

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

I. Seasonal Patterns and the Influence of Meteorological Variables

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In order to obtain actual data about the qualitative and quantitative occurrence of airborne fungi in The Netherlands, a viable-volumetric sample survey with the Andersen sampler was undertaken. From April 1981 to the end of June 1983, one day a week samples were taken on the unobstructed rooftop of a hospital in the N-E Netherlands. An average of 268 Colony Forming Units (CFU), per sample, per cubic metre of air were found. Almost three-quarters of the total catch consisted of seven genera, *Cladosporium* predominating. *Botrytis* showed in surprisingly high numbers when compared with other surveys, while *Alternaria* occurred in very low concentrations. Most atmospheric airborne spora were found between May and September. *Aspergillus* and *Penicillium* prevailed in the autumn and winter months, although were present the year-round. Momentary weather conditions seemed less important for the overall spore picture than the average meteorological characteristics for a whole season. The obtained aero-mycological information may be useful in determining clinical strategies for skin testing and serological investigations in patients with suspected mould allergies.

Key words: aerobiology; Andersen sampler; *Botrytis*; fungal airborne spora; fungal allergy.

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CLINICAL ASPECTS

The determination of prevailing fungal airborne spora in a region, or country where allergic, e.g. asthmatic, patients are living is useful for estimating the most relevant skin tests and serological investigations, in order to avoid unnecessary examinations. Also, specific seasonal allergic complaints can be better appreciated, and differentiated from suspected pollen allergy.

In the Netherlands, between 1920 and 1930, Storm van Leeuwen and his co-workers (26) demonstrated the importance of airborne allergens as a possible cause of bronchial asthma. In addition, they suggested the existence of an as yet unknown factor or organism, found particularly in damp dwellings in a humid climate. They called it "miasmata", or "klima-antigenen". Cultures from air and mouldy places, especially kapok mattresses, pointed to *Aspergillus fumigatus* as being this particular organism, and a high percentage of sufferers

from severe asthma showed positive skin tests (27).

Some 20 years later, aerobiological surveys with the open-plate method were carried out by Van der Werff at three different locations in the mid-west region of The Netherlands (29). He found mainly *Cladosporium*, Yeasts, *Penicillium*, and *Fusarium*. *Aspergillus*, however, was only occasionally cultured. Up to the present no volumetric data are available on airborne moulds, as they appear all the year round in the North East Netherlands. Skin testing with

extracts of moulds is still being carried out with those genera that presumably have the highest incidence. The purpose of this study was to obtain actual data about the qualitative and quantitative occurrence of fungi in this part of the Netherlands, and the influence of seasonal and meteorological factors on this occurrence.

MATERIAL AND METHODS

Weekly collections of fungal airborne spora were carried out from April, 1981 to the end of June, 1983, using an Andersen sampler (3), model 0101 (2000 Inc., Atlanta, Ga.). The sampler was calibrated periodically to draw 28.3 l/min (= one cubic foot of air) over six standard Petri dishes containing YM (yeast-morphology) agar. This agar contains 3 g yeast extract, 5 g peptone, 10 g glucose and 20 g agar. Lactic acid is added to produce a pH of 3.5. In comparison with sabouraud, malt, and corn meal agars the YM medium maintained easy but not too rapid growth of colonies which had good sporulating properties. (Personal observations).

One day a week three 10-min samples were taken between 0900 and 0910, 1300 and 1310, and 1800 and 1810 hours, leaving the six Petri dishes in the same position. The sampler was placed on its side in a holder, mounted on a vane and therefore moved freely with the orifice directed into the wind. At the end of the sampling day the plates were removed, inverted in their covers and incubated at 28°C for 2 weeks. The emerging colonies were counted every 3 days and determined as quickly as possible. After 2 weeks no new colonies emerged. The total Colony Forming Units (CFU) per plate were calculated according to the positive hole conversion table (3) of Andersen, and additionally converted to numbers per cubic metres of air. Determination was performed to Genus level only, and all genera were rendered to numbers per cubic metre for the whole sampler (all stages together). Identification of the fungal genus was carried out macroscopically, with the aid of a stereo-microscope,

and by light-microscopy with slide preparations from spores and hyphae.

In case of doubt the culture was isolated and sent to the "Centraalbureau voor Schimmelcultures" (Dr. G. A. deVries, Baarn, the Netherlands) for determination. Due to the method chosen, some CFU were not identifiable because of overgrowth by other, faster emerging, colonies. However, the overall results are not influenced by this identical complication which randomly occurred in the culture-plates.

Sampling took place on the unobstructed rooftop of Beatrixoord-Hospital in Haren (3 km south of the province capital Groningen), 20 m above ground level. The hospital is situated in an agricultural area consisting mainly of grassland and a few small woody areas and with small scale horticulture to the west and a small village to the south.

Meteorological data were derived via the KNMI (Royal Dutch Meteorological Institute), from the airport of Eelde, situated some 5 km from the sampling site. Mean temperature (°C), rough radiation (J/cm²), rainfall or snow (mm), relative humidity (%), and mean wind velocity (1/2 m/sec), plus main wind direction were recorded.

For statistical analysis, regression-analysis was used to detect a possible relationship between the recovered fungi and meteorological variables. Average meteorological data (relative humidity, temperature, and wind speed) from the sampling day and the two preceding days were used for this purpose. The wind direction and precipitation at the time of sampling were also taken into account.

RESULTS

Overall mould recoveries

Table 1 shows the average monthly numbers of CFU/m³ in each sampling year for seven prominent fungi, a group of less frequently occurring but identifiable fungi, and for *Mycelia sterilia*, and unidentified moulds. Total figures and percentages of the individual fungi are also shown. The sampling period covered 117 weekly measurements with the Andersen

sampler. A total of 30,152 CFU/m³, or an average of 258 viable airborne spora/sample/m³, were found. Twenty-one genera were present, listed in Table 2. *Cladosporium*, *Botrytis*, yeasts, *Penicillium*, Basidiomycetes, *Aspergillus*, and *Alternaria* made up 74.3% of the total catch.

Table 1
Average values of airborne mould-genera for every month of the year of sampling

Month/ year	<i>Asp.</i>	<i>Peni.</i>	<i>Alt.</i>	<i>Clado.</i>	<i>Botr.</i>	Yeast	Bas.	Rest identi- fiable*	Undeter-** mined + <i>Myc. ster.</i>	Total
Jan. 1982	16	10	0	0	0	1	5	0	24	56
1983	21	13	0	3	5	3	8	1	39	93
Feb. 1982	7	11	0	0	1	2	11	0	10	42
1983	6	19	0	11	0	1	5	0	9	51
Mar. 1982	10	8	0	0	1	1	16	0	12	48
1983	1	10	0	8	3	1	47	0	34	104
Apr. 1981	11	24	0	2	4	1	13	4	41	100
1982	3	3	0	0	4	7	16	0	29	62
1983	3	5	0	6	6	0	63	0	60	143
May 1981	8	17	0	60	22	10	13	4	72	206
1982	3	24	0	1086	9	6	13	2	81	1225
1983	2	5	0	8	33	7	55	0	115	225
June 1981	5	9	0	248	7	307	10	3	200	789
1982	4	6	1	1	10	18	6	0	65	111
1983	2	10	0	20	78	16	4	1	79	210
July 1981	13	14	8	5	44	5	20	3	42	154
1982	1	3	4	695	79	7	4	0	84	877
Aug. 1981	3	11	12	3	36	6	7	1	48	127
1982	24	22	14	838	93	14	2	2	186	1195
Sept. 1981	13	20	7	2	27	13	3	3	83	171
1982	3	23	3	210	75	3	2	3	103	425
Oct. 1981	6	13	13	0	5	8	11	3	26	85
1982	16	29	2	16	64	6	16	2	77	228
Nov. 1981	10	30	0	1	2	2	31	3	35	114
1982	26	29	0	32	15	5	9	1	49	166
Dec. 1981	34	16	0	0	1	1	2	0	26	80
1982	8	21	0	16	2	1	9	1	39	97
Average/m ³ per sample										
1981	11	17	4	36	16	39	12	3	65	203
1982	10	16	2	241	29	6	9	1	64	378
1983	6	10	0	9	21	5	30	0	56	137
Overall mean										
	10	15	2	110	22	18	15	2	64	258
Percentage of mean										
	3.7	5.8	0.9	42.6	8.6	7	5.7	0.9	24.8	100

* "Rest identifiable", stands for the mould-genera listed from No. 8 down to No. 21 in Table 2.

** Undetermined: these colonies were countable but not identifiable, due to overgrowing by other colonies. (See also Material and Methods).

Undetermined colonies, and sterile Mycelia another 24.8%. The remaining 0.9% was composed of 14 genera, listed from Nos. 8 to 21 in Table 2. A seasonal preference was found (Fig. 1), showing most fungi in summer, with a mean of 485 CFU/m³. From September up to and including November (autumn) there were more than 100 but less than 300 CFU/m³, and in winter less than 100. Spring showed values lying between those of summer and autumn.

Occasionally very high concentrations of airborne spora were sampled, e.g., June 23, 1981: 3388 CFU, May 18, 1982: 4638, and August 11, 1982: 3069 CFU/m³. (Also represented in Table 1 by the high mean numbers in these months).

Yearly differences were noted (Fig. 1, Table 3): 1982 yielded more fungi in spring (414 CFU/m³) and summer (725 CFU/m³), almost double the concentrations of 1981 and 1983. The autumn of 1982 showed more than double the numbers of airborne fungi compared with

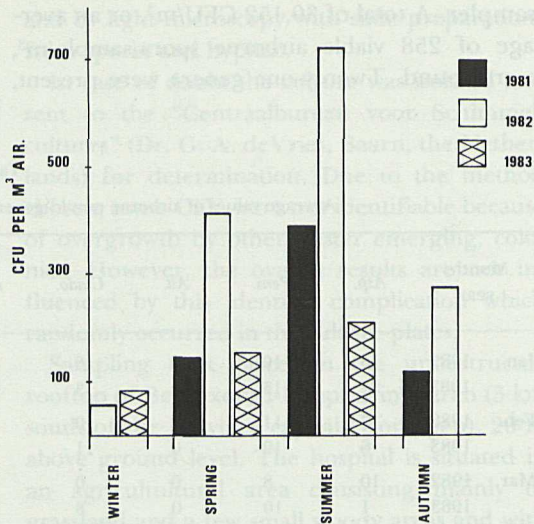


Fig. 1. Yearly seasonal differences of atmospheric mould recoveries in the North-East Netherlands.

* Winter = December, January, February. Spring = March, April, May. Summer = June, July, August. Autumn = September, October, November.

** Summer 1983 = June only. Spring 1981 = April and May.

Table 2

Rank order of atmospheric mould-genera as sampled in the air of the North-East Netherlands

1. <i>Cladosporium</i>	}	74.3 %
2. <i>Botrytis</i>		
3. Yeasts		
4. <i>Penicillium</i>		
5. <i>Basidiomycetes</i>		
6. <i>Aspergillus</i>		
7. <i>Alternaria</i>		
8. <i>Epicoccum</i>	}	0.9 %
9. <i>Rhizopus</i>		
10. <i>Mucor</i>		
11. <i>Beauveria</i>		
12. <i>Fusarium</i>		
13. <i>Paecilomyces</i>		
14. <i>Trichoderma</i>		
15. <i>Oidiodendron</i>		
16. <i>Scopulariopsis</i>		
17. <i>Ulocladium</i>		
18. <i>Aureobasidium</i>		
19. <i>Streptomyces</i>		
20. <i>Stemphylium</i>		
21. <i>Phoma</i>		
22. <i>Mycelia sterilia</i>	}	24.8 %
23. Undetermined colonies		

the same period in 1981 (276 and 120 CFU/m³, respectively).

Table 3 shows the meteorological data covering the three sampling years, together with the seasonal overall sample results. In 1982 the mean temperature, as well as the mean maximum and minimum temperatures, were higher than in 1981 and 1983 for all seasons. Also the number of "absolute warm days" (> 25°C) and "warm days" (> 20°C) was higher in 1982 than in the other years. (Note: the figures for spring 1981 are higher because the survey started April 1, thus omitting March as one of the "spring" months). 1982 was not a very dry year, with relatively large amounts of mm precipitation in summer and winter.

Individual mould recoveries (Table 1, Fig. 2)

1. *Cladosporium* had a yearly mean of 110 CFU and predominated from May to the end of December, although spores are occasionally found in the other months as well. May and August showed the highest concentrations (≥

500/m³), followed by June, July, and September (100/m³). In 1982 very high counts of more than 4000/m³ were sampled on May 18, and in July, August, and September counts were also higher in 1982 compared with the other years.

2. *Botrytis* were found with an average of 22/m³ for all measurements. Low numbers (0–4/m³) were present from December to May, after which they sharply increased to a maximum of 68/m³ in July. The highest score, was on August 18, 1982, with 235 CFU/m³. Mean scores for the seasons are 48 in summer, and 31/m³ in autumn.

3. *Yeasts* are mainly represented by *Rhodotorula* spp., with an overall mean of 18/m³. A high concentration was found in June 1981, with 1533 CFU/m³. From May to November the yeasts persisted between 7 and 10 CFU/m³, with the exception of June (all years) with 113/m³. From December to May, very low concentrations of around 1/m³ were found. The seasonal mean figures were therefore only substantial for the summer, with 63/m³.

4. *Penicillium* was airborne in almost equal numbers all the year round, with an average of

15 CFU/m³. In winter, spring, and summer, concentrations were between 10 and 15/m³; rising to 24/m³ in autumn. In 1982, particularly from August to November, more spores were airborne than in the other 2 years. In July 1982, however, only 10 CFU/m³ were caught, being the lowest value for all months. The highest numbers were found on November 11, 1981 (72 CFU/m³).

5. *Basidiomycetes* show only rudimentary growth on most nutrient agars. Nevertheless, several CFU were caught with a mean year-round concentration of 15 CFU/m³. Highest counts were met in March, April, May, and November, and lowest in September with only 3/m³. A record count was made on May 3, 1983, with 149 CFU/m³.

6. *Mycelia-sterilia*. These white colonies fail to sporulate under artificial growth conditions, hence their name. An average of almost 13 CFU/m³ was found all the year round.

7. *Aspergillus* showed airborne concentrations of almost 10 CFU/m³, and proved to be present during every month of the year. In winter months, concentrations rose to 16/m³, in

Table 3

Mean meteorological data and the corresponding airborne mould concentrations for every season during the sampling study

Meteorological variables	Summer				Autumn			Winter			Spring			
	1981	1982	1983*	Normal	1981	1982	Normal	1981	1982	Normal	1981**	1982	1983	Normal
Mean temperature	15.8	17.6	16.4	16.1	10.0	11.6	10.0	1.1	3.5	2.5	10.8	8.5	8.3	8.3
Mean max. temp.	20.0	22.4	21.5	20.8	13.8	15.3	13.9	4.0	6.1	5.2	15.4	13.4	12.2	12.7
Mean min. temp.	11.5	12.7	11.0	11.1	5.9	7.8	6.2	-2.1	0.7	-0.3	6	3.6	4.3	3.8
Days > 20°C	40	59	18	49	11	18	12	—	—	—	12	11	1	9
Days > 25°C	9	25	7	13	0	4	1	—	—	—	5	3	0	2
Precipitation (hours)	119	78	29	122	202	156	155	168	193	186	81	120	258	137
Precipitation, mm														
on sample site	167	181	60	220	302	158	214	130	203	186	74	147	330	147
Percentage sun	32	41	48	40	30	27	30	28	23	20	36	46	24	37
Days < 0°C	—	—	—	—	9	2	8	60	37	43	3	12	8	18
Days with snow	—	—	—	—	3	0	2	19	22	19	1	8	6	7
Total amount of CFU (average per season)	390	724	210	×	120	276	×	58	80	×	147	414	153	×

(Data kindly obtained from the Royal Dutch Meteorological Institute, De Bilt, the Netherlands).

* Figures for June only.

** Figures for April and May.

(Summer = June, July and Aug.; autumn = Sept., Oct. and Nov.; winter = Dec., Jan. and Feb.; spring = March, April and May).

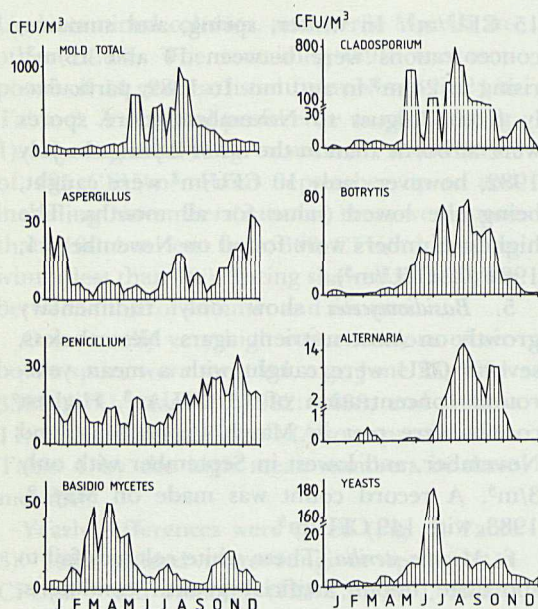


Fig. 2. Three weeks moving averages of the seven most frequently occurring mould genera and the total amount of moulds per m^3 air, as they appeared during the sampling period from April 1981 to the end of June 1983.

autumn $12.5/\text{m}^3$. Spring and summer showed an almost 50% reduction in numbers, although, occasionally, high counts were recorded, e.g. $88 \text{ CFU}/\text{m}^3$ on August 11, 1982. Only once, on December 15, 1981, was a higher score recorded ($124/\text{m}^3$), and this month also had the highest mean concentration with $22.5/\text{m}^3$. Generally, the lowest figures were found in June with only $3.5/\text{m}^3$.

8. *Alternaria*. This member of the *Fungi imperfecti*, with a relatively large spore size ($> 20 \mu\text{m}$), was only temporarily present, from July to the end of October. No spores were found in the other months. The annual average was $2 \text{ CFU}/\text{m}^3$, which was less than 1% of the whole catch. Figures rose to a mean of $13/\text{m}^3$ in August, the seasonal average for the summer months being $5/\text{m}^3$. Only once was a relatively high concentration recorded: October 1, 1981 with $51/\text{m}^3$.

9. *Other fungal genera*, listed from No. 8 down in Table 2, were occasionally detected, mainly in the months from spring to winter. These 14 moulds together represented only 0.9% of all

airborne spores capable of developing a mycelium on the nutrient agar.

Meteorological correlations

Regression-analysis of weekly total, and individual mould recoveries were correlated with meteorological data, and a significant relationship ($P < 0.05$), was found for the following variables: 1) a higher temperature yields greater total concentrations, *Cladosporium*, and *Botrytis*, 2) higher relative humidity was correlated only with a higher concentration of Basidiomycetes, 3) there was a higher concentration of *Cladosporium* if the wind was not from the west, and 4) wind speed and precipitation on the sampling day were *not* significantly correlated to the quantitative or qualitative aspects of mould recovery.

DISCUSSION

Fungal prevalence

In this survey the overall atmospheric fungus-spore concentration in the Northern-Netherlands was sampled during 117 weeks, from April 1981 to the end of June 1983.

The average total number of $258 \text{ CFU}/\text{m}^3$ per sample is in good agreement with a comparable viable-volumetric survey carried out in Copenhagen, Denmark, by Larsen (16) who noted an average of $231/\text{m}^3/\text{sample}$.

Both the Copenhagen and our study showed *Cladosporium* to be the most prevalent mould, while the percentages of *Penicillium*, *Aspergillus*, yeasts, and sterile mycelia were almost equal. The differences between *Botrytis* and *Alternaria*, however, are notable. The prevalence of *Alternaria* was $< 1\%$ in The Netherlands as opposed to 9.4% in Denmark, while for *Botrytis* the prevalence was 8.6% in The Netherlands and 0.8% in Denmark. Other European studies (1, 4, 5, 6, 8, 11, 14, 20, 29) are difficult to compare with, because they either concentrate on one, or only a few airborne fungi, or use different sampling techniques, such as the non-quantitative gravimetric "jelly-slide", or open-plate method, or the non-viable Burkard-trap

surveys. However, these surveys clearly show that *Cladosporium* ranks first in most European countries, followed by high concentrations of hyaline and coloured Basidiomycetes, which are not detectable by our method. The high concentrations of *Botrytis* found in our survey are not equalled by others, only Morrow Brown (20) has shown this fungus to peak in late July, 1980.

In comparison with overall atmospheric surveys in the United States, there are important differences, although here again only few viable-volumetric samplings have been carried out (18, 23, 28). *Cladosporium* is also reported present in the highest concentrations, but on the other hand, the high prevalence of *Alternaria*, *Epicoccum*, *Aureobasidium*, and *Helminthosporium*, is in sharp contrast to the data from the present and other European studies (2, 7, 17, 19, 24). *Botrytis* is only seldom mentioned in non-European surveys. Concerning *Aspergillus* and *Penicillium*, only viable methods are comparable, and results showing between 3 and 6% of the total catch, are similar to the results in our study (7, 15, 17, 19, 25).

Seasonal fluctuations and meteorological influences

Botrytis and *Alternaria* demonstrated the clearest seasonal occurrence, both being present from mid-summer to mid-autumn. Notably, the concentration of *Botrytis* was five times higher than that of *Alternaria*. Yeasts and *Cladosporium* were mainly airborne from late spring through autumn, making their "season" more prolonged. *Aspergillus*, *Penicillium*, and the Basidiomycetes showed no pronounced seasonal activity. Mainly in spring and summer, rare fungi, such as *Paecilomyces*, *Oidiodendron*, *Epicoccum*, *Aureobasidium*, and others, were found, but never in high airborne concentrations. Other European studies covering year-round mould sampling (1, 8, 10, 16) showed essentially the same seasonal trends as in our survey, although *Alternaria* and *Cladosporium* occurred earlier in summer and spring when samples were taken in the more southern areas of Europe (1, 4, 8, 16).

Different reports point to the influence of the prevailing climate on airborne spore recoveries

(9, 11, 12, 13, 22, 28). From these studies it seems obvious that meteorological factors in approximate order of importance are: rainfall, wind direction, daily maximum temperature, sunshine, windforce, and convection. In our survey the presence or absence of rain had no clear influence on the spore count. We may have missed the "rain-wash-out" phenomenon, because of sampling three times a day with the same set of petri-dishes in the sampler. Thus, the number of spores could be reduced or even be absent during precipitation, only to return at another sampling occasion after the rain had stopped. Indeed, a day of continuous snowfall reduced the amount of spores to zero.

Statistical analysis of the correlations between spore numbers and meteorological variables showed a positive relation between Total Airspora, *Cladosporium*, and *Botrytis* with higher temperatures. This finding is not surprising as these two fungi are typical summer airborne spora and, thus, substantially influence our total numbers. The humidity factor was correlated with the presence of Basidiomycetes only, and this could be due to the frequent foggy days in spring and autumn when these spores are abundant. A typical "local" correlation was found between the occurrence of *Cladosporium* and easterly-winds. This might be because the area to the east of the sampler was largely grassland (10, 21). An earlier report from Leiden, the Netherlands (4) also showed high *Cladosporium* counts on days with an easterly wind. This could be because fewer spores come from the west owing to the North Sea to the north-west. Our study also demonstrated annual differences in spore counts. All seasons in 1982, except winter, showed almost double CFU concentrations compared with 1981 and 1983. It seems likely that meteorological differences, such as a higher mean temperature, or differences in the amount of precipitation, and variations in agricultural activities, could be responsible for this finding.

In conclusion

The principal airborne spora in the North East Netherlands, measured with a viable-volume-

tric airsampler, are *Cladosporium*, *Botrytis*, yeasts, *Penicillium*, *Aspergillus*, and some Basidiomycetes.

In comparison with other European and non-European studies, *Botrytis* occurs in high numbers, while *Alternaria*, *Epicoccum*, and *Helminthosporium* are but rarely found.

Summer showed the highest concentrations for airborne spora, mainly through the high *Cladosporium* and *Botrytis* counts. *Aspergillus* and *Penicillium* were always present in the air, with maxima during autumn and winter.

The average number of airborne spores for a given season are more dependent on the average meteorological situation, than on momentary weather phenomena present during sampling.

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