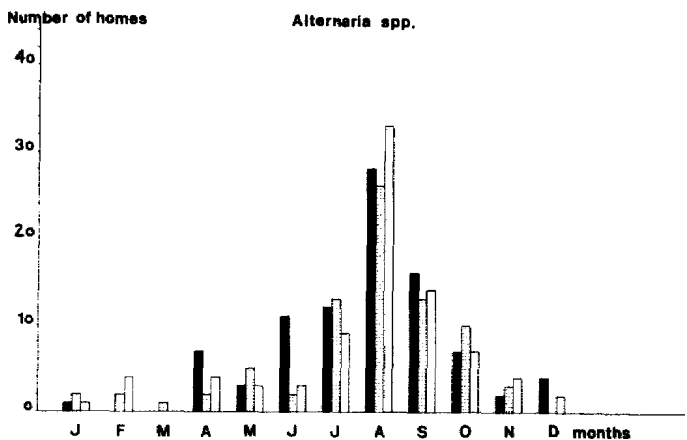


Fig. 3 g.

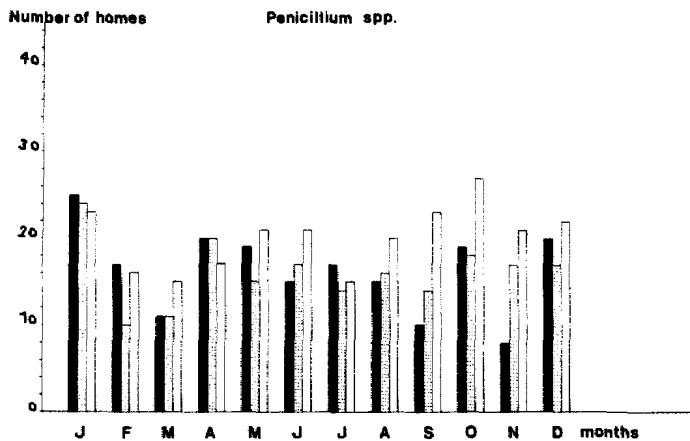


Fig. 3 h.

Fig. 3.

Monthly variation of some of the most frequently occurring genera and species of the observed micro-fungi from the 44 investigated homes.

TABLE 1

The qualitative and quantitative occurrence of micro-fungi in the 1584 agar-plates examined.

sp. stands for *species*, spp. for *species pluralis*.

Fungi observed	Qualitative occurrence	Percentage	Number of colonies
<i>Cladosporium herbarum</i>	718	45 %	3398 (4253)
<i>Penicillium</i> spp.	595	37 %	1652 (2653)
<i>Mycelia sterilia</i>	493	28 %	744
<i>Alternaria</i> spp.	268	16 %	532
<i>Rhodotorula rubra</i>	215	13 %	416 (502)
<i>Aspergillus</i> spp.	186	11 %	297 (327)
<i>Yeast and yeast-like</i>	146	9 %	271 (301)
<i>Botrytis cinerea</i>	135	8 %	159
<i>Cladosporium macrocarpum</i>	134	8 %	313
<i>Trichosporon pullulans</i>	94	6 %	134
<i>Chrysosporium</i> spp.	89	5 %	177
<i>Phoma</i> spp.	70	4 %	105
<i>Scopulariopsis</i> spp.	45	3 %	96 (129)
<i>Verticillium</i> spp.	42	2 %	50
<i>Trichoderma viride</i>	40	2 %	51
<i>Mucor</i> spp.	33	2 %	35
<i>Epicoccum</i> spp.	32	2 %	38
<i>Arthriniun</i> spp.	26	2 %	28
<i>Fusarium</i> spp.	23	1 %	24
<i>Paecilomyces</i> spp.	19	1 %	19
<i>Rhizopus</i> spp.	18	1 %	31
<i>Cephalosporium</i> spp.	18	1 %	20
<i>Aureobasidium</i> (<i>Pullularia</i>) <i>pullulans</i>	17	1 %	24
<i>Trichothecium roseum</i>	14		16
<i>Capnodium</i> sp.	13		20
<i>Gliocladium</i> spp.	13		13
<i>Echinobotryum atrum</i>	12		17
<i>Doratomyces</i> spp.	12		12
<i>Ulocladium</i> spp.	12		14
<i>Tritirachium</i> spp.	11		24
<i>Thamnidium</i> sp.	8		8
<i>Microsporum canis</i>	7		30
<i>Pyrenochaeta</i> spp.	7		11
<i>Humicola</i> spp.	6		6
<i>Chaetomium</i> spp.	6		12
<i>Pithomyces</i> sp.	3		3

TABLE 1, continued.

Fungi observed	Qualitative occurrence	Percentage	Number of colonies
<i>Stachybotrys sp.</i>	2		3
<i>Monilia spp.</i>	2		3
<i>Oedocephalum sp.</i>	2		4
<i>Pestalotia sp.</i>	2		2
<i>Bipolaris sp.</i>	2		7
<i>Acrospeira sp.</i>	2		2
<i>Gonatobotrys simplex</i>	2		3
<i>Absidia sp.</i>	1		1
<i>Arthrobotrys sp.</i>	1		1
<i>Botryosporium sp.</i>	1		1
<i>Graphium sp.</i>	1		1
<i>Helicosporina sp.</i>	1		1
<i>Helminthosporium sp.</i>	1		1
<i>Trichocladium asperum</i>	1		1

Monthly number of colonies



Fig. 4.

Number of colonies observed for each month on the 132 agar-plates examined.

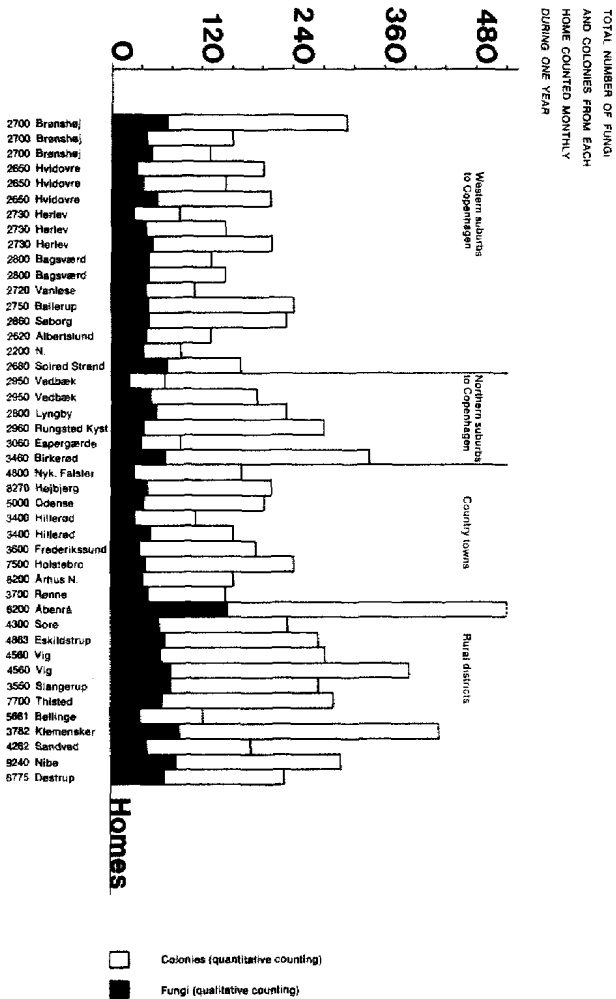


Fig. 5.

Qualitative and quantitative presence of the fungi in the 44 homes examined.

micro-fungi of growth upon painting, tapestry, isolation-materials, furniture etc. (8, 16, 29). The question that these homes may not be typical of the population will be elucidated by supplementary investigation of homes of normal persons.

No special instructions were given concerning persons present in the rooms, cleaning, open or closed windows and radiations during the plate exposure as it was considered too comprehensive for the individual homes to pay regard to such factors.

The variation of the circumstances mentioned have a certain influence upon the results, and must be considered with the factors mentioned below:

A possible selective action of the culture-medium.

This possibility seems insignificant, as most culturable micro-fungi grow and sporulate well on V-8-agar.

The way of growth of individual fungi.

Presumably weaker and more slow-growing fungi will be ousted. Analyses of e.g. *Botrytis*, *Mucor*, *Rhizopus* or *Trichoderma* will often cause uncertainty concerning the quantitative estimation, as these fungi are very rapidly growing and often produce a widespread fluffy mycelium which may cover a whole agar-plate in very few days and nights.

Questions about expedition by mail.

If the plates were not returned by the patient immediately after the exposure, a colony may have had time to develop. Conidia germinating from such a colony and producing new colonies itself can cause quantitative misunderstanding. The problem was relevant largely for colonies of *Aspergillus*, *Penicillium* and *Cladosporium*, all of which are rapidly growing. Numbers of colonies in brackets express this uncertainty. Due to the two latter restrictions the number of colonies were re-counted several times. But new colonies very rarely appeared 1-2 weeks after the incubation, and the counting after 1-2 weeks of incubation, therefore, gives a representative colony-measure.

As material and to a certain degree also the procedure are different, a direct comparison between other investigations and the present one will not be attempted.

Outside Scandinavia a difference of the composition of the micro-fungal flora will be present depending on difference of climate, soil and flora of higher plants. Qualitative differences will be evident in the micro-flora of different geographical regions according to the phanerogamus plants often being hosts to saprophytic or parasitic fungi, which are mostly species-specific.

The previous Scandinavian works correspond reasonably to the present study in respect to the occurrence of the fungi.

Still *Rennerfelt* (26) and *Winge Flensburg & Samsøe-Jensen* (12) count *Aureobasidium* (*Pullularia*) *pullulans* as second in frequency. In the present work it was only observed in the homes at a rate of 20 per cent. (Fig. 2). An explanation may be that preliminary decomposition of dead leaves of beech in the forest is started by *Aureobasidium*. The fungus attacks the pectine of the middlelamella of the cellwalls of the leaves and uncovers cellulose for the next wave of attack by micro-organisms (20). Even if *Aureobasidium* is able to grow on painting or other indoor materials, it will be far more manifest outdoor (9).

A fungus not previously mentioned in connection with air-analyses is the asporogen yeast *Trichosporon pullulans* which was observed in about 65 per cent of the homes. The fungus is often isolated from mucous membranes of the nose but apparently it is not pathogenic.

Candida albicans and *Neurospora* (*Monilia*) *sitophila* are two easily culturable fungi often mentioned as allergenic; they were not observed in the present material. The absence of *Candida albicans* is concordant to a Danish investigation of yeast on mushrooms including 300 isolates from different yeasts cultivated on five different medias. Many species of *Candida* were obtained but no *Candida albicans* (14). About 90 species of *Candida* have until now been described.

Targow (30) and *Prince & Morrow* (23) are of the

opinion that the reactions of the patients refer to the botanical systematic groups. The assertion which is opposed by *Mandell* (18) may be problematic, as the group *Fungi imperfecti*, to which a considerable part of the micro-fungi belongs, is an artificial group of fungi with asexual reproduction.

From Fig. 3 it appears that no variation of a given fungus in the investigated three rooms can be stated. To obtain uniform experimental conditions as far as possible in future investigations instructions considering exact hour, persons present, windows etc. will be desirable. Further, with a view to the rapid growth of colonies, expeditions by mail must not exceed four days.

From Fig. 3 it will be seen that a significant seasonal variation can be stated concerning the most frequently occurring fungi with the exception of *Penicillium*. On beforehand the indoor flora could be imagined to be more constant during the year as compared to the outdoor flora, as many fungi are found in housedust, furniture upholstery, etc. (8, 29). With the exception of *Aspergillus* the maximum rates were found in the months of high summer or late summer, which is corresponding to previously made outdoor investigations. Thus it can be stated that to a great extent the micro-flora follows the macro-flora which often serves as hosts for the fungi in question. The common occurrence during the year of *Penicillium* similarly is corresponding to previous investigations (2, 26, 27).

The present material is too small to give a picture of the seasonal variation in respect to every single home. The fungi most often observed appear in almost all homes (Fig. 2). Among the homes the numbers of observations and colonies vary considerably. Fig. 5 shows that a greater number of colonies and observations have been counted in group IV, the country houses. The most rare genera and species were likewise observed in this group.

CONCLUSION

It can be concluded that the seasonal variation of the indoor airborne micro-fungi correspond with the variation of the outdoor flora. Out of the eight most frequently occurring fungi six have their maximum at high summer and late summertime and have their greatest extension in the rural districts. These must be reasonable objects for preliminary investigations of mould-allergy, later followed by other fungi.

The question about cross-reactions, specificity of species and strains must be solved in co-operation with treating clinics. Since many questions about allergy to fungi still need further investigation and understanding, an examination of the homes of patients with suspected hypersensitivity to fungi is recommended.

SUMMARY

44 children referred to the Pediatric Allergy Department of the University Hospital of Copenhagen were subjects to a preliminary investigation of hypersensitivity to micro-fungi. This investigation was followed by an examination of the indoor micro-flora of the patients homes performed by Allergologisk Laboratorium, with the agar-plate-method. A seasonal variation of the indoor airborne micro-flora corresponding to the outdoor flora was demonstrated. Out of the eight most frequently occurring genera and species of fungi six have their maximum at high and late summertime and have their greatest extension in the rural districts. As long as the problems of mould-allergy are not thoroughly understood, an investigation of the homes of patients suspected to be hypersensitive to fungi is recommended.

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REFERENCES

1. *Aas, K.*: Allergiske barn. J. W. Cappelens Forlag, Oslo, 1969.
2. *Agarwal, M. K., Shivpuri, D. N. & Mukerji, K. G.*: Studies on the allergenic fungal spores of the Delhi, India, metropolitan area. *J. Allergy* 44, 193, 1969.
3. *Austwick, P. K. C.*: The role of spores in the allergies and mycoses of man and animals. Colston Papers. Proc. 18th Sym. Colston Res. Soc. Bristol 18, 321, 1966.
4. *Barnett, H. L.*: Illustrated Genera of Imperfect Fungi. Second Edition. Burgess Publ. Company, Minneapolis, 1967.
5. *Barron, G. L.*: The Genera of Hyphomycetes from Soil. The Williams and Wilkins Company, Baltimore, 1968.
6. *Bruun, E. & Schwartz, M.*: Svampeallergi. *Nord. Med.* 26, 121, 1945.
7. *Christiansen, M. Skytte*: Vejledning til kursus i mikrobiologi. Second Edition, Danmarks Ingeniør Akademi, København.
8. *Conant, N. F. & al.*: Fungi found in pillows, mattresses and furniture. *J. Allergy* 7, 234, 1936.
9. *Cooke, W. B.*: An ecological life history of *Aureobasidium pullulans* (de Bary) Arnaud. *Mycopathologia*, 12, 1, 1959.
10. *Davies, R. R., Denny, M. J. & Newton, L. M.*: A comparison between the summer and autumn air-spores of London and Liverpool. *Acta Allergol.* 18, 131, 1963.
11. *Feinberg, S. M.*: Mould allergy its importance in asthma and hay-fever. *Wisc. Med. Jour.* 34, 254, 1935.
12. *Flensburg, E. Wingé & Samsøe-Jensen, T.*: Studies in mold allergy. *Acta Allergol.* 3, 49, 1950.
13. *Goodman, D. H. & al.*: A study of airborne fungi in the Phoenix, Arizona, metropolitan area. *J. Allergy* 38, 1, 56, 1966.
14. *Hartmann, A. L.*: Unpublished study. Copenhagen. 1964.
15. *Jimenez-Diaz, C. & al.*: The aethiologic role of molds in bronchial asthma. *Acta Allergol. suppl.* 7, 139, 1960.
16. *Liebeskind, A.*: Diagnostic value of culture procedures and provocation tests in suspected mold allergies. *Acta Allergol.* 26, 106, 1971.
17. *Lodder, J. & Kreger-van Rij, N. J. W.*: The Yeasts. A. Taxonomic Study. North-Hollands Publ. Company, Amsterdam, 1952.
18. *Mandell, M.*: Proof of genus specific mold allergens. *Ann. Allergy* 25, 48, 1967.
19. *Merksamer, D. & Sherman, H.*: An evaluation of atmospheric mold spores as allergens in New York metropolitan area. *J. Allergy* 29, 60, 1958.

20. *Nissen, T. Vincents*: Mikroorganismernes liv. Teknisk Forlag, København, 1966.
21. *Nilsby, I.*: Allergy to moulds in Sweden, a botanical and clinical study. *Acta Allergol.* 2, 57, 1949.
22. *Pepys, J.*: Hypersensitivity Diseases of the Lungs due to Fungi and Organic Dusts. Monographs in Allergy. Vol. 4, S. Karger, Basel, New York, 1969.
23. *Prince, H. E. & Morrow, M. B.*: The diagnosis of mold allergy. *Ann. Allergy* 25, 337, 1967.
24. *Raper, K. B. & Fenell, D. I.*: The genus *Aspergillus*. The Williams & Wilkins Company, Baltimore, 1965.
25. *Raper, K. B. & Thom, C.*: A Manual of the *Penicillia*. The Williams and Wilkins Company, Baltimore, 1949.
26. *Rennerfelt, E.*: Några undersökningar över luftens halt av svampsporer. *Svensk Bot. Tidsk.* 41, 2, 1947.
27. *Rooks, R. & Shapiro, R. S.*: The value and limitations of mold surveys by the culture plate method. *J. Allergy* 29, 24, 1958.
28. *Solomon, W. R. & Gilliam, J. A.*: A simplified application of the Anderson-sampler to the study of airborne fungus particles. *J. Allergy* 45, 1, 1970.
29. *Sweably, M. A. & Christensen, C. M.*: Molds in house dusts, furniture stuffings and the air within homes. *J. Allergy* 23, 3, 1952.
30. *Targow, A. M.*: Studies on atopic skin-test reactions: I. Survey of reactions to the fungi: Evidence for existence of two different group allergens. *Ann. Allergy* 24, 112, 1966.
31. *Shivpuri, D. N. & Agarwal, M. K.*: Studies on the allergenic fungal spores of the Delhi, India, metropolitan area. *J. Allergy* 44, 204, 1969.
32. *Tuft, L. & Mueller, H. L.*: Allergy in Children. W. B. Saunders Company, Philadelphia, 1970.
33. *Turner, P. D.*: The fungal air spora of Hong Kong as determined by the agar plate method. *Trans. Brit. Mycol. Soc.* 49, 255, 1966.
34. *de Vries, G. A.*: Contribution to the Knowledge of the Genus *Cladosporium* Link ex Fr. (Diss.), Baarn, 1952.
35. *Van der Werff, P. J.*: Mould Fungi in Bronchial Astma. H. E. Stenfert Kroese, N. V. Leiden, 1958.
36. *Van Leeuwen, V. S.*: Bronchial astma in relation to climate. *Proc. Roy. Soc. Med.* 17, 19, 1924.
37. *Zycha, H., Siepmann, R. & Linnemann, G.*: *Mucorales*. Verlag von J. Cramer, Hann. Münden, 1969.

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