Aerobiological and clinical studies in mould allergy
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SUMMARY

This thesis details an investigation into the occurrence of mould spores in the surrounding atmosphere, both in- and outdoors. Data was gathered on the influence of seasons and meteorological factors. The recorded types and concentrations of spores were related to the sensitization of patients with CARA (Chronic Aspecific Respiratory Disease), and ABPA (Allergic Bronchopulmonary Aspergillosis).

Chapter 1 introduces the mycology of fungi, especially those which could be of importance in the respiratory system.

In Chapter 2 a number of different methods used to sample the air for fungal spores, are reviewed. The advantages and disadvantages of the various spore traps are discussed. The following arguments are given for the choice of the Andersen-sampler in this investigation: 1. the sampler is easy to transport, which is important when sampling in the patients' immediate surroundings; 2. determination of the many fungal colonies is more accurate than with the 'non-viable' techniques. This is especially true for small spores, such as Aspergillus and Penicillium; 3. if necessary the colonies can be further processed for obtaining extracts for allergy tests; 4. the sampler separates spores according to size which corresponds to the manner of deposition in the human airways; 5. in the international literature the use of the Andersen-sampler is frequently reported in aerobiological studies, which facilitates the comparison of results.

The Burkard-trap, which is also frequently used, was considered less suitable for this investigation for the following reasons: 1. the sampler is difficult to transport for in-home studies and it is not possible to sample in several rooms sequentially; 2. accurate identification of many spore types is difficult and further processing to obtain extracts is impossible.

In Chapter 3 the author's experiences with the Andersen sampler during the preparatory stage of the aerobiological study are reported. The optimal sample time was found to be 10 minutes. The use of one Petri dish in the sixth 'stage' of the sampler gave comparable results with res-
to counts and identification, as did six plates in the sampler (the advantage lay in a decrease of work involved; the disadvantage was that no longer possible to separate particles according to size). Sampling in rooms gave highly variable results depending on air turbulence and possibly also on the size of the room. The most reproducible scores were obtained when the air was still, and it is therefore important to avoid disturbing the air during sampling. New colony growth in the Petri dishes was found up to 10-14 days after incubation at 28°C.

Chapter 4 is a literature review of aerobiological studies done in and outside Europe since 1958. The literature up until 1958 has been reviewed in detail by Van Der Werff in the monograph 'Mould fungi and bronchial asthma'. In Europe the most frequently occurring fungi, ranked according to frequency of occurrence, are:

The studies performed outside Europe show in general the following most frequently occurring spores listed according to order of occurrence:

The differences between the European and non-European studies can be partly explained by the different samplers used, but one could also expect real differences in the pattern of spore occurrence. In Europe 'non-viable' techniques are mostly used, whereas outside Europe the 'viable' methods are more common. For this reason Asco- and Basidio-spores would be less frequently observed in the non-European studies, which does not mean that these moulds actually occur less frequently. The importance of using comparable sampling techniques is stressed.

Chapter 5 is a literature review of clinical investigations into the sensitization to fungi. Those studies that report corresponding aerobiological data are summarized in table form. The studies differ greatly in purpose and design and comparison of results is potentially hazardous. Es-
establishing a list ranking the degree and frequency of sensitization in an atopic population is also difficult owing to the differences in patient selection, moulds to be tested, strength of the extracts, and the scoring of the skin tests. For example, in the United States, positive skin tests for Cladosporium and Alternaria are reported far more frequently than in Europe. The frequency of the positive skin test is also highly variable, from 4% to more than 50%, depending on the patient selection. European studies generally report between 5 and 10%.

An investigation into the occurrence of airborne moulds in the Northeast of the Netherlands is reported in Chapter 6. From April 1981 until July 1983, weekly air samples were taken on the roof of the Hospital ‘Beatrixoord’ in Haren, Groningen. The average number of colony forming units (CFU) was 258 per m² per sampling. Seventy five percent of the total catch was found to consist of 7 fungal types, as follows, listed in order of frequency: 1. Cladosporium, 2. Botrytis, 3. Yeasts, 4. Penicillium, 5. Basidiomycetes, 6. Aspergillus, and 7. Alternaria. This order of occurrence is in reasonably good agreement with other European studies, although finding Botrytis in second place differs from other reports, and is worthy of comment. The remaining twenty-five per cent consisted of sterile mycelia and unidentified colonies, the latter due to overgrowth by other fast growing colonies. Fourteen other mould genera were found in the atmosphere; together, however, they were less than one per cent of the total.

A clear seasonal influence was found, the highest total mould counts were found in the summer, with increases in Cladosporium and Botrytis especially. In the autumn Penicillium, Aspergillus, and Basidiomycetes were increased, while Aspergillus was the most dominant spore during the winter months. Momentary weather changes had only short-lived influence on the occurrence of airborne moulds and had no effect on the typical seasonal averages.

In chapter 7 a study of mould spores in and around the homes of 28 CARA patients with positive skin tests for moulds is described. Penicillium was found to be the most frequently occurring mould, making up 80.5% of the indoor fungal population, and, surprisingly, 52% of that immediately outside the house. In absolute numbers Penicillium occurred twice as frequently indoors as outdoors. Also commonly found in-
doors were Botrytis, Cladosporium, and Aspergillus, and once again seasonal changes were observed. Penicillium, for example, scored an average of 654 CFU/m$^3$ in the winter as opposed to only 96 CFU/m$^3$ during the summer period.

Exposure to mould spores in the immediate living surroundings of the patients was found to be of quite a different nature i.e. both in spore type and quantity, to that in the 'general' outside air as was described in Chapter 6. Also the 'peak-load' in mould allergens showed differing patterns in- and outdoors. Indoors, Penicillium-'clouds' were sometimes found while outdoor it was often Cladosporium found in this form. On the other hand, Aspergillus can be inhaled throughout the year, indoors as well as out.

Chapter 8 reports a study of the possible correlation between moulds in the prevailing atmosphere, as described in Chapter 6, and the sensitization of a group of patients suspected of CARA and allergy (N = 833). The direct reaction to subcutaneous skin tests were measured and only a reaction greater than 10 mm (2+) was recorded as positive and as possibly clinically relevant. It was found that the degree of sensitization to fungi did not correspond to the frequency of occurrence in the local atmosphere. For example, the number of positive reactions to Cladosporium was only 2.3% and to the relatively rare Beauveria, 6.8% (in 72 patients). Aspergillus scored comparatively high in the skin tests with 4.7%, while the percentage in the atmosphere was 3.7%. In the total patient group the percentage of positive skin reactions for one or more moulds was 4.6%.

The difference in the ability of the various fungal 'air spora' (viable and non-viable spores, spore clusters and mycelium threads) to cause sensitization is remarkable. The following factors could possible play a role: the size of the spore; the ability to germinate in airways - a temperature dependent process (e.g. 37°C is optimal for Aspergillus but not for Penicillium) - the readiness with which the spore can be phagocytosed by the alveolar macrophages and polymorph nuclear granulocytes which is, in turn, possibly dependent on the composition of the spore wall.

It is concluded that moulds in general play only a minor role as possible allergens responsible for CARA complaints, certainly when one takes into account simultaneous sensitization to other inhalation allergens.
In Chapter 9 an investigation into the possible relationship between changing concentrations of airborne mould spores and changing degrees of bronchusobstruction is reported. Eight CARA patients with positive skin tests and a positive RAST (Radio allergosorbent test) for one or more fungi were followed. Regular sampling of the air in their immediate living environment was performed over a period of 1-2 years. The patients recorded any complaints and their Peak-flow (PF) values. In seven out of the eight cases Penicillium dominated; Alternaria was the most frequently found spore type in one patient. Sixty-six per cent of the reported increases in bronchusobstruction were in the period from July to November, while in the same period 56% of all high spore counts (> 500 CFU/m³) were recorded. There was a significant relationship between low PF values on days with the highest spore counts compared with PF values on days with low concentrations. Occasionally a particular mould could be assumed responsible for simultaneously occurring obstructive complaints. However, generally this was not the case.

It is concluded that bronchusobstructive reactions occur more frequently in periods with higher concentrations of moulds spores. It is possible that a summation effect occurs, in which other allergens also play a role. The proof that a particular mould is responsible for the obstructive reaction at that moment, could only be obtainable by inhalation provocation studies with the moulds that were found at that particular time in the immediate environment of the patient.

Chapter 10 reports an exploratory study into the occurrence of Aspergillus spores in the immediate living environment of two patients with allergic-bronchopulmonary aspergillosis (ABPA). The aim of the investigations was twofold: is there a specific source from which large numbers of Aspergillus spores disperse, and is there a connection between exacerbations of the illness and the occurrence of particular mould concentrations? A remarkable observation was the absence, during the period of investigation, of exacerbations that were frequently seen in earlier periods. This could be a result of the patients obtaining information about the source of the mould as discovered during the course of the study. At the first patient's home, a haysted was found to contain large numbers of Aspergillus, and high scores were sometimes found in a greenhouse belonging to the second patient. Apart from this, low
counts were generally registered at this patient’s house, most likely too low to cause aggravation of the illness. The patients did, however, register lower PF values during periods of higher numbers of Aspergillus in the outside air.

The conclusion is drawn from this study that undertaking an aerobiological investigation in the immediate living environment of patients with ABPA is useful in tracking down a potential source of allergen, thus leading to its elimination or avoidance of exposure.

In Chapter 11 a more detailed study of immunological changes in serum and broncho-alveolar lavage fluid in a patient with ABPA is presented. IgG, IgA, IgM antibody concentrations were determined by the ELISA method (Enzyme-linked immunosorbent assay). The ELISA-IgG antibody levels were higher than IgA and IgM and ran clearly parallel with the various stages of illness, i.e. from an acute phase to remission to exacerbation. It was concluded that IgG-ELISA measurements were preferred as an indicator of sickness activity over an extended period, and these measurements appeared to be more sensitive than the double-immuno-diffusion technique which used to be employed for this purpose. In addition, the levels of specific IgE in serum remained elevated in the remission phase, possibly as a reflection of continuing exposure to low numbers of Aspergillus spores.

The data from broncho-alveolar lavage were obtained during the acute and the remission phase of the ABPA. Proteins such as albumine, IgG, IgA, and IgM transudate to the broncho-alveolar compartment during the acute phase. During a non-active phase, normal values were found, except for the specific anti-Aspergillus IgA. This antibody is produced locally and remains increased, possibly as a result of continued exposure to spores. Further indication that the local immune reactions do not completely disappear is shown by the continued presence of polymorph nuclear granulocytes in the lavage fluid and the above mentioned specific IgE in serum.

**General conclusion:**
Aerobiological investigations into the occurrence of mould spores shows a difference between indoors and out: a. indoors the total spore count per m² is generally higher than outdoors, b. the type of mould most frequently occurring also differs, i.e. more *Penicillium* is found
indoors and immediately out of doors, and more *Cladosporium* and *Botrytis* in the overall outside atmosphere. These results show that the occurrence of moulds is largely dependent on the domestic conditions and that aerobiological measurements outdoors (in the 'general' atmosphere) are not directly relevant. This is in contrast to aerobiological pollen studies.

Sensitization to fungi was found in 4.6% of patients with CARA. Those genera that frequently give positive skin tests are not necessarily those most frequently occurring in the inhaled air. A relationship between the occurrence of bronchusobstructive complaints and the (increased) occurrence of certain moulds in the air could only be shown in part. However, in patients with ABPA, exposure to *Aspergillus* spores appeared to be an important factor in the course of the illness.

Continuous sampling of the air in the immediate surroundings of the patient and the identification of all spore types is important in order to determine which types and concentrations of moulds can initiate bronchusobstructive reactions. For practical reasons such a study is difficult to carry out. However, the recently introduced immuno-chemical determination of antigen levels in the atmosphere should greatly help in achieving this goal.