

Sources and incidence of airborne *Aspergillus fumigatus* (Fres)

J. MULLINS, R. HARVEY and A. SEATON

Asthma Research Unit, Sully Hospital, Penarth and Botany Department, University College, Cathays Park, Cardiff

Summary

Specific surveys of the air for *Aspergillus fumigatus* were carried out in rural and urban situations over a 2-year period. Overall, low concentrations of spores were recorded with a higher incidence during the 'winter' months. Counts in the open air and in a hospital ward showed similar fluctuations, the indoor counts being consistently lower. Plant debris in the form of compost heaps and stacks of hay and straw baled with a high moisture content in which self-heating occurs, produces large numbers of spores which may be liberated into the air causing high but localized counts if disturbed. The widespread distribution of decaying leaves following leaf fall represents a potential source of smaller concentrations of spores but over a much larger area. This availability of decaying plant debris with high water content fulfils the growth requirements of *Aspergillus fumigatus* and is the probable explanation of its winter seasonality.

Introduction

The medical importance of *A. fumigatus* was summarized in the monograph by Pepys (1969). As a cause of asthma this organism is unusual in its ability to grow saprophytically within the bronchial tree, making it virtually impossible for the patient to avoid his allergen. *Aspergillus fumigatus* is also unusual as a mould in having an optimum temperature for growth of 40°C with limits of 12°C and 53°C (thermotolerant) in comparison with the majority of moulds whose optimum temperature is below 28°C (mesophilic).

In a review of skin test results to *A. fumigatus* in patients with chronic lung diseases, Henderson, English & Vetch (1968) reported that the incidence of positive results varied between 5 and 100%. More recently, positive results were obtained by Pepys (1969) in 13% of asthma patients attending the Brompton Hospital and by Warren *et al.* (1975) in 50% of forty-three cystic fibrosis patients. In Cardiff we have obtained positive prick test results to *A. fumigatus* (1/10 w.v. Bencard) in 6.6% of 1039 asthmatic patients (unpublished data). These results are difficult to compare directly due

to differences in antigen and patient selection but they provide some indication of the relative importance of the organism as an allergen.

The air spora has been extensively surveyed but most of the culture plate surveys undertaken have been for mesophilic fungi using an incubation temperature of about 25°C, at which temperature *A. fumigatus* may be obscured by more rapidly growing fungi; in most cases identification has proceeded only to the genus level. Similarly, in surveys using the Hirst automatic spore trap (Hirst, 1952) where spores are impacted onto a sticky glass slide which is subsequently examined microscopically, *A. fumigatus* spores are indistinguishable from those of other species of the genus or even from spores of *Penicillium*. Results for these surveys can only be given for *Aspergillus*-*Penicillium* type spores, and do not therefore provide a reliable indication of prevalence of the *Aspergillus* spore. The present study was designed to measure the seasonal prevalence in the air of *A. fumigatus* spores and to investigate their sources.

Methods and materials

Malt extract agar (2%) (Blakeslee's formula, 1915; Raper & Fennel, 1965) was used throughout this investigation. Plates were incubated at 37°C to exclude the mesophilic moulds and examined after a minimum of 48 hr.

(a) Air spora surveys

Settle culture plates (gravity plates), were exposed for 10-min periods each hour between 09.00 and 17.00 hours, one day each week over a period of 2 years. Three such surveys were carried out in urban situations, the first on the roof of the Botany Department, University College (15 m above ground level) and the remaining two (one indoors in a hospital ward, and one outdoors) at St David's Hospital. Both sites are adjacent to parks but otherwise surrounded by built-up areas to a distance of about 1.7 km.

Once a month over the 2-year period the hospital surveys were extended to cover a 24-hr period, from 09.00 to 08.00 hours the following day.

During the second year a mains operated Andersen Sampler Model 0604 (Andersen, 1958) was used once weekly at both urban sites and also at the gardens of the Botany Department. The latter lie outside the city, surrounded by mixed farming and deciduous woodland. Air was sampled onto agar plates at the rate of 28.3 l/min for 10-min periods. At the gardens, samples were taken at distances of 1 m and 31 m from a compost heap.

(b) Substrate investigations

(1) The compost heap used in the Andersen sampler surveys was also used in the substrate investigations. It was composed of mixed deciduous leaves and was built in a concrete bin of 1 m³ capacity. At weekly intervals small samples, each equivalent to five or six decaying leaves, were removed from the centre and from the surface of the heap. The samples were washed and following serial dilution, the washings were plated out to provide estimates of the spore product per gram weight of the compost material.

(2) Scattered leaf debris was collected at fortnightly intervals over an area of grassland in a municipal park. Each sample, consisting of five leaves, was collected in sterile jars. Each leaf was shaken for 10 min with 10 ml sterile distilled water, and the washings from each sample were collected. Following serial dilution the washings were plated out to obtain estimates of spore product per unit area of leaf sample.

Results

(a) Air spora

Gravity plate surveys showed relatively low concentrations of *A. fumigatus* spores in the air throughout the year, with higher values in the winter months (Fig. 1). The average number of isolates per day was higher in the hospital grounds than in the wards during both summer and winter, although similar fluctuations were observed

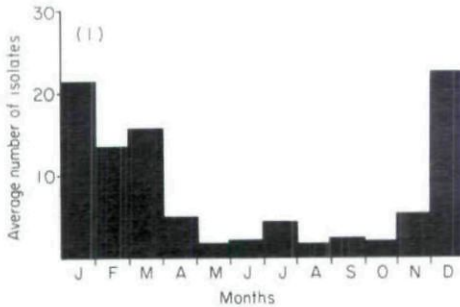


Fig. 1. Average monthly *A. fumigatus* counts from a weekly gravity plate survey taken over a 2½-year period.

in both sets of results (Fig. 2). In the Andersen Sampler urban surveys (Fig. 3) average concentrations were also higher in the winter than in the summer.

The highest maximum and average concentrations in the air were recorded alongside the compost heap during the winter. The summer average at that position was also higher than those recorded at the urban surveys. Sampling at positions adjacent to (1 m) and 31 m from the compost heap gave results which clearly indicated the very localized effect of the compost heap as a source of spores (Fig. 4).

(b) Substrate investigations

(1) *Compost heap*. Numbers of isolates rose very sharply during the fourth week of sampling (Fig. 5) especially at the centre of the compost heap where a maximum count of 170,000 spores/g of compost was recorded. The temperature at the centre of the

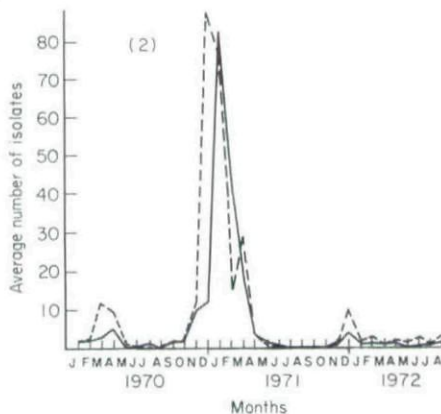


Fig. 2. Average monthly *A. fumigatus* counts from a weekly gravity plate survey in a hospital ward and grounds. ---, Grounds; —, ward.

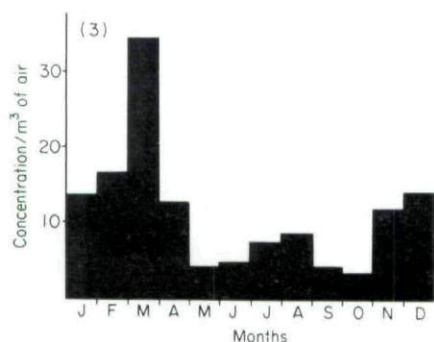


Fig. 3. Average monthly *A. fumigatus* concentrations recorded in weekly urban surveys with the Andersen sampler over a period of 19 months.

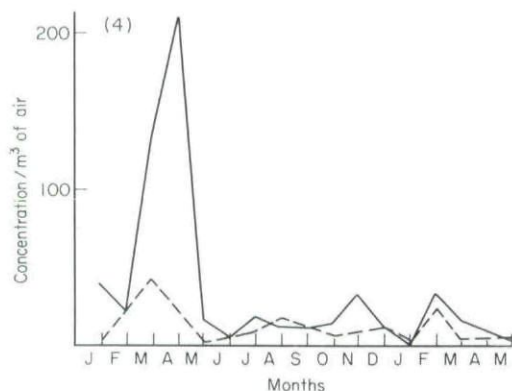


Fig. 4. Average monthly *A. fumigatus* concentrations recorded weekly with an Andersen sampler at distances of 1 m and 31 m from a compost heap. —, 1 m; ---, 31 m.

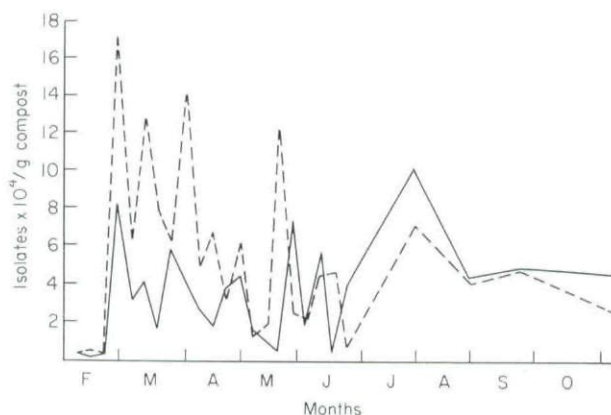


Fig. 5. Numbers of *A. fumigatus* isolates obtained from surface and internal samples from a compost heap over a 10-month period. ---, Internal; —, surface.

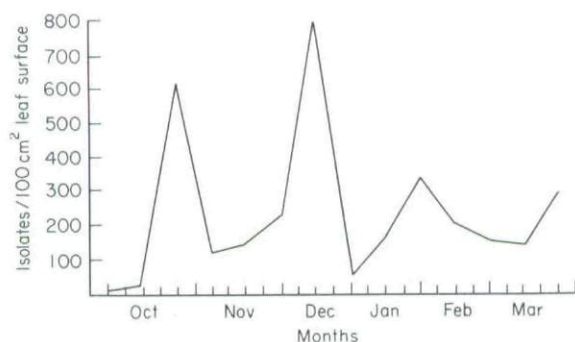


Fig. 6. Numbers of *A. fumigatus* isolates obtained from scattered leaf litter from October to April.

compost reached its maximum (65°C) within the first 3 days of composting and fell steadily to that of ambient air during the following 4 weeks. By the eleventh day the temperature had fallen to 54°C and during the fourth week, when peak numbers of isolates were obtained, it fell from 36.5 to 26.5°C. In succeeding weeks isolates showed considerable fluctuations in numbers but counts from the centre of the compost were approximately twice as high as those from the surface until the twenty-sixth week of sampling. The increase in surface isolates at that time coincided with an increase in air temperatures and duration of sunshine, but showed no obvious correlation with rainfall and humidity.

(2) *Scattered leaf debris*. As in the samples taken from compost, there were considerable fluctuations in the number of isolates obtained by washing samples of scattered leaf debris (Fig. 6). Nevertheless, significant numbers were recovered throughout the survey period, reaching a peak of 885 isolates/100 cm² of leaf surface during January.

Discussion

Hinson, Moon & Plummer (1952) drew attention to bronchopulmonary aspergillosis at a time when it was still considered to be relatively rare. They described three categories of the condition—allergic, saprophytic and invasive. The clinical significance of *A. fumigatus* was further clarified by later publications, particularly the monograph by Pepys (1969), which included descriptions of five types of pulmonary disease in man caused by the fungus, namely asthma, asthma and pulmonary eosinophilia, saprophytic infection, extrinsic allergic alveolitis and invasive aspergillosis. In recent years there has been an increase in the number of reports of invasive and disseminated aspergillosis especially in patients on immunosuppressive therapy (Rifkino *et al.*, 1967) and with leukaemia (McCormick *et al.*, 1975) and of *Aspergillus* endocarditis following cardiac surgery (Kammer & Utz, 1974). In these cases the increasing incidence of *Aspergillus* infection is related to the larger numbers of patients now receiving such forms of therapy. The factor in the physiology of the mould which makes it of such marked clinical importance in contrast to most other saprophytic moulds is its ability to grow at body temperature, at which temperature the growth of most moulds is inhibited.

Despite the importance of the mould very little work has been carried out specifically on the occurrence of *A. fumigatus*. Most of the air spora surveys have taken identification to genus level only. An analysis of ninety-four surveys of world wide origin (Mullins, 1974) showed that the genus *Aspergillus* was recorded between first

and fifteenth in order of incidence of airborne moulds and varied in frequency from 0.1 to 22% of the total air spora sampled. In the majority of surveys either no seasonality of incidence was recorded or it was described as 'low', 'sporadic' or 'relatively constant throughout the year'. Where seasonal variations were recorded the periods of maximum incidence in different surveys included all the seasons, depending upon geographical location and the climate of the sampling site.

Notable exceptions in their specificity include the reports by Austwick (1963) of *A. fumigatus* concentrations of 0.3–7.0/m³ at London zoo and by Noble & Clayton (1963) of concentrations in a hospital ward reaching 2400/m³ with peak concentrations during the winter. Hudson (1969) sampling for Aspergilli in Cambridge found them to be more abundant during the winter months. Evans (1972), by exposing Petri dishes for 8-hr periods and incubating at 45°C for thermophilic fungi, found *A. fumigatus* in 50% of the total catch and relatively constant throughout the year. Hudson (1973) at Cambridge found *A. fumigatus* spore concentrations from 0.3 to 13/m³ comprising 69% of the total catch (incubated at 45°C) with predominantly higher counts during December and January and suggested that this might reflect the lack of foliage at that time to serve as a natural filter for spores. Solomon & Burge (1975) found *A. fumigatus* in less than 5% of outdoor samples at Ann Arbor, Michigan and rarely indoors. The spores rarely exceeded 100/m³ and formed spring and autumn peaks.

Although *A. fumigatus* appears to be fairly ubiquitous in its occurrence, having been recorded in small numbers from such varied sites as mattresses and coal tips (Van Leeuwen *et al.*, 1925; Evans, 1971a, b) its association with hay and straw has long been recognized (Renon, 1897; Lucet, 1897). Large numbers of spores have been recorded from these substrates (Austwick, 1963; Gregory & Lacey, 1963) and from compost heaps (Emmons, 1962). Hugh-Jones & Austwick (1967) established a correlation between the incidence of mycotic abortion caused by *A. fumigatus* and the number of rainy days the previous June. This appears to relate to the difficulty of making good hay in a wet June, and hence to the numbers of spores associated with such hay subsequently fed to cows.

Previous investigations of the mycoflora of compost heaps (Gregory *et al.*, 1964; Chang, 1967) stopped on completion of the composting process. In the present study sampling has been extended over a longer period of time and has shown that, although subject to considerable fluctuations, compost remains a potential source of large numbers of spores for at least several months. However, the effect of such a source on the air spora is strictly localized and is maximal when the heap is disturbed. Similar observations of increasing spore concentrations have been noted by Baruah (1961) during the feeding of hay to livestock, and by Gregory and Lacey (1963) from the sampling of hay and straw in wind tunnel experiments.

The results from the present study confirmed the low incidence of the mould and the tendency for counts to be higher during the winter months, giving an average concentration around Cardiff of 15/m³. The number of isolates obtained from within the ward was consistently lower than the number obtained from the hospital grounds, the results from both sampling sites showing similar fluctuations. No counts were obtained within the ward comparable with the maximum recorded by Noble & Clayton (1963) of 2400/m³. In comparison with other aeroallergens the concentration of *A. fumigatus* spores in the air is low. Over the same period the following concentrations were recorded using the Hirst trap in Cardiff, e.g. *Cladosporium*, 2133/m³;

Sporobolomyces 262/m³; ascospores, 1156/m³ and basidiospores, 1343/m³. In a survey run alongside the present survey using cornmeal agar plates (Harvey, unpublished) very much lower concentrations of *A. fumigatus* were recorded, confirming the need for the use of a selective technique for the effective census of this mould, and indicating why such variable results have been obtained from previous surveys of both the air and possible substrates.

The high temperature requirements of *A. fumigatus* appear to conflict with its high incidence during the coldest months of the year. However, Ayerst (1969) reported that in addition to a temperature requirement of 40°C, optimum growth requirements include a high water activity of the substrate which is more likely to occur during the winter months.

The disparity between the numbers of isolates recovered from the compost (up to 170,000/g wt) and from the air alongside (maximum 527/m³; average 33.2/m³) implies that in spite of the favourable nature of the compost as a substrate, the liberation of spores by wind erosion is relatively inefficient and largely confined to more superficial regions of the heap. This conclusion was tested during one sampling period by turning the compost with a garden fork. The concentration of *A. fumigatus* in the vicinity of the heap rose to 527/m³, considerably higher than the maximum in undisturbed conditions.

The overall fall in the number of isolates recovered from the centre of the compost heap cannot be related to a release of spores to the atmosphere, and it must be concluded that there is a net loss of spores by washing away by rain, animal ingestion, senescence and germination. Fluctuations in the later months of the survey seemed to be associated with meteorological conditions, particularly rainfall and sunshine.

Significant numbers of isolates were obtained from scattered leaf litter which is distributed over such a large area that its potential as a source of *A. fumigatus* spores may be considerably greater than is apparent from the numbers of isolates obtained. Dickinson (1967) has suggested that isolates from such litter are derived from spores deposited from the atmosphere rather than from the growth of the fungus on the litter; however, we found it was possible to isolate *A. fumigatus* over a period of months from leaf litter covered with polythene to prevent the deposition of airborne spores (Mullins, 1974). The discrepancy between these findings may be related to the fact that Dickinson (1967) incubated culture plates at 25°C whereas in the present study culture plates were incubated at 37°C to exclude growth of mesophilic moulds and to prevent colonies of *A. fumigatus* from being either inhibited or overlooked. As Kendrick & Burges (1962) pointed out, it is not possible to use a single non-selective technique in sampling for all types of isolates and in the case of *A. fumigatus* the use of a high incubation temperature (40°C) is necessary to reveal a wide and abundant distribution (Apinis, 1963a, b; Evans, 1971a, b).

Our studies show that the majority of potentially allergic subjects are exposed only to low levels of *A. fumigatus* in the air, unless they are in the immediate proximity of decaying organic matter. However, the ubiquity of the organism allied with its small size make it ideal to penetrate into the bronchial tree, while its optimum temperature requirement gives it an advantage over other moulds in allowing it to germinate therein.

Acknowledgments

We thank Dr D. A. Williams for help and encouragement, Professor G. F. Asprey

and Professor A. G. Smith for facilities in the Botany Department, and Mrs J. Ashton for technical assistance. This paper forms part of the work accepted for the degree of Ph.D. conferred on J.M. by the University of Wales.

References

- ANDERSEN, A.A. (1958) New sampler for the collection, sizing and enumeration of viable airborne particles. *Journal of Bacteriology*, **76**, 471.
- APINIS, A.E. (1963a) Thermophilic fungi of coastal grasslands. Soil organisms. *Proceedings of the colloquium on soil fauna, soil microflora and their relationships* (Ed. by J. Doekson & I. van der Rift), pp. 427–438. North-Holland, Amsterdam.
- APINIS, A.E. (1963b) Occurrence of thermophilic microfungi in certain alluvial soils near Nottingham. *Nova Hedwigia*, **5**, 57.
- AUSTWICK, P.K.C. (1963) Ecology of *Aspergillus fumigatus* and the pathogenic phycomycetes. *Recent Progress in Microbiology*, **8**, 644.
- AYERST, G. (1969) The effects of moisture and temperature on growth and spore germination in some fungi. *Journal of Stored Produce Research*, **5**, 127.
- BARUAH, H.K. (1961) The air spora of a cowshed. *Journal of General Microbiology*, **25**, 483.
- BLAKESLEE, A.F. (1915) Linder's roll tube method of separating cultures. *Phytopathology*, **5**, 68.
- CHANG, Y. (1967) The fungi of wheat straw compost. III. Biochemical and physiological studies. *Transactions of the British Mycological Society*, **50**, 667.
- DICKINSON, C.H. (1967) Fungal colonisation of *Pisum* leaves. *Canadian Journal of Botany*, **45**, 915.
- EMMONS, C.W. (1962) Natural occurrence of opportunistic fungi. *Laboratory Investigations*, **11**, 1026.
- EVANS, H.C. (1971a) Thermophilic fungi of coal spoil tips. II. Occurrence, distribution and temperature relationships. *Transactions of the British Mycological Society*, **57**, 255.
- EVANS, H.C. (1971b) Thermophilic fungi of coal spoil tips. III. Seasonal and spatial occurrence. *Transactions of the British Mycological Society*, **57**, 267.
- EVANS, H.C. (1972) Thermophilous fungi isolated from the air. *Transactions of the British Mycological Society*, **59**, 516.
- GREGORY, P.H. & LACEY, M.E. (1963) Liberation of spores from mouldy hay. *Transactions of the British Mycological Society*, **46**, 73.
- GREGORY, P.H., LACEY, M.E., FESTENSTEIN, C.N. & SKINNER, F.H. (1964) Microbial and biochemical changes during the moulding of hay. *Journal of General Microbiology*, **33**, 147.
- HENDERSON, A.H., ENGLISH, M.P. & VETCH, R.J. (1968) Pulmonary aspergillosis. *Thorax*, **23**, 513.
- HINSON, K.F.W., MOON, A.J. & PLUMMER, N.S. (1952) Bronchopulmonary aspergillosis. *Thorax*, **7**, 317.
- HIRST, J.M. (1952) An automatic volumetric spore trap. *Annals of Applied Biology*, **39**, 257.
- HUDSON, H.J. (1969) Aspergilli in the air spora at Cambridge. *Transactions of the British Mycological Society*, **52**, 153.
- HUDSON, H.J. (1973) Thermophilous and thermotolerant fungi in the air spora at Cambridge. *Transactions of the British Mycological Society*, **61**, 595.
- HUGH-JONES, M.E. & AUSTWICK, P.K.C. (1967) Epidemiological studies on bovine mycotic abortion. I. The effect of climate on incidence. *Veterinary Record*, **81**, 273.
- KAMMER, R.B. & UTZ, J.P. (1974) *Aspergillus* species endocarditis. The new face of a not so rare disease. *American Journal of Medicine*, **56**, 506.
- KENDRICK, W.B. & BURGESS, A. (1962) Biological aspects of the decay of *Pinus sylvestris* leaf litter. *Nova Hedwigia*, **4**, 313.
- VAN LEEUWEN, W.S., BIEN, Z., KREMER, W. & VAREKAMP, H. (1925) Ueber die Bedeutung Kleinsporiger *Aspergillus* arten (typus *Aspergillus fumigatus*) für die Aetiologie des Asthma Bronchiale. *Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, **44**, 19.
- LUCET, A. (1897) De l'*Aspergillus fumigatus* chez animaux domestiques et dans les oeufs en incubation. In: *Etude Clinique et Experimentale*, p. 108. Ch. Mendel, Paris.
- MCCORMICK, W.F., SCHOCHET, S.S., WEAVER, P.R. & MCCRARY, J.A. (1975) Disseminated aspergillosis. *Archives of Pathology*, **99**, 353.
- MULLINS, J. (1974) *The ecology of Aspergillus fumigatus* (Fres). Ph.D. thesis, University of Wales.
- NOBLE, W.C. & CLAYTON, Y.M. (1963) Fungi in the air of hospital wards. *Journal of General Microbiology*, **32**, 397.

- PEPYS, J. (1969) Hypersensitivity diseases of the lungs due to fungi and organic dusts. In: *Monographs in Allergy*. S. Karger, Basel.
- RAPER, K.B. & FENNEL, D.I. (1965) *The genus Aspergillus*. Williams and Wilkins, Co., Baltimore.
- RENON, L. (1897) *Etude sur l'aspergillose chez les animaux et chez l'homme*. Masson, Paris.
- RIFKINO, D., MARCHORIO, T.L., SCHNECK, S.A. & HILL, R.B. (1967) Systemic fungal infections complicating renal transplantation and immunosuppressive therapy. *American Journal of Medicine*, **43**, 28.
- SOLOMON, W.R. & BURGE, H.P. (1975) *Aspergillus fumigatus* levels in and out of doors in urban air. *Journal of Allergy and Clinical Immunology*, **55**, 90.
- WARREN, C.P.W., TAI, E., BATTEN, J.C., HUTCHCROFT, B.J. & PEPYS, J. (1975) Cystic fibrosis—immunological reactions to *A. fumigatus* and common allergens. *Clinical Allergy*, **5**, 1.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.