

Ten types of microscopically identifiable airborne fungal spores at Leiden, The Netherlands

A.H. Nikkels, P. Terstegge, F.Th.M. Spieksma*

Laboratory of Aerobiology, Department of Pneumology, University Hospital, P.O. Box 9600, NL-2300 RC Leiden, The Netherlands

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Abstract

A universal method for the complete assessment of atmospheric fungal spores does not exist, which is continuous, volumetric and non-selective, and offers at the same time reliable identification of the collected spores. To perform a survey of airborne fungal spores, a choice has to be made between a viable and non-viable method. For the study carried out in Leiden, the non-viable, continuous volumetric method has been employed, showing the results over a period of 10 years, for 10 microscopically identifiable fungal spore types. Of this selection, *Cladosporium* spores have by far the highest airborne quantities, with an average annual total of the daily averages of over 700 000. *Botrytis*, *Ustilago* and *Alternaria* follow with much lower spore concentrations of between 20 000 and 30 000 as annual totals. The spore types of *Epicoccum*, *Erysiphe*, *Entomophthora*, *Torula*, *Stemphylium*, and *Polythrincium* are represented with annual sums lower than 10 000. A spore calendar shows the overall seasonal appearance of the 10 selected types.

Keywords: Aerobiology; Fungal spores; Spore calendar

1. Introduction

In the last 25 years, surveys of airborne spore concentrations have been carried out in many places. Examples of some European studies are by Bagni et al. (1977), Stix (1977), Larsen (1981), D'Amato et al. (1984), Rubulis (1984), Beaumont et al. (1985), Spieksma et al. (1987), Kersten and von Wahl (1989), Cosentino et al. (1990), Larsen and Gravesen (1991), Ballero et al. (1992), and Simeray et al. (1993). Results of a comparative survey in 11 European cities by various authors have been published by Wilken-Jensen and Gravesen (1984). Some North American studies were by Levetin and Horowitz (1978), Sneller et al.

(1981), Al-Doory et al. (1982), and Anderson (1985), and Asian surveys have been reported by Tuchinda et al. (1983), Singh et al. (1987), Vittal and Krishnamoorthi (1988), Shaheen (1992), and Satheesh and Rao (1994).

However, all these studies are incomplete in one way or another, because not all fungal spores are completely identifiable with a single sampling method (Solomon, 1984). As a matter of fact with the Hirst-type sampler (Hirst, 1952) the identification is morphological, whereas with the Andersen sampler (Andersen, 1958) it is cultural.

So, depending on the type of technique employed, reports on airborne fungal spores always give incomplete information about the actual presence of spores in the air. Probably the best solution for this dilemma should be a combined use of both methods (Burge et al., 1977; Rubulis, 1984).

* Corresponding author. Tel.: +31 71 5262495; fax: +31 71 5248118.



Fig. 1. Colour photographs of 10 fungal spore types assessed in this study: A: *Ustilago*; B: *Erysiphe*; C: *Cladosporium*; D: *Entomophthora*; E: *Torula*; F: *Alternaria*; G: *Stemphylium*; H: *Botrytis*; I: *Polythrincium*; J: *Epicoccum*.

In practice, however, most researchers choose one or the other technique, mostly based on pragmatic rather than scientific grounds. This report is a typical example of such a choice: fungal spores were included in the routine monitoring of outdoor airborne biological particles, and added to the counting procedure of pollen grains, which had started 10 years earlier. For microscopical identification use has been made of pictorial aids as published by Gregory (1973) (plates 6 and 7), Southworth (1973), Nilsson (1983), and Grant Smith (1986). To illustrate the feasibility of reliable identification of these selected types with the compound light microscope, colour photographs are shown (Fig. 1A–J).

2. Materials and methods

The site of the observations is located at Leiden (52°10' N; 4°29' E; elevation 1 m below sea level) in the west of The Netherlands, about 8 km from the North

Sea coast. The trapping instrument was placed at about 20 m above street level, on top of the roof of one of the University Hospital buildings, to the west of the city centre of Leiden, in a semi-urban area.

The vegetation in the direct vicinity of the trap (within 250 m) is not dense, and consists of a wide variety of ornamental plants, shrubs and trees. Most of the area between the buildings is occupied by streets and parking lots. There are a few ditches. At some greater distance, there is some pasture land and meadows, small patches of mixed deciduous woods, some parks, and many small private gardens. There is horticulture at more than 5 km distance, predominantly the cultivation of bulb flowers in the north-west, and of mixed summer flowers in the west. There is also some waste land and disturbed soil in the south-west, where building activities are taking place.

The climate is moderate Atlantic, with rain all year round, and prevailing wind from the west.

Airborne particles are sampled continuously, 12 months a year, with a Hirst-type spore trap (Hirst,

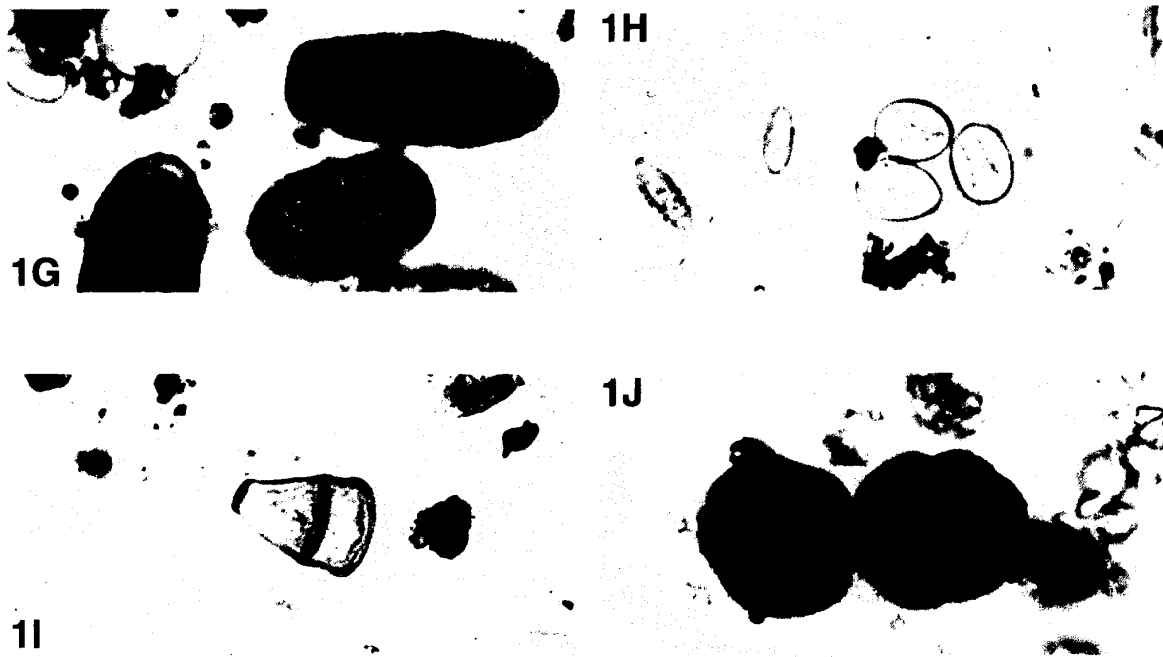


Fig. 1G–1J.

1952) (manufacturer Burkard, Rickmansworth, UK). The assessment of airborne fungal spores is carried out on one longitudinal band of 0.2 mm wide and 48 mm long, with a light microscope at $\times 650$. More detailed descriptions of the trap and of the methods used for the preparation and scanning of the slides can be found in Leuschner (1974), and Ogden et al. (1974).

For this survey, 10 types of fungal spores were arbitrarily selected, partly based on the relative ease of their identification with the compound light microscope, and partly on the abundance of their airborne occurrence. They are, in the order of their seasonal peaks: *Ustilago*, *Erysiphe*, *Cladosporium*, *Entomophthora*, *Torula*, *Alternaria*, *Stemphylium*, *Botrytis*, *Polythrincium*, and *Epicoccum*.

Results from a period of 10 years (1980–1989) are presented as annual totals of the daily average numbers per cubic meter of air. A so-called calendar shows the annual appearance of the 10 spore types over the 36 10-day periods of the year. In this presentation, the logarithmic means for the 10 years of the average daily spore counts for the 10-day periods are calculated. Subsequently, these means are placed in exponential classes, which are plotted as columns in the graphically presented calendar. The key to the classes and the column heights is given in Fig. 2. With this method of logarithmic transfer of data it is possible to construct a

calendar chart with identical scales for all spore types, showing in one figure the data from periods with low and high concentrations, and the data of types with low and high frequencies, like *Epicoccum* and *Cladosporium*, respectively. The principle of this presentation technique, calculated for 10 m^3 of air, was introduced by Stix (1971) for pollen grains, and a little later by Stix and Ferretti (1974) for fungal spores. It was also employed by Spieksma (1984).

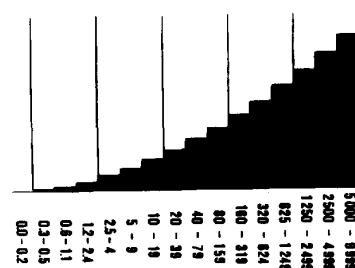


Fig. 2. Key to pollen calendar chart (Fig. 3): relation between column heights and exponential classes of average daily airborne concentrations of fungal spores.

Table 1

Annual totals of the average daily airborne concentrations of 10 types of morphologically identifiable fungal spores, Leiden, The Netherlands, 1980–1989

Year	<i>Ustilago</i>	<i>Erysiphe</i>	<i>Cladosporium</i>	<i>Entomophthora</i>	<i>Torula</i>	<i>Alternaria</i>	<i>Stemphylium</i>	<i>Botrytis</i>	<i>Polythrincium</i>	<i>Epicoccum</i>
1980	12 565	7435	834 890	2660	3115	11 590	1620	26 855	1195	5090
1981	10 825	1975	451 120	3080	2400	10 745	720	27 800	1080	3975
1982	47 470	1930	633 895	1865	1880	15 740	1510	29 010	1830	5875
1983	28 120	3255	667 160	2380	1450	22 115	1260	22 010	560	5320
1984	15 965	2205	459 885	2340	1870	21 195	650	33 980	650	4420
1985	17 005	2970	559 985	6650	4695	23 020	960	32 845	1440	8860
1986	12 705	3250	723 915	1955	2055	15 850	1135	25 830	965	9680
1987	8275	2275	448 005	3710	4740	15 765	700	51 515	1705	8355
1988	3780	3950	583 240	2760	4020	10 735	845	30 370	740	5790
1989	23 995	6515	1 895 250	6465	5790	31 580	1675	39 735	640	8925
Total	180 705	35 760	7 257 345	33 865	32 015	178 335	11 075	319 950	10 805	66 290
Trend (%) ^a	−6.3	−1.4	+8.2	+7.9	+10.3	+5.5	−1.5	+4.9	−3.3	+7.1
Coefficient ^b	−0.27	+0.08	+0.42	+0.46	+0.66	+0.45	−0.13	+0.56	−0.24	+0.67
	(n.s.)	(n.s.)	(n.s.)	(n.s.)	(<i>P</i> < 0.05)	(n.s.)	(n.s.)	(n.s.)	(n.s.)	(<i>P</i> < 0.05)

^aTrend is the year-to-year increase or decrease averaged over the 10 years of observation, expressed as a percentage of the average annual total.

^bCoefficient is the correlation coefficient of the regression line of the trend, for which the statistical significance is also given.

3. Results

3.1. Annual totals

The annual totals of the average daily concentration of airborne fungal spores in the 10 years of observation 1980–1989 are given in Table 1, showing the 10 selected spore types in the order of the appearance of their seasonal peak.

Within this selection, *Cladosporium* clearly has the highest concentrations with an average annual total of over 700 000. Second in this selection comes *Botrytis* with over 30 000, followed by *Ustilago* with 18 000, and *Alternaria* with 17 800. *Epicoccum* is represented with 6600, then *Erysiphe* with 3570, *Entomophthora* with 3380, and *Torula* with 3200. *Stemphylium* with 1100 and *Polythrincium* with 1080 have the lowest average annual totals of this selection of fungal spore types.

An analysis of increasing or decreasing trends in the annual totals by calculation of the regression over the 10 years reveals statistically significant (*P* < 0.05) regressions for *Torula* with an average year-to-year increase of 10.3%, and for *Epicoccum* with an increase of 7.1%. All other trends, five increasing, three decreasing are statistically not significant at the 5% probability level. The non-significant trend for *Cladosporium* (+8.2%) is caused by the extremely high concentrations in the year 1989; without this year the trend for *Cladosporium* would be decreasing.

3.2. Spore calendar

Fig. 3 shows the seasonal course in the airborne concentrations of the 10 selected spore types, presented as a so-called spore calendar based on the data of 10 years of observation. The numerical key to the calendar, as explained in Section 2, is given in Fig. 2.

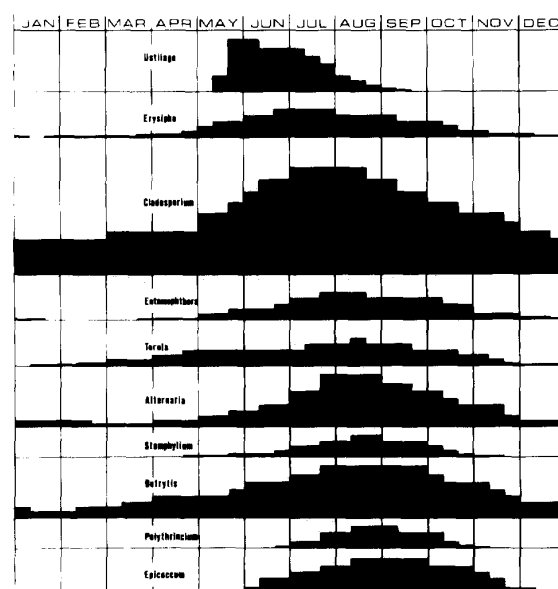


Fig. 3. Spore calendar chart of 10 types of microscopically identifiable fungal spores, for Leiden, The Netherlands, 1980–1989.

It appears that *Ustilago* has the earliest peak, in the months of May and June, followed by *Erysiphe* with its highest numbers in June and July. *Cladosporium* is well presented the whole year round with average daily airborne concentrations never under 40 spores per cubic meter, during January and February. The peak period for *Cladosporium* is during July and August with average daily airborne concentrations always exceeding 5000 per cubic meter. Spores of *Entomophthora*, *Torula*, *Stemphylium*, and *Polythrincium* have their highest concentrations in late summer, between July and October. Peak numbers of *Epicoccum* spores are found in August and September accounting for numbers between 40 and 80 per cubic meter as average daily concentrations. *Alternaria* and *Botrytis* are represented the whole year around with highest daily averages between 160 and 320 in August, and August/September, respectively.

4. Discussion

As already argued in Section 1, this report is restricted to an arbitrary selection of spore types which can be collected continuously and are easily identifiable with the compound light microscope. It should be realized that abundant spore types like *Aspergillus*, *Penicillium*, *Sporobolomyces*, many types of Ascospores and Basidiospores, and many more, are not taken into consideration. It is, therefore, impossible to relate these amounts of airborne spores to the total quantities of spores present in outdoor air. The data shown here just demonstrate when and in what volumetric quantities the selected spore types were found.

With growing experience and skill, the selection of microscopically identifiable fungal spore types could be extended without too much difficulty with other types (von Wahl and Kersten, 1991) like *Curvularia*, *Didymella*, *Drechslera*, *Fusarium*, *Ganoderma*, *Leptosphaeria*, *Pithomyces*, *Pleospora*, *Puccinia*, Uredospores, and *Urocystis*, which can also be common in the atmosphere.

This quantitative survey of 10 identifiable types over a period of 10 years confirms the dominant airborne abundance of *Cladosporium* spores, with daily averages during the summer peak period of between 5000 and 10 000 per cubic meter of outdoor air. In the year 1989, when the summer was very hot and humid, *Cladosporium* spore concentrations were extraordinarily high with concentrations of more than 50 000 on six individual days. The maximum daily value was 105 250 spores during the 24 h of 6 July. Also the airborne quantities of the other spore types of this selection are basically in agreement with most of the findings reported by others, for instance at 11 European cities (Wilken-Jensen and Gravesen, 1984). Some more European studies, using volumetric continuous samplers and microscopical

identification, with comparable results are by D'Amato et al. (1984), Spieksma et al. (1987), Kersten and von Wahl (1989), and Cosentino et al. (1990). Results from non European studies are also not very different (Anderson, 1985; Vittal and Krishnamoorthi, 1988; Shaheen, 1992).

As appears from these surveys, the regional and even continental differences in the spectrum of airborne fungal spore types are small, certainly smaller than the differences with respect to airborne pollen. This finding would lead to the proposition that a monitoring network for (this selection of) fungal spores could be less dense than a pollen monitoring network.

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