

Volumetric Aerobiological Survey of Conidial Fungi in the North-East Netherlands

II. Comparison of Aerobiological Data and Skin Tests with Mould Extracts in an Asthmatic Population

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A study was undertaken to see whether the principal airborne fungi in the North-East Netherlands were also found to be the most reactive in skin testing. Atmospheric samples were taken weekly with the Andersen sampler, from April 1981, up to and including, June 1983. At the same time skin tests of 833 patients referred to the outpatient Departments of Pulmonology and Allergology, because of recurrent bronchial obstructive complaints and a suspected allergy, were studied for strongly positive skin reactivity to fungi. 4.6% of the patients reacted with a wheal of 10 mm diameter or more to one or more of the tested fungi. Almost three-quarters of the airborne fungal "flora" was composed of seven genera, namely (in order of occurrence): 1) *Cladosporium* (42.6%), 2) *Botrytis* (8.6%), 3) Yeasts (7%), 4) *Penicillium* (5.8%), 5) Basidiomycetes (5.7%), 6) *Aspergillus* (3.7%), and 7) *Alternaria* (0.9%). In skin-testing, however, a different order of occurrence existed: namely: 1) *Beauveria* (6.8%), 2) *Botrytis* (6.1%), 3) *Aspergillus* (4.7%), 4) *Mucor* (3.8%), 5) *Epicoccum* (3%), 6) *Cladosporium* (2.3%), and 7) *Alternaria* (1.1%). It is concluded that the most prevailing airborne moulds are not necessarily the most potent allergens, at least in skin testing. *Aspergillus* and *Botrytis* showed a high sensitization rate, while *Cladosporium* and *Alternaria* did not. *Botrytis* deserves further study because of its frequent airborne occurrence and marked allergenic properties.

Key words: aerobiology; airborne fungi; Andersen sampler; skin testing; fungal allergy; allergens.

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CLINICAL ASPECTS

It may be important to know that fungi with low airborne concentrations may be potent allergens (e.g. *Aspergillus*), the reverse may also be true (e.g. *Cladosporium*). Thus, fungi have different sensitizing capacities.

Whereas pollen are well-studied aeroallergens, much less is known about fungal spores (13, 14, 26, 27). The class of the Deuteromycetes, or *Fungi imperfecti*, is the best studied, mainly because they are easily recognized by the current viable air-sampling techniques, and develop rapidly on artificial culture media. Inhalation-provocation studies demonstrated the capacity to incite bronchial obstructive reac-

tions in sensitized persons (9). In our clinic, skin testing is performed with those fungi assumed to be present in the air, a choice which is principally based on historical (34) and foreign information (7, 10, 11). Although Storm van Leeuwen in the second decade of this century (30, 31), and Van der Werff in the late 1940's (34), performed some aerobiological surveys in the Netherlands with the open-plate method,

no clinical studies were undertaken to evaluate the significance of fungal allergy in the asthmatic population.

In the present study results of airborne fungal spore sampling, as presented in an accompanying paper (5), are compared with skin test results in a group of bronchial obstructive patients referred to our clinic because of a suspected allergic involvement in their disease. It appeared that the most prevalent airborne moulds were not necessarily the most positive in skin reactivity.

MATERIAL AND METHODS

Aerobiological sampling for fungal spores was carried out as described in an accompanying paper (5). In short: from April 1981 to the end of June 1983, one-day samples were taken weekly on the rooftop of a hospital (Beatrixoord, Haren) in the North-East Netherlands, with an Andersen viable volumetric sampler (2). Petri dishes with yeast morphology (YM) agar were incubated at 28°C, the emerging colonies counted every 3 days, and determined to genus levels as quickly as possible. The total number of colony forming units (CFU) per plate was calculated according to the positive-hole conversion table of Andersen (2), and additionally converted to numbers per cubic metre of air, as described before (4).

Intracutaneous skin test (ICT) from 692 patients who visited the outpatient Department of Pulmonology and Allergology, or who were hospitalized, between May 1980 and December 1981 were evaluated. Almost all these patients were referred because of recurrent bronchial obstructive complaints with a suspected allergic background. Additional investigations were done to establish the diagnosis of chronic obstructive lung disease (COLD), e.g. extensive lung function studies, histamine and acetylcholine thresholds, and if necessary, inhalation provocation and additional RAST tests. Very few patients with primarily nose and eye problems were seen, because they were initially sent to other departments. All ICT were carried out with fungal extracts, and other standard inhalation allergens (Diephuis Laboratories, Gro-

ningen, The Netherlands). Fungal extracts contained 0.02 mg dry lyophilized antigenic extracts per ml of Coca's solution, and the following genera were used: 1) *Aspergillus* spp., 2) *Penicillium* spp., 3) *Cladosporium* spp., 4) *Alternaria alternata*, 5) Mixed group consisting of: *Aureobasidium*, *Botrytis*, *Mucor*, *Epicoccum* and *Abidia*. The latter group 5 was again tested with the separate genera when a positive reaction of 5 mm or more was observed. Additionally, two smaller groups of patients were tested in a later phase of the study and after obtaining more aerobiological information. The first group of 73 patients was studied with *Beauveria bassiana*, *Rhodotorula*, and *Fusarium*, a second group of 68 patients with *Botrytis cinerea*. Histamine (0.01 mg/ml), 48/80 (0.01%), and Coca's solution were used respectively as positive and negative standard reference ICT. All tests were read and the wheal recorded 15–20 min after the ICT. Only wheals of 10 mm or more in diameter are reported in our results, as they were regarded to be of clinical relevance.

RESULTS

In Table 1, the occurrence of airborne fungi is shown, listed according to the numbers of CFU encountered during the entire sampling period. Additionally, the ratio of percentages occurring as positive skin test and airborne presence is shown. In Fig. 1 percentages of airborne occurrence compared to skin reactivity are shown for eight moulds.

Three quarters of the total catch is represented by seven genera, in which *Cladosporium* ranks first with 42.6%, followed by *Botrytis* (8.6%), the Yeasts (7%), *Penicillium* (5.8%), the Basidiomycetes (5.7%), *Aspergillus* (3.7%), and *Alternaria* (0.9%). Thirteen genera of rare occurrence, accounted for only 0.9% when taken together. Almost one quarter of all recovered fungi consisted of *Mycelia sterilia* and undetermined colonies. A detailed description of the individual moulds and their seasonal occurrence and yearly differences, as well as the relationship to meteorological phenomena has been presented in an accompanying paper (5).

Table 1 also shows the results of skin tests for the whole group of patients. Wheals of ≥ 10 mm ($\geq 2+$) to one or more fungal genera were found in 4.6%.

The incidence of reactions to the individual genera was: *Penicillium* 3%; *Cladosporium* 2.3%; *Alternaria alternata* 1.1%; *Aspergillus* 4.7% and the mixed group 4.9%.

Botrytis cinerea tested in a separate group of patients induced a strong reaction in 7.3% (5 of 68 patients). *Beauveria bassiana* showed 6.8% strong reactions in the third separate group of patients (5 of 73). *Rhodotorula* and *Fusarium* showed only weak or negative results in this population. The "skin-air" ratios were low for

Cladosporium and high for *Aspergillus*. *Alternaria* also showed a high ratio, but numbers were too small to be of value. As a comparison with the fungi, skin test results for some other inhalant allergens were also noted in the group of 692 patients (≥ 10 mm); house dust 44%, house dust mite 31.6%, animal danders 34%. No reactions whatsoever to allergens were found in 18.5%.

DISCUSSION

Many allergic bronchial obstructive patients demonstrate broad patterns of wheal and flare skin reactivity to multiple allergens, sometimes including fungi. However, the clinical significance of the different allergens in any given

Table 1
Occurrence of fungal airspora sampled weekly from April 1981 to the end of June 1983, percentage positive skin tests, and "skin-air" ratio of some fungi

Genus rank order	Total CFU* per m ³ air	Average CFU per sample	Percentage of total	Percentage pos. skin test	Ratio % skin/air
1. <i>Cladosporium</i>	12858	109.9	42.6	2.3 (16/692)	0.054
2. <i>Botrytis</i>	2608	22.3	8.6	4.9 (34/692)** 7.3 (5/68)	0.64
3. Yeasts	2131	18.2	7	0	0
4. <i>Penicillium</i>	1761	15	5.8	3 (21/692)	0.52
5. Basidiomycetes	1719	14.7	5.7	1	—
6. <i>Aspergillus</i>	1138	9.7	3.7	4.7 (31/692)	1.30
7. <i>Alternaria</i>	261	2.2	0.9	1.1 (8/692)	1.22
8. <i>Epicoccum</i>	27			4.9 (34/692)**	
9. <i>Rhizopus</i>	20				
10. <i>Mucor</i>	18			4.9 (24/692)**	
11. <i>Beauveria</i>	18			6.8 (5/73)	
12. <i>Fusarium</i>	17			0 (0/73)	
13. <i>Paecilomyces</i>	16				
14. <i>Trichoderma</i>	13				
15. <i>Oidiodendron</i>	11				
16. <i>Scopulariopsis</i>	8				
17. <i>Ulocladium</i>	6				
18. <i>Aureobasidium</i>	4			4.9 (34/692)**	
19. <i>Streptomyces</i>	3				
20. <i>Stemphylium</i>	2				
21. <i>Phoma</i>	1				
<i>Absidia</i>	0			4.9 (34/692)**	
22. <i>Mycelia sterilia</i>	1488	12.7	4.9		
23. Undetermined	6024	51.5	19.9		
Total	30152	257.7	100	4.6 (38/833)	

* CFU = colony forming units.

** () = Results in the mixed mould extract.

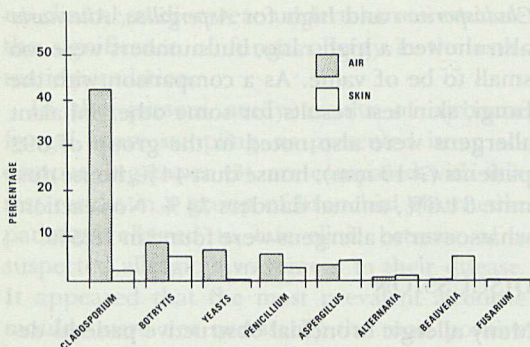


Fig. 1. Percentage occurrence in the air of some fungal spores (stippled bars) shown together with the percentage of positive skin tests (≥ 10 mm) with these moulds (open bars).

patient often remains uncertain (26). Many members of the *Fungi imperfecti* elicit positive inhalation-provocation reactions and specific IgE tests in atopic individuals, and IgE-mediated respiratory allergic disease to these fungi is well known (9, 10, 16).

In the present survey, only 4.6% of all patients showed a whealing reaction of 10 mm or more, indicating a low overall sensitization level, especially when compared with strong reactions to, e.g., house dust (44%) and animal danders (34%) in the same group. Our figures are comparable to other European studies; Wales (12): 5%, Sweden (20): 10%, Germany (8, 16): 5–10%. Surveys from the United States reported a higher sensitization trend towards fungi; Prince (22), Roby (23), and Street (32) noted between 20 and 60% positive skin tests, the most important moulds being *Cladosporium*, *Alternaria*, *Epidermophyton* and *Helminthosporium*.

An explanation for the different skin reactivity might be the variability in mould extract potency. As was recently demonstrated by Aas et al. (1) the incidence of positive skin tests with *Cladosporium* varied from 12.5% to 65%. The fungal extracts we used were tested and compared with other commercial extracts, and they showed high IgE-binding properties as well as comparable results (personal observations). Also, the finding that 18.5% of the suspected allergic patients did not show any reaction in ICT favours the purity of the extracts used.

When looking at the individual mould genera

in our survey, the discrepancy between airborne occurrence and skin test reactivity is remarkable. The difference is very pronounced for *Cladosporium*: accounting for almost half of all airborne numbers, but eliciting only 2.3% positive skin tests. This finding may indicate that *Cladosporium* spores contain but low allergenic properties, also illustrated by the low "air-skin" ratio of 0.054. Other European reports too, showed low sensitization levels for *Cladosporium* (6, 8, 16). In contrast to American studies (22, 23, 32), *Alternaria* has hardly any significance in our country, either as an important airborne component, or as an allergen. We know of an occasional patient, living near grain fields, with *Alternaria* sensitivity (4).

The Yeasts, most often represented by *Rhotorula* in our country, accounted for some 7% of the total airborne "flora", in skin testing; however, this micro-organism elicited no strong reactions. *Aspergillus* and *Botrytis*, on the other hand, were met occasionally in the samples, but were relatively important as inhalational allergens.

Although *Botrytis* is but seldom mentioned in aerobiological surveys, it played an important role in our sampling studies (4, 5), ranking second, after *Cladosporium*, with 8.6% of the total catch. The exact role of *Botrytis* in respiratory allergy is at present determined by means of inhalation-provocation studies and RAST technique. Our figure of 6% strongly positive skin tests indicates some importance. Due to its frequent occurrence in greenhouses and on vegetables and fruits, more and more people, both professionally and recreationally, come into close contact with this so-called "grey-mould".

Aspergillus spp. has long been recognized as an outstanding aero-allergen (31). Its capacity to induce a number of lung diseases, from allergic asthma and Allergic bronchopulmonary aspergillosis (ABPA) to invasive and disseminated forms, clearly demonstrates its clinical importance. Due to the year-round presence of its tiny spores (1–5 μ m) in the air (4, 5, 17), *Aspergillus* forms a continuous threat to the respiratory system. Atmospheric concentrations are on the average only 10 CFU per m³, or 4%

of all airborne fungi, but skin testing was positive in almost 5% of all patients, indicating a high skin- versus air-percentage ratio (Table 1). From former studies (3, 18, 23, 28, 33) it appears that especially high concentrations of *Aspergillus* are found in agricultural surroundings, where hay, straw and other decaying organic matter is stored. People living next to these environments are more prone to the allergic and/or saprophytic properties of *Aspergillus* (3, 5).

When *Aspergillus* is compared with *Penicillium*, important similarities may be observed in airborne behaviour (5) and spore size, but, clinically, large discrepancies exist. *Penicillium* is hardly ever demonstrated in situations comparable to aspergillus-asthma, ABPA, or extrinsic allergic alveolitis, not to mention aspergilloma. The conditions in the human bronchial tree therefore, clearly favour optimal growth for *Aspergillus*, and possibly temperature plays a key role in this context. In a study by Mullins (19) the relative frequency of *Aspergillus* cultured from lung tissue was explained by its special resistance to the lung defences. Schaffner (24) demonstrated that the natural immunity to resting spores of *Aspergillus* largely depends on the alveolar macrophage, while a second line of defence is formed by the polymorphonuclear cells, which are largely directed to killing mycelia. Many factors, e.g. smoking, corticosteroids, viral infections, etc. (24) may influence these defence mechanisms, rendering the lung more vulnerable to fungal invasion.

Other airborne moulds did not seem to play an important role, either in atmospheric sampling, or as aero-allergens. However, patients living near a specific source of such a fungus may be apt to respiratory manifestations, and local sampling may reveal important information (3, 4). In fact, aerobiological studies reporting "average" background figures at rooftop level should be completed by surface source sampling in and around patients' dwellings (29). We have found another rank-order of fungi inside the homes of patients with a fungal allergy, although skin test results did not parallel the indoor exposition (4). Fungi not capable of growing on artificial media, as is used in such

volumetric-viable sampling techniques as Andersen and Biap slit-samplers (17), are less well clinically studied because of difficulties in extract preparation. However, some Basidio-, Asco- and Phycomycetes may induce allergic respiratory complaints (15, 25). Further simultaneous aerobiological, laboratory, and clinical research is needed to define their exact place in bronchial obstructive and related allergic disorders.

In conclusion, the present study showed that airborne fungi induced strongly positive skin reactivity in only 4.6% of patients with bronchial obstructive complaints, thus their clinical relevance seems rather small. Atmospheric spore concentrations do not necessarily reflect the types of fungi which most frequently induce sensitization, hence, the individual allergic capacities of fungi are different. *Aspergillus*, in particular, showed a high sensitization rate in comparison with its low airborne concentrations. *Botrytis* deserves further clinical study because of its frequent airborne occurrence and marked allergenic properties.

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REFERENCES

1. Aas, K., Leegaard, J., Aukrust, L. & Grimmer, Ø.: Immediate type hypersensitivity to common moulds. *Allergy* 35, 443-451, 1980.
2. Andersen, A. A.: A new sampler for the collection, sizing and enumeration of viable airborne particles. *J. Bacteriol.* 76, 471-484, 1958.
3. Beaumont, F., Kauffman, H. F., Sluiter, H. J. & de Vries, K.: Environmental aerobiological studies in allergic bronchopulmonary aspergillosis. *Allergy* 39, 183-193, 1984.
4. Beaumont, F., Kauffman, H. F., Sluiter, H. J. & de Vries, K.: A volumetric-aerobiological study of seasonal fungus prevalence in the indoor and outside air of dwellings of asthmatic patients living in the North-East Netherlands. *Ann. Allergy* 1984. (In press).

5. Beaumont, F., Kauffman, H., Van der Mark, T. H., Sluiter, H. J. & de Vries, K.: A volumetric-aerobiological survey of conidial fungi in the North-East Netherlands. I. Seasonal patterns and the influence of meteorological variables. *Allergy* 40, 173–180, 1985.
6. Buisseret, P. D.: Seasonal allergic symptoms due to fungal spores. *Br. Med. J.* 11, 507–508, 1976.
7. Charpin, J., et al.: Presentation des moisissures atmosphériques. *Lille Med.* 11, 832–837, 1966.
8. Debelic, M. & Virchow, Ch.: Schimmelpilz Allergie bei Asthma bronchiale und asthmoider Bronchitis. *Schweiz. Med. Wochenschr.* 98, 1450–1453, 1968.
9. Feinberg, S.: Mold allergy: its importance in asthma and hay fever. *Wis. Med. J.* 34, 254–260, 1935.
10. Hamilton, E. D.: Studies on the airspora. *Acta Allergol.* 13, 143–175, 1959.
11. Hirst, J. M.: Changes in the atmospheric spore content. Diurnal periodicity and the effects of weather. *Trans. Br. Mycol. Soc.* 36, 375, 1953.
12. Hyde, H. A., Richards, M. & Williams, D. A.: Allergy to mold-spores in Britain. *Br. Med. J.* 1, 886, 1965.
13. Hyde, H. A.: Atmospheric pollen and spores in relation to allergy I. *Clin. Allergy* 2, 153–179, 1982.
14. Hyde, H. A.: Atmospheric pollen and spores in relation to allergy II. *Clin. Allergy* 3, 109–126, 1973.
15. Jenkins, P. F., Mullins, J., Davies, B. H. & Williams, D. A.: The possible role of aero-allergens in the epidemic of asthma deaths. *Clin. Allergy* 10, 611–620, 1980.
16. Kersten, W. & Hoek, G. T.: Schimmelpilz Allergie. *Wien. Med. Wochenschr.* 130, 275–282, 1980.
17. Larsen, L. S.: A three year survey of micro fungi in the air of Copenhagen 1977–1979. *Allergy* 36, 15–22, 1981.
18. Marsh, P. B., Millner, P. D. & Ula, J. M.: A guide to the recent literature on aspergillosis as caused by *Aspergillus fumigatus*, a fungus frequently found in self-heating organic matter. *Mycopathologia* 69, 67–81, 1979.
19. Mullins, J. & Seaton, A.: Fungal spores in lung and sputum. *Clin. Allergy* 8, 525–533, 1978.
20. Nilsby, I.: Allergy to moulds in Sweden. A botanical and clinical study. *Acta Allergol.* 2, 57–90, 1949.
21. Pepys, J.: Hypersensitivity diseases of the lungs due to fungi and organic dusts. Monographs in allergy, Vol. 4. S. Karger, A. G., Basel-New York, 1969.
22. Dematte, H. E. & Morrow, M. B.: Skin reaction patterns to dermatiaceous mold allergens. *Ann. Allergy* 29, 535–538, 1971.
23. Roby, R. R. & Sneller, M. R.: Incidence of fungal spores at the homes of allergic patients in an agricultural community. II. Correlations of skintests with mold frequency. *Ann. Allergy* 43, 286–288, 1979.
24. Schaffner, A., Douglas, H. & Braude, A.: Selective protection against conidia by mononuclear and against mycelia by polymorphonuclear phagocytes in resistance to aspergillus. *J. Clin. Invest.* 69, 617–631, 1982.
25. Salvaggio, J. & Seabury, J.: New Orleans asthma IV. Semiquantitative airborne spore sampling. 1967 and 1968. *J. Allergy Clin. Immunol.* 48, 82–95, 1971.
26. Salvaggio, J. & Aukrust, L.: Mold induced asthma. *J. Allergy Clin. Immunol.* 68, 327–346, 1981.
27. Solomon, W. & Mathews, K.: Aerobiology and inhalant allergens. In Middleton, E., Reed, C. & Ellis, E. (eds.): *Allergy, principles and practice*. C. V. Mosby Co., St. Louis, 1978.
28. Solomon, W. R. & Burge, H. P.: *Aspergillus fumigatus* levels in and out-of-doors in urban air. *J. Allergy Clin. Immunol.* 55, 90–91, 1975.
29. Solomon, W. R.: Sampling airborne allergens. *Ann. Allergy* 52, 140–147, 1984.
30. Storm van Leeuwen, W. & Kremer, W.: Schimmelpilz Allergene als Krankheitsursachen. *Klin. Wochenschr.* 6, 408–412, 1927.
31. Storm van Leeuwen, W., Bien, Z., Kremer, W. & Varenkamp, H.: Über die Bedeutung kleinsporiger *Aspergillus*arten für die Ätiologie des Asthma bronchiale. *Z. Immunitätsforsch.* 44, 1, 1925.
32. Street, D. H. & Hamburger, R. N.: Atmospheric pollen and spore sampling in San Diego, California, I. Meteorological correlations and potential clinical relevance. *Ann. Allergy* 37, 32–40, 1976.
33. de Vries, G. A.: *Aspergillus fumigatus* and *Actinomyces* in air. *Acta Allergol.* 15, 99–106, 1960.
34. Van der Werff, P. J.: Mould-fungi and bronchial asthma. H. E. Stenvert Kroese, N. V., Leiden, 1958.

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