

Microorganisms

In Home and Indoor Work Environments

Diversity, Health Impacts, Investigation and Control



edited by
Brian Flannigan
Robert A. Samson
J. David Miller

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To the memory of John Lacey DSc
1937–1998

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PREFACE

While much of the concern about air pollution in the past has been focused on the outdoor environment, in recent years indoor air quality (IAQ) has moved up the agenda. Over the period between 1987 and 1999, more than \$1 billion of federal government money was spent on research into indoor air pollution in USA. In March 2000 the Environmental Protection Agency released a report on “Healthy Buildings, Healthy People: A Vision for the 21 st Century”, which set the objective of achieving major health gains by improving indoor environments. The National Institute for Occupational Health (CDC) has studied the impact of poor indoor air quality on productivity. The median estimate of these losses is \$100 billion per year. Other countries, including Canada, Denmark, Finland, Netherlands and Sweden also have substantial programmes on residential housing and health. However, there is wide variation in the research effort and expenditure on measures to improve IAQ, and IAQ in some is very much lower on the order of priorities.

A document produced in July 2000 by a WHO European working group has further emphasized the global importance of IAQ as a determinant of population health and well being. This document, “*The Right to Healthy Indoor Air*”, sets out nine principles (derived from the general principles in the International Bill of Human Rights). These are intended to inform all those who have an influence on public health of their obligations to honour the right of every individual to breathe healthy indoor air, and influence those national governments that do not have plans for future action on healthy indoor air to put it on their agenda.

Despite the large amount of money spent on research into pollution of the indoor environment, the US General Accounting Office has confirmed that what has been done has pointed to the complexity of the problem and to major gaps in knowledge. Among these gaps are accurate knowledge of the identities and sources of pollutants and of the effects of prolonged exposure to indoor pollutants on health. This book considers one such group of pollutants, namely microorganisms, and more particularly heterotrophic bacteria and fungi. Advances have certainly been made in our knowledge of microorganisms in the home and indoor work environment as research has accelerated in the last decade. Fully elucidating their effects on human health has however been bedevilled by problems of accurate assessment of exposure to microorganisms and precise identification of those present in the environment. The first three sections of the book review the types of microorganism in outdoor and indoor air, their growth and control in home and work environments, and their role in respiratory disease. The remaining sections of the book are given over to addressing the twin problems of exposure assessment and identification, discussing the methodology for and conduct of investigations of indoor environments and providing keys and colour illustrations to

assist in the identification of approaching 100 mould, yeast and actinomycete contaminants.

We think that the availability of information on the microorganisms that grow in the built environment, together with information on the limitations of the methods currently available to measure them, will be useful to researchers, public health officials and industrial hygienists. Together with reviewers, the authors from Canada, Sweden, the Netherlands, the United Kingdom and USA have worked hard to produce material that is accurate and timely. In addition to thanking reviewers and authors for their efforts, we should also like to express our gratitude to Margaret Flannigan for editorial assistance, Karin van den Tweel for helping with the drawings and Ans Spaapen-de Veer for preparing the index. Thanks are given to Dr Brian Crook (HSL, Sheffield, UK) for substantive assistance in the preparation of Chapters 3.2.

We have dedicated this book to the memory of a fellow microbiologist, John Lacey, who was internationally recognized for his unique expertise at the interface of stored product microbiology, aerobiology, occupational hygiene and medicine, and was the author of more than 300 publications. The aerobiological, taxonomic and ecological studies of John and his co-workers not only clarified the role of microorganisms in a number of occupational lung diseases, but have a relevance to home and non-industrial work environments as well as to the storage and processing of the particular agricultural materials with which they were first concerned. In his friendly collaboration and links with many workers in overseas institutes, he was generous in passing on his knowledge and expertise to others, and his work and the influence that he has had are reflected in this book.

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Chapter 1.

Microorganisms in air

Chapter 1.1

MICROORGANISMS IN OUTDOOR AIR

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EARLY STUDIES OF OUTDOOR AIR

Man has long believed that the air could bring disease to man and crops, but it was not until the invention of the microscope in the 17th century that it was possible to observe the array of particles that are carried in the air. With his lens, Antonie van Leeuwenhoek (Dobell, 1932) was just able to observe bacteria. It gradually became recognized that the air carried bacteria, yeasts, fungal spores, spores of mosses and ferns, algae, pollen grains and even protozoa. Initial studies were concerned with the controversy surrounding spontaneous generation of organisms and it was Pasteur (1861) who, by drawing air through gun cotton and after dissolving the gun cotton examining the deposit under a microscope, discovered that the air contained a variety of different particles. However, he did not pursue these studies and the realisation that the air contained a variety of microbes resulted in a concerted effort by medical men to discover the microbes that caused disease (Bulloch, 1938). The original work of Miquel (1899) in Paris into airborne bacteria stands as one of the most sustained series of volumetric measurements of the microbial population of the air ever attempted. Samples were collected over a 16-year period in plugs of gun cotton and after this was dissolved the filtrate was cultured in flasks of filtered saline beef extract. From his studies he discovered that in a park 5 km from the centre of Paris bacteria were nearly three times as numerous in summer as in winter; in the centre of Paris counts were twice as many as in the park, but with a similar seasonal fluctuation. He also sampled a narrow unhygienic street and the main sewer of Paris, which proved to be no more contaminated than the air of the streets outside. In the park there were on average 290 bacteria m^{-3} air, in the centre of Paris 7480 bacteria m^{-3} , in the unhygienic street 5550 m^{-3} and in the main sewer 3835 m^{-3} . He also noted a steady annual decline which he attributed to improved street cleaning and washing to lay dust. Miquel came to the conclusion that the source of most outdoor airborne bacteria is the surface of the ground. He also studied the variations during the day and attributed increases during the course of the day to mechanical causes such as road sweeping and traffic. Miquel lost interest in fungal spores and developed media which selectively discouraged mould growth. Fungal spores in the air were then largely ignored until investigated by Cadham (1924), who confirmed spores of cereal rust fungi as a cause of asthma and re-kindled an interest in airborne fungal spores driven by allergists.

FUNGAL SPORES IN OUTDOOR AIR

Types and sources of fungal spores

Fungal spores are present in outside air throughout the year (the air spora) with scarcely any exceptions, and in the author's experience concentrations in the city centre at Cardiff have reached as high a 24-h mean as nearly 85000 m^{-3} air. It is virtually impossible to take a breath without inhaling a quantity of fungal spores. Estimates of the volume of air inspired at rest suggest a figure of 10 L min^{-1} , the rate of sampling adopted for Hirst spore traps (Hirst 1952). At this rate, 1 m^3 air would be inspired in 100 min, but any increase in activity would dramatically increase the volume of air inspired, resulting in a greater intake of fungal spores.

The presence of so many fungal spores in the air is a consequence of the mechanism in many fungi as a means of dispersal, namely the production and release into the air of enormous numbers of spores. The spores of some fungi are dispersed in water and may only become airborne in spray thrown up by wave action. Insects also are responsible for the dispersal of some fungal spores, but the majority of fungi release their spores directly into the air.

Fungi produce both sexual and asexual spores: some produce only asexual spores, some produce only sexual spores, and some produce both. Asexual spore types include sporangiospores, conidiospores, pycnidiospores, and the teliospores, uredospores and aeciospores produced at different stages of the life cycle of the rust fungi. The sexual spores include zygosporangia (which are mostly sessile), ascospores and basidiospores.

Release and aerosolization of spores

As fungi are relatively small, certain barriers to spore dispersal exist which have to be overcome. There is a static layer of air known as the laminar boundary layer varying in thickness from 1 m to 10 cm from the ground; it is thicker in still conditions and becomes thinner with increased wind speed. If spores are released into this layer they will not be dispersed. Accordingly, mechanisms have evolved in the fungi to ensure that their spores are released into the turbulent layer, which extends above the laminar layer.

Much extensive work has been carried out by Ingold and his co-workers on spore release mechanisms, to whom reference should be made for a more exhaustive treatment of the subject (Ingold 1971). Most of the asexual spores are released relatively passively and rely on wind currents and turbulence to carry the spores away. Among these, sporangiospores are produced within a sporangium, which is raised up on a sporangiophore, exposing spores to the scouring effects of air currents as the sporangial wall bursts on maturity exposing the spores for dispersal. In some species of *Mucor* the sporangial wall appears to dissolve leaving a mass of spores in a sporangial drop exposed to drying air currents. In the majority of sporangial fungi the sporangial wall ruptures as the sporangiospores initially packed into the sporangium in polyhedral shapes, round off as they mature and increase the pressure on the sporangial wall. Although this method of

spore release is found in *Mucor* and other fungi in the order Mucorales within the Zygomycetes (see Chapter 1.2), despite the abundance of this group in nature relatively few of their spores are isolated from outside air.

The asexual aeciospores of rusts are released by a similar method of rounding off from a polyhedral shape as they mature, exposing them to erosion by air currents. However, by their situation as biotrophic parasites on the leaves of plants, rusts and their spores are effectively raised above the laminar layer, facilitating spore release.

Perhaps the most common asexual spore produced by fungi is the conidiospore or conidium. Such spores are produced externally, rather than within a sporangium, and the spores are individually budded off or may be formed in chains but raised up on conidiophores, allowing for erosion of the spores by wind currents. This latter mechanism is found in *Cladosporium*, which is the genus which contributes most to the air spora in temperate countries, including UK (Harvey 1967, 1970). *Cladosporium* and a number of other common airborne fungi such as *Alternaria* and *Epicoccum* colonize the surface of leaves (the phylloplane), stems and other plant organs, particularly as they senesce, so that these fungi are often referred to as phylloplane fungi

Sexual spores are produced by Ascomycetes and Basidiomycetes. In Ascomycetes (the cup fungi), the spores are produced in groups of eight within a sac known as an ascus. Asci may be produced within, or over the surface of, a fruiting body. In response to the appropriate stimuli the ascus releases its spores explosively into the air. The stimulus may be a change in relative humidity or even a response to light (Walkey and Harvey 1966, 1968a). In Basidiomycetes (mushrooms and toadstools) the whole of the fruiting body may be raised above the ground on a thick stalk or it may grow on decaying wood, as in the bracket fungi. The basidiospores develop externally, usually as a group of four, each on a separate sterigma, on a basidium. Depending on the type of basidiomycete, the basidia cover the surface of sheet-like gills or surround pores in the fruiting body. They are forcibly ejected by a mechanism which is not yet fully understood into the space between the gills or into pores, and thereafter fall by gravity into the turbulent air flow below the cap. In puffballs and the earth star fungi a different mechanism has evolved in which the spores are produced inside a thin papery capsule with an apical opening, the ostiole. The dry spores are either forced out of the capsule by the impact of raindrops on the surface of the capsule or are drawn out as the air passes across the ostiole.

Once airborne, the length of time spores remain in the air will depend in part on the size of the spore, as large spores will naturally tend to be deposited from the air more rapidly than small spores. The rate of fall of a spherical spore in still air is given by Stoke's Law:

$$V = \frac{\sigma - \rho}{\mu} g r^2$$

Where V is terminal velocity in cm sec⁻¹

σ is the density of the spore

ρ is the density of air

g is acceleration due to gravity (981 cm sec⁻¹)

μ is the viscosity of air ($1.8 \times 10^{-4} \text{ g cm}^{-1} \text{ sec}^{-1}$ at 18°C)

r is the radius of the spore

Since the density of most spores is 1.0 and the density of air is so small that it can be ignored, this can be simplified to:

$$V = \frac{2gr^2}{9\mu}$$

Then the rate of fall of a spore is proportional to the square of the radius, giving rates of fall of $2.0\text{--}2.8 \text{ cm sec}^{-1}$ for the very large spores of *Cochliobolus sativus* (*Helminthosporium sativum*), which are $80 \times 15 \text{ }\mu\text{m}$, compared with 0.05 cm sec^{-1} for the spores of *Lycoperdon pyriforme* ($4 \text{ }\mu\text{m}$ diameter).

Long distance transport of spores

Fungal spore distribution from a point source has always been of interest to plant pathologists who wish to predict the distance which spores will travel and to workers attempting to predict concentrations of smoke screens, gas clouds, radioactive particles or pollen released from genetically modified crops (Emberlin *et al.* 1999).

The extensive work done in this field was described by Gregory (1973), both from his own studies at Rothamsted and from the work of others. It is apparent that producing a mathematical model to predict the distance to which a spore will travel is extremely difficult. Unlike gas clouds, spores are eroded from the cloud by deposition, but Chamberlain (1966) considered that for grass pollen (with grains around $20 \text{ }\mu\text{m}$ in diameter) and other pollen released at about knee height the evidence suggests ranges in the order of 1 km, and for pollen released at tree top level distances of travel in the order of tens of kilometres. Raynor *et al.* (1970) found that 1% of ragweed pollen released from a point source remained airborne at a distance of 1 km from the point of release. As agents by which plant disease may spread, spores and the distances they may travel have also long been of economic interest. Stakman and Hamilton (1939) reported uredospores of stem rust of wheat (*Puccinia graminis tritici*) at distances of some 970 km from the source, which was a vast area of winter wheat in the southern USA. Hirst *et al.* (1967) had the opportunity to sample airborne particles from an aircraft travelling over the North Sea, and some 400–500 km from the English coast recorded unexpected clouds of fungal spores. These corresponded to spores which had been released the previous day over the land and had been carried eastward by the prevailing winds. There was a further increase between 500 and 600 km from the coast of spores characteristic of those released at night, again which had been released over land, but the previous night. As Gregory (1973) has noted, the extent of spore dispersal is sufficient to spread plant diseases between countries and across continents.

Sampling the air spora

Estimates of spore concentrations in the air are obtained by air sampling. Two methods are widely used to carry out a census of the atmosphere. Spores may be collected onto culture plates and the fungi, which develop into colonies, can be identified and counted. Although spores may comprise the bulk of airborne fungal material, there can also be fragments of the vegetative mycelium (see Chapter 1.2), hyphal fragments, which may give rise to colonies. Aggregated clumps of spores, not just individual spores (or in the case of yeast, not only individual cells but clumps), may also be trapped on culture plates. To allow the spores simply to settle on the plate is to invite sampling errors because of the different rates of fall of different spores. Consequently, an efficient sampling device such as the Andersen sampler (Andersen 1958), based on the cascade impactor devised by May (1945), is required to ensure volumetric sampling. Because of the danger of overloading the culture plates, the time for which the plates can be exposed for sampling purposes is limited. Therefore, sampling has to be restricted to “spot” or “grab” samples or a series of samples taken at intervals throughout the day and night, with the attendant problems of incubating and subsequently examining a large number of culture plates. There is also the problem that different culture media tend to be somewhat selective in the fungi which will grow on them, and many fungal spores will not germinate on culture plates, including most of the sexual spores which may form a significant part of the air spore. As one cannot be certain as to what exactly colonies have arisen from—individual spores, clumps or hyphal fragments—counts on culture plates are expressed volumetrically as colony forming units (CFU) m^{-3} air.

The alternative is to collect the spores on a glass microscope slide using a volumetric trap such as the Hirst trap (Hirst 1952) or the Burkardt automatic volumetric spore trap (AVST) into which it evolved. Irrespective of whether the spores collected for visual identification under the microscope are viable (culturable) or nonviable, identification is carried out and is dependent on the morphological characteristics of the spores. Counts are expressed as spores m^{-3} air, and not as CFU m^{-3} as in culture-based methods. Some spores are easy to identify but others are very difficult to distinguish, either because of their small size or their lack of distinguishing features. In consequence, many spores tend to be counted according to categories such as colour and shape. This is particularly true of the ascospores and basidiospores, which frequently occur in the air in high numbers and are likely to have originated from many different species. It may therefore be necessary to use a culture method when it is the only way of confidently estimating the concentration of airborne spores of a particular species of fungus, particularly when visual identification is not possible (Mullins *et al.* 1976). With the advent of PCR (polymerase chain reaction) and other modern molecular methods, advances have been made in identifying specific organisms which occur in the air in small numbers (Wakefield *et al.* 1998), so that such techniques may prove to have more widespread applications.

Factors affecting the composition of the air spora

The variety and concentration of spores in the air is subject to continuous diurnal and seasonal variation. Contributory factors include availability of substrate, activities such as mowing grass and harvesting grain, and climatic factors, particularly temperature and rainfall, which have a direct effect on the release of spores into the air. Rain and warmth also promote the development of vegetation on which parasitic and saprophytic fungi subsequently develop. Broad correlations between the composition of the air spora and climatic factors enable predictions to be made that certain spore types will be more abundant in warmer, drier summers whereas others will be more abundant during damp weather.

The outdoor air spora is largely derived from spores produced by moulds and other fungi growing on natural and cultivated vegetation and on surface vegetable debris. Surveys of the air spora at any site tend to be dominated by spores of local origin, with others of more distant origin forming only a smaller part of the total census. Some species are practically ubiquitous, whereas others are more or less confined to certain localities. It is therefore not surprising that the air spora of towns and cities will tend to be less abundant than that of the surrounding countryside. In addition to spores, hyphal fragments are also present in the air. Obviously the source of these fragments cannot be identified, but it has been observed that they are made airborne particularly by such activities as mowing and grain harvesting.

AVST surveys of fungi in outdoor air

Local variation in the temperate air spora

To illustrate the main characteristics of the outdoor air spora and some factors which have a bearing on it, this chapter will examine data from air sampling in and around Cardiff in UK. Cardiff, the capital of Wales, lies in the southern part and is a large seaport on the north side of the Bristol Channel, approximately 220 km west of London. In Cardiff, the air has been sampled continuously since 1954 using an AVST at rooftop level in the centre of the city.

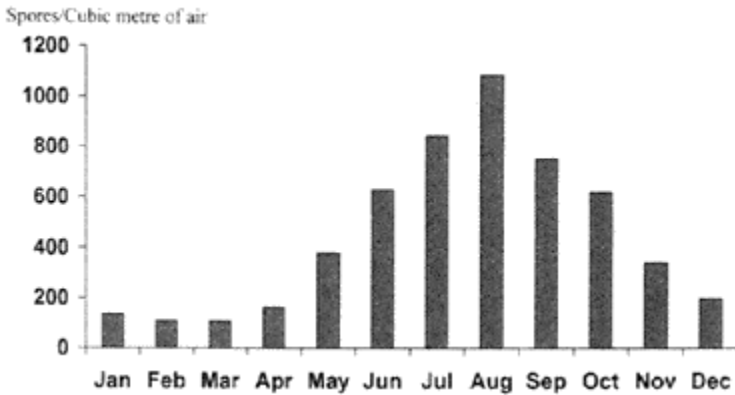


Fig. 1. Incidence of airborne *Cladosporium* spores trapped by an AVST at roof top level in Cardiff.

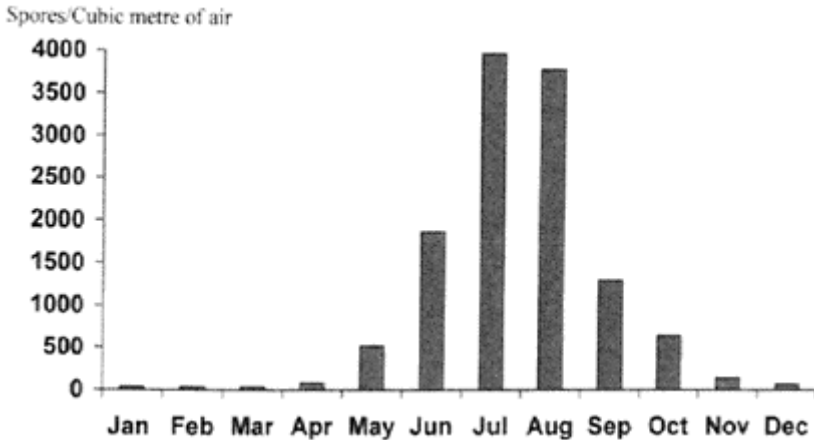


Fig. 2. Incidence of airborne ascospores trapped by an AVST at roof top level in Cardiff.

The data obtained can be regarded as being typical for a north temperate climate and the incidence of different spores throughout the year in the UK and comparable climatic areas of mainland Europe are broadly similar.

City centre site

In Figs 1–5 the incidence in the air of *Cladosporium*, ascospores, basidiospores, rust and smut spores, and particles of hyphae throughout the year is shown. It will be noticed that *Cladosporium* reaches its peak in July and August (Fig. 1), hyphal fragments reach their highest levels in August (Fig. 5), and rusts and smuts achieve their highest levels in June when smut fungi infect the flowers of grasses (Fig. 4). Ascospores also reach their

highest levels in August (Fig. 2), but basidiospores continue at high levels through the months of September and October (Fig. 3).

Counts obtained during the summer months from various outstations at woodland, coastal, upland valley and coastal plain sites can be compared with the Cardiff city centre site so as to show the influence of local factors on spore concentrations. Since only one site distant from the city centre site was sampled in any particular year, it is not possible to make direct comparisons between the outstations except as they contrast with Cardiff.

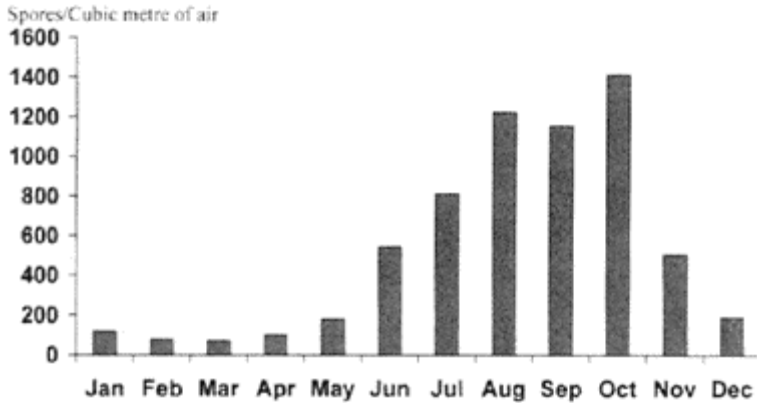


Fig. 3. Incidence of airborne basidiospores trapped by an AVST at roof top level in Cardiff.

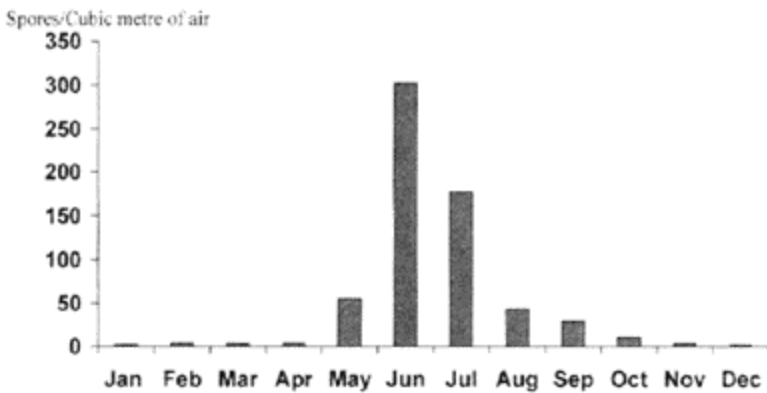


Fig. 4. Incidence of airborne rust and smut spores trapped by an AVST at rooftop level in Cardiff.

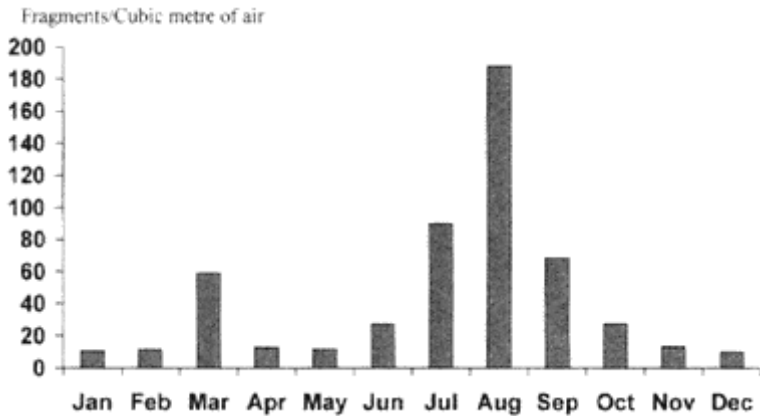


Fig. 5. Incidence of airborne hyphal fragments trapped by an AVST at roof top level in Cardiff.

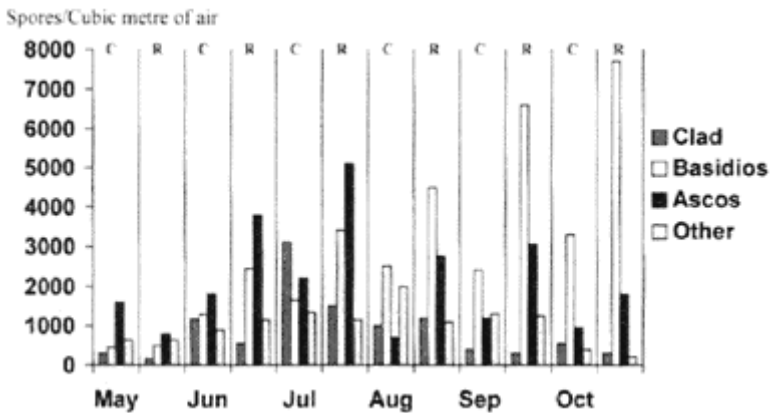


Fig. 6. Comparison between airborne fungal spores at a city centre site in Cardiff and at a coniferous woodland site at Resolven.

At Resolven, where the trap was positioned on the edge of a field of rough grass surrounded by plantations of Sitka spruce, Douglas fir and Japanese larch, spore concentrations were consistently higher than at Cardiff after May (Fig. 6), because of the release of large numbers of ascospores and basidiospores in the plantations. Overall, the basidiospore totals were 217% and ascospore totals 206% of those at Cardiff. By comparison, *Cladosporium* totals at Resolven were 61% of those at Cardiff and the other categories of fungi, including other imperfect fungi and rusts and smuts, were 83% of those recorded at Cardiff. This was due to relative paucity of local substrates for growth of these fungi within the plantations.

At Tintern, alongside a stream in a field surrounded by mixed deciduous and

coniferous woodland with intervening fields and small orchards, the total spore concentrations (Fig. 7) were greater than at the city centre site each month with the exception of August. During that month there was a very high urban concentration of *Cladosporium*, which was 175% of that at Tintern, with an average daily concentration of 7000 spores m^{-3} air. Over the survey period basidiospore totals were 337% and ascospore totals 254% of those in Cardiff.

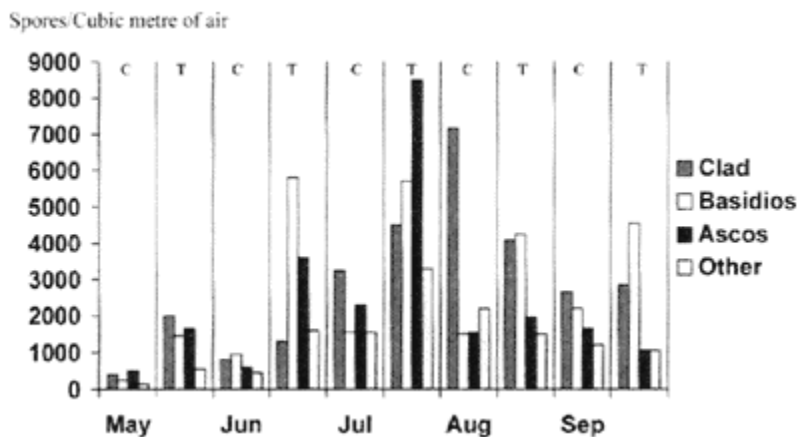


Fig. 7. Comparison between airborne fungal spores at a city centre site in Cardiff and at a mixed deciduous/coniferous woodland site at Tintern.

The trap at Cefn Mably was surrounded for a distance of 500–900 m by mixed deciduous woodland, parts of which had been neglected for some time and included large quantities of decaying timber. Total spore concentrations at this site were >300% greater than at Cardiff (Fig. 8). Basidiospore totals were 755% greater than at Cardiff and ascospores 375% greater. *Cladosporium* totals were also greater at this woodland site, averaging 130% of those at Cardiff. Owing to the proximity of so many fruiting bodies of Basidiomycetes, the usual annual periodicity seen in Cardiff over a period of years was not replicated at Cefn Mably, where basidiospore levels were consistently high throughout the summer months.

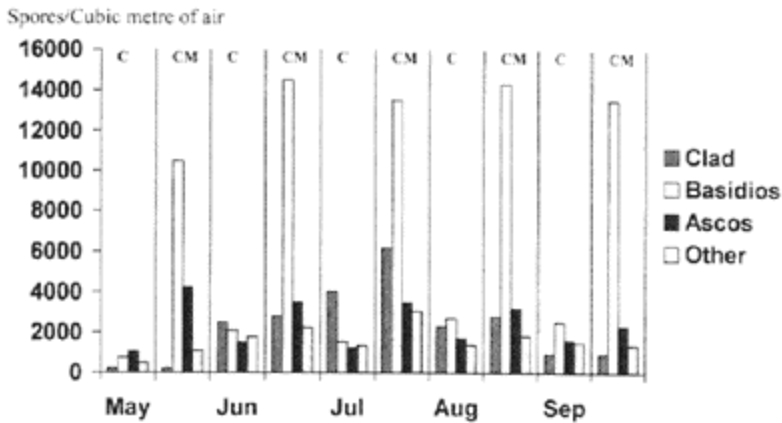


Fig. 8. Comparison between airborne fungal spores at a city centre site in Cardiff and at a mixed deciduous woodland site at Cefn Mably.

These woodland surveys indicate that woodlands are, as might be expected, a prolific source of ascospores and basidiospores, the unmanaged mixed woodland (Fig. 8) being a greater source of spores than the managed coniferous or managed mixed coniferous and deciduous woodland. Herxheimer *et al.* (1969) demonstrated that basidiospores and ascospores can cause allergy and induce asthma attacks. Many cases of late summer asthma can be attributed to the inhalation of spores originating in woodlands.

Coastal site

Spore concentrations over the sea are generally much lower than those over the land, particularly at some distance from the land, owing to deposition of the spores on the sea without any compensating addition of spores (Hirst *et al.* 1967), so that traditionally a sea cruise was considered an effective way of avoiding seasonal allergens.

As the prevailing wind direction in UK is from the west, a west-facing coast should benefit from on-shore air currents comparatively free of spores, unlike the east coast, which will receive the spore load picked up as the air currents cross the land. Fig. 9 shows a comparison between Cardiff city centre and a west coast site on Cardigan Bay.

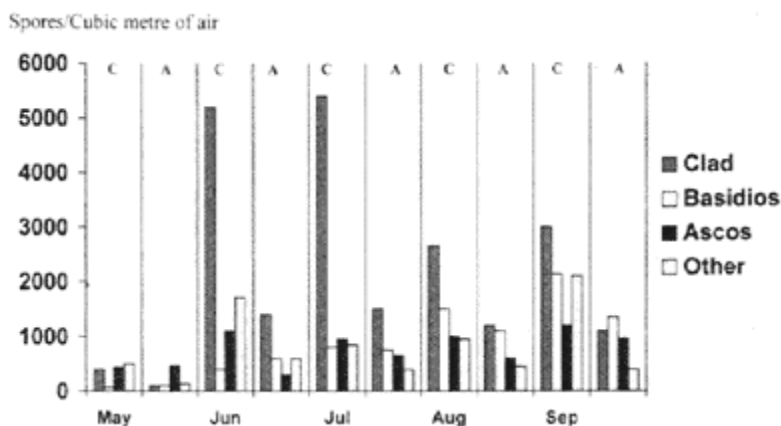


Fig. 9. Comparison between airborne fungal spores at a city centre site in Cardiff and at a seafront site at Aberystwyth.

This roof top site was at Aberystwyth, 120 km NW of Cardiff, and faced west near the seafront, from which town streets containing relatively few trees and gardens extended 600–900 m to the east, with agricultural land lying beyond that. Spore concentrations were consistently lower over the period of the survey, with total concentrations being only 44% of those in Cardiff. While both ascospore and basidiospore concentrations in Aberystwyth were around 80% of those in Cardiff, *Cladosporium* totals were only 32% of the corresponding Cardiff totals. These results confirm the trend to lower spore counts near a west facing coast, particularly so in the case of *Cladosporium* spores.

Upland valley site

At Llwynypia Hospital, in the Rhondda Valley 25 km NW of Cardiff, a sampler was sited on a roof top of a building on a west facing slope covered for the most part by rough pasture and scrub vegetation with some hedgerows. On the opposite side of the valley was an extensive conifer plantation, as well as more pasture and scrub vegetation. The valley, like all South Wales valleys, runs roughly in a north-south direction, and Davies (1969a,b) suggested that such a valley might mimic the situation of upland valleys in Switzerland. The relatively poor vegetation plus wind flows across the Swiss valleys produce environments with low spore concentrations, which have favoured the construction of sanatoria in these areas for the relief of asthma.

During the period from May to September (Fig. 10), overall spore counts were found to be 57% of those recorded in Cardiff, with basidio spores 49%, ascospores 73% and *Cladosporium* 65% of the corresponding Cardiff totals. When Williams and Higgins (1959) carried out a survey of the incidence of asthma in the Rhondda Valley they did in fact observe a lower incidence of asthma compared to Cardiff, and in our survey we also noted lower concentrations of grass pollen, another cause of asthma.

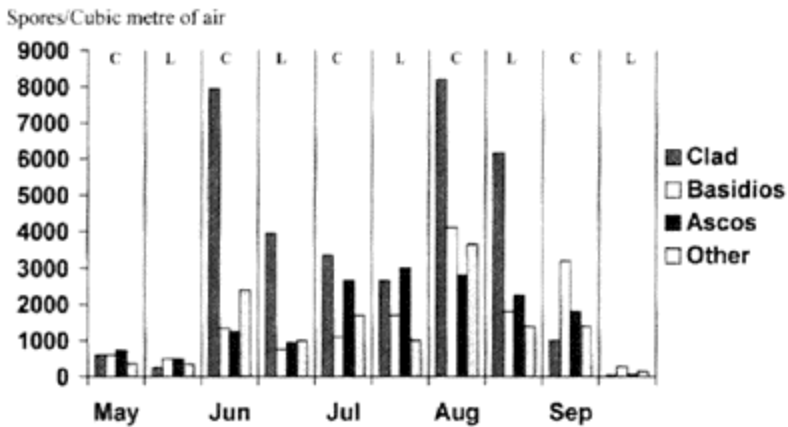


Fig. 10. Comparison between airborne fungal spores at a city centre site in Cardiff and at an upland valley site at Llwynypia.

Coastal plain site

South of the valleys in South Wales is a coastal plain leading to the Bristol Channel. A trap was situated at Cleppa Park which is a rural site 14 km East of Cardiff. The trap was surrounded by fields used for cereal and vegetable production and for pasture, with small woodlands of mixed conifer and broad-leaved trees some 550–1190 m away.

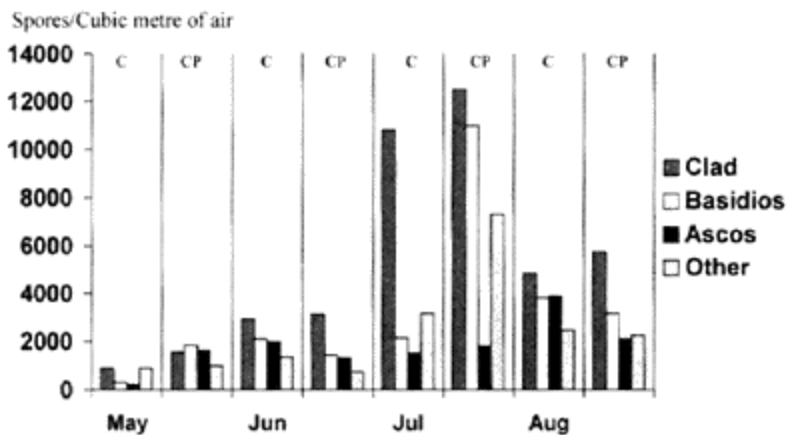


Fig. 11. Comparison between airborne fungal spores at a city centre site in Cardiff and at a farmland site at Cleppa Park.

The incidence of spores (Fig. 11) was higher than at Cardiff overall, with counts of basidiospores being 209%, ascospores 90% and *Cladosporium* 118%

of those in Cardiff. However, in June and August, ascospore and basidiospore concentrations at Cardiff exceeded those at Cleppa Park. Surprisingly for a rural site, rust and smut spores in total were marginally lower than in the city, but together rust and smut spores accounted for only some 2–3% of the total air spora recorded at both sites. During July, the spore concentration at Cleppa Park was particularly high in relation to Cardiff, being some 186% of the city concentration.

The results from these different outstations around Cardiff illustrate, within one geographical area, the importance of local sources of spores in the concentration and make-up of the air spora. When Kramer and Eversmeyer (1984) sampled the air at four sites within the city of Manhattan, Kansas, and two sites outside the city, all within a radius of 10 km of the city, they found no significant difference between mean spore concentration in major spore types at the different sites when tested with Duncan's multiple range test (Table 1). However, the authors noted that variations in concentrations of the air spora between different sites were primarily due to differences in environmental conditions which influence the numbers and kinds of fungi that develop, sporulate and release spores, although the site descriptions do not indicate large vegetational differences between the environs of the different sites. However, the differences noted by Kramer and Eversmeyer (1984) were most pronounced in periods during which rain was sufficient to allow for abundant growth and sporulation of fungi locally. Conversely, during dry periods development of fungi in the area is slowed severely, if it occurs at all, so that overall the greatest percentage of the air spora at all these sites is composed primarily of spores transported through the atmosphere from remote sources. Unfortunately, the time of year during which the concentrations shown in Table 1 were recorded is not given, so comparisons cannot be made with data from other sites. The high numbers of *Alternaria* spores in the air suggest that the prairie is the likely source of spores found in the city.

Table 1. Mean concentrations of airborne fungal spores collected outdoors by Kramer-Collins drum sampler in and around Manhattan, Kansas, over a 10-day sampling period in 1979 (Kramer and Eversmeyer 1984).

Location	Mean concentration (spores m ⁻³ air)			
	<i>Cladosporium</i>	<i>Alternaria</i>	Ascospores	Basidiospores
Prairie	3852a	307ab	509ab	426a
Experimental farm	995b	183b	94b	121b
City Park	2750a	452ab	894a	468a
New suburbs	4751a	561a	689ab	503a
Old suburbs	2872ab	403ab	688ab	465a

City Centre	4063a	608a	685ab	555a

Mean values in the same column followed by the same letter are not significantly different from each other

Seasonal and diurnal variation in the air spora

Seasonal variations similar to those in Cardiff (Figs 1–5) have been recorded in Waterloo, Ontario (Table 2), where Li and Kendrick (1995) sampled outside air on a balcony over a period of a year using a particle sampler which took 10-min samples of the air every two hours. Compared with the spore concentrations recorded in Cardiff in 1992, the concentrations of *Cladosporium*, ascospores and basidiospores recorded in Ontario were all higher than those recorded in Cardiff, with respective average concentrations of 1425, 368 and 845 spores m^{-3} air in Ontario and 871, 161 and 248 m^{-3} in Cardiff, and the percentages of the total air spora respectively 41, 10.6 and 24.1% in Ontario compared with 55, 10.2 and 15.7% in Cardiff. However, concentrations for Cardiff in 1992 differ from the 30-year averages for the city, in which *Cladosporium* accounts for 41% of the total air spora, ascospores for 17.2%, and basidiospores 20.6%.

Li and Kendrick (1995) also noted diurnal periodicity in ascospore and basidiospore concentrations were demonstrated, with an increased prevalence during the early hours of the morning, similar to that for the prevalence of *Didymella* ascospores (Allitt, 1986) observed in UK at Edinburgh (Richardson 1996). Richardson derived his data using an AVST for continuous sampling on a rooftop and found an association between increased spore concentrations and relative humidity during the early hours of the morning. In addition to the diurnal periodicity of ascospores and basidiospores, Li and Kendrick (1995) observed periodicity in the prevalence of conidia of *Cladosporium* and other imperfect fungi which are released passively. These conidia tended to increase in number during the afternoon, with the highest concentration of *Cladosporium* at 1600 h and of *Alternaria* at 1800 h.

Table 2. Dominant fungal spores during 1992 from outdoor air of Waterloo, Ontario, expressed as mean concentration and percentage of total airspora (after Li and Kendrick 1995).

Type	Whole year		May-October		November-April	
	Spores m^{-3}	% of total	Spores m^{-3}	% of total	Spores m^{-3}	% of total
<i>Alternaria</i>	62	1.8	139	1.9	13	1.3
<i>Aspergillus/Penicillium</i>	130	3.8	139	1.9	125	12.4
<i>Cladosporium</i>	1425	41.0	3018	40.8	421	41.9
<i>Epicoccum</i>	46	1.3	64	0.8	35	3.5

<i>Leptosphaeria</i>	183	5.3	454	6.1	12	1.1
Unidentified ascospores	185	5.3	403	5.5	48	4.7
Coprinaceae	174	5.0	444	6.0	5	0.5
<i>Ganoderma</i>	249	7.2	641	8.7	0.8	0.1
Unidentified basidiospores	422	12.1	992	13.4	62	6.2
Other unidentified spores	294	8.5	563	7.6	125	12.4
<i>Hyphal fragments</i>	171	4.9	258	3.5	116	11.6

Culture plate surveys of fungi in outdoor air

Temperate climates

The limitations of trapping airborne inoculum on agar plates has already been mentioned, but it is worth considering the mistaken impressions of the air spora which may result from relying on sampling methods which are based on culture. plate sampling by a wind-orientated Andersen When comparing AVST and volumetric culture sampler, Burge *et al* (1977) noted that *Cladosporium* spore concentrations assessed with both methods varied directly. However, as AVST spore levels rose the culture plate data progressively underestimated prevailing concentrations, giving low estimates of prevalence (20–40%) at levels below 100 spores m⁻³ and falling to below 5% at levels above 500 spores m⁻³. Several additional taxa were also substantially understated in abundance and regularity. Sampling for culturable airborne fungi in Copenhagen using a slit sampler (Table 3) indicated a much higher percentage incidence of *Cladosporium* at 68.9% (Larsen 1981) and 77.8% (Larsen and Gravesen 1991) than was found using the AVST in Cardiff or the particle sampler in Ontario (Li and Kendrick 1995). Whereas it is possible to differentiate a much greater number of imperfect fungi in the air and to record a range of yeasts by culture-based methods, it is important to realise that the large numbers of ascospores and basidiospores in the air are not recorded on culture plates, and so sampling onto agar medium is likely to have biased the values for the percentage incidence of *Cladosporium* recorded by the Danish workers.

As mentioned above, culture-based sampling methods do enable a wider range of yeasts to be identified than can be differentiated by cell morphology after AVST. For example, when Rantio-Lehtimäki (1988) sampled the air specifically for yeasts in southern Finland over a one-year period using an Andersen sampler with yeast-peptone-D-glucose agar culture plates, she found that concentrations of yeasts in the air never exceeded 50 colony forming units (CFU) m⁻³ air. The predominant yeast genus isolated on the agar plates was *Sporobolomyces*, followed by *Rhodotorula* and *Cryptococcus*, all of which are basidiomycetous yeasts. Ascomycetous yeasts including *Candida* were caught infrequently.

Table 3. Principal viable fungi collected by slit sampler from outdoor air in Copenhagen, as percentage of total colonies on slit-sampler collection plates.

Type	1977–79 ^a	1978–87 ^b
<i>Cladosporium</i>	68.9	77.8
<i>Alternaria</i>	9.4	3.0
<i>Penicillium</i>	6.0	2.5
<i>Aspergillus</i>	2.5	0.8
Non-sporing filamentous fungi	6.3	n.s.
Yeasts	4.1	n.s.

^a Larsen (1981); ^b Larsen and Gravesen (1991)

Employing a slit sampler, Larsen (1981) found that yeasts comprised only 4.1% of the airspora in Copenhagen. The mirror yeasts *Sporobolomyces* and *Tilletiopsis* can be identified on AVST slides or tapes, and Gregory and Sreeramulu (1958) reported *Sporobolomyces* concentrations of up to 10^6 spores m^{-3} air over an estuary in the south of England; and routine monitoring of the air at Cardiff over the last 30 years has shown an average *Sporobolomyces* spore concentration of 167 m^{-3} air, with a maximum of 27297 m^{-3} , and an average for *Tilletiopsis* of 48 m^{-3} air, with a maximum of 7920 m^{-3} . This reinforces the view of Burge *et al.* (1977) that culture plate methods seriously underestimate the spore content of the atmosphere.

Tropical and subtropical climates

The air spora in tropical and subtropical regions is likely to be substantially different from that in temperate regions of the world. This is exemplified by a study of Oliveira *et al.* (1993), who used settle plates to study the air spora at five locations around the city of Natal in tropical Brazil over a one-year period. A similar incidence was observed at all of the sites, each of which was sampled for 15 min every two weeks. The lack of volumetric sampling did not allow estimations of spore concentrations, and the incidence of fungi is expressed as percentage frequency of fungal isolation (Table 4), but from this it is apparent that the relative incidence of fungi in Natal is quite different from that reported in Copenhagen (Larsen 1981, Larsen and Gravesen 1991). *Aspergillus* and *Penicillium* were isolated with a very high percentage frequency especially during the dry season and there was a dramatic increase in *Fusarium* during the rainy season.

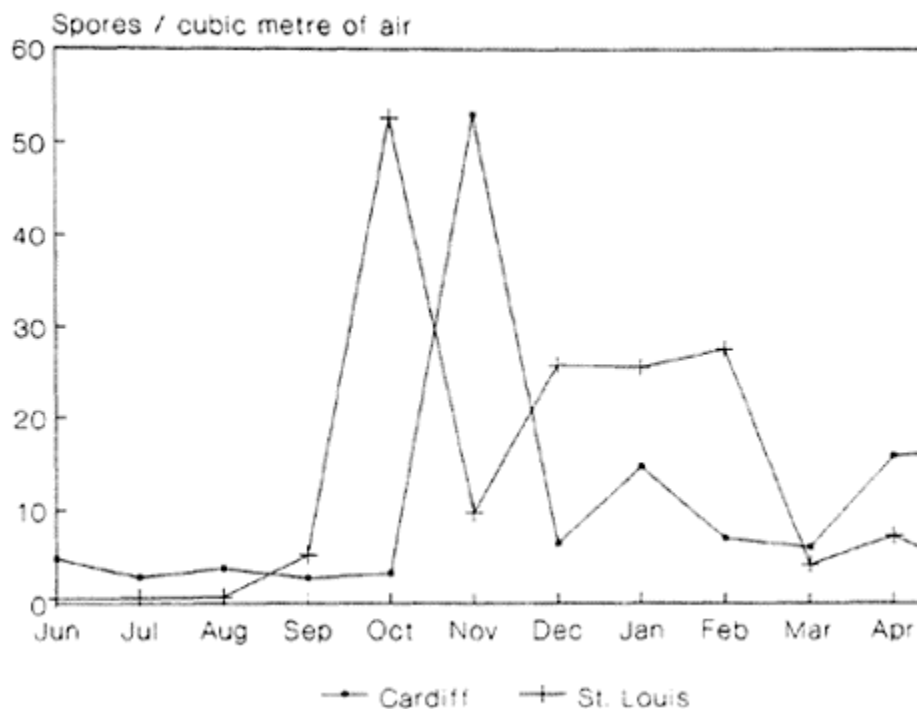


Fig. 12. Outdoor cultivable *Aspergillus fumigatus* concentrations in Cardiff and St. Louis (after Mullins, Hutcheson and Slavin 1984)

Table 4. Percentage frequency of viable airborne fungi collected during 1988 on settle plates exposed to outdoor-air in the city of Natal in tropical Brazil (after de Oliveira *et al.* 1993).

Type	Dry season	Rainy season
<i>ASPERGILLUS</i>	77.7	55.3
<i>Penicillium</i>	62.9	40.0
<i>Fusarium</i>	16.6	44.6
<i>Cladosporium</i>	25.9	10.7
<i>Curvularia</i>	11.1	20.0
<i>Rhizopus</i>	11.1	16.9
<i>Rhodotorula</i>	12.9	9.2
<i>Neurospora</i>	12.9	3.0

<i>Drechslera</i>	1.8	10.7
<i>Aureobasidium</i>	5.5	4.0
<i>Trichoderma</i>	-	6.1
<i>Cunninghamella</i>	1.8	3.0

In contrast, *Cladosporium*, which dominates the air spora in temperate regions had a comparatively low frequency, although this was not true for all cities in Brazil and the authors quote percentage frequencies from other cities where the incidence of *Cladosporium* was much greater, but overall the incidence of *Aspergillus* and *Penicillium* through-out the surveys quoted tends to be higher than that reported in temperate regions.

In subtropical Taiwan, Li and Hsu (1995) used an Andersen sampler with malt extract agar culture plates to compare spore concentrations within and outside the Taipei homes of atopic and nonatopic children between July and September (Table 5). The fungal spore concentrations recorded were comparatively low, with *Cladosporium* having a lower incidence than *Aspergillus* and *Penicillium*. Hurtado *et al.* (1989) sampled the air in the mildly tropical climate of Bogota in Colombia for pollen and spores for a year and found that *Cladosporium* was the most frequently recorded genus, with the combination *Penicillium/Aspergillus* being next. The findings in the tropical and subtropical surveys show a different pattern of fungal spore incidence, and spores of *Aspergillus* and *Penicillium* appear to be more prevalent in the air than in temperate regions of Europe and North America.

Although aspergilli and penicillia are generally less abundant in outdoor air in temperate regions, a range of species in both genera may be isolated by culture plate sampling for spores in the atmosphere (Hudson 1969, Fradkin *et al.* 1987). The opportunistic respiratory pathogen *Aspergillus fumigatus*, for example, may be detected. In a comparative study of *A. fumigatus* in the air of St Louis in USA and Cardiff in UK (Fig. 12) over a 12-month period using Andersen samplers, sampling onto Blakeslee's malt extract agar culture plates which were incubated at 37°C to eliminate other fungi, average concentrations

Table 5. Concentration of viable fungi (colony forming units) collected by Andersen N6 impaction sampler from outdoor air of homes of asthmatic, atopic and control children in Taiwan (after Li and Hsu 1995). Geometric mean concentration (CFU m⁻³ air)

Type	Asthmatic (n=46)	Atopic (n=20)	Control (n=26)
<i>Alternaria</i>	1.7	1.5	1.8
<i>Aspergillus</i>	17.2	22.5	23.6

<i>A. clavatus</i>	1.1	1.1	1.2
<i>A. flavus</i>	4.7	2.7	5.9
<i>A. fumigatus</i>	1.1	1.1	1.2
<i>A. niger</i>	4.7	6.7	5.9
<i>Cladosporium</i>	19.5	7.3	3.8
<i>Curvularia</i>	1.7	1.3	1.9
<i>Fusarium</i>	4.3	1.8	5.5
<i>Paecilomyces</i>	2.2	1.5	2.0
<i>Penicillium</i>	47.6	55.5	101.0
<i>Trichoderma</i>	1.1	1.1	1.2
Yeasts	70.3	73.8	72.5
Total	547	449	668

recorded were 13.5 CFU m⁻³ in St Louis and 11.3 CFU m⁻³ in Cardiff and the seasonal incidence of the fungus was similar at both sites (Mullins *et al.* 1984).

In order to sample for *A. fumigatus* in the air both culture media and incubation conditions were modified to favour growth of this fungus at the expense of any competing fungi. In particular, richer media, such as Blakeslee's malt extract agar, favour *Aspergillus* and *Penicillium*. Blakeslee's 1915 formula for the agar contains 2% malt extract, 2% glucose and 0.1% peptone, as opposed to the unamended 2% malt extract usually recommended for isolation of the general run of fungi from air. In all surveys of the air spora the sampling method chosen will favour sampling of certain fungi and it should be remembered, as mentioned previously, that the sampling techniques used can have a major influence on the results of the survey.

Owing to the important effect of weather conditions on spore release, daily spore counts can vary considerably; in some fungi spore discharge is associated with drying whereas in others damp conditions are required. Raindrops can discharge spores from puffballs, and Walkey and Harvey (1968a,b) observed that ascospore discharge in the Pyrenomycete group was stimulated by rainfall. Many spores are thrown up by rain splash, e.g. in *Chaetomium* (Harvey *et al.* 1969), so that certain spore types are characteristically associated with damp conditions and others are associated with dry conditions. The variability of conditions during the day can affect spore concentrations and it was found in UK that fungi such as the yeast *Sporobolomyces* tend to occur in high concentrations during the night when humidity is highest, whereas *Cladosporium* has its highest concentration in the day when humidity is at its lowest (Hirst 1953, Gregory and Sreeramulu 1958), as was also noted in Ontario by Li and Kendrick (1995). Even light can act as a contributory factor in spore release, and Ingold (1971) has described light and dark induced rhythms of spore discharge in a number of fungi.

The regular seasonal cycles of the year determine which fungal spores are likely to be available for release into the air, thereafter the development of the fungi and the

subsequent release of their spores will depend on weather conditions. The concentration will further be dependent on location and prevailing winds.

BACTERIA IN OUTDOOR AIR

Surveys of the air for bacteria are much less common than for fungi. The interest in airborne bacteria tends to be confined to the possible presence of pathogens, but even when sewage sludge is being applied to land (Pillai *et al.* 1996) bacterial pathogens are absent from the air. Kock *et al.* (1998) sampled at 7 sites around Graz in Austria for fungi and bacteria using an Andersen sampler and found that, at 327 CFU m^{-3} air, bacterial counts in an agricultural area were four times higher than in a suburban residential area. They were also higher by 29% in the vicinity of a composting facility, but at an industrial and business site affected by heavy traffic, the bacterial counts were 146 CFU m^{-3} , twice the level found at the composting facility.

Lighthart & long (1998) sampled in midsummer for total and culturable bacteria in the outdoor air of the mid-Willamette River Valley in Oregon using a wet cyclone bioaerosol sampler. Total bacteria varied between 5.9×10^5 and 8.8×10^2 cells m^{-3} air, and culturable cells between 1.3×10^4 and 3.1 CFU m^{-3} . They noted a diurnal periodicity of bacteria, with highest concentrations during the day and lowest concentrations at dawn and dusk. The percentage of the total, which was culturable, was maximal at midday (39%) and lowest in the early morning and late evening (0.5–2%). When sampling the dust plume downwind of a grain seed combine, however, they recorded that 73% of a total concentration of 2.9×10^6 cells m^{-3} air were culturable.

Clearly, these findings show that the differences in results which are associated with employment of different sampling techniques for fungi apply equally to sampling for bacteria in air. They also confirm the conclusions of Miquel (1899) from his sampling in Paris, that the main source of outdoor bacteria is the surface of the ground.

CONCLUSION

Hyde (1969) characterized the air spora as being an “expression of climate, a reflex of the vegetation as a whole, and an essential factor or complex of factors in the general environment”. As this chapter has shown, such essential factors include weather, prevailing winds, the local vegetation, topography and human activity.

What we learn about the outdoor air spora depends very much on sampling techniques, which have a major influence on the results obtained. The use of different techniques in different surveys can make comparisons difficult. There is, as yet, and there may never be, a sampling technique to satisfy all the requirements of aerobiologists, and the sampling technique chosen will depend on resources, the nature and number of sites to be sampled, the facilities available and the information which is sought from the survey. It continues to be necessary to have regard to the sampling technique when considering the results from any survey, and to be cautious when making comparisons between surveys.

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Chapter 1.2

MICROORGANISMS IN INDOOR AIR

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INTRODUCTION

Bioaerosols in indoor environments comprise a range of microscopic biological particles of animal, plant and microbial origin which may be inhaled and impinge on human health. Allergenic animal particulates include dander and dried saliva of pets, such as cats and dogs, and other furred animals; fragments of insects such as cockroaches, fleas and clothes moths; fragmented bodies and faeces of house dust and storage mites; and amoebae and other unicells belonging to the Protozoa. Plant particulates include minute fragments and pollen grains of higher plants; spores of lower plants such as ferns and mosses; and both unicellular algae and fragments of multicellular filaments. Although protozoans and unicellular algae are microorganisms, the most medically important microbial particulates in indoor air belong to neither of these groups, but are the cells and spores of fungi and bacteria, and viruses. In this chapter, the different categories of microorganism (including algae and Protozoa) present in indoor air are described in general terms. Because of the diversity of forms, fungi are dealt with in more detail than the other categories. Where the health implications of individual groups are not dealt with in detail in later chapters, they are discussed briefly here. Since pollen grains resemble spores in many respects, the opening section of the chapter is a brief discussion of pollen in indoor air.

COMPONENTS OF BIOAEROSOLS IN INDOOR ENVIRONMENTS

Pollen

Pollen in indoor air

As mentioned in the first chapter, grass and tree pollens are well-known causes of hay-fever and claim many victims as a result of high species-specific airborne concentrations at different times of the growing season. It is patent that workers in commercial greenhouses producing, for example, tomatoes may be affected by high concentrations of pollen, as well as spores of the mould *Botrytis cinerea* growing on senescent and dead leaves and soil debris. While allergic responses to the pollen of ornamental plants such as sunflowers, goldenrod and chrysanthemums has been noted in about one-quarter of the

general atopic population, among commercial flower growers the incidence is more than 50% (Goldberg *et al.* 1998). In homes, offices and other indoor environments we can also be exposed to pollens, but the pollen of house plants and cut flowers, which are mostly insect-pollinated, tends not to become aerosolized to the same extent as that of wind-pollinated grasses and trees outdoors. However, pollen originating outdoors infiltrates buildings. Even if intact pollen grains do not infiltrate, sub-microscopic particles present in quantity in outdoor dusts after the pollen season (Pehkonen *et al.*, 1993) may enter in substantial amounts, especially if windows are opened periodically (Holmquist and Vesterberg, 1999). During the pollen season indoor concentrations of pollen antigens in naturally ventilated buildings may be 40–60% of contemporaneous outdoor concentrations (D'Amato *et al.* 1996), and occasionally about as high (Kvernes *et al.* 1999). Keeping windows and doors closed (Adams and Hyde 1965), fitting of filters (Unger and Myers 1961) or air conditioning (Spiegelman *et al.* 1963) can however reduce the airborne load to at most a few percent of that outdoors.

Pollen in house dust

Since pollen grains and antigenic particles released from them may relatively rapidly sediment out of the air, both outdoor and indoor dusts become reservoirs of pollen allergens. This material can become resuspended as a result of physical disturbance and so be inhaled. For example, Fernandez-Caldas *et al.* (1986) peaks of airborne red oak pollen allergens out of the pollination season and concluded that this was due to reentrainment of allergens adsorbed to soil particles when wind direction and velocity changed. However, Platts-Mills *et al.* (1987) reported that in Virginia concentrations of ryegrass (*Lolium*) pollen allergen in house dust which had increased rapidly during the grass flowering season in May and June then fell back in July to levels similar to those before. They ascribed this to the efficacy of cleaning in removing pollen that had settled on furniture, but birch (*Betula*) and timothy grass (*Phleum pratense*) antigens have been found in sporadic, low concentrations in settled dust in Norwegian schools during both the growing season and also in December-January (Dybendal *et al.* 1989a,b). Kvernes *et al.* (1999) also found that three months after the birch pollen season there were birch pollen allergens in dust samples taken in a Swedish pre-school, although there were none in corresponding samples of indoor air.

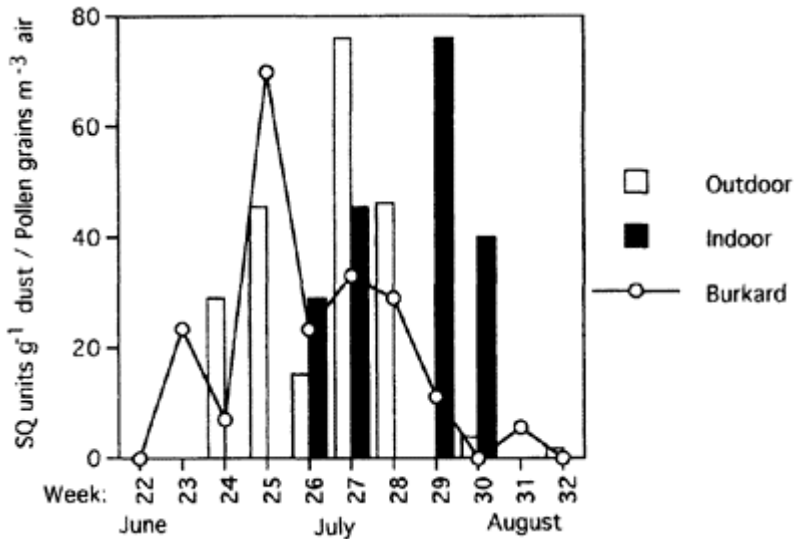


Fig. 1. Mean allergenic activity (SQ units g⁻¹ dust) of grass pollen in settled dust collected outdoors and from within five urban homes, together with weekly Burkard sampler airborne pollen counts, during the 1995 summer in Turku, Finland (after Yli-Panula 1997).

In Finland, the grass pollen allergenicity of settled domestic dust (Yli-Panula 1997) was found to be low in comparison with the corresponding birch pollen allergenicity (Yli-Panula and Rantio-Lehtimäki 1995). The sporadic peaks of allergenic activity of grass pollen in indoor dust followed those of outdoor dust (which in turn correlated with airborne pollen counts outdoors) with a lag of 1–5 weeks (Fig. 1). Since it has been reported that grass pollen maintains its antigenic activity throughout and after the pollen season (Yli-Panula 1997), it has been considered that pollen and released particles in outdoor dust carried in by people and pets (Yli-Panula 1998) or blown in over extended periods may be the main source of the allergenicity of settled dust in homes and other buildings (Yli-Panula and Rantio-Lehtimäki 1995). There may be sufficient grass pollen allergen present in house dust to cause allergic symptoms after the outdoor pollen period is over (Yli-Panula 1998). A recent study of Japanese cedar (*Cryptomeria japonica*) pollen has indicated that, after airborne pollen has disappeared and the main allergen (Cry j 1) is no longer detectable in outdoor dust exposed to rain, Cry j 1 can still be present in house dust and allergic symptoms may persist (Takahashi *et al.* 1998).

Algae

Nature of algae in indoor air and house dust

Although it has been shown that they can behave as inhalant allergens and cause rhinitis and asthma, microalgae comprise a largely ignored category of microorganism in indoor

environments. These organisms contain chlorophyll and are therefore photosynthetic, unlike the heterotrophic fungi and bacteria to be considered later. The types most often isolated are green (the plant division Chlorophyta) and blue-green algae (now recognized as having stronger affinities with bacteria and therefore classified as Cyanobacteria), and diatoms (Chrysophyta). They may take the form of unicells, simple chains of cells, cylindrical filaments, or colonies (composed of up to 50,000 unicells organized into a coherent structures). Some are macroscopic membranous or tubular structures composed of very large numbers of cells. Unlike the other types, the unicellular diatoms are enclosed by a two-piece wall of crystalline silica, which has been likened to a Petri dish.

Although they may be generated indoors, the most usual sources of algae in indoor environments are unicells or fragments of filaments in air infiltrating from outside and dust blown or carried in on clothing or animal fur. These algae normally grow in water or in soil, and on rocks and the bark of trees. They are aerosolized by wind, mechanical disturbance of soil or dust, bubble phenomena or forced aeration of aqueous environments ranging from aquaria to sewage disposal plants. The numbers in air are not likely to exceed those of pollen grains or fungal spores (Tiberg 1987). In United States, the most prominent types detected by cultural methods in house dust collected during the indoor heating season in homes were the unicellular green algae *Chlorella*, *Chlorococcum*, *Chlamydomonas* and *Plankosphaeria* and the filamentous Cyanobacteria *Anabaena* and *Schizothrix* (Bernstein and Safferman 1970). In a more recent study in Sweden (Tiberg *et al.* 1984), 88% of dust samples collected from carpets and beds in the homes of atopies contained viable algae. The genera found were mostly the same as those found outdoors, but with Cyanobacteria being found in slightly higher frequency. The most frequent types were the unicellular green algae *Chlorococcum*, *Chlorella* and *Stichococcus* and the Cyanobacteria *Nostoc*, *Phormidium* and *Anabaena*. The same types dominated in dust from a health care centre, a recreation centre and several nursery schools. In the nursery schools, the diatoms *Hantzschia* and *Navicula* were occasionally isolated. Although counts of green algae in these schools peaked in June, the cyanobacteria showed no marked seasonal differences.

Health effects of algae

Since the allergenic properties of algae are discussed in Chapter 3.1, it is sufficient to note here that it is more than 30 years since it was first reported that they were a cause of respiratory allergy in children and that in skin tests they reacted positively in patients with histories of inhalant allergies (McElhenny *et al.* 1962; McGovern *et al.* 1966; Bernstein and Safferman 1967). It is now recognized that, in addition to members of the Chlorophyta such as *Chlorella* and *Chlorococcum*, Cyanobacteria such as *Anabaena* are allergenic (Tiberg *et al.* 1984; Tiberg 1987).

Although there appear to be no reports of algae in air or house dust causing dermatitis, a case of contact dermatitis has been reported in a swimmer exposed to *Anabaena*, one of a number of species forming so called "algal blooms" in freshwater lakes (Cohen and Reif 1953). There are also rare human cases of cutaneous or disseminated infections by unicellular *Prototheca*. For example, a case of disseminated infection in a male with a specific deficiency in cell-mediated immunity by achlorophyllous *P. wickerhami* was

reported from New Zealand (Cox *et al.* 1974). However, *Prototheca* is generally found in soil and it is thought that soil contact is the major cause of infection.

Protozoa

Nature of Protozoa in indoor environments

Motile free-living members of this group of unicellular animals are found scavenging on organic debris in soil and water outdoors, and may also be found indoors in bodies of water such as aquaria, humidifier reservoirs and HVAC drainage pans (Schlichtung 1969), and also in plumb-ing systems (Steinert *et al.* 1997). Although some have cilia and others flagella which propel them through water, most of the free-living protozoans that impinge on human health are amoeboid, moving and engulfing nutrient particles by means of protoplasmic extensions, pseudopodia. They become aerosolized by mechanisms ranging from scouring of drying soil by wind to forced aeration of aquatic environments such as sewage ponds or aquarium tanks and dispersion of water droplets by humidifiers. The types of protozoan which have been isolated from air in a variety of investigations have been collated by Schlichtung (1969), and a recent paper (Rohr *et al.* 1998) indicates the types that may be present in indoor environments. Amoebae in hospital hot water systems were identified as *Hartmannella vermiformis* and species of *Echinamoeba*, *Saccamoeba* and *Valkampfia*, and species of *Acanthamoeba*, *Hartmannella*, *Naegleria*, *Valkampfia* and *Vanella* were isolated from moist areas in bathrooms and showers, sink drains and water taps (Rohr *et al.* 1998). *Hartmannella castellani* has been isolated from the air of a respiratory care unit (Kingston and Warhurst 1969).

Health effects of Protozoa in indoor environments

As far as health is concerned, the best-known protozoans affecting human health are those parasites that are spread by insect-bite, causing diseases such as malaria, sleeping sickness, Chaga's disease and leishmaniasis. However, there are free-living protozoans that may occasionally cause disease. The widespread species *Naegleria fowleri* is occasionally pathogenic, causing a fatal meningoencephalitis. It is an amoeboflagellate, existing in flagellate form in water and as an amoeba in human tissues. In nature, it also forms cysts, which are protected from adverse environmental conditions by a thick wall. Species of *Acanthamoeba* are also widespread and may cause encephalitis, most particularly in immunocompromised individuals. They also cause ulceration of the skin and cornea (Baron *et al.* 1994). The cysts of these amoebae are readily dispersed by air currents, contaminating aqueous environments ranging from brackish water to hot tubs, and also soil and dust. It seems probable that infection with *N. fowleri* is most often the result of contaminated water entering the nose, for example during swimming, but it is considered that in the case of acanthamoebae inhalation may be responsible as well as direct contact with contaminated waters or soil. (Rohr *et al.* 1998) found six potentially pathogenic strains of *Acanthamoeba*, but none of *Naegleria*, colonizing shower heads, drains, walls and tiles in bathrooms. They postulated that infection might occur by inhalation of aerosolized *Acanthamoeba* in sprayed water.

Members of the Protozoa growing within buildings have also been implicated in

humidifier fever. Edwards *et al.* (1976) isolated both amoebae and ciliates from a contaminated humidifier serving an office in which a number of workers suffered this febrile disease. Extracts of the amoebae, but not the ciliates, reacted with the sera of affected workers, and antigenic identity between extracts of the amoebae, dust on a suspended ceiling and a culture-collection strain of *Naegleria gruberi* was demonstrated. Protozoa such as *Acanthamoeba*, *Hartmannella* and *Naegleria* have been isolated from plumbing systems contaminated with bacteria in the genus *Legionella* (Steinert *et al.* 1997) and the presence of the bacterium in amoebae such as *A. polyphaga* has been demonstrated (Newsome *et al.* 1998). Some 13 amoebae and two ciliates have been shown to provide an environment in which legionellae can replicate prolifically (Kwaik *et al.* 1998), and it has recently been shown that two species of *Acanthamoeba* can expel vesicles between 2.1 and 6.4 μm in diameter containing living *L. pneumophila* (Berk *et al.* 1998). Aerosolization of such vesicles, which are of respirable size, may be a means of transmission of this agent of legionellosis. Another bacterium for which *Acanthamoeba* can act as environmental host is *Mycobacterium avium*, a pathogen which like *L. pneumophila* is widespread in aquatic environments, including municipal drinking water. However, *M. avium*, a primary health threat to AIDS patients, is found in the cyst wall of the amoeba rather than in vesicles (Steinert *et al.* 1998).

Table 1. Abbreviated classification of some fungi found as spores in indoor air.

<i>Phylum</i>	<i>Class</i>	<i>Genera</i>
Ascomycota		<i>Emericella</i> , <i>Eurotium</i> , <i>Peziza</i> , <i>Saccharomyces</i>
Basidiomycota	Basidiomycetes	<i>Coprinus</i> , <i>Ganoderma</i> , <i>Sistotrema</i> , <i>Serpula</i>
Zygomycota	Zygomycetes	<i>Absidia</i> , <i>Cunninghamella</i> , <i>Mucor</i> , <i>Rhizopus</i>
Deuteromycota	Hyphomycetes	<i>Alternaria</i> , <i>Botrytis</i> , <i>Epicoccum</i> , <i>Stachybotrys</i>
	Coelomycetes	<i>Phoma</i>
	Agonomycetes	Sterile (non-sporing) fungi

Filamentous fungi

Structure and classification of filamentous fungi

Like those in outdoor air, the fungi, which are found in indoor air as spores and cells, fall into basic two categories, filamentous fungi and yeasts. Yeasts are regarded conventionally as unicells and are dealt with in the next section of this chapter. The typical filamentous fungus is regarded as developing from a germinating spore, which extends into a **germ tube**. As a result of continued apical growth this develops into a roughly tubular **hypha**, which branches as it grows, forming an extending network of hyphae known as a **mycelium**.

The hyphae are incompletely divided by septa into communicating compartments (the

equivalent of cells) along their entire length, giving septate mycelium. In the case of Zygomycetes (Table 1), however, the mycelium is described as non-septate, although occasional septa occur. These are in older areas of mycelium and where reproductive structures are formed. Mycelium of the Basidiomycetes is characterized by clamp connections at the septa which give the hyphae a nodular appearance absent in other septate fungi.

An extremely important fundamental characteristic of fungi is that they produce dispersible spores. In **microfungi**, including those frequently referred to as moulds, a microscope is needed to examine the spores and the specialized structures on or in which they are produced and thus enable the species to be identified. In other fungi, there may arise from a vast mycelium aggregations of hyphae organized into complex macroscopic sporing structures. Because fungi were originally regarded as plants and were described and classified by botanists, these macroscopic structures tend still to be known as fruiting bodies. They include the cups, mushrooms and brackets (or conks) of species that are referred to as **macrofungi**.

The greatest number of filamentous fungi found indoors are allocated to an artificial grouping of species for which no sexual reproductive phase has been found, but there are in indoor air also spores of a much more limited number of species which can be assigned to one or other of three phyla with a sexual phase (Table 1). The group of fungi lacking sexual (or perfect) stages have been called the Deuteromycota (Deuteromycotina, Deuteromycetes), but in the latest edition of Ainsworth and Bisby's Dictionary of the Fungi (Hawksworth *et al.* 1995) this taxonomic category is not considered acceptable, since the species in it have not evolved from a common ancestral group. Nevertheless, currently this group is usually referred to as the Deuteromycetes, and indeed sometimes by the earlier term Fungi Imperfecti (fungi lacking the perfect or sexual stage). Hawksworth *et al.* (1995) use the term "mitosporic fungi" instead of Deuteromycota for these fungi because the spore here is a mitospore, its nuclei being the product of the type of nuclear division associated with vegetative growth and asexual reproduction, i.e. mitosis, and not of the reduction division (meiosis) which is part of sexual reproduction. However, the spore is most often called a **conidium**, and the structure bearing conidia as a **conidiophore**. The wide range of morphological variation in conidia and conidiophores which enables different Deuteromycetes isolated from the air or from surfaces in buildings to be identified is fully evident from the illustrated descriptions in Chapter 5. They are classed according to whether the conidiophores are naked (Hyphomycetes) or are enclosed in sporing bodies (Coelomycetes). There are also some well-characterized fungi which have never been found to produce spores, and these sterile fungi (or Mycelia Sterilia as they were formerly known) are allocated to the Agonomycetes.

As mentioned, filamentous fungi in the other three relevant phyla (Table 1)—usually referred to as Ascomycetes, Basidiomycetes and Zygomycetes—are recognizable by the characteristics of their sexual phase, or **teleomorph**. They may also have an asexual phase, or **anamorph**, in which they produce mitospores. Because the anamorph may be more frequently encountered the anamorphic name may be more widely used than that of the teleomorph, e.g. in the two Ascomycetes mentioned later in this paragraph. It is considered that most members of the Deuteromycetes are either fungi which in the

process of evolution have lost their teleomorphic phase, but they might be anamorphs for which no teleomorph has yet been found. As is shown in more detail in Chapter 4.2, the sexual phase of members of the Ascomycetes, Basidiomycetes and Zygomycetes results in the production of, respectively, ascospores, basidiospores and zygospores. Ascospores and basidiospores are dispersed by air currents, but zygospores are larger and their function is regarded as being survival rather than dispersal. Under suitable conditions, a zygospore will germinate to produce an anamorphic structure, the sporangium, which contains sporangiospores that are dispersed. Although the reproductive structures in Ascomycetes such as *Emmericella nidulans* (anamorph: *Aspergillus nidulans*) or *Eurotium herbariorum* (anamorph: *Aspergillus glaucus*) are microscopic, the teleomorphs of many others are characterized by complex macroscopic fruiting bodies, or ascocarps, e.g. the cup fungi in the genus *Peziza*. Likewise, although the teleomorphs of Basidiomycetes like *Sistotrema brinkmannii* are microscopic, those of the dry rot fungus *Serpula lacrymans* indoors and of the beech bracket *Ganoderma applanatum* outdoors are conspicuously macroscopic basidiocarps.

Outdoor air as a source of filamentous fungi in the indoor air spora

The wide diversity of fungal spores in outdoor air between early summer and autumn, when vegetative growth and sporulation of fungi are at their greatest, has been mentioned in Chapter 1.1. The outdoor air is for indoor air an important source of living spores and hyphal fragments which behave as **propagules** from which mycelium can develop. These propagules infiltrate naturally ventilated buildings and strongly influence the indoor fungal burden both qualitatively and quantitatively (Sneller and Roby 1979). Factors such as the proximity of a building to organic debris which supports fungal growth and spore production, and shade which enhances survival, lead to increased numbers of airborne propagules penetrating buildings (Kozak *et al.* 1979). Indoor levels of mould propagules broadly parallel those outdoors during the summer, but as with pollen there is a time lag before counts within buildings reflect peak outdoor concentrations, and again the counts are usually lower than outdoors (Ackermann *et al.* 1969; Sneller and Roby 1979). In Arctic and temperate climates, of course, outdoor fungal growth and sporulation is minimal in winter because of the lower temperature. Consequently, in winter counts of spores indoors are normally higher than those outdoors, particularly when there is snow cover (Reponen *et al.* 1992) which precludes re-entrainment of settled spores on outdoor surfaces and in dust and soil.

Naturally, the degree and type of ventilation employed greatly affects the air spora in buildings. An investigation in UK (Adams and Hyde 1965) found that, as with pollen, simply closing the windows and doors of a room in a naturally ventilated home during summer could exclude 98% or more of the spores present in outdoor air. Provided that it is not circumvented by opening doors and windows, central air-conditioning reduces spore counts in houses by 50% or more (Spiegelman *et al.* 1963). Window airconditioning units can reduce numbers of *Alternaria* and *Ganoderma* spores, and also pollen grains, to around 5% of the outdoor levels (Solomon *et al.* 1980). Kozak *et al.* (1979) reported that central electrostatic filtration could reduce indoor air concentrations of total propagules to 3%, and *Cladosporium* to less than 1%, of those experienced

without such filtration (Kozak *et al.* 1979).

Table 2 Abundance of principal spore types in indoor and outdoor air of 15 homes in Ontario, presented as mean concentration for 132 sampling days over 22 months and percentage of total air spora (after Li and Kendrick 1995).

	Indoor		Outdoor	
	No. m ⁻³ air	%	No. m ⁻³ air	%
<i>Alternaria</i>	44	1.9	74	2.1
<i>Aspergillus/Penicillium</i>	457	19.8	131	3.8
<i>Cladosporium</i>	895	38.8	1479	42.5
<i>Coprinus</i> (basidiospores)	41	1.8	78	2.3
<i>Epicoccum</i>	7	0.3	20	0.6
<i>Ganoderma</i> basidiospores)	59	2.6	111	3.2
<i>Leptosphaeria</i> (ascospores)	182	7.9	547	15.7
Unidentified ascospores	65	2.8	138	4.0
Unidentified basidiospores	152	6.5	310	8.9
Other unidentified spores	206	8.9	301	8.7
Hyphal fragments	146	6.3	112	3.2

The lower numbers of airborne spores indoors relative to outdoor air in summer is, with some exceptions, well illustrated in the results of a recent Canadian study (Table 2) where spores were collected on coated glass slides and counted under a microscope (Li and Kendrick 1995). It should be said here that Table 2 also shows that air contains spores of types which do not appear in counts obtained by more frequently employed methods involving culture of trapped fungi, so-called viable sampling methods. Although there are large discrepancies which will be discussed later (see Chapter 4.1), counts of the phylloplane fungi *Alternaria*, *Cladosporium* and *Epicoccum* (fungi growing widely in nature on leaves and other plant surfaces) can be made by either method. However, numbers of basidiospores of the macrofungi *Coprinus* and *Ganoderma* (although in theory they could be) and the ascospores of *Leptosphaeria* (an important cause of late summer asthma) in indoor air are not enumerated by viable sampling methods. More detailed analysis of the Canadian data (Li and Kendrick 1996) showed that from May to October the relationships between numbers of airborne spores and hyphal fragments, and the diversity of fungi, indoors and outdoors were very strong (especially so for *Alternaria* and *Leptosphaeria*), probably because of windows being opened during the summer months. Path analysis indicated that, as might have been expected, *Alternaria*, *Leptosphaeria*, unidentified ascospores, *Coprinus* and *Ganoderma* came mainly from outdoor sources.

Effect of growth of filamentous fungi indoors on the air spora

As indoor temperatures are usually favourable for fungi, growth and sporulation of fungal contaminants are likely to occur in any damp areas in buildings and consequently modify the relationship between the indoor and outdoor aeromycota (see Chapter 2.1). The major exceptions to the tendency for indoor counts in summer to be lower than the corresponding outdoor counts are *Aspergillus* and *Penicillium*. The failure of Li and Kendrick (1996) to detect any functional or causal relationship, winter or summer, between *Aspergillus*/*Penicillium* con id in indoor and outdoor air is in line with the generally held belief that the conidia of fungi in these two genera are primarily of endogenous origin. Path analysis also suggests indoor sources of *Cladosporium*, *Epicoccum*, unidentified basidiospores and "other unidentified spores" (Table 2), although they also have outdoor origins (Li and Kendrick 1996). Along with *Penicillium*, *Cladosporium* is usually one of the commonest genus isolated from mould patches in damp homes (Grant *et al.* 1989).

The category *Aspergillus*/*Penicillium* was used by Li and Kendrick (1996) because the spores of only a very few species in these two large genera are sufficiently distinctive for them to be recognized in a field sample under the microscope, and even then only by an extremely skilled mycologist. Although the quantitative data indicate the relative abundance of aspergilli and penicillia (taken together), it must be realized that different species of *Aspergillus* and *Penicillium* are quite different in their physiology, ecology and significance for health. It is therefore important that in investigating indoor environments species should be accurately identified, and that involves culture of the fungi isolated. An example of diverse species of *Aspergillus* and *Penicillium* that may be encountered in indoor air can be seen in Table 3, which presents the results of two studies carried out in the same city using different samplers and different agar isolation media.

Table 3. Species of *Aspergillus* and *Penicillium* reported present as airborne viable particles in homes of asthmatic children and adults in Mexico City (Garcia *et al.* 1995; Rosas *et al.* 1997).

Category	Frequency	Percentage of samples)
	Adults (n=30)†	Children (n=8)*
<i>Aspergillus</i> spp.	32	48
<i>A. Candidus</i>	3	-
<i>A. f lavus</i>	9	10 ^a
<i>A. fumigatus</i>	3	-
<i>A. glaucus</i> (<i>Eurotium herbariorum</i>)	1	63
<i>A. melleus</i>	-	27 ^b
<i>A. niger</i>	10	16

<i>A. ochraceus</i>	6	-
<i>A. parasiticus</i>	2	-
<i>A. versicolor</i>	4	56 ^c
<i>A. wentii</i>	1	-
<i>Penicillium</i> spp.	97	100
<i>P. aurantiogriseum</i>	58	88
<i>P. brevicompactum</i>	13	-
<i>P. chrysogenum</i>	15	79
<i>P. citrinum</i>	9	-
<i>P. crustosum</i>	1	1
<i>P. griseofulvum</i>	4	-
<i>P. janthinellum</i>	2	-
<i>P. mineoluteum</i>	-	2
<i>P. oxalicum</i>	1	-
<i>P. purpurogenum</i>	3	7
<i>P. spinulosum</i>	-	41
<i>P. verrucosum</i>	2	-
<i>P. viridicatum</i>	20	-

* Burkard personal sampler, malt extract agar; † Andersen 2-stage sampler, dichloran-18% glycerol agar; Not reported; ^aPlus *A. sydowi*; ^bPlus *A. flavo-furcatis*; ^cPlus *A. petrakii*

It is important to realize, however, that not all species contributing to total counts of *Aspergillus* + *Penicillium* obtained in investigations such as that of Li and Kendrick (1995), or the collective viable counts in many other published investigations, may actually be more abundant indoors. Fradkin *et al.* (1987) found that, whilst the collective viable counts of penicillia indoors were twice those outdoors, the very common species *Penicillium chrysogenum* was more abundant outdoors. This study also found that some *Cladosporium* spp. were less abundant indoors during the summer, but the concentrations of others were double those outdoors, again pointing to the fundamental importance of species identification in aerobiological studies.

Effect of human behaviour and activity on the indoor air spora

Spores and hyphal fragments that have infiltrated buildings by the airborne route are added to those brought in on contaminated clothes and fur by humans and their animal pets. Li and Kendrick (1996) suggested that firewood and fuel wood chips, vegetables and clothes and tools brought indoors after garden work were possible sources of *Epicoccum*.

Unwashed root vegetables and birch firewood were found to be abundant sources of

Cladosporium and *Penicillium* spores contaminating indoor air (Lehtonen *et al.* 1993) and fuel wood chips have been reported as a potential source of spores of the toxigenic fungus *Trichoderma* (Miller *et al.* 1982). Pasanen *et al.* (1989) presented circumstantial evidence for carriage on clothes of *Acremonium*, *Alternaria*, *Botrytis* and *Chrysosporium* spores from cow sheds into farm houses during the Finnish winter, and (Lehtonen *et al.* 1993) noted transport of *Aspergillus* on riding clothes. By whatever route they enter, or whatever their indoor source, airborne propagules are rapidly disseminated throughout naturally ventilated buildings by air currents (Christensen 1950), although the closer to an endogenous source the higher are airborne counts likely to be (Hunter *et al.* 1988).

Since house dust contains large numbers of microorganisms, one can expect that indoor activities that raise dust will also affect the indoor air spora. This was demonstrated more than a century ago in a study of houses and schools in a Scottish mill town, Dundee (Carnelley *et al.* 1887). Comparison of corresponding male and female classes showed that under normal conditions “boys tend to make the air of a room more impure than girls do” because they “are more restless, and so raise more dust, which necessarily contains micro-organisms”. When Carnelley *et al.* (1887) subsequently got a class of boys to stamp on their classroom floor, the total number of microorganisms in the air increased 15-fold! This paper, which incidentally made observations on antibiosis that pre-date Fleming’s serendipitous discovery by half a century, should be prescribed reading for anyone carrying out surveys on indoor air. In more recent times, various investigators have shown that constructional work (Maunsell 1952; Hunter *et al.* 1988), floor sweeping ((Lehtonen *et al.* 1993), cleaning carpets using a vacuum cleaner lacking an exhaust filter (Hunter *et al.* 1988) and changing bed-clothes (Lehtonen *et al.* 1993) all temporarily elevate airborne mould counts. Variations in the degree and type of activity of adults, children and pets in occupied buildings cause temporal and spatial differences in the air spora that are reflected in large differences between counts of bacteria and fungi in indoor air, even when counts are taken within minutes of each other (Hunter *et al.* 1988; Verhoeff *et al.* 1990; Mouilleseaux and Squinazi 1991).

Health effects of filamentous fungi in indoor environments

The health impact of filamentous fungi, as sources of allergens, mycotoxins and β -glucans, and as pathogens is dealt with in Chapter 3.1–3.3 and Chapter 3.6 of this book.

Yeasts

Nature of yeasts in indoor environments

Yeasts are usually defined as unicellular fungi which reproduce vegetatively by budding, i.e. producing buds which develop into daughter cells which either separate from the mother cell or remain adherent so that clusters or branched chains of cells are formed. In some genera (*Sterigmatomyces* and *Fellomyces*), the buds are produced on short stalks. Not all yeasts bud, however. In *Schizosaccharomyces*, reproduction is by fission (one or more cross-walls split the cell into segments which split off and develop independently). In some budding yeasts, such as *Candida*, under a range of growth conditions the budded cells are elongated and remain attached in chains, forming pseudohyphae. In *Geotrichum*

and *Trichosporon*, there are true hyphae which split up into loosely joined chains of cylindrical cells.

As well as reproducing vegetatively, some yeasts reproduce sexually, forming ascospores, and are therefore classified as Ascomycetes, e.g. *Saccharomyces*. Other sexually reproducing yeasts are Basidiomycetes. One such is *Rhodosporidium sphaerocarpon*, best known because of its anamorphic state *Rhodotorula glutinis*. *R. sphaerocarpon* produces teliospores from which basidiospores are derived. The genera *Sporobolomyces* and *Bullera*, which multiply vegetatively by budding, also produce spores which are forcibly discharged (ballistospores). These two genera are also classified as Basidiomycetes; teleomorphs have been found for some species of *Sporobolomyces*.

Although yeasts are frequently mentioned in reports of investigations of the indoor air spora, they are seldom identified even to generic level. The reason for this is that identification of individual species, and even genera, requires both experience and skill and is extremely time-consuming, involving morphological examination and a battery of physiological or biochemical tests (See Chapter 4.5). Consequently, many reports present only total viable counts, sometimes subdividing them according to colony colour into "pink" and "white" yeasts. Mention of pink or reddish yeasts as *Sporobolomyces* or *Rhodotorula* in reports should always be treated with caution, unless full identification procedures have been followed.

A detailed examination of three urban apartments and a rural farm-house in Finland did, however, revealed that members of the basidiomycetous genera *Cryptococcus*, *Rhodotorula* and *Sporobolomyces* were the most numerous airborne yeasts indoors (Rantio-Lehtimäki 1988). Both indoors and outdoors, other yeasts (mostly *Debaryomyces hansenii*, *Williopsis californica* and *Wingea robertsii*) only accounted for around 2% of the total trapped on Andersen sampler plates. As with outdoor air, numbers of yeasts were greater in the autumn than at other times of the year. *S. roseus*, *S. holsaticus* and *S. salmonicolor* were found indoors during autumn, and small numbers of *S. hispanicus* were noted. Cryptococci appeared to be more abundant in indoor air than outdoors (or similar in the case of the farmhouse) and showed little seasonal variation, with *C. albidus* and *C. laurentii* as the most frequent species. *Rhodotorula* spp. were also more frequently isolated from indoor air, with *R. graminis* and *R. glutinis* being isolated in summer and *R. pilimanae* in late autumn. Elsewhere, Mouilleseaux and Squinazi (1994) found that airborne yeasts were primarily unspecified *Rhodotorula* and *Torulopsis*, although *Candida* also appeared occasionally, and Solomon (1974) reported that in USA *Sporobolomyces roseus* and *Rhodotorula* spp., and also *Geotrichum candidum*, were abundant in air humidified by a cold-mist vaporizer.

Various authors have reported that yeasts are abundant in house dust, e.g. Flannigan *et al.* (1993) and Verhoeff *et al.* (1994), and the predominant species found in dust from both mattresses and floors have been *R. glutinis*, *R. minuta*, *R. mucilaginosa*, *Cryptococcus albidus* and *C. laurentii* (Hoekstra *et al.* 1994).

Health effects of yeasts in indoor environments

In general, it appears that very few of the yeasts isolated in homes or non-industrial work

places are either fermentative or ascomycetous (Rantio-Lehtimäki 1988), but in work environments such as bakeries, breweries and distilleries, the fermentative Ascomycete *Saccharomyces cerevisiae* (baker's or brewer's yeast) may be detected in the air spora together with cereal-borne fungi. The enolase of this species is a major allergenic component, and a wider range of patients than bakery workers with inhalant allergies to fungi show sensitivity to this enzyme (Baldo and Baker 1988), which cross reacts with that from *Candida albicans*. Cross reactivity between allergens from different yeast genera has also been reported by Koivikko *et al.* (1988). Although the range of yeasts which have been implicated in allergic disease is much smaller than for filamentous fungi, *Rhodotorula*, *Sporobolomyces* and *Tilletiopsis* are other yeasts which have been reported to cause allergic reactions (Jackson 1984; Rantio-Lehtimäki and Koivikko 1984; Burge 1989). Hodges *et al.* (1974) noted precipitating antibodies to both *Rhodotorula* sp. and *Cryptococcus* sp. (and a range of other fungi isolated from a home cold-mist vaporizer) in the serum of a patient with hypersensitivity pneumonitis (HP), and a *Rhodotorula* sp. has been recorded as a cause of HP in the occupant of a house with a heavily contaminated basement (Gravesen 1994). Sensitivity to *Candida* has been recorded in children with asthma (Koivikko *et al.* 1991), and *Trichosporon cutaneum* is the cause of a summer hypersensitivity pneumonitis in Japan (Yoshida *et al.* 1989).

Bacteria

Most often, but not always, there are more bacteria than fungi in the air of homes (Nevalainen *et al.* 1988), schools (Mouilleseaux *et al.* 1993) and non-industrial workplaces such as airconditioned offices (Mouilleseaux *et al.* 1993). More than a hundred years ago in their study of homes and schools in Dundee, Carnelley *et al.* (1887) demonstrated the effect of ventilation and overcrowding on bacterial numbers. They noted that numbers of viable airborne bacteria were higher in naturally ventilated than in mechanically ventilated schools, with the ratio of bacteria to fungi being 132:1 as opposed to only 29:1. Counts of viable bacteria in domestic air were shown to rise with occupation density and the ratio of bacteria:fungi also increased, from 21:1 in spacious houses to 49:1 in overcrowded houses. Dust-raising activities were also observed to increase numbers of airborne bacteria relative to those of fungi (Carnelley *et al.* 1887). Some-what more recently, it has been shown that even in much more exalted places than Dundee houses and schools human numbers and activity have an important bearing on counts of airborne bacteria.

Despite air filtration and conditioning, counts of airborne bacteria (and fungi) correlate positively with the numbers of visitors to and their presence in the Sistine Chapel (Montacutelli *et al.* 1998).

Table 4. Viable bacteria isolated from air in airconditioned and naturally ventilated sites in two office buildings (after Austwick *et al.* 1989)

Gram-positive	Gram-negative
<i>Micrococcus</i> spp.	<i>Acinetobacter</i> calc var. <i>Iwoffii</i>
<i>Staphylococcus aureus</i>	<i>Aeromonas hydrophila</i>
<i>Staph. epidermidis</i>	<i>Flavobacterium</i> sp.
<i>Streptococcus</i> spp.	<i>Moraxella</i> sp.
	<i>Pasteurella haemolytica</i>
	<i>P. pneumotropica</i>
	<i>Pseudomonas aeruginosa</i>
	<i>Ps. cepacia</i>
	<i>Ps. fluorescens</i>
	<i>Ps. paucimobilis</i>
	<i>Ps. vesicularis</i>

As Table 4 shows, any investigation of airborne bacteria in buildings generally leads to the isolation of a diverse range of bacteria, but a limited number of types shed by the occupants normally predominate. These dominant types are principally Gram-positive members of the Micrococcaceae, among which *Staphylococcus epidermidis* associated with skin scales is frequently most prominent. The shedding of staphylococci by humans accounts for their airborne numbers increasing proportionally more than other bacteria during visiting hours at the Sistine Chapel (Montacutelli *et al.* 1998). Other bacteria in the Micrococcaceae which are most likely to be present are *Micrococcus* spp. such as *M. luteus* (Flannigan *et al.* 1999), again originating on the skin, and *Staph. aureus* shed from the nasal membranes and skin. Although Gram-positive endospore-forming rods in the genus *Bacillus* are commonly isolated from a wide range of indoor (and outdoor) environments, they are seldom isolated in number from indoor air. Two other types of Gram-positive bacteria which may be found in indoor air (Austwick *et al.* 1986) are coryneforms, which are irregularly shaped, non-spore-forming rods, and actinomycetes, which are mycelial forms producing asexual spores on the ends of filaments or in sporangia.

Although some coryneforms are harmless saprotrophs in soil and water, many are pathogens. These include *Corynebacterium diphtheriae*, which like *Mycobacterium tuberculosis* is considered to invade the host mainly as a result of inhalation of droplet nuclei from a carrier of the disease and probably does not survive long in indoor air. Although only rather small numbers of mesophilic *Streptomyces* and other actinomycetes are normally found in indoor air (Nevalainen *et al.* 1988; Nevalainen 1989), they are commoner in homes with dampness problems, and have been particularly associated with

complaints of odour in a range of types of building (Nevalainen *et al.* 1990). Ström *et al.* (1990) found streptomycetes in 25% of building material samples taken from sick buildings. It is not rare to isolate thermophilic species such as *Saccharopolyspora* (Faenia) *rectivirgula* and *Thermoactinomyces candidus* or *Th. vulgaris* from HVAC equipment (Fink *et al.* 1971; Kreiss and Hodgson 1984), and spores dispersed from massively contaminated systems may cause hypersensitivity pneumonitis among building occupants (Banaszak *et al.* 1970; Fink *et al.* 1971).

Gram-negative bacteria are usually much less abundant than Gram-positive species in indoor air. Elevated counts of Gram-negatives are indicators of conditions wet enough to allow proliferation, e.g. in HVAC humidifier reservoirs and drainage pans or on very damp surfaces. In such cases, *Pseudomonas*, *Acinetobacter*, *Alcaligenes* and *Flavobacterium* are likely to be among those most commonly isolated, but other Gram-negative rods such as *Achromobacter*, *Aeromonas*, *Agrobacterium*, *Enterobacter*, *Klebsiella*, *Moraxella* and *Proteus*, and also Gram-negative cocci, may also be present (Flannigan *et al.* 1999).

Health effects of bacteria in indoor environments

Inhalation of the lipopolysaccharide or endotoxin present in the wall of Gram-negative bacteria is known to induce a range of health effects which will be discussed later in Chapter 3.6. The Gram-negative bacterium of greatest concern is the respiratory pathogen *Legionella pneumophila*, which has been mentioned above in relation to free-living protozoa. *Legionella* is dealt with more fully in Chapter 3.4, together with Gram-positive and Gram-negative bacteria causing nosocomial pneumonia. A major portion of Chapter 3.4 is given over to the Gram-positive bacteria, *Mycobacterium tuberculosis* and related species, which are responsible for tuberculosis, a disease now reported to claim more victims than malaria and AIDS. The importance for health of exposure to various bacteria in work environments is discussed in Chapter 2.2, and again in Chapter 3.3.

Viruses

Relatively little is known about viruses in indoor air, but it is generally considered that they do not survive in infective form for any length of time outside the host organism. They are assumed under normal circumstances to pass from person to person by direct contact or in the case of viral disease of the upper respiratory tract by droplet infection. Nevertheless, there is some evidence that viruses can be distributed by mechanical ventilation systems. A report indicating spread via a ventilation system concerned an outbreak of acute febrile respiratory disease among trainee soldiers (Brundage *et al.* 1988). Significantly greater numbers of recruits in recently constructed mechanically ventilated barracks were affected than in similar, but older, naturally ventilated barracks. It was later found that the risk in mechanically ventilated barracks was reduced significantly by prophylactic treatment with adenovirus vaccine. In an earlier report of an outbreak of measles in a school, the spread of infection from infected children in one classroom to others in different classrooms was also taken to have been via the school ventilation system (Riley *et al.* 1978). Since the evidence for their presence in indoor air is limited and investigation of them is so specialized, further detail on viruses in this

volume is restricted to a short discussion of influenza in Chapter 3.4.

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Chapter 2.

Microorganisms in homes and work environments

CHAPTER 2.1

MICROBIAL GROWTH IN INDOOR ENVIRONMENTS

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MICROBIAL NUTRITION

In Chapter 1.2, it was noted that algae differed in their nutrition from the bacteria, fungi and protozoans in indoor environments. Algae are autotrophic; they obtain the carbon that is required for the biosynthesis of the organic molecules that make up their structure solely from carbon dioxide. This they are able to do because they are photosynthetic, utilizing light energy for the reduction and incorporation of the carbon dioxide. Although most of the algae in indoor air are of outdoor origin, they are able to grow indoors on perpetually damp surfaces exposed to light, e.g. on wet window reveals or on the soil of potted plants.

In contrast, bacteria, fungi and protozoons are heterotrophic; they cannot use carbon dioxide as their sole carbon source, but utilize preformed organic molecules as both sources of carbon and energy. The source of these organic molecules is normally other organisms. The Protozoa have two types of heterotrophic nutrition. As indicated in Chapter 1.2, they can engulf and digest organic particles, including bacteria—holozoic nutrition. However, they can also take up nutrients such as amino acids and sugars through their surface—saprozoic nutrition. The two types of nutrition are not mutually exclusive and operate side by side in aquatic environments rich in both particulate and dissolved organic matter, e.g. in heavily contaminated drainage pans and humidifier reservoirs of heating, ventilation and air-conditioning (HVAC) systems.

The bacteria and fungi in buildings are usually saprotrophs; their nutrients are obtained from dead organic matter. Laboratory studies have shown that bacteria such as *Pseudomonas cepacia* can utilize any of more than a hundred different organic compounds as sole carbon source. Saprotrophs can take up any free organic molecules, such as simple sugars or amino acids, which may be present in the environment. Most of the organic molecules available to microorganisms are, however, bound up in polymers that have been synthesized by other organisms. For example, plant polysaccharides such as cellulose, hemicellulose, pectin and starch are constructed from simple sugars, but the sugars only become available when cleaved from the polymers by the hydrolytic action of microbial extracellular enzymes. Amino acids are similarly released from proteins in dead plants, animals and microbes. Because of the particular importance of cellulose in

constructional and finishing materials, the breakdown of this polymer by cellulolytic microorganisms will be examined in more detail later in the chapter.

In addition to being able to lead a saprotrophic existence, some bacteria and fungi that grow in indoor environments can also behave as parasites, i.e. they can obtain their nutrients from living organisms. One such facultative parasite is the mould *Aspergillus fumigatus*. It can grow as a saprotroph under a wide range of circumstances, but is particularly notable in indoor environments where there are organic materials that are or have been at temperatures well above ambient, e.g. mushroom compost or self-heated (farmer's lung) hay or grain. In its role as a parasite it behaves as an opportunistic pathogen (see Chapter 3.5); it does not normally invade the healthy human body, but can be invasive in debilitated or immunosuppressed individuals.

WATER RELATIONS OF MICROORGANISMS

Moisture in materials

Irrespective of the nutrient status of any material, whether a microorganism grows on and in that material or not depends on the availability of water in the material. Each and every microorganism has its own particular moisture requirements. Its growth depends on enough "free" water being available. This water does not include the bound water or water of constitution held tightly in chemical union with the material, but is comprised of the adsorbed water that is less strongly held by molecular attraction and the absorbed water loosely held in spaces by the weak forces of capillary action.

In older texts, e.g. Block (1953), water availability was expressed in terms of relative humidity (RH), but most microbiologists consider that it is wrong to describe water availability in a material using a term that in the strict sense applies to the moisture status of air. Instead, most biologists now use "water activity" (a_w) for the amount of free or available water in a substrate. This is defined as the ratio of the vapour pressure exerted by water in the (hygroscopic or porous) material to the vapour pressure of pure water at the same temperature and pressure. In effect, it is equivalent to 1/100th of the RH of the air in equilibrium with the material, i.e. a material in equilibrium with an atmosphere of 95% RH has a a_w of 0.95. The a_w can be determined indirectly by using a dew point meter to measure the RH of the atmosphere within a sealed container in which a sample of the material has been allowed to equilibrate. The RH of the atmosphere in this equilibrated system is of course the "equilibrium relative humidity" (ERH), and this term is occasionally used in microbiological publications rather than a_w , e.g. by Rowan *et al.* (1999).

ERH is a term better appreciated by engineers, most of whom use not a_w but "equilibrium moisture" or "equilibrium moisture content" (EMC) to describe the moisture status of a material. Alternatively, the engineer may use "fibre saturation point" or maximum sorption moisture content (Richards *et al.* 1992), EMC or merely MC to describe the moisture-holding properties of materials. As Richards *et al.* (1992) have noted, fibre saturation point (or maximum sorption point) is a useful indicator of the

relative amount of water in material. However, because it is difficult to measure directly, this point is usually determined either by extrapolation to 100% RH or at an arbitrarily defined lower RH, say 98%.

The problem with using MC to describe moisture status is the confusion that arises because different materials take up different amounts of water from the ambient atmosphere. Materials with the same MC may have quite different a_w or ERH values (Flannigan 1993). It follows that materials with the same a_w or ERH may have entirely different water contents. An example illustrating this was quoted by an International Society for Indoor Air Quality task force on moisture problems in buildings (Flannigan *et al.* 1996): at ERH 80% (a_w 0.80) the MC of softwood is around 17%, wallpaper 11.3%, cement render 1%, brick 0.1–0.9%, and gypsum plaster 0.7%. Provided there has been sufficient soiling of the last three with organic nutrients, at a_w 0.80 all of these materials are susceptible to growth of some moulds, despite the large differences in MC between them. In a building context, this disparity in MC is very well illustrated by Oxley and Gobert (1983), who described a hypothetical wall with wooden skirting board affixed to gypsum plaster-finished brick, with the three components being in moisture equilibrium. Under normal dry conditions, the brick might have a MC of about 1%, the plaster 0.5% and the skirting 12%. If, however, the wall became wet due to rain penetration and the components equilibrated the corresponding MC's would be 2–5 (brick), 1–3 (plaster) and 22% (skirting).

Clearly there would be no confusion if there were clear documentation available to show the relationship between MC and ERH or a_w for every material present in buildings, but information here lags well behind that available to the food industry concerning microbiologically safe storage of food commodities. There have, however, been useful studies carried out by various workers, e.g. Richards *et al.* (1992). Richards and his colleagues constructed adsorption curves which show the relationship between RH and EMC for two types of softwood, several types of board and sheathing, kraft paper and vinyl covered gypsum board. Bearing in mind that a_w is expressed as 1/100th of the ERH and knowing the minimum a_w for particular types of fungi, it is theoretically possible to predict from any such curve which fungi might colonize the material that had absorbed water and reached a particular MC. However, as mentioned above, for many materials in buildings curves showing the relationship between MC and RH are not readily available. Where they are available they require to be treated with caution since they are usually only based on values for MC arrived at by uptake of water from the ruling atmosphere.

Naturally, the relationship between a_w (or ERH) and MC follows the same pattern as that for RH and EMC (see Richards *et al.* 1992). As an example, Fig. 1 displays the relationship between a_w and MC of a plywood sample over a microbiologically relevant range. The measured EMC data (ordinate) are plotted against corresponding a_w values (abscissa). The a_w values at any point on the sorption curves correspond to the RH in the sealed vessels in which the plywood was incubated. As applies to all material, there is a difference (hysteresis) between this adsorption curve, where the dry material has taken up water from the ambient atmosphere, and the corresponding desorption curve, where the same material fully imbibed with water has come to equilibrium by losing water to the

ambient atmosphere. Consequently, a porous or hygroscopic material, say paper or wood, taking up water from a humid atmosphere and reaching a particular MC will have a slightly higher a_w than the same material drying down to that MC after flooding. This has implications as far as the susceptibility to microbial invasion is concerned. Depending on the precise MC, some organisms that are just able to grow on a material which has arrived at that MC by absorbing water from the atmosphere may not be able to grow on that material when it has reached that MC by drying down from a wetter state.

Moisture requirements of microorganisms

Each type of microorganism has a minimum and an optimum a_w for growth. Although the a_w minima for different species may differ by 0.30 or more, the differences between the optima are much smaller, with most optima being in the range 0.90–0.99 (Ayerst 1969). For example, the optimum a_w for a great many species of bacteria is c. 0.99, although for some staphylococci it may be around 0.93. Scott (1957) reported that the minimum a_w for *Staphylococcus aureus* was 0.86, but many other bacteria, such as members of the genera *Escherichia* and *Pseudomonas*, fail to grow at $a_w < 0.95$. Although the optimum for growth of filamentous fungi is usually appreciably greater than a_w 0.90, the minimum is normally much lower than for the majority of bacteria.

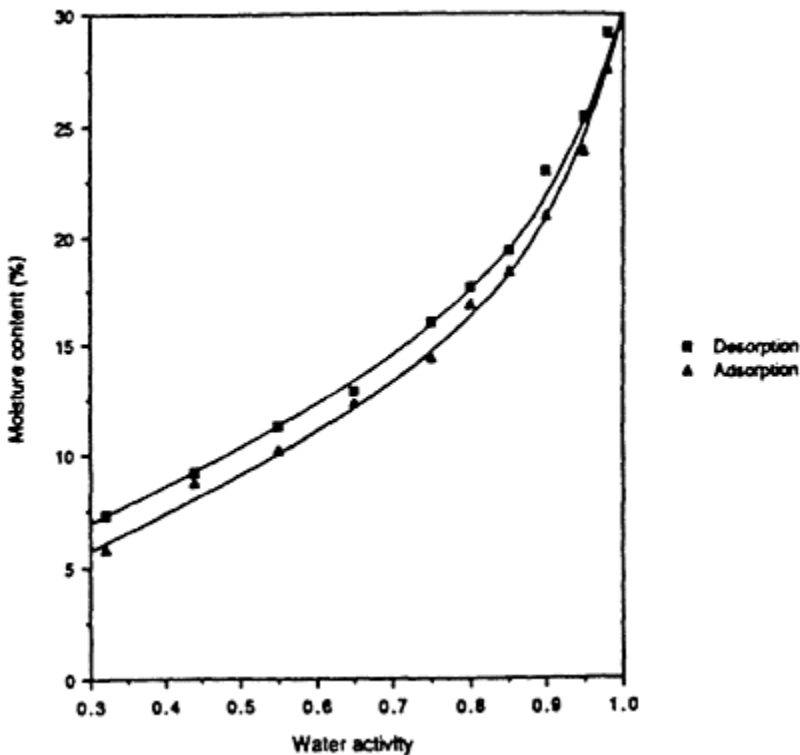


Fig. 1 Relationship between moisture content and water activity of plywood faced with Douglas fir under adsorption and desorption conditions, based on data provided by L. Geraldine Lea, Building Research Establishment, Garston, Watford, UK (after Flannigan 1993).

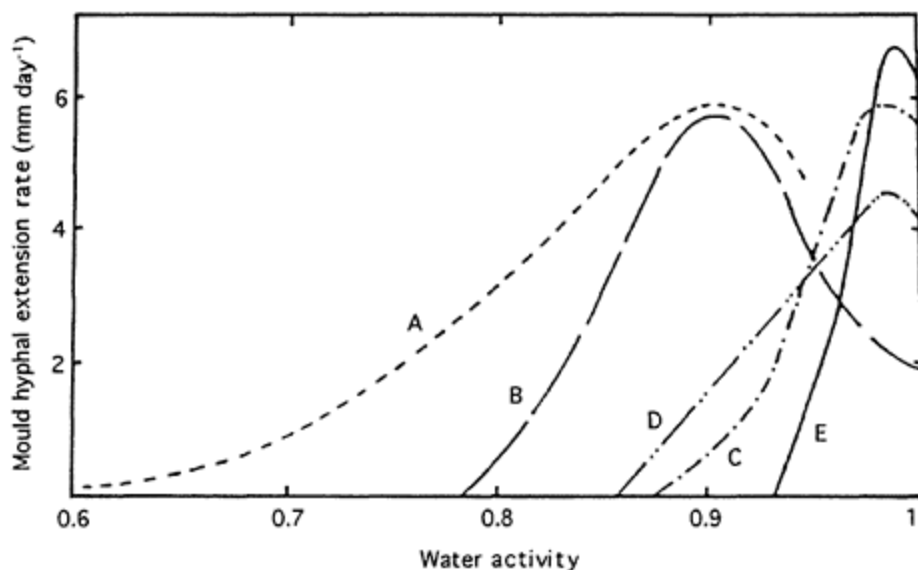


Fig. 2 Relationship between water activity and growth rate of moulds and bacteria (after Scott 1957): A, *Xeromyces bisporus*; B, *Eurotium herbariorum* (*A. glaucus*); C, *Aspergillus niger*; D, *Staphylococcus aureus*; E, *Salmonella* sp.

A range of fungi found in indoor environments are able to grow at a_w 0.70–0.80 (Ayerst 1969), and some even at $a_w < 0.65$. Although the minimum for most yeasts is a_w 0.88–0.92, the minimum for *Zygosaccharomyces rouxii* is c. 0.65. Fig. 2 shows the overall effect of a_w on the growth of a number of microorganisms, ranging from bacteria in the genus *Salmonella* to the food-borne mould, *Xeromyces bisporus*, which is able to grow at a a_w as low as 0.61.

Since filamentous fungi are generally more of a problem in buildings than other microorganisms, species for which minimum a_w values for growth have been published and which are encountered in buildings are listed in Table 1. They have been grouped according to a widely accepted scheme of categorization (Lacey *et al.* 1980). Those which have minima greater than a_w 0.90 are classified as **hydrophilic** (Gr., “water-loving”) as they are only suited to growing under conditions of high water availability. Those classified as mildly, moderately and extremely **xerophilic** (Gr., “dry-loving”) are in that order increasingly tolerant of, and able to grow in, conditions of reduced a_w . At opposite ends of the range of moulds in Table 1 are the extreme xerophile *Eurotium*

echinulatum with a minimum a_w of 0.64 and the hydrophilic basidiomycete microfungus *Sistotrema brinkmannii* with its minimum of a_w 0.96–0.97.

In a building, of course, the distribution of moisture within any material is seldom if ever uniform. Consequently, different areas of a structural element such as a wall may well select for the growth of different microorganisms. Grant *et al.* (1989) cited the case of a dwelling where due to a structural fault there was direct ingress of water to the interior surface of a boundary wall. Around the fault, where the wall was extremely wet, the hydrophilic mould *Stachybotrys chartarum*, grew profusely, but it was replaced nearer the drier margins of the affected area by more xerophilic species, viz. *Aspergillus versicolor* and various penicillia. Grant *et al.* (1989) also recorded that the mycobiota of the papered surface of a kitchen wall changed as it became progressively wetter with condensation during winter.

Table 1. Minimum a_w for growth at 25°C of filamentous fungi isolated from building interiors, after Northolt *et al.* (1995) with additional data from authors cited below.

Extremely xerophilic		
(Minimum a_w <0.75)	<i>Aspergillus penicillioides</i>	0.73–0.77
	<i>A. restrictus</i>	0.71–0.75
	<i>A. wentii</i>	0.73–0.75
	<i>Eurotium amstelodami</i>	0.71–0.76
	<i>E. chevalieri</i>	0.71–0.73
	<i>E. echinulatum</i>	0.64
	<i>E. repens</i>	0.72–0.74
	<i>E. rubrum</i>	0.70–0.71
	<i>Wallemia sebi</i>	0.69–0.75
Moderately xerophilic (Minimum a_w 0.75–0.79)		
	<i>Aspergillus candidus</i>	0.75–0.78
	<i>A. flavus</i>	0.78–0.80
	<i>A. ochraceus</i>	0.76–0.83
	<i>A. sydowii</i>	0.78; 0.81
	<i>A. tamarii</i>	0.78
	<i>A. terreus</i>	0.78
	<i>A. versicolor</i>	0.78; 0.74 ^e ; 0.75 ^d ; 0.79 ^b
	<i>Exophiala werneckii</i>	0.77–0.78

	<i>Paecilomyces variotii</i>	0.79–0.84
	<i>Penicillium aurantiogriseum</i>	0.79–0.85
	<i>P. brevicompactum</i>	0.78–0.82
	<i>P. chrysogenum</i>	0.78–0.81; 0.85 ^d
Slightly xerophilic (Minimum a_w 0.80–0.89)	<i>Absidia corymbifera</i>	0.88–0.89 ^d
	<i>Alternaria alternata</i>	0.85–0.88
	<i>A. clavatus</i>	0.85; 0.88 ^d
	<i>A. fumigatus</i>	0.85–0.94
	<i>Aureobasidium pullulans</i>	0.87–0.89 ^b
	<i>Chrysonilia sitophila</i>	0.88–0.90 ^a
	<i>Cladosporium cladosporioides</i>	0.86–0.88; 0.83–0.84 ^b
	<i>C. herbarum</i>	0.85–0.88
	<i>C. sphaerospermum</i>	0.83–0.84 ^b
	<i>Epicoccum nigrum</i>	0.86–0.90
	<i>Fusarium culmorum</i>	0.87–0.91
	<i>F. graminearum</i>	0.89
	<i>F. moniliforme</i>	0.87 ^{*a} ; 0.89–0.91 ^b
	<i>F. solani</i>	0.87–0.90
	<i>Mucor plumbeus</i>	0.87–0.93 ^b
	<i>Penicillium citrinum</i>	0.80–0.82
	<i>P. commune</i>	0.83
	<i>P. expansum</i>	0.82–0.85
	<i>P. fellutanum</i>	0.80 ^e
	<i>P. oxalicum</i>	0.88
	<i>P. rugulosum</i>	0.85; 0.80 ^d
	<i>P. viridicatum</i>	0.81 ^c
	<i>Ulocladium chartarum</i>	0.89 ^b
Hydrophilic (Minimum a_w $\geq$ 0.90)	<i>Botrytis cinerea</i>	0.93–0.95
	<i>Geomyces pannorum</i>	0.92; 0.89 ^b
	<i>Mucor racemosus</i>	0.94; 0.92 ^d
	<i>Neosartorya fischeri</i>	0.925

<i>Rhizopus stolonifer</i>	0.93
<i>Sistotrema brinkmannii</i>	0.96–0.97 ^b
<i>Stachybotrys chartarum</i>	0.94; 0.91–0.93 ^b
<i>Ulocladium chartarum</i>	0.89 ^b
<i>Verticillium lecanii</i>	0.90

*Spore germination, but growth not observed; ^a Armolik and Dickson (1956); ^b Grant *et al.* (1989); ^c Mislivec and Tuite (1970); ^d Panasenکو (1967); ^e Snow (1949).

The first colonists of the paper, when it was still comparatively dry, were *Penicillium* spp. and *A. versicolor*, but with continued condensation they were gradually overtaken by other species, culminating in hydrophiles such as *Ulocladium* spp. and *S. chartarum* when the wallpaper was very wet.

Based on field observations and laboratory studies of a_w minima, Grant *et al.* (1989) classified moulds successively colonizing materials into primary, secondary and tertiary colonizers (Table 2). Naturally, if a material is rapidly and massively wetted, so that its a_w is well above 0.90, it is open to immediate colonization by all types of mould, from xerophiles to hydrophiles. Under such immediately favourable conditions, so-called tertiary colonizers can act as primary invaders of the material.

Carlisle and Watkinson (1994) quote a lower limit for growth of most major basidiomycetous wood-rotting fungi as being about a_w 0.97, i.e. they are hydrophilic, but data on decay is usually quoted in terms of MC of the wood in question. A common cause of 'wet rot' of timber is *Coniophora puteana*; it is believed to account for around 90% of wet rot in buildings in UK. The optimum MC for decay of wood by this species is 50–60% and the minimum in spruce wood is 24% (Eaton and Hale 1993). Although rare in North America, another widespread wood-rotting basidiomycete is *Serpula lacrymans*, known as the dry rot fungus. The optimum MC of wood for decay by this species is variously quoted as somewhere between 30% and 80% and the minimum at 20% (Schmidt and Moreth-Kerberník 1990).

Although *S. lacrymans* does not grow in extremely wet wood, i.e. above about 90% MC, the 'dry' epithet is derived from the fact that the decayed wood breaks up into dry cubes which can easily be reduced to a powder. Although fibre saturation may be needed for initiation of *S. lacrymans* growth, once it is established in wood the organism itself can generate enough moisture metabolically not to require an external supply of water. It also has a capacity for translocating water from wetter areas into previously dry wood, causing localized wetting.

It is generally held that only where wood can be guaranteed to remain at 18% (Eaton and Hale 1993) or 20% MC (Oxley and Gobert 1983) is it safe from decay by wood-rotting Basidiomycetes. Connolly (1993) has, however, recommended adoption of a rather higher maximum safe level, 30% MC (fibre saturation), for prevention of fungal attack and decay.

Table 2. Moisture levels for colonization of construction, finishing or furnishing materials by selected microorganisms (Grant *et al.* 1989; Flannigan 1992; Nevalainen 1993).

MOISTURE LEVEL IN MATERIAL	CATEGORY OF MICROORGANISM
High ($a_w > 0.90$, ERH > 90%)	TERTIARY COLONIZERS <i>Mucor plumbeus</i> <i>Alternaria alternata</i> <i>Stachybotrys atra</i> <i>Ulocladium consortiale</i> Yeasts, e.g. <i>Rhodotorula</i> , <i>Sporobolomyces</i> Actinomycetes
Intermediate (a_w 0.80–0.90, ERH 80–90%)	SECONDARY COLONIZERS <i>Cladosporium cladosporioides</i> <i>C. sphaerospermum</i> <i>Aspergillus flavus</i> <i>A. versicolor</i> *
Low ($a_w < 0.80$, ERH < 80%)	PRIMARY COLONIZERS <i>A. versicolor</i> † <i>Eurotium</i> spp. <i>Penicillium brevicompactum</i> <i>P. chrysogenum</i> <i>Wallemia sebi</i>

*At 12 °C; †At 25 °C

Just as a_w affects the growth rate of moulds (usually measured as the rate of linear extension of hyphae), so it also influences germination of mould spores. At any given temperature, a reduction in a_w depresses the rate of germination, firstly by increasing the latent period, i.e. the time before germ tubes initially appear (see Chapter 1.2), and secondly by slowing the rate of elongation of the germ tubes once they have appeared (Scott 1957). The limiting a_w for germination of spores is usually lower than for linear growth of hyphae at optimum temperatures. For example, Magan and Lacey (1984) noted that spore germination of *Alternaria alternata* and other field (phylloplane) fungi occurred at $a_w > 0.85$, but for linear growth of hyphae in colonies of these fungi $a_w > 0.88$ was required.

TEMPERATURE RELATIONS OF MICROORGANISMS

Like a_w , environmental temperature also has a great bearing on microbial growth. Microorganisms can be allocated to different classes depending on the temperature range over which they grow.

Psychrophiles are defined as growing well at 0°C, with an optimum temperature for growth at 15°C or less, and a maximum in the region of 20°C. The bacterium *Micrococcus cryophilus* is a good example of a psychrophilic microorganism. Its cardinal temperatures are -4°C (minimum), 10°C (optimum) and 24°C (maximum).

Thermophiles are defined as being able to grow at temperatures of 50–55°C or higher. Bacteria classed as being thermophilic usually have minima around 45°C, but the thermophilic label is applied to fungi when they cannot grow below 20°C. Hence, the bacterium *Bacillus stearothermophilus* (cardinal temperatures 30°, 60–65° and 75°C) and the mucoraceous mould *Rhizomucor pusillus* (21–23°, 45–50° and 50–58) are both in the thermophilic category. In buildings, thermophilic *Bacillus* spp. and Actinomycetes such as *Saccharopolyspora* (*Faenia*) *rectivirgula*, *Thermoactinomyces candidus* and *T. vulgaris* may be isolated (and dispersed) from some types of heating system, and from the air during handling of self-heated agricultural materials (Chapters 1.2, 2.1 and 3.2).

Mesophiles are microorganisms that are neither psychrophilic nor thermophilic. By far the majority of bacteria and fungi in buildings fall into this category. Some of these mesophiles can grow at 0°C, but their optima are higher than for psychrophiles (in the range 20–30°C) and their maxima are around 35°C. The heterotrophic microorganisms that fall into this category are therefore described as **psychrotolerant mesophiles** (although the noun is often dropped from the term). Both psychrotolerant and psychrophilic microorganisms are able to grow in refrigerated environments. Some such bacteria and fungi, the latter including some species of *Cladosporium* and *Penicillium*, are major causes of spoilage in refrigerated foods. Some mesophiles are able to grow at temperatures of 50–55°C, but are excluded from the thermophilic category because their minima are <20°C. They are classified as **thermotolerant mesophiles**. The opportunistic fungal pathogen *Aspergillus fumigatus* (10–12°, 37–40° and 53–57°C) is one such thermotolerant species.

INTERACTIVE EFFECTS OF WATER ACTIVITY AND TEMPERATURE ON MICROORGANISMS

The minimum a_w for growth of the species listed in Table 1 were mostly derived from experiments in which the fungi were grown on agar media at 25°C, the optimum temperature for a large number of mesophilic moulds. In buildings, however, the materials on which growth occurs are almost invariably less nutritionally rich than such agar media. Also, at least in cold temperate climates, the temperature of substrates on which moulds grow, such as hardwall gypsum of outer walls, may be well below the optima for the moulds in question. It has been shown (Grant *et al.* 1989) that the a_w

minima for growth of moulds on wallpaper are higher than on agar media. However, experiments in which additional nutrients were provided by coating the paper with a cellulose ether, carboxymethyl cellulose (CMC), to simulate soiling with wallpaper paste, reduced the differential. Painting with mould-susceptible, water-based emulsion paint in which cellulose ethers are the thickening agents also promoted growth at a lower a_w than on unamended wallpaper (Grant *et al.* 1989). Earlier experiments showed that temperature has an important bearing on the minimum a_w for growth. In common with other investigators, Ayerst (1969) observed that the greatest tolerance to low a_w in all the fungi that he studied occurred at temperatures close to the optimum temperature at optimum a_w . For most of these fungi, the optimum a_w for growth was lowest at temperatures close to the maximum for the species concerned. Magan and Lacey (1984) noted that, for a selection of fungi encompassing both hydrophilic and xerophilic mould species, the more divergent the temperature from the optimum the less tolerant of lower a_w were these moulds. Table 3 illustrates this reduced tolerance in a range of fungi with temperature optima around 25°C but growing on wallpaper at temperatures that may be encountered at the internal surfaces of perimeter walls during winter in cold temperate regions. Nutrient availability and temperature are seldom likely to be optimal in buildings, so that if heterotrophic moulds and bacteria are to grow there they will almost invariably need damper conditions than indicated by their minimum a_w as determined in the laboratory on agar medium.

Bearing in mind these cautions, detailed observations of spore germination and hyphal growth on malt extract agar (Ayerst 1969) can be used to indicate the limiting conditions for a range of mesophilic mould species which can be found growing in buildings.

Table 3. Effect of temperature on minimum water activity at which selected moulds isolated from air and walls in houses grow on emulsion-painted woodchip paper (after Grant *et al.* 1989).

Species	12°C	18°C
<i>Aspergillus versicolor</i>	0.87	0.79
<i>Penicillium brevicompactum</i>	0.87	0.83
<i>Penicillium chrysogenum</i>	0.87	0.85
<i>Cladosporium sphaerospermum</i>	0.93	0.92
<i>Ulocladium consortiale</i>	0.94	0.92
<i>Stachybotrys atra</i>	0.98	0.97

Figs 3–7 have been reprinted from the original paper of Ayerst (1969) with permission from Elsevier Science. Each individual diagram depicts (a) a series of isopleths, or lines connecting points of equal value, for rates of linear extension of hyphae up to 10 mm day⁻¹, and (b) a series of points indicating the time taken for spore germination to occur.

The effect of reduced a_w in increasing the time required for germination has already

been mentioned, but is illustrated very clearly in the diagrams. For example, in *Eurotium chevalieri* strains tested at 25°C (Fig. 3) germination at a_w 0.95 took at most two days, but at a_w 0.85 the time required was 2–4 days and at a_w 0.75 8–16 days. The rates of linear extension of hyphae in Figs 3–7 show that growth was generally most rapid under the same conditions as the delay in germination was shortest (Ayerst 1969). Also, linear growth was slowest where the preceding delay in germination was greatest.

The relatively wide range of a_w and temperature under which *Eurotium* spp. can grow (Figs 3 and 4) is clearly seen. For *E. chevalieri*, *E. intermedium* (Fig. 3) and *E. amstelodami* (Fig. 4) the minimum a_w for growth was 0.71 and the lower and upper temperature limits were 10°C and 42°C. For *E. herbariorum* (Fig. 3) and *E. rubrum* (Fig. 4), the limiting a_w was also 0.71, but the temperature limits were 7°C and 38°C. Standing in sharp contrast to the diagrams for these xerophiles are those showing the somewhat more restricted conditions under which certain species grew, e.g. *Penicillium islandicum* and *Stachybotrys chartarum* (Fig 7). The different temperature relations of psychrotolerant and thermotolerant mesophiles are well illustrated by comparing Fig. 7 for *Penicillium aurantiogriseum* (cardinal temperatures <5°C, 23°C and 32°C) with Fig. 5 for *Aspergillus fumigatus* (12°C, 40°C and 53°C).

The net effect of moisture availability and temperature on growth is, of course, the outcome of their effects on the individual energy release and synthetic processes of primary metabolism in microorganisms. However, it is not only growth-related processes that are subject to their influence.

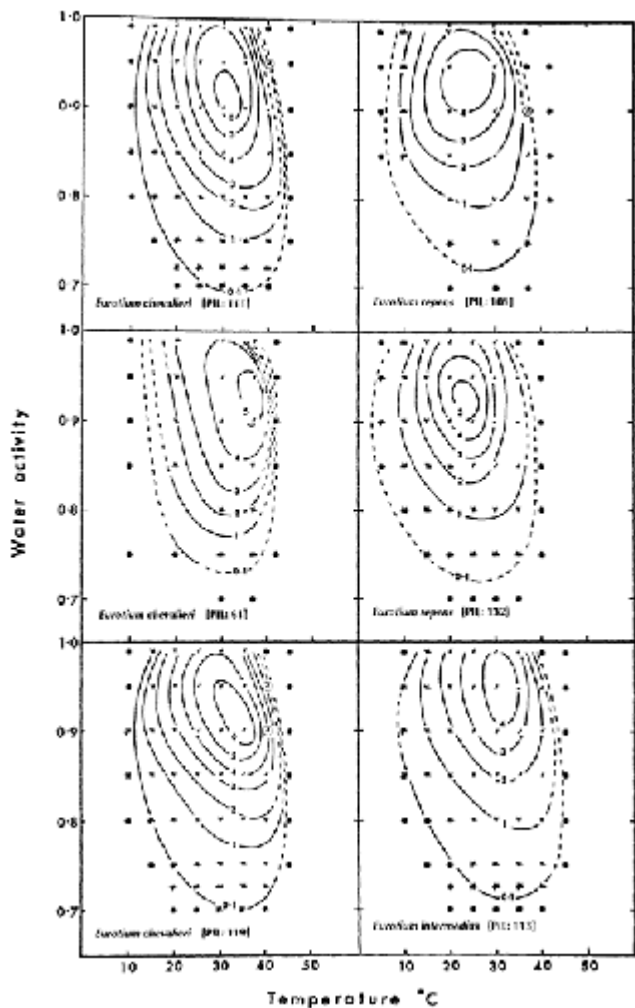


Fig. 3. Effect of temperature and water activity on spore germination time and rate of linear extension of hyphae in strains of *Eurotium chevalieri*, *E. herbariorum* and *E. intermedium*. Reprinted from Ayerst (1969), with permission from Elsevier Science. Key to symbols used in Figs 3–7 to indicate germination times. If circled, germination occurred but any further growth did not continue at a steady rate.

Explanation of symbols on growth rate diagrams

Symbol		•	♣	♠	♣	♠	♠	♠	●
Days to	minimum	–	1	2	4	8	16	32	95

germination maximum 1 2 4 8 16 32 95 43

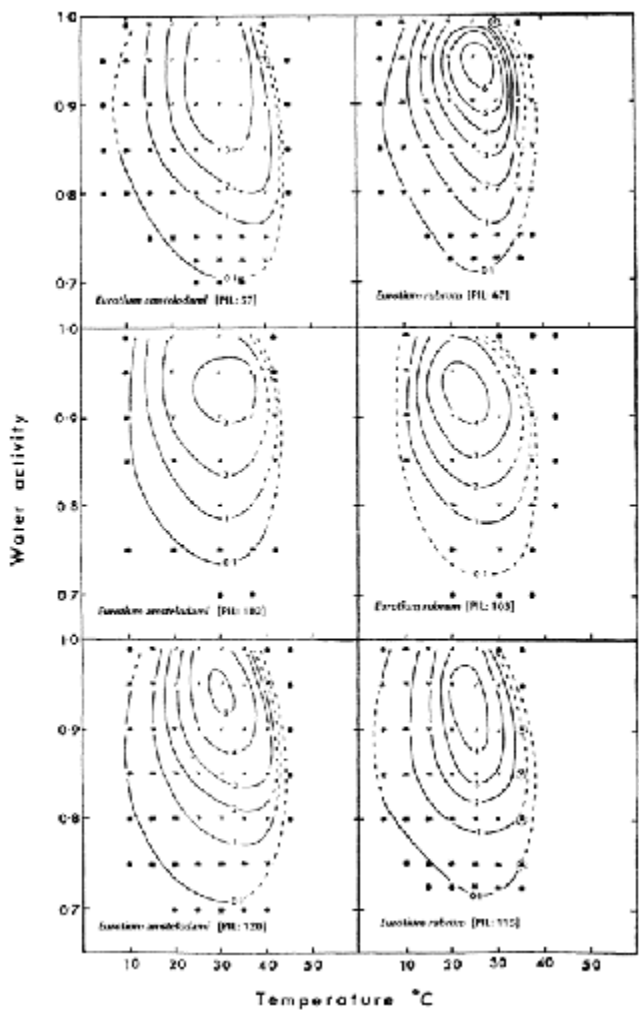


Fig. 4. Effect of temperature and water activity on spore germination time and rate of linear extension of hyphae in strains of *Eurotium amstelodami* and *E. rubrum*. Reprinted from Ayerst (1969), with permission from Elsevier Science.

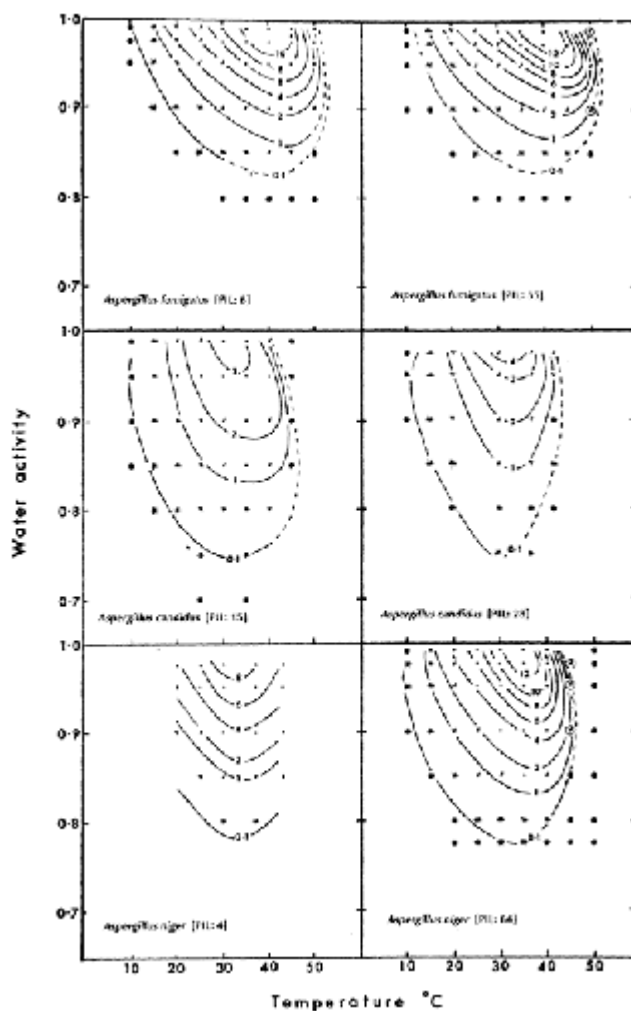


Fig. 5. Effect of temperature and water activity on spore germination time and rate of linear extension of hyphae in strains of *Aspergillus fumigatus*, *A. candidus* and *A. niger*. Reprinted from Ayerst (1969), with permission from Elsevier Science.

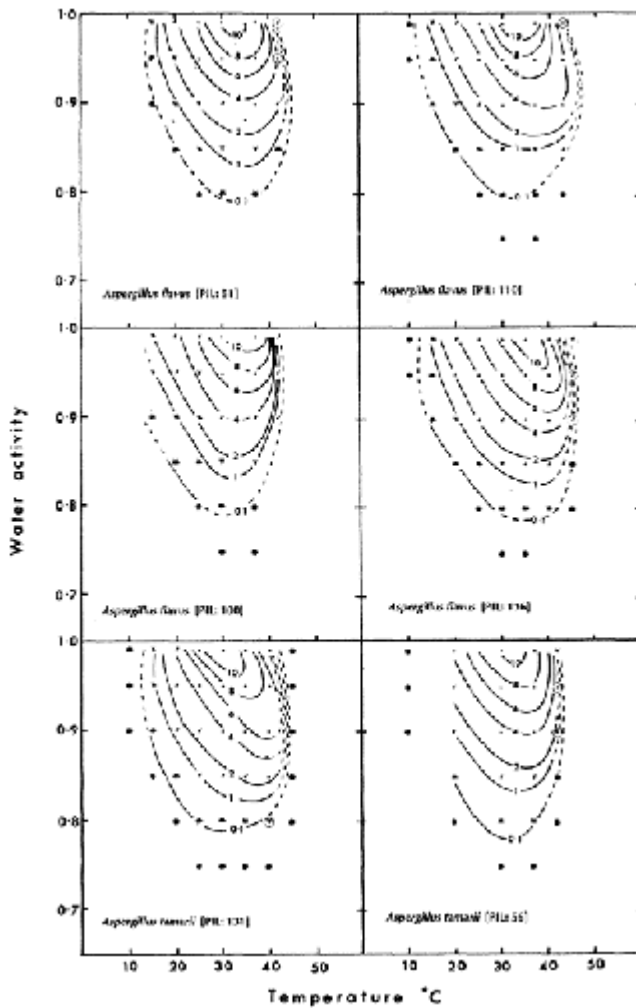


Fig. 6. Effect of temperature and water activity on spore germination time and rate of linear extension of hyphae in strains of *Aspergillus flavus* and *A. tamarii*. Reprinted from Ayerst (1969), with permission from Elsevier Science.

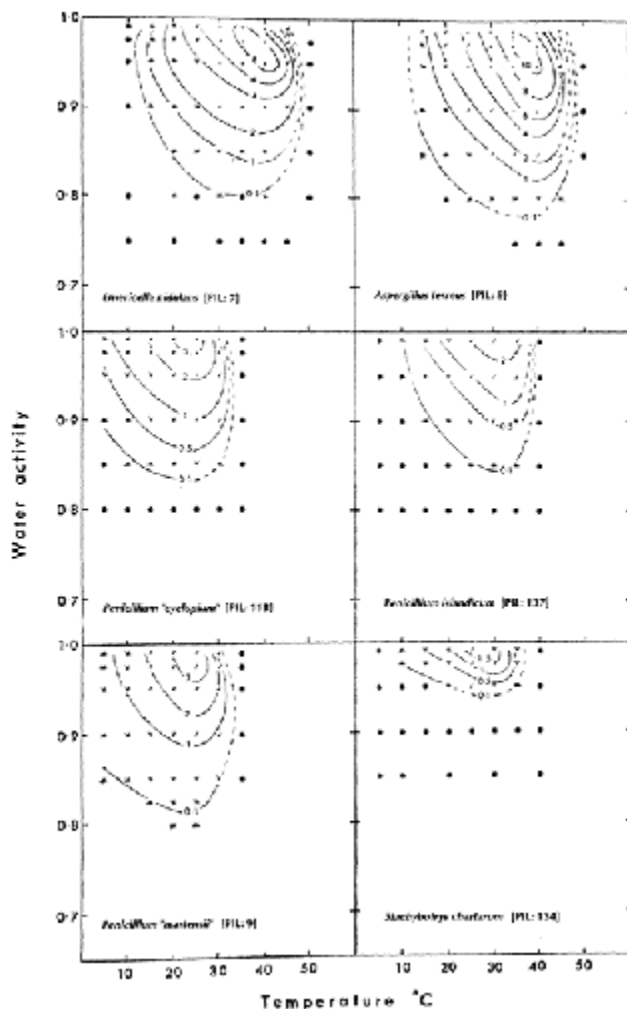


Fig. 7. Effect of temperature and water activity on spore germination time and rate of linear extension of hyphae in strains of *Emericella nidulans*, *Penicillium "cyclopium"* and "*martensii*" (= *P. cf aurantiogriseum*), *Aspergillus terreus*, *P. islandicum* and *Stachybotrys chartarum*. Reprinted from Ayerst (1969), with permission from Elsevier Science.

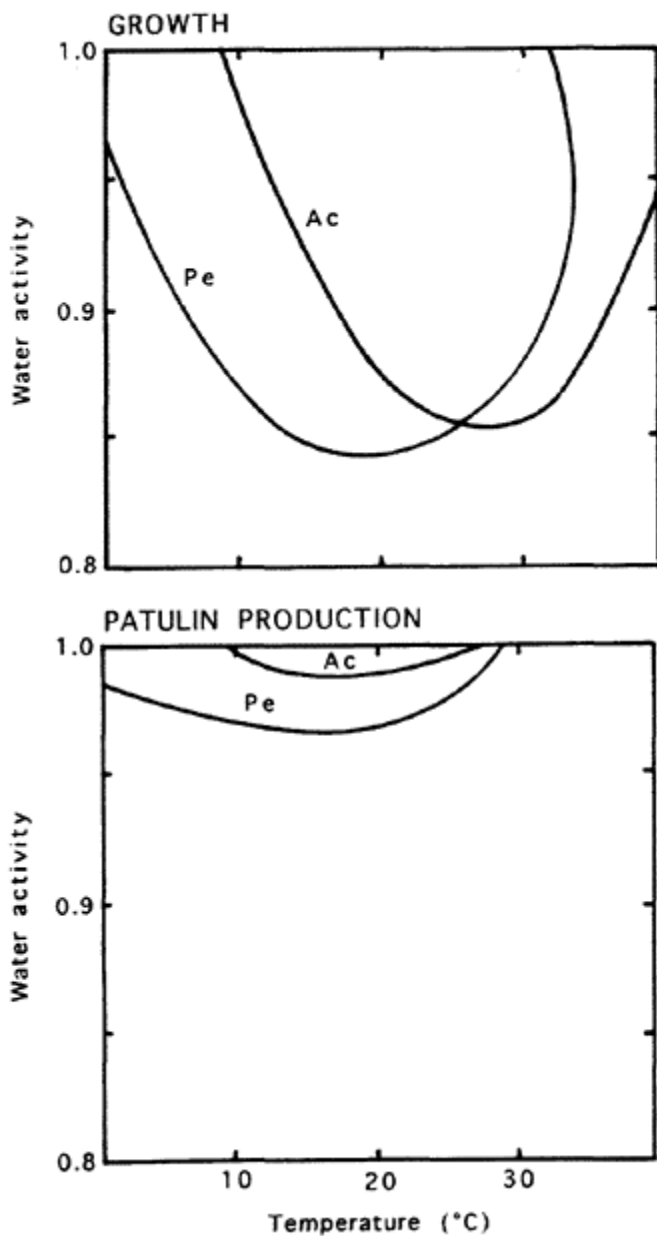


Fig. 8. Temperature and water activity (a_w) limits for hyphal growth and patulin production in *Aspergillus clavatus* (Ac) and *Penicillium expansum* (Pe) (after Northolt 1979).

Also affected is the production of compounds that are not essential for growth but may serve some other role, i.e. secondary metabolites such as mycotoxins. When Northolt (1979) examined the effect of a_w and temperature on the growth of a range of toxigenic moulds and the toxins that they produced, in some cases the limiting conditions appeared to be near to those for mycelial growth. For example, the limits for production of aflatoxin B1 by *Aspergillus flavus* (and the related species *A. parasiticus*) were relatively close to those found by Ayerst (1969) for growth of *A. flavus* (Fig. 6). In contrast to this, however, the a_w and temperature range within which penicillic acid and patulin accumulated was much more restricted than for growth of the producers (Fig. 8). The restriction of penicillic acid production by *Penicillium aurantiogriseum*, and of patulin by *A. clavatus* (Chapter 3.2) and *P. expansum* (Fig. 8), to conditions of extremely high a_w was particularly marked.

SOURCES OF WATER FOR MICROBIAL GROWTH IN BUILDINGS

Condensation and microbial growth

Condensation on walls

Before discussing the conditions under which growth occurs in buildings, the reader should be cautioned that what we deduce from the presence of different types of microorganism is based very largely on steady-state experiments under different moisture regimes, rather than from well-designed experiments in which there are temperature and RH fluctuations like those that actually occur in buildings. One of the few investigations that has revealed the effects of high: low temperature and RH cycling on fungal growth is presented in the thesis of Adan (1994), who examined the growth of *Penicillium chrysogenum* on gypsum and drew attention to the “time of wetness” for this growth.

The first sign of a condensation problem in a naturally ventilated building is often the appearance of mould growth on the inside surface towards the top of external walls, particularly in winter. This condensation occurs because the warm moist room air coming into contact with the surface is cooled to (or below) the dew point temperature, i.e. the temperature at which it is saturated with water vapour and dew forms. Psychometric charts will show that the dew point of air at 20°C and 70% RH is 14.5°C. For air at the same temperature but with a RH of 60% the dew point is about 12°C. Under these circumstances, condensation will make possible growth of a wide range of moulds, ranging from xerophiles to hydrophiles, and also yeasts and Gram-negative bacteria. However, mould growth can also occur where there is no condensation. Even if the temperature of a surface is only 5° lower than room air at 20°C and 60% RH, the RH at that surface will be 80%. Any porous finish to a wall, such as wallpaper, will equilibrate to a_w 0.80 (ERH 80%), a level at which xerophilic moulds could grow (Table 1), although not hydrophilic species.

In practice, there are many poorly insulated buildings in cold-temperate climates where the temperature of at least some of the inner surface of exterior walls is at temperatures close to dew point for much of the winter. For example, during winter in UK mean

weekly temperatures of 9–10°C and RH's of 85–91% were recorded at a kitchen wall surface, with those for the air adjacent to the wall being 11.0–11.2°C and 77–83% RH. The surface RH was >70% for 85–95% of the time, and 100% for 15–25% of it; penicillia, *Aspergillus versicolor*, *Cladosporium*, *Ulocladium*, *Stachybotrys* and other moulds were isolated from the wallpaper (Hunter 1989). Although mould growth may be widespread, it can also be limited to scattered patches. This is usually because there is thermal bridging between inner and outer layers of the building envelope at these sites, and as a consequence there are cold spots and condensation or high a_w . Structural members such as metal studs or ties, which have a high thermal conductivity, can act as thermal bridges. Irrespective of the cause of condensation, particular areas which favour mould growth, because ventilation is locally limited and the air is stagnant, include corners of external walls; areas behind large items of furniture placed up to external walls; and cupboards or closets built onto external walls. Another site is of course at the junction of the glass and wooden frame of single-glazed windows, where water accumulates and *Aureobasidium pullulans* and *Sistotrema brinkmannii* are notable. The former has also been found to predominate at the junction in triple-glazed windows, owing to the metal beading forming a thermal bridge to the cold exterior (Käpylä 1985).

Condensation in basements, cellars and underfloor crawl spaces

Condensation may be a problem in both occupied basements and unoccupied cellars. In basements, throughout the year the temperature of the surrounding ground is significantly lower than that indoors. In humid summers, the RH may frequently be high enough for mould to appear on paper and wooden surfaces, and condensation may be found in the envelope and collecting as puddles on the floor (Rowley and Walkinshaw, 1995). The temperature difference between cellars and the occupied space above is one which accounts for much of the frequency of mould growth in cellars. As Kelly (1988) observed, even where the occupied space in a house is at 21°C and the RH of the air is only 50%, the cellar temperature is commonly as low as the dew point, i.e. about 11°C. Another problem area is the crawl space under suspended floors, where evaporation from walls below the damp-proof course and from the soil can produce a very high RH. As a result of taking up water from highly humid sub-floor air, joists and floorboards may have a MC that is anything from 18 to 30%. The higher levels occur where the floor is covered by a waterproof material like linoleum, vinyl or tiles, and at these levels decay by Basidiomycetes is common (Oxley and Gobert 1983). Without a waterproof covering, water evaporates from the floor into the rooms above, where it can contribute to condensation problems. Christian (1993) indicated that 40–72 litres water day⁻¹ can enter the occupied space from the underfloor space by this route, but where there is a waterproof solum or ground cover it is likely to be only about 10% of this.

Condensation in the roof space

Another area in which condensation may be a problem is the roof space. As Oxley and Gobert (1983) have pointed out, in naturally ventilated houses which have been draught proofed, or sealed, and decorative vinyl wall coverings applied, the roof space may be virtually the only area to which water vapour can escape. Water vapour will pass up though the ceilings and thermal insulation and, especially where ventilation is impeded,

condense on the roof lining whenever its temperature is at or below dew point. Where the lining is impermeable, the condensate may drip onto the rafters, thermal insulation, uninsulated gypsum board ceilings or stored materials, which may consequently be colonized by moulds. Where the lining absorbs the condensation it may also become mouldy. For example, the first named author has examined a house where the lower surface of the gypsum board lining the roof bore profuse growth of *Acremonium* and *Stachybotrys*.

Interstitial condensation

What is known as interstitial condensation can occur within the thickness of a porous wall through which water vapour can pass when there is a temperature gradient between the inner and outer sides. Water vapour in warm air passing out from the occupied space through the fabric of the wall condenses when the dew point is reached. If this is near the outer surface it is not usually a problem, because it evaporates at times when there is no condensation. In cavity walls, the condensation occurs in the space between the inner and outer leaves of the wall, and again is not normally a problem if the cavity is ventilated. If however the cavity is filled by insulation, dew point may be reached somewhere in the insulation and mould growth may ensue (Oxley and Gobert 1983). Although not necessary for most masonry or brick walls, a vapour retarder applied to the inner surface of the wall reduces the risk of interstitial condensation. However, for wood frame and other building envelope structures correctly sited vapour retarders are essential. For a more detailed discussion of this the reader is directed to Oxley and Gobert (1983), TenWolde (1993) and Lstiburek (1994).

Origin of water vapour for condensation

Major sources of water vapour which condenses in homes are bathing, clothes washing/drying and cooking (Oxley and Gobert 1983; Christian 1993). In combination, they can more than double the amount that enters the air as a result of the respiration and perspiration of the occupants, but the total will vary widely. Obviously, high occupancy load can be a factor contributing to condensation problems (Merrill and TenWolde 1989). Among other factors which will boost the total are gas cookers or heaters which discharge their combustion gases inside, rather than through a flue); kerosene heaters, which generate virtually as much water as the amount of fuel that they consume; storing green wood (which itself may support mould and be a source of airborne spores) indoors for use as fuel; and drying of clothes on a rack, rather than in a drier venting to the outside. As Flannigan *et al.* (1996) have noted, estimates of the total amount of water vapour generated daily in the home of a family of four range from 7.5, through 11.4 to 23.1 litres.

A further point to be borne in mind is the time for which a building is heated. A problem that has emerged in recent years in UK is that many homes are now unoccupied and unheated for much of the day. The house may be heated for only a short period in the morning, during which moisture is generated by showering and cooking, before the occupants leave for work and school. The house then cools, with resultant condensation, before the occupants return in the evening and it is heated again and more moisture is generated prior to overnight cooling. This mode of occupancy has led to an increasing

number of condensation and mould problems in the British housing stock. Similar problems may arise in public and other larger buildings where heating is only periodic. In this context, an interesting observation, which might not be received with equal concern by both sides of any community divided by religion, was made by Wälchli and Raschle (1983). They noted that over a 25-year period 75% of cases of dry rot (*Serpula lacrymans*) in Swiss churches occurred in Protestant churches and only 25% in Roman Catholic churches. This was attributed to the fact that public services of worship were held throughout the week in the latter, but only on Sunday in the former. That is, Protestant churches were heated for less time and condensation was greater.

It should also be borne in mind that in newly built or renovated buildings considerable amounts of water evaporate from green timber, concrete, gypsum plaster, and types of insulation that are applied wet. It has been estimated that in a North American house the average volume of water released into the indoor air during the first year is 10 litres day⁻¹ (Christian 1993), an amount which may equal or exceed the total amount from all other internal sources. However, this input of construction water is halved in the second year and is thereafter negligible. Another source to be mindful of is water that either accumulates in condensate pans of air conditioning systems or drains into the underfloor crawl space, instead of to the outside or into the waste water system. In hot humid summers, reevaporation of this water can add considerably to problems, with the resultant water vapour migrating into the occupied space (Christian 1993).

Condensation is also a problem in tropical and subtropical climates, but there it occurs on internal surfaces of external walls because of infiltration of the building envelope by humid air from outdoors. In air-conditioned buildings, the cooled conditioning air can lower the temperature at the surface of gypsum board to round about or below the dew point of the infiltrating air. Microbial growth may therefore occur. For example, in Singaporean buildings a wide range of fungi contribute to the major problem of biodeterioration and discoloration of painted walls, wallpaper, ceiling tiles and even glass panels (Lim *et al.* 1989). The abundance of the hydrophiles *Fusarium decemcellulare* on ceiling tiles and *F. solani* on wallpaper are witness to conditions of extreme moisture. Where vinyl or other low-permeability decorative covering on the gypsum board impedes inward transfer of moisture, there may be extensive growth of xerophilic and hydrophilic fungi, and Gram-negative bacteria, below the covering. Morey (1993b) has reported that in southern USA growth may be extreme enough to render some buildings unfit for occupation. Further discussion of building envelope problems in tropical and subtropical climates, and also in cold climates, can be found in Flannigan *et al.* (1996).

Standing water

Standing water in evaporative condensers, shower stalls, whirlpool baths and evaporative humidifiers are among aquatic environments listed in Chapter 3.4 as having been linked epidemiologically to Legionnaire's disease episodes, although quite clearly any body of static water, including water in inadequately maintained drain pans, drains, cooling towers and cold water humidifiers, is a potential environment for microorganisms. Dust particles and organic debris (including dead microorganisms) in the water will provide a

supply of nutrients. Unless the water is severely oxygen depleted, it will support the growth of nearly all types of saprotrophic bacteria and microfungi that are likely to be found indoors. Gram-negative bacteria, yeasts and moulds can grow suspended in the water, in accumulated deposits at the bottom of the vessel and as biofilms on the surfaces. For example, Airoltd and Litsky (1972) reported that cold water humidifiers in use as an adjunct to respiratory therapy in a hospital were heavily colonized by *Flavobacterium* and other Gram-negative bacteria. Staphylococci, *Bacillus*, *Alternaria*, *Aspergillus*, *Mucor* and *Penicillium* were also isolated from the reservoir water, and Gram-negative bacteria were present in the mist emitted by the humidifiers. As indicated in Chapters 1.1 and 3.4, amoebae can ingest bacteria that are present in standing waters. In doing this they may become hosts in which *Legionella pneumophila* amplifies (Chapter 3.4), particularly where the water temperature is greater than 20°C and favours its growth. Also, as noted in Chapter 3.5, members of the medically important fungal genus *Exophiala* are found in bath water and in the sludge in bathroom drains in Japan, which are considered important habitats and perhaps significant sources of human infection by these organisms (Nishimura *et al.* 1987).

Other Sources of Water

Indoor sources

Apart from providing an environment for microbial growth, as a result of evaporation standing water adds to water vapour that may condense and dampen the fabric of the building so that it is affected by mould. However, it is only if large amounts of standing water are discharged, e.g. as a result of breakage, that it directly causes readily visible water damage to the fabric of a building. Slow persistent leakages in plumbing which go undetected may have a similar effect, but it is sudden failures leading to widespread flooding of ceilings, floors and walls that are likely to have a catastrophic effect. Not only do they cause clearly visible mould growth on surfaces but they also are likely to lead to colonization of concealed elements such as insulation (Chapters 2.3 and 4.4). The report of Fergusson *et al.* (1984) illustrates the effect that a persistent leak in pipework may have. A fine jet of water spraying onto the underside of a wooden floor from a domestic central heating system led to profuse local growth of two penicillia on the floor boards and their linoleum cover and was responsible for hypersensitivity pneumonitis in the occupants. At the other end of the scale, Morey (1993a) recorded that 3 months after 10^6 litres of water had been used to extinguish a serious fire on the ninth floor of a 10-storey office building carpets, gypsum board, wood panelling, ceiling tiles, and plaster walls and ceilings in the lower floor of both the fire-damaged building and another building connected to it were still moist. Species of *Cladosporium*, *Penicillium* and *Aspergillus* were widespread on surfaces and airborne propagule concentrations ranged up to $1.3 \times 10^6 \text{ m}^{-3}$ air (above ceilings) 7 months after the fire.

Outdoor sources

Water can enter the fabric of a building from the outside both above and below ground. Infiltration of the building envelope by water vapour in humid outdoor air has been

mentioned as a source of moisture which may condense within. To this source of moisture can be added liquid water in the form of rain, melting snow and ice, and soil water.

The most obvious form of water input above ground is rain penetration. Rain striking and wetting the surface of porous construction materials—concrete, bricks and mortar—moves through the material by capillary action (wicking) and can reach the inside surface of that element of the building envelope. In the normal run of events, during dry weather the wall will lose water to the atmosphere by evaporation from the outer surface and, as a consequence, the water in the pores will move towards the surface because of the difference in vapour pressure. However, this drying process takes much more time than wetting. When a porous wall is exposed to extended periods of rain, the pores may fill entirely with water (capillary saturation) and then the inner surface may be visibly wet. In a solid wall this will lead to mould growth, but a cavity wall is designed to prevent this happening. Any water reaching the inside of the outer layer evaporates into the ventilated space and thence to the outside. However, water will reach the inner layer if the cavity is bridged by mortar or other porous material that acts as a wick. Where the cavity is filled by insulating material, that material should not allow movement of water across the cavity (Oxley and Gobert 1983), nor should it support microbial growth. As Table 4 indicates, and as will be discussed later, significant problems have occurred in the past with urea-formaldehyde foam insulation.

Table 4. Common fungi in 1529 samples of urea formaldehyde foam insulation, expressed as percentage of samples contaminated.

Species	%
<i>Penicillium fellutanum</i>	49
<i>Cladosporium cladosporioides</i>	36
<i>Alternaria alternata</i>	35
<i>Penicillium viridicatum</i>	19
<i>Ulocladium chartarum</i>	10
<i>Penicillium brevicompactum</i>	9
<i>Penicillium decumbens</i>	7
<i>Trichoderma viride</i>	7
<i>Paecilomyces variotii</i>	6
<i>Cladosporium herbarum</i>	5
<i>Penicillium glabrum</i>	4
<i>Penicillium janthinellum</i>	4

Other factors which may be the cause of, or exacerbate, ingress of rainwater (or melt

water from snow and ice) include defects in brick- or stonework, mortar and rendering; badly installed windows; defective sealant joints or caulks; inadequate flashing of walls and roofs; faulty design and maintenance of roofs; and faults in rainwater disposal—undersized, choked or leaking rain gutters and down pipes (Oxley and Gobert, 1983; Christian, 1993; Connolly 1993).

Below ground, improper damp-proofing of foundations and flooding are important causes of moisture problems (Oxley and Gobert 1983; Christian, 1993). Rising damp, the capillary rise of water up through walls from the foundations, occurs where a damp-proof course is faulty, bridged or lacking. In extreme cases water may rise to a height of 50 cm above a suspended floor, resulting in a band of mould growth extending from the skirting board upwards, and occasionally the development at skirting level of fruiting bodies of “plaster fungi” such as *Peziza* (Ascomycetes).

Christian (1993) has listed sources of water which may penetrate the underground building fabric, including those caused by building and drainage defects. As well as entering by capillary action, water can be forced into foundations and basements under the hydrostatic pressure exerted by a high water table or flood and melt water. Whatever the source of water, flooded basements are subject to bacterial and fungal growth, particularly if the water is contaminated by backed-up sewage. In addition, just like crawl spaces, the basement can behave as a large humidifier (Christian 1993), supplying water vapour which can migrate upwards into the remainder of the building.

GROWTH OF MICROORGANISMS ON CONSTRUCTIONAL, FINISHING AND OTHER MATERIALS IN BUILDINGS

As discussed above, anything that becomes mouldy does so because it naturally contains, or has gained, enough moisture to permit growth and sporulation. Which fungus or fungi dominate on sufficiently moist substrates depends on both the a_w and composition of the building material. Just as, on a worldwide basis, there is surprisingly little variation in the fungi recovered from outdoor air, there appears to be little variation in the mycobiota of building materials in different parts of the world. This reflects (1) the widespread adoption of particular building materials (e.g. gypsum board), (2) the general reduction of air change rates in buildings and (3) the increase in conditioning the indoor environment to 20–25° C regardless of outdoor conditions. As more careful studies of the mycobiota are made there will be a greater understanding of the common fungi associated with mould-damaged building materials. The intention in this section is to illustrate the ecology of the built environment, first considering growth on cellulosic materials in general before turning to gypsum board and then other materials and situations in which growth may occur.

Cellulosic Materials

Types of cellulosic material

The constructional and finishing materials in buildings that are most susceptible to microbial growth and biodeterioration are those materials containing natural organic polymers encountered and degraded by microorganisms in their role of recycling nutrients in their natural environments. These polymers include the starch, cellulose, hemicellulose, pectin and lignin in plants. A great many microbes are able to degrade starch, hemicelluloses and pectins; a narrower range can degrade cellulose; and lignin degradation is confined to an even more limited range, mainly wood-rotting Basidiomycetes such as those mentioned earlier. Under damp conditions water-based adhesives such as starch and animal glues containing the proteins casein and gelatin are highly susceptible to attack by microbial deteriogens. The ability of wallpaper and cellulose ether adhesives to sustain growth of moulds has already been mentioned (in the previous section), and it is microbial growth on these and the wide range of other materials containing cellulose that gives the greatest cause for concern. The microbial biodeterioration of structural, finishing and furnishing materials such as timber, wood composites, jute, cotton, cardboard and wallpaper in buildings may have not only serious economic consequences, but may also result in the amplification of species that are of significance for health. For example, in investigating the etiology of asthma in a number of patients Kozak *et al.* (1980) noted the importance of damp wallpaper, jute carpet backing, and wicker and straw baskets as environments for the growth of the causative moulds in their homes. Ando *et al.* (1991) reported that damp wood and tatami mats were associated with the development of a summer-type hypersensitivity pneumonitis caused by *Trichosporon cutaneum*.

Cellulolytic microorganisms in air and on exposed cotton in outdoor environments

To get a good idea of the types of airborne microorganism, excepting major wood-rotting species, that may contaminate and cause damage to cellulosic materials in buildings one really has to go back some fifty years to the investigations of the US Army Quartermaster Corps during and after World War II (Siu 1951). The need for field and laboratory investigations rapidly became apparent because of the conflict in Southeast Asia and the Pacific, where it was found that the useful life of untreated tarpaulins, tents, ropes and sandbags in areas with high temperature and rainfall was very short. For example, in New Guinea (annual rainfall, 250 cm) army tents began to leak after 3–4 months and had to be replaced after 6–8 months, and in the Dutch East Indies the average life of a sandbag was only 6 weeks. The Quartermaster Corps amassed a culture collection of some 5000 isolates from microorganisms present on deteriorated materials gathered from military bases in the South and Southwest Pacific, others collected on expeditions in Panama and Florida and cultures from earlier programmes. Among 4500 isolates of fungi from exposed cotton textiles (Siu 1951), the most abundant categories were the genera *Penicillium* (18.2% of total) and *Aspergillus* (14.6%). In rank order, the next most abundant were non-sporing isolates (11.2%), *Fusarium* (8.6%), *Trichoderma* (8.5%), *Pestalotia* (4.4%), *Botryodiplodia* (3.2%), *Aureobasidium* (3.2%), *Chaetomium* (2.9%),

Phoma (2.2%), *Cladosporium* (1.9%), *Gliocladium* (1.6%), *Curvularia* (1.6%), *Memnoniella* (1.2%) and *Paecilomyces* (1.1%).

The most common species shown in tests to be cellulolytic was *Trichoderma viride* (Table 5), which accounted for 7.2% of all isolates. The only other species that individually comprised 2% or more of the total were cellulolytic *Penicillium citrinum* series isolates; *Botryodiplodia theobromae*, some isolates of which were cellulolytic and others not; and three non-cellulolytic species, *Aureobasidium pullulans*, *Aspergillus niger* and *A. sydowii*. As indicated by the results for *B. theobromae*, whatever the type of function or enzyme under study there is always variation in the degree of activity exhibited by different isolates of an individual species. Based on the collective results for the cellulolytic isolates in Table 5 and many other species not shown, Siu (1951) divided the cellulose-degraders on exposed cotton textiles into five groups. These are presented in decreasing order of activity in Table 6. Since isolations were made from cotton textiles exposed to air and dust or in contact with soil, not unsurprisingly, the mycobiota of exposed cotton was found to be similar to that of soil (Siu 1951).

In a later survey at the Tropical Research Unit in North Queensland, Australia, Upsher (1972) found strong similarities between the fungi on exposed cotton and soil. Most fungi in the 46 genera recorded were regarded as being soil-borne. Among the 46 genera, 30 were recovered from soil and vegetation and 31 from exposed cotton fabric, with 24 genera being common to vegetation and cotton fabric. However, only 17 genera were isolated from exposed timber and 13 from paints and plastics.

Breakdown of cellulose

Truly cellulolytic microorganisms have the ability to degrade native cellulose, in which the antiparallel long-chain molecules of the glucose polymer are aligned in micelles. These in turn are aligned in microfibrils that form the skeletal strengthening of the walls of plant cells ranging from thin-walled parenchyma in herbaceous plants to thick-walled fibres in woody plants. Briefly, cellulose molecules are composed of up to 10^4 D-anhydroglucose units and can be described as a (1→4)-β-D-glucan. Laterally contiguous cellulose molecules are linked by hydrogen bonding, giving highly ordered crystalline regions within microfibrils, but there are also paracrystalline and non-crystalline (amorphous) regions in which the degree of order is less (Eaton and Hale 1993). Complete breakdown of native cellulose by fungi is currently held to depend on their ability to produce three types of hydrolytic enzyme, viz. exoglucanase, endoglucanase and β-D-glucosidase, which act synergistically. Exoglucanase, or (1→4)-β-D-glucan cellobiohydrolase (CBH), cleaves off disaccharide residues (cellobiose) step-wise from the non-reducing end of cellulose molecules in crystalline cellulose; endoglucanase (EG) or endo-(1→4)-β-D-glucan 4-glucanohydrolase, randomly cleaves celluloligosaccharides from amorphous cellulose; and β-D-glucosidase, or β-D-glucoside glucohydrolase, hydrolyses cellobiose, cellotriose and lower

Table 5. Order of abundance of commonest taxa among 4500 isolates of cellulolytic and non-cellulolytic fung from exposed cotton fabrics (after Siu 1951).

Rank	Name	% of total	Cellulolytic activity
1	<i>Trichoderma viride</i>	7.2	+
2	<i>Penicillium citrinum</i> (series)	5.1	+
3	<i>Botryodiplodia theobromae</i>	3.2	+/-
4	<i>Aureobasidium pullulans</i>	3.0	-
5	<i>Aspergillus niger</i>	2.7	-
6	<i>Aspergillus sydowii</i>	2.0	-
7	<i>Aspergillus flavus</i>	1.9	-
8	<i>Penicillium luteum</i> (<i>Talaromyces luteus</i>)	1.7	-
9	<i>Aspergillus versicolor</i>	1.7	-
10	<i>Aspergillus oryzae</i>	1.7	-
11	<i>Chaetomium globosum</i>	1.4	+
12	<i>Fusarium moniliforme</i>	1.2	+
13	<i>Penicillium funiculosum</i> (series)	1.1	+/-
14	<i>Memmoniella echinata</i>	1.1	+
15	<i>Penicillium waksmanii</i>	1.0	-
16	<i>Pestalotia virgatula</i>	0.9	?
17	<i>Paecilomyces variotii</i>	0.9	-
18	<i>Aspergillus terreus</i>	0.8	+
19	<i>Fusarium semitectum</i> var. <i>majus</i>	0.6	+/-
20	<i>Curvularia lunata</i>	0.6	+

oligosaccharides to glucose. A (1→4)-β-D-glucan 4-glucohydrolase may also be involved; this breaks down to glucose any soluble oligomers released during breakdown of cellulose (Eaton and Hale 1993).

Non-cellulolytic microorganisms

As can be seen in Table 5, far from all of the isolates that can be made from deteriorating cotton are cellulolytic. *Aureobasidium pullulans* is a very good example of a fungus that is found on plant surfaces, in leaf litter and soil, and on canvas and timber, and yet appears unable to break down cellulose to any extent (Upsher 1972). Species of *Eurotium* and many aspergilli and penicillia are also widespread fungi that are not cellulolytic. Siu (1951) commented that, although *Memmoniella echinata*, *Stachybotrys chartarum* and

Chaetomium spp. were the predominant and destructive fungi on tents and tarpaulins after long exposure in New Guinea, the earliest colonists on the inside of tents (and to some extent on the outside) were species of *Aspergillus*, *Penicillium* and *Rhizopus*. He considered that these latter fungi utilized detritus or finishing substances (waterproofing) as nutriment.

Another explanation for the prevalence of types that lack the full array of synergistic enzymes required to degrade native cellulose to sugars that can be metabolized is that they may, however, be able to degrade soluble or modified forms of cellulose. For example, CMC and other cellulose ethers made from wood cellulose and used as adhesives, as thickeners in paints or for sizing are susceptible to degradation by a wider range of fungi than those classified as cellulolytic (Flannigan 1970; Flannigan and Sellars 1972). Any of the species possessing endoglucanases (and glucosidases) which degrade CMC should be able to hydrolyse oligosaccharides released from fibres by fully cellulolytic fungi such as those in Table 6. Members of the genus *Mucor*, which ranked eighteenth in order of abundance of the genera isolated from exposed cotton textiles in the US Army studies (Siu 1951), have no activity against even the most susceptible forms of cellulose and have been described as "sugar fungi".

Table 6. Relative degradative activity of cellulolytic fungi isolated from exposed cotton fabrics (after Siu 1951).

VERY STRONG	<i>F. oxysporum</i>
<i>Chaetomium funicola</i>	<i>F. solani</i>
<i>C. globosum</i>	<i>P. luteum</i> (<i>Talaromyces luteus</i>)
<i>C. indicum</i>	<i>P. sclerotiorum</i>
<i>Myrothecium verrucaria</i>	<i>P. simplicissimum</i>
<i>Sordaria fimicola</i>	<i>Ulocladium consortiale</i>
	DEFINITE
STRONG	<i>Alternaria alternata</i>
<i>Aspergillus fumigatus</i>	<i>Aspergillus oryzae</i> var. <i>effusus</i>
<i>Chaetomium atrobrunneum</i>	<i>A. (Fennellia) flavipes</i>
<i>C. cupreum</i>	<i>A. rugulosus</i>
<i>C. succineum</i>	<i>A. sulphureus</i>
<i>C. brasiliense</i>	<i>A. ustus</i>
<i>Corynascus sepedonium</i>	<i>Chrysomilia sitophila</i>
<i>Curvularia lunata</i>	<i>Cladosporium herbarum</i>
<i>Fusarium lateritium</i>	<i>Fusarium scirpi</i>
<i>Memnoniella echinata</i>	<i>F. semitectum</i>

<i>Myrothecium roridum</i>	<i>P. funiculosum</i>
<i>Stachybotrys chartarum</i>	<i>Penicillium glabrum</i>
<i>Trichoderma koningii</i>	<i>P. implicatum</i>
<i>T. viride</i>	<i>P. janczewskii</i>
MODERATE	WEAK
<i>Aspergillus (Neosartorya) fischeri</i>	<i>Aspergillus japonicus</i>
<i>Aspergillus niger</i>	<i>Aspergillus awamori</i>
<i>A. terreus</i>	<i>Aspergillus (Emericella) nidulans</i>
<i>Chaetomium aureum</i>	<i>Aspergillus ochraceus</i>
<i>C. cochliodes</i>	<i>C. murorum</i>
<i>Botryodiplodia theobromae</i>	<i>Penicillium atramentosum</i>
<i>C. spirale</i>	<i>Penicillium purpurogenum</i>
<i>Cladosporium cladosporioides</i>	<i>Penicillium miczynskii</i>
<i>Eupenicillium javanicum</i>	<i>Penicillium waksmanii</i>
<i>Fusarium avenaceum</i>	<i>Scopulariopsis brevicaulis</i>
<i>F. equiseti</i>	<i>Trichothecium roseum</i>
<i>F. moniliforme</i>	

After having utilized free sugars in fresh substrates and rapidly colonized them, for further growth they are dependent on sugars released from cellulose and hemicelluloses by the extracellular hydrolytic enzymes of other fungi present on the substrate. Their relative abundance and that of *Rhizopus* and other sugar fungi on exposed canvas may reflect the availability of sugars released by cellulolytic fungi that assimilate the products of degradation less rapidly than they degrade the cellulose substrate.

Major cellulolytic fungi

Because of their "very strong" or "strong" cellulolytic properties (Table 6), *Chaetomium globosum*, *Memnoniella echinata*, *Myrothecium verrucaria* and *Stachybotrys chartarum* have all been used in testing the decay resistance of mould-proofed canvas and other cellulosic materials. However, Siu (1951) noted that, despite its being the most powerful cellulose-destroying species in laboratory tests, *Myrothecium verrucaria* was practically never found growing or sporulating on cotton fabrics in the field. In contrast, *Memnoniella echinata* was the dominant species on deteriorated canvas tents throughout the Southwest Pacific area, despite pure-culture tests showing that it was much more fastidious in its growth requirements than *S. chartarum*. Further laboratory tests indicated the reason for the dominance of *M. echinata* in the field; it competed well against other species. When cotton duck was inoculated with a mixed spore suspension of *S.*

chartarum and *Chaetomium* sp., the two species grew in well-defined areas and did not invade areas already colonized by the other. However, when the suspension contained spores of *M. echinata* as well as *S. chartarum* and *Chaetomium* sp., *M. echinata* became dominant, excluding the other species. Other tests showed that sporulation in *M. echinata* was in fact stimulated by *Stachybotrys*, *Chaetomium* and another 11 fungi (both cellulolytic and non-cellulolytic). These may have provided an exogenous source of biotin, a vitamin found to be a requirement for growth of *M. echinata* (Siu 1951).

Growth on gypsum board

One of the structural components of modern buildings which frequently becomes damp and also provides an ample source of cellulose on which microorganisms can grow is gypsum board, also known as wallboard in North America and plasterboard in UK. It is manufactured by sandwiching a paste of gypsum (hydrated calcium sulphate) between cardboard layers that are held to the paste by starch glue and by gypsum crystals which penetrate the surface of the cardboard as the sheets dry. By the mid-1960's, this cheap material had almost entirely replaced traditional lath and plaster in wall construction. Although gypsum plaster is still applied directly to the inner "leaf" of cavity walls constructed from building blocks or brick, gypsum board is used as a wall component in much of the present built environment.

The cardboard and starch glue of gypsum board are of course highly biodegradable sources of carbon for moulds, and relatively little water is required to raise the a_w of the wrapper to a level which will sustain mould growth. This water can come from condensation or, as cardboard is hygroscopic, can be taken up from the atmosphere. Where there is gradual moistening of the wrapper, as condensation worsens over a winter, for example, a succession of moulds may appear. Given sufficient input of moisture, the succession may culminate in *Stachybotrys chartarum* or other hydrophilic fungi, as described by Grant *et al.* (1989) for wallpaper and discussed earlier in relation to moisture relations.

A representative range of fungi that grow on gypsum board can be gauged from Table 7, which presents records of fungal isolations from more than 2000 samples of mouldy wallboard in six buildings in North America. Four of these buildings were in mid-continental Pacific coastal areas and two in the Atlantic Northeast. Although the samples were taken in areas the width of the continent apart, no major geographical differences were evident in the species found, which are recognized as being cosmopolitan (Ellis 1976; Klich and Pitt 1988; Pitt 1988). However, Table 7 shows that there were differences in the prevalence of some fungi found in the buildings in the Maritime Pacific climatic zone. For example, *Penicillium citrinum* was relatively more prevalent in Building 1 than in the others, as were *P. glabrum* and *Memmoniella echinata* in Building 5. Pitt (1988) has suggested that *P. citrinum* may well be one of the most common eukaryotic life forms on Earth and noted that it is a powerful biodeteriogen and is found worldwide in soil, decaying vegetation and air. He also states that although most monographers consider that *P. glabrum* is also cosmopolitan, and it is widely distributed in soils, he believes that this biodeteriogen is much less common than *P. spinulosum*. As

mentioned earlier, *M. echinata* is a strongly cellulolytic species which can occupy the same niche as *Stachybotrys chartarum*, and indeed supplant it on cellulosic substrates in tropical climates.

When Pearson correlations (Ludwig and Reynolds, 1988) were run comparing the six previously mentioned North American buildings and their species lists, an association was noted between the gypsum wallboard mycobiota of two buildings in Maritime Pacific climatic areas, viz. Buildings 1 and 4 ($r^2=0.648$).

Table 7. Filamentous fungi isolated from 21 34 samples of mouldy gypsum wallboard taken in six buildings in USA and Canada, with taxa in descending order of prevalence overall.

Building:	Samples contaminated by listed taxa (%)					
	#1 ^{a*}	#2 ^{b**}	#3 ^{at}	#4 ^{at}	#5 ^{at}	#6 ^{b†}
<i>Chaetomium globosum</i>	55.2	27.5	8.9	66.7	12.9	7.0
<i>Penicillium viridicatum</i>	32.1	60.5	49.1	22.2	58.1	-
Non-sporulating isolates	41.7	9.5	14.3	94.4	35.5	51.0
<i>Eurotium herbariorum</i>	36.3	27.5	5.4	27.8	22.6	8.5
<i>Penicillium aurantiogriseum</i>	22.8	47.3	19.6	22.2	-	45.1
<i>Penicillium citrinum</i>	32.1	3.8	4.5	5.6	3.2	2.8
<i>Stachybotrys chartarum</i>	25.8	1.7	11.6	11.1	77.4	60.1
<i>Aspergillus sydowii</i>	12.7	2.0	0.9	44.4	3.2	-
<i>Penicillium chrysogenum</i>	6.6	12.9	4.5	2.8	12.9	10.0
<i>Penicillium commune</i>	0.7	37.0	-	2.8	35.5	4.2
Basidiomycetes (clamp connections)	3.1	15.2	25.9	19.4	-	-
<i>Eurotium repens</i>	7.4	0.9	-	8.3	6.5	-
<i>Memmoniella echinata</i>	4.3	1.7	4.5	2.8	22.6	-
<i>Aspergillus versicolor</i>	4.2	-	0.9	30.6	16.1	25.4
<i>Paecilomyces variotii</i>	1.9	4.6	5.4	8.3	-	7.0
<i>Cladosporium sphaerospermum</i>	2.4	1.1	3.6	-	9.7	5.6
<i>Penicillium oxalicum</i>	2.5	0.3	-	19.4	-	-
<i>Aspergillus niger</i>	1.1	5.7	-	8.3	9.7	15.5
<i>Penicillium corylophilum</i>	2.2	1.4	2.7	2.8	3.2	11.3
<i>Penicillium decumbens</i>	0.7	4.3	5.4	-	-	2.8
<i>Trichoderma harzianum</i>	0.7	-	15.2	-	3.2	1.4

<i>Penicillium fellutanum</i>	0.7	0.6	10.7	-	-	4.2
<i>Penicillium glabrum</i>	0.4	2.6	-	-	19.4	1.4
<i>Aspergillus ustus</i>	0.8	-	-	-	22.6	12.7
<i>Penicillium variable</i>	0.7	-	8.0	-	-	10.0
<i>Talaromyces flavus</i>	0.3	1.4	-	25.0		

^a Located in mid-continental Pacific coastal area; ^b Located in Atlantic Northeast

* Single water event taking approx. 3 months to dry, exposed to rainwater during this period

** Single water event taking approx. 3 months to dry

† Subjected to more or less chronic water infiltration for months to years

As noted in Table 7, Building 1 underwent a major flood, which was followed by rainwater penetration for several months afterwards, whereas Building 4 had a chronic water leak. The mycobiota of Buildings 2 and 3 were also associated ($r^2=0.740$). In this case, Building 2 had suffered a single water incursion, which was followed by slow drying without any further ingress. The history of Building 3 was quite different, over several years there had been chronic cycles of water incursion followed by periods of dryness.

Analysis of the more xerophilic species isolated (Table 7) showed that *Eurotium repens* was associated with *Aspergillus sydowii* ($r^2 = 0.614$) and *E. herbahorum* ($r^2=0.749$), apparently reflecting similarities in a_w and substrate preferences. Among less xerophilic species (Table 1), *Penicillium commune* was associated with *P. chrysogenum* ($r^2=0.821$) and *Paecilomyces variotii* ($r^2=0.649$). The less xerophilic species *P. chrysogenum*, *Paecilomyces variotii* and *P. aurantiogriseum* were negatively correlated to the more xerophilic *A. sydowii*, *A. versicolor* and *E. repens* ($r^2=-0.666$, -0.688 , -0.600 respectively). *A. sydowii* was not associated with *A. versicolor*, indicating that these closely related species, which are common on gypsum wallboard, have different a_w requirements for growth on this substrate. The published minimal a_w for growth of the former under laboratory conditions is 0.78–0.81, and the latter 0.74–0.79 (Table 1).

Among four species which were more hydrophilic and were frequently isolated from the wallboard in the six buildings, viz. *Chaetomium globosum*, *Cladosporium sphaerospermum*, *Memmoniella echinata* and *Stachybotrys chartarum*, the first-mentioned was negatively correlated to *C. sphaerospermum* ($r^2=-0.603$) and not associated with either *M. echinata* or *S. chartarum*. Although *C. sphaerospermum* was isolated less frequently than the other three species, it was strongly associated with both *S. chartarum* and *M. echinata* ($r^2=0.919$ and 0.778 , respectively). *S. chartarum* was associated with the closely related species *M. echinata* ($r^2= 0.649$). This suggests a clear ecological difference between the most common species on gypsum wallboard, *C. globosum*, and the other species. For both hydrophilic species and moderate xerophiles the statistical analyses divided up the clusters of organisms according to their known biology. The clear separation of *C. globosum* from the other hydrophilic species is surprising, however.

When Gravesen *et al.* (1997) investigated water-damaged buildings they reported that

the materials most vulnerable to attack by moulds were those containing cellulose. The genera of microfungi that they isolated most frequently from gypsum board and other materials were *Aspergillus* and *Penicillium* (Table 8). They were present in 56 and 68% of samples, respectively. About one-fifth of samples were contaminated by *Ulocladium* and the strongly cellulolytic genera *Chaetomium* and *Stachybotrys*. The species encountered most frequently were *S. chartarum*, *P. chrysogenum* and *A. versicolor*.

In subsequent studies in Copenhagen, Nielsen *et al.* (1998a,b) obtained evidence that gypsum board could support mycotoxin production as well as growth of *S. chartarum*. Hydrolysed extracts of heavily *S. chartarum*-contaminated gypsum board from either side of a wall frame in a school and from a basement wall in a house contained verrucarol, a strong indicator of toxic macrocyclic trichothecenes, probably satratoxins (Nielsen *et al.* 1998a). When old and new gypsum board was artificially inoculated with this species, there was again evidence of toxin production by *S. chartarum* (Nielsen *et al.* 1998b), but none of a range of trichothecenes was produced by eight strains of another cellulolytic genus, *Trichoderma*, which were also found on gypsum board in the field (Tables 7 and 8). Although not notable as a cellulolytic species, *A. versicolor* grew and produced large quantities of sterigmatocystin and 5-methoxysterigmatocystin on gypsum board, and also on wallpaper, pine wood and chipboard pieces (Nielsen *et al.* 1998b).

Table 8. Principal mould genera isolated from 72 samples of mould-affected building materials (Gravesen *et al.* 1997).

Genus	Samples (%)
<i>Penicillium</i>	68
<i>Aspergillus</i>	56
<i>Chaetomium</i>	22
<i>Ulocladium</i>	21
<i>Stachybotrys</i>	19
<i>Cladosporium</i>	15
<i>Acremonium</i>	14
<i>Mucor</i>	14
<i>Paecilomyces</i>	10
<i>Alternaria</i>	8
<i>Verticillium</i>	8
<i>Trichoderma</i>	7

Also in Europe, the species composition of mould growths on gypsum board and gypsum-plastered solid walls finished with wallpaper or cellulose ether-based paints in damp houses was recorded by Hunter and Bravery (1989). They observed that

Cladosporium was present in about 70% of mould patches (and a predominant component in more than four-fifths of these), in contrast to occurring in only 15% of samples of mould-affected materials examined by Gravesen *et al.* (1997). Hunter and Bravery (1989) recorded that *Ulocladium* spp. (74%) and *Penicillium* spp. (58%) were also frequently isolated (Table 9), but *Aspergillus* (mainly *A. versicolor*) and *Stachybotrys* were much less frequent. *Chaetomium* was apparently absent, but it is possible that its isolation would not have been favoured by the method employed, i.e. surface swabbing followed by plating of serial dilutions prepared from the swabs (Hunter *et al.* 1988).

Although there are differences in the prevalence of different taxa between these studies it is not surprising that there are strong qualitative resemblances in the mycobiota on

Table 9. Principal fungi in mould growths on walls in dwellings, according to percentage frequency of isolation from growths (after Hunter and Bravery 1989).

Species	Predominant (%)	Present (%)
<i>Acremonium strictum</i>	8	11
<i>Alternaria alternata</i>	3	3
<i>Aspergillus</i> spp. (mainly <i>A. versicolor</i>)	8	18
<i>Aureobasidium pullulans</i>	8	16
<i>Cladosporium</i> spp. (<i>C. cladosporioides</i> and/or <i>C. sphaerospermum</i>)	61	71
<i>Fusarium moniliforme</i>	-	13
<i>Geomyces pannorum</i>	5	18
<i>Geotrichum candidum</i>	-	5
<i>Mucor</i> spp.	-	3
Non-sporing isolates	3	24
<i>Paecilomyces variotii</i>	-	5
<i>Penicillium</i> spp. (incl. <i>P. brevicompactum</i> , <i>P. chrysogenum</i> , <i>P. glabrum</i> , <i>P. jancweskii</i> and <i>P. viridicatum</i>)	39	58
<i>Phoma herbarum</i>	5	18
<i>Scopulariopsis brevicaulis</i>	-	3
<i>Stachybotrys chartarum</i>	5	13
<i>Ulocladium</i> spp. (<i>U. chartarum</i> and/or <i>U. consortiale</i>)	39	74
Yeasts	26	76

gypsum board and other cellulosic constructional and finishing materials. As in the studies

of fungi on exposed cotton fabrics in tropical environments (Siu 1951; Upsher 1972), there may be some that are regarded as “hot climate” types (Upsher 1972), but most are ubiquitous. What develops on the substrate depends not only on nutrient availability, but also on the presence of toxic substances in the substrate, on temperature and on moisture. These factors also modify the inter-specific interactions that are important in the development of the mycobiota.

Other Materials

Plastics

It was stated earlier that the materials which are most susceptible to microbial growth and biodeterioration contain organic polymers found in the natural environments. This does not mean, however, that synthetic polymers will not support or be degraded by microbes. For example, various types of synthetic rubber and plastic suffer microbial biodeterioration. Although natural rubber is one of the most resistant of natural polymers, some microorganisms are able to metabolize its long chains of repeating isoprene units. Based on experiments with a nocardioform member of the Actinomycetes and two other coryneform bacteria (Linos and Steinbüchel 1996), synthetic styrene-butadiene rubber appears to be more susceptible to attack than other types of synthetic rubber tested. Nitrile, silicone and neoprene rubber appear to be extremely resistant to microbial attack. Among plastics, polyesters such as polycaprolactone and polybutylene adipate are susceptible to microbial attack, but others such as those derived from phthalates or aromatic hydrocarbons are not. Polyurethanes comprise a wide range of materials—elastomers, surface coatings, adhesives and both flexible and rigid foams—in which urethane is the predominant component and other groups such as esters and ethers are incorporated. Polyether-containing polyurethanes are more susceptible than polyester polyurethanes, with fungi being known to cause cracking in some of the latter type (e.g. polycaprolactone polyurethane) in as little as 10 days. Localized cracking round fungal colonies may be the result of loss of plasticity caused by attack on susceptible plasticizers based on adipates of sebacates which are included in the finished product (Allsopp and Seal 1986).

Urea-formaldehyde foam insulation

Another example of a substance “new” to microorganisms is urea-formaldehyde resin, which is used in adhesives, laminates, and finishes for textiles. Another form, urea-formaldehyde foam insulation (UFFI), has been noted earlier in this chapter as being colonized by fungi. Because of a possible health hazard due to off-gassing, in Canada use of UFFI was banned at the end of 1982, but not before it had been installed in an estimated 80,000 homes, either in the cavities in masonry walls of older houses or the spaces between studs in frame houses. The reaction between the urea-formaldehyde resin and the acidic hardening agent in the injected mixture is reversible, and the foam slowly deteriorates after installation. Although this may contribute to fungal growth, freshly injected UFFI may contain 75% water by weight and the release of water during curing, condensation on the surface of the foam and/or leaks are considered to be the main

reasons for growth. Urea is of course a source of nitrogen, which is usually the limiting nutrient for the growth of fungi in nature, and can be used as such for the growth of a wide range of microorganisms.

As the report of Bissett (1987) and Table 4 illustrate, UFFI may be extensively contaminated by moulds. Table 4 shows that, as in a variety of insulation materials (Dudney *et al.* 1982), in UFFI which had been in service for 5–10 years the common phylloplane species *Cladosporium cladosporioides*, *Alternaria alternata*, and *C. herbarum* were frequently present (Miller *et al.* 1984). However, one-half of the samples were contaminated by *Penicillium fellutanum*, an organism associated with decaying organic material and, by inference, tolerant of high concentrations of formaldehyde. The presence of *P. fellutanum* on the samples was negatively correlated with wall cavity formaldehyde concentration. As Table 7 shows, this species also occurs on gypsum board, but is not a major component of its mycobiota. In contrast, the most common species associated with wallboard, *P. viridicatum*, was only present on about 20% of the UFFI samples, and there was lesser contamination by other species. This illustrates that fungal growth on wetted building materials is modulated by not just nutrients and water, but also by the presence of toxic compounds, in this case formaldehyde.

Paint

Mould growth on in-service films of water-based paints in which cellulose ethers are the thickeners has already been mentioned, but it can also occur on oil or solvent-based paints. Moulds which have been isolated include *Alternaria alternata*, *Aspergillus versicolor*, *Aureobasidium pullulans*, *Cladosporium* spp., *Fusarium* spp., *Paecilomyces variotii*, various *Penicillium* spp. and *Ulocladium consortiale* (Ross *et al.* 1968; Allsopp and Seal 1986). As Allsopp and Seal (1986) have pointed out, however, it may not be clear whether moulds on the surface are actually attacking the paint film or not. While some may be utilizing paint components, others may only be taking nutrients from dirt on the surface and disfiguring the film because of their inherent pigmentation (e.g. *Alternaria*, *Cladosporium* or *Ulocladium*) or soluble pigment taken up by the paint (e.g. *Aureobasidium*). In other cases, the damage may be largely physical, with mycelium which is growing on the substratum under the paint, e.g. wood, erupting through and sporulating on the film. Bacteria which have been isolated from oil paint include *Alcaligenes*, *Bacillus* spp. (*B. cereus*, *B. mycoides* and *B. sphaericus*), *Flavobacterium* and *Micrococcus* (Ross *et al.* 1968). The most common form of paint failure caused by bacteria appears to be peeling, i.e. loss of adhesion to the substratum due to biodeterioration of resinous binders in the paint. Where conditions are extremely wet and light is available, paint films of all types may be disfigured by green algae such as *Pleurococcus*, *Stichococcus* and *Trentepohlia*, and by Cyanobacteria such as *Oscillatoria* (Allsopp and Seal 1986).

Dust

While a selection of materials that are susceptible to microbial biodeterioration has been

discussed above, it has to be remembered that virtually no material in a building is immune from microbial growth if there is sufficient moisture available.

Table 10. Filamentous fungi (in descending order of occurrence) isolated from settled dust in 369 homes in Wallaceburg, Ontario, Canada, using intermediate and high a_w culture media.

COMMON	FREQUENT	RARE
<i>Aureobasidium pullulans</i>	<i>Stachybotrys chartarum</i>	<i>Penicillium oxalicum</i>
<i>Alternaria alternata</i>	<i>Ulocladium chartarum</i>	<i>Penicillium implicatum</i>
<i>Penicillium chrysogenum</i>	<i>Paecilomyces variotii</i>	<i>Aspergillus ustus</i>
<i>Aspergillus versicolor</i>	<i>Penicillium griseofulvum</i>	<i>Penicillium crustosum</i>
<i>Eurotium herbariorum</i>	<i>Aspergillus sydowii</i>	<i>Pithomyces chartarum</i>
<i>Epicoccum nigrum</i>	<i>Penicillium citreonigrum</i>	<i>Scopulariopsis Candida</i>
<i>Cladosporium cladosporioides</i>	<i>Penicillium raistrickii</i>	<i>Penicillium echinulatum</i>
<i>Penicillium spinulosum</i>	<i>Penicillium vulpinum</i>	<i>Eurotium repens</i>
<i>Penicillium corylophilum</i>	<i>Scopulariopsis brevicaulis</i>	<i>Penicillium miczynskii</i>
<i>Penicillium commune</i>	<i>Aspergillus fumigatus</i>	<i>Penicillium italicum</i>
<i>Cladosporium sphaerospermum</i>	<i>Penicillium simplicissimum</i>	<i>Penicillium digitatum</i>
<i>Trichoderma viride</i>	<i>Penicillium decumbens</i>	<i>Fusarium oxysporum</i>
<i>Aspergillus niger</i>		<i>Aspergillus candid us</i>
<i>Cladosporium herbarum</i>		<i>Chaetomium globosum</i>
<i>Penicillium expansum</i>		<i>Penicillium glandicola</i>
<i>Penicillium viridicatum</i>		<i>Penicillium purpurogenum</i>
<i>Penicillium brevicompactum</i>		<i>Penicillium islandicum</i>
<i>Wallemia sebi</i>		<i>Geomyces pannorum</i>
<i>Phoma herbarum</i>		<i>Penicillium verrucosum</i>
<i>Aspergillus ochraceus</i>		<i>Penicillium variable</i>
<i>Penicillium aurantiogriseum</i>		<i>Emericella nidulans</i>
<i>Penicillium citrinum</i>		<i>Alternaria tenuissima</i>

In the previous paragraph and earlier in the chapter, the role of dirt, viz. dust and organic debris, in supplying nutrients for microbial growth has been referred to. It enables

microorganisms that cannot degrade particular organic materials, e.g. cellulose, to grow on the surface of these materials. Even where the material is entirely inorganic (e.g. steel, glass, brick, concrete or stone), soiling with dust, organic debris and volatile substances which condense on its surface can allow microbial growth to take place. As well as being a source of nutrients, settled dust is an important reservoir of dormant microorganisms which may be dispersed into the air. For example, Cole *et al.* (1994) isolated 1.6×10^4 – 1.2×10^5 colony forming units (CFU) of fungi g^{-1} carpet dust in a 4-storey multi-use building, as well as 1.4×10^6 – 8.8×10^6 mesophilic bacteria and 5×10^2 – 7×10^3 thermophilic bacteria (including Actinomycetes). The mixture of fungi present in settled dust includes species exhibiting varying degrees of xerophily and also others regarded as being more hydrophilic.

Table 10 shows that the species prevalence in settled dust in family dwellings is normally dominated by fungal propagules originating outdoors. However, three kinds of change appear. Firstly, since these data are gathered from studies of culturable fungi, species with long-lived propagules gain in apparent importance, including various types of yeast. Secondly, soil-borne fungi are tracked into buildings. Thirdly, food-associated fungi become important components of the mycobiota. In the list of common fungi in settled dust in Table 10, *Penicillium expansum*, *P. digitatum* and *P. islandicum* are most likely to have come from fruit brought into the house. Another category in Table 10 comprises species such as *P. chrysogenum*, which may originate from food, but may sometimes also grow on building materials. Among the houses surveyed, the ratio of the combined total of *Aspergillus*, *Eurotium* and *Penicillium* to the phylloplane species or of all others to the phylloplane species approached unity in the settled dust of those in which fungal growth was absent (Miller 1995). Concentrations of fungi in settled dust on media for xerophilic fungi are typically 10^4 – 10^5 CFU g^{-1} dust m^{-2} floor (Lawton *et al.* 1998, Dales *et al.* 1997, Verhoeff 1994), with concentrations on carpeted floors (CFU m^{-2}) being 3–4 times higher (Verhoeff 1994). Comparison of the list in Table 10 with, for example, species isolated in Japan (Takatori *et al.* 1994) and Netherlands (Hoekstra *et al.* 1994; Verhoeff 1994) confirms that there is a strong similarity in the floor dust mycobiota of buildings in different geographical regions.

Notably absent from Table 10 is *Chaetomium globosum*, although it is common on mouldy gypsum board (Table 7). This may reflect its relative absence from the particular set of houses surveyed, but what is more likely is that it reflects the difficulty of isolating this organism and also the short survival time of its propagules. In contrast, one of what are known as black yeasts, *Aureobasidium pullulans*, and various other yeasts are exceptionally common in house dust, as it appears that their propagules survive for very long periods. Black yeasts are actually yeast-like states of various filamentous fungi such as *Aureobasidium*, *Cladosporium*, *Moniliella* and especially anamorphs of ascomycete genera in the Herpotrichiellaceae, including the pathogen *Exophiala*. Among phylloplane fungi, *Alternaria alternata* and *Epicoccum nigrum* propagules must also survive longer than *Cladosporium cladosporioides* and *C. herbarum* (and *C. sphaerospermum*), because their prevalence is much greater than that indicated in outdoor air. Data such as these strongly reinforce the importance of evaluating the relative persistence of fungal propagules when considering the order of species in laboratory reports.

Aspergillus versicolor, *Eurotium herbariorum*, *Penicillium commune*, *P. viridicatum* and *P. aurantiogriseum* are all common in dust (Table 10) and also on mouldy gypsum board (Table 7), indicating the likelihood that this wall material is an important source of these species in the settled dust. Although it is not certain, moulds such as *P. spinulosum* and *P. brevicompactum* probably grow on some other damp material, such as carpets. The last of the common moulds in house dust (Table 10), *Wallemia sebi*, is an extreme xerophile. This species has been reported as common in house dust from Japan (Sakamoto *et al.* 1989) and the Netherlands (Verhoeff 1994). In Japan, it was associated with floor mats (Torii *et al.* 1990), but the source of this fungus in the Canadian and Dutch investigations is not known.

Qualitatively, the culturable mycobiota of dust in upholstered furniture (Schober 1991) and mattresses (Hoekstra *et al.* 1994) is similar to that of floor dust. Schober (1991) did, however, note that hydrophilic fungi such as *Alternaria* and *Rhizopus* were more abundant in carpet dust. Counts of slightly xerophilic types (dominated by *Penicillium brevicompactum* and *P. chrysogenum*) were significantly greater in the case of upholstered furniture, where conditions were drier and more closely related to the ambient climate than in carpets, hence fewer hydrophiles in upholstery. Notwithstanding, Schober (1991) did make the point that regular use of upholstered furniture modifies conditions as a result of uptake of moisture generated by the users. This would clearly apply to mattresses, although Hoekstra *et al.* (1994) found that the higher frequency of isolation of *Scopulariopsis brevicaulis* from mattress dust was the only noteworthy difference from the moulds and yeasts isolated from floor dust.

Although many of the microorganisms in dust on floors, shelves, upholstered furniture, etc., are there because their propagules have sedimented out of the air, they may also have proliferated in the dust. Not only are skin scales, animal dander and other organic debris a source of nutrients for growth, but to varying degrees the debris is hygroscopic and can absorb moisture from a humid atmosphere. In water saturated atmospheres, total numbers of viable bacteria in floor dust have been found to increase 100-fold in 8 weeks (Flannigan *et al.*, 1992). In addition to such quantitative change, the qualitative nature of the microbiota changes, with pseudomonads replacing as the predominating bacteria the Gram-positive types (*Staphylococcus*, *Micrococcus* and *Bacillus*) characteristic of dry dust. Although the species of *Penicillium* and *Eurotium* in dry dust may still dominate the mycobiota, the range of moulds widens as the dust absorbs increasing amounts of water from the atmosphere, with members of the Mucorales being prominent where the atmosphere is saturated.

Just as counts of culturable fungi increase when dust is damp enough to allow proliferation of moulds already resident in it, so they also increase when propagules of fungi growing on damp building materials sediment out into dust. For example, in houses where an area of mould growth visually assessed as 1.2 m² was present there was a 100-fold increase in counts of culturable propagules enumerated on media for xerophilic fungi relative to where there was no such mould growth (Lawton *et al.* 1998). Paralleling observations that fungi growing on building materials alter the ratio of phylloplane to other fungi in the air (Nathanson and Miller 1989; Miller 1993). Lawton *et al.* (1998) found that such growth also had an effect on the mycobiota of settled dust. The ratio of

the combined total of *Aspergillus*, *Eurotium* and *Penicillium* to the total for phylloplane fungi rose to around 10.

Heating, ventilation and air-conditioning (HVAC) systems

This final section on microbial growth material in buildings turns to HVAC systems because of the range of niches that they present, ranging from standing water to absorptive porous material.

Although central and window air-conditioning has the general effect of reducing the number of airborne microorganisms relative to outdoor air (see Flannigan *et al.* 1991), if incorrectly operated or maintained the complex HVAC systems such as are installed in many large public buildings and non-industrial workplaces may become a source of microorganisms which contaminate the conditioned space. Surfaces such as those of the filters, coils, mix box areas, drainage pans, areas where flow is not continuous, and exposed porous insulation, are sites where dust and other particles may accumulate and be a nutrient base for microorganisms. Where there is standing water, as in drain pans and humidifiers, or moisture is absorbed by porous insulation or dust, particularly in the vicinity of cooling coils and humidifiers and on airstream surfaces downstream of cooling coils, microbial growth becomes possible.

Table 11 illustrates the viable bacteria isolated from a range of sites and in an air-handling system lacking humidifiers and duct insulation. The range of bacterial types may be regarded as typical of air-handling systems, although it is the first record of the budding bacterium *Blastobacter*, an organism associated with outdoor aquatic environments. Among bacteria that may be found in humidifiers and other HVAC equipment are *Saccharopolyspora* (*Faenia*) *rectivirgula* and *Thermoactinomyces vulgaris*, thermophilic members of the Actinomycetes which have also been isolated from domestic heating systems (Fink *et al.* 1971).

Table 11. Bacteria in a well-maintained air-handling system (AHS) in Brisbane, Australia (after Hugenholtz and Fuerst 1992).

Category of Bacterium	Location in system
<i>Blastobacter</i> sp. (pink)	Surface of supply coils
<i>Blastobacter</i> sp. (yellow)	Drain pan water
	Post-coil air
	Supply air
<i>Flavobacterium</i> spp. (esp. <i>Fl. odoratum</i>)	Evaporative condenser sump water
	Surface of air intake grill
	Return-fresh air mix

	Surface of return coils
	Drain pan water
	Supply air
<i>Bacillus</i> spp., <i>Pseudomonas</i> spp. Species of <i>Acinetobacter</i> , <i>Arthrobacter</i> <i>Cedecea</i> , <i>Corynebacterium</i> <i>Staphylococcus</i>	Various sites—air, water and/or surfaces Sporadically throughout AHS— air, water and/or surfaces

Although they did not find thermophilic Actinomycetes among the bacteria on filters, Heinemann *et al.* (1994) noted that *T. candidus*, a species related to *T. vulgaris*, was more abundant than the latter in HVAC spray humidifiers. As with the bacteria, fungi isolated from filters were not numerous, but included the thermotolerant opportunistic pathogen of man, *Aspergillus fumigatus*. Another such pathogen, *Exophiala jenselmei*, was isolated from nearly 50% of humidifier reservoirs, but along with *Phoma*, *Acremonium* and other species in the humidifier water was never found in the air of the offices served by the HVAC systems.

Porous acoustic or thermal insulation installed on the airstream surface of HVAC components and ducts may be colonized by moulds. Where the insulation is covered by a metal foil or plastic-type facing material, and this remains intact, mould growth appears to be limited to the surface. Relatively few fungi develop on metal foil, but plastic facings may be densely covered by heat resistant and xerophilic fungi such as *Byssoschlamys* and *Eurotium* (Ahearn *et al.* 1993). However, where the facings are breached the exposed fibreglass matrix becomes contaminated with dust and organic debris and will absorb moisture from the system. It has been reported that in atmospheres with a RH>90%, such insulation can be colonized by the moulds *Acremonium obclavatum*, *Aspergillus niger*, *A. versicolor*, *Cladosporium herbarum* and *Penicillium miczynski* (Ezeonu *et al.* 1994). Laboratory experiment also showed that aqueous extracts of fibreglass insulation, containing urea, formaldehyde and unidentified organic compounds, supported spore germination and growth in a representative species, *A. versicolor*, with urea serving as a nitrogen source (Ezeonu *et al.* 1995).

With the potential of HVAC systems for dispersing microorganisms throughout buildings, the importance of preventing and controlling microbial contamination of HVAC cannot be stressed enough. For further reading on this, the reader should consult Shaughnessy *et al.* (1999). More extended treatments of moisture problems in general are to be found in Rose and Tenwolde (1993) and Flannigan *et al.* (1996).

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Chapter 2.2

BACTERIA AND OTHER BIOAEROSOLS IN INDUSTRIAL WORKPLACES

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INTRODUCTION

At the outset, it is probably of value to define what shall and shall not be covered in this chapter in the context of other chapters. Firstly, the definition of “industrial workplaces” may overlap with other environments. For example, there may be no clear distinction between indoor work environments handling produce and livestock, i.e. the point at which they cease to be agricultural and become food production and/or industrial work is unclear. Similarly, hospitals and laboratories as work environments may have much in common with non-industrial (domestic and office) environments with regard to air quality, while at the same time overlapping with industrial workplaces if these are taken to include pilot- and small-scale biotechnology operations.

The potential health consequences of exposure to bioaerosols in industrial workplaces remain the same across the broad spectrum of the definition, respiratory infection, allergic respiratory disease and toxicological response to microbial cell components. The mechanisms by which these diseases occur will be described in detail in Section 3.

This chapter aims to describe:

- the circumstances in which workers may be at risk of exposure to bioaerosols responsible for these respiratory syndromes;
- the agent(s) involved; and
- typical concentrations, as measured in various research investigations into respiratory ill-health.

The methods used to measure exposure to bioaerosols in industrial workplaces have recently been reviewed elsewhere (Crook and Olenchock 1995; Eduard and Heederik 1998) and therefore will not be repeated here.

RESPIRATORY INFECTIONS CAUSED BY EXPOSURE TO BIOAEROSOLS IN INDUSTRIAL WORKPLACES

With the exception of ubiquitous and not work related colds and influenza, respiratory infections likely to be encountered in industrial workplaces are rare. However, some

infectious agents transmitted via bioaerosols can be encountered in the workplace and in some circumstances may be life-threatening.

Legionnaires' Disease

The infection probably causing the greatest impact in industrial workplaces is Legionnaires' Disease, a life-threatening pneumonia caused by inhalation of the bacterium *Legionella pneumophila*. More detailed descriptions of the biology of *L. pneumophila* lie outside of the scope of this section but can be found in detail in Chapter 3.4. This ubiquitous water-borne bacterium is readily capable of multiplying in ambient to hot water systems. Therefore, it presents a significant hazard in industries where hot processes are controlled using cooling waters (e.g. in cooling towers and evaporative condensers), if the workplace or its surroundings is exposed to aerosols or airborne droplets of contaminated cooling water. Extensive research has increased understanding of the ecology and pathology of this organism, and therefore should help to control exposure. It is still a major cause of respiratory infection and death each year, however, as exemplified by occasional outbreaks in industrial premises (Castellani-Pastoris *et al.* 1997; Heng *et al.* 1997; Anon. 1998; Breiman and Butler 1998). The bacterium can proliferate in biofilms which give protection from physical and chemical control agents. In industrial water sources, particular problems are associated with stagnation points such as dead-legs in pipework and unused taps or faucets where *L. pneumophila* can multiply, or in poorly maintained packing elements in cooling towers.

Zoonotic agents causing pneumonias

Other bioaerosols capable of causing respiratory infection in industrial workplaces are limited and are found especially at the interface between industry and agriculture, where workers may be exposed to zoonotic agents, i.e. those capable of spreading from animals to humans. Examples are described below.

Q fever is a life-threatening respiratory disease caused by the rickettsial bacterium *Coxiella burnettii*, and workers may be at risk where they are exposed to contaminated dusts. Q fever presents acutely with fever, headache and other influenza-like symptoms, or as atypical pneumonia. Less frequently, it may present as chronic infection including endocarditis. *C. burnettii* can survive for a long period in dust, which can assist its spread and infectivity. It is highly infective, as is evident from an outbreak attributed to windborne spread of dust from agricultural land into a conurbation (Hawker 1998). However, industrial exposure is limited, although a cluster of cases in postal workers was attributed to their exposure to contaminated dust in mail sacks from rural postal delivery (Winner 1987).

Another zoonotic bacterium, *Chlamydia psittaci*, can cause respiratory infection in humans exposed to dusts contaminated by infected birds, especially psittacines and pigeons. As with Q fever, psittacosis presents as 'flu-like symptoms or an atypical pneumonia. Outbreaks have been reported in poultry process workers whose work involved evisceration (Newman *et al.* 1992) and infrequent individual cases occur in pet

shop workers exposed to infected cage birds (Moroney *et al.* 1998). Investigations have been made in the authors' laboratory into other possible industrial cases of psittacosis (Higham, unpublished). The etiology of three separate instances of work related respiratory complaints were studied. These included a worker at a carpet factory exposed to dust from imported jute; the custodian of a building whose task it was to sweep up dust contaminated by pigeon droppings; and demolition workers who were clearing a roof space of several kilograms of pigeon droppings. No direct causal link was established, despite the use of sensitive molecular-based assays to search for the infectious agent in both airborne and settled dust, but it nevertheless highlights areas of potential industrial exposure.

Inhalation anthrax

Industrial anthrax can be contracted via the inhalation route. The spore-forming Gram-positive bacterium *Bacillus anthracis*, the causal agent of anthrax, causes endemic infection in domestic animals, especially cattle, sheep and goats, in a broad belt of the tropical zones spreading from Africa and Eastern Europe, through Asia to China (Hugh-Jones 1996). Spores of *B. anthracis* are shed into soil and vegetation by infected animals, where they can survive for many years (Turnbull 1996), and their presence in dust can result in contamination of animal hair, especially goat hair, and hides. The spores can become airborne in dust during manufacturing processes and goat hair, such as cashmere, has been particularly associated with anthrax spore contamination in the past (Crook *et al.* 1996).

In the early 1900s, anthrax was an important industrial disease in UK, especially in the Yorkshire woollen mills, where it was referred to as "wool sorter's disease". Inhalation anthrax affects the mediastinal lymph system and the lungs (Brachman 1986). Inhaled spores are phago-cytised by alveolar macrophages and transported into the lymph system where they germinate, multiply and produce toxins which cause toxæmia. Clinical effects include non-specific upper respiratory tract infection, a general malaise, fatigue and mild fever with cough, followed by sudden onset of severe respiratory distress with a rise in temperature and in respiratory and pulse rates, and a fall in blood pressure. Death usually occurs within 24 hours of the onset of this acute phase. The workers who were at greatest risk were those sorting fleeces by hand to remove debris. However, improved hygiene conditions and diligent control measures, including exhaust ventilation, reduced to a minimum the incidents of reported human infections in industrialised countries. Only seven cases of the infection, these being cutaneous rather than inhalation infection, have been reported in the last 10 years in UK (Turnbull *et al.* 1996). However, earlier case reports and classic animal inhalation exposure studies verified its position as an infection potentially caused by bioaerosol exposure in the industrial workplace.

An example of incidental exposure was the death from inhalation anthrax of a man working in a factory next door to a goat-hair processing plant (LaForce 1969). Spores were recovered not only from machinery, hair and the air in the plant, but also from the factory where the infected worker was employed. In Switzerland, during the late 1970s and early 1980s, 25 workers in one textile factory handling goat hair from Pakistan

contracted anthrax (Pfisterer 1991). Of these, 24 had cutaneous anthrax and one inhalation anthrax. Studies at a mill in USA where outbreaks of anthrax had occurred revealed airborne *B. anthracis* at concentrations up to 300 spores m^{-3} air, this being between 0.1 and 0.9% of the total number of airborne bacteria detected (Dahlgren *et al.* 1960). Infectivity was demonstrated by drawing contaminated air from the process into exposure chambers housing monkeys. The disease therefore remains a hazard in the textile industry, although because of improved control measures it is one with low risk. Other potential sources of industrial anthrax are contaminated animal skins and hides in tanneries, and, because of the long survival potential of the spores, also in dusts created during remediation of old tannery sites. The presence of anthrax spores has been detected in soil along drainage courses from old tanneries (Bohm and Otto 1996). In building work, it was the practice until less than 100 years ago to reinforce plaster work with animal hair. Therefore, any demolition of old buildings may expose workers to anthrax spores surviving in dusts if the animal hair was contaminated. Although the likelihood of exposure is small, it should nevertheless be recognized and suitable control measures taken, since spores have been identified in plasterwork (Turnbull *et al.* 1996).

Other infectious bioaerosols

Other potential sources of infectious bioaerosols arise from waste water treatment. Here, workers may be exposed to coliform bacteria and viruses. Incidents of infections in waste water workers have been reported recently, such as hepatitis A infection (Brugha *et al.* 1998). Previously, an association was made between higher levels of antibody to Norwalk virus and inexperience in wastewater treatment workers (Clark *et al.* 1985), but in all cases the role of inhalation (as distinct from ingestion) as a route of infection is not clear. Waste water treatment may also represent a toxigenic bioaerosol hazard which will be discussed later.

Although we are concerned in this chapter primarily with bacteria, it should be noted at this point that fungal bioaerosols represent an unusual respiratory infection hazard, mainly associated with immunocompromised hosts. Probably the most prominent is *Aspergillus fumigatus*, a fungus ubiquitous in the environment. Occasional cases are reported of infection following exposure to contaminated dusts generated during building demolition or renovation (Anderson *et al.* 1996b). This can create an infection risk for hospitalized patients exposed to bioaerosols generated from such building work. However, construction work can also lead to exposure to bioaerosols which have been implicated in 'flu-like illness, but this was considered not to be infection but allergic response (Epling *et al.* 1995). *Penicillium* spores were isolated from the air at various points during reconstruction work at an airport in concentrations up to 4000 colony forming units (CFU) m^{-3} air sampled. It was speculated that exposure to these spores, or mycotoxins associated with them, or to some other bacterial bioaerosol present, contributed to workers' respiratory ill-health.

BIOAEROSOLS IN INDUSTRIAL WORKPLACES AS ALLERGENS AND TOXICANTS

Any industrial work process that can expose the workforce to large concentrations of airborne micro-organisms or their products potentially can elicit respiratory syndromes such as occupational asthma, bronchitis or rhinitis, mucous membrane irritation, allergic alveolitis (hypersensitivity pneumonitis) or inhalation fever (ODTS). The potential sources therefore are many and varied. Broadly, industrial workplace biological hazards can be divided into three categories:

- workplace activities which involve purposeful handling of products containing biological agents, for example, in biotechnology, or composting;
- where workplace materials are contaminated by biological agents; or
- where sources of bioaerosols are incidental to the main work activity, e.g. contamination of humidifiers and air conditioners.

Biotechnology

In industrial biotechnology, workers may be exposed to the process microorganisms or their components at various stages, particularly at the downstream processing stage, including centrifugation, product concentration and waste handling (Crook 1996). Occasional cases of occupational asthma and mucous membrane irritation have been reported (Topping *et al.* 1985; Carvalheiro *et al.* 1994) in “low technology” biotechnology, i.e. more conventional brewing and enzyme production. Exposure to bacterially derived proteases in detergent production is a recognized cause of industrial respiratory allergy (Flindt 1995; Johnsen *et al.* 1997). Reports of ill-health among workers in biotechnology are rare, even though the potential exists because of the process organisms used (Bennett *et al.* 1991). For example, a common process organism is *E. coli*. This could give rise to exposure to endotoxin (See Chapter 3.6), a component of the cell walls of Gram-negative bacteria (Palchak *et al.* 1988; Crook 1996). However, because containment is a requirement, not only to ensure product purity but also for environmental safety, especially if the process organism is genetically modified, this provides the worker with protection.

Composting

Exposure to bioaerosols is less controlled in compost production, where the presence of microorganisms is fundamental to the composting process. Composting involves enhanced multiplication of a broad range of thermotolerant fungi and thermophilic Actinomycetes to degrade the organic matter present. A succession of microbial groups predominate as the microorganisms present break down lignocelluloses in the substrate and their metabolic activity increases the heat within the compost. Temperatures can reach 80°C in active composting and enhance the growth of thermotolerant and

thermophilic species, many of which are respiratory allergens. Workers handling the compost therefore are potentially exposed to these microorganisms (Lacey *et al.* 1992). Exposure can occur at the following points:

- Turning of windrows (heaps of composting material) to ensure efficient composting. This is likely to be done using tractors or mechanical diggers, but even where these have integral cabs with air filters, they may provide limited protection from bioaerosols. Recent studies highlighted these limitations in agricultural vehicles, where workers may be exposed to similar bioaerosol concentrations (Thorpe *et al.* 1997).

- In mushroom production, the mechanical mixing of composts with mushroom spawn. Composts typically are prepared from horse manure and straw and undergo a two-phase composting process before being inoculated with spawn (mycelium of the common mushroom *Agaricus bisporus*). Spawning may involve mechanical agitation of the compost, which can generate bioaerosols of the compost microbiota. Later in the mushroom production process, disturbance of the compost during mushroom picking can generate bioaerosols of the compost microbiota and contaminant moulds on the surface of the compost, as well as spores from the mushroom themselves as their caps mature and open (Crook and Lacey 1991). Other mushroom species, such as shiitake (*Lentinus edodes*) and *Pleurotus*, may themselves contribute greatly to the bioaerosol as they liberate their spores early in the growth phase (Michils *et al.* 1991; Tarvainen *et al.* 1991; Lenhart and Cole 1993).

- Sorting and bagging of prepared composts. Wood bark is subject to a composting process to stabilize its metabolic activity prior to being prepared for horticultural use. This may involve mechanical screening into different sizes—the largest chips for soil cover and the finer material for soil conditioner—then being put into bags for sale. The screening, bagging and any other movement by tractor or conveyor belt can generate bioaerosols of the compost microbiota (Crook *et al.* 1994).

- In northern European countries and North America, wood chips and bark are used for fuel in homes and as energy sources for power stations. Workers handling the fuel chips may be exposed to bioaerosols generated from microorganisms degrading the wood chips (Hedenstierna *et al.* 1986; Eduard *et al.* 1994; Millner *et al.* 1994).

The microbiota, in which thermophilic Actinomycetes may be prominent, and typical concentrations in bioaerosols measured in composting-associated activities are summarized in Table 1.

Waste disposal

Microbiological activity naturally occurs in any organic waste material, therefore workers disposing of waste may be exposed to bioaerosols generated at various stages of the process. Bioaerosols may be created by domestic waste disposal during collection from domestic and industrial premises and when the material is emptied from waste collection vehicles at waste transfer stations. Subsequently, material may be transferred into bulk containers and taken for disposal at landfill sites, or may be disposed of by incineration. Each process will involve further mechanical handling and the potential for generation of dust aerosols, and any delays during this process will increase the opportunity for multiplication of microorganisms present.

Table 1. Bioaerosols associated with composting. Total no. m⁻³ air.

Activity	Bacteria, incl. Actinomycetes	Fungi taxa	Predominant	Reference
Mushroom compost—preparation and mixing	10 ⁷ –10 ⁸	10 ⁵	<i>Thermomonospora</i> ; <i>Actinomadura</i>	Crook and Lacey 1991; Van de Bogart <i>et al.</i> 1993
Mushroom picking— <i>Agaricus</i>	10 ⁵	10 ⁵	<i>Penicillium</i> ; <i>Trichoderma</i> , <i>Plicaria</i>	Crook and Lacey 1991
Mushroom picking— <i>Pleurotus</i>	ND	ND	<i>Pleurotus</i> spores	Mori <i>et al.</i> 1998
Mushroom picking—shiitake (<i>Lentinus</i> sp.)	ND	ND	>10 ⁶ <i>Lentinus</i> spores	Sastre <i>et al.</i> 1990
Bark composting—screening	10 ⁵	10 ⁷	<i>Rhizomucor</i> , <i>Paecilomyces</i>	Crook <i>et al.</i> 1994
Bark composting—bagging	10 ⁴	10 ⁶	<i>Rhizomucor</i> , <i>Paecilomyces</i>	Crook <i>et al.</i> 1994
Fuel chip preparation	ND	10 ⁴ –10 ⁷	<i>Penicillium</i>	Blomquist <i>et al.</i> 1984; Hedenstierna <i>et al.</i> 1986
Domestic waste	10 ⁴ –10 ⁶	10 ⁴ –10 ⁶	<i>Aspergillus fumigatus</i> , <i>Thermoactinomyces</i> , <i>Thermomonospora</i>	Lacey <i>et al.</i> 1992; Marchand 1995; Nielsen <i>et al.</i> 1997b

ND, not determined

Table 2. Components of domestic waste bioaerosols as airborne concentrations in CFU m⁻³

Airborne concentrations CFU m ⁻³					
Activity	Total bacteria	Gram-negative bacteria	Total fungi	Airborne endotoxin (EU m ⁻³)	Reference

Door-to-door collection	10^3 – 10^4	ND	10^4 – 10^5	50	Nielsen <i>et al.</i> , 1997a; Heldal <i>et al.</i> , 1997
Waste transfer station	up to 10^6	10^5	10^6	ND	Crook <i>et al.</i> , 1987
Incineration	10^7	10^4	10^7	ND	Crook <i>et al.</i> , 1987
Materials recycling (unseparated waste)	10^5	10^4	10^5	9.9×10^3	Poulsen <i>et al.</i> , 1991; Malmros <i>et al.</i> , 1992
Materials recycling (paper source separated)	10^4	10^4	10^4	1.1×10^2	Poulsen <i>et al.</i> , 1991; Würtz and Breum 1997
Landfill	10^6	10^4	10^5	ND	Rahkonen <i>et al.</i> , 1987
Background	10^3	10^1	10^3	ND	Crook <i>et al.</i> , 1987

ND, not determined

Recent initiatives to increase resource recovery have a potential impact on worker exposure. In the European Union, targets have been set for recycling of disposed waste (Department of the Environment 1995; Commission of the European Communities 1996) which have meant that more waste streams are being diverted into labour intensive activities such as materials recycling and reclamation. Some schemes involve separation at source of recyclable materials such as plastics, glass and metal, but further sorting is required at materials recovery facilities (MRF's). In other schemes recyclable materials are separated from organic materials.

Much of this involves manual sorting, which can expose workers to bioaerosols. The organic fraction of domestic waste may be composted, as described earlier. Table 2 summarizes typical reported exposures to bioaerosols during all aspects of domestic waste disposal.

Wastewater treatment

Treatment of wastewater and human sewage involves many processes that are capable of generating bioaerosols, e.g. pumping, screening and aeration, much of which is outdoors, but some of which can be indoors or in confined areas, therefore there is the potential for workers to be exposed to toxigenic bacteria. In a study of airborne bacteria and endotoxin at wastewater treatment plants, total bacterial levels as measured by direct fluorescence microscopy were in the range of 10^7 – 10^9 cells m^{-3} air, viable bacteria 10^2 – 10^5 CFU m^{-3} and up to 3000 endotoxin units (EU) m^{-3} air (Laitinen *et al.* 1992; Melbostad *et al.* 1994). They found good correlation between airborne viable bacteria and endotoxin concentrations.

Disposal of de-watered sewage sludge has mainly been by incineration or landfill, but as the costs of these methods increase and more environmentally sound disposal routes are increasingly required by legislators, composting has emerged as another option for treatment of sewage sludge. However, this may increase the potential for workers' exposure to bioaerosols similar to that described earlier. Recent measurements taken at a sewage sludge composting facility in USA showed that it was a very dusty process, exceeding occupational standards for general dust exposure, and that endotoxin levels were high, ranging from 28.9 ng m^{-3} (289 EU m^{-3}) to 5930 ng m^{-3} (59300 EU m^{-3}) when screening and sweeping operations took place (Darragh *et al.* 1997). Therefore, as sewage sludge composting develops as a disposal option, further consideration will need to be given to reducing workers' exposure to bioaerosols, especially in enclosed facilities as described in this study.

Engineering and manufacturing industries

In manufacturing industry, exposure to bioaerosols may result either from working with contaminated materials or from indirect sources, such as humidifiers or air conditioning where contaminated water sources can lead to gross exposure to waterborne bacteria other than legionellae. Complex fever symptoms and immunological responses have been associated with contaminated air conditioning and humidification systems used in some printing and textile works, the syndrome being described generally as "humidifier fever" (Finnegan *et al.* 1985; Burge *et al.* 1985). However, although in some instances a relationship has been demonstrated between symptoms and antibody response to microbial contaminants (McSharry *et al.* 1993), in other instances exposure to microbial contaminants and endotoxin is low, suggesting a multifactorial etiology, in which microbial exposure may be a single component (Kateman *et al.* 1990).

Studies by one of the authors examined the relationship between water sources and bioaerosols. Bioaerosol samples taken next to humidifiers in textile mills yielded 10^2 – 10^4 CFU bacteria m^{-3} , the lowest concentrations being where water was delivered directly from a piped supply and the greatest where a reservoir of standing water was involved. Airborne fungi were in the range of 10^2 – 10^3 CFU m^{-3} (Crook 1995). In a print works, bioaerosol samples taken immediately prior to a reservoir-fed humidifier being switched on yielded 10^2 CFU m^{-3} , but shortly after the humidifier was switched on airborne bacterial concentrations in the vicinity rose to 10^5 CFU m^{-3} . This demonstrated the potential influence that a source of contamination can have upon bioaerosol concentrations (Crook 1995; Crook and Sherwood-Higham 1997). Gram-negative bacteria predominated in humidifier water and bioaerosol in both instances, typical isolates being pseudomonads and *Flavobacterium* spp.

Exposure to bacterial contaminants occurs in engineering where metals are being cut, drilled and shaped. This is because metalworking emulsions (MWE) are employed; these oil-in-water emulsions are used to cool and lubricate machine tools. They are highly susceptible to attack by bacteria, typically waterborne Gram-negative bacteria such as *Pseudomonas* spp. (Kriebel *et al.* 1997). Because the MWE are recirculated via a sump which either supplies a single machine or which services a number of machines the

opportunity arises for bacterial contamination to multiply in the MWE. This is exacerbated by continued use of the same recirculated MWE, with periodic topping up, often for several weeks. Aerosols of the MWE are generated as it is delivered to the machine parts. Contamination levels have been measured in sumps in the order of 10^7 – 10^9 microorganisms ml^{-1} MWE, of which Gram-negative bacteria accounted on average for 65% of the total (Thorne *et al.* 1996). Similar prevalence was found in the air (Woskie *et al.* 1996). Bioaerosols in engineering facilities measured in the same study showed workers to be exposed to these bacteria at up to 550 CFU m^{-3} of air sampled. The predominant bacteria are pseudomonads, including *Ps. pseudoalcaligenes* and *Ps. alcaligenes*, and Gram-positive *Micrococcus luteus*. Exposure to the pseudomonads present has been associated with immunological response in engineering workers (Matsby-Balzer *et al.* 1989; Travers-Glass and Crook 1994).

Because of the predominance of Gram-negative bacteria, endotoxin is present in large quantities in such systems. Endotoxin levels in sumps may be as high as 166000 EU ml^{-1} MWE and airborne endotoxin in engineering works has been reported at concentrations of 787 EU m^{-3} (Thorne *et al.* 1996). This has been associated with workers respiratory ill health such as bronchitis and fever and flu-like symptoms (Kriebel *et al.* 1997; Rosenman *et al.* 1997).

Fungal contamination is not usually a problem in MWE, because fungi are out-competed by bacteria. In the study of Thorne *et al.* (1996), most bulk samples yielded ≤ 50 CFU ml^{-1} , with airborne levels up to 3800 CFU m^{-3} , but mostly less than 60 CFU m^{-3} air. However, biocidal additives or changes to some formulations, including the use of synthetic oil emulsions, to suppress Gram-negative bacteria could encourage the growth of fungi or even Gram-positive bacteria and acid-fast bacilli. Recent studies in USA have identified other potential etiological agents associated with clusters of hypersensitivity pneumonitis (allergic alveolitis) among workers exposed to MWE. Strong candidates from this study were the acid-fast Gram-positive bacilli *Mycobacterium chelonae*, *M. fortuitum* and *M. abscessus* (Kreiss and Cox-Ganser 1997; Fox *et al.* 1999).

Other industrial process waters

Other process waters in industry have led to workers being exposed to bioaerosols consisting of bacteria and endotoxin. A detailed study was made at a fibreglass manufacturing plant in USA (Milton *et al.* 1995, 1996a,b). Here cooling water and wash water used in the manufacturing process was found to be highly contaminated with bacteria and endotoxin. Gram-negative bacteria predominated, although no legionellae were detected. Gram-positive *Bacillus* spp. were few, no thermophilic Actinomycetes were detected and fungi were few. The process waters were found to be contaminated by $>10^7$ Gram-negative bacteria ml^{-1} and 340000 EU ml^{-1} . Workers were found to be exposed to concentrations of more than 10^3 bacteria and 278000 EU m^{-3} air. This exposure was associated with the symptoms of severe flu-like illnesses and asthma.

A similar problem was found with cooling water held in a large sump in an electronics facility (Anderson *et al.* 1996a). The water was found to be heavily contaminated by the

Gram-negative bacteria, including *Pseudomonas putrefaciens*, *Ps. testosteroni* and *Alcaligenes* spp., yielding endotoxin concentrations in the sump of up to 57000 EU ml⁻¹. Exposure of workers to bacterial bioaerosol concentrations in excess of 10⁴ CFU m⁻³ air was associated with work taking place in the process area, and exposed workers suffered fever symptoms, a syndrome described by the authors as “sump bay fever”. Few fungi were isolated—in the region of 50–100 CFU m⁻³ air—and these were mainly *Penicillium* spp.

Soluble materials from paper and wood pulp provide a rich nutrient source for multiplication of bacteria, creating slimes in process waters. These comprise a complex mixture of bacteria (Vaisanen *et al.* 1994, 1998). Recycled process water in paper and pulp mills, and in saw mills, have led to workers being exposed to considerable endotoxin levels (Liesivuori 1994). As well as 10⁴ bacteria m⁻³ air, endotoxin at levels up to 360 EU m⁻³ air was measured in the air during a study of workers complaining of respiratory and mucosal symptoms at a paper mill where they were exposed to recycled water (Sigsgaard *et al.* 1994). Other studies have revealed in nasal swabs taken from mill workers the presence in excess of *Klebsiella pneumoniae*, a bacterium predominant in paper mill slime (Niemala *et al.* 1985).

Contamination of process waters in food production has led to workers being exposed to bacteria and endotoxin in bioaerosols. Forster *et al.* (1989) reported that water used to transport sugar beet from storage hoppers to chopping machines and on into sucrose extraction equipment was recycled via settling lagoons. However, as the beet-harvesting season progressed the bacterial contamination levels built up in the water, and were added to by the deterioration in microbial quality of the stored beet. Workers were exposed to airborne Gram-negative bacteria and endotoxin concentrations up to 2×10⁵ total bacteria, 4×10³ Gram-negative bacteria and 320 CFU m⁻³ air before installation of local exhaust ventilation, which controlled exposure.

In potato processing, workers have suffered flu-like symptoms and acute lung-function changes. The processing of potatoes for industrial starch, protein and fibre production is similar to that described above for sugar beet, and creates several points where workers can be exposed to bacterial bioaerosols. Again, recycling of process water increases the likelihood of bacterial contamination. As a consequence, workers may be exposed to as many as 9×10⁴ bacterial CFU m⁻³ air, of which up to 3×10⁴ m⁻³ can be Gramnegative bacteria, and 62000 EU m⁻³ inhalable and 1400 EU m⁻³ respirable endotoxin (Hollander *et al.* 1994; Zock *et al.* 1995, 1998).

Textile industries

Raw materials used in textile manufacture are contaminated by soil bacteria from harvesting, and the numbers may increase during storage if conditions allow. The most widely researched aspect of this is the role of inhaled bioaerosols in the etiology of a chronic bronchitis affecting textile workers in the cotton industry, byssinosis. Related to this is a syndrome, referred to as Monday morning fever, in which there is an increased bronchial reactivity following initial exposure after a period of absence from work, and progressive reduction in symptoms with regular repeated exposure. This response is

similar to the symptoms of chronic exposure to endotoxin and studies have proved the link between the exposure of workers to endotoxin in airborne cotton dust and their respiratory symptoms (Rylander *et al.* 1985; Rylander and Bergstrom 1993; Niven *et al.* 1997). Especially when handling raw cotton at the early stages of the process (combing, carding and weaving), workers may be exposed to up to 10^5 total bacteria, 10^5 Gram-negative bacteria (Enterobacteriaceae) and 10^5 fungi m^{-3} air, and endotoxin at a level of 7000 EU m^{-3} air has been recorded (Haglund and Rylander 1984; Castellan *et al.* 1987; Kennedy *et al.* 1987; Sigsgaard *et al.* 1992). In contrast, wool processing exposes workers to bioaerosols containing predominantly fungal spores. Up to 800 CFU m^{-3} have been reported (Sigsgaard *et al.* 1992). Byssinosis was not diagnosed in wool workers, although other studies have reported respiratory ill health among wool textile workers (Love *et al.* 1991; Zuskin *et al.* 1995), possibly stimulated more by the dust than the microbial component in this instance (Brown and Donaldson 1996).

Processing of hemp leads to intense exposure to dust and bioaerosols for workers, causing immunological response and reduction in lung function (Zuskin *et al.* 1992, 1994). A recent study of a British hemp processing factory revealed inhalable bacteria ranging in number from 4.7×10^6 to $190 \times 10^6 \text{ CFU m}^{-3}$ air and endotoxin at levels between 4700 and 59800 EU m^{-3} air (Allan *et al.* 2000).

Flax, used for weaving linen, relies on growth of bacteria during the early stages of preparation (retting) to soften the fibres. Subsequent handling of the flax can expose workers to bioaerosols, predominantly bacteria and endotoxin (Cinkotai *et al.* 1988). Although airborne concentrations were not measured, settled dust was examined microbiologically by Buick *et al.* (1994), who found Gram-negative bacteria at 10^9 CFU g^{-1} and endotoxin at $920000 \text{ cfu g}^{-1}$ dust.

Stored food and other products

Bagasse, sugar cane fibre used to produce constructional board, is much like flax, in that microbial activity at an early stage of the process softens the fibres prior to use. This means, however, that workers can be exposed to bioaerosols, in this case predominantly thermophilic Actinomycetes. Khan *et al.* (1995) reported that spores of the *Thermoactinomyces sacchari* account for >50% of the total population of thermophilic Actinomycetes, followed by *T. vulgaris*, *T. thalophilus*, *Saccharomonospora viridis* and *Saccharopolyspora rectivirgula*. Both this investigation and other studies demonstrated correlation between exposure to these spores and immunological response in exposed workers (Phoolchund 1991).

The storage of products in bulk quantity, and subsequent handling and movement, mean that any microbial contaminants present may be aerosolized. Also, if storage conditions allow, multiplication of the contaminants can occur resulting in workers being exposed to very high bioaerosol concentrations. This is particularly the case in agriculture, e.g. stored hay and grains, but in other more industrially related work areas there is the potential for bioaerosol exposure. Organic dust from coffee beans, stored and subsequently handled for roasting and preparing coffee on an industrial scale, has resulted in workers reporting allergic respiratory symptoms. The cause is likely to be a

combination of a number of factors, including antigens from the coffee beans and from castor beans, as old castor bean sacks are sometimes recycled for use to store coffee (Romano *et al.* 1995). However, bioaerosol measurements in a large-scale coffee factory revealed that workers were exposed to airborne fungi at a concentration of 2×10^4 CFU m^{-3} air, predominantly species of *Penicillium* and *Aspergillus*, and bacteria at 2×10^3 CFU m^{-3} (Thomas *et al.* 1991).

Handling of stored products in animal feed mills has resulted in workers being exposed to endotoxin at concentrations up to 4700 EU m^{-3} air, and this exposure is strongly related to reduction in lung function (Smid *et al.* 1992, 1994; Heederik *et al.* 1994). Bakery workers may be exposed to large concentrations of flour dust and enzymes such as amylases which can trigger asthmatic response (Burdorf *et al.* 1994; Baur *et al.* 1998; Houba *et al.* 1998), but when the raw products are handled there may also be exposure to microbial aerosols in which fungal species such as *Cladosporium* and *Penicillium* predominate (Crook *et al.* 1988; Musk *et al.* 1989).

Some other examples of bioaerosols to which workers can be exposed during handling of particular stored products are given in Table 3.

Table 3. Bioaerosols associated with handling of stored products. No. of microorganisms m^{-3} air.

	No of microorganisms	m ⁻³ air		
Stored Product	Total bacteria, incl. Actinomycetes	Total fungi	Taxa present	Reference
Tea	10 ²	10 ³	Predominantly <i>Aspergillus</i>	Cartier and Malo 1990; Muller <i>et al.</i> 1991; Zuskin <i>et al.</i> 1993; Jayawardana and Udupihille 1997
Tobacco	10 ³	10 ⁴	Includes <i>A. fumigatus</i> and <i>Rhizomucor pusillus</i>	Huskonen <i>et al.</i> 1984; Ogundero and Aina 1989; Uitti <i>et al.</i> 1998
Citrus fruits	ND	10 ⁵ – 10 ⁷	Predominantly <i>Penicillium</i>	Ström and Blomquist 1986
Spices	10 ²	10 ³	Predominantly <i>Aspergillus</i>	Zuskin <i>et al.</i> 1993; Delcourt <i>et al.</i> 1994; Sastre <i>et al.</i> 1996
Cork	ND	10 ⁶	Predominantly <i>Penicillium</i>	Lacey 1973; Avila and Lacey, 1974; Alegre <i>et al.</i> 1990
ND, not determined				

SUMMARY

A diversity of industrial work environments can expose workers to a wide range of bioaerosols. The extent of exposure is dependent on the contamination source and the work activity. This is a function of the potential for creation of aerosols and the amount of protection the worker has from the aerosols, e.g. engineering control or personal protective equipment. Exposure to bioaerosols can cause infection, allergy or toxicosis, dependent on the microorganisms in the bioaerosol and the exposure dose. Only limited data are available on dose-response relationships for bioaerosols, and no occupational standards exist at present, although limits have been proposed in Europe for endotoxins (Heederik and Douwes 1997). In the absence of such standards, it is important to raise awareness of the work practices associated with significant exposure to bioaerosols and the composition of those bioaerosols. These data can then be used both to correlate with development of respiratory disease and to use in risk assessment tools to control disease.

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CHAPTER 2.3

REMEDICATION AND CONTROL OF MICROBIAL GROWTH IN PROBLEM BUILDINGS

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INTRODUCTION

In the context of microbial growth, a problem building almost certainly means that chronic leaks or damp conditions exist. Thus filamentous fungi may grow on biodegradable water-damaged or damp finishing and construction materials. In addition to fungi, mites may reproduce on damp interior surfaces with abundant human skin scales. Fungi such as *Fusarium* and yeasts, as well as Gram-negative bacteria, may grow in water or on wet surfaces in components of heating, ventilation, and air-conditioning (HVAC) systems, and of course *Legionella* may grow in poorly maintained potable water systems and cooling towers.

The necessity for microbial remediation in a building implies that extensive biodeterioration or growth has already occurred. Almost all microbial problems in buildings are caused by failure to keep the infrastructure clean and dry, and/or by failure in the design, operation and maintenance of building systems. This chapter first reviews microbial remediation procedures, with the emphasis being on fungal contamination. Actions which can be taken to prevent microbial colonization, primarily through moisture control, are then described. The chapter concludes with checklists of questions that can be asked when inspecting a building for moisture and microbial growth problems. Sampling as a component of the building evaluation is reviewed in Chapter 4.4 (Morey 1999).

REMEDICATION OF FUNGAL GROWTH IN BUILDINGS

It has long been recognized that extensive growth of fungi on interior surfaces is unacceptable. Over 3000 years ago, the Book of Leviticus (Blomquist 1994) described the removal of fungus-affected materials from buildings. It is written in Chapter 14 of Leviticus that materials affected by the “plague” should be removed to an unclean place outside the city. The plaster, mortar and timbers should be removed if the plague continues to grow. Additionally, persons living in the unclean house should wash their clothing. The unacceptability of fungal growth in interior environments and the general

principles listed in Leviticus for dealing with have been reaffirmed in modern documents (NYC 1993; Health Canada 1995; ISIAQ 1996; ACGIH 1999).

While fungal growth problems have been known in buildings since ancient times, some modern constructional practices have presented new habitats for colonization. Traditional construction practice prior to about 1950 in North America emphasized the use of stone, brick, plaster and wood. These materials are broadly resistant to microbial growth.

Surfaces in pre-1950 buildings were mostly nonporous and therefore easily cleaned. In recent decades the use of amorphous cellulose products, porous coverings for floors, walls, and ceilings, porous insulation and pressed-wood products have greatly increased. These modern construction and finishing products are susceptible to biodeterioration when wetness and dampness occur chronically in building system components. In addition, porous construction and finishing materials are difficult to clean. The presence of dirt in some porous materials allows for microbial growth to occur when moisture is non-limiting.

The location of modern buildings in warm, moist geographical locations such as Florida and Singapore increases the potential for microbial growth on susceptible construction and finishing materials. Vigilance in keeping modern building systems dry and clean is essential if microbial growth is to be prevented.

Review of consensus publications

A number of publications during the past decade have called attention to the growth of fungi on building materials and have presented guidance on clean-up of microbial contamination.

International workshop on health implications of fungi in indoor environments

This workshop held in Baarn in the Netherlands in 1992 (Samson *et al.* 1994) made the following statements or recommendations regarding fungal growth in buildings:

- The growth of fungi on interior surfaces of non-industrial buildings is unacceptable on both health and hygiene grounds.
- Condensation on building surfaces and penetration of water into building envelopes should be prevented.
- The health risks of biocides are not fully understood and therefore biocides should be used only as a last option for controlling fungal growth indoors.
- The inhalation of fungal spores and other products such as microbial volatiles should be avoided when handling contaminated materials.

NYC Stachybotrys guidelines

A panel in 1993 in New York City discussed appropriate assessment and remediation actions to be taken when visible *Stachybotrys chartarum* growth occurs on interior surfaces (NYC 1993). The panel was convened because of several cases of *Stachybotrys* colonization of paper-fibre gypsum board and library materials in New York City buildings. Panel recommendations included:

- Visibly mouldy materials should be removed by persons equipped with appropriate

personal protective equipment including respirators and gloves.

- The use of containment barriers (plastic sheeting) and negative pressurization is required for removal of mouldy materials with a surface area greater than about 3 m² (the approximate surface area of one side of a paper-fibre gypsum board used in North American buildings). Smaller patches of mouldy materials can be removed by simpler methods.
- Building maintenance personnel can remove small patches (<3 m²) of colonized materials. Persons trained in the removal of hazardous dusts should be employed when larger areas of colonized materials are removed.
- Defects causing water incursion into building infrastructure must be repaired.

Health Canada (1995) Fungal contamination guide

In 1995, Health Canada published a guide to assist investigators in recognizing and managing fungal contamination issues in buildings. An appendix in the guide reaffirmed the necessity for using personal protective equipment by persons carrying out clean-up of mouldy materials. When large-scale clean-up operations are necessary (colonized surfaces >10 m² approximately), physical isolation from, and negative pressurization relative to, the work area from both the HVAC system and the interior spaces are required. Evacuation of occupants from the building should be considered in complex large-scale fungal remediations.

ISIAQ guide

This guide (ISIAQ 1996) reviewed previous publications on fungal remediation and recommended additionally that:

- Soft porous materials that are visually colonized should be discarded (see also CMHC 1993).
- The object of clean-up/remediation is not to render interior surfaces sterile, but rather to return the building to a condition where normal (background) types and concentrations of fungi occur.
- There should be greater urgency for remediation when toxigenic fungi colonize interior surfaces. However, the extensive growth of any fungus on interior surfaces is undesirable and this growth should be removed.

ACGIH Bioaerosols Committee

This publication (ACGIH 1999) affirms the general principles of other guides (NYC 1993; Health Canada 1995; ISIAQ 1996) such as removal of porous materials with extensive colonization, removal of surface colonization from non-porous materials, and elimination of moisture problems that allowed growth to initially occur. The ACGIH committee classifies the extent of fungal colonization as minimal, moderate, and extensive without assignment of numerical surface-area guidelines. It states that plastic-sheeting barriers and negative pressurization should be used to contain dusts when extensive colonization is removed; that, at a minimum, N-95 respirators should be used by those performing remediation regardless of the extent of fungal colonization; and that personnel employed in large-scale fungal remediations should be informed of the potential health risks associated with exposure to bioaerosols.

Basic principles for fungal remediation

Important components of fungal remediation are (a) the physical removal of mycelium from interior surfaces, (b) removal of spores in settled dusts that may have previously been dispersed from mouldy surfaces, (c) prevention of dusts associated with clean-up from entering occupied or clean areas, and (d) use of personal protective equipment by clean-up workers. A plan to prevent future leaks or dampness that caused colonization is essential for effective remediation.

Porous materials such as paper-fibre gypsum board, ceiling tiles, insulation, wallpaper, carpet, etc., that are visually mouldy should be discarded. Because it is difficult to see fungal micro-colonies, it is prudent to remove some of the sound material adjacent to the visibly colonized surface. Cellulose-tape sampling may be used to distinguish colonized surfaces (hyphae or fruiting structures present) from sound surfaces that have been casually contaminated by spores of phylloplane fungi that settle out on all surfaces from the ambient air.

Fungal colonies that may be present on nonporous surfaces such as sheet metal, ceramic tiles, glass, etc., are physically removed by cleaning. Tap water with detergents should be effective for most cleanings.

The method used to remove growths from semi-porous materials such as wood depends on the degree to which hyphae have penetrated the substrate. Wood that is rotted by dry- or wet-rot basidiomycete fungi (Lloyd and Singh 1994) is discarded. Wood that is sound except for colonization of the outer surface (to approximately 1 mm depth) may be sanded, planed, refinished, and reused. The guiding principle for reuse should be absence of hyphae and fruiting structures (over and above those normally present in sound timber) in the wood cells of the timber being salvaged.

The airborne spore concentration can exceed 10^6 spores m^{-3} air when mouldy materials are disturbed (Morey and Ansari 1996; Rautiala *et al.* 1996). Consequently, those persons involved in clean-up activities must use personal protective equipment.

An N-95 respirator and gloves are adequate during the clean-up of minimal colonization (ACGIH 1999). For remediations involving extensive colonization, the use of disposable full-body protective clothing and respirators (P-100 or HEPA cartridge) must be considered. The use of containment barriers and dust suppression techniques during removal of mouldy materials is essential for preventing dissemination of spores into occupied or clean areas. The extent of the surface colonized (minimal, moderate, extensive) in a room or in one area of a building is the most important factor to be considered in relation to use of containment barriers or dust suppression methods.

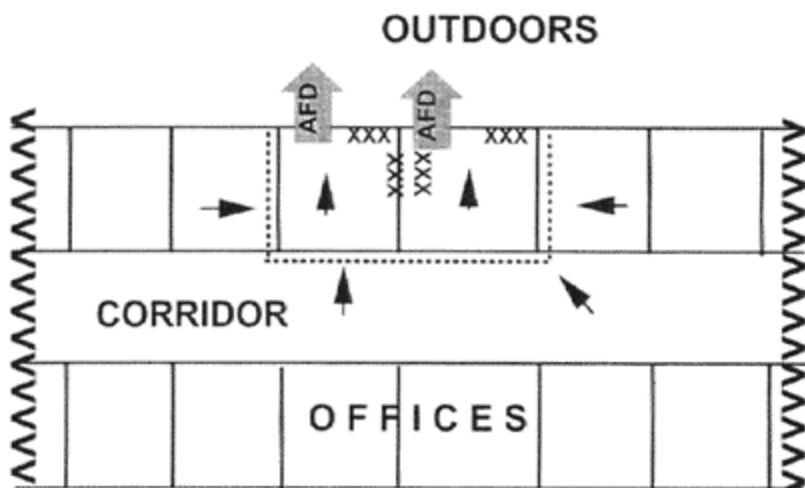


Fig. 1. A floor plan of a large scale remediation where dusts from colonized surfaces in two rooms are prevented from entering the rest of the building by a contaminant barrier. Key: (-----), barrier; xxx, colonized surfaces; AFD, negative-air machine or air filtration device; → direction of air flow.

Other factors important in determining the dust containment methods to be employed during a remediation include (a) the presence of highly susceptible occupants (Streifel 1988) and (b) the likelihood that hidden colonization may be uncovered within building components.

A dust containment method used when extensive fungal growth is removed from two adjacent rooms is illustrated in Fig. 1. The colonized surfaces are isolated from the rest of the building and the HVAC system using plastic sheeting (containment barriers). The rooms are depressurized through use of one or more negative-air machines which remove spore laden air and discharge it outdoors through HEPA filters, on which the spores are trapped. The flow of conditioned air is always from clean areas into the rooms undergoing remediation.

A principle common to guidelines on fungal remediation (NYC 1993; ISIAQ 1996) is that building maintenance personnel can perform remediation of minimally and moderately colonized surface areas. Fig. 2 illustrates an example where 1–2 m² of visible fungal colonization is being removed in a mini-enclosure. The colonized area is isolated from room air by plastic sheeting and negatively pressurized by insertion of the nozzle of a HEPA vacuum into the enclosure.

Wearing appropriate personal protective equipment (full-body disposable suit, gloves, full face respirator with N-95 or better cartridge), the clean-up person (a) enters the enclosure, (b) removes and bags mouldy materials, and (c) prior to leaving the enclosure uses the nozzle of a second HEPA vacuum cleaner to remove settled dust from the outer

surface of the bagged trash, from the surfaces of sound finishing material, from the inner surface of the plastic sheeting and from the surfaces of protective clothing and equipment.

Interior surfaces with minimal fungal growth (e.g. one or two ceiling tiles or 0.1–0.2 m² paper-fibre gypsum board) can be removed by persons wearing gloves and a respirator. Items such as mouldy ceiling tiles can be carefully placed and sealed in a plastic bag and then removed.

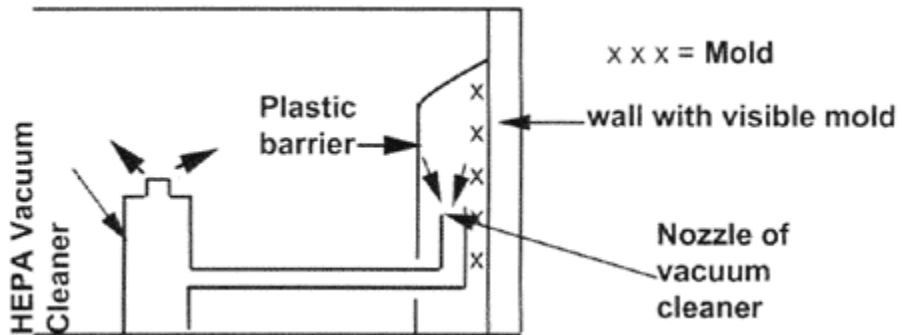


Fig. 2. A simple containment (mini-enclosure) used to control dust emissions when moldy surfaces of moderate extent are remediated. Key: xxx, colonized surfaces: → direction of air flow.



Fig. 3. Water being used for dust control during remediation on an upper floor wets a paper-fibre gypsum board wall, providing an opportunity for new colonization.

The colonized paper surface of gypsum board can be covered with a sticky sheeting (the sticky surface against the colonized surface), extracted from the wall in one piece, bagged, and removed.

Any technique that reduces dust emission from the colonized surface should be considered. Thus, application of an encapsulant to a colonized surface or damp-wiping of the surface may be useful. The application of a gentle mist to colonized surfaces may be effective in dust suppression as long as hydrophobic spores are not dispersed into the air by impacting water droplets. Where used, water mists and sprays must not wet sound infrastructure (Fig. 3).

Spores from colonized surfaces may have previously been dispersed on air currents into areas of the building unaffected by moisture problems. A combination of damp-wiping (non-porous surfaces) and HEPA vacuum cleaning should be adequate to remove most of the dusts containing these dispersed spores.

The objective of remediation is to remove colonization and associated mould laden dusts, but not to sterilize or disinfect interior surfaces. The use of biocides and

disinfectants is unnecessary. The physical removal of mouldy materials plus the removal of associated dusts by vacuum cleaning and damp wiping should be adequate for cleaning (ISIAQ 1996; ACGIH, 1999).

MICROBIAL REMEDIATION—SPECIAL SITUATIONS

Hospitals

For more than two decades it has been known that the incidence of fungal infection (aspergillosis) among immunosuppressed patients is reduced when air is mechanically ventilated (filtered) as compared to natural ventilation (Rose and Hirsch 1979). Other studies have shown that epidemics of aspergillosis in immunocompromised patients were associated with the presence of fungal growth on surfaces in HVAC systems and patient rooms, and with emissions of dusts during soil excavation and/or new construction (Streifel 1988; Walsh and Dixon 1989).

As a result of aspergillosis outbreaks in some medical centres very conservative guidelines have been recommended for controlling and preventing exposure of immunocompromised patients to essentially all culturable fungal spores (Streifel 1988, 1996). Procedures such as the following are recommended during hospital renovation or remedial work:

- (a) isolation and negative-pressurization of the remediation/clean-up area; impervious, with rigid floor-to-ceiling barriers to isolate patient areas from potential sources of viable fungi;
- (b) employment of administrative procedures to prohibit tracking of dusts by clean-up workers into patient areas;
- (c) provision of high quality air (spores absent) in patient areas by point-of-discharge filtration in supply air ductwork; and
- (d) Positive pressurization of patient rooms relative to areas in which there is fungal colonization or construction and renovation dusts.

The conservative actions used to reduce incidence of fungal infection among highly immunocompromised patients provide a framework for remediation guidelines that may be necessary in special clean-up situations arising when highly susceptible people may be present in non-medical facilities.

HVAC airstream surface

A niche exists for fungal growth in HVAC air-conditioning systems when airstream surfaces are both moist and dirty (Yang 1996). Remediation of fungal growth in HVAC systems is dependent on the extent and location of the colonized surface. Dirt and fungal growth on non-porous surfaces such as sheet metal can be removed by cleaning (Morey 1996). However, fungal colonization of a porous substrate, such as insulation, cannot be removed by cleaning (Morey 1996). Physical removal of the porous material itself is

required if hyphae have penetrated into the substrate.

When fungal growth in HVAC systems has to be removed, it must be recognized that air-handling units and ductwork are designed to transport ventilation air to building occupants. Therefore, provision must be made during cleanup to deactivate and isolate HVAC system components and to prevent dusts and spores from following air pathways into occupied spaces. When fungal growth is removed from airstream surfaces where colonization is minimal in extent, an N-95 respirator, gloves and a HEPA vacuum cleaner to contain dusts and fibres are usually adequate. However, when the fungal colonization on the airstream surface is moderate or extensive a mini-enclosure or full containment is generally necessary to prevent dispersion of spores and fibres. Because of the difficulty associated with remediation of fungal colonization on porous HVAC insulation materials, interest has been shown in the incorporation of antifungal compounds into insulation (Yang and Ellringer 1996) and the manufacture of insulation surfaces that are smooth, cleanable, hydrophobic, and resistant to biodeterioration (Morey 1994).

Books, paper and archives

The clean-up of books, paper and archives damaged by floods and dampness involves discarding mouldy items, drying out wet materials and removing settled dusts containing spores. Fungi can grow rapidly on these materials because of the adhesives, gums, starch, etc., often present in jackets and bindings and also because of the presence of delignified cellulosic (paper) substrates.

Because of the susceptibility of books, paper, and archives to biodeterioration, the drying of water damaged or damp materials is of critical importance in restoration. Special techniques such as freeze-drying of water soaked material are important in restoration because low temperatures arrest fungal colonization and evaporation of water molecules (subliming) lowers available moisture so that growth can not recur (Peterson 1993; Conservation Center 1996). A fundamental goal of restoration is to lower the moisture content of paper to its normal range, 5–7% (Waters 1979), so that fungal growth does not occur.

Conservators have developed several simple techniques for removing superficial colonization from valuable paper materials. Miniature aspirators are used to provide gentle suction through a pipette nozzle, which is used to carefully remove spores from the surface of the paper (Conservation Center 1996). A larger vacuum device can be used to remove spores if a fine screen is placed firmly over the paper to provide protection to the fragile material being cleaned (Conservation Center 1996). All restoration activities involving manual removal of surface colonization should be performed by persons with adequate personal protective equipment, and preferably in a biosafety cabinet.

The cleaning of library materials which are not visually colonized but which have been stored in buildings with problems of moisture and mould growth on ceilings, walls and floors is a challenge because of the enormous amount of paper surface potentially involved. Books, for example may be covered with a fine layer of dusts containing *Stachybotrys* and other non-phyllplane fungi that originated on water-damaged cellulosic ceiling tiles overhead. The following activities can be effective in cleaning

non-colonized library materials that had been stored in dusty, mouldy environments:

- HEPA-vacuum cleaning of the top, bottom and sides of books and files to remove settled dusts.
- HEPA-vacuum cleaning and damp-wiping of the surfaces of shelves, file cabinets, desks and other non-porous surfaces in the library. The presence of visible dust on books and on nonporous surfaces such as library shelves indicates unsuccessful cleaning.
- Fanning the pages of the books, files, and other paper materials in the immediate vicinity of the suction orifice of a HEPA vacuum cleaner. The object is to reduce the amount of settled dust present on surfaces of library materials. However, this cleaning will probably not change the qualitative make-up of fungi in residual dust that remains on surfaces.

Residences

Remediation of fungal colonization in residences differs from that in most commercial and public buildings for reasons of occupancy and construction. In homes, occupants may be present for the entire day, every day of the week. In some residences, occupants may be present who are especially sensitive or susceptible, such as infants, the elderly and those with immunosuppressive conditions.

Most single-family residences are smaller in volume than commercial and public buildings. This means that compared with a large office building a residence has a greater ratio of envelope surface (roof, exterior walls, basement) to air volume. Thus, there is a greater envelope surface where moisture may enter from precipitation or the soil. It is possible that the association between dampness, mould growth, and respiratory symptoms in homes (Health Canada 1995) is related to the relatively large envelope surface in small residential buildings. Problems of fungal growth in residences are exacerbated by the use of porous biodegradable materials in damp locations, such as basements, where moisture control is difficult.

An important difference between residences and office buildings is the greater use of timber and pressed wood products in residences. Consequently, wood-rotting fungi such as the dry rot fungus *Serpula lacrymans* and soft-rot fungi such as *Trichoderma* spp. are more likely to be present in residences with persistent moisture problems. Remediation of fungal growth in residences is often logistically difficult because of problems with access in crawl spaces and attics to biodeteriorated wood structural members.

Sewage backflow

Backflow of sewage can make a residence or commercial building uninhabitable. Effective restoration requires that sewage waters be removed from building infrastructure and that potentially pathogenic viruses, bacteria (e.g. *Escherichia coli* O157/H7), fungi, protozoa (e.g. *Giardia lamblia*) and invertebrates be inactivated by disinfection (Berry *et al.* 1994). Residual moisture from sewage waters in building finishing materials must be removed to prevent the subsequent growth of filamentous fungi.

During building restoration, attention must be given to the porosity of building

materials directly contaminated by sewage waters. When porous materials of minimal value such as insulation, paper-fibre gypsum board, clothing and carpet are contaminated by sewage waters, they should be discarded (Berry *et al.* 1994). Semiporous materials such as timber, wood furniture, concrete, and pressed wood products can usually be cleaned and disinfected. Some semiporous materials such as particle board flooring may have to be replaced as sewage waters may have penetrated beneath this material. Nonporous materials such as metal, glass, linoleum can be easily cleaned and disinfected (Berry *et al.* 1994).

A difficult aspect of building restoration following a backflow is to identify areas where sewage waters have penetrated into building infrastructure such as wall cavities, floor systems, ventilation ductwork, and crawl spaces. Some removal of undamaged and nonporous finishes may be required to gain access for disinfection.

Uncertainties associated with fungal remediations

Hidden colonization

Fungal colonization may be hidden within the building infrastructure. Colonization may occur on paper-fibre gypsum board or on wood in wall cavities, on insulation materials in ceilings, walls, and HVAC systems, and in flooring systems beneath the carpet or in the carpet pad or underlay. In buildings with an exterior insulation finishing system (EIFS) in the envelope, colonization may occur on surfaces of paper-fibre gypsum board (exterior sheathing) when water leaks penetrate into the outer portion of the EIFS wall. Rigid insulation in the outermost part of the EIFS prevents water in the sheathing from evaporating or drying to the outside. Water becomes trapped in the paper-fibre gypsum board and fungi grow on the moist paper surfaces (Fig. 4). Destructive opening of the envelope may be required to estimate the extent of biodeterioration.



Fig. 4. Fungal growth on the internal surface of exterior gypsum board sheathing of an envelope wall. Gypsum board on the interior side of metal studs has been cut away revealing the growth within the wall cavity. Rainwater which wet the exterior sheathing could not evaporate to the outside because of a thick layer of rigid plastic insulation in the exterior part of the envelope.

Dogs trained to detect microbial volatile organic compounds (MVOC's) have been used in investigations to locate areas of active dry rot (*Serpula lacrymans*) growth in inaccessible areas in roofs, walls and floors (Lloyd and Singh 1996). However, this method of detection is less reliable when dry rot is inactive and MVOC's are not being emitted. Fibre optic devices (boroscopes) may be used to detect colonization in inaccessible areas. However, it may be difficult to identify colonized surfaces because of the dirt, debris and soot that has also accumulated. A starting point for all investigations of potentially hidden colonization is location of areas in building systems where both biodegradable materials and conditions of chronic water incursion or dampness occur.

Personal protective equipment

There is uncertainty concerning the adequacy of personal protective equipment worn by workers performing clean-up activities. The protection factors for respirators used by

remediation workers vary from 10 to 100. This implies that in an atmosphere containing 10^7 spores m^{-3} the spore concentration in inhalation air (air that passes through the filter) could be 10^5 – 10^6 spores m^{-3} . At present, it is not known what fraction of spores in a highly contaminated atmosphere penetrates the respirator filter or the face seal.

All remediation guidelines recommend use of protective clothing for persons performing cleanup in order to keep microbial particulates off street clothes and skin. For large-scale remediations where extensive colonization is being removed it is uncertain if two layers of protective clothing provides a significant advantage over one layer, or whether showering of work personnel should be required to remove microbial particulates that may penetrate as far as the skin.

Because of these uncertainties the usage of personal protective clothing recommended by some practitioners during fungal remediation may be overly conservative.

FUNGAL REMEDIATION GUIDELINES—GENERAL CONSIDERATIONS

Unlike guidelines on removal of hazardous chemical and physical agents, e.g. asbestos, where rigid inspection and removal protocols are followed and specific numerical guidelines are used to document remediation effectiveness, the removal of fungal growth from buildings is a complex process affected by many variables including the potential growth of the organisms that may be present. In order to carry out building restoration successfully, a considerable amount of information must be obtained, including all sites of chronic moisture incursion and visible fungal growth. The location of occupants, including those with special susceptibilities, must also be established. The position of biodegradable and porous finishing and construction materials affected by chronic dampness and water incursion must also be established. Sampling procedures which will demonstrate whether or not clean-up activities are effective should be determined prior to onset of remediation (Chapter 4.4; Morey and Sawyer 1999).

The object of fungal remediation is *not* to disinfect or sterilize interior surfaces but rather to restore surfaces to conditions characteristic of non-problem buildings. Surfaces in non-problem buildings are characterized by the occurrence of settled dusts containing spores derived from outdoor sources such as vegetation. The fungi of settled dusts in non-problem buildings generally exhibit a diversity of species normally characteristic of outdoor air (Chapter 4.4; AIHA 1996). The finding of colonization by any fungus on interior surfaces and the predominance of species that do not normally occur outdoors are indicators of unsuccessful cleaning or restoration.

Because of variation in building construction and the differences in moisture damage that may occur in components of building systems, a considerable degree of judgement is required in choosing the appropriate remediation procedure for an individual building (Morey and Sawyer 1999).

REMEDICATION OF MICROBIAL GROWTH IN DRAIN PANS, SUMPS, HOT WATER SYSTEMS AND COOLING TOWERS

The presence of stagnant water in drain pans, humidifier sumps, and water spray systems almost certainly results in the formation of biofilm on submerged or wet surfaces. Procedures for remediation are well known (Brundrett 1979) and include (a) turning off the HVAC component, (b) draining stagnant water, (c) physically removing the biofilm, using detergents and disinfectants in the cleaning process, (d) removing cleaning chemicals, and (e) re-commissioning the HVAC component. The aerosolization of biocides and disinfectants into ventilation air by an active HVAC system is not appropriate (Brundrett 1979; ASHRAE 1996; ISIAQ 1996).

Considering the large number of buildings with heat rejection (cooling towers, evaporative condensers) and hot water systems, outbreaks of legionellosis are rare. Principles for appropriate design and preventative maintenance and for emergency disinfection of these systems are well known (WDH 1987; HSE 1994). Emergency disinfection measures that may be necessary if legionellosis is associated with a building water system include:

- Thermal or chemical disinfection of hot water tanks and piping; removal of sludge and dirt from tanks; operation of the system temporarily at elevated temperatures to kill *Legionella*; preventing conditions of stagnation in piping.
- Decommissioning the heat rejection system; closure nearby outdoor air inlets; addition of biodispersant and disinfectant and circulation of these chemicals in the water system; draining the water system into an approved discharge pathway; and repeat disinfection as necessary to remove *Legionella* reservoirs. During emergency remediation of building water systems it is essential to protect clean-up workers from *Legionella* aerosols (at a minimum an N-95 respirator is required, and it should be frequently changed); from disinfectant splash or aerosols (an acid gas respirator cartridge is required for chlorine disinfectants, and skin and mucous membrane protection are required for disinfectant splash); and from scalding (administrative controls on use of the hot water system during thermal disinfection are required).

HOUSE DUST MITES

In buildings where hypersensitivity disease is caused by house dust mite allergen, remediation involves reduction of relative humidity (RH) near or on interior surfaces, removal of nutrients (human skin scales) and less reliance on use of fleecy finishing materials (IOM 1993). Mites reproduce optimally in the 70–80% RH range (Arlian 1992; IOM 1993; ACGIH 1999). Lowering the RH below 50% by air-conditioning, dehumidifier operation or raising the dry bulb temperature of surfaces results in desiccation and death of mites (Arlian 1992; ACGIH 1999). Use of non-porous covering for bedding and upholstery and non-porous floor covering reduces the number of niches for accumulation of skin scales and mite faeces. Mite allergens are also reduced by removal

of settled dust from the building by cleaning with a highly efficient vacuum cleaner.

PREVENTION OF FUNGAL GROWTH

General requirements

As has been mentioned in Chapter 2.1, adequate temperatures, nutrients (substrate) and moisture are required for fungal growth. Since the range of temperatures in buildings that is acceptable for human comfort is also suitable for microbial growth and since virtually all buildings contain some biodegradable materials, control of moisture in construction and finishing materials is the most critical variable for preventing fungal growth in indoor environments. There is a large number of publications on moisture control in buildings (e.g. Rose and TenWolde 1993; Trechsel 1994; ISIAQ 1996).

In Chapter 2.1, the critical role of moisture was discussed primarily in relation to colonization of materials by microorganisms, so that it is important here to consider it in relation to remediation. A case of flooding can be taken as an example. If the RH in a room that has been recently flooded remains consistently in the 90–100% range, equilibrated porous finishing materials will have a water activity (a_w) >0.90 or equilibrium relative humidity (ERH) >90%, respectively. Consequently, Actinomycetes and other bacteria, as well as hydrophilic fungi (Chapter 2.1) such as *Fusarium*, *Phoma*, *Stachybotrys*, *Chaetomium*, *Trichoderma* and yeasts, might be expected to grow on the nearly saturated (a_w >0.9) materials. Since xerophilic fungi such as *Eurotium* spp. (*Aspergillus glaucus*) or *Wallemia sebi* are less demanding for water and can grow on surfaces that are consistently damp (a_w 0.65–0.85) rather than wet, they could also be expected to grow, but they would not compete well with the faster growing hydrophilic species.. Since virtually no fungi can grow on interior surfaces at a_w <0.65, even with optimal nutrient conditions (ISIAQ 1996), the prevention of fungal growth is achieved by as rapidly as is feasible lowering the available moisture to levels equivalent to an a_w <0.65. Failure to dry materials rapidly enough or completely enough will result in only partial control, with growth of hydrophiles being prevented, but not xerophiles. Hunter and Sanders (1991) have noted that when the a_w of biodegradable material exceeds 0.80 for several days, fungal growth is likely, although it will be of xerophilic rather than hydrophilic species. Thus, if wallpaper in the flooded room is only dried to the extent that its a_w is 0.80 (moisture content approximately 11.3%; Flannigan 1992) it will support growth of *Eurotium* spp. or *Wallemia sebi*, but not *Fusarium*, *Phoma*, *Stachybotrys*, *Chaetomium* or *Trichoderma*.

Prevention of colonization of biodegradable materials that have become water saturated by flooding (assume normal building temperatures to be 20–30°C) is achieved by drying the materials within 24–48 h so that the a_w is $\leq$ 0.65 (ASHRAE 1996; ISIAQ 1996). In the case of wallpaper, this means until the moisture content is <7%. Simply lowering the RH in room air to 50% does not mean that at the surface of biodegradable materials the a_w will be below 0.65. On one occasion the author encountered an actively photo-synthesizing green plant growing in the flooring of a building subject to chronic

flooding. Although the RH of the air was $< 65\%$ and the surface of the carpet felt dry to the touch, the presence of the unwilted plant indicated that the base of the carpet and the underlying flooring were thoroughly soaked. The roots of plants require an a_w of at least 0.97–0.98 for root hairs to carry out their normal function of extracting from the substrate the water necessary for growth and survival (Flannigan 1992). This example therefore illustrates the practical necessity of removing moisture from hidden layers within building components if biodeterioration of susceptible materials is to be avoided.

Preventing fungal growth in the building envelope

Fungal growth can occur in the envelope of buildings in both so-called heating and cooling climates, i.e. where in the first case (winter) heating of the indoor environment is necessary for comfort, and in the second where cooling is necessary during at least part of the year, as in tropical or sub-tropical regions. Moisture from rain or snow if retained in the envelope is likely to cause biodeterioration. Fungal growth may also occur in the envelope when water vapour from a warm source—which can be either indoors or outdoors—encounters a relatively cool surface resulting in localized dampness and condensation. In a cold climate, this kind of localized dampness or condensation occurs on or in the envelope when moisture from warm room air encounters a surface that is cool because of thermal bridging or poor insulation (White 1990). In tropical climates or seasons, moisture-laden warm air can migrate through the envelope of an air-conditioned building causing dampness or condensation and consequent fungal colonization of the cool inner surface of the wall.

Regardless of geographical location, buildings must be constructed so that any water entering the envelope drains to the outside. Buildings should be designed so that water drains away from the foundations and so that below-grade portions of the envelope are adequately damp-proofed to prevent entry of capillary moisture (Lstiburek and Carmody, 1994). When wet materials such as concrete, and plaster are used in construction, they must be allowed to dry adequately before closing the envelope in order to protect biodegradable materials that may also be present.

If a vapour diffusion retarder is used in a cold climate it should be installed on the insulation facing the occupied space. Dampness and condensation in cold (heating) climates can be prevented by reducing indoor moisture-producing activities and by mechanical ventilation with relatively dry outdoor air.

In air-conditioned buildings in warm humid climates vapour diffusion and air retarders if used should be installed in the external portion of the envelope. Buildings should be positively pressurized to minimize the infiltration of humid outdoor air into the envelope. Indoor air must not be cooled to a temperature below that of the mean monthly outdoor dew-point. Finishes that are impermeable to water vapour should not be used on the inner surface of the envelope.

Prevention of microbial growth in HVAC systems

Detailed information on the prevention of microbial growth in HVAC systems is available in a number of publications (Brundrett 1979; Morey 1994; ASHRAE 1996). Control of microbial growth in operating air-conditioning systems is challenging because of wet surfaces near dehumidifying cooling coils and because the relative humidity in ventilation air downstream of these coils approaches 100%.

Design considerations

HVAC systems should be operated so that there is no carry-over of water droplets from dehumidifying cooling coils. Drain pans should be designed with proper sloping and trapping to assure self drainage during fan operation (ASHRAE 1996; ISIAQ 1996). Filters should have at least 65% efficiency in collecting 1–3 μm particles so as to prevent soiling of airstream surfaces. The use of porous materials should be avoided on any airstream surface subject to wetting. Hinged access panels should be installed in locations requiring inspection and cleaning (ASHRAE 1996).

Operation considerations

HVAC systems should be operated according to design principles. Thus, the temperature of chiller water should not be arbitrarily raised to save energy costs as this will compromise the dehumidification capability of the air-conditioning system. In buildings with mechanical outdoor air inlets the dehumidification cooling coils should be operated continuously during humid weather conditions. The practice of de-energizing coils in operating HVAC systems at night or over week-ends, and introduction of humid outdoor air into interior spaces can result in fungal growth throughout the building. Simply turning off the mechanical system in air-conditioned buildings during vacation periods in warm humid seasons can also result in widespread fungal growth.

Maintenance

Microbial growth can occur in well designed HVAC systems if maintenance is neglected. A written maintenance programme should be followed, with particular attention being given to inspection of HVAC components, such as drain pans and coils, for evidence of microbial growth and for soiling which can lead to growth (ASHRAE 1996). Physical removal of biofilms or fungal growth is required. Filters that are wet should be discarded. Application of biocides or disinfectants instead of physical removal of microbial growth is not appropriate.

Commissioning for moisture control

Most microbial growth problems can be prevented by giving attention to moisture issues during design and construction. The issues that should be considered prior to construction include the location of vapour diffusion and air retarders (where used) in the envelope; provision for drainage of water that by chance enters the envelope; and provision for drainage of water away from building foundations. The use of highly biodegradable materials in building locations that are likely to be chronically damp or wet should be

avoided. Provision must be made at the construction site for protection of biodegradable materials such as paper-fibre gypsum board, insulation, carpet, and pressed wood products from moisture damage. Dust and debris (from sanding, sawing, and cutting of finishing materials, for example) must be kept out of the HVAC system by use of construction filters (ASHRAE 1996). A written maintenance programme for the cooling tower and potable hot water system should be developed in coordination with the manufacturer or installer.

HVAC components should be tested for adequate performance prior to occupancy. Thus drain pans should self-drain when the fan is operating (ASHRAE 1996). Water droplets from dehumidifying cooling coils should not be carried onto downstream surfaces by ventilation air. Filters installed should be those specified by design plans. Access openings in the HVAC system should be present at locations where inspection and cleaning will occur.

A plan for water disaster management should be developed during building commissioning. The plan should include provision for water removal in the event of natural disasters such as floods and hurricanes, as well as water disasters associated with breakage of fire suppression piping and plumbing lines. Because fungal growth can occur on surfaces within 24 h of wetting, a plan should be available as part of building commissioning for rapid drying of materials that may be affected by a water disaster.

THE BUILDING INSPECTION

Prevention and remediation of microbial growth begin with an inspection of the building and its infrastructure. The location and nature (e.g., porous or non-porous; biodegradable or resistant to biodeterioration) of water-damaged materials and the extent of damage must be determined, in light of the realisation that damaged components may be hidden from view. The location and extent of visible colonization must also be determined during the inspection (Morey and Sawyer 1999). Literature on identification of moisture and fungal growth problems in buildings (Health Canada 1993; Lstiburek and Carmody 1994; Singh 1994; Trechsel 1994; ISIAQ 1996; ACGIH 1999) should be consulted as part of the inspection process. Principles, which are important in the building inspection, and questions to be answered, are listed in the sections below.

Moisture in occupied spaces

1. Make an inventory of water damaged materials distinguishing between those which are porous and non-porous. Does the location and nature of the moisture damage provide clues on the source of the water problem? For example, bowed ceiling tiles may indicate conditions of chronically elevated relative humidity in interior spaces.
2. Are there musty odours, due to MVOC's which may indicate microbial growth (Wessen *et al.* 1995)? Are scented air fresheners used in the building to mask MVOC's?
3. Have interior finishes previously suffered water damage? Past water damage may have

only been superficially cleaned up. Look for signs of hidden water damage within wall cavities, beneath carpeting, above ceiling tiles, etc.

4. Do seasonal moisture sources exist? For example, poorly insulated cold or chilled water pipes in walls and ceiling plenums may condense and drip water only during the humid season. Are humidifiers used during the heating season?
5. Are unusual water sources present such as waterfalls in atrium landscaping? Aerosolization of bioparticles can arise from poorly maintained water systems (Rose 1996). Wicker baskets, carpet and bark chips that are wetted by watering of indoor plants are potential sources of indoor bioaerosols.
6. Are wet methods used for cleaning of carpet or other interior finishes? Wet or damp finishes are niches for possible microbial growth.
7. Use a moisture meter to determine whether some finishes and construction materials which appear superficially dry actually contain significant amounts of moisture (Lloyd and Singh 1994).
8. Are materials which are highly susceptible to moisture damage, e.g. photographic archives, books and clothes, stored in chronically damp or wet areas?
9. Find out whether there is a plan for drying interior finishes and constructional materials in the event of water damage arising from, for example, ruptured pipes or backflow of sewage.

Biodeterioration

1. Make an inventory of visibly mouldy interior surfaces including the extent (in m²) and locations of colonized materials. This is essential for development of a remediation plan (Morey and Sawyer 1999).
2. Determine whether or not there are materials which are highly susceptible to biodeterioration, such as those containing amorphous cellulose, hidden in moist niches in building components. In moisture-damaged buildings, the extent of hidden colonization is almost always larger than that which is readily visible. Therefore, in order to estimate the extent of hidden deterioration of susceptible materials it may be necessary to open up and inspect floor, wall and ceiling systems in the most heavily water-damaged portions of the building.
3. Determine whether there are unusual or strong sources of bioaerosols (composting, wastewater treatment, etc.) outdoors. Can the outdoor bioaerosols impact on the indoor environment?
4. In buildings where extensive colonization has occurred determine whether or not fungal spores have been dispersed to the extent that they have settled as dust into other interior spaces. What kind of cleaning is needed to remove dispersed spores?
5. Are interior building surfaces (in attics, for example) or surfaces near or in outdoor air inlets covered by bird or bat droppings? Bird or bat droppings are almost certain to be a source of pathogenic fungi and must be carefully removed by personnel wearing appropriate protection.

Moisture in the building envelope

1. Does condensation or dampness occur on the inner surface of the envelope in air-conditioned buildings in hot humid climates or seasons? Conditions of overcooling, negative pressurization, and use of impermeable wall coverings are associated with this kind of moisture problem.
2. Does condensation or dampness occur on or near the inner surface of the envelope in buildings in cold climates or seasons? Thermal bridges or poor insulation in the wall, poor ventilation in occupied spaces, and unusually strong moisture sources (such as humidifiers, respiration where occupancy rates are high, and boiling of foods) are associated with this kind of moisture problem.
3. Are damp niches and biodeteriorating materials (e.g. rotted timbers) found in below-grade locations such as crawl spaces? Are walls below grade damp-proofed adequately enough to prevent entry of capillary moisture? Does water drain away from the building foundations?
4. Is there evidence that the envelope retains moisture from rain or from artificial sources such as wetting by an outdoor sprinkler system? The presence of rusted metal studs or utility outlets indicates chronically wet conditions. Are materials in wet portions of the envelope readily biodegradable (e.g. amorphous cellulose or pressed wood products) or resistant to biodeterioration (e.g. brick, concrete, plaster, terracotta)?
5. Are windows, expansion joints and roof drainage channels properly flashed so as to avoid chronic leakage into the building infrastructure?

Moisture and the HVAC system

1. Are there problems of water stagnation in and around drain pans and dehumidifying coils? Standing water in drain pans indicates that there is a need for redesign to give complete drainage. Wet or rusty airstream surfaces downstream of coils indicate carry over of water droplets.
2. Are porous materials in HVAC systems wet or chronically damp? Porous materials susceptible to biodeterioration include insulation and filter materials. Are airstream surfaces in chronically damp or wet HVAC locations inherently resistant to water uptake and biodeterioration? Are they smooth and easily cleaned?
3. Determine whether there are humidifiers in air-handling units or supply ductwork. Does the operating humidifier wet airstream surfaces? Do the humidifiers operate by aero-solization of water droplets from open, stagnant water sumps? Do humidifiers emit moisture in molecular form or as water droplets?
4. Do damp or wet niches occur where there is no access for inspection and cleaning? Fungal colonization and biofilms may occur in such hidden locations.
5. Are HVAC systems in the air-conditioned buildings in humid seasons or climates operated so that unconditioned humid outdoor air enters air-handling units when dehumidifying cooling coils are inactive? The introduction of humid air into an air-conditioned building can result in massive condensation on cool surfaces and can raise the amount of moisture available for support of fungal growth in interior finishes.

6. Does fungal growth occur on surfaces of diffusers or on surfaces near diffusers? If so, this suggests inadequate dehumidification of room air or localized overcooling of surfaces.
7. Determine whether or not the HVAC system design relies on large numbers of distributed fan coil units, induction units or unit ventilators. Maintenance of a distributed HVAC system is more difficult than a system with a few large central air handling units. In a distributed HVAC system find out if individual units are characterized by water leaks, dampness, and musty odours. Water leaks from individual units and associated piping systems may result in hidden water damage and hidden colonization in building components.

Biofilms and *Legionella*

1. Can drift (water droplets) from cooling towers enter HVAC outdoor air inlets and other building openings? Airflow patterns around the building and the direction of the prevailing wind must be considered before selecting the locations of cooling towers and inlets for outdoor air (HSE 1994; ASHRAE 1996).
2. How rigorous is cooling tower maintenance? The likelihood of *Legionella* amplification in cooling towers can be reduced by a maintenance programme involving scale, corrosion, and microbial control. Periodic testing for culturable *Legionella* can be carried out to evaluate the effectiveness of microbial control.
3. Is there a maintenance programme for control of *Legionella* growth in hot water system components such as calorifiers, storage tanks, piping and piping outlets?
4. In the event of a legionnaire's disease outbreak occurring in a building, is there a plan for emergency disinfection of the cooling tower or hot water system (HSE 1994; WDH 1987)?
5. Are HVAC components such as drain pans, dehumidification cooling coils and sump type humidifiers inspected periodically for presence of stagnant water and biofilm? Is biofilm physically removed and affected surfaces cleaned? Are disinfectants placed in active HVAC components instead of biofilm being physically removed?

CONCLUSION

This chapter has described general principles for prevention of microbial growth, as well as for remediation of growth that may have occurred in or on building components. Limiting available moisture (keeping ERH <65%) is central to prevention of fungal colonization on construction and finishing materials. In building systems, including HVAC components and cooling towers, preventive maintenance of a high standard is essential for prevention of microbial amplification. In buildings where prevention has failed, attention must be focused both on establishment of preventive maintenance activities and on moisture attenuation. Microbial growth that has occurred, such as fungal colonization, must be physically removed using approaches that prevent dispersion of bioaerosols and protect workers performing remediation activities.

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Chapter 3.

Airborne microorganisms and disease

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Chapter 3.1

ALLERGENIC MICROORGANISMS AND HYPERSENSITIVITY

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INTRODUCTION

Allergy may be defined as an untoward response to a specific antigen mediated by an immunological reaction. It may take different forms. The commonest clinical manifestation of an allergic reaction to moulds is IgE-mediated as represented by symptoms of rhinitis, conjunctivitis and asthma. Hypersensitivity pneumonitis has both IgG- and cell-mediated features and other fungal conditions have even more complex immunological relationships.

The microorganisms capable of producing hypersensitivity responses in humans are represented in large proportion by the fungi and to a lesser degree by bacteria, algae, etc. Because mites are so frequently encountered in indoor environments, and their allergens are often encountered together with those of fungi, e.g. in house dust, they will be discussed in addition to microbial allergens. Fungi are the main offenders among microorganisms and will consequently be covered in more detail.

HYPERSENSITIVITY TO FUNGI

The earliest report of an allergic response to moulds occurred in 1726; Sir Floyer reported severe asthma in a patient who had just visited a wine cellar. In 1873, Blackley described “bronchial catarrh” and severe chest tightness following inhalation of *Penicillium* spores (Blackley 1873). Since these early beginnings, research has shown that exposure to fungal products generates a wide range of adverse responses, including hypersensitivity. Reactions occur not only to the spores and mycelia of fungi, but also to the mycotoxins produced as secondary metabolites, the volatile organic compounds (VOC’s) emitted by growing fungi, enzymes produced by the organisms, and (1→3)-β-D-glucan, a component of the fungal cell wall.

Epidemiological evidence for mould-related respiratory disease

A great many studies have been conducted on the association of dampness, mould and respiratory health in residential housing. In the Harvard University six-cities programme, the respiratory health of 4600 children was examined in relation to mould and dampness in their homes. The outcomes were correlated to several respiratory and non-respiratory symptoms. The deleterious health effects on the children were demonstrated to be similar to those found in situations of parental smoking (Brunekreef *et al.* 1989).

Similar conclusions were drawn from a 30-community study involving 15,000 children and 18,000 adults (Health Canada 1995). This suggested that a non-allergenic mechanism was involved (Dales *et al.* 1991a,b). A dose effect was also noted in that more visible mould yielded more symptoms.

Table 1. Moulds and other fungi noted in the literature for which IgE-mediated allergy has been demonstrated (adapted from Gumowski *et al.* 1991).

<i>Absidia</i>	<i>Cryptococcus</i> ²	<i>Leptosphaeria</i>	<i>Puccinia</i> ⁴
<i>Agaricus</i> ¹	<i>Cryptostroma</i>	<i>Leptosphaerulina</i>	<i>Rhizopus</i>
<i>Agrocybe</i> ¹	<i>Dacrymyces</i> ¹	<i>Lycogala</i> ²	<i>Rhodotorula</i> ²
<i>Alternaria</i> (<i>Macrosporium</i>)	<i>Debaryomyces</i> ²	<i>Lycoperdon</i> ¹	<i>Saccharomyces</i> ²
<i>Amanita</i> ¹	<i>Didymella</i>	<i>Malassezia</i> ⁵	<i>Scleroderma</i> ¹
<i>Armillaria</i> ¹	<i>Drechslera</i>	<i>Merulius</i> (<i>Serpula</i>) ¹	<i>Scopulariopsis</i>
<i>Arthrinium</i> (<i>Papularia</i>)	<i>Epicoccum</i>	<i>Mucor</i>	<i>Sphaerotheca</i> ⁴
<i>Aspergillus</i>	<i>Epidermophyton</i> ⁵	<i>Mycogone</i>	<i>Spondylocadium</i>
<i>Aureobasidium</i>	<i>Erysiphe</i> ⁴	<i>Neurospora</i> (<i>Chrysonilia</i>)	<i>Sporobolomyces</i> ²
<i>Boletus</i> ¹	<i>Eurotium</i>	<i>Nigrospora</i>	<i>Sporotrichum</i>
<i>Botrytis</i>	<i>Fomes</i> ¹	<i>Paecilomyces</i>	<i>Stemonitis</i> ³
<i>Calvatia</i> ¹	<i>Fuligo</i> ³	<i>Phytophthora</i> ⁴	<i>Stemphylium</i> (<i>Pleospora</i>)
<i>Candida</i> ²	<i>Fusarium</i>	<i>Penicillium</i>	<i>Tilletiopsis</i> ²
<i>Cantharellus</i> ¹	<i>Ganoderma</i> ¹	<i>Phoma</i>	<i>Torulopsis</i> ²
<i>Cephalosporium</i> (<i>Acremonium</i>)	<i>Geotrichum</i> ²	<i>Piptoporus</i> ¹	<i>Trichoderma</i>
<i>Chaetomium</i>	<i>Gibberella</i> ^{4*}	<i>Pisolithus</i> ¹	<i>Trichophyton</i> ⁵
<i>Chlorophyllum</i> ¹	<i>Gliocladium</i>	<i>Pleurotus</i> ¹	<i>Trichothecium</i>

<i>Cladosporium</i>	<i>Graphium</i>	<i>Podaxis</i> ¹	<i>Ustilago</i> ⁴
<i>Claviceps</i>	<i>Helminthosporium</i>	<i>Polyporus</i> ¹	<i>Verticillium</i>
<i>Coniosporium</i>	<i>Hypholoma</i> ¹	<i>Polystictus (Coriolus)</i> ¹	<i>Xylaria</i> ¹
<i>Coprinus</i> ¹	<i>Inonotus</i> ¹	<i>Psilocybe</i> ¹	<i>Xylobolus</i>

Key: ¹Macrofungus; ²Yeast; ³Slime mould; ⁴Plant pathogen; ⁵Human pathogen (skin disease, dermatomycosis);

*Teleomorph of some *Fusarium* spp.

Data from a total of 13,000 children in 24 cities in the USA and five in Canada demonstrated the same pattern (Spengler *et al.* 1994). A review of nine cross-sectional studies assessing the association between exposure to fungi in the home environment and health effects of the occupants revealed one or more positive associations between the two in seven of the nine studies (Verhoeff and Burge 1997). A comparable study of 400 homes in Wallaceburg, Ontario, Canada, demonstrated that self-reported mould, water damage and mouldy odours were associated with elevated concentrations of indoor fungi. However, inaccuracy was high and a systematic reporting bias appeared to be present; respondents with respiratory symptoms were more likely to report the presence of fungi than respondents without symptoms (Dales *et al.* 1997). The authors recommended that objective measures, not questionnaires, be used to clarify the health effects of indoor fungi.

Other epidemiological evidence for mould-related respiratory disease comes from studies relating the effect of outdoor fungal spore concentrations to daily asthma severity. A study in Southern California demonstrated that when controlling for weather, total fungal spore concentrations were associated with increased asthma symptom scores, inhaler use and decreases in peak expiratory flow rate (Delfino *et al.* 1997). This study confirmed earlier findings by the same group (Delfino *et al.* 1996). Evidence also exists to link skin sensitivity to fungal spores as a risk factor for severe or fatal asthma attacks (O'Hollaren *et al.* 1991).

Allergenic species of fungi and their allergens

A vast number of fungi have been identified in the medical literature as being implicated in allergic disease (Table 1). Most commonly involved are the anamorphic fungi, including species in the genera *Alternaria*, *Cladosporium* and *Aspergillus*, as well as *Candida albicans*, *Penicillium* spp., *Stachybotrys* spp. and members of the Basidiomycetes, Zygomycetes, and Ascomycetes.

Progress in understanding fungal allergy has been impeded by the great variety of potentially allergenic fungi; the instability and variability of their allergens; lack of agreement on current fungal nomenclature; and the selection of adequate source material for the preparation of extracts (Horner *et al.* 1995). Additional problems arise in that allergic effects can be induced by both spores and mycelium, the former more so than the latter, although it is in fact the mycelium which is extracted in conventional extraction

media. Further, extraction media differ from one laboratory to another, and storage of certain extracts has proven extremely difficult (Bousquet 1997). As a result, strain-to-strain and batch-to-batch variations occur in the processing of allergenic extracts. For these reasons, characterization of fungal allergens has lagged behind characterization of other aeroallergens. The exceptions—*Alternaria alternata*, *Cladosporium herbarum*, *Penicillium chrysogenum* and *Aspergillus fumigatus*—are discussed here.

Alternaria alternata

A. alternata is one of the most widely distributed moulds in nature and is the most important allergenic fungus in USA (Day 1996). The major allergenic fraction of *A. alternata* is heterogeneous and can induce allergenic reactions at very low concentrations in sensitized individuals (Yuninger *et al.* 1980). Investigators have identified 32 antigens and 19 different allergens (Bush *et al.* 1983; Kroutil and Bush 1987; Steringer *et al.* 1987). Alt a 1 is considered to be the major allergen. It is a heat-stable acidic glycoprotein (Yuninger *et al.* 1980; Deards and Montague 1991; Matthiesen *et al.* 1992) consisting of two disulphide-linked chains of MW 15 kDa (Deards and Montague 1991; Matthiesen *et al.* 1992) present in the mycelium, spores and extracellular components (Pariss *et al.* 1991; Cruz *et al.* 1995). The N-terminal sequence was defined as an epitope with IgE-binding ability (Matthiesen *et al.* 1992; Zhang *et al.* 1995), and the complete cDNA sequence has been elucidated (DeVogue *et al.* 1996). Minor allergens, Alt a 2 to Alt a 10, have also been characterized (Cruz *et al.* 1997).

Cladosporium herbarum

This species has been studied extensively and approximately 60 antigens revealed in extracts (Gutman and Bush 1993). Two major allergens have been described, Cla h 1 and Cla h 2 (Aukrust and Borch 1979; Sward-Normo *et al.* 1988). Their biological functions are still unknown and their sequences have not yet been determined. Spores contain a greater amount of Cla h 1 than mycelium, and Cla h 2 is found in small amounts in both (Sward-Normo *et al.* 1988). These allergens have been found to vary widely from strain to strain (Day 1996). Minor allergens from *C. herbarum* have been defined as Cla h 3 to Cla h 9. Of these, cDNA's encoding for Cla h 3 to Cla h 6 have been cloned and sequenced (Achatz *et al.* 1995).

Aspergillus fumigatus

Two antigens (18 and 20 kDa) from *A. fumigatus* have been purified by chromatography (Latge *et al.* 1991; Samuelsen *et al.* 1991). The 18 kDa antigen was isolated from mycelium and its characteristics suggest that it is similar to Ag-3 (Longbottom 1986) or Ag-10 (Horner *et al.* 1995), which were described earlier. The 20 kDa allergen is a glycoprotein that is currently held to be the major allergen, Asp f 1. Culture filtrate extracts of this allergen demonstrate more allergenic potential than those extracted from mycelium (Cruz *et al.* 1995). Another glycoprotein allergen, designated gp55, has been isolated and determined to be a novel protein (Teshima *et al.* 1993).

Penicillium

In this genus, 11 different allergens have been identified (Shen *et al.* 1991), and a 68 kDa allergen has been proposed as a major allergen (Shen *et al.* 1995), but this is still open for discussion as there are only a few articles regarding *Penicillium* allergens in the literature. A 67 kDa component capable of binding IgE from patients sensitized to extracts from *P. chrysogenum* (Cruz *et al.* 1994) is probably the same component previously described by the above mentioned authors (Cruz *et al.* 1997).

HYPERSENSITIVITY TO ALGAE

A full list of algae known to be associated with hypersensitivity is provided in Table 2 overleaf.

MITE HYPERSENSITIVITY

Two major types of mite are associated with allergy i.e. dust mites and storage mites. Dust mite allergy is far more prevalent, due to ubiquitous exposure, whereas storage mites pose problems only for individuals in certain environments.

Table 2. Algae reported as being allergenic by authors cited in the section Hypersensitivity to Algae, (p.), including blue-green algae now known as Cyanobacteria and some macroalgae from which allergenic particles may be aerosolized on fragmentation (see Henderson *et al.*, 1984).

<i>Anabaena</i> ¹	<i>Microcystis</i> ¹
<i>Ankistrodesmus</i> ²	<i>Myremecia</i> ²
<i>Aphanothece (Synechococcus)</i> ¹	<i>Myxosarcina</i> ²
<i>Bracteacoccus</i> ²	<i>Nostoc</i> ¹
<i>Chlamydomonas</i> ²	<i>Oocystis</i> ²
<i>Chlorella</i> ²	<i>Oscillatoria</i> ¹
<i>Chlorococcum</i> ²	<i>Phormidium</i> ¹
<i>Coccomyxa</i> ²	<i>Porphyridium</i> ⁴
<i>Ecklonia</i> ³	<i>Scytonema</i> ¹
<i>Fucus</i> ³	<i>Spyrogia</i>
<i>Hormidium (Klebsormidium)</i> ¹	<i>Stichococcus</i> ²
<i>Laminaria</i> ³	<i>Tetracystis</i> ²
<i>Lessonia</i> ³	<i>Trebouxia</i> ²

*Lyngbya*¹*Ulva*²*Mesotaenium*²*Westiellopsis*¹

Key: Cyanobacteria; ²Chlorophyta (Green Algae); ³Phaeophyta (Brown algae); ⁴Rhodophyta (Red algae)

That dust mites produce allergic responses in individuals was first suspected in 1928 (Dekker, 1928), but not confirmed until more than 20 years later by Voorhorst *et al.* (1964). The most common species is *Dermatophagoides pteronyssinus*, which predominates over all other species in most countries. The occurrence of pyroglyphid mites such as *D. farinae*, *D. microceras* and *Euroglyphus maynei* varies according to climate (Fain 1979).

Certain mites infesting stored products such as cereal-based foods (storage mites) and belonging to the genera *Acarus*, *Tyrophagus* (Acaridae), *Glycyphagus*, and *Lepidoglyphus* (Glycyphagidae) have also been implicated in allergy (Guerin 1995). The persons exposed to these mites are primarily farmers and their families who, through their work, are in regular contact with the dust in barns containing hay, straw or grain. The existence of common allergenic determinants in these various mites seriously complicates the interpretation of skin or blood responses, measured by various provocation tests (Guerin 1995).

Dust mite allergens

Mite allergens have been divided into groups on the basis of immunological and physicochemical similarities. They have been assigned specific names and seven groups have been delineated by the WHO/IUIS Allergen Nomenclature Sub-committee (King *et al.* 1994). However, other mite allergens have been identified and isolated but have yet to be assigned to specific groups.

Group 1 allergens

The group 1 allergens are 25 kDa acidic to neutral proteins recognized by most mite-allergic individuals (Chua *et al.* 1998). The allergens are found in both whole body and faecal extracts, and are synthesized by cells lining the gastrointestinal tract of the mite (Tovey and Baldo 1990; Thomas *et al.* 1991). The complete sequences of the group 1 allergens from *D. pteronyssinus* (Der p 1) and *D. farinae* (Der f 1), and an almost complete sequence for the group 1 allergen from *E. maynei* (Eur m 1) have been reported (Chua *et al.* 1998; Dilworth *et al.* 1991; Kent *et al.* 1992). These data indicate that the group 1 allergens are synthesized as preproteins comprising a leader peptide (18 residues) and a peptide (80 residues) together with the mature protein of 222–223 residues (Dilworth *et al.* 1991). Analysis of the available sequence data indicate that the group 1 allergens are very similar (81–84% identity). Homology searches indicate that the group 1 allergens belong to the cysteine group of proteolytic enzymes (Stewart 1995).

Group 2 allergens

The group 2 allergens are 14 kDa nonglycosylated allergens recognized by the majority of mite-allergic individuals (Lind 1985; Heymann *et al.* 1989). They are neutral to basic proteins and are resistant to denaturation by heat, extremes of pH (Lombardero *et al.* 1990) and digestion by proteases (Oshika *et al.* 1994). However, reduction and alkylation of the disulphide bonds significantly reduces the allergenicity of the protein. The complete amino acid sequences of the group 2 allergens from *D. pteronyssinus* and *D. farinae* and the storage mite *L. destructor* have been obtained either by conventional means or from cDNA clones (Stewart 1995).

Analysis of the three sequences indicates that the group 2 allergens from *D. pteronyssinus* and *D. farinae* are about 88% identical, whereas the degree of identity between the allergens of the latter and that of *L. destructor* is about 40% (Varela *et al.* 1994). Sequence homology studies have not thus far revealed the identity of the group 2 mite allergens, although they share some overall properties with lysozyme, an enzyme associated with defence against Gram-positive bacteria and with digestion of food. This suggestion is based on comparative biochemical and physicochemical data derived from studies performed on enzymes present in mite faecal and whole-body extracts (Stewart *et al.* 1992b).

Group 3 allergens

The group 3 allergens are 30 kDa proteins that have been shown on the basis of substrate specificity and amino acid sequence homology to be trypsin-like enzymes (Stewart 1995). These enzymes cleave at the C-terminal side of the basic amino acids arginine and lysine. At present, the data suggest that the frequency of reactivity in mite-allergic populations varies from 16% with Der f 3 to 100% with Der p 3, the reason for which is unclear (Heymann *et al.* 1989; Stewart *et al.* 1992c; Yasueda *et al.* 1993). The DNA coding for Der p 3 has been cloned (Smith *et al.* 1994), and the deduced sequence shown to code for a preprotein with a 232-residue mature protein. The allergen showed approximately 40–50% identity with both vertebrate and invertebrate trypsins.

Group 4 allergens

The group 4 allergens are 60 kDa proteins that appear to be intermediate or minor allergens. Physicochemical studies have shown these allergens to be amylases (Lake *et al.* 1991). Group 4 equivalents have been demonstrated so far in *D. pteronyssinus*, *D. farinae*, *E. maynei* and storage mites (Bowman 1984; Stewart *et al.* 1992a). Studies indicate that the allergenicity of Der p 4 is dependent on the integrity of the disulphide bonds. The allergenicities of the enzymes from the other species have yet to be determined.

Group 5 allergens

The group 5 allergens are approximately 14 kDa proteins that are recognized by about 50% of mite-allergic individuals (Tovey *et al.* 1989). A cDNA clone for the allergen from *D. pteronyssinus* has been isolated and shown to code for a protein of 148 amino acid residues with a molecular weight slightly greater than the native allergen. The

biochemical identity of this allergen is unknown.

Group 6 allergens

The group 6 allergens are 25 kDa proteins that are recognized by about 40% of mite-allergic individuals (Yasueda *et al.* 1993). They have been shown to be serine proteases with specificities similar to vertebrate and invertebrate chymotrypsins.

Group 7 allergens

The group 7 allergens are 22 kDa proteins recognized by just over 50% of the mite-allergic population. The cDNA for the group 7 allergen from *D. pteronyssinus* has been cloned and shown to code for a 198-residue mature protein with a predicted molecular mass of 22177 Da (Shen *et al.* 1993). At present, the biochemical identity of this allergen is unknown. There is the potential presence of a glycosylation site, so that it is feasible that the allergen is glycosylated, explaining why it appears in forms of different MW.

Heat shock protein

Recently, a clone coding for an IgE binding protein from a *D. farinae* cDNA library was isolated and sequenced (Aki *et al.* 1994a). The partial deduced amino acid sequence showed marked homology with the C-terminal region of the heat-shock cognate protein Hcs 71 from the heat shock protein 70 family. This protein is highly conserved and can be found in bacteria, insects, plants and mammals. The native protein was determined to be about 67 kDa and the frequency of reactivity in a mite-allergic population was about 10%.

Glutathione-S-transferase

A cDNA clone coding for glutathione-S-transferase has been cloned from a *D. pteronyssinus* library and the expressed protein showed to be allergenic (O'Neill *et al.* 1994). These homodimeric enzymes are ubiquitous and catalyze the conjugation of reduced glutathione with a number of electrophiles. They are thought to play a major role in detoxification.

Other mite allergens

A 39 kDa protein has been isolated from *P. farinae* (Aki *et al.* 1994b) and preliminary studies indicate that *D. pteronyssinus* contains at least three other allergens. They include a serine protease which is biochemically distinct from the group 3 and group 6 enzymes (King *et al.* 1996); glucoamylase; and an allergen designated DPt 36 (Stewart *et al.*, 1998). The terminal sequence of this last allergen has been determined and it shows some homology with a bacteriolytic enzyme from *Listeria monocytogenes*, the bacterial agent of listeriosis.

Mite-fungus relations

It was once thought that fungi enhance rates of population increase in mites by providing a food source. This was disputed by Hay *et al.* (1993), whose research showed that *D. pteronyssinus* reared over two generations on diets with fungi had reduced survival,

prolonged development time and shorter adult length compared to mites reared on diets without fungi. It is now established that fungi are of no nutritional significance to house dust mites, and also that the presence of mites does not influence the growth of fungi (Hay 1991).

In stored products, however, fungi are thought to be more beneficial to mites (Rodriguez and Rodriguez 1984). Indeed, *Acarus* and *Tyrophagus* can be reared on a diet consisting exclusively of stored product fungi (Stewart 1995).

Fungi are an important source of dust allergens (Bush and Yunginger 1987), and since they are ingested by mites it is possible that fungi could contribute to mite allergenicity if allergenic spores, mycelium or fungal enzymes are present in mites or their faeces. This does not, however, appear to be the case in house dust mites. Hay *et al.* (1992) demonstrated that larval *D. pteronyssinus* which lack fungi have allergen profiles indistinguishable from fungus-bearing adult mites. Whether a similar situation exists for storage mites is not known.

Hypersensitivity to bacteria and their products

Investigations of the role of bacteria in allergic diseases has focused mainly on the respiratory hypersensitivities. Bacterial species are suspected of playing a significant role in asthma exacerbations and other upper respiratory symptomatology. More research, however, has focused on the role of bacterial endotoxin in hypersensitivity diseases. Endotoxin, a component of the outer cell wall in Gram-negative bacteria consists of a polysaccharide chain linked with a core polysaccharide to a lipid moiety (lipid A) which consists of fatty acids. Significant epidemiological data exists to support the association between exposure to bacterial endotoxin and severity of asthma (Michel *et al.* 1991, 1996), demonstrated not only by subjective symptom worsening and use of medication, but also by objective decreases in lung function (i.e. FEV1 and VC). Recently it has become apparent that infection with *Chlamydia pneumoniae* can precede asthma exacerbations or worsen asthma severity (Johnston 1997; Laurilla *et al.* 1997). In addition, exposure to Gram-negative bacteria in an occupational setting has been associated with symptoms of chest tightness, flu-like symptoms, and itchy eyes, nose and throat (Sigsgaard *et al.* 1994). Norn and others have demonstrated that bacteria release histamine from human basophils and mast cells via both IgE dependent and independent pathways (Norn *et al.* 1986, 1987). Furthermore, bacteria and endotoxin have been shown to enhance histamine release caused by allergens (Norn 1994). While hypersensitivity to bacteria does exist, the non-allergic mechanisms are thought to predominate, given that asthmatic symptoms occur in non-atopic individuals during infection with bacterial pathogens.

Hypersensitivity to algae

As mentioned in Chapter 1.2, algae were first reported as a possible cause of inhalant allergy by McElhenney *et al.* (1962), whose findings were based on the results of skin-prick testing. Since then, several authors have reported positive skin-prick test findings in

both atopic children (Tiberg 1987; Tiberg *et al.* 1995) and adults (McGovern *et al.* 1966; Champion 1971; Ng *et al.* 1994). These results are supported by studies in which intradermal testing (Mittal *et al.* 1979; Mittal 1981), nasal provocative challenges (Hosen 1968; Lunceford 1968), bronchial provocation challenges (Mittal *et al.* 1979; Henderson *et al.* 1984) and RAST for algae-specific IgE (Tiberg *et al.* 1990, 1995) were completed. Negative responses in non-atopic controls demonstrated that the reactions are immunological and not irritative in nature (Mittal *et al.* 1979). The frequency of skin reactivity to algae varies geographically and probably depends on the choice of algal strain, extract concentration, patients and test method (McGovern *et al.* 1966). The algal genus most extensively investigated is the unicellular green alga *Chlorella*, but *Chlorococcum* and the blue-green *Anabaena* are among others studied (see Chapter 1.2). *Chlorella* has a worldwide distribution, being found in all kinds of environment, often in soils as an aerophyte (Round 1981).

Allergic clinical syndromes

Fungi and fungal products cause a wide range of hypersensitivity responses, some which have been recognized for centuries (e.g. asthma) and others which have been just recently been identified as clinical entities (e.g. allergic fungal sinusitis). Mite hypersensitivity has also been implicated in almost every range of clinical syndrome, while bacterial endotoxins are associated with asthma. Algae play a minor role in many different allergenic responses, particularly inhalant allergy. All are detailed below.

Allergic rhinitis

Rhinitis occurs as an emergence of nasal mucous membrane inflammation leading to nasal discharge, sneezing, and congestion. Mould spores are an important cause of nasal allergy because they are so widespread. The particles are much smaller than pollen and are easily inhaled and absorbed. Allergic rhinitis due to moulds can be seasonal, as found with outdoor species, and perennial as found with indoor and some outdoor species.

In a large study of 3371 patients, 25.8% of those with a diagnosis of rhinitis were sensitized to moulds, as were 27.4% of those with a combined diagnosis of asthma and allergic rhinitis (Boulet *et al.* 1997). Exposure to *Alternaria* (Granel *et al.* 1993), *Penicillium*, *Cladosporium*, *Aspergillus* (Shah and Sircar 1991), *Helminthosporium*, *Fusarium* (Kumar *et al.* 1984), *Mucor*, *Pullularia* (*Aureobasidium*), *Rhizopus* (Collins-Williams *et al.* 1972) and *Candida* (Gumowski *et al.* 1987) have all been implicated in allergic rhinitis to varying degrees. The role of the Basidiomycetes is controversial, and the literature presents conflicting evidence of their involvement (Santilli *et al.* 1985; Lehrer *et al.* 1994).

Often, patients with allergic rhinitis symptoms associated with exposures to substances at work are in fact mould-allergic, and it is the contamination by the moulds that leads to their symptoms, as opposed to the obvious agent. For example, an investigation of wood furniture workers with nasal hypersensitivity symptoms were demonstrated to have a higher incidence of sensitivity to the moulds isolated from the factories where they worked than to the wood dust in these workplaces (Wilhelmsson *et al.* 1994).

In addition, mould allergy has been implicated in the clinical entity of persistent cold-

like symptoms (PCLS) in children, characterized by persistent symptoms of mouth breathing, rhinorrhoea, nasal voice, bloodshot eyes and headache during the winter months (Huang and Kimbrough 1997). Clinically this is very similar to perennial allergic rhinitis, yet is symptomatically much worse, with statistically significant differences in symptom scores.

Prior sensitization to dust mites is a well-documented risk factor towards the development of chronic allergic rhinitis (Hagy *et al.* 1976). In the above mentioned study of 3371 atopic patients, 54.2% were sensitized to house dust mite antigens (Boulet *et al.* 1997). Of those whose diagnosis was that of allergic rhinitis alone, 52.6% demonstrated dust mite sensitivity. Among patients with a dual diagnosis of allergic rhinitis and asthma 60.0% were dust mite sensitive.

Bacteria and bacterial products such as endotoxins are rarely cited in the literature as significant players in allergic rhinitis. One study suggested an epidemiological link between the two, as refuse workers who were exposed to significantly more endotoxin than control workers in a water supply plant had a significantly increased prevalence of rhinoconjunctivitis symptoms, viz. itchy eyes and itching nose (Sigsgaard *et al.* 1994). Another similar study of poultry slaughterhouse workers exposed to endotoxins and organic dust suggested an increase in nasal irritation and rhinorrhoea in those workers with endotoxin exposure (Hagmar *et al.* 1990).

Green algae, specifically *Chlorella*, have been implicated in the clinical syndromes of both seasonal and perennial allergic rhinitis in children (Tiberg *et al.* 1995). The etiological role of algae in allergic rhinitis has also been replicated in adults as well in case report form (Champion 1971; Ng *et al.* 1994), and in specific nasal challenge studies (Hosen 1968; Lunceford 1968).

Asthma

This is a reversible airway obstruction which is characterized by inflammation and hyper-irritability of the bronchial mucosa and marked changes in lung volumes and expiratory flow rates. Asthmatic reactions can be immediate, late, or dual. Clinically, asthma is typified by dyspnoea (shortness of breath), with a notable wheeze or cough.

It has been suggested that as fungal spores are smaller than pollen grains, they can be more readily deposited in smaller bronchi and thus more easily induce asthmatic symptoms.

Not only have multiple mould species been demonstrated to provoke symptoms and objective finding of bronchial asthma, but exposure and sensitivity to fungal aeroallergens have been associated with respiratory arrest (O'Hollaren *et al.* 1991). The severity of asthma symptoms has been clearly linked to the concentrations of outdoor fungal spores (Delfino *et al.* 1996, 1997). A number of studies have documented the link between increases in atmospheric spore counts and hospital admissions for acute asthma exacerbations (Hasnain *et al.* 1985; Packe *et al.* 1985), and in the number of deaths due to asthma attacks (Targonski *et al.* 1995). Also, evidence exists to suggest that asthmatics become increasingly sensitive to moulds over time (Szantho *et al.* 1992).

The mould species implicated in asthma are similar to those associated with allergic rhinitis (Kumar *et al.* 1984; Santilli *et al.* 1985; Gumowski *et al.* 1987; Granel *et al.*

1993; Lehrer *et al.* 1994), with *Alternaria* (Granel *et al.* 1993; Halonen *et al.* 1997) having the strongest correlation.

The role of the dust mite in asthma is considerable. Indeed, it has been estimated and widely quoted that, worldwide, 85% of asthma can be attributed to dust mite exposure (Platts-Mills 1990). Indeed, sensitization to house dust mite has been shown to be an independent risk factor for the development of asthma (Platts-Mills and Chapman 1987; Ulrik *et al.* 1996). The increasing prevalence of asthma has been associated mainly with changes in lifestyle and housing which have lead to increased exposure to indoor allergens, with dust mite being the primary offender (Custovic and Woodcock 1996). Studies have clearly demonstrated that the severity of asthma in patients sensitized to house dust mites is related to the level of exposure to mite allergens in patients' beds (Custovic *et al.* 1996). Yet, the relationship between exposure and asthma symptoms in already sensitized individuals is complex. Some sensitized patients will react to a very low dose of allergen, while in others the level required to cause symptoms will be considerably higher (Hagy and Settupane 1976). Exposure threshold values have been proposed, but the levels of exposure to which individuals are susceptible vary widely, and no absolute value that could generally ensure minimum risk has been identified (Wahn *et al.* 1997).

Bronchial provocation studies confirm an important role for storage mites among allergens responsible for asthmatic reactions (Ingram *et al.* 1979; VanHage-Hamsten *et al.* 1988). Reduction in FEV1 due to inhalation of storage mites extract is species-specific, and does not occur in patients with asthma caused by *D. pteronyssinus*. In poultry farmers, respiratory allergy as confirmed by specific bronchial provocation tests may develop as a result of exposure to northern fowl mite, a prevalent external parasite of chickens (Lutsky 1984).

As mentioned previously, several studies have suggested that bacterial endotoxin may play a significant role in asthma and its exacerbations. Acute inhalation of lipopolysaccharides (the major constituents of endotoxins) induced in asthmatics, but not normal subjects, a bronchial obstruction associated with an increase in histamine bronchial responsiveness (Michel *et al.* 1989).

In association studies it was found that, compared to those who had low exposure to endotoxin, patients whose homes contained a high concentration of endotoxin demonstrated a significant increase in dyspnoea, requirement for medication and lower FEV1/FVC values (Michel *et al.* 1991). These findings were confirmed in later studies by the same researchers, as well as by another group studying the relationship between endotoxin exposure and asthma severity in children (Rizzo *et al.* 1997).

Bacteria have been shown to cause the release of histamine and other mediators from mast cells and basophils in both IgE-dependent and independent pathways (Norn *et al.* 1986, 1987; Norn 1994). This bacterially induced histamine release has been observed in human pulmonary and tonsillar mast cells (Church *et al.* 1987), in nasal mucosa (Baenkler *et al.* 1985), and in superficially lying mast cells in the airway epithelium obtained by bronchoalveolar lavage (Clements *et al.* 1989). Further, bacteria and bacterial endotoxin have been shown to enhance the allergic histamine release, exacerbating existing allergic responses (Norn *et al.* 1986, 1987; Norn 1994).

Epidemiological associations have been shown between atmospheric counts of green algae and asthma exacerbations in children (Khot *et al.* 1988). This has been confirmed in studies where the allergy was documented via positive skin-prick testing (Tiberg *et al.* 1995). The role of algae in asthma has also been demonstrated in bronchial provocation studies; decreases in pulmonary function occurred in subjects allergic to algae as shown by skin-prick or intradermal testing (Ng *et al.* 1994). Pulmonary hypersensitivity to aerosols from macroalgae is an occupational health concern in workers in the alginate industry, and has been documented by bronchial challenge (Henderson *et al.* 1984).

Hypersensitivity pneumonitis

Also known as extrinsic allergic alveolitis (EAA), hypersensitivity pneumonitis (HP) is a syndrome which is caused by a broad spectrum of inhaled organic dusts or chemical products which cause an immunologically mediated inflammatory response of the alveoli and bronchioles frequently accompanied by systemic symptoms. This expression depends on several factors, including immunological responsiveness of the host, intensity of exposure, antigenicity of inhaled biological dust. Despite extensive studies, the exact immunological mechanisms are not known. Antigen exposure is associated with the presence of circulating IgG antibodies in exposed individuals. Histological changes in lung tissue and cell findings in bronchoalveolar lavage support the involvement of delayed-type hypersensitivity (Fink 1993).

Various forms of expression of HP are related to three different factors (Table 3). The classic, and most widely studied form of HP known as "Farmer's Lung" is caused by contamination of hay, compost or silage by thermophilic Actinomycetes. *Penicillium brevicompactum* and *P. olivicolor* have also been proposed as probable sources of antigens (Nakagawa-Yoshida *et al.* 1997). Ventilation HP can be caused by thermophilic Actinomycetes, fungi such as *Aspergillus fumigatus*, *Aureobasidium* and possibly Protozoa (Pitcher 1990). HP related to a covered and heated swimming pool environment has been reported, with thermophilic Actinomycetes and a mould, *Neurospora* sp. (*Chrysonilia*), suggested as the causative agents (Moreno-Ancillo *et al.* 1997). Summer-type HP, a unique disease form in Japan (Kawai *et al.* 1984), is caused by *Trichosporon* (Yoshida *et al.* 1989; Nishiura *et al.* 1997). Bacterial endotoxins could play a role as an initiator of the acute inflammatory reactions preceding the onset of HP (Rylander 1987), but this has not been proved.

Clinical presentation can vary and is affected by the duration and nature of exposure. Three forms have been identified. The acute form includes symptoms of headache, arthralgia, malaise, fever, lethargy, chills, cough and dyspnoea. Patients typically experience symptoms 4–6 h after exposure. These subside 18–48 h later (Stites *et al.* 1987). Chest radiographs may be normal or

Table 3. Principal antigens or agents in aerosols from materials associated with hypersensitivity pneumonitis?

Disease	Source of Aerosol	Principal Antigen/Agent
Actinomycete-induced		
Farmer's Lung	Mouldy (heated) hay	<i>Saccharopolyspora rectivirgula</i> , <i>Thermoactinomyces vulgaris</i>
Bagassosis	Mouldy sugar cane bagasse	<i>T. vulgaris</i> , <i>T. sacchari</i> , <i>S. rectivirgula</i> (?)
Mushroom worker's lung	Mouldy mushroom compost	<i>Excellospora flexuosa</i> , <i>Thermomonospora</i> spp (?)
Mould-induced		
Malt-worker's lung	Mouldy malt	<i>Aspergillus clavatus</i>
Sequoiosis	Mouldy wood dust	<i>Aureobasidium pullulans</i>
Suberosis	Mouldy cork dust	<i>Penicillium frequentans</i> (<i>P. glabrum</i>)
Cheese worker's lung	Cheese mould	<i>Penicillium casei</i>
Summer-type pneumonitis	House dust	<i>Trichosporon</i> spp.
Ventilation pneumonitis	Contaminated ventilation systems	Various fungi, Actinomycetes and amoebae
Protein-induced		
Bird-breeder's lung	Avian species	Avian dusts
Pituitary snuff-taker's lung	Bovine/porcine pituitary powder	Pituitary proteins
Animal handler's lung	Laboratory animals	Urinary proteins

show reticulonodular changes in the early stages of disease and possibly hyperinflation. In the subacute form, symptoms tend to appear over a period of weeks and are characterized by cough and dyspnoea. Tachypnoea, tachycardia, fever and bibasilar respiratory rales might occur as evidence of acute exacerbation (Stites *et al.* 1987). Chest films may be normal or show soft reticular or patchy infiltrates with or without small poorly defined nodules. Pulmonary function tests usually reveal restrictive defect, decreased compliance, and at the end stage, diminished diffusion capacity. The insidious form is typified by progressive symptoms with possible intermittent acute periods. Prolonged avoidance of the offending antigen is required for effective management. Physical findings are compatible with those observed in the subacute form. Chest x-rays indicate interstitial fibrosis, mainly of the peripheral lung fields; honeycombing may occur

at the periphery.

HP is discussed further in relation to episodes occurring in indoor environments in Chapter 3.2.

Allergic bronchopulmonary aspergillosis (ABPA)

Allergic bronchopulmonary aspergillosis (ABPA) is a pulmonary disease which is caused by *Aspergillus fumigatus* which has colonized the lower respiratory tract. Patients are usually atopics with asthma, and those individuals with cystic fibrosis are particularly at risk (Ricketti *et al.* 1984; Vaughan 1993). The *A. fumigatus* spores are inhaled and trapped in the respiratory tract, where they can grow at body temperature in airway mucus. The spores germinate to form mycelium, and they continuously release antigens and mycotoxins within the segmental airways. In pre-disposed individuals, antigen-specific IgE, IgG and IgA antibodies are produced, leading to an array of immunological responses. Damage to the bronchial wall and surrounding pulmonary tissue by eosinophilic consolidation occurs, but the precise immunological mechanisms responsible for the changes are unknown (Rohatgi and Rohatgi 1984; Detjen *et al.* 1991). The diagnostic criteria for ABPA include asthma, immediate cutaneous reactivity to *A. fumigatus*, elevated total serum IgE ($>1000 \text{ mg ml}^{-1}$), precipitating antibodies to *A. fumigatus*, proximal bronchiectasis, chest x-ray infiltrates, peripheral eosinophilia at the time of chest film infiltrates, and elevated serum IgE and IgG antibodies to *A. fumigatus* (Vaughan 1993).

As early diagnosis has become more common, clinicians have begun to use the notations ABPACB and ABPA-S (Patterson *et al.* 1986) to differentiate patients with ABPA that has progressed to the bronchiectatic form (CB=central bronchiectasis) from those in whom it has not (S=serological). Bronchiectasis is defined as an abnormal segmented dilation of the central and distal airways. Patients with ABPA-S are seropositive and exhibit the first four diagnostic criteria discussed above. Those with ABPA-CB exhibit all eight of the aforementioned criteria; ABPA-CB signifies more permanent lung damage resulting from ABPA.

Recent evidence (Roig *et al.* 1997) suggests that *Candida albicans* IgE and IgG subclasses may participate in the pathophysiology of ABPA by exacerbating pulmonary infiltrates (IgE) and inducing eosinophil-mediated inflammatory reaction (IgG₁, IgG₃). This remains to be confirmed in follow-up studies.

Allergic fungal sinusitis

Allergic fungal sinusitis (AFS) is a recently recognized clinical entity that occurs in up to 7% of patients with chronic sinusitis (Ence *et al.* 1990). APS should be suspected in patients with atopy and chronic, often intractable, sinusitis and nasal polyposis (DeShazo *et al.* 1997). Most have pansinusitis, and many have had multiple sinus operations by the time of diagnosis (Goldstein *et al.* 1985; DeShazo and Swain 1995). Patients tend to be young and immunocompetent (Spring and Amedee 1995). At surgery, involved sinuses contain brown or greyish-black material with the consistency of peanut butter or cottage cheese. This material has been dubbed "allergic mucin" and contains laminated accumulations of intact and degenerating eosinophils, Charcot-Leyden crystals, cellular debris, and sparse hyphae rarely visualized without fungal stains (Katzenstein *et al.*

1983). The sinus mucosa contains a mixed cellular infiltrate of eosinophils, plasma cells and lymphocytes. The allergic mucin and polyps obstruct sinus drainage and perpetuate the bacterial sinusitis often associated with AFS (DeShazo *et al.* 1997). Demonstration of elevated levels of fungal-specific IgE in previous studies helped to confirm that AFS is a true allergic, rather than infectious, disease (Manning *et al.* 1993; Mabry and Manning 1995).

The most common etiological agents associated with AFS are the dematiaceous (dark-pigmented) fungi, including *Curvularia*, *Bipolaris* and *Pseudallescheria*, and the non-dematiaceous moulds such as *Aspergillus* and *Fusarium* (DeShazo and Swain 1995; Walsh *et al.* 1996). *Penicillium* has also been implicated (Perez-Jaffe *et al.* 1997). Patients with APS often have asthma and usually have allergic rhinitis, eosinophilia, and elevated total and fungus-specific IgE concentrations. AFS appears to represent an IgE-mediated hypersensitivity reaction to fungi resembling that occurring in the bronchi in ABPA (Katzenstein *et al.* 1983).

The diagnostic criteria for AFS consist of five clinical features: radiologically confirmed sinusitis; the demonstration of fungal hyphae in the mucin; the absence of fungal invasion of submu-cosa, blood vessels or bone; and the absence of diabetes, immunodeficiency states, or recent treatment with immunosuppressive drugs (Bent and Kuhn 1994; DeShazo and Swain 1995). AFS does not become invasive. It must be distinguished from other infectious, neoplastic and inflammatory conditions causing sinusitis (Brandwein 1993). The short term prognosis for AFS is good, but patients and physicians need to understand that it currently appears to be a chronic disease, with objective findings recurring prior to those of subjective clinical symptoms after treatment (Kupferberg *et al.* 1997).

Baker's asthma

While this occupational illness has a wide diversity of allergens associated with it, fungal allergens play a substantial role in the etiology. Naturally, an important component of baking is the baker's yeast, *Saccharomyces cerevisiae*. It produces a 52 kDa enolase enzyme, a potent fungal allergen against which IgE response has been demonstrated in sera from asthmatic bakers (Baldo and Baker 1988). Alpha-amylase is a starch-cleaving enzyme of fungal origin (*Aspergillus oryzae*) often used as a flour additive and recognized as an occupational allergen in baker's asthma (Baur *et al.* 1988; Blanco Carmona *et al.* 1991). However, the cereal α - and β -amylases may be more important allergens than fungal α -amylase in baker's asthma, as demonstrated by an increased incidence of IgE to cereal amylase, relative to IgE to fungal amylase, in a study of 30 subjects with baker's asthma (Sandiford *et al.* 1994).

In addition to amylase, fungal cellulase has been found to be an occupational allergen by skin testing, histamine release test, reverse enzyme-immunoassay for specific IgE antibodies and bronchial provocation test (Quirce *et al.* 1992). In two cases of baker's asthma, pulmonary hypersensitivity was found to the moulds *Alternaria* and *Aspergillus* which were also present in the room air of the bakeries (Klaustermeyer *et al.* 1977).

Even although storage mites may be present in bakeries as contaminant allergens of the working environment, a survey of respiratory symptoms and occupational allergens

among 226 bakers and pastry makers in Italy revealed that wheat allergens and α -amylase must be considered as causative agents, but sensitization to storage mites is not important in this occupational allergic response (De Zotti *et al.* 1994).

Compared to the *Aspergillus* enzymes used as baking additives, sensitization to proteases from the paw-paw *Carica papaya* (papain) and the bacterium *Bacillus subtilis* has proved to be a rare phenomenon among symptomatic bakers (Baldo and Baker 1988).

Byssinosis

This occupational related disease is thought to be largely due to inhaled endotoxin. It is acquired by workers exposed to aerosols of dust generated in the early stages of processing raw cotton. Although this dust certainly contains many other bio-active impurities, the weight of evidence for the major inciting agent leans heavily towards endotoxin, either alone or in synergy with other agents (Burrell 1994). Several studies have exposed cotton workers, both with and without symptoms, and normal volunteers to various cotton dusts containing different amounts of endotoxin (Castellan, *et al.* 1984, 1987; Rylander *et al.* 1985). All of these studies agree that the presence/degree of symptoms and decline in FEV1 were not related to the total dust concentration, but were directly correlated with the endotoxin concentration.

Clinical manifestations of byssinosis include fever, flu-like symptoms, increased respiration rate, shortness of breath, and chest tightness.

Cutaneous manifestations

Finally, evidence is beginning to appear in the literature to indicate the role of various microorganisms and microbial enzymes in the etiology of eczema (atopic dermatitis) and allergic contact urticaria. A study of 30 children with atopic dermatitis demonstrated a mould mixture as a sensitizer (in patch testing) in 50% of those studied (Wananukul *et al.* 1993). A recent case report documented an eczema due to airborne *Penicillium* and *Cladosporium* (Kanny *et al.* 1996). Fungal α -amylase has been identified as a cause of contact dermatitis in bakers also (Morren *et al.* 1993).

Urticaria, or hives, has been shown to be produced in response to the same fungal α -amylase as implicated in baker's asthma, rhinitis, and dermatitis (Kanerva *et al.* 1997). A role is also suggested for *Candida albicans* as a factor in chronic urticaria (James and Warin 1971), defined as urticaria lasting longer than 6 weeks.

Fungal allergy can complicate the clinical picture of tinea pedis, commonly known as "athlete's foot". Tinea pedis can be caused by infection with either *Trichophyton rubrum* or *T. mentagrophytes*. Intense inflammation in *T. mentagrophytes* infections is the result of an immune, contact allergic response to the fungal antigens of that species (Leyden 1994).

The house dust mite *D. pteronyssinus* is widely suspected of being an important factor in the pathogenesis of atopic dermatitis, yet its role remains controversial. Epicutaneous challenge with dust mite allergen in patients with atopic dermatitis results in an eczematous reaction in which allergen-specific CD₄ T-cells are found (Reitamo *et al.* 1986; Bruynzeel-Koomen *et al.* 1988). A pilot, open trial of house dust mite avoidance demonstrated a significant reduction in symptom scores after 8 weeks of avoidance in 83% of participants (Roberts 1984). At least one double-blind controlled trial of dust mite

allergen avoidance has shown reduction in atopic dermatitis activity in those patients who received Goretex™ bedcovers, benzyl tannate spray and use of a high-filtration vacuum cleaner as dust mite reduction/avoidance techniques (Tan *et al.* 1996). Thus, dust mite allergy and is clearly contributory to the symptomatology of eczema.

The prominent role of skin colonization by *Staphylococcus aureus* as a contributing factor to atopic dermatitis exacerbations has been established. As demonstrated first by Leyden *et al.* (1974), and confirmed by others (Aly *et al.* 1977; Hanifin and Rogge 1977), *S. aureus* could be isolated from the skin in more than 80% of atopic dermatitis patients. Further studies have suggested a link between *S. aureus* colonization and the intensity of skin inflammation (Hauser *et al.* 1985), as the density correlates with severity, exacerbation and IgE levels (Herz *et al.* 1998). In contrast, only about 5–10% of non-atopic individuals carry *S. aureus* on their skin. Also, about 25% of atopic dermatitis patients have circulating IgE antibodies to *S. aureus* cell wall products (Leyden *et al.* 1974). The precise pathogenic role of *S. aureus* remains unknown, however.

Skin colonization by another bacterial species, *Helicobacter pylori*, has been related to chronic urticaria, but no case-control studies have been performed as yet (Rebora *et al.* 1995).

The medical literature contains one case report linking algal hypersensitivity and atopic dermatitis (Champion 1971); this represents a possible, but rare, manifestation of allergy to algal species.

Volatile organic compounds, mycotoxins and β -glucan

Over the last decade, a mounting body of evidence has accumulated to implicate the metabolic products and cell wall components of fungi and bacteria as key players in the allergic response of susceptible individuals. These products are discussed in more detail in Chapters 3.6 and 4.5 of this text, but their mention in this context is noteworthy.

Volatile organic compounds

Some VOC's are the metabolic by-product gases emitted by growing fungi. Multiple compounds have been isolated, including hexane, methylene chloride, benzene and acetone. The VOC's have been demonstrated in experimental settings to induce mucous membrane irritation (Mølhave *et al.* 1986) and asthmatic symptoms (Norbäck *et al.* 1995; Becher *et al.* 1996), and decrease FEV1 values significantly (Harving *et al.* 1991). They have been implicated (Hodgson *et al.* 1994; Nordsrom *et al.* 1994; Anon. 1996) in the etiology of sick building syndrome (SBS)/building related illness (see Chapter 3.6).

Mycotoxins

The spores of some fungal species have a high content of mycotoxins, including trichothecenes, ochratoxins and aflatoxins. These toxins have been shown to disregulate macrophage function *in vitro* and *in vivo* (Sorenson 1990; Jakab *et al.* 1994). As well, monocyte function has been shown to be impaired (Cusumano *et al.* 1996). *Stachybotrys chartarum* (*S. atra*) produces the most potent members of the large family of mycotoxins called trichothecenes. *S. chartarum* has been considered a cause of pulmonary haemosiderosis in infants, which in some cases was fatal (Dearborn *et al.* 1994, 1996,

1997). It is thought that trichothecenes may also have a role in SBS (Croft *et al.* 1986; Smoragiewicz *et al.* 1993). It may be that the mycotoxins and the moulds themselves have an effect on the airways in producing respiratory symptoms. There is a questionable role for them in ABPA suggested by the detection of specific IgE to ribotoxins of *Aspergillus* in 75% of a group of ABPA patients (Kurup *et al.* 1994).

Bacterial endotoxin

This component of bacterial cell walls is mentioned in this context for completeness. Full details have been covered in earlier sections of this chapter.

Microbial enzymes

Discussed earlier in detail in this chapter are the mite enzymes which behave as allergens in humans. Also allergenic, however, are enzymes of microorganisms. In particular, the yeast species, *Candida albicans*, secretes an acid protease which has been reported to induce atopic asthma (Akiyama *et al.* 1994). In addition, in a study of demonstrated a positive histamine-release response of peripheral blood leukocytes to *C. albicans* acid protease, as well as a conjunctival 49 *C. albicans*-allergic patients by skin-test, many clinical response (Akiyama *et al.* 1996). These studies suggest that *C. albicans* acid protease is an important allergen of itself.

β -Glucan

As discussed in Chapter 3.3, (1 $\rightarrow$ 3)- β -D-glucan is a component of the fungal cell wall. Human monocytes possess β -glucan receptors, which provide a mechanism by which a heightened state of host responsiveness is initiated (Czop *et al.* 1989). Pulmonary alveolar macrophage function and the immune system response to (1–3)- β -D-glucan is only partially understood. Fungal glucan appears to decrease phagocytosis and macrophage numbers (Rylander and Goto 1991; Rylander and Peterson 1993). Subjects exposed to the glucan in experimental challenges reported symptoms of thirst and nose irritation, headache, fatigue and cough (Rylander *et al.* 1992; Rylander 1993).

Clinical history with allergenic microorganisms

Mould Allergy

In all allergy evaluations the history is extremely important in elucidating the possible allergenic agents at work. It is important to gain an exposure history, and the clinical symptom profile will match one or both patterns of airborne mould allergy or indoor mould allergy.

Exposure history

Questions regarding the surrounding vegetation can provide useful information on likely allergenic microorganisms or pathogens. Patients may be able to describe obvious mouldy odours and/or visible mould growth in the home, work or school environment. Signs of visible water damage to occupied environments and the relative humidity in these environments heighten suspicion of involvement of moulds. The air may feel damp, surfaces may be wet, or the only hint may be the presence of carpeting in the basement.

Enquiries about the ventilation of the environment may provide important clues, as not only may a lack of adequate ventilation lead to mould growth, but ventilation sources may be contaminated with fungi, and filters may need cleaning or replacing.

Allergy to airborne moulds

The symptoms tend to follow a seasonal profile, with certain times of the year (spring and late summer/early autumn) being worse than others. The moulds involved are dominant and ubiquitous. Allergy to these moulds is frequently associated with pollinosis and prolongation of the hay fever season for sufferers. The diagnosis of this type of allergy is facilitated by specific, good quality extracts that are commercially available.

Allergy to moulds in the home

Little or no seasonal fluctuation of symptoms exists in patients with allergies to primarily indoor species. A large number of different fungal species fit into this category. Each habitat has associated with it its own characteristic fungal species, but the commercially available range of extracts remains incomplete and therefore insufficient to categorically exclude the possibility of fungal allergy.

Fungal extracts

Part of the challenge in the diagnosis of fungal allergy is the variability of commercially available fungal extracts used in skin-prick testing. The reasons for such wide variation are mycological and immunological. Moulds are highly adaptable, with the result that there are very wide variations in cellular and constitutional structures. There is highly marked pleomorphism in the recognition of these structures by the immune system; not only are many antigenic molecules recognized by allergic patients, but the degree of recognition of each of these antigenic sites differs from one individual to another (Gumowski 1997). Production of a reference antigen is extremely complicated in that, unlike other allergenic agents, moulds are whole living cells with remarkable powers of adaptation. In addition, each genus of mould usually comprises at least several species, and in the case of genera like *Aspergillus* or *Penicillium* many different species, and a number of somewhat different strains may exist within each individual species. Depending on cultural conditions, immediate environment and available nutrients, and the constitutional and metabolic nature of the fungus, the antigenic expression may change completely for a single strain. This may be demonstrated by cross-over immunoelectrophoresis (Aukrust 1979). The main problem is that the methods devised by researchers for the adequate production of purified ex-tracts are unusable at a commercial level, since preparation of the starting materials alone in practice requires an individual and specific protocol for each species (Platts-Mills *et al.* 1987). A complete list of the difficulties is provided below in Table 4.

Table 4. Difficulties encountered with preparation of fungus material for *in vitro* or *in vivo* diagnostic tests.

-
- Accurate identification of the organism
 - Selection and storage of the suitable strain
 - Adequate culture methods, metabolism, growth kinetics
 - Choice of morphological form: spores or mycelium
 - Extraction techniques
 - Standardization techniques
 - Qualitative variations between batches
 - Spontaneous enzymatic degradation
 - Choice of substrate and adaptation to specific tests
 - Control and elimination of toxins
 - Insufficient species studied and characterized
-

Mite allergy

The clinical history in patients with dust mite allergy is typically one of symptoms following a perennial pattern. In temperate climates, seasonal fluctuations in numbers of mites found in house dust are evident, numbers being low at the beginning of the summer and reaching a peak in late summer before dropping again in late autumn to low winter levels. This fluctuation results from a combination of outdoor temperature and humidity, along with the use of central heating. Despite this, the allergenic faeces produced by these mites remain in the environment, and the amount fluctuates less obviously than the number of mites (Platts-Mills *et al.* 1987; Kalra *et al.* 1992). Although mite numbers show seasonal trends, allergic symptoms to mite allergens are perennial in nature. However, patients may be more symptomatic in the morning, having spent the night sleeping on a dust mite-filled mattress. Mattresses provide favourable growing conditions for mites while occupied. Within 2 h, the heat and transpiration of the occupant cause the temperature to rise to between 2° and 30° C, regardless of the ambient temperature, and relative humidity increases to 80–95% (Hughes and Maunsell 1973; Arlian 1992). Furthermore, if the bed is made immediately on rising, it can take up to 16 h for the temperature and humidity of the mattress to fall to, and come into equilibrium with the drier, cooler bedroom air (Taylor 1971). Corresponding with this diurnal fluctuation in the humidity of the mattress, a diurnal vertical migration of mites within the mattress has been observed (DeBoer 1990).

Mite extracts

Extracts used in the testing of allergy to dust and storage mite are free of the component difficulties associated with fungal species. These have proved to be reliable in testing for allergy to dust mite, and are used on occasion in immunotherapy.

Allergy to bacteria and algae

As both of these types of organism are more uncommon contributors to allergenic hypersensitivity, no unique clinical history is attributed to them, save for specific obvious occupational exposures. As with the dust mite, the extracts used for the diagnosis of algal hypersensitivity appear to be reliable and reproducible.

Therapeutic recommendations

Avoidance of the allergen is the mainstay of all allergy therapies. Obviously, this is a difficult undertaking given the ubiquitous nature of allergenic microorganisms. However, some personal and environmental modifications can be initiated that may prove beneficial. Firstly, adequate outdoor air ventilation with filtration must be maintained throughout the year. Elimination of dampness and water leaks indoors is essential. The use of dehumidifiers (cleaned regularly) to maintain a relative humidity no greater than 50% may assist in inhibiting mould growth, but only if the substrate on which the growth occurs dries out with the dehumidification (see Chapter 2.1). For pre-existing obviously mouldy areas, cleaning with a hypochlorite bleach and detergent solution will eliminate the colonies, but re-growth may occur if resistant spores remain. It is imperative that persons working with such solutions protect their respiratory system by use of a mask and abundant ventilation of the room being cleaned.

Personal exposure to moulds can be reduced by wearing well-fitted particle masks (1 μm) while handling compost, vacuuming and cleaning fungus-contaminated areas.

Avoidance of dust mite allergens is a widely discussed topic, with many strategies open for employment, so they will be reviewed only briefly here. The most successful studies on dust avoidance in the home have emphasized very strenuous efforts in cleaning of bedrooms, including removal of carpeting, covering of mattresses and regular washing of bedding. Zippered vinyl covers for pillows, mattresses, and box spring beds have been shown at the end of a 1-month study to cause statistically significant reductions in symptoms (Murray and Ferguson 1983). It is important to note that washing of bedding is only effective in killing mites at temperatures greater than 70° C.

Investigations suggest that even with enthusiastic vacuum cleaning the efficacy is limited to removal of mite allergen on the surface. The live mites generally live away from the surface areas and are unaffected by vacuum cleaning. Because mite recolonization usually occurs within months, replacement or renewal of bedding, mattresses or carpets is for mite-allergic patients only a short-term solution. In a study of allergy avoidance that utilized regular vacuum cleaning of the bedrooms as the major avoidance measure, there was no significant improvement in either the patients'

symptoms or the mite antigen levels (Burr *et al.* 1980). It is important to note that mite-allergic patients who do their own cleaning should wear surgical masks during cleaning and for 10–15 min after cleaning.

In undisturbed rooms it is difficult to detect airborne mite allergen even after prolonged sampling. Whole mite bodies and fragments of mite can become airborne during bed-making and vacuuming, but very little allergen is associated with particles that will remain airborne for more than a few minutes. Therefore it is unlikely that air filtration can significantly reduce mite allergen exposure. Bowler *et al.* (1985) were unable to show any additional clinical benefit from combining existing dust control measures with electrostatic and high efficiency particulate air (HEPA) filters. Air filters, however, can remove very small particles, and some patients may feel helped by air filtration because of a reduced irritant effect of small non-allergenic particles (Pollart *et al.* 1987).

The use of dehumidifiers and/or air conditioning can reduce mite numbers by as much as ten times. Limiting relative humidity indoors to less than 50% is not only beneficial in reducing mite growth, but may also the control growth of mould species, as mentioned earlier.

Three basic acaricidal chemical compounds, benzyl benzoate, pirimiphos methyl and PARAGERM have passed from successful *in vitro* assessment on to trials in houses. Several different solutions exist, some combining the above ingredients, others involving combinations with other substances, e.g. tannic acid. All showed some effect, none achieved complete control of mites, and all would require application every two months if control was to be maintained (Pollart *et al.* 1987; Wickman 1997).

As bacteria and their products are essentially ubiquitous and unavoidable, no specific strategies other than frequent hand-washing and good hygiene will reduce exposure. No strategies have been identified in the literature for avoidance of exposure to algae.

Medical therapies include oral antihistamines (H1 receptor antagonists) for symptoms of allergic rhinitis. The first generation antihistamines, such as diphenhydramine, are very effective for symptom control but have a high incidence of significant drowsiness as a side effect. Thus, the second generation H1 antagonists (e.g. cetirizine, fexofenadine and loratadine) are useful non-drowsiness alternatives. Also of benefit for perennial symptomatology are the intranasal glucocorticosteroids, e.g. fluticasone propionate, budesonide and mometasone furoate. These products have low systemic absorption (approximately 1%) and thus provide safe, efficacious therapy in patients with chronic, daily symptoms. Prevention of symptoms may be achieved by the intranasal sodium cromoglycate preparations, e.g. Nasalcrom®, through their mast cell stabilizing properties.

Patients with asthmatic symptoms in response to allergenic microorganisms will achieve symptomatic relief from the inhaled β_2 -agonists (e.g. salbutamol) but daily use of an anti-inflammatory inhaled sodium cromoglycate or corticosteroids are essential in stabilizing the underlying inflammatory reaction that produces the airway irritability and narrowing in asthma. A specific study of sodium cromoglycate in lipopolysaccharide (endotoxin)-induced bronchial obstruction in asthmatics demonstrated that acute treatment with sodium cromoglycate blocks the endotoxin-induced FEV₁ response (Michel *et al.* 1995).

Systemic corticosteroids are the mainstay of treatment in HP, after avoidance, as they may prevent fibrotic scarring. Bronchodilators are not indicated. The treatment of ABPA is the same as for hypersensitivity pneumonitis, but with the possible inclusion of other anti-asthmatic agents. The positive effects of the corticosteroid therapy is thought to result from the dampening of the inflammatory response and an increase in efficiency of killing the responsible fungi (Kauffman *et al.* 1995). Antifungals such as ketoconazole are not beneficial and have no place in treatment (Day 1996).

Treatment of AFS requires endoscopic removal of polyps and inflammatory material to establish aeration and drainage of the involved sinuses, followed by oral corticosteroids for 2 weeks post-operatively (DeShazo *et al.* 1995). Patients should receive intranasal corticosteroids on a long-term basis (Corey *et al.* 1995). Antifungal agents have not been shown to be useful.

Immunotherapy

The best characterized fungi for immunotherapy are *Alternaria* and *Cladosporium*. The therapeutic benefits of immunotherapy with these fungi have been demonstrated in both open-label and controlled double-blind trials. Standardized extracts of *Alternaria* were prepared for diagnosis and immunotherapy to improve symptoms in 24 patients with allergic rhinitis, and with or without asthma (Horst *et al.* 1990). When 39 children with asthma and/or rhinitis were followed prospectively for 3 years while receiving immunotherapy for *A. alternata* allergy, 80% reported excellent results when they reached the 80,000 PNU, the remaining 20% claiming good results. While at the intermediate dose of 40,000–80,000 PNU, only 51% reported excellent results. While open-label, a recent trial comparing injective versus sublingual immunotherapy in *A. alternata*-allergic patients demonstrated the clinical efficacy of the two treatments, with a better safety and tolerability profile for the sublingual form (Bernardis *et al.* 1996). A clear tendency towards a beneficial role of *Cladosporium* immunotherapy was demonstrated in a study of 22 adult asthmatics (Mailing *et al.* 1986), as well as one involving 30 children with asthma (Dreborg *et al.* 1996).

Immunotherapy has been shown to have a role in the treatment of AFS also, with early results demonstrating a decrease in objective findings in patients as well as diminished need for corticosteroids, systemic or topical (Mabry and Mabry 1997).

Despite these promising trials, caution must be exercised in the implementation of immunotherapy, particularly in asthmatic patients. A prospective study by Oostergaard *et al.* (1986) demonstrated that in asthmatic children receiving immunotherapy for various allergens mould extracts were responsible for the most frequent and serious side effects (*Alternaria*, 3/106 patients; *Cladosporium*, 8/106) and the grass *Phleum pratense* responsible for 2 of 6 anaphylactic reactions. Another analysis of children receiving immunotherapy with mould extracts for asthma documented the withdrawal of seven (19%) children due to serious side effects (Kaad and Oostergaard 1982). The report went on to suggest that hyposensitization of children with mould extracts may be hazardous in children who may have a primary “immune balance”, as suggested by an increased number of circulating precipitating antibodies to the injected extracts in the children

requiring withdrawal.

The other challenge in interpreting the findings of immunotherapy trials is that the approach in the studies has been to use potent, and in some cases, standardized extracts prepared by the investigators themselves for the studies. Skin testing with the extracts is correlated by history and the patients are challenged with this material to document a state of hypersensitivity. Patients then receive immunotherapy and are sometimes re-challenged with the same extract to document efficacy (Dhillon 1991). Such standardization of extracts both for testing and for therapy is not widely available commercially, and further research directions should focus on the production of such extracts, so that trials with these allergens can be conducted.

Immunotherapy for dust mite allergy has so far been proved effective for asthma in patients in whom the dust mite allergy is isolated (Hedlin 1995). A review by Bousquet and Michel (1994) has suggested that children tended to improve more than adults do, and the improvement from mite immunotherapy was greater in children who had normal or close to normal lung function. A number of studies have shown the late phase response in bronchial reactivity to be attenuated by immunotherapy. The effects on nonspecific bronchial hyper-reactivity have been contradictory, however, with both beneficial effects (Gruber *et al.* 1994) and lack of effect (Hedlin 1995) being seen in different studies. The role of immunotherapy for allergic rhinitis induced by dust mite has not yet been firmly established. Bacterial antigen immunotherapy is not indicated as a treatment for allergic respiratory disease.

The role of immunotherapy in hypersensitivity to algae is as yet unpublished in the medical literature. Given the relative rarity of hypersensitivity to algae, immunotherapy extracts are not likely to be widely produced and/or available.

CONCLUSION

Fungi are the commonest microorganisms responsible for allergic disease. Exposures to various fungal products produce a wide range of reactions and these may occur in response to spores, mycelium or components of the cell wall, viz. (1 $\rightarrow$ 3)- β -D-glucan. Metabolic by-products, including VOC's and mycotoxins, also produce adverse consequences which are not allergic.

In spite of important new knowledge, clinical management of symptoms, including testing and treatment, is hampered by inadequate information regarding the composition of fungal allergens and the role of toxic compounds produced by fungi and bacteria. Dust mites induce allergic responses, while bacteria and their endotoxins augment hypersensitivity reactions. Algae are likely contributors in certain instances, but their specific role is unknown.

Further studies are required to add to accumulated information which will help to define actions and interactions of these biological aerosols in production of allergic disease.

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Chapter 3.2

OCCUPATIONAL RESPIRATORY DISEASE: HYPERSENSITIVITY PNEUMONITIS

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INTRODUCTION

Among the diseases related to the built environment, hypersensitivity pneumonitis (HP) is the one most clearly recognized by health practitioners as attributable to some specific source, despite its rarity. In that, it differs dramatically from asthma, despite evidence that building-related asthma is a far more widespread problem (Sieber *et al.* 1996; Hodgson *et al.* 1998). A single case of HP suggests the need for a broader public health approach, as the conditions leading to a single case are likely to put additional members of the exposed cohort at risk (Rutstein *et al.* 1983). In fact, the one well-recognized published report of building-related asthma is associated with additional cases of hypersensitivity pneumonitis, bronchiolitis obliterans and usual interstitial pneumonitis (Hoffmann *et al.* 1993). In addition, in at least one outbreak of HP, a substantial proportion of affected individuals developed onset of wheezing 1–3 h after exposure (Hodgson *et al.* 1987). This suggests that outbreaks of one form of building-related allergic disease are not infrequently associated with other forms. In fact, a recent publication presents evidence indicating that buildings are the single most common source of work-related asthma in the USA (Jajoski 1999), so pointing to the need for far more intensive scrutiny of building-associated disease.

The “sentinel health events” mode of practice in occupational and environmental medicine (Rutstein 1984; Matte *et al.* 1990; Mullan and Murthly 1991) is based on the recognition that diseases may be related to specific risk factors, which may guide specific actions for individual cases and lead to broad-based intervention strategies by identifying both work exposures that are remediable and exposed cohorts that require some screening for further disease. The common failure to seek or recognize additional cases of disease leads to the dramatic under-recognition and reporting of occupational disease throughout the developed countries. A single case HP should always lead to scrutiny of the group of individuals exposed to the same environment, since identification of the source is of paramount importance.

HYPERSENSITIVITY PNEUMONITIS

History and Epidemiology

Since publication of the report of Fink *et al.* (1971), HP has been recognized as one of the potential consequences of contaminated ventilation systems. The interstitial lung diseases—HP, sarcoidosis and others—occur at a prevalence rate of about 70 per 10⁶ persons and an incidence of 30 per 10⁶ person years. Less than 3% of these cases represent HP, the disease most clearly associated with moisture and mould; over 80% are considered idiopathic interstitial pneumonitis. Data presented by the National Institute for Occupational Safety and Health (NIOSH) suggest that HP in North America represents primarily a disease of farmers (NIOSH 1996). Pigeon breeding is recognized as the second most frequent cause of disease, at least in the UK. In USA, buildings and ventilation systems are frequently recognized as a source of HP, in parallel with their being commonly contaminated by bioaerosols (Batterman and Burge 1995), although they rarely lead to fatalities.

HP is not a new disease. Ramazzini (1713) recognized that chest symptoms were occupational consequences among workers with organic dust exposures. The clinical syndrome of HP was first described by Campbell (1932). Replacement of square baling of hay with round baling has led to a dramatic decrease in mouldiness of hay and a dramatic decrease in the incidence of disease. Subsequently, HP has been attributed primarily to large molecular weight antigens associated with bioaerosols in a wide range of environments. Etiological lists often attribute disease to both particular industries, which can range, for example, from floor malting to enzyme detergent manufacture, and to a specific microbial agent. Occupational processes have consistent features from workplace to workplace, so that similarities in materials being processed, the processes themselves and the use of water lead to characteristic combinations of nutrient media and wetness. These similar conditions are then associated with predictably similar microorganisms. As discussed in Chapter 2.1, in buildings where nutrients and moisture conditions (the water activity of the substrate) are similar, predictably similar microorganisms will grow. Residential heating systems, mushroom compost and badly stored (moist) hay or grain may not seem to be similar. However, they can all provide the nutrients, moisture and elevated temperatures which favour the growth of the thermophilic Actinomycetes, *Saccharopolyspora (Faenia) rectivirgula* and *Thermoactinomyces vulgaris*, responsible for the HP disease known as farmer's lung. Less frequently, small molecular weight antigens are associated with HP. Characteristic of these agents is their reactivity; most are intermediaries or monomers in manufacturing, such as isocyanates and anhydrides.

Mechanisms

The mechanisms underlying HP have been reviewed elsewhere (Salvaggio 1997; Daroowalla and Raghu 1997); they include aspects of both Type III and Type IV

reactions. The presence of granulomas clearly documents the importance of cell-mediated phenomena, as do animal models. The presence of symptom onset 4–8 h after exposure, complement-fixing antibodies, and Arthus reactions after skin testing support the importance of Type III mechanisms.

Clinical presentation and diagnostic strategies

Patients generally present with one of three forms—acute, subacute and chronic. Acute episodes occur presenting either as symptoms in a temporal pattern or as acute hospitalization, sometimes with hypoxia (hypoxaemia) requiring mechanical ventilation. With well-established disease, patients generally present with both chest (chest tightness, coughing, dyspnoea, and wheezing) and systemic symptoms (generalized aching, feverishness, and chills). Fatigue is a prominent symptom and in some early disease fatigue is the only presenting symptom, even in individuals who are subsequently shown to have biopsy-confirmed disease. Therefore, prominent fatigue with some nausea should at least trigger the question of moisture and potential exposure to bioaerosols.

The usual diagnostic strategies rely on documentation of disease (restrictive changes on objective lung function testing), documentation of exposure (see later), and linkage.

Documentation of disease

Imaging

Increasing evidence suggests that chest radiography, the standard primary technique for documentation, is quite insensitive (Hodgson *et al.* 1989). In the one recent population-based investigation, <10% of chest x-rays and <50% of thin-section CT scans were abnormal (Lynch *et al.* 1992). Data from this outbreak suggest that few patients had abnormal lung function tests, the second usual justification for diagnosis (Rose *et al.* 1998). This same phenomenon has recently been documented in another large outbreak of HP associated with exposure to metal-working fluids (Hodgson *et al.*, 2000).

Physiology

The diagnostic tests most commonly relied upon are those that yield results consistent with restrictive lung disease. In lung function testing, decrements in forced vital capacity, total lung volume, and functional residual capacity are commonly encountered. Decreased carbon monoxide diffusing capacity, either single breath or preferably steady state, are frequently seen. Antibodies to agents in the environment are of interest but remain very difficult to interpret.

Biopsy

A relatively invasive technique, lung biopsy, is the only specific diagnostic technique to characterize disease identified through sensitive screening instruments such as questionnaires. Lung biopsy is rarely employed in UK, where the general view of respiratory physicians is summarized by Murphy *et al.* (1995): “Lung biopsy is neither necessary nor justifiable in most cases of allergic alveolitis. However, the disease may

occasionally be suspected but no allergen identified. In such cases, a biopsy may be necessary to prove the diagnosis or to exclude other possibilities". Nevertheless, in a recent outbreak among life-guards at a recreational pool in USA, a substantial proportion demonstrated disease on biopsy without other evidence of clinical disease, including early airways closure. Similar findings have been encountered in other outbreaks (Hodgson *et al.* 2000), and there is currently disagreement on the need for, and utility of, biopsies across the USA, especially in early cases where disease is most readily completely reversible.

Documentation of exposure

In industries clearly recognized to be at risk for HP, the combination of moisture and typical nutrients leads to a characteristic microbial growth. In most forms of HP, either the microbiota or the characteristic agents, such as avian proteins, have been identified and may be sampled in standard ways. Exposure documentation is then useful primarily in the context of scientific work. On the other hand, outbreaks of hypersensitivity pneumonitis, and even individual cases (Kreiss and Hodgson 1984), have generally been associated with unwanted moisture in the built environment. As part of the Centers for Disease Control in USA, NIOSH therefore suggested seven steps that should be taken to prevent moisture incursion into buildings (Anon. 1984). Including types other than HP, outbreaks of interstitial lung disease described since 1984 have generally confirmed the paramount importance of moisture. Because many outbreaks actually present with multiple forms of lung disease (asthma, HP, bronchitis, mucosal irritation), it is instructive to examine building factors associated with the various described outbreaks rather than focusing on HP alone. Detailed engineering analyses, which systematically reviewed all potential causes of building moisture, have been carried out in only a few outbreaks of HP associated with buildings.

Unwanted water may result from failures in construction management, from deterioration in buildings, from inadequate maintenance from internal water sources, from site planning problems, or from enthalpy problems related to ventilation system sizing.

The effect of such moisture problems on microbiological air quality has been discussed in Chapter 2.1. Such problems may be classified according to the system to which they are attributed (mechanical ventilation, building envelope, plumbing, etc.) or to the phase of the building's life (construction, commissioning, operations, maintenance, etc.). As mentioned in Chapter 2.1, common causes of construction moisture include inadequate drying time for wet building materials, inadequate curing times for concrete, and construction defects related to envelope integrity (roof flashing, joint sealing on internal drains, etc.). Inadequate maintenance includes failure to detect and repair breaks in pipework, either within walls or slabs, or in other hidden locations; to detect and remedy contamination in ventilation system filters, sound insulation liners and inadequately draining drain pans; and to deal with carpet contamination. A recently recognized problem relates to building siting and drainage, in USA a problem commonly encountered in schools. There, because developers frequently give to the community for

the construction of schools the most poorly draining land, as it less desirable for property owners, the schools are often built in areas with high water tables, i.e. swampy areas. Consequently, moisture seeping up through concrete slabs has been identified as a problem in several recent outbreaks of building-related lung disease.

Older investigations generally failed to present detailed descriptions of the HVAC system or of the spectrum of moisture problems in the affected building. In general, they did identify a specific mechanical system or set of conditions that served as a reservoir and amplifier for bioaerosols. Because investigators in the past stopped after identifying a single apparent source, it remains unclear whether other contributory sources would have been revealed by fuller investigation. Careful scrutiny of the built environment in recent outbreaks has generally identified more than one source, or problem, arising from inadequate moisture control (Welterman *et al.* 1998; Hodgson 1998).

Welterman *et al.* (1998) described a single case of HP associated with a building on the basis of a biopsy and convincing clinical improvement and recurrence associated with removal from and re-exposure to the building. The patient had IgG antibodies to commercial preparations of *Aspergillus* strains that were identified on surface sampling, but not in air samples. Ratios of indoor: outdoor microorganisms in bioaerosols established using an Andersen N-6 sampler were not elevated. In addition, excess symptoms consistent with HP were seen in a comparison with four control buildings, but no clear evidence of additional clinical HP was identified. The building was constructed in an area with a high water table; employed an oversized ventilation system with inadequate enthalpy control; and had water collecting in puddles on the roof, which also had some leaks.

Together with an excess of wide-ranging symptoms consistent with a systemic illness, Hodgson *et al.* (1998) observed obstructive and restrictive lung disease among occupants of a mould-affected building in Florida. This court building had evidence of moisture incursion through the envelope (leaks in flashing on the roof, and in drains within wall cavities); insulation problems, with vinyl wallpaper acting as a moisture barrier on interior surfaces; and ventilation system design problems (inadequate dehumidification), with elevated indoor relative humidity. Growth of *Aspergillus versicolor* and *Stachybotrys atra* was widespread under the wallpaper and on surfaces, and spores of these fungi were present in the indoor air. Indoor: outdoor ratios of *Aspergillus* were elevated. "Semi-aggressive" air sampling, i.e. sampling after opening law books and various other routine indoor activities (described in the paper), demonstrated that there were would be substantial increases in indoor bioaerosol concentrations as a result of the natural disturbance created by occupants of the building.

In general, air sampling has not been useful in the diagnosis of immunological lung disease associated with buildings. Walkthroughs have generally allowed the identification of potential reservoirs, either by smell or visual evidence of biological contamination. On the other hand, growth and characterization of the organisms identified in the workplace is often undertaken, but is painstaking, time-consuming and often less than helpful, as is described later.

Linkage

Disease can be linked to environmental causes in one of three ways, epidemiologically, toxicologically or clinically. Of these, the latter two are most persuasive to physicians, although not necessarily to non-health care practitioners. The classic forms of HP disease, in pigeon breeders and farmers, are generally linked to the cause in a relatively straightforward fashion. Disease attribution related to buildings, where most of us spend over 90% of our time, is far more difficult.

Linkage of acute and subacute forms generally relies on documentation of temporal changes related to exposure. This may require energetic participation on the part of the patient and the employer or the family. The patient should be evaluated, some pertinent outcome measure should be determined, and the patient removed from the source of exposure being re-exposed, with repeated determination of the same outcome. Such strategies may help distinguish home from work-related disease, with major consequences for whichever group of individuals should also be scrutinized (family or work colleagues). This will generally narrow down the site of exposure considerably. In chronic forms, with temporal resolution in several weeks, patients may not present with such clean repeated exposure scenarios and results.

Although some investigators have attempted to use antibodies, these have long been considered markers of exposure rather than effect (Burrell and Rylander 1982). Equally importantly, in some cases, patients with disease related to a specific agent in the workplace have not demonstrated precipitins to agents identified in the air in workplaces. This has occurred for two reasons. Firstly, the immunological characteristics of some agents change as they are cultured, so that any individual strain of organism may produce responses in the laboratory different from those when it is growing in the field. Secondly, some agents are simply not identified because of the time required for sampling for such organisms. Despite frequent assumptions that precipitating antibodies to an agent grown at the implicated site are necessary, the first author has found them less than useful. The linkage to an exposure generally relies on a history of exposure to an agent that has been implicated epidemiologically. This takes a great deal of time and makes the recognition of new causes problematic. Nevertheless, for interstitial lung disease in most of its forms this is the only available technique. For sarcoidosis from beryllium (Newman *et al.* 1997), an alternative relies on the demonstration of the actual immunological response documented through lymphocyte transformation tests. Despite much discussion about the utility of such tests for the diagnosis of cell-mediated immunity in HP, this author is unaware of any cases of HP where such tests have been used or useful.

Clinical Course

When detected early, HP may resolve completely. The more frequently that episodes of acute disease occur, the more likely there is to be some longer-lasting chronic damage, as has been demonstrated in farmer's lung disease. Nevertheless, it is clear that even in the absence of repeated episodes, decrements in carbon monoxide diffusing capacity (Milton *et al.* 1995) and exercise testing (K.Kreiss, personal communication) have been

demonstrated.

Overlap/differential diagnosis

In a recent case-control study of non-granulomatous interstitial lung disease (excluding the pneumoconioses), which represents 80% of the burden of all lung disease, individuals with exposure to mould in their workplace, including those in buildings, were approximately 15 times more likely to develop such disease (Mullen *et al.* 1998). Sarcoidosis is meanwhile considered likely to represent an inhalation disorder, with a different immunology from HP (Newman 1997). A recent report of sarcoidosis appeared related to a wet school (Thorn *et al.* 1997), although the disease in this and a similar case were subsequently called HP after linkage to a building (Forst and Abraham 1995). In addition to this, a replication of the case-control study mentioned above using sarcoidosis patients as cases revealed that subjects were eleven times more likely than controls to have exposure to moulds in their workplaces, including those indoors (Ortiz *et al.* 1998). Case reports of non-specific interstitial pneumonitis, distinct from HP, have been associated with fungal exposure (Lonneux *et al.* 1995).

Some evidence exists that office workers may develop yet another form of interstitial pulmonary response, possibly more frequently than commonly assumed, viz. organic dust toxic syndrome (Chapter 3.3), the same disease as humidifier fever (Milton 1996). Beginning with a search for humidifiers as a cause of symptoms noted in office workers (Finnegan *et al.* 1984), excess rates of chest tightness and flu-like illness have been reported. A reanalysis of several older data sets (Apter *et al.* 1997) suggested that a symptom cluster of chest tightness, difficulty in breathing and flu-like illness that was common among office workers without recognized HP may have been associated with exposure to endotoxin (Gyntelberg *et al.* 1994; Teeuw *et al.* 1994) or fungal glucan (Rylander *et al.* 1992).

TYPES OF OCCUPATIONAL HYPERSENSITIVITY PNEUMONITIS

As mentioned earlier in this chapter and Chapter 3.1, and discussed more fully in Murphy *et al.* (1995), the causes of HP are various. The remainder of this chapter is given over to four diseases drawn from the partial list of those caused by microorganisms presented in Table 3 of Chapter 3.1. Three of these diseases are related to each other in being caused by thermophilic Actinomycetes, and the causal organism in the fourth is a mould. The occupational and microbiological aspects of these diseases, and measures which can be taken to reduce or eliminate respiratory exposure to causal organisms in the indoor work environment, are discussed.

Farmer's Lung

Farmer's lung is probably the best-known example of an occupational HP. However, although HP associated with handling of mouldy hay was first described in the early 1930's (Campbell 1932), it was not until 30 years later that thermophilic Actinomycetes were recognized as a source of "farmer's lung hay" antigens (Pepys *et al.* 1963; Pepys and Jenkins 1965). The disease is caused by inhalation exposure to spores in the dust released not just from "self-heated" or "mouldy" hay but also from similarly deteriorating grain. Hay that is damp when it goes into store, i.e. with a moisture content of 35% or more, provides a substratum on which microbial contaminants acquired during the growing season and at harvesting can grow. The potential health hazards from microorganisms associated with production of composts from various plant materials have been discussed in Chapter 2.2; the biodeterioration, or spoilage, of damp hay is the result of unintentional composting.

The microbiological changes which occur in deteriorating hay (Gregory *et al.* 1963) and moist-stored grain (see Lacey, 1989) were established by P.H.Gregory, M.E.Lacey, J.Lacey and other workers at Rothamsted Agricultural Station in UK. Since damp bulk hay or bales provide a well insulated environment, the heat generated by microbial metabolism inexorably builds up; the types that are less tolerant of rising temperatures give way to thermotolerant and thermophilic species. Among the thermotolerant fungi which develop, *Aspergillus fumigatus* is prominent, but the upper limit for its growth under ideal circumstances is 50° C or so. However, thermophilic Actinomycetes can grow at temperatures above this and become the dominant microorganisms, raising the temperature to around 65° C. *Saccharopolyspora (Faenia) rectivirgula* and *Thermoactinomyces vulgaris* are usually cited as the principal causative agents of farmer's lung, but *T. vulgaris* has been subdivided into two antigenically different species. One retains that name and the other is *T. thalpophilus* (Lacey, 1989). Two other actinomycete species which have been implicated in the disease are *Saccharomonospora viridis* and *T. candidus* (Kurup 1989).

Huge numbers of spores present in self-heated hay and grain are released into the air during handling. Opening hay bales in an open shed may result in concentrations of nearly 10^8 Actinomycetes/Eubacteria m^{-3} air; shaking baled hay inside a farm shed may give close to $1.6 \times 10^9 \text{ m}^{-3}$ air; and unloading moist-stored barley from an unsealed silo release almost $1.8 \times 10^9 \text{ m}^{-3}$ (Lacey 1989). It is in winter when farm workers open bales indoors and fork out rations for stock that they most commonly experience HP symptoms. The incidence of farmer's lung is obviously influenced by climate. For example, in UK the disease is commoner in the wetter, milder west than in the drier east (Grant *et al.* 1972). However, differences in agricultural practice are also important; clearly, the prevalence of farmer's lung among populations engaged in dairy and beef farming is likely to be higher than among those in areas where arable farming predominates.

Typically, farmer's lung is seen as an acute disease that develops a few hours after exposure to the airborne dust from self-heated grain or hay. The initial symptoms—fever, chills, dry cough, joint pains and breathlessness—together resemble influenza, and

because they do not occur at the time of the exposure may not be associated in the victim's mind with massive exposure to dust from badly deteriorated hay or grain. Basal crepitant rales, decreased pulmonary diffusing capacity, characteristic changes in radiographs and the development of precipitating IgG antibodies (Pepys and Jenkins 1965) may accompany these symptoms. Repeated exposure to the dust results in loss of weight and increasing breathlessness as the lung is increasingly infiltrated by granulomatous and fibrous tissue and, in extreme cases, is eventually transformed into a multitude of dilated air spaces surrounded by fibrous tissue ("honey-comb lung"). However, it is considered that, depending on the pattern of exposure and natural susceptibility of those exposed to the actinomycete allergens, it is more likely that in a high proportion of exposed subjects the development of the disease is more insidious (Murphy *et al.* 1995).

Farmer's lung is, at least theoretically, a wholly avoidable occupational disease. Since 1964, it has been officially recognized as such in UK, where affected workers are eligible for industrial compensation, but in many countries there is no such compensation. The first step to control is to educate and advise the farming community on the hazards and how to avoid them. The prime avoidance measure is the adequate and rapid drying of hay and grain, and where grain for animal feed is stored moist maintenance of a depleted-oxygen atmosphere in the silo, so that microbial growth is largely prevented. Clearly, there are years when it is difficult to dry hay satisfactorily, and in such cases mechanical handling can reduce, and the wearing of effective respirators (HSE 1998a,b) prevent, inhalation exposure to injurious spores. In climatic regions where drying is perpetually a problem, abandoning hay making in favour of silage has been advocated.

Mushroom worker's lung

A related HP occurs among workers involved in commercial production of the common cultivated mushroom, *Agaricus bisporus*, was first described in the 1950s (Bringhurst *et al.* 1958). As has been mentioned in Chapter 2.2, it is necessary to produce compost suitable for the growth of the mushroom. In the initial phase of the two-phase process, the usual mixture of moistened cereal straw and horse manure is composted outdoors. It is then heated indoors with humidified air to 60° C in special chambers and composted for a further 10 days or so at this temperature. A thermotolerant/thermophilic microbiota rapidly dominated by Actinomycetes develops during this phase, and the temperature of the compost may rise locally to 70° C. The final propagule count for fungi may be around 10^6 g⁻¹ compost, and for the Actinomycetes and other bacteria as high as 10^{10} g⁻¹ compost. The elevated temperature has a pasteurizing effect on the compost, killing mesophilic fungi which might otherwise grow during the *A. bisporus* cultivation stage. The spawn of mushroom mycelium is mechanically mixed with the compost at a rate of approximately 0.5% and incubated at 25°C, the optimum for mycelial growth, for a further two weeks. After this, the compost is dispensed into trays, covered with a layer of peat and chalk and maintained at 16–18°C in a highly humid atmosphere, the first crop being harvested after about 3 weeks and further harvests taken over the next 5 weeks or so (Carlisle and Watkinson 1994).

During the mixing of the mushroom spawn with the compost, huge numbers of thermophilic actinomycete spores may be released into the air, i.e. $>10^9$ CFU m^{-3} air, but the count for fungi, including the genera *Aspergillus*, *Penicillium* and *Scytalidium*, may be only 10^3 CFU m^{-3} (van den Bogart *et al.* 1993). The predominant thermophilic Actinomycetes recorded by van den Bogart *et al.* (1993) were *Excellospora flexuosa*, *Thermomonospora alba*, *T. curvata* and *T. fusca*. In stationary-bed mushroom houses Kleyn *et al.* (1981) found that the total count for spent compost was 16×10^8 spores g^{-1} , of which more than 90% were Actinomycetes and only about 5% were moulds. The total count for dust emanating from spent compost during was 0.33×10^6 m^{-3} air. A worker called upon to dump spent compost could inhale a total of 6.4×10^7 microorganisms during the 2.5 h taken to complete the operation (Kleyn *et al.* 1981).

Although Sakula (1967) reported the occurrence of precipitins against *Saccharopolyspora faeni* and *Thermoactinomyces vulgaris* in the sera of MWL patients, when van den Bogart *et al.* (1993) tested sera of 10 Dutch mushroom growers with MWL against spores for antibodies of these microorganisms by a qualitative dot-ELISA all were positive for one or more of the four species mentioned in the previous paragraph. No antibodies were found among these sera in tests against other actinomycete species (*Streptomyces thermovulgaris*, *Thermoactinomyces vulgaris* and *T. sacchari*) or fungi (*Aspergillus fumigatus*, *Penicillium brevicompactum*, *P. chrysogenum*, *Scytalidium thermophilum* and *Trichoderma viride*). While the sera of 19 non-exposed individuals were negative for thermophilic Actinomycetes, in 11 of 14 workers routinely involved in spawning the compost in tunnels the sera reacted positively with one actinomycete or more, the titres increasing with length of employment. On inhalation, air rich in spores of *E. flexuosa* and the three species of *Thermomonospora* provoked reaction in workers with MWL. This provocation and the elevated serum titres in the workers led van den Bogart *et al.* (1993) to conclude that these organisms contribute to the occurrence of MWL. In an earlier Dutch study, Cox *et al.* (1991) observed that measures taken to reduce the exposure to these allergenic spores included supplying pre-treated compost requiring no preparation by mushroom growing personnel, wetting compost to reduce aerosols, improving air-conditioning at the work sites, and wearing masks.

Bagassosis

Bagassosis in the cane sugar industry

Bagassosis is also a disease in which the presenting symptoms are basically the same as in farmer's lung (Murphy *et al.* 1995). It occurs among workers in cane sugar mills or in industries that utilize the fibrous waste product, bagasse, which remains after the sap is extracted from crushed sugar cane (Phoolchund 1991). With around 50% of its fresh weight of bagasse being water and roughly 4% being sugar (Hunter and Perry 1946), bagasse is moist enough and contains sufficient nutrients to support microbial growth. When stacks of the baled by-product, perhaps as much as 1000 tons in a stack (Hearn 1968), are left at the sugar mills to dry outdoors in the hot and humid climate for up to 12 months, microbial activity raises the temperature. As noted in Chapter 2.2, a mixed microbiota of fungi and Actinomycetes, but ultimately dominated by thermophilic

Actinomycetes, develops in the "self-heated" bales (Lacey 1974). If bagasse is later sent for paper or fibreboard manufacture, the badly weathered and mouldered bales to the outside are discarded and the remaining bales are fed manually into an indoor hydraulic ram, which compresses the material into smaller bales for despatch (Hearn 1968).

A recent investigation found that in two Indian sugar cane mills these Actinomycetes were *Thermoactinomyces sacchari*, *T. vulgaris*, *T. thalpophilus*, *Saccharomonospora viridis* and *Saccharopolyspora rectivirgula* (Khan *et al.* 1995). The principal cause of bagassosis is considered to be *T. sacchari*, but *S. rectivirgula* also appears to be involved. Khan *et al.* (1995) found that among the 22% of workers with precipitating antibodies against thermophilic Actinomycetes, more than one-half had positive precipitin reactions to *T. sacchari* alone, and approaching one-third to *S. rectivirgula*. In symptomatic workers the mean absorbance values for IgG antibody activity against *T. sacchari* and *S. rectivirgula* were significantly higher than in asymptomatic workers and unexposed controls. Khan *et al.* (1995) concluded that *T. sacchari* and *S. rectivirgula* are the major species causing sensitization of bagasse workers in India.

Where most of the fresh bagasse is used immediately as fuel for generating the electricity and steam needed during the seasonal 5–6 months of sugar crushing and extraction, the total airborne bacterial counts are not likely to be excessively high, e.g. in Australia (Dawson *et al.* 1996) and in Japan (Ueda *et al.* 1992). Dawson *et al.* (1996) found that in two Australian mills these counts were in general below the voluntary industry standard of 10^6 m^{-3} air, and even the highest counts were substantially lower than the suggested level of concern for the general population, i.e. 10^8 m^{-3} air. All workers in sugar mills are exposed to spores associated with sugar cane, but it is where they are engaged in re-baling stored bagasse that they have the highest exposure to thermophilic Actinomycetes. Hearn (1968) noted in the West Indies that the work of re-baling stored bagasse for onward transport work fell to full-time employees, whereas seasonal workers handled only bales of fresh bagasse and were therefore at less risk of exposure to these agents of bagassosis.

Ueda *et al.* (1992) observed that dust levels in an Okinawan sugar refinery were relatively low, except for a storage room in which 1–3% of the bagasse was processed and bagged for use as fertilizer, feed and a mulch for seedbeds. Those engaged in this work were "exposed to some mouldy bagasse". Although 10% of the refinery workers had abnormal chest radiographs, 6% had positive precipitin tests to bagasse extracts and 20% were positive to at least one of seven antigens (including *Aspergillus fumigatus*, *S. rectivirgula* and *T. vulgaris*), there were no confirmed cases of bagassosis. In Queensland, when bagasse stored since the end of the previous season was reclaimed for fuel at the beginning of the cane crushing season the total counts for airborne bacteria were $>10^6 \text{ m}^{-3}$ air (Dawson *et al.* 1996). During the rest of the season the counts were lower than this. In general, high counts were restricted to the boiler station and bagasse transport system. Personal breathing zone spore counts were highest among workers who spent significant periods of time near the transport system. In the cane crushing season, viable counts of *T. sacchari* were equivalent to only 0.01–1.0% of the corresponding total counts for airborne bacteria. Although total counts for *T. sacchari* were not obtained, Dawson *et al.* (1996) concluded from the aerobiological and medical data gathered that

workers in Australian sugar mills were not exposed to sufficient spores of this actinomycete to constitute a significant risk of contracting either acute or chronic bagassosis.

As indicated above, using as much of the fresh bagasse as possible for fuel during the crushing season, and thereby avoiding the need to store bagasse, greatly reduces exposure to airborne spores of *T. sacchari* and other thermophilic Actinomycetes. Storing fresh bagasse loose rather than in bales improves aeration, and limits the development of these organisms by encouraging some degree of drying and slowing the build-up of heat. Although most fresh bagasse was used as boiler fuel within hours in the mills examined by Dawson *et al.* (1996), the excess was stockpiled on crushed rock and covered by tarpaulins to prevent the saturation by rain responsible for the hazardous state of the outer bales in exposed bagasse stacks (Hearn 1968). Propionic acid may be applied to fresh bagasse to inhibit microbial growth and has been shown to prevent deterioration of the fibre (Wright 1970; Lacey 1974).

Bagassosis in other industries

The use of bagasse in the manufacture of paper and particle board means that the disease is not necessarily confined to regions of the world where sugar cane is grown. For example, it occurred in UK among workers manufacturing board for interior decorating and thermal insulation from bagasse imported from Louisiana (Hunter and Perry 1946). Initially, to keep down dust the imported bales of bagasse were broken under water, but when more tightly packed bales were received this was no longer possible. The bales had to be broken by pick-axe and sections fed dry into a shredder, resulting in a very dusty atmosphere and an outbreak of respiratory illness, largely among those working at this stage of the manufacturing process. After water was routinely directed at the shredder wheel and exhaust ventilation was installed there was only one further case of what was at that time described as bronchiolitis.

Ueda *et al.* (1992) refer to the first case of bagassosis occurring in Japan within two months of the opening of a particle board factory in 1966. Within a year, 10 of the 134 employees were affected, and a further 24 cases were diagnosed later. Although it was noted that there had been no further cases of bagassosis in Japan since the factory closed, Ueda *et al.* (1992) indicated that there was a need for surveillance in lacquerware factories (where bagasse is used as a cheaper substitute for wood), as well as cane sugar factories.

Lehrer *et al.* (1978) considered that apparent absence of bagassosis among workers in a Louisiana paper mill, where there had been a considerable number affected in the past, was due to increased management awareness of the nature of the problem and the need for its control. Measures were introduced to try to retard microbial growth and reduce the amount of airborne organic dust. These included storing raw bagasse loose or unpacked and continuously sprinkling it with water to reduce airborne dust as it was moved for shredding and hammer milling. The importance of respiratory protection was also recognized and workers were provided with "face masks".

Malt worker's lung

Although the higher than normal incidence of respiratory problems in the malting industry had been recognized much earlier, it was only in 1968 that the first case of the HP known as malt worker's lung was described (Riddle *et al.* 1968). The malt worker affected exhibited symptoms similar to those in farmer's lung, and it was later shown that about 5% of workers in Scottish maltings had symptoms, although not usually severe (Grant *et al.* 1976). The causative agent was identified as the mould *Aspergillus clavatus*. At 3.0–4.5*2.5–4.5 μm , the elliptical conidiospores or conidia of this species are somewhat larger than actinomycete spores (<2 μm), but nevertheless reach and provoke reaction at the acinar (alveolar) level. They are even more readily detached from the conidiophores that bear them than in other species of *Aspergillus*, and have walls that are particularly rich in allergens (Blyth 1978).

The minimum water activity (a_w) for growth of this mould is approximately 0.88, much higher than, for example, *A. versicolor* and more xerophilic species of *Aspergillus* and *Eurotium* (Chapter 2.1). Its optimum a_w is around 0.98 (Flannigan and Pearce 1994). It is a species, which therefore requires a substrate with a very high level of hydration if it is to colonize the substrate and sporulate on it. The malting of cereals provides a substrate, which is both nutritious, and has the degree of hydration needed by *A. clavatus*.

In traditional floor malting (Flannigan and Pearce 1994), high-quality dried barley steeped until it is fully hydrated (M.C. around 45%) is spread over a malting floor as a layer 10–15 cm deep, allowing germination of the grain. At intervals during seven days or longer, maltsmen traverse the floor, turning the germinating grain by shovel or mechanical turner to ensure uniform aeration, prevent matting of developing rootlets and maintain the temperature throughout the bed of grain close to ambient (usually 13–16° in temperate countries). The resulting green (meaning fresh) malt is then kilned at temperatures up to 80° C to reduce its M.C. to 3–4% and the rootlets are then screened off mechanically for animal feed.

As *A. clavatus* is rarely found on dry grain elsewhere, individual consignments of substandard grain and the presence of feral pigeons in grain stores have been suggested as sources of contamination in maltings. Whatever the source, *A. clavatus* is extremely difficult to eliminate from maltings. Conventional cleaning procedures and control measures such as adding hypochlorite to steep do not appear to be effective. Consequently, any dry grain arriving or stored at malting premises is likely to become contaminated by airborne *A. clavatus* spores in the air of the premises. The spores on the grain contaminated in this way are not killed at the concentration of hypochlorite that may be added to the steep as a surface disinfectant (Flannigan *et al.* 1984). During germination of the grain, conditions are almost ideal for the growth and speculation of *A. clavatus*, and of *Alternaria*, *Aureobasidium*, *Fusarium*, *Geotrichum*, *Rhizopus* and other fungi which comprise the normal mycobiota of cereals. However, where it is present *A. clavatus* becomes predominant. In extreme cases, large rafts of mycelium bearing blue-green *A. clavatus* spores may appear on the germinating barley (Shlosberg *et al.* 1991). When these areas are disturbed, the visible "smoke" of released spores may be dense enough to impede visibility (Riddle *et al.* 1968). In another case where a worker

developed HP, $>2 \times 10^5$ *A. clavatus* spores m^{-3} air were recorded (Nolard *et al.* 1988). The acts of stripping green malt from the malting floors, loading it into the kiln and then unloading it from the kiln when dry all expose maltsmen to high concentrations of airborne spores. Although mostly inactivated during kilning, the spores are still allergenic, and workers cleaning the residual rootlets from storage bins have been found to have serious respiratory symptoms. Not only that, but the spores of *A. clavatus* are so readily and widely dispersed that they may be isolated from the sputum of office workers and other employees who seldom enter the malthouse (Channell *et al.* 1969).

In recent years, cost-driven mechanization of malting has seen floor malting largely replaced by mechanically turned box (Saladin) malting or enclosed systems such as germination vessels, rotating drums or continuous malting plant. These partially or fully mechanized systems speed up the process, and therefore limit the time available for fungal growth and sporulation, so reducing exposure of workers to this respiratory hazard. Witness to the salutary effects of this increased mechanization is that 1.1 % of workers in makings with enclosed systems showed symptoms of malt worker's lung, compared with 6.8% in floor maltings (Grant *et al.* 1976). However, floor malting has not been eliminated and *A. clavatus* is still a problem in both the Southern (Rabie and Lübben 1984) and Northern Hemisphere (Gilmour *et al.* 1989; Shlosberg *et al.* 1991). In southern Africa, *A. clavatus* is one of the principal moulds encountered during the malting of sorghum in both outdoor commercial floor maltings and in enclosed industrial Saladin maltings (Rabie and Lübben 1984). The abundance of *A. clavatus* in sorghum malt produced outdoors in South Africa is not unexpected as the ambient temperature may reach 28° C, close to the optimum for growth of this species. The profuse growth of *A. clavatus* in a floor maltings in Israel appeared to be the result of a heat wave (Shlosberg *et al.* 1991). The first report of malt worker's lung (Riddle *et al.* 1968) noted that the problem arose after the temperature in the green malt was allowed to rise well above the normal 16°C (in order to speed up the malting process) and drew attention to the importance of controlling temperature to limit growth of *A. clavatus*.

In UK, a Health and Safety Executive Guidance Note (HSE 1993) on grain dust in maltings set a maximum exposure limit for inhalable grain dust at 10 mg m^{-3} air for an 8-hour time-weighted average, and recommended engineering and operational control measures for reducing exposure to grain dust. Although HSE has stated that respiratory protective equipment (RPE) is necessary for plant maintenance and cleaning operations for which other control measures are either not practicable or adequate, it stresses that RPE should normally only be employed as a last resort (HSE 1993). The note does not address the specific problem of *A. clavatus* in maltings, but did stress the importance of health surveillance of workers and the obligation to notify HSE of any cases of occupational asthma or HP. Although it is unlikely that *A. clavatus* can be eliminated entirely from the malting industry, a combination of scrupulous cleanliness, enclosed malting systems, effective temperature control and use of only high-quality, undamaged grain will reduce possible exposure to *A. clavatus* and other airborne moulds.

The importance of controlling this mould is added to by the fact that it is toxigenic and has been shown to produce patulin and cytochalasin E during malting (Lopez-Diaz and Flannigan 1997). Tremorgens are among the other mycotoxins that the species can

synthesize (Flannigan and Pearce 1994), but whether these are actually produced during malting and account for neurological symptoms seen in some victims of malt worker's lung is not known.

Other microorganisms causing hypersensitivity pneumonitis

The role of thermophilic Actinomycetes in HP has been discussed by Kurup (1989) and Murphy *et al.* (1995), but the latter have also reviewed a range of occupation-related types of HP where the causal organisms are fungi, and Flannigan *et al.* (1991) briefly noted cases of HP caused by fungi in domestic environments. These serve to confirm that HP problems arise whatever the environment, work or home, when that environment provides a blend of substrate, moisture and temperature that matches the needs of particular allergenic microorganisms. As indicated earlier, this is perfectly illustrated by thermophilic Actinomycetes, which will grow in badly maintained heating, ventilation and air-conditioning systems (Kurup 1989) as well as in hay, grain, mushroom compost and bagasse.

Murphy *et al.* (1995) have reviewed a range of other well-known types of HP caused by exposure to non-microbial and also non-biological agents, as well as by microorganisms or their products. However, as new technologies are used, as construction materials and their use changes and as health practitioners remain alert, HP is being recognized in new environments. For example, the disease has been identified in household-waste recycling and attributed to *Aspergillus fumigatus* (Allmers *et al.* 2000); it has been linked to contamination of tatami mats and attributed to *Trichosporon* and *Cryptococcus* species (Ando *et al.* 1991); and it has been associated with avian proteins tracked indoors (Saltoun *et al.* 2000). Disease follows the trails of exposure, which in the case of biological agents are generally moisture and organic matter, including dirt and dust. Of this physicians and patients should beware.

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Chapter 3.3

OCCUPATIONAL RESPIRATORY DISEASE: ORGANIC DUST TOXIC SYNDROME

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INTRODUCTION

Organic dust toxic syndrome

Organic dust toxic syndrome (ODTS) and hypersensitivity pneumonitis (HP) are associated with inhalation of high concentrations of organic materials, especially materials such as grain dust, hay, or silage, when they are heavily contaminated with microorganisms (Lecours *et al.* 1986; May *et al.* 1986; Pratt and May 1984; Pratt *et al.* 1990). Although concentrations of these organisms might be expected to be much lower in home and indoor work environments than in agricultural dust, a number of investigations have shown that humidifiers and air conditioning systems can act as aerosol generators, creating aerosols contaminated with fungi, amoebae, bacteria, and associated products such as endotoxin (Edwards 1980; Mamolen *et al.* 1993; Parrott and Blyth 1986; Patterson *et al.* 1981; Rylander *et al.* 1978). ODTS has been known by a number of names, including “humidifier fever”, “atypical farmer’s lung disease”, and “silo unloader’s disease” (Parker *et al.* 1994). More recently, Rylander (1994) has suggested the term “toxic pneumonitis” for this syndrome. HP, also known as extrinsic allergic alveolitis, has been recognized for a very long time and occurs in a variety of occupational settings (Parker *et al.* 1992). The term “pulmonary mycotoxicosis” was applied to ODTS to differentiate it from farmer’s lung disease (FLD, the most familiar form of HP in agriculture) and to reflect the possibility that mycotoxins might be involved in the etiology (Emanuel *et al.* 1975). To date, mycotoxins have not been demonstrated in dust samples collected from outbreaks of ODTS (May *et al.* 1986). Interestingly, however, Blyth and Hardy (1982) reported that spores of a sputum-derived *Aspergillus clavatus* yielded mutagen(s) when extracts of the spores were fractionally precipitated with ethanol following alkaline hydrolysis. Unimmunized mice showed an inflammatory response with severe perivascularitis and peribronchiolitis, attributed to pulmonary mycotoxicosis. Nearly 80% of the mice had developed lung tumours 8 months after the treatment. HP due to *A. clavatus* is relatively common among maltworkers, and

volunteers have been found to remain sputum-positive for this species for at least 1 month after a single exposure to contaminated malt (Channell *et al.* 1969).

ODTS is a noninfectious illness resembling influenza and is characterized by fever, malaise, myalgia, and a neutrophilic inflammation of the lower respiratory tract (Lecours *et al.* 1986; Parker *et al.* 1992). ODTS and HP have many features in common including similar exposure settings and clinical symptoms (Emanuel *et al.* 1975; Pratt and May 1984; Parker *et al.* 1992) but the illnesses may not be mediated by the same mechanisms. Although both illnesses appear to involve inflammation of lung parenchyma, HP is characterized by a lymphocytic infiltrate into the lower airways, suggesting that it may be due to a cell-mediated hypersensitivity reaction, and ODTS is characterized by neutrophilic infiltration and can occur in nonsensitized persons. Outbreaks of ODTS are characterized by a much higher "attack rate" than is observed in HP; there is usually no correlation between presence of precipitating antibodies and illness; and clinical findings suggest that ODTS results from nonspecific immune mechanisms (Von Essen *et al.* 1990). An interesting example of such a clustered outbreak was provided by Brinton *et al.* (1987) in a case report of a college fraternity party in which baled straw had been used on the basement floor of the fraternity house. Of the 67 students who attended the party and answered a questionnaire, 82% became ill. Risk of illness was related to length of time spent at the party and duration of illness ranged from 4.5 h to 7 days.

There is no sharp distinction between HP and ODTS and therefore diagnosis and differentiation may be difficult, particularly in a single affected individual (Parker *et al.* 1992; Weber *et al.* 1993). Mamolen *et al.* (1993) described an outbreak of humidifier fever which illustrates this difficulty. The outbreak was associated with operation of a humidifier which had not been cleaned for several months. The humidifier was set to operate automatically and it began to run at a time when the ventilation system was shut down because of a plugged intake filter. There were positive serological reactions among print shop workers to extracts of organisms isolated from the humidifier, but the presence of antibodies could neither distinguish ill from well workers nor identify causative organisms. Furthermore, although the evidence favoured a diagnosis of ODTS in this instance, previous exposure to the humidifier contaminants appeared to be a risk factor in that "those who worked in the shop for at least 3 months showed a 4-fold increase in relative risk."

Fungi found in organic dust associated with ODTS

In agricultural dust, the fungi most commonly associated with ODTS are the so-called storage fungi, i.e. those species which develop with time under the conditions of reduced water availability generally prevailing during grain storage.

These are predominantly species of *Aspergillus* and *Penicillium*, but the mycobiota of moist or heated grain may be richer and more diverse. Species of *Aspergillus* commonly isolated from stored grains include *A. candidus*, *A. flavus*, members of the *A. glaucus* group (*Eurotium* spp.), *A. ochraceus*, *A. restrictus*, and *A. versicolor*. Among the most frequently isolated *Penicillium* spp. are *P. aurantiogriseum* (as *P. cyclopium*), *P. brevicompactum*, and *P. viridicatum* (Mislivec and Tuite 1970).

Studies of the mycobiota of grain dust are limited, both for settled and airborne dust. As expected, the species found in grain dust reflect those found in the corresponding grains and varies with the type of grain examined and the moisture and heating history of storage (Farant and have shown that *A. fumigatus*, *A. niger*, *A. terreus* Moore 1978). Previous studies in our laboratory and *P. spinulosum* can occur as the predominant fungi in samples associated with outbreaks of ODS (Dutkiewicz *et al.* 1989; Olenchock *et al.* 1990; Pratt *et al.* 1990), and *A. candidus*, *Eurotium amstelodami*, and *C. cladosporioides* are often the predominant fungi in agricultural dust. Particularly striking in many of these samples are *Eurotium* spp. referred to in many previous studies as members of the *A. glaucus* group. These are xerophilic fungi common on cereal grains (Raper and Fennell 1965). *E. umbrosum*, a member of this group, has been reported to be particularly abundant in hays associated with FLD in Finland and was found to react with most sera from FLD patients in precipitin tests in that country (Terho and Lacey 1979). *E. umbrosum* is regarded by some authors (Pitt 1985; Pitt and Hocking 1985) as a synonym of *E. herbariorum*, which is closely related to *E. rubrum*. *E. amstelodami* and *E. rubrum* were reported to be both toxic and common in industrial feed mixtures (Borkowska-Opacka and Truszczyński 1979). Eduard *et al.* (1993) reported that *A. fumigatus*, *Paecilomyces variotii* and *Rhizopus rhizopodiformis* were the predominant species of fungi collected on filters in the sorting and trimming plants of Norwegian saw mills.

Table 1. A partial list of mycotoxins found in spores.

Species	Mycotoxin	Reference
<i>Alternaria alternata</i>	alternariol	Häggblom 1987
<i>Alternaria alter nata</i>	alternariol monomethylether	Häggblom 1987
<i>Aspergillus fumigatus</i>	tryptacidin	Parker and Jenner 1968
<i>Aspergillus fumigatus</i>	fumitremorgen	Land <i>et al.</i> 1994
<i>Aspergillus fumigatus</i>	verruculogen	Land <i>et al.</i> 1994
<i>Aspergillus fumigatus</i>	fumiclavine C	Palmgren and Lee 1986
<i>Aspergillus flavus</i>	aflatoxin	Wicklow and Shotwell 1983
<i>Aspergillus parasiticus</i>	aflatoxin	Wicklow and Shotwell 1983
<i>Fusarium graminearum</i>	deoxynivalenol, T-2??	Miller 1992
<i>Fusarium sporotrichioides</i>	deoxynivalenol, T-2??	Miller 1992
<i>Stachybotrys chartarum</i>	satratoxin G, H	Sorenson <i>et al.</i> , 1987

Land *et al.* (1989) reported that isolates of *A. fumigatus* isolated from sawmills in Sweden produced tremorgenic mycotoxins.

In contrast to outdoor air, where spores of *Cladosporium*, *Alternaria* and Basidiomycetes may predominate, the most common fungi in indoor air are similar to

those observed on grains, i.e. soil or storage fungi such as species of *Aspergillus* and *Penicillium*. Therefore, although ODS has been less extensively studied in homes and indoor work environments than in agriculture, it appears that similar fungi are involved.

POTENTIAL ROLE OF FUNGAL SPORES

Mycotoxins and mycotoxigenic fungi

At present, approximately 350–400 fungal metabolites are considered to be toxic and the majority of these are relatively small molecules of >200 but <500 mass units (Samson 1992). Excluding the mushroom toxins, perhaps the most important mycotoxins are the aflatoxins, the 12,13-epoxytrichothecenes, and the fumonisins. The most important mycotoxigenic species belong to the genera *Aspergillus*, *Penicillium* and *Fusarium* (Samson 1992). Many toxigenic species produce multiple mycotoxins. For example, the *P. verrucosum* complex (*P. verrucosum*, *P. aurantiogriseum*, *P. viridicatum*, *P. crustosum*, and *P. solitum*) has been shown to produce nearly 20 different mycotoxins (Frisvad and Filtenborg 1989). These fungi are common contaminants of agricultural commodities and some of the mycotoxins that they produce are known to be produced by fungi common in house dust (Tobin *et al.* 1987). Other toxigenic fungi include species of *Alternaria*, *Paecilomyces*, *Rhizopus*, *Trichoderma* and *Trichothecium*. All of these fungi are commonly found in soil, agricultural products, grain dust and house dust (Tobin *et al.* 1987).

Although few investigators have examined spores of various fungi for mycotoxins, the presence of mycotoxins has been demonstrated in the spores of several species of fungi (Table 1) and current evidence suggests that their presence in spores of toxigenic species is much more common than has been appreciated. The vast majority of mycotoxins are not volatile and therefore mycotoxin exposure by inhalation is most likely to occur via inhalation of spores. Several studies have linked inhalation of mycotoxins with human disease (Table 2). One of the difficulties of epidemiological studies relating to inhalation of spores and mycotoxins has been the lack of adequate dosimetry data on intake, excretion and metabolism, but recently new tools for this purpose have been developed for the aflatoxins (Autrup *et al.* 1991; Zarba *et al.* 1992). At the present time very little information is available on the levels of any mycotoxin in indoor air. A much more extensive database will be needed before it will be possible to assess the role of mycotoxins in ODS.

Table 2. Health outcomes associated with inhalation of spores

Species	Disease	Reference
<i>Aspergillus flavus</i>	lung cancer	Hayes <i>et al.</i> 1984
<i>Aspergillus flavus</i>	colon carcinoma	Deger 1976
<i>Aspergillus flavus</i>	lung cancer	Dvorackova 1976
<i>Stachybotrys chartarum</i>	malaise	Croft <i>et al.</i> 1986
<i>Aspergillus flavus</i>	lung cancer	Olsen <i>et al.</i> 1988
<i>Aspergillus ochraceus</i>	acute renal failure	Di Paolo <i>et al.</i> 1993
<i>Aspergillus fumigatus</i>	tremorgenic encephalopathy	Gordon <i>et al.</i> 1993
<i>Stachybotrys chartarum</i>	infant haemosiderosis	Etzel <i>et al.</i> (1999)

Toxicity of mycotoxins for alveolar macrophages

Several mycotoxins have been shown to be acutely toxic to alveolar macrophages (AM) *in vitro* (Ayril *et al.* 1992; Gerberick and Sorenson 1983; Gerberick *et al.* 1984; Sorenson *et al.* 1985; Sorenson *et al.* 1986; Vidal and Mavet 1989). Trichothecene mycotoxins are also known to have immunosuppressive effects (Tryphonas *et al.* 1984, 1986), and gliotoxin is a potent immunosuppressive metabolite of *A. fumigatus* (Eichner *et al.* 1986). Jakab *et al.* (1994) reported that phagocytosis by alveolar macrophages (AM) from rats exposed to aflatoxin B1 (AFB1) in aerosols (estimated dose=16.8 fg kg⁻¹ body weight) was suppressed and the effect persisted for approximately 14 days. AFB1 administered by intratracheal instillation (75 fg microcrystalline AFB1 also suppressed release of tumour necrosis factor (TNF) and impaired phagocytosis by peritoneal macrophages as well as primary splenic antibody responses. Therefore, it is clear that mycotoxins can have a profound toxic effect on AM.

Cell wall composition

Fungal cell walls typically are rigid structures able to withstand a variety of environmental insults and are composed of several different components which include polysaccharides, proteins, lipids, pigments and inorganic salts. Of these, polysaccharides commonly make up some 80% of cell wall dry weight, proteins range from 3 to 20%, and lipids and the other components are present in smaller amounts (Ruiz-Herrera 1992). Cell wall components can be considered to belong to either of two main functional components: structural components, consisting of chitin, cellulose, and (1→3)-β-D-glucans, and cementing agents consisting of α-glucans, β-glucans, chitosans, polyuronides, lipids, inorganic salts and pigments (Ruiz-Herrera 1992). Hydrolysis of cell wall polysaccharides indicates that glucose is the most abundant and most prevalent sugar in cell walls of organisms representative of all fungal taxa, followed by glucosamine,

galactose and mannose. Galactosamine, glucuronic acid, rhamnose and xylose are present in lower amounts and are not universally present in the various taxonomic groups (Ruiz-Herrera 1992).

Mannans/mannoproteins

Galactomannans (GM) have been found to be important immunogens of several species of *Aspergillus* and antibodies specific for purified galactomannans have been used successfully for the detection of fungal contamination of foodstuffs (Kamphuis *et al.* 1989), and serum and urine (Rogers *et al.* 1990). GM is a major cell wall component in *Aspergillus* spp. and is also secreted into the growth medium as an exoantigen.

Mannoproteins of the cell wall of *Candida albicans* have been shown to be powerful immunomodulators in the peritoneal cavity of mice, inducing natural killer and macrophage-mediated cytotoxicity (Scaringi *et al.* 1988).

β -Glucans

(1–3)- β -D-Glucans are glucose polymers consisting predominantly of chains of glucopyranosyl rings joined by β -(1–3) linkages and with varying degrees of β -(1–3) side chains. They are thought to exist as stable triple helices under physiological conditions, with three glucose polymers held in close proximity in a rigid helical rod-like structure by intramolecular hydrogen bonds (Williams *et al.* 1991). Numerous investigators have demonstrated the immunomodulating activities of (1–3)- β -D-glucans (Morikawa *et al.* 1985; Patchen *et al.* 1987; Sherwood *et al.* 1987; Williams *et al.* 1987). It is believed that macrophage activation is the central event in the β -glucan immune stimulatory effect (Williams *et al.* 1983). Glucans enhance both the number and function of macrophages (Patchen *et al.* 1984; Williams *et al.* 1987), inducing release of haemopoietic growth factors and stimulating proliferation of haemopoietic progenitors (Patchen *et al.* 1984). Soluble glucans also enhance production of the interleukins IL-1 and IL-2 by splenic macrophages (Sherwood *et al.* 1987) and stimulate production of monocyte chemoattractant protein 1 (MCP-1), which is preceded by an early, transient rise in TNF (Jones and Warren 1992). The rise in bronchoalveolar lavage (BAL) fluid monocyte chemotactic activity could be nearly completely neutralized with antibody directed against MCP-1. Abel *et al.* (1989) demonstrated that lentinan treatment of resident and thioglycollate-elicited mouse peritoneal macrophages resulted in a dose-dependent stimulation of phagocytosis. Hoffman *et al.* (1993) demonstrated that fungal (1–3)- β -D-glucans stimulate rat macrophage release of tumour necrosis factor- α (TNF α) if the cells were treated with <500 fg β -glucan ml⁻¹. Treatment with concentrations of β -glucan >500 fg ml⁻¹ resulted in TNF release comparable to untreated cells, with no effect on cell viability. When cells were treated with lipopolysaccharide (LPS) or interferon- γ in the presence of high concentrations of β -glucans, TNF α release was also inhibited. These results suggest an immunomodulating role for fungal β -glucans.

Current concepts for the etiology of ODTS

A number of possible mechanisms have been suggested for ODTS including direct activation of complement (Lewis and Olenchock 1989; Olenchock and Burrell 1976;

Olenchok *et al.* 1990) and the presence of “polyclonal cell activators”, i.e. agents which stimulate cells of the immune system in a nonspecific way, resulting in production of various mediators of inflammation (Willoughby *et al.* 1985). Polyclonal cell activators (PCA's) may stimulate by as much as 10^6 times the number of cells with 60-fold greater mediator release than by antigen stimulation (Willoughby *et al.* 1985). PCA's may be especially significant with respect to macrophages because, whereas antigen can only stimulate these cells indirectly via lymphokines from sensitized lymphocytes, most PCA's can activate macrophages both directly and indirectly (Willoughby *et al.* 1985). Activation of macrophages can result in enhanced glucose oxidation, secretion of enzymes, synthesis and release of complement components, release of prostaglandins and reactive oxygen intermediates, release of cytokines and enhanced phagocytosis, as well as enhanced bactericidal and tumouricidal activities (Willoughby *et al.* 1985). Potential inflammatory agents in grain dust and hay include fungi and bacteria and their metabolites, plant lectins, insects, mites and animal dander. Thus, there is strong evidence that the etiological mechanism of ODTS involves inhalation of organic materials containing numerous inflammatory substances.

Both Gram-negative and Gram-positive bacteria are consistent components of organic dust associated with ODTS and may be present in large numbers. These organisms are the source of a number of compounds such as peptidoglycan, muramyl dipeptide (MDP), N-formyl-methionylleucyl-phenylalanine (FMLP) and LPS, which are potent inflammatory agents that can act as mitogens, adjuvants and chemoattractants, and can activate inflammatory cells (Malmberg and Rask-Andersen 1993). Although bacteria and their products are beyond the scope of the present chapter, they are omnipresent and must be considered to play a role in the etiology of ODTS. Several excellent reviews on the properties of endotoxin in relation to its inflammatory and immunomodulating properties have been published (Morrison and Ryan 1979; Morrison 1983; Morrison and Ryan 1987; Wilson 1985; Burrell 1990).

In a study of 143 farms in Sweden, Malmberg *et al.* (1990) reported that some ODTS reactions occurred when dusts contained very low levels of endotoxins, suggesting that factors other than endotoxin could contribute to toxic febrile reactions. Since mould and actinomycete spores are usually present in large numbers in dust associated with ODTS, these authors suggest that excessive activation of alveolar macrophages and the subsequent release of acute phase reaction mediators may cause ODTS reactions. The remainder of this chapter will focus on specific aspects of the interaction between fungal spores and the alveolar macrophage, the first line of defence in the lung.

Spore interactions in the lung

Spore diffusates

To prepare spore “diffusates”, Robertson and co-workers incubated live conidia of *A. fumigatus* in tissue culture medium for up to 3 h and then removed the conidia by centrifugation. The diffusates inhibited chemotaxis of human polymorphonuclear leukocytes (PMN) and spreading of mouse peritoneal macrophages (Robertson *et al.* 1987c); inhibited phagocytosis of antibody-coated sheep erythrocytes (Robertson *et al.* 1987b);

decreased superoxide anion and hydrogen peroxide production (Robertson *et al.* 1987a); and inhibited transcription factors NF κ B and AP-1, leading to loss of cytokine expression in rat alveolar macrophage cultures (Nicholson *et al.* 1996). The authors showed that the active material in the diffusates, termed AfD for diffusible substance from the surface of *A. fumigatus* spores (Nicholson *et al.* 1996), is dialysable and thus has a molecular weight of <14,000 daltons (Robertson *et al.* 1987a). Subsequent work suggested that the material is not gliotoxin or any of the other known toxins derived from the mycelium (Mitchell *et al.* 1997). *A. fumigatus* is an important opportunistic fungus capable of producing life-threatening lung infections in immunosuppressed persons (Warren and Warnock 1982). Release of substances from the spores which inhibit phagocytic cells during early contact in the lung may have great importance in ensuring survival of the spores in the lung and enhancing their pathogenic potential (Robertson *et al.* 1987c). Sorenson *et al.* (1994; 1995) reported that diffusates of *A. niger*, but not of several other common fungi, had a direct chemotactic effect for human PMN and rat AM. This would be expected to facilitate phagocytic clearance of the fungus. Diffusates from *A. niger* and *A. fumigatus* strongly inhibited superoxide anion production (Sorenson 1995), confirming the observation by Robertson *et al.* (1987a) for *A. fumigatus* and noting similar activity in *A. niger*.

Activation of complement

Sorenson *et al.* (1994) demonstrated that conidia of several fungi have the ability to activate complement. Complement activation has several important consequences including production of complement factor C3b which coats bacteria and fungi and facilitates phagocytosis because of the presence of C3b receptors on the surface of PMN and AM. Activation of complement also results in increased concentrations of C3a and C5a, which are potent chemotactic agents and lead to recruitment of large numbers of PMN to the site of activation. C3a and C5a have anaphylatoxic activity, i.e. they can cause contraction of smooth muscles and degranulation of mast cells and basophils with the release of vasoactive amines such as histamine. Olenchock and Burrell (1976) have shown that unimmunized rabbits exposed to small and massive aerosols of *Aspergillus* spores demonstrated post-challenge arterial hypoxia as well as decreased haemolytic complement activity. Unimmunized animals treated with cobra venom factor in a manner known to achieve complement depletion did not respond in this manner.

Production of superoxide anion

Production of reactive oxygen intermediates has been postulated to contribute to the tissue damage seen in pulmonary inflammation. Fungal spores alone were found to stimulate a modest increase in O₂⁻ production (Sorenson *et al.*, 1994; 1995). However, if macrophages were primed by pretreatment with LPS and then exposed to the fungal spores, significant enhancement of O₂⁻ production was observed (Shahan *et al.* 1994). Both live and killed spores were active. These results support the concept that the combined exposure to both bacterial and fungal agents may be important in the pathogenesis of ODS.

Stimulation of leukotriene LTB₄ production

LTB₄ is a potent chemotactic factor for PMN (Smith *et al.* 1980) and would contribute to recruitment of PMN to the lung following exposure to agents eliciting LTB₄ release. The observation that several species of fungi common in agricultural dust stimulate release of LTB₄ from alveolar macrophages (Sorenson *et al.* 1994, 1995) suggests that exposure to spores of these species would result in recruitment of PMN to the lung. LTB₄ possesses potent calcium ionophore activity and, in addition to recruiting inflammatory cells to the lung, it can upregulate their function and modulate the immune response (Bjornsdottir and Bush 1993).

Stimulation of cytokine production

Development of fever is an important clinical sign of ODS and is usually thought to result from IL-1 production. In early experiments to assess the ability of either live or heat-killed spores to stimulate IL-1 production by AM *in vitro*, Sorenson *et al.* (1994) were consistently unable to show an increase in IL-1 activity. Endotoxins and their major component, LPS, are well known stimulators of IL-1 production. Natural exposure to organic dust in concentrations sufficient to cause ODS commonly (perhaps invariably) includes exposure to Gram-negative bacteria and endotoxin. The natural occurrence of fungal spores and endotoxin together in grain dust raises the question of whether the presence of spores could modulate LPS-induced IL-1 production by AM. When AM were treated with heat-killed spores in combination with suboptimal concentrations of LPS, there was little enhancement of IL-1 production, and IL-1 production was inhibited by *A. fumigatus*, *A. niger*, and *E. amstelodami* (Sorenson *et al.* 1995). These results suggest that fungal spores may lack the innate ability to activate IL-1 production/release, at least *in vitro*, without the presence of T- and/or B-lymphocytes, and that fever associated with ODS may be due to other mediators or to indirect mechanisms. For example, complement factor C5a can stimulate IL-1 release (Lambris 1988). Inhibition of IL-1 production would be expected to diminish, rather than contribute to, symptoms of ODS. The inhibition of IL-1 production by *A. fumigatus*, *A. niger*, and *E. amstelodami* (Sorenson *et al.* 1995) suggests the possibility that mycotoxins in the spores may have been responsible for this inhibition. Sorenson and his colleagues observed striking inhibition of LPS-stimulated IL-1 production at ratios of 5:1 or 50:1 spores per AM. When one considers that spore concentrations in heavily contaminated agricultural dusts often reach 10⁸ spores m⁻³ air and that during moderate activity a worker's ventilation could be ≤ 3 m³ h⁻¹, exposures of up to 3–10⁸ spores h⁻¹ or 5–10 min⁻¹ could be reached. Thus, it is easy to imagine that ratios of 50:1 spores per macrophage may not be uncommon among workers exposed to heavily contaminated dusts.

Shahan *et al.* (1998) demonstrated by northern blot analysis that the C-X-C chemokines, macrophage inflammatory protein-2 (MIP-2) and KC, are upregulated in rat alveolar macrophage cultures when the macrophages are co-incubated with heat-killed conidia of *A. fumigatus*, *A. niger*, *A. candidus*, *E. amstelodami*, and *C. cladosporioides*. The magnitude of this response was species-dependent and was not observed in *A. terreus* and *P. spinulosum*. Specific m-RNA's for these chemokines reached peak levels after incubation for 1 h and then decreased. The peak was somewhat delayed for *A.*

candidus and *E. amstelodami*, occurring between 1 and 3 h. KC is thought to be the rat physiological equivalent of interleukin-8 (Huang *et al.* 1992). In addition, *A. fumigatus* also stimulated upregulation of MIP-1 α and TNF α . This latter activity was confirmed by bioassay, i.e., of the seven species studied, TNF α was only observed in macrophage cultures treated with conidia of *A. fumigatus* (Shahan *et al.* 1998). The potential of MIP-2 and KC to contribute to neutrophil chemotaxis and activation during pulmonary inflammation was demonstrated by the ablation of neutrophil accumulation following LPS challenge in the rat lung by anti-KC (Schmal *et al.* 1996) and anti-MIP-2 antibodies (Frevert *et al.* 1995; Ulich *et al.* 1995). In the work of Nicholson *et al.* (1996), which demonstrated that cytokine expression was inhibited as a result of the inhibition of transcription factors NF- κ B and AP-1, the direct effect of spores of *A. fumigatus* on macrophages was not investigated. Shahan *et al.* (1998) reported stimulation of mRNA transcripts for several cytokines as well as significant TNF α protein. *A. fumigatus* conidia also stimulated MIP-2, KC, and MIP-1 α as determined by ELISA (unpublished data).

Modulation of phagocytic activity

Phagocytosis of heat-killed *Saccharomyces cerevisiae* was shown to be mediated predominantly by β -glucan receptors in murine peritoneal macrophages with little involvement of mannose receptors or complement receptors (Goldman 1988). Similar results were found for zymosan induced arachidonic acid release from rabbit alveolar macrophages. This release was blocked by soluble β -glucan while soluble mannan had no effect (Daum and Rohrbach 1992), indicating that the release was at least in part via a β -glucan receptor. The fact that β -glucan is a major component of the cell wall of a wide variety of fungi suggests that stimulation of alveolar macrophage β -glucan receptors with subsequent release of arachidonic acid may play a role in the pathogenesis of ODS.

Review of spore-macrophage interactions

The studies reviewed above indicate that the spores of fungi implicated in episodes of ODS all possess activities which suggest a potential to initiate inflammation, although there are major differences between species. These activities include the production of substances on the surface of the spore which have direct chemotactic and/or inhibitory properties, activation of complement leading to the production of the chemotactic complement components C3a and C5a, stimulation of superoxide anion production, a reactive oxygen intermediate associated with tissue damage, modulation of IL-1 production, and stimulation of a variety of chemotactic substances including LTB $_4$, MIP-2 and KC. Whereas readily diffusible substances on the surface of the spores were only demonstrable in *A. fumigatus* and *A. niger*, all of the species which were studied activated complement, and stimulated LTB $_4$ and superoxide anion production, but there were major differences in their ability to upregulate mRNA's for chemokines. For example, MIP-2 and KC were differentially upregulated in rat alveolar macrophage cultures by conidia of *A. Candidus*, *A. niger*, *E. amstelodami* and *C. cladosporioides* whereas conidia of *A. terreus* and *P. spinulosum* failed to stimulate an increase of any of the cytokines studied. Conidia of *A. fumigatus* were the most active of the species studied, stimulating upregulation of MIP-2, KC, and MIP-1 α .

SUMMARY

ODTS is associated with agricultural and other dust contaminated with microorganisms. Although originally called "pulmonary mycotoxicosis", there is little evidence to support mycotoxin etiology and considerable evidence that other components of microorganisms, such as endotoxin and components of the fungal cell wall, are abundant in organic dust and are able to activate components of the innate immune system (phagocytic cells and complement). Inflammation is an important part of this response and involves an increased blood supply to the area; increased vascular permeability, permitting entry of large molecules such as soluble mediators of immunity; and recruitment of circulating neutrophils into the site of inflammation by chemotaxis. Therefore, current evidence strongly suggests that ODTS is the result of stimulation of immune cells leading to inflammation. Fungal spores are commonly present in homes and indoor work environments and these spores and several of their components, e.g. (1→3)- β -D-glucan and manno-proteins, have been shown to possess potent inflammatory activity.

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Chapter 3.4

RESPIRATORY TRACT INFECTIONS— BACTERIA

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INTRODUCTION

The most important bacterial respiratory tract infections in indoor environments are tuberculosis, nosocomial pneumonia, and legionellosis. These are discussed in this chapter. Inhalation exposure to bacteria such as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Saccharopolyspora rectivirgula* (*Faenia rectivirgula*, *Micropolyspora faeni*), and *Thermoactinomyces vulgaris* has been associated with hypersensitivity pneumonitis (Flannigan *et al.* 1991). Hypersensitivity pneumonitis is discussed in Chapter 3.2. *P. aeruginosa* is a Gram-negative organism and as such contains endotoxin. Health effects resulting from endotoxin exposure are discussed in Chapter 3.6 The bacteria that cause tuberculosis, legionellosis and bacterial nosocomial pneumonia represent a substantial human health hazard. All can be acquired both nosocomially and occupationally. Tuberculosis is subject to occupational health and safety standards and impending regulation. Unlike legionellosis, tuberculosis and many bacterial pneumonias can be transmitted person-to-person. In addition to standard public health measures, engineering controls and considerations can minimize occupational and nosocomial transmission of legionellosis and tuberculosis. Although airborne transmission prevails in tuberculosis and legionellosis, air sampling is not a useful tool for evaluating exposure or the effectiveness of control measures.

TUBERCULOSIS

Epidemiology

Mycobacterium tuberculosis (MTB) is the agent responsible for one of the most prevalent infections in the world, pulmonary tuberculosis (TB). Some 30–50% of adults in developing countries have been exposed to tuberculosis. There are an estimated 8.8 million new cases per year (Bloom *et al.* 1992) and TB has re-emerged as one of the leading causes of death with nearly three million deaths per year (WHO 1995a,b).

Tuberculosis affects man in two ways, as a latent infection or as an active disease. Most often, people exposed to MTB become infected (“latent infection”) but are not

contagious and do not manifest any symptoms. For immunocompetent people infected with MTB, the lifetime risk of progression from subclinical infection to clinical disease (usually pneumonia) is approximately 10%, 5% in the first year and 5% for the remaining lifetime. For the immunocompromised, the lifetime risk of progression is approximately 10% per year.

Active TB indicates symptomatic disease, and is contagious if untreated. Symptoms of TB include fatigue, malaise, weight loss, fever, night sweats, cough, bloody sputum, chest pain, and hoarseness. Only people with active TB are considered true cases of TB. Clinically active TB in sites other than the lungs (so-called extrapulmonary TB) is less common and includes such sites as the larynx, lymph nodes, pleura, pericardium, kidneys, bones and joints, skin, intestines, peritoneum, and eyes. Miliary tuberculosis is a form of the active disease reflecting disseminated infection throughout the body.

Mycobacterial agents other than MTB, such as *M. africanum*, *M. bovis* and the *M. avium* complex, also cause human disease. Man is the principal reservoir for MTB and *M. africanum*, while *M. bovis* can be transmitted from its natural reservoir, cattle, to man by ingestion of unpasteurized milk or dairy products. *M. avium* is especially common among people infected with human immunodeficiency virus (HIV). Infections with other mycobacteria will not be reviewed in this chapter

Screening

Skin testing is the primary means of screening for the TB infection. The two available methods of screening are the Mantoux tuberculin skin test and the two-step Mantoux skin test. The standard test involves injecting a small dose of Purified Protein Derivative Standard (PPD-S) into the epidermis of the forearm. Most people infected with MTB will respond with a localized skin swelling within approximately 48 h. Some infected individuals do not respond to a single test, but may show a positive test 1–3 weeks later; this represents the “booster effect”. Such persons may erroneously be labelled skin-test converters. A converter is an individual who at one-point tests negative and later tests positive. Such false-positive conversions can be avoided by initial testing with the two-step procedure. Persons showing “boosting” should be classified as reactors, not converters

According to CDC (1995b) and American Thoracic Society (ATS 1990) guidelines, the cutoff for a positive TB skin test varies with the health status of the individual tested. For example, in healthy people, a swelling of more than 15 mm in diameter is considered positive. In patients with HIV or in patients with household contacts to TB, a swelling >5 mm in diameter is considered positive.

A positive skin test generally indicates previous exposure, but not clinically active disease. People immunized with Bacillus Calmette Guerin (BCG) may also have positive skin tests, but are not necessarily infected with TB. Similarly, people who are immunocompromised as a result of HIV infection, corticosteroid use, old age or disseminated TB infection, for example, may also have negative skin tests.

Under certain circumstances, the chest x-ray may be considered an important screening tool. Incarcerated individuals, individuals in homeless shelters and other transient

populations may have poor follow-up for skin testing. In addition, some patients, such as elderly patients in long-term care facilities, HIV-infected individuals or patients on immunosuppressive drugs, may be anergic, i.e. have false negative skin tests due to poor immune status.

Screening for TB using the Mantoux skin test is commonly used in the following situations:

- Baseline and periodic screening of potentially exposed employees, e.g. health care providers at risk
- Post-exposure screening of contacts
- High-risk populations.

In the absence of BCG immunization, a skin-test conversion implies recent exposure to and/or infection with TB. Because treatment may have significant adverse effects (see below), treatment for skin test converters is recommended only for people under age 35.

Diagnosis

Diagnosis of active pulmonary TB involves chest x-ray, staining of sputum specimens for AFB (acid-fast bacilli), sputum culture, drug susceptibility testing, and, on occasion, DNA fingerprinting. Persons with symptoms suggestive of pulmonary tuberculosis should receive a chest x-ray despite negative skin test results. If the chest x-ray is abnormal, or if the person has symptoms indicative of extrapulmonary tuberculosis, additional diagnostic procedures should be performed. These could include AFB analysis and culture of cerebrospinal fluid, urine, blood, and histological staining of lung, lymph node, bone marrow or liver tissue. Drug susceptibility testing should be performed on all positive cultures.

Characteristics of transmission

The principal route of infection is inhalation of infective tubercle bacilli contained in respiratory droplet nuclei. Droplet nuclei are airborne particles with partial evaporation of the extracellular material in which the bacilli are aerosolized, e.g. saliva or mucus. Although much less common, transmission can also occur through direct invasion of mucous membranes or breaks in the skin. The probability of mycobacterial transmission depends on characteristics of the index case, the environment and the health status of potential contacts. The presence of acid-fast bacilli in sputum is the primary indicator of transmissibility (Loudon *et al.* 1969). Other characteristics associated with contagion include: cough (involuntary or induced), poor respiratory hygiene (failure to cover nose and mouth during coughing or sneezing), high volume or watery respiratory secretions, singing, sneezing, prolonged duration of respiratory symptoms, isolation of mycobacteria from sputum, cavitation evident in the chest x-ray, and inadequate antimicrobial chemotherapy.

The probability of transmission is also influenced by characteristics of the index individual's environment, including low air volume shared between index and contacts,

degree of environmental ventilation (i.e. of outdoor air), degree of recirculation without air treatment, the presence and efficacy of air treatment (e.g. high efficiency particulate air [HEPA] filters, ultraviolet germicidal irradiation [UVGI]), absence of engineered source control (e.g. an enclosed booth specifically designed as a sputum booth) and lack of personal protective equipment. High-risk health care activities include performing cough-inducing procedures in patients, bronchoscopy, endotracheal suctioning, and aerosolized pentamidine treatment.

Other risk factors increasing the probability of infection include increased time with the index case, physical proximity to the index case, failure to take prophylactic chemotherapy (if indicated) and host factors, such as the contact's age, race, and immunological status.

Treatment

Clinically active disease

Anti-tuberculous chemotherapy is the primary treatment for active TB. At least ten drugs have been approved in USA to treat active TB. The currently accepted first-line regimen for initial intensive treatment of US patients is four-drug therapy—isoniazid (isonicotinic acid hydrazide or INH), rifampin (rifampicin), ethambutol and pyrazinamide—until susceptibility results are available. The combination of drugs dictates the duration of chemotherapy and therefore potentially impacts on patient compliance. If only isoniazid and rifampin are given, the regimen should be continued for at least nine months. Most regimens are a combination of four drugs daily for 15 doses, then four drugs twice a week for 15 doses, then two drugs twice a week for 32 doses. Treatment guidelines for HIV-positive patients with TB have also been published (CDC 1998b).

With adequate chemotherapy, almost all patients with clinically active TB become sputum-negative, recover and remain well. Continuous administration of the chosen drug regimen for a sufficient period of time is critical, because relapse rates are higher if chemotherapy is stopped prematurely (CDC 1994c). TB must be treated for a minimum of 6 months; multiple drug resistant TB (MDR-TB) may be treated for up to 2 years (CDC 1994a).

If three anti-tuberculosis drugs are used, isoniazid and rifampin need only be given twice per week without adversely affecting relapse rates (Dutt *et al.* 1982). Such a regimen allows directly observed therapy (DOT). In DOT, health care workers (usually nurses) travel to meet the patient in the community in order to monitor the supervised administration of anti-tuberculous medications. DOT improves patient compliance and reduces the overall cost of treatment.

Prophylaxis

Prophylactic chemotherapy with isoniazid is administered to infected, but not clinically ill, individuals to reduce the risk of clinical disease. The duration of prophylactic treatment is 6 months for HIV-negative persons and 12 months for HIV-positive persons (ATS 1994). There is now an approved 2-month regimen (rifampin and pyrazinamide) for HIV-positive patients (CDC 1998a). Isoniazid treatment, if completed successfully, is

effective in up to 88% of individuals (CDC 1990). Isoniazid is recommended for the following patients: recent infection (reflected by recent skin test conversion), close or household contact with a current case, tuberculin skin test reactors with chest x-rays consistent with non-progressive tuberculosis in whom there are neither positive bacteriological findings nor a history of adequate chemotherapy, and positive tuberculin skin test reactors with diabetes, silicosis, prolonged therapy with adrenocorticosteroids or other immuno-suppressants, leukemia, Hodgkin's disease, HIV infection and after gastrectomy.

Adverse effects of TB treatment

The most common adverse effects of isoniazid are neurological and hepatic. Peripheral neuropathy, the most common neurological reaction, is dose-related and occurs most often in the undernourished, in alcoholics and in diabetics. Other neurotoxic effects are uncommon at conventional doses, but can include convulsions, toxic encephalopathy, psychosis, optic neuritis and atrophy, and memory impairment.

Hepatic reactions range from asymptomatic elevations in serum levels of liver enzymes to fatal hepatitis. Early symptoms suggestive of hepatotoxicity are anorexia, nausea, vomiting, fatigue, malaise and weakness. Mild and transient elevation of serum transaminase levels occurs in 10–20% of those receiving isoniazid. Elevations usually occur in the first 4–6 months of treatment, but can occur at any time during therapy. Administration of the drug should be discontinued immediately in the presence of hepatotoxicity. Significant hepatotoxicity with isoniazid is age-dependent (Table 1); it is rare in individuals under 20, but occurs in up to 2.3% of those over 50 years of age. Haematological reactions to isoniazid can include agranulocytosis, haemolytic, sideroblastic or aplastic anaemias, thrombocytopenia and eosinophilia. Hypersensitivity reactions can include fever, skin eruptions, lymphadenopathy and vasculitis. Metabolic and endocrine reactions can include pyridoxine deficiency, pellagra, hyperglycaemia, metabolic acidosis and gynaecomastia. Miscellaneous

Table 1. The risk of isoniazid hepatitis for specific age categories (PDR 1993).

Age group	Cases per 1 000
<20	0
20–34	3
35–49	12
50–64	23
>65	8

reactions can include rheumatic syndrome and systemic lupus erythematosus-like syndrome.

Although no isoniazid-related congenital anomalies have been found in reproduction

studies in mice, rats and rabbits, the drug should be prescribed in pregnancy only when therapeutically necessary. Because isoniazid crosses the placental barrier and can be found in breast milk, neonates and breast-fed infants of mothers on isoniazid should be carefully monitored for adverse effects.

Finally, animal carcinogenicity studies suggest that isoniazid may be carcinogenic in humans (Engbaek *et al.* 1965). In addition, six epidemiological studies have found excess cancers (predominantly lung cancer) in humans receiving isoniazid therapy compared to those who did not (Campbell and Guilfoyle 1970; Miller 1975; Glassroth *et al.* 1977a,b). However, in its review, the International Agency for Research on Cancer (IARC) was unable to come to a firm conclusion regarding the carcinogenicity of isoniazid (IARC 1985).

Ultimately, the risk of isoniazid-related hepatitis and other adverse reactions must be individually weighed against the health risk arising from non-treatment of TB. The benefit of treatment for active TB is clear. The benefit of isoniazid prophylaxis in asymptomatic skin-test converters is more controversial. Several quantitative analyses weighing the risks and benefits of INH prophylaxis among different age, sex, and race groups have been published. (Koplan *et al.* 1980; Taylor *et al.* 1981; Rose *et al.* 1986; Tsevat *et al.* 1988; Colice 1990; Jordan *et al.* 1991 a, b; Dolan *et al.* 1994; Sarasin *et al.* 1995; Sterling *et al.* 1995; Salpeter *et al.* 1997; Rose 1998). CDC recommends that asymptomatic skin test converters under age 35 be given isoniazid prophylaxis, while those over age 35 should not; the cutoff age remains controversial. From a public health perspective, a better strategy is to prevent TB transmission using all means possible.

Although it has few major adverse effects, rifampin can cause heartburn, nausea, vomiting, anorexia, stomach cramps, flatulence and diarrhoea. Headache, fever, drowsiness and fatigue have also been reported. More rarely, cholestatic hepatitis, flu-like symptoms, immune-mediated thrombocytopenia and renal failure have been reported. Rifampin may cause an annoying, but harmless, red-orange colouring of urine, stool, saliva, sweat and tears. Rifampin has many pharmacological and pharmacokinetic interactions with other drugs. It may make birth control pills and contraceptive implants less effective.

Drug resistance

Primary drug resistance

The recent development of drug resistance, the inability of typical antituberculous drugs to kill MTB, has complicated the treatment of TB. Primary drug resistance (PDR) occurs when typical drugs are ineffective in previously untreated patients. Risk factors for PDR include exposure to a patient who has drug-resistant tuberculosis, being from a country with a high prevalence of drug resistance, and residence in a community where PDR is high (ATS 1994). For many years the PDR rate was thought to be 2–5%. However, an on-going surveillance programme initiated by the US Public Health Service in 1975 has shown considerable variation in the incidence of PDR among patients in different geographical areas of the country, as well as in various ethnic groups. Rates of PDR as high as 15% (Los Angeles) and 19% (Texas) have been reported (Kopanoff *et al.* 1978).

High PDR rates have been observed among Hispanics and Asians.

Multiple drug resistance

Multiple drug resistance tuberculosis (MDR-TB) occurs when TB bacteria are resistant to both isoniazid and rifampin. Some strains are resistant to as many as nine drugs. Clearly, MDR makes the treatment of TB much more difficult. Risk factors for MDR include exposure to someone with MDR-TB disease, poor compliance with medication regimens, recurrence of previous clinical disease and residence in areas where MDR-TB is common, i.e. South East Asia, Latin America, Haiti and the Philippines (CDC 1994d). In USA, most (61.4%) MDR-TB originates in New York City. Surprisingly, the three-month population-based incidence rate of MDR-TB in New York City was found to be 52.3 times higher than in the rest of the nation. Non-Hispanic whites in New York City were 39 times more likely to develop MDR-TB than those nationwide, and Hispanics 299.3, Asian/Pacific Islanders 420.9 and non-Hispanic blacks 701 times more likely (Bloch *et al.* 1994). Of patients with MDR-TB, 50% are cured, 25% remain infectious for life, and 25% die.

Outbreaks of MDR-TB have been documented among hospitalized patients with HIV infection (Di Perri *et al.* 1989; Edlin *et al.* 1992). MDR-TB outbreaks have also been documented in a substance-abuse centre (CDC 1991), a residential facility (Daley *et al.* 1992) and a homeless men's shelter (Torres *et al.* 1990).

Aerobiology

Infectious dose of MTB

Riley and O'Grady (1961) showed a strong correlation between the number of organisms contained in droplet nuclei and the number of pulmonary lesions among people exposed (Riley *et al.* 1961). The term "quantum of infection" had been introduced earlier by Wells (1955) to refer to the minimal number of organisms necessary to produce infection, and Riley and O'Grady (1961) concluded that the quantum dose was a single infectious particle, or a very few particles. CDC has reported that the infectious dose of MTB by inhalation is <10 mycobacteria (CDC/NIH 1993), and may be as small as one bacillus.

Mycobacterial aerosols

A bioaerosol of mycobacteria can be generated by an individual with pulmonary or laryngeal tuberculosis who coughs or sneezes, or is talking or singing. Coughing is by far the most effective mechanism for developing aerosols that yield droplet nuclei of MTB (Hopewell 1986). Coughs and sneezes have characteristic aerosol size distributions. Humans generate bacterial aerosols from the normal flora of the respiratory tract or oral cavity, and characteristics of aerosols produced by subjects in an aerosol test chamber have been determined using particle size-segregating cascade impactors (Riley *et al.* 1961; Miller 1973). In aerosols generated during coughing 30% of all viable bacteria are ≤ 5 μ m in diameter (Miller 1973). During sneezing, 50% of all bacterial aerosol particles are ≤ 5 μ m in diameter. There is no evidence to suggest that the particle size would be significantly different if the individual were tuberculosis-infective.

Droplet nuclei of tubercle bacilli are approximately 1–10 µm in diameter (Volk *et al.* 1986; CDC 1994b); particles in the range of 1–5 µm (such as those containing tubercle bacilli and those produced by coughing and sneezing) can remain airborne for long periods. Particle size is the most important characteristic of aerosols and is an essential component of inhalational exposure and environmental controls.

Airborne concentrations of MTB

Case histories suggest that the numbers of MTB bacteria generated by coughing patients or during bronchoscopy are orders of magnitude higher than that required for infection (Falkinham 1994). However, no air-sampling data documenting this has been published. Mycobacteria have been successfully sampled using an Andersen six-stage impactor and an all-glass impinger (Loudon *et al.* 1969; Riley *et al.* 1976) in the laboratory. However, efforts to collect mycobacteria in a waiting room of patients at high risk of developing communicable respiratory infections, including TB, failed (Macher *et al.* 1992).

The number of tubercle bacilli generated from the oral cavities of known TB patients has been determined using settle plates (Table 2). Bacterial growth plates placed 0.5–4 ft (15–120 cm) from the mouths of five patients showed positive MTB cultures during different treatments in a dental office (Belting *et al.* 1964). Although MTB colonies could have resulted from splatter rather than aerosol, these data document their generation and successful culture from the air. These studies suggest that successful recovery of MTB from air samples depends critically upon knowledge of the infection status of individuals tested.

Other air sampling methods which are not culture-based may be available. The National Institute for Occupational Safety and Health (NIOSH) used the Roche Amplicor™ MTB assay, based upon polymerase chain reaction (PCR) technology, to detect aerosols of *M. bovis* BCG artificially generated in the laboratory and collected from the air on polytetrafluoroethylene (PTFE) filters (Schafer *et al.* 1998).

Table 2. Numbers of MTB colonies on settle plates placed 6 in (15 cm), 2 ft (60 cm) and 4 ft (120 cm) from patients' mouths during dental procedures (Belting *et al.* 1964).

Patient	Air rotor-water on	Air rotor-water off	Mouthwash spray
1	0–2	3–17	0–2
2	0–2	0	0–1
3	0–1	0–25	1–3
4	2–15	0–2	0–5
5	0–5	0–2	0–25

Although mycobacteria were detected with the method, the limited data suggested that culture-based sampling with the Andersen method was more sensitive than PCR.

Additional work is needed to determine the reliability of PCR to detect and quantify airborne concentrations of mycobacteria.

Calculated generation rates of MTB

Generation rate refers to the rate of release of MTB bacteria into the air by actively infected patients. Several generation rates have been reported in the literature. In a series of mostly untreated, infectious TB patients, Riley (1988) reported a generation rate of 1.25 infectious units h^{-1} . One patient with laryngeal TB released 60 infectious units h^{-1} . However, examination of this author's original work (Riley *et al.* 1972) suggests that the reported per person generation rate was probably overestimated because the medical ward on which the study was carried out was simultaneously occupied on average by 3.7 infected patients. Adjusting for average ward occupancy produces a crude generation rate of 0.34 infectious units h^{-1} per infectious TB patient. Other investigators have reported much higher generation rates. Catanzaro (1982) reported a rate of 249 infectious units h^{-1} and Miller (1973) reported generation rates for the normal oropharyngeal flora as high as 1000 and 3400 min^{-1} during coughing and sneezing, respectively.

In their review, the American Thoracic Society has summarized these findings as follows (ATS 1992):

- There is great variation in the concentrations of droplet nuclei generated by various patients, estimated to range from as low as one nucleus in 11,000 ft^3 (approximately 311 m^3) to as high as one in 70 ft^3 (2 m^3) air for a highly infectious patient.
- Because humans inhale about 18 ft^3 air h^{-1} (approximately 0.5 m^3), the probability of a person becoming infected during a 1-h exposure can thus be between 1-in-4 and 1-in-600.
- Therefore, although months of exposure are usually required for infection to occur, under extraordinary circumstances, when the concentration of droplet nuclei has been much higher, extensive transmission has been observed during exposures as brief as 2 h.

Bacterial survival in aerosols

Several factors unique to MTB enhance its survival in aerosols. Such factors include:

1. *Hydrophobicity*. The hydrophobicity of mycobacterial cell walls causes mycobacteria to aggregate, a characteristic that enhances aerosolization. Hydrophobicity also promotes survival by increasing resistance to antibiotics and disinfectants.
2. *Slow growth*¹. Compared to most other bacteria, mycobacteria reproduce slowly. Most disease-associated mycobacteria require culture for 2–6 weeks on complex media at 37° C. Because many antibiotics work by inhibiting growth (rather than by direct killing), slow growth results in slow antibiotic-mediated cell death.
3. *Lipid-rich walls*. Mycobacterial cell walls are rich in lipids, which account for approximately 40% of the total cell dry weight (Volk *et al.* 1986) and render MTB impermeable and thus resistant to chemical agents and drying. Delayed desiccation allows mycobacteria to survive for long periods in rooms, bedding and sputum.

Prevention of disease

The principal strategies for reducing the transmission of tuberculosis are (1) vaccination,

(2) aggressively treating clinically active cases, (3) interrupting the development of disease after infection and (4) preventing exposure. Each strategy is reviewed below.

Vaccination

Although used routinely for children in UK, vaccination with BCG is not in routine use in USA, primarily because the population-based risk of infection is still low and the effectiveness of BCG remains controversial. CDC has noted that in clinical trials, observed protection in children has varied from 2 to 80% for pulmonary TB and from 52 to 100% for tuberculous meningitis and military TB (CDC 1996). Efficacy rates varied with the quality of the vaccine and the strain of BCG used. Vaccination with BCG makes the screening skin test more complex. Many, but not all, vaccinated subjects show positive reactions when tested with the standard PPD test; however, skin swelling wanes with time. The size of the skin reaction does not distinguish previous BCG reaction from TB infection. However, an increase exceeding 15 mm in the size of the skin test reaction in vaccinated persons suggests recent TB infection. CDC recommends BCG vaccination for selected populations (CDC 1996), viz.

- children who cannot be placed on isoniazid preventive therapy, but have continuous exposure to persons with active disease;
- children who have continuous exposure to patients infected by organisms resistant to isoniazid or rifampin; and
- children who belong to groups with annual rates of new infection greater than 1 % per year.

BCG vaccination is contraindicated for individuals with symptomatic HIV infection, but can be administered to asymptomatic individuals infected with HIV. BCG vaccination has also been recommended for health care professionals if the annual skin test conversion rates in the particular health care setting exceed 1% (Gantz 1983).

¹ A new non-HIV related strain has been reported in the US with a rapid doubling time of 14 h (compared with the more typical 24 h), and a growth rate 1000 times that of standard strains (Valway *et al.* 1998).

Contact investigation and prophylactic chemotherapy

Tuberculosis is a reportable disease in most states in USA, meaning that new cases must be reported to local health authorities. Reporting disease is essential so that contacts can be examined for evidence of infection and/or disease and can then be given appropriate chemotherapy as quickly as possible.

All household contacts and close contacts should be tested with skin tests. If negative, a repeat skin test should be performed 2–3 months after exposure has ended. CDC recommends presumptive prophylactic chemotherapy (isoniazid prophylaxis) for children and adolescents who are close contacts with an active case until a repeat skin test 12 weeks after presumed contact is negative (CDC 1990). If the 12-week test is positive, a chest x-ray should be taken and chemotherapy should be continued.

Preventive chemotherapy is indicated for close contacts. Adults should receive isoniazid daily for 6 months; children, 9 months; and HIV-infected persons, 12 months

(CDC 1994a). A 9-month regimen has recently been approved for HIV-positive individuals (CDC 1998a). Preventive therapy with rifampin should be considered for close contacts of persons with isoniazid-resistant tuberculosis (CDC 1994a).

Treatment of clinically active cases

The best control measure for the prevention of transmission of pulmonary TB is prompt identification and treatment of infected individuals. Effective drug therapy renders contagious persons uninfected within a few weeks. Hospitalization is only necessary for patients who are extremely ill and when treatment at home is impossible. Hospitalized patients with culture-positive sputum should be placed in isolation rooms. Only when patients' sputa become culture-negative, when patients are on adequate chemotherapy and no longer cough, should they be removed from isolation. At a minimum, patients in isolation rooms should be required to cover their mouths and noses when they cough or sneeze. Health care professionals and others entering isolation rooms should wear respirators, not a surgical mask, since respirators are the only proven means of personal protection from MTB droplet nuclei (see below).

Environmental Controls

Introduction

Apart from the identification and treatment of infected and clinically active cases, the most important element of a hospital-based occupational TB control programme is the use of environmental controls (Nicas 1998). Environmental controls include dilution ventilation and local exhaust ventilation. Implementation of environmental controls is particularly important in facilities such as health care settings, correctional facilities, drug and alcohol treatment centres, and shelters for the homeless and HIV-infected individuals with a high prevalence of TB or a high risk of TB transmission. Health care workers may be at particularly high risk for MDR-TB. In CDC investigations of MDR-TB outbreaks in hospitals between 1990 and 1992, more than 100 health care workers were infected (ascertained by skin test conversions), 17 developed MDR-TB disease, and seven died.

The sections that follow discuss critical aspects of environmental controls for MTB.

Dilution ventilation

Dilution ventilation is the mixture of fresh air with contaminated air. Failure of dilution ventilation (e.g. recirculation of untreated air from spaces occupied by infectious individuals) has resulted in micro-epidemics of MTB infection and disease (Houk et al. 1968; Ehrenkranz *et al.* 1972). However, although dilution ventilation lowers the concentration of contaminants with fresh air, it can never reduce or eliminate the total amount of contaminants released. Sound industrial hygiene practice dictates that dilution ventilation be used to control exposure to occupants only if the following criteria are met:

1. Small quantities of contaminants are released at uniform rates.
2. There is sufficient distance from the source of the contaminant to the potentially exposed worker.
3. The contaminant has a low toxicity or is present at non-toxic concentrations.

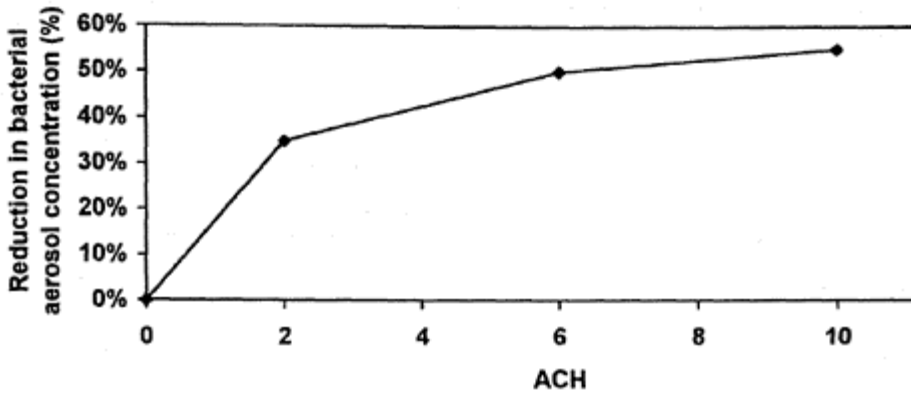


Fig. 1 Percentage reduction in bacterial aerosol concentration as a function of effective room air changes per hour (ACH).

No air-cleaning device is needed to collect contaminants before the exhaust air is discharged to the outside or recirculated. Because none of these criteria apply in occupational exposure to mycobacteria, dilution ventilation is not an adequate means of controlling exposure to MTB. Furthermore, supplying large volumes of dilution air to the space can be costly relative to other, more effective measures. Dilution ventilation can be used to reduce the concentration of droplet nuclei generated intermittently by patients over time in an isolation room. However, it is extremely difficult to control worker exposure near the source of the contaminant where dilution has not yet occurred.

CDC has stipulated that an effective tuberculosis infection-control programme includes isolation rooms designed to achieve ≤ 6 air changes h^{-1} (ACH) for existing facilities and ≤ 12 ACH in new or renovated facilities. The minimum of 6 ACH was chosen based on comfort and odour control considerations rather than health and safety considerations. In fact, the relative effectiveness of increasing ACH in reducing TB transmission has not been documented.

CDC allows the use of portable HEPA filtration units in tuberculosis isolation rooms as a means of achieving ≤ 6 ACH by augmenting the mechanical ventilation system. However, NIOSH-supported research indicates that ventilation rates ≥ 6 ACH, whether achieved by HEPA filtration units, by the mechanical ventilation system, or a combination of the two, do not significantly diminish bacterial aerosol concentrations (Fig. 1). The data, however, did indicate that local filtration by the in-room HEPA filtration units was as effective as the ventilation system, i.e. 6 ACH from the ventilation system reduced the mean room concentration to the same level as a combination of 2 ACH from the ventilation system and 4 ACH from the in-room HEPA filtration units.

NIOSH researchers reported that the optimal flow rate for ventilation exchange alone is 12 ACH. This figure applied for all but the unidirectional configuration, which was equally efficient at all flow rates. Strong conclusions regarding optimal flow rate cannot be drawn from this study because of its limited sample size (only one data point for each

tracer gas injection rate and ventilation rate).

The American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) has promulgated indoor air quality requirements for ventilation with outdoor air for three types of buildings, i.e. commercial facilities, institutional facilities, and residential facilities. Table 3 gives the outdoor air requirements for the institutions where there is an increased risk of occupational tuberculosis (ANSI/ASHRAE 1989).

Ventilation configuration

The term ventilation configuration refers to the location of supply and exhaust vents in a room. NIOSH has conducted research to determine the best ventilation configuration by studying the dispersion of a tracer gas (sulphur hexafluoride) throughout a room. In this experiment, four ventilation configurations were studied: (1) a typical hospital room with the air supply in one corner and the exhaust in the opposite corner; (2) a ceiling supply with perimeter exhaust near the floor; (3) a unidirectional flow with supply vents covering one wall and exhaust vents covering the opposite wall; and (4) a unidirectional flow with only three slots in opposing walls. The unidirectional ventilation configuration appeared to be approximately 100 times better than the others, suggesting that this configuration is most appropriate for the control of exposure to MTB.

Air mixing

Poor air mixing markedly decreases the effectiveness of dilution ventilation, and increases the risk of exposure to MTB. Failure to take into account poor air mixing usually results in overestimation of the effectiveness of a given number of ACH. Consider dilution ventilation within an occupied space. Unlike plug-flow, air entering an occupied space mixes with air already in the room by turbulent diffusion. The degree of mixing depends upon the air velocity, the geometry of the interior of the room (taking into account its furnishings), and the placement of air supply and exhaust vents. In the well-mixed case, after 1 h at one ACH, only 63% of the original room air is actually replaced. In a less well mixed (or “short-circuited”) space, the amount of air replaced will be substantially lower.

Air mixing can be modelled by assuming the existence of several room air zones. A poorly mixed room probably consists of several zones, each subject to different replacement rates. Simple modelling of room zones, such as the two-zone model, makes unrealistic assumptions and may generate spurious results. Two-zone models assume that droplet nuclei concentration is uniform throughout the lower zone when, in reality, the concentration is probably a monotonic function of distance from the source. Given that proximity to the source increases the likelihood of infection, the two-zone model may substantially underestimate exposure intensity and the risk of infection, and overestimate the efficacy of upper room air ultraviolet germicidal irradiation UVGI (see section below). For example, in a room with an upper- to lower-zone volume ratio of 2:3, the underestimation error can reach 40% (Nicas 1996a).

Local exhaust ventilation

Local exhaust systems capture and remove airborne contaminants at or near their source

before they are dispersed into a space. Local exhaust also requires less airflow than dilution ventilation. Local exhaust ventilation is theoretically preferred to dilution ventilation as a means of minimizing exposure to airborne contaminants. Local exhaust ventilation systems are designed for specific applications. A system typically includes a hood, ductwork, an air cleaner and a fan. The process or equipment is enclosed as much as possible, and air is withdrawn at a rate sufficient to assure unidirectional airflow and contaminant entrainment into the hood.

There are three types of hood for local exhaust ventilation: receiving, capturing and enclosure hoods. Receiving hoods catch contaminants that rise or are thrown into them.

They are not practical for preventing exposures unless the process emits hot air or air with sufficient velocity to carry contaminants into the hood. Receiving hoods therefore play no role the control of exposure to droplet nuclei.

Table 3. Outdoor air requirements for ventilating specified health care and correctional facility settings (ANSI/ASHRAE 1990).

Application	Estimated Maximum Occupancy	cfm* per person	cfm* per ft ³ room air
Hospitals, nursing and convalescent homes			
Patient rooms	10	25	
Medical procedure	20	15	
Operating rooms	20	30	
Recovery and intensive care	20	15	
Autopsy rooms			0.5
Physical therapy	20	15	
Correctional facilities			
Cells	20	20	
Dining halls	100	15	
Guard stations	40	15	

*cfm=ft³ min⁻¹ (28.3 L min⁻¹)

Capturing hoods “reach out” to capture contaminants provided that their capture velocity is sufficient. Their advantage lies in the convenience of their placement alongside a process rather than enclosing it. However, large air volumes are needed to generate sufficient capture velocities and the capture distance is limited to about 2 ft (60 cm) from the hood opening (McDermott 1985).

Capturing hoods are used to prevent the release of droplet nuclei during cough-inducing procedures such as sputum induction and aerosolized drug therapy. CDC

guidelines refer to them as exterior devices (CDC 1994b). Typically, the patient sits within a capture hood “cart”. The patient’s face is surrounded on five sides, the far side being the surface of a HEPA filter through which air is circulated. Droplet nuclei may escape from the device if the air velocity into the filter is not sufficient to overcome crosscurrents near the patient’s face or if the patient does not face directly into the hood when coughing or sneezing. CDC guidelines (CDC 1994b) state that this velocity should be $\geq 200 \text{ ft min}^{-1}$ (approx. 1 m sec^{-1}). However, because peak bronchial airflow during sneezing and coughing approaches 300 m sec^{-1} (Riley and O’Grady 1961), it is unlikely that a device designed with a capture velocity of 200 ft min^{-1} (1 m sec^{-1}) could effectively capture aerosol because the air velocities are so mismatched.

The enclosing hood design is superior to both receiving and capturing hood designs because it surrounds the contaminant maximally. Enclosing devices used to control exposure to droplet nuclei include biological safety cabinets, sputum induction booths, and isolation tents or hoods.

Biological safety cabinets

Biological safety cabinets are specialized local-exhaust ventilation devices that should be used in diagnostic laboratory settings when necessary. Because MTB may be present in many body secretions, including sputum, gastric lavage fluids, cerebrospinal fluid and urine, and other tissues, laboratory personnel who process such samples are at substantial risk. In one report, the incidence of tuberculosis in laboratory personnel working with MTB was three times higher than in those not working with it (CDC/NIH 1993).

Exposure to aerosols generated by laboratory manipulations of potentially infected materials pose a substantial hazard to laboratory personnel. Aerosol-generating procedures include preparation of frozen sections, manipulation of liquid cultures, and even the addition of undiluted bleach to sputum samples prior to acid-fast staining. These and any other aerosol-producing procedures must be performed in a Class II biological safety cabinet. The cabinet should also be used whenever high concentrations or large volumes of MTB are used.

Class II biological safety cabinets are designed to protect both personnel and materials within the cabinet. They are designed with an air velocity measured in the vertical plane of the sash of $75\text{--}100 \text{ ft min}^{-1}$ (roughly $0.4\text{--}0.5 \text{ m sec}^{-1}$), HEPA-filtered vertical laminar airflow and HEPA-filtered exhaust air. They are classified into two types, Type A and Type B, based on construction, airflow velocities and patterns, and exhaust systems.

In USA, biological safety cabinets are tested and certified by specialists certified by the US National Sanitation Foundation. Testing and certification of Class II biological safety cabinets are required upon installation, annually thereafter, and whenever relocated. Such testing is meant to ensure that users and the immediate environment are protected. Of course, protection also depends upon the work practices of the user.

Sputum induction booths

A sputum induction booth, used for obtaining sputum samples from patients who cannot produce sufficient sputum spontaneously, typifies an enclosing device. It is a miniature,

free-standing room that should be designed to pull room air past the breathing zone of a patient seated in the booth into a pre-filter and HEPA filter. The booth design should not allow room air to be pulled directly from the air supply inlet to the exhaust outlet, nor should it allow exfiltration, i.e. air leakage outward through cracks and interstices or through ceilings, floor, and walls of a space or building. Air should be exhausted only through the HEPA filter into the room or outdoors. The integrity of the system can be checked using a smoke tube at all exfiltration points while the unit is running.

Isolation tents

Isolation tents are enclosing devices commonly used to create an isolated environment within a room, such as a negative-pressure bed enclosure for infectious patients; for aerosolized drug therapy, such as for ribavirin treatment in a tented crib; or for isolation of an infected patient being transported. The typical design draws air upward from the area around the bottom of the skirt through HEPA filters prior to recirculation into the room.

Air filtration

Filtration has long been used as an effective means of cleaning air. Air filters remove particles by five known mechanisms, including interception, inertial impaction, diffusion, gravitational settling, and electrostatic attraction. Filtration efficiency and its reciprocal, penetration, are dependent upon the size of particles in the airstream passing through and on the air velocity. Maximum penetration of most filters occurs when particles are 0.3 μm in size or perhaps smaller (Cambridge Filter Corporation 1982; Hinds, 1982). Particle sizes larger than 0.3 μm will be removed with greater efficiency.

There are several types of filter. HEPA filters are expendable extended-media dry-type filters in rigid frames. HEPA filters should have a minimum particle collection efficiency of 99.97% for 0.3 μm thermally generated dioctyl phthalate (DOP) particles or specified alternative challenge aerosol and a maximum clean-filter pressure drop of one inch water column when tested at rated airflow capacity (Institute of Environmental Sciences, IES 1986). This requirement takes into account variables affecting testing results, including particle size, method of particle generation, airflow, and pressure drop. HEPA filtration is endorsed by CDC as a method of air cleaning that supplements dilution ventilation (CDC 1994b).

HEPA filters fall into five performance classes and two construction classes. Together, these class combinations provide a range in construction, performance and cost suitable for different application requirements. Performance classes A to E are based on DOP penetration and resistance to airflow (CDC 1994b). Type A filters meet the minimum performance level or removal efficiency that satisfies the basic definition of a HEPA filter. Type B filters meet the minimum efficiency of Type A filters, but are certified to be free of pinhole leaks that would cause penetration at low flow rates. Type C filters meet the minimum efficiency of Type A filters, but are tested using aerosols at 80–100 ft min (0.406–0.508 m sec^{-1}) face velocity with an upstream smoke concentration of approximately 10–20 mg L^{-1} . They are fully scanned to detect and eliminate all significant leakage streams having concentrations greater than 0.01% of the upstream DOP smoke concentration at these concentrations. Type D filters must meet the

requirements of Type C filters, but must also yield a penetration of $\leq 0.001\%$ at rated flow. Type D HEPA filters can suffice when ultra-low penetration is required. Type E filters are designed, constructed and tested in accordance with two US military standards for use in air cleaning systems involving toxic chemical, carcinogenic, radioactive or hazardous biological particles. HEPA filters are also categorized into two construction classes, Grades 1 and 2 (CDC 1994b). Grade 1 construction class is fire resistant, whereas Grade 2 is semi-combustible.

Additional performance and construction considerations should be taken into account when selecting HEPA filters, viz.

- Single filters having a depth of less than one-quarter of the minimum side dimension may not meet pressure loading requirements in standardized test methods. Consideration should be given to the desired separator direction to avoid media failure. Where filter faces are mounted vertically, pleats and separators should be vertical. Sagging of the pack may occur in filters installed with horizontal separators, increasing the risk of media failure. Installation of lower efficiency pre-filter(s) upstream of the HEPA filter can extend the life of a HEPA filter up to 25%. Similarly, following the disposable filter by a 90% extended surface filter can extend the life of the HEPA filter by nearly 900% (ACGIH 1992). Both used prefilters and HEPA filters should be handled and disposed of as if contaminated with droplet nuclei.
- A manometer or other pressure-sensing device should be installed in the filter system to monitor the need for filter replacement. The manufacturer should be able to describe the pressure drop characteristics of the filter as it loads particles. The installation design for the filter should allow for maintenance that will not contaminate the ventilation system or the area it serves.
- A quantitative leak test should be performed when the filter(s) is initially installed, when it is changed or moved, and every six months. There are several test procedures, including the leak test, the scan test, the two-flow test, and a laser test (Cambridge Filter Corporation 1982).
- Proper filter installation should prevent leakage between filter segments and between the filter mat and its casing.
- Manufacturers of equipment containing HEPA filters provide a maintenance schedule and recommended procedures for installation, removal, and disposal of filter elements. Only properly trained personnel should do such maintenance. Improper design, installation, or maintenance of HEPA filters or HEPA filtration units can result in exposure to MTB droplet nuclei.

Filter configuration

HEPA filters can be used in a number of configurations, including the following: in booths or enclosures recirculating air back into a room; within a room either in free-standing (portable) air cleaning units or in wall-mounted recirculation units; in HVAC ductwork prior to recirculation to other areas; or in exhaust ducts prior to exhaust to the outside.

In-room filtration

Whether or not HEPA filters effectively remove MTB from the air has not been studied. However, CDC guidelines state that they are likely to be effective, since they have been shown to remove spores of the fungus *Aspergillus*, which range in size from 1.5–6.0 µm (Opal *et al.* 1986; Sherertz *et al.* 1987). Unfortunately, the volumetric flow rate conditions under which these studies were conducted were not well described. The ability of a HEPA filter to remove particles of a certain size (e.g. 1.5–6.0 µm) only addresses the issue of filter efficiency. Any properly manufactured HEPA filter should also perform according to its performance and construction rating when installed correctly. More importantly, the ability of HEPA filters, either in freestanding or in-room mounted units, to remove particles of a given size from an occupied space effectively should be documented because variables unique to the room may reduce the unit's effectiveness. Such variables include ventilation configuration, air-mixing patterns in the room, placement of the unit, unit design, proximity of health care workers to the infectious patient and the duration of exposure.

Miller-Leiden *et al.* (1996) have evaluated the effectiveness of free-standing HEPA room air units to exhaust aerosol within a mock hospital room. Three commercially available freestanding HEPA filtration units tested performed equally well. As expected, the highest concentration of particles occurred closest to the source, and the placement within and the flow geometry of the room had a significant impact on effectiveness. Unfortunately, because care-giving demands close proximity to the infectious patient (where particle concentration is highest), it is likely that exposure will occur despite adequate ACH provided by the ventilation system, the presence of a free-standing HEPA filtration unit, and, for that matter, the presence of UVGI. Clearly, adequate respiratory protection is still required when caring for patients with clinically active TB.

Miller-Leiden *et al.* (1996) also investigated the effectiveness of ceiling-mounted filtration units. The experimental protocol involved simultaneous aerosol generation and sampling for 2 h and determination of concentrations in the air as a function of space and time. Three units were evaluated using various combinations of ventilation and equivalent ACH filtration². Ceiling-mounted filtration units appeared to work well. The units provided 6 equivalent ACH and reduced the average room concentration to a value less than that achieved by 6 ACH with general ventilation only. However, there was substantial within-unit and between-unit variation. Moreover, the concentration nearest the source was always higher than the average room concentra

² The term equivalent ACH equates air that has been treated by filtration to remove droplet nuclei with clean outdoor air.

tion. Increasing the airflow rate beyond 12 equivalent ACH resulted in little change in airborne concentration.

The preferred method of CDC for recirculating HEPA-filtered air within a room is a stand-alone system. Air is exhausted from the room into a duct separate from the HVAC ductwork, passes through a HEPA filter in the duct, and returns to the room. Such a design requires placement of ductwork in the ceiling cavity of the room. The reasoning

behind CDC's preference for this system (over portable or wall-mounted units) is not readily apparent, as installation and maintenance is more difficult.

Filtration in HVAC ductwork

HEPA filters can also be placed within HVAC ductwork. However, this practice is not justified. Air from tuberculosis isolation and treatment rooms should be exhausted to the outside in accordance with applicable federal, state and local regulation. Consequently, there is no reason to filter the air exhausted from such rooms for recirculation purposes³. Only in existing facilities, where recirculation of the air into the general ventilation system from such rooms is unavoidable, should HEPA filters be installed in the exhaust duct leading to the general ventilation system. Note that HEPA filtration within the HVAC system does not lower the airborne concentration of droplet nuclei *within* the room from which it is being exhausted.

Ultraviolet germicidal irradiation (UVGI)

Ultraviolet (UV) radiation is non-ionizing radiation in the electromagnetic spectrum within three wavelength bands, viz. UV-C (100–280 nm), UVB (280–315 nm), and UV-A (315–400 nm). Commercially available UV lamps used for germicidal purposes are low-pressure mercury vapour lamps that emit radiant energy in the UV-C range, predominantly at 253.7 nm.

UV lights can be mounted on a wall or ceiling of a space to irradiate the upper portion of the air, in HVAC ductwork to irradiate air passing through ducts, in freestanding UVGI units or combination UVGI/HEPA units, or in self-contained units mounted in ceiling cavities. CDC recommends the use of UVGI lamps in general-use areas in which the risk of transmission is relatively high as an adjunct to ventilation for reducing the airborne concentration of MTB. However, the effectiveness of this practice has not been adequately evaluated (CDC 1994b).

UV lights mounted on a wall or ceiling of a space are passive; they do not contain air-moving devices that bring MTB droplet nuclei to the UV beam for a germicidal dose. As air-handling unit(s) of the HVAC system draw contaminated air from an occupied space past the lamps mounted on the interior duct surface, UV lamps mounted in HVAC ductwork may be more effective. Freestanding UVGI units or combination HEPA/UVGI units contain pumps to move contaminated room air past UV lamps. Actively moving large volumes of room air past UV lamps may result in more effective MTB control than passive UVGI, assuming sufficient residence time in the zone of irradiance. UV lamps must be installed properly and maintained according to manufacturer's instructions. Only specially trained employees should handle and maintain these lamps.

Effectiveness of UVGI

The biocidal properties of ultraviolet light have been studied. *M. tuberculosis*, *M. phlei*, and BCG are less susceptible to loss of viability than *Staphylococcus albus*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis* and *Serratia marcescens* (Collins 1971). These data contrast with those of Riley *et al.* (1976), who found that virulent tubercle bacilli and BCG were equally susceptible to ultraviolet irradiation but that *M. phlei* was 10 times more resistant. It is important to note that these

studies involved the generation of artificially generated aerosols in test rooms. It has not been demonstrated that the rates of inactivation observed in test rooms can be attained in occupied rooms in mechanically ventilated buildings.

Chang *et al.* (1985) measured the biocidal dose-response of UV-C to other human pathogens such as viruses and spores and cysts of amoebae, and also other pathogenic bacteria. Greater doses of UV-C were required to kill cysts and spores than viruses (Chang *et al.* 1985). Jen

³ Notably, if air exhausted to the outside can re-enter the building ventilation system supply, then HEPA filtration is needed.

sen (1963) demonstrated that several strains of virus were effectively inactivated by UV exposure in an experimental chamber, the rates of kill being high (99.6–100%).

Biocidal effectiveness of UVGI is a function of relative humidity (RH). Under experimental conditions, a sharp decrease in the number of *S. marcescens* test organisms was observed above 60–70% RH (Riley *et al.* 1972). The practical implication of this finding is that RH, an environmental condition that is often not tightly controlled, may reduce effectiveness of UVGI.

Total room ventilation and air exchange between portions of a room are also important considerations. One study found that upper air irradiation with a passive UVGI device in the waiting room of an outpatient HIV clinic reduced the concentration of ambient airborne bacteria by 15–30% (Macher *et al.* 1992). However, the authors subsequently indicated that the total ventilation of the room might have been underestimated, resulting in an overestimate of the reduction attributable to UVGI. Air currents of approximately 20 ft min⁻¹ (approx. 0.1 m sec⁻¹) are sufficient to waft MTB droplet nuclei into an upper zone of irradiation. However, the amount of air exchanged between upper and lower room zones is not known. Moreover, the effectiveness of passive upper air UVGI in reducing MTB exposure due to close proximity to infectious patients has not been documented.

The current position is that research performed to date has not determined the value of UV-C in “real world” applications such as hospitals and other institutions, where variables such as temperature, RH, and volumetric flow rate cannot be or are not controlled. Although UVGI was shown to be effective in preventing the transmission of measles and influenza viruses in classrooms (Wells 1955), its subsequent employment in schools was unsuccessful (Macher 1993). Additional studies are needed to determine accurately the extent of UVGI-attributable droplet nuclei reduction and, more importantly, to evaluate whether the resulting reduction is sufficient. It is also unclear whether the inherent health risks (reviewed below) of exposure to UV light emitted by UVGI sources are justified by their mycobacteriocidal effectiveness. It is likely that passive UVGI reduces both the concentration at the source and the average room concentration much more slowly, and to a lesser degree, than HEPA filtration units or UVGI units equipped with an air-moving device.

Comparison of UVGI designs

Ultraviolet lamps are sometimes mounted in HVAC ductwork, but in this location they only disinfect (to an unknown degree) the air upstream of the lamps. Thus, CDC recommend that UVGI devices should not be regarded as adequate substitutes for HEPA filters in ducts that discharge air from TB isolation rooms into the general ventilation system (CDC 1994b).

HVAC duct-mounted, freestanding UVGI, HEPA/UVGI units over passive wall-mounted UV lamps, and units recessed in a ceiling cavity offer several distinct advantages. Firstly, they minimize room occupant exposure to UV radiation. Secondly, recessed units do not distort room air mixing by taking up room space and, thirdly, permanently mounted systems probably have greater biocidal effectiveness than free-standing floor units, the effectiveness of which is subject to where they are positioned on the room floor. Portability of freestanding UVGI or HEPA/UVGI units is only advantageous if TB isolation rooms are not dedicated, permanent rooms. Freestanding UVGI or combination HEPA/UVGI and recessed (ceiling cavity) UVGI all provide some level of “equivalent” ACH of ventilation⁴.

Critics of combination HEPA/UVGI units claim that there is no need for both HEPA filtration and UVGI capability within the same device. If both were equally effective, that claim would be true. However, combination units are primarily built as HEPA filtration units and typically provide only a fraction of the UV intensity of dedicated UVGI units. If the filter fails, the effectiveness of the UVGI alone may be minimal. While combined units offer the advantage of redundancy, in that if one technology fails the other is still present, their incremental effectiveness is probably low.

⁴ Strictly speaking, ventilation implies supplying outdoor air. Because these units contain an air moving device, they in effect provide ACH in addition to those of the mechanical ventilation system because the air is presumed disinfected and, therefore, in theory, as fresh as outdoor air.

Health effects of ultraviolet radiation exposure

The adverse effects of human exposure to ultraviolet light have been well documented. Prolonged exposure to UV radiation causes several dermatological reactions. It causes an erythema, similar to a sunburn delayed by 2–10 h. Some subjects may experience photosensitivity reactions, abnormal skin reactions to UV light in the presence of certain chemical agents. Direct phototoxins—substances directly activated in the skin by exposure to UV light—include coal tar, colognes, lipsticks, hair preparations, psoralens and sunscreens. Photoallergic reactions, resulting from immune responses to radiation-induced chemical reactions in the skin, can occur with some salicylanilides and antibiotics, hexachlorophene, cosmetics, and colognes. Urticaria (hives) or eczema (scaly skin reactions) have also been reported.

In the eye, UV-C and UV-B of wavelengths <300 nm are absorbed to a high degree by the cornea, and at wavelengths >300 nm are increasingly absorbed by the lens. The result is dose-dependent photokeratitis (inflammation of the cornea) and conjunctivitis (inflammation of the outer epithelial lining of the eye). Signs and symptoms include pain, itchy eyes, blepharospasm (uncontrollable blinking), tearing, congestion of the

conjunctiva, photophobia, blurred vision and occasionally blepharitis (red, swollen eyelids). Although these effects may be incapacitating for up to 2 days, permanent damage is rare.

Because of these and other adverse health effects of UV exposure, the American Conference of Governmental Industrial Hygienists (ACGIH 1995), NIOSH (1972) and the International Radiation Protection Association (IRPA 1985) recommend occupational exposure limits for wavelengths between 180 and 400 nm. ACGIH occupational exposure limits are called threshold limit values (TLV's). TLV's are given as a measure of irradiance and radiant exposure⁵. The ACGIH TLV's for radiant exposure at 254 nm are 60 J cm^{-2} (6.0 mJ cm^{-2}). The product of a TLV radiant exposure at a specified wavelength and its relative spectral effectiveness (RSE)⁶ is the limiting dose. The use of RSE's allows irradiance data to be weighted to the limiting TLV at 270 nm of 30 J cm^{-2} , which is based on the potential for photokeratitis.

The Recommended Exposure Limit (REL) of NIOSH (1972) requires that the measured irradiance level be multiplied by the specific wavelength to obtain the effective irradiance. The maximum permissible exposure time can then be calculated. Neither the REL or TLV exposure limits should be used to determine acceptable exposure of photosensitive individuals or regarded as protective for individuals lacking a crystalline lens.

Experienced, qualified personnel should measure irradiance levels using a radiometer. Prior to measurement, personnel should determine source characteristics, operator-source interaction, and the location of the source relative to reflective objects such as aluminum surfaces. Should the measurement data warrant use of personal protective equipment for the eyes, qualified, experienced personnel can determine which protective eyewear is compatible with the spectral distribution and intensity of the UV light source.

Negative room pressure and directional airflow

CDC guidelines recommend that tuberculosis isolation rooms be maintained under negative pressure to prevent the escape of droplet nuclei into surrounding areas. Pressure differentials, such as created by negative pressure in an enclosed space, cause air to flow into the space. To maintain negative pressure, it is recommended in the guidelines that doors to isolation rooms be kept closed as much as possible. The guidelines also recommend that negative pressure in the room be monitored daily while the room is being used for TB isolation.

⁵ Irradiance is the level of radiation arriving at a point in space, the quotient of the radiant power and the cross-sectional area, or time-averaged energy flow. Radiant exposure is the change of the incremental radiant energy in a given cross-sectional area, the energy dose of radiant energy. Irradiance is measured in watts per square metre (W m^{-2}), and radiant exposure in Joules per square metre (J m^{-2}).

⁶ RSE is a hazard weighting function indicating the biological efficacy of that wavelength.

Although the guidelines do not require an anteroom, they state that this design may increase the effectiveness of the isolation room by minimizing the potential escape of droplet nuclei into the corridor when the door is opened. To work effectively, the

anteroom should have positive air pressure relative to the isolation room. The pressure relationship between the anteroom and the corridor may vary according to ventilation design.

CDC states that the minimum pressure difference necessary to achieve and maintain airflow into a room is 0.001 in (0.025 mm) water column. Negative isolation-room pressure is usually achieved by exhausting air at a rate higher than the air being supplied to the room. However, this small pressure differential may be insufficient to prevent exfiltration during normal disturbances, such as opening the door. Rather than balancing the system using room pressure differential, a better approach is to balance the ventilation so that the exhaust flow is 10% greater than the air supply.

The procedure for establishing sufficient isolation room pressure differentials involves:

1. Inspecting the room for leakage through doors, windows, plumbing, and equipment wall penetrations. Sealing leaks.
2. Balancing the ventilation system to achieve an exhaust flow of 10% more from the room than the air supply to the room.
3. Monitoring with static pressure differential gauges or sensors, or visually with a smoke tube.

Such flow proportioning achieves better control of average infiltration into a room than does the static pressure method (ASHRAE 1984).

CDC guidelines give alternate means of achieving negative room pressure, including use of fixed room-air recirculation systems, use of portable room-air recirculation units, use of a small centrifugal blower (fan), or pressurizing the corridor. If a fan is used to exhaust isolation room air to achieve negative pressure, it should not be near any other window or door that could be contaminated by the exhaust. Pressurizing the corridor involves balancing the ventilation system to create a higher air pressure in the corridor than in the isolation room. This is usually accomplished by increasing the supply rate, but not the exhaust rate (although it may be necessary to decrease the exhaust rate depending upon the ventilation system design). Positively pressurizing the corridor and rooms off the corridor may be difficult to achieve with existing designs of ventilation system. Some rooms, such as bronchoscopy rooms, are required to be positively pressurized.

Recirculation of isolation room air

Air from TB isolation rooms and treatment rooms should not be recirculated into the general ventilation recirculation, but rather be exhausted to the outside. In some facilities, the ventilation system or facility configuration makes venting exhaust to the outside impossible. In such facilities, CDC guidelines recommend that HEPA filters be installed in the exhaust duct leading from the room to the general ventilation system. The obvious economic disadvantage of exhausting isolation air to the outside is the loss of conditioned air, i.e. air that has been heated, cooled, humidified or dehumidified. However, use of devices such as free-standing or mounted HEPA filtration units to increase the number of ACH in a isolation room can actually recover some of this loss by cleaning contaminated air and then recirculating it.

Personal protective equipment

In regard to MTB, personal protective equipment (PPE), i.e. devices worn by workers as barrier protection against hazards in the environment, is designed to minimize exposure to bioaerosols such as MTB droplet nuclei. Surgical masks provide only limited protection and are fundamentally different from respiratory PPE, a fact often misunderstood by health care professionals.

Surgical masks

Surgical masks provide a barrier for splashes and splatter. The mask material filters large particles ($>5\text{ }\mu\text{m}$) fairly well. However, because of their generally poor fit to the face, surgical masks provide little protection against MTB. Small particles can easily travel with the airstream around the non-sealing periphery of the mask into the breathing zone. For example, under experimental conditions, Tuomi (1985) showed that 5% of $5.5\text{ }\mu\text{m}$ particles passed through sealed and 45% through unsealed masks. Notably, 94% and 98%, respectively, of $1\text{ }\mu\text{m}$ particles passed through sealed and unsealed masks (Tuomi 1985). Mask wearers, especially those with poorly fitting masks, are thus at substantial risk of exposure to 1–5 μm MTB droplet nuclei.

Respiratory personal protective equipment

Respiratory PPE is designed to provide substantial protection from the inhalation of hazardous atmospheres (ANSI Z88.2–1992), including MTB droplet nuclei. Respirators do not remove contaminants from the work area, as do local exhaust and general ventilation systems. Rather, they reduce the airborne concentration of contaminants at the breathing zone. There are two basic types of respirator, air-purifying and atmosphere-supplying. An air-purifying respirator uses the inhalational force of the wearer to bring ambient air through an air-purifying element that removes the contaminant(s). Atmosphere-supplying respirators supply a respirable atmosphere, independent of the workplace air. Both types are further differentiated by their respiratory inlet covering (half-mask, full, helmet/hood or loose-fitting respirators).

Each respirator is assigned a protection factor (assigned protection factor, APF) depending upon the type of respirator and respiratory inlet covering (see Table 4). APF is the expected workplace level of respiratory protection that would be provided by a properly functioning respirator or a class of respirators to properly fitted and trained users (ANSI Z88.2–1992). APF is calculated as

$$C_1 = C_A / \text{APF},$$

where C_1 is the contaminant concentration in inspired (respirator) air and C_A is the contaminant concentration in ambient air. APF is essentially the magnitude of reduction of airborne contaminant concentration provided by respirators; the higher the APF, the higher the protection of the wearer.

All respirators of the same class are given the same APF value. For example, the APF for an approved disposable air-purifying half mask HEPA respirator is 10. The APF is

also 10 for a non-disposable elastomeric half-mask air-purifying respirator equipped with dust/mist filters or with HEPA filters.

Table 4. Assigned protection factor of several respirators (ANSI 1 992).

Respiratory inlet covering				
Type of respirator ^a	Half mask	Full respirator		
Air-purifying	10	100		
Atmosphere supplying				
Self-contained breathing apparatus (demand) ^b	10	100		
Airline (demand)	10	100		
Respiratory inlet covering				
Type of respirator	Half mask	Full respirator	Helmet/Hood	Loose-fitting respi
Powered air purifying	50	1000 ^c	1000 ^c	25
Atmosphere supplying airline	50	1000	-	-
pressure demand	50	1000	1000	25
continuous flow				
Self-contained breathing apparatus	-	d	-	-
Pressure demand				
Open/closed circuit				

^aIncludes quarter mask, disposable half masks and half masks with elastomeric respirators.

^bDemand SCBA shall not be used for emergency situations such as fire fighting. ^cProtection factors listed are for high-efficiency filters and sorbents (cartridges and canisters). With dust filters, an assigned protection factor of 1 000 is to be used owing to the limitations of the filter.

^dAlthough positive-pressure respirators are currently regarded as providing the highest level of respiratory protection, a limited number of recent simulated workplace studies concluded that all users may not achieve protection factors of 10,000. Based on this limited data, a definitive assigned protection factor could not be listed for positive-pressure SCBA's. For emergency planning purposes where hazardous concentrations can be estimated, an assigned protection factor of no higher than 1 0,000 should be used.

NOTE. Assigned protection factors are not applicable for escape respirators. For combination

respirators, e.g. airline respirators equipped with an air-purifying filter, the mode of operation in use will dictate the assigned protection factor to be applied.

Such respirators would be ineffective against an airborne concentration of 200 tubercle bacilli m^{-3} air, since the concentration of MTB in respired air would be 20 bacilli m^{-3} or 2–10 times the infectious dose.

Regulatory standards

Health care providers, CDC, NIOSH and health and safety professionals have engaged in intense debate since CDC issued its recommendation for a disposable particulate respirator (Dooley et al. 1990). That recommendation was based on the particle size distribution of MTB aerosols and the filtration efficiency of air-purifying respirators. Both the 1994 CDC guidelines and the OSHA proposed rule (Federal Register 1997) require negative pressure air-purifying or more protective respirators which:

- are capable of HEPA or N95 filtration
- can be qualitatively fit-tested in a reliable way to obtain a face seal leakage of no more than 10% for most workers
- can be fit-checked by the wearer each time he/she puts on the respirator.

Implied in these recommendations is that a total leakage of 15% (5% filter leakage and 10% face-seal leakage) is acceptable. However, when considering the potency of MTB infection, this level of leakage is most likely unsafe. If the ambient airborne concentration were 100 MTB bacilli m^{-3} and the APF 6.7, then the concentration in inhaled air would be 15 bacilli m^{-3} , because the infectious dose will be exceeded in <1 h. The most obvious flaw is the assumption that a respirator with a low APF value (6.7) is suitable in a wide variety of situations involving MTB exposure. The recommendations also imply that APF's of 50, 100, and 1,000 are not needed, an assumption which is not warranted.

The debate over the minimum required level of respiratory protection was so intense that NIOSH responded with revised 42 CFR Part 84 regulations on testing and certification of respirators to account for the CDC criteria described immediately above. NIOSH plans to revise its regulations in three modules, including APF's, which will be addressed at a later date.

In the revised 42 CFR Part 84, NIOSH created three series of particulate filters (Types N, R and P) with three filter efficiencies for each series (95, 99 and 100%). Type N filters may be used against solid particles in workplaces free of oil aerosols, while Type R and Type P filters may be used against aerosols of both solid particles and liquids, including oil. Type N filters are tested against a sodium (NaCl) aerosol, while Type R and P filters are tested against a dioctyl phthalate (DOP) aerosol. The Type R and P filters differ in that the latter filters are more rigorously tested. Both the NaCl and DOP challenge aerosols have a mass median aerodynamic diameter of approximately 0.3 μm . Efficiencies of 95, 99 and 100% indicate that the filters are able to remove at least 95, 99 and 99.7%, respectively, of the challenge aerosol particles. A labelled filter efficiency of 100% is misleading because the filter need not remove every particle to pass the test. In

its introduction to the new regulations, NIOSH stated that all nine classes of particulate filter respirator meet or exceed the respirator performance criteria in the CDC guidelines for preventing TB transmission. Interestingly, NIOSH did not require quantitative determinations of the amount of face-seal leakage as part of the respirator certification process. Instead, they presumed that if a health care worker wearing a Type N95 respirator passed a qualitative fit test, the worker would experience less than 10% face-seal leakage. At present, it appears that many health-care institutions may use disposable, filtering-respirator Type N95 respirators against MTB aerosols. A filtering respirator is a one-piece disposable device similar in appearance to a surgical mask. The experience of the present author is that disposable respirators cannot be reliably fit-tested.

A subcommittee of the American National Standards Institute (ANSI) Z88 committee on respiratory protection (Z88.12) has been charged with the task of developing a standard for the selection and use of respirators for protection against infectious aerosols. ANSI standards are consensus documents that involve due process requirements of openness and balance, ensuring that the process is not dominated by any single interest category. The ANSI subcommittee is using a risk-based approach despite the fact that neither air sampling data nor a legally defined acceptable risk level is available. This risk-based approach relies, in part, on a modification of the traditional Poisson probability model for airborne MTB exposure and infection, also known as the Wells/Riley equation (Nicas 1996b). The user/employer can consult tables based on infectious dose, anticipated exposure concentration, and a personally acceptable level of risk and choose a class of respirator (represented by APF) based upon those parameters. Although it may take several years to finalize this standard, such a standard may take less time than collecting air-sampling data. The legal determination of an "acceptable risk level" may take even longer still, since the US Occupational Safety and Health Administration (OSHA) appears to be unable or unwilling to address the issue. CDC has also avoided the issue of acceptable risk, stating only that the infection control staff must individually estimate risk and may, based upon this assessment or best judgment, determine that respiratory protection exceeding their criteria may be considered appropriate (CDC 1994b).

A complete respiratory protection programme is required in work environments where respirators are needed and used. OSHA has set out the elements of a minimally acceptable programme (29 CFR 1910.134). Such a programme includes training in the proper use of respirators and their limitations (29 CFR 1910.134). For the reasons cited above, it is essential that the user be properly instructed in the selection, use, and maintenance of a respirator. Training must provide users with the opportunity to handle the respirator, have it fitted properly, test its face-piece-to-face seal, wear it in normal air for a long familiarity period, and finally, to wear it in a test atmosphere (CDC 1994b). Properly conducted respiratory protection training takes time; it cannot be done in 15 min. The legal requirements represent a baseline minimum level of training to ensure adequate understanding of the selection and use of respiratory protective equipment. As such, the requirement is not excessive.

Individuals should not be assigned to tasks requiring use of respirators unless it has been determined that they are physically able to perform the work and use the equipment

properly (CDC 1994b). The local health physician shall determine what health and physical conditions are pertinent and should review the respirator user's medical status periodically (CDC 1994b). There are medical conditions that contraindicate the use of a respirator, e.g. diabetes, anaemia, lower respiratory tract disease such as emphysema, asthma and bronchitis (ANSI Z88.6 1984). The institution's occupational health physician should perform appropriate (according to ANSI standards) spirometry testing and examinations. Expense involved in meeting this requirement can be offset by scheduling the examination/testing at the time of the employee's annual physical check-up.

Fit testing is required for all air-purifying respirators and tight-fitting positive pressure respirators. ANSI stipulates that the protocol given in the OSHA Asbestos Standard, 29 CFR 1910.1001, should be followed.

A common misconception is that respirators should be used to the exclusion of other control measures, including engineering controls. That is, a risk manager may choose a single strategy, e.g. dilution ventilation, UVGI, HEPA filtration or respiratory protective equipment, when it is likely that none of these controls on its own will prevent transmission of occupational TB. The limitations of each method described in this chapter argue that a combination of controls must be used.

With the widespread use of the infection control practices of *Universal Precautions and Body Substance Isolation*, the health care industry has become accustomed to the use of disposable supplies. Disposable negative-pressure air-purifying respirators may therefore be appealing to the health care industry and others. Disposable respirators have the advantage in being less expensive than elastomeric half-mask respirators, and also do not require a scheduled maintenance programme as is necessary for other respirators. However, disposable respirators have several disadvantages. In practice, most are reused until the excessive air resistance develops or there is physical damage. The number of times these devices may be safely reused has not been determined. At issue are the sanitary condition of the respirator and maintenance of the physical integrity of the respirator. Disposable respirators cannot be chemically sanitized or disinfected like non-disposable respirators, including disposable ones, must be stored in a manner to prevent distortion, contamination and damage due to physical agents such as vibration, electric shocks, sunlight, cold and moisture. While such storage is possible with a disposable respirator, it is likely that its storage would be handled with less care than a non-disposable respirator.

Airline travel and tuberculosis infection

Because of the relationship between proximity and risk of exposure, crowding and snaring air with infectious individuals, especially for extended periods of time, has long been recognized as a risk factor for the transmission of TB. Because aircraft are enclosed spaces providing only partially recirculated air, air travel imposes greater risk of infection for numerous communicable diseases, including TB.

Several outbreaks of TB infection occurring as a result of air travel have been reported in the literature. Among 760 travellers in the same plane as a Korean woman with

confirmed MDRTB, 29 individuals subsequently tested skin-test positive (Kenyon *et al.* 1996). The woman had taken four different flights, the longest of which lasted 8.75 h. This flight had the highest proportion of positive skin tests (15/249, or 6%). Of these 15, nine were seated at various locations throughout the aircraft and had other risk factors for tuberculosis. The remaining six had no other known risk factors and were seated within two rows of the index case. It should be pointed out that in this and other investigations, the only way to prove that fellow passengers were infected during air travel is to demonstrate, using molecular biology techniques, that the same MDR strain isolated from the index case produced infection in fellow passengers (Aguado *et al.* 1996).

Miller *et al.* (1996) conducted a contact investigation of 120 passengers and crew from two flights after a man from Moscow was discovered to have active TB. Only two of the 29 contacts without other identified risk factors were PPD-positive, they were not seated in close proximity to the index case.

Another contact investigation involved a highly infectious airplane passenger with pulmonary and laryngeal tuberculosis on two 1.25-h flights (Moore *et al.* 1996). Of the 100 passengers and 10 flight crew subsequently tested, five were PPD-positive. However, each had a risk factor other than shared air on the aircraft. In addition, this prevalence rate (5%) corresponds to the estimated prevalence of tuberculosis infection among unselected US adults (CDC 1995b).

Infected crew members may expose other crew members, as well as passengers, to risk. Driver *et al.* (1994) surveyed 212 crew members and 59 frequent-flier passengers who were exposed to an infected flight attendant during a 6-month period. Among US-born, those who recalled exposure to the flight attendant's coughing during the second 3 months had significantly higher rates of positive PPD tests than did contacts exposed in the first 3 months (30% versus 5.8%, $p < 0.001$). The strongest variable associated with positive PPD was duration of exposure (Driver *et al.* 1994).

Incidence data describing the risk of in-flight infection with TB have not been published. Olcerst (1996) calculated a theoretical risk of in-flight transmission of 71.8 per 100,000 for passengers and orders of magnitude higher for flight attendants. This theoretical calculation agrees with the epidemiological evidence cited above, suggesting that the in-flight risk of transmission of TB is low. Other investigators have reported a lack of transmission. McFarland *et al.* (1993) found no skin-test conversions among 91 US citizens exposed to a smear-positive passenger with pulmonary MDR tuberculosis on a flight between London and Minneapolis. Moore *et al.* (1996) also reported no transmission. Others have postulated that the risk of infection is no greater on board aircraft than on other forms of public transportation (Wick and Irvine 1995). CDC has suggested three criteria to guide decisions regarding notification of passengers and crew of possible exposures to TB during commercial air travel, namely the infectiousness of the index case at the time of the flight, the duration of the flight and the seating proximity to the index case (CDC 1995a).

Concern regarding the quality of aircraft air has increased to the point that ASHRAE has established a committee (SPC 161 P) to write guidelines for commercial passenger air-carrier aircraft certified under Title 14 CFR Part 25. The guidelines will address chemical, physical and biological contaminants.

US Occupational Safety and Health Administration

OSHA regulation concerning occupational exposure to tuberculosis

In 1993, OSHA was petitioned by a labour coalition to initiate rulemaking for a standard to protect employees from occupational transmission of TB. Despite the publication of the CDC *Guidelines for the Prevention of Transmission of M. tuberculosis in Health Care Facilities* in 1990, TB outbreaks preceding the petition involved non-compliance with those guidelines. Thus, in 1993 the director of compliance programmes issued a memorandum to all OSHA regional administrators that established enforcement policy and procedures for occupational exposure to TB. The memorandum clarified policy and procedures for inspections for the private sector as well as federal agencies. All requirements set forth in the policy are still in effect. In essence, the compliance directive requires compliance with CDC's updated *Guidelines for Preventing the Transmission of Tuberculosis in Health Care Settings* (CDC 1994b).

OSHA citations

Inspections are conducted for occupational exposure to TB only in response to employee complaints or as a part of industrial hygiene inspections in five types of facility identified by CDC as having a higher incidence of TB than the general population. One of the types, comprising health care settings, does not include private physicians' and dental offices. Citations have been issued under the General Duty Clause of the 1970 Occupational Health and Safety Act where employees had potential exposure to the exhaled air of a confirmed or suspected case of TB, or were exposed during a high hazard procedure performed on an individual who may have had TB. High-hazard procedures include aerosolized medication treatment, bronchoscopy, sputum induction, endotracheal intubation, suctioning procedures and autopsies. The presence of the hazard is the basis of a general duty clause citation. A violation of the General Duty clause requires proof that the employer failed to keep the workplace free of a hazard to which his or her employees were exposed; that the hazard was recognized; that it was causing or likely to cause death or serious physical harm; and that a feasible and useful method to abate the hazard existed.

Inspection guidance under the general duty clause

This section provides information potentially useful to employers who must be prepared for OSHA inspection. An employer's TB control programme (or generic infection control programme) can serve as evidence of knowledge and recognition of the General Duty Clause. Feasible and useful TB control methods include the following:

1. *A protocol for the early identification of individuals with active TB.* Anyone with symptoms of TB (including productive cough, coughing up blood, weight loss, loss of appetite, lethargy/weakness, night sweats, or fevers) should have diagnostic testing and close medical follow-up.
2. *Training and information.* Employers must ensure employee knowledge of the hazard of TB transmission, its signs and symptoms, medical surveillance, therapy, and site-

specific protocols including the purpose and proper use of exposure controls. In addition, the training requirement of the OSHA standard for the selection and use of respiratory protective equipment (29 CFR 1910.134) can be cited for absent or inadequate respirator training.

3. *Medical surveillance at no cost to the employee.* Employers should offer pre-placement evaluation, administration and interpretation of skin tests, as well as periodic employee testing. All employees should undergo a baseline skin test. Workers potentially exposed to TB owing to performance of high-risk procedures should have biannual tests. Employees who are definitely exposed to an individual with infectious TB should be managed according to CDC 1990 recommendations (CDC 1990).
4. *Post-exposure evaluation and follow-up.* Employees with a positive skin test or a history of positive skin tests who are exhibiting symptoms of TB must be evaluated and managed. Proper management includes work restrictions for infectious employees.
5. *Respiratory isolation rooms and special procedure areas.* Individuals with suspected or confirmed TB must be placed in respiratory isolation rooms in the hospital. Respiratory isolation rooms for suspected or confirmed TB patients and areas where high-hazard procedures are performed must be maintained under negative pressure and be exhausted either directly to the outside away from intake vents or through properly designed, installed and maintained HEPA filters. Despite not knowing the efficacy of available engineering controls, OSHA instructs compliance officers to encourage the employer to follow the CDC guidelines with respect to local exhaust and ultraviolet lights.

OSHA-proposed rule on tuberculosis

OSHA published *Occupational Exposure to Tuberculosis: Proposed Rule* in the US Federal Register in October 1997. At the time of this writing, rule-making hearings have been held. The proposed rule applies to occupational TB exposure occurring in hospitals, long term care facilities, correctional facilities and other facilities that house inmates or detainees, hospices, shelters for the homeless, drug abuse treatment facilities and laboratories that handle specimens that may contain MTB or perform activities that result in MTB aerosolization. Key elements of the rule include the following requirements: employee exposure determination, written exposure control plans, work practice and engineering controls, clinical and research laboratories, respiratory protection, medical surveillance, training, labelling, and record-keeping. Issues still being contended by stakeholders include requirements for two-step versus one-step skin testing, laboratory biosafety, respirator fit testing and monitoring pressure relationships in isolation rooms. Until the final rule is promulgated, OSHA can issue citations under existing OSHA rules, including the respiratory protection rule (29 CFR 1910.134), accident prevention signs and tags (29 CFR 1920.145), access to employee and medical records (29 CFR 1910.20), and the OSHA log and summary of occupational injuries and illnesses (29 CFR 1904).

IHA has provided OSHA with testimony at hearings and post-hearing comments supporting all of these requirements. Many industrial hygienists believe that the CDC 1994 guidelines (CDC 1994b) are more stringent than the rule proposed by OSHA, but CDC guidelines are solely guidelines and are not legally enforceable. At least one study

demonstrated that voluntary compliance with CDC guidelines is not widespread (Sutton *et al.* 1998). In this study, 28% (7/25) of isolation rooms were under positive pressure; air moved toward, rather than away, from likely worker locations in almost all (26/27) rooms tested; and none of the hospitals regularly checked the performance of engineering controls. Although 83% (20/24) of the isolation rooms tested had ≥ 6 ACH (nominal), supply air did not mix rapidly. Thus, the nominal ACH overestimated the effective ACH and the protection provided. Voluntary compliance is clearly lacking.

In conclusion, TB is a widespread, debilitating and sometimes fatal disease. Technology for exposure control exists and augments non-engineering public health interventions. While regulations in USA may be promulgated, voluntary compliance with control measures presently must be relied upon for prevention of disease transmission.

NOSOCOMIAL PNEUMONIA

General aspects

Nosocomial infections affect approximately two million patients annually in acute care facilities in the United States at a direct annual patient care cost of approximately \$3.5 billion⁷. ATS (1995) defines nosocomial pneumonia (NP) as pneumonia occurring <48 h after hospital admission and excluding any infection that is incubating at the time of admission. The US National Nosocomial Infection Surveillance System indicates that NP accounts for approximately 15% of all nosocomial infections and is the second most common type of nosocomial infection (CDC 1997). NP has been associated with high fatality rates. Among all cases, the crude mortality rate is 20–50% and the attributable mortality rate is 30–33%. In critical care units, mortality rates as high as 50–70% have been reported (Bassin and Niederman 1995). NP increases morbidity, can pro-

⁷ National Center for Infectious Diseases, CDC, Mission Statement

long hospitalization, and costs an estimated \$1.2 billion per year in direct costs in USA. Those affected are primarily infants, young children, and persons over 65 years of age. Risk factors for NP include persons with severe underlying disease of any type, cardiopulmonary disease, immunosuppression, depressed sensorium, and/or cardiopulmonary disease and persons who have had recent thoracoabdominal surgery. A prospective study of bacterial NP in a pediatric intensive care unit identified immunodeficiency, immunosuppression, and neuromuscular blockade as three independent risk factors for NP (Fayon *et al.* 1997). Respiratory tract infection was the second most common nosocomial infection (31% of all infections) in a study of 13 nursing homes in USA (Beck-Sague *et al.* 1994).

Etiological agents causing nosocomial pneumonia differ between different hospitals and patient populations. Differences in diagnostic methods also affect the characterization of NP. This section discusses various nosocomial bacterial pneumonias transmitted by inhalation. Pneumonia caused by moulds in the genus *Aspergillus* and the

influenza virus will also be briefly discussed. Respiratory syncytial virus, a common virus causing NP, is transmitted by direct inoculation of the conjunctivae or nasal mucosa by contaminated hands; it is not transmitted by inhalation and for this reason, it will not be discussed further.

Bacterial agents of nosocomial pneumonia

Gram-positive and Gram-negative bacteria

Bacterial NP is transmitted by three known routes: aspiration of bacteria colonizing the oropharynx or upper gastrointestinal tract, inhalation of aerosols and spread from a distant body site via the bloodstream. The first of these, aspiration, is the most common and the last is rare. When the agents of NP are transmitted by inhalation, they are in the form of aerosols generated primarily by devices used in respiratory therapy, e.g. contaminated nebulizers or anaesthesia-breathing equipment. Thus, physical conditions requiring prolonged use of mechanical ventilatory support with potential exposure to contaminated respiratory equipment are potential risk factors for nosocomially acquired pneumonia. The appropriate infection-control measure involves proper cleaning, sterilization or disinfection, and handling of all such devices. When the device is no longer needed, its use should be discontinued.

Bacterial NP's frequently result from infection with a mixture of bacteria rather than a single bacterial species. Gram-negative isolates from NP cases include *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Enterobacter* spp., *Klebsiella pneumoniae*, *Escherichia coli*, *Serratia marcescens* and *Proteus* spp. Gram-positive isolates, such as *Staphylococcus aureus* (especially methicillin-resistant strains) and *Streptococcus pneumoniae*, are somewhat less common.

The control of bacteria responsible for NP (for which strategies are presented below) is all the more important given the increasing frequency of antimicrobial resistance in general (Stamm *et al.* 1993; Jones *et al.* 1994; Maniatis *et al.* 1997) and specifically among bacteria causing NP (Bassin and Niederman 1995) and respiratory tract infections (Mandigers *et al.* 1994; Rikitomi *et al.* 1994). A study based in Hong Kong demonstrated statistically significant increases in penicillin resistance of *Strep. pneumoniae* and ampicillin resistance of *H. influenzae* (Ho *et al.* 1995). Reinforced hygienic procedures and segregation of types of patient has been shown to reduce the number of lower respiratory tract infections and crude mortality rate due to resistant staphylococci (Lingnau and Allerberger 1994).

Transmission by aerosols

As mentioned previously, transmission by aerosols usually involves contaminated devices used for respiratory therapy, e.g. contaminated nebulizers or anaesthesia-breathing equipment. This may, in part, add to the risk associated with prolonged use of mechanical ventilatory support. Appropriate infection control measures involve proper handling, cleaning, and sterilization or disinfection of all such devices. When the device is no longer needed, it should be discarded.

Theoretically, any bacteria contaminating respiratory equipment might be implicated in

nosocomial transmission of pneumonia. Nebulizer reservoirs have long been shown to be amplification sites of Gram-negative bacteria (Reinarz *et al.* 1965; Griebble *et al.* 1970). *Pseudo-monas* was the most common organism isolated from aerosol nebulizer fluid samples taken from six hospitals (Reinarz *et al.* 1965).

Infected large-volume nebulizers pose the greatest NP risk because, naturally, they are capable of generating a large amount of aerosol. Large-volume nebulizers are those with reservoirs of >500 ml and include those used in intermittent positive-pressure breathing machines and ultrasonic or spinning-disk room air humidifiers. Reservoirs may become contaminated from the hands of health care workers, use of unsterile humidification fluid, or inadequate sterilization or disinfection between uses. Large numbers of bacteria, including *Legionella* spp. (which will be discussed separately below) can multiply to reach concentrations high enough to pose a substantial risk to patients receiving inhalation therapy. Good infection control procedures, such as hand washing and sterilization or high-level disinfection, are effective in breaking the chain of transmission. The medical benefits of nebulizers attached to intermittent positive-pressure breathing machines clearly outweigh the costs, unlike room air humidifiers, the effectiveness of which has not been demonstrated and which are costly to maintain. Small-volume, hand-held medication nebulizers have also been associated with bacterial NP, including Legionnaires' disease. Use of sterile water for rinsing and filling the reservoir eliminates legionellae-contaminated potable water as a source.

CDC recommends that only sterile water be used for filling and rinsing nebulizers and other semi-critical respiratory care equipment. Large-volume room-air humidifiers that create aerosols should not be used unless they can be sterilized or subjected to high-level disinfection procedures daily and again should be filled only with sterile water.

Aspergillosis

Although *Aspergillus* spp. are fungi, not bacteria, aspergillosis is of interest as an aerosol-mediated nosocomial infection, the most important of which is pneumonia. The primary route of transmission is by inhalation of fungal spores. Once inhaled, dissemination of spores to other organs may occur via the bloodstream. The most frequently isolated species in patients with laboratory-confirmed aspergillosis are *A. fumigatus* and *A. flavus*. Especially in the immunocompromised, such as patients undergoing chemotherapy and/or organ transplantation, nosocomial pulmonary aspergillosis can kill up to 94% of infected individuals (Denning and Stevens 1990). Individuals with pre-existing lung disease are more susceptible to aspergillosis, including those with chronic obstructive lung disease, cystic fibrosis, or inactive TB.

Aside from predisposing health status, the presence of *Aspergillus* spp. in the air of hospital environments is the most important risk factor for aspergillosis. Increased airborne concentrations of aspergilli resulting from construction and/or renovation activities in hospitals are associated with nosocomial aspergillosis (Anderson *et al.* 1996). Presumably, aspergilli originate either from the non-sterile building materials used during original construction or renovation, or from disturbance of settled dust not associated with building materials. Other environmental sources include fireproofing material, water-damaged building materials, air-handling systems, and bird droppings (Mehta

1990).

Appropriate intervention strategies to prevent aspergillosis in high-risk patients during construction and renovation and HVAC maintenance are:

- High efficiency particulate air (HEPA at $\geq 99.7\%$ efficiency) filtration of supply air from the HVAC system or use in recirculation of air.
- Directional air flow
- Positive room pressure relative to the corridor
- “Air-tight” construction
- High rates of air changes, i.e., >15 ACH
- Laminar flow rooms.

Many of these precautions overlap with those used for TB control. Laminar flow rooms are those which provide HEPA-filtered air from a bank of filters along one wall. This configuration does not ensure truly laminar airflow, but is at least unidirectional across the patient’s bed and out through the opposite wall. Such rooms have been shown to be effective (Wald *et al.* 1997; Akiyama *et al.* 1994), but not completely so (Bretagne *et al.* 1997), in decreasing or eliminating the risk for nosocomial aspergillosis in high-risk patients (Wald *et al.* 1997). The disadvantages of laminar flow rooms are the high initial investment and the high cost of maintenance cost. Maintenance costs include the expense of filter replacement and of energy to provide essentially sterile room air at extremely high exchange rates (100–400 ACH). The effectiveness of alternative room air supply and equipment, including cross-flow ventilation, in-room HEPA filtration of recirculated room air and more realistic rates of room air changes (i.e. 10–15 ACH) is not known.

Influenza

While this chapter concerns bacterial respiratory disease, brief comments on the influenza virus are warranted because it is one of three viruses responsible for 70% of viral NPs. All people are susceptible to influenza pneumonia. Individuals at highest risk include the very young and old, and those who are immunosuppressed or have chronic underlying medical conditions. Influenza virus is transmitted from person to person by direct inhalation of droplet nuclei or small aerosols or direct deposition of droplets containing virus onto mucosal surfaces of the upper airways resulting from close contact with an infected person. There have been many outbreaks of nosocomial influenza and its epidemiology is fairly well understood (Blumenfeld *et al.* 1959; Bean *et al.* 1983).

The most effective control for influenza pneumonia is immunization, which usually takes place in the fall, prior to the ‘flu season (December–April). Immunization is indicated for several risk groups including health care workers, children aged 6 months to 18 years who are receiving long-term aspirin therapy, people over the age of 65, people in long-term care facilities, and those with chronic pulmonary or cardiovascular disorders, diabetes mellitus, renal dysfunction, haemoglobinopathies or immunosuppression. The availability of more convenient immunization routes, such as nasal inhalation, may greatly expand the indication for influenza immunization.

The antiviral agents amantadine and rimantadine are effective in treating ill individuals and those infected with influenza type A virus. They are also indicated as a prophylactic

adjunct to vaccination against the type A virus in the setting of an outbreak. Neither is effective against influenza type B virus. The Advisory Committee on Immunization Practices (US) recommends the use of these antiviral agents as prophylaxis in the following persons exposed to influenza:

1. High-risk persons after receiving late vaccination.
2. Persons for whom vaccination is contraindicated.
3. Individuals who might not produce a sufficiently high antibody titre after vaccination.
4. Health care providers who administer to patients at high risk for infection.
5. Any indicated individual when vaccine strains do not closely match epidemic viral strain(s).

Preventive measures other than vaccination and chemotherapeutic prophylaxis include surgical mask protection of the breathing zone and infection control procedures such as contact-isolation precautions, placement of infected persons in private rooms, and segregation of infected persons by ward. The effectiveness of these and other measures has not been determined.

Legionellosis

Clinical syndromes and epidemiology

Infection with *Legionella* spp. causes two distinct clinical syndromes with distinct epidemiological features. Legionnaires' disease, discovered after a 1976 outbreak among those attending an American Legion convention (Fraser *et al.* 1977), presents clinically as pneumonia often accompanied by multi-organ dysfunction. It may occur both as outbreak-related epidemics of pneumonia or NP. Pontiac fever is an acute febrile, self-limiting illness, without pneumonia. Legionnaires' disease has a low attack rate but a high fatality rate (15 to 39%), if untreated. The fatality rate is generally higher among the immunocompromised. Pontiac fever has a high attack rate but a low fatality rate. It is believed to be a reaction to inhaled bacterial antigen rather than true infection. Risk factors for Legionnaires' disease are:

- Male sex
- Age greater than 65 years
- Smoking
- Diabetes mellitus
- Chronic obstructive lung disease
- Congestive heart failure
- Chronic renal disease
- Malignancy
- Immunocompromised, including AIDS, organ transplant recipients, and use of systemic corticosteroids.

Initially, both diseases present with fever, chills, anorexia, malaise, myalgia and headache. The incubation period is generally 2–10 days for Legionnaires' disease and 48–72 h for Pontiac fever. Diarrhoea, abdominal pain, and a non-productive cough are

common. In Legionnaires' disease, chest x-ray shows patchy or focal areas of consolidation that may progress to bilateral involvement and respiratory failure. Diagnosis depends on isolation of legionellae, direct immunofluorescent stain in involved tissue or respiratory secretions, by radioimmunoassay detection of antigens of *L. pneumophila* serogroup 1 in urine, or by a four-fold or greater rise in levels of convalescent serum antibodies. So-called finger-printing techniques of multilocus enzyme assay (MEA), ribotyping, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), and pulsed-field-gel electrophoresis (PFGE) can be used to correlate the specific *Legionella* strain clinically isolated to that isolated from a suspected environmental source. Antibodies to serogroup-specific antigens are useful for diagnostic or epidemiological studies, but they do not appear to confer immunity.

Although more than 60 serogroups within at least 39 species of legionellae have been identified, 85 to 90% of all cases of legionellosis have been caused by a single species, *L. pneumophila*. Most (65–70%) of these involve one of 15 serogroups, namely *L. pneumophila* serogroup 1, *L. micdadei*, *L. bozemanii*, *L. longbeachae* and *L. dumofii* have also been isolated, primarily from immunocompromised patients with pneumonia.

The epidemiology of NP due to legionellae is less well understood. Because the incubation period for Legionnaires' disease is generally 2–10 days, pneumonia attributed to *Legionella* spp. occurring more than 10 days after hospital admission is considered to be nosocomial. A single case of laboratory-confirmed definite Legionnaires' disease or two or more cases of laboratory-confirmed possible nosocomial Legionnaires' disease occurring within 6 months of each other should trigger an investigation. CDC recommends (1) reporting the disease to local health departments, (2) taking steps to protect high-risk patients, e.g. eliminating showers and providing sterile water for drinking and baths, and (3) conducting an epidemiological and environmental investigation (see section below).

Legionnaires' disease is under-reported. Approximately 10,000 to 100,000 of the 2.4 million cases of pneumonia occurring annually in USA are Legionnaires' disease. However, only 1,000–3,000 of these are actually reported. Buffered charcoal yeast extract agar, which contains the necessary L-cysteine cofactor, is required for successful cultivation of *Legionella* spp. Many laboratories may not have access to such media. Also, physicians often may not perform appropriate diagnostic tests in patients with pneumonia (May *et al.* 1994).

Transmission of Legionella

Legionellosis is transmitted primarily by inhalation. Some investigators believe that the disease may also be acquired by drinking *Legionella*-contaminated water, especially if legionellae are aspirated into the lungs. Aspiration into the lungs following on from colonization of the oropharynx (Yu *et al.* 1982) may be possible, but has not been proved conclusively.

Recently it has been proposed that airborne transmission may involve inhalation of vesicles containing legionellae released from amoebae (Berk *et al.* 1998). These vesicles measure 2.1–6.4 µm in diameter, a size range that can penetrate and deposit in the lungs.

Each vesicle may contain several hundred legionellae. Interestingly, *L. pneumophila* is more invasive after growth with amoebae (Cirillo *et al.* 1994). This theory is consistent with the epidemiology and aerobiology of *Legionella* infections; cooling towers as far as 1.2 km away have been documented as sources. Moreover, bacteria-containing vesicles may survive air transport better than bacterial aerosols, which are prone to desiccation.

Environmental biology of legionellae

Legionellae are poorly staining, Gram-negative bacteria found primarily in aquatic environments. Water environments epidemiologically linked to Legionnaires' disease include domestic water systems, cooling towers and evaporative condensers, respiratory care equipment, showers, faucets, whirlpool baths, humidifiers, decorative fountains, an air-perfused footbath (Den Boer *et al.* 1998) and a grocery store mist machine. It has also been isolated from hot and cold-water taps and showers, hot tubs, and from creeks (streams) and ponds and surrounding soil.

Many natural environments are low in nutrients or have none available to support bacterial growth (Morita 1988). Bacteria that do not form spores may show specific starvation responses to survive (Lappin-Scott and Costerton 1990). A fraction of the bacterial population can enter and remain in a viable, starved state until favourable growth conditions are restored (Lappin-Scott and Costerton 1990). There is some evidence that legionellae can remain viable (and possibly infectious), but not culturable for up to 16 months in the absence of nutrients. For a fresh culture of *L. pneumophila* in tap water for 14 days at 4°C and 37° C, the LD₅₀ values decreased to 0.5 and 0.25 CFU, respectively. The investigators concluded that these results were due to the cells going into a viable but non-culturable (VBNC) state (Hussong *et al.* 1987). This phenomenon has been observed for *Vibrio cholerae* (Xu *et al.* 1982) and *Campylobacter jejuni*, both bacteria found in faeces-contaminated water.

There are several other problems with using culture as a method for detecting legionellae bacteria. As with other bacteria, legionellae can form biofilms. Not only do legionellae colonize various metal and non-metal surfaces but they are found preferentially on surfaces, i.e. adherent rather than free-living (Lee and West 1991; Wright *et al.* 1991). Assessing only the free-living populations, such as is done with typical swab sampling, would therefore underestimate total populations. Moreover, although standard methods of isolation have been developed, legionellae are difficult to culture on synthetic media and require at least three to seven days at 37° C. Not all laboratories may have access to appropriate growth media.

Many microorganisms can either support or inhibit the growth of *Legionella*. Microorganisms reported to support growth include the alga *Fischeriella* (Bohach and Snyder 1983a,b), green algae (Pope *et al.* 1982), cyanobacteria (Tison *et al.* 1980), amoebae (Rowbotham 1980; Tyndall and Domingue 1982; Wadowsky *et al.* 1988), ciliated protozoa (Anand *et al.* 1983; Fields *et al.* 1984; Fields *et al.* 1986; Wadowsky *et al.* 1988; Fields *et al.* 1989) and non-*Legionella* heterotrophic bacteria (Stout *et al.* 1985). Growth of legionellae on synthetic media is inhibited by numerous heterotrophic bacteria commonly present in the same environmental sample (Wadowsky and Yee, 1983, 1985; Stout *et al.* 1986). Other investigators have documented the inhibition of legionellae by

bacterial strains isolated from water samples (Makin 1986) and by bacterial strains isolated from heterotrophic plate counts of drinking water (Toze *et al.* 1990). Inhibition by other bacteria is important in that a water sample containing both legionellae and bacterial strains which inhibit their growth will show a reduced plate count for the legionellae. While heat and acid pre-treatment of environmental samples is recommended (APHA 1992) to kill undesirable non-legionellae bacteria, such treatment can potentially affect survival of legionellae and reduce plate counts.

Intracellular growth of legionellae cells has also been demonstrated in ciliated protozoa and amoebae. Species of ciliated protozoa in the genera *Tetrahymena* (Nahapetian *et al.* 1991) and *Cyclidium* have been shown to support intracellular multiplication of *L. pneumophila*. Legionellae can grow in trophozoites of amoebae and become incorporated into their cysts. Species of *Acanthamoeba*, *Hartmanella*, *Valkampfia* and *Naegleria* can all be infected and, depending on conditions, may coexist with legionellae for prolonged or indefinite periods (Rowbotham 1980, 1986; Tyndall *et al.* 1982; Anand *et al.* 1983; Wadowsky *et al.* 1988). Tens or hundreds of legionellae cells may exist within the vesicles of amoebae. At low temperature (20° C), only limited growth of legionellae occurs, but at 35° C they rapidly multiply intracellularly (Anand *et al.* 1983; Rowbotham 1984). The protozoa feed on legionellae, but cannot digest virulent strains. Instead of being metabolized, virulent strains multiply within protozoa just as they do in human phagocytic cells (Fields *et al.* 1990; Horwitz *et al.* 1981). Multiplication within certain protozoa has been linked to pathogenicity (Fields *et al.* 1986; Rowbotham 1984).

Sodium hypochlorite is often used as a biocidal control strategy (see section below). However, legionellae may remain viable, i.e. metabolically active by the INT method (Zimmerman *et al.* 1978), but non-culturable at hypochlorite concentrations up to 100 ppm (Bej *et al.* 1991). Amoebae and protozoa are also more resistant to hypochlorite than free-living legionellae, suggesting that water disinfected with hypochlorite can be re-inoculated when amoebae and protozoa release legionellae within them.

In summary, negative culture or low CFU counts, particularly of cooling tower water samples, may be obtained for any of the following reasons:

1. Legionellae are difficult to grow and require an appropriate L-cysteine-containing medium.
2. Legionellae can remain viable, but nonculturable, and may therefore be more resistant to standard biocides.
3. Other microorganisms present in environmental samples may inhibit the growth of legionellae on agar plates. Pretreatment of such samples may also kill legionellae.
4. Legionellae living within amoebae cysts and protozoa are protected from disinfection and are then free to re-colonize cooling tower water and surfaces. This provides a mechanism whereby viable legionellae can be released into cooling tower waters unpredictably.
5. Free-living legionellae are more effectively killed by biocidal treatment than adherent populations, which remain to colonize surfaces.

All of these findings suggest that hypochlorite may have reduced biocidal effectiveness as a biocide in cooling towers and water distribution systems, and culture analysis may be

an insensitive indicator of adequate maintenance and contamination.

Legionella control strategies

There are several strategies useful in the control of *Legionella* infections. Common techniques used to control the growth of legionellae are cooling tower maintenance, increased heat and heat shock treatments, and the use of oxidizing and non-oxidizing chemical biocides. ASHRAE has emphasized the importance of good preventive maintenance of cooling towers and other evaporative condensers. Appropriate cooling tower maintenance procedures include water treatment to control biological activity, prevention of scaling, periodic cleaning, maintenance of a written record of procedures performed, and periodic inspections (ASHRAE 1988; Barbaree 1991). ASHRAE has recently updated its recommendations to include having effective drift eliminators and maintaining mechanical components in working order (ASHRAE 1988). While maintenance does not control legionellae growth with certainty (Swann *et al.* 1982), lack of adequate preventive maintenance leads to inefficient system operation and conditions optimal for transmission of legionellosis. ASHRAE maintains that more emphasis should be placed on clean equipment in excellent repair than on periodic testing with concurrent system neglect (ASHRAE 1988).

Although legionellae are capable of growth and survival under the range of physical and chemical conditions commonly encountered in most man-made water systems, they prefer warm water. Cooling tower water counts of legionellae have been found to correlate positively with water temperature, pH, and protozoan counts (Yamamoto *et al.* 1992). However, legionellae are killed by hot water. Heat shock treatment and maintenance of water distribution systems above a minimum temperature have both been used as techniques to control legionellae growth in potable water due to their well-known dependence on temperature for optimal growth. However, the effectiveness of heat shock was questioned when investigators demonstrated that the strains isolated after heat shock were not the same as those detected in the original water (Colbourne *et al.* 1988). These data spawned the theory that *L. pneumophila* may possess heat shock genes analogous to those in *E. coli* and that heat shock can alter monoclonal expression and virulence. The effectiveness of increasing the water temperature to 50–70° C has been challenged since *L. pneumophila* has been isolated in two domestic hot water systems that delivered water at temperatures of over 50° C, and in one instance, at 70° C (Cameron *et al.* 1991). One possible explanation for this finding is that the temperature is not homogeneous throughout the system (Colbourne *et al.* 1984).

Cooling Towers

Cooling towers play an important role as environmental sources of Legionnaires' disease (Garbe *et al.* 1985). Cooling towers are mass heat-transfer devices; their basic function is that of evaporative cooling and exchange of sensible heat. Water is lost via discharge to the atmosphere as hot moist water vapor and aerosol mist, resulting in a cooling effect of the remaining water. For each 10° F (5.5° C) of cooling range⁸, approximately 1.2% of the design flow is lost (Burger 1982). "Drift" refers to the whole water droplets released to the atmosphere and includes water, minerals, and presumably microorganisms.

Cooling towers are of two basic types, direct and indirect contact. In the former, air

and water to be cooled are in direct contact; in the latter, they are kept separate. Only direct contact between the ambient air and legionellae-containing water is of interest as an environmental exposure source for community-acquired legionellosis and for occupational exposure involving cooling tower maintenance.

Both inspection and water treatment are critical aspects of an effective cooling tower maintenance programme. Water treatment is generally aimed at controlling deposition of scale and biomass. Scale is the deposition of inorganic compounds in the tower. Biofouling results from the growth of algae and the production of slime by bacteria and fungi. Both scale and slime interfere with the proper functioning of the cooling tower by reducing heat transfer. A water treatment programme may be effective in controlling scaling and biofouling, but may not control legionellae. Conversely, a water treatment programme ineffective against scaling and biofouling will definitely be ineffective for control of legionellae.

Although attempts should be made to control the level of all aquatic bacteria in cooling towers (including legionellae), it is not feasible to attempt to eradicate them completely. Because cooling towers provide an environmental niche for legionellae, it is inevitable that cooling towers, by design and function, will release legionellae to the ambient air. The systematic use of biocides is generally coordinated with all other maintenance activities such as mechanical cleaning of the cooling tower. Continuous feed of biocides is preferred to slug (intermittent) treatment as it is more likely to provide a consistent biocide concentration. *Legionella* control strategies should also include control of cyanobacteria (blue-green algae), which both enhance growth of legionellae in aquatic reservoirs and improve their survival in aerosols.

Cooling tower maintenance

Disinfection is important in cooling tower maintenance. The goal of disinfection is to control growth of bacteria, algae, and protozoa that may contribute to the growth of legionellae. Of the oxidizing biocides, chlorine is the most effective in controlling legionellae, but only if the appropriate concentration and pH are maintained (Barbaree 1991). However, corrosion and pH maintenance make use of chlorine difficult. Legionellae have been shown to be inhibited or killed by a wide range of oxidizing and non-oxidizing biocides (Lee *et al.* 1991), even at low concentrations (Wang *et al.* 1979). Free-chlorine at 0.4 mg L⁻¹ kills *L. pneumophila* (Domingue *et al.* 1988). Chlorination of cooling towers at 15 mg L⁻¹ for routine cleaning and 50 mg/L for decontamination has been recommended (Kilvington and Price 1990). The Wisconsin Division of Health has recommended a free-residual chlorine concentration of 10 ppm for maintenance and 50 ppm in an emergency protocol (WDH 1987). However, many biocides that showed promising results in the laboratory have been less efficacious *in situ* (England *et al.* 1982; Fliermans and Harvey 1984). Thus, it is not surprising that ASHRAE has recommended that biocidal efficacy be determined in the field under actual working conditions rather than relying entirely on laboratory testing under controlled conditions (ASHRAE 1998).

Three mechanisms have been proposed as underlying the decreased efficacy of chemical biocidal treatment: (1) decreased biocide susceptibility of legionellae within protozoa, (2) decreased biocide susceptibility of legionellae within biofilms, and (3) a

biocidal stimulatory

⁸ Cooling range=water temperature entering tower—water temperature leaving the tower.

effect on amoebae. Protozoa that support intracellular growth of legionellae are resistant to normal levels of biocide. *L. pneumophila* has been recovered from *Acanthamoeba polyphaga* cysts after exposure for 18 h at 25° C to 50 mg free chlorine L⁻¹ in Page's amoebal saline (Kilvington and Price 1990). Trophozoites of *Acanthamoeba* were sensitive to 1.25 mg free chlorine L⁻¹ survive concentrations of up to 50 mg L⁻¹ (Kilvington and Price 1990). Encysted legionellae may therefore be able to survive cooling tower disinfection and re-colonize the system. While biocides are relatively effective against free-living *L. pneumophila* over a short period of time (minimum of 9–12 h), substantially longer periods (at least 48 h) were required to reduce the number of culturable bacteria to below detectable levels in the population that had colonized cooling tower construction materials (Wright *et al.* 1991). Cooling tower isolates of *Acanthamoeba hatchetti* and a species of *Cochliopodium* resembling *C. minus* may both be more resistant to biocides than laboratory grown species and actually be stimulated by exposure to recommended concentrations of biocides (Srikanth and Berk 1993). These results suggested that cooling tower amoebae adapt to biocidal treatment and that the use of biocides may actually enhance populations of amoeba host organisms for pathogenic legionellae. Finally, legionellae within vesicles of *Acanthamoebae* remained viable after amoebicidal biocide exposure (Berk *et al.* 1998). Assessing only the number of legionellae with source waters where amoebae may be present may greatly underestimate the number of legionellae within vesicles.

Chemical treatment involving the addition of ionic copper and silver to free chlorine has been used to enhance inactivation of *L. pneumophila* (Landeem *et al.* 1989a,b). Water disinfection systems utilizing electrolytically generated copper and silver ions and low levels of free chlorine were evaluated at room and elevated temperature for their ability to kill *L. pneumophila*. Significantly increased inactivation rates were observed at a 0.4 mg free L⁻¹ chlorine when 400 mg copper L⁻¹ and 40 mg silver L⁻¹ were added, regardless of temperature (Landeem *et al.* 1989a,b). Copper and silver ions are believed to interfere with enzymes involved in cellular respiration and bind specifically to DNA (Liebe and Stuehr 1972a, b; Shinohara *et al.* 1983).

Domestic water system design

Domestic water systems have also been sources of human infections with legionellae. General control strategies for domestic water systems should prevent stagnation of water, use materials that do not provide nutrients for growth of legionellae, and address conditions that allow for buildup of scale and sediment. Examples of specific strategies for control of *Legionella* in domestic water systems are to:

- Remove dead-legs that harbour stagnant water
- Use neoprene or other synthetic material gaskets rather than natural rubber
- Use copper rather than plastic piping, which promotes *Legionella* colonization
- Avoid faucet aerators

- Replace heavily-scaled faucets and shower heads
- Ream all pipe end burrs.

As in cooling towers, heat shock is an appropriate general control strategy for domestic water systems. However, hot water tanks in hot water generators may have cool zones since only about 65% of the water is maintained at the set temperature and because hot water is not constantly demanded from the system. Efforts to minimize such cool zones include relocating the supply and return taps or installing a top-to-bottom circulating pump. Sediment and warm temperatures at the bottom of a tank beneath the heating element are ideal conditions for legionellae growth.

High-temperature semi-instantaneous steam heaters, available in newer hot water tanks, are advantageous as they are not conducive to growth of legionellae. If there is insufficient steam capacity, consideration should be given to a tank that will keep a high percentage of water at the set temperature, as well as one which can produce water at 70°C for heat-shock disinfection. A vertical tank should be considered rather than a horizontal one, and it should be set so that it can be drained completely.

Domestic water system operation and maintenance

Water temperatures between 55 and 60°C in tanks and 50°C at all taps will control legionellae in the absence of scale and biofilm. Domestic cold water should be maintained below 25°C. Because the complete absence of biofilm is unlikely, temperature control only minimizes but does not eradicate colonization or amplification of legionellae.

The temperature of water drawn from several sources, including the drainage valve, water returning to the tank, and water taken from the drain of cold water storage tanks, should be monitored on a regular basis. The hot water tap should read 50°C within 1 min of full flow and the cold water tap should read $\leq 20^\circ\text{C}$ after running for 2 min, even at the most distal locations. Points farthest from the point of distribution should be routinely checked and other locations should be included.

Recommended water temperatures for controlling the growth of legionellae in tanks and at taps varies by geographic locations. Hospitals in the United Kingdom are required to keep water at or above 50°C. In contrast, regulations in USA are based on a maximum temperature, usually about 60°C, that will not result in scalding. The temperature of cold-water storage and distribution should be $\leq 20^\circ\text{C}$. The appropriate choice and use of thermostatic mixing valves and anti-scald valves is essential in achieving optimum temperatures.

Environmental sampling

There is considerable debate in USA as to whether domestic water systems and cooling tower water should be routinely sampled. Even if performed, sampling should not substitute for either preventive measures or patient surveillance. Preventive maintenance should always be performed.

Routine sampling for legionellae may not be cost-effective because the results cannot always be interpreted clearly enough to guide action (Freije and Barbaree 1996). Taking corrective action based on “true positive” sampling result may save lives, avoids lawsuits and therefore ultimately saves money. However, false-negative results provide a

misplaced sense of security and may result in preventable cases of Legionnaires' disease. In addition, falsely positive sampling results may reflect temporal variability rather than constitute a real hazard (Freije and Barbaree 1996), triggering a substantial but ultimately wasteful investigation and remediation. On the other hand, the routine collection of data, even if subject to some error, is usually viewed favourably in times of litigation.

Before environmental sampling is initiated, it is critical to define its objectives and to understand its limitations. The CDC has stated that "the relationship between the results of water cultures and the risk of legionellosis remains undefined" and that "[sampling] data are insufficient to assign a level of risk of disease". ASHRAE's current position is consistent with that of CDC (ASHRAE 1998). However, even in the absence of a fully defined risk relationship, routine sampling may accomplish particular objectives. For example, *in situ* efficacy of biocides can only be determined by taking routine samples. In doing so, the sampling strategy should be planned so that (1) a sufficient number of sites are sampled to assess an entire system, e.g. a domestic hot water system, (2) data from one sample location can be compared with samples taken at the same location over time, and (3) the data are examined as a whole, e.g. the percentage of samples which are "positive". Objective data can be useful in determining whether operations at the facility are achieving its maintenance responsibilities.

Bulk water and swab samples are most useful. Recommended sampling locations are given in Table 5. Air sampling, discussed below, is less useful. Bulk water samples best address free-living populations of legionellae, which probably comprise the major inhalational exposure. Swab samples best address sessile populations residing in biofilms, such as at a faucet. While both free-living and sessile populations may contribute to potential exposure, it is a water sample that is probably more closely related to aerosol exposure. Swab samples generally yield higher counts than bulk water samples (Ta *et al.* 1995), providing an example of the potential for unpredictable release of legionellae from amoebae into the water.

Water sample bottles should be polypropylene, sterile, of 250–1000 ml capacity, leakproof and autoclavable for reuse. Aseptic technique should always be used. The required sample volume varies by laboratory according to the desired limit of detection. Sterile swabs should be within the expiration date. Because differences in temperature greatly affect growth, the water temperature should be consistently measured, either before or after a sample is taken. The thermometer used should be disinfected and dried before use. It may be necessary to neutralize residual chlorine by having the laboratory add sodium thiosulphate to the sample bottle. One approach is to determine if residual chlorine is present before adding sodium thiosulphate. While, in theory, residual chlorine can adversely affect the concentration of culturable legionellae, one commercial laboratory has not found that residual chlorine decreases the concentration of several isolates (verbal communication, George Morris, PhD., PathCon Laboratories, Norcross, GA, US). Both ASTM (1996) and APHA (1992) describe rinsing sample bottles with the subject water prior to taking the actual sample. Rinsing is unnecessary if the sample bottles are truly clean and sterile; it should not be done if sterile sample bottles contain sodium thiosulphate. Samples should be shipped at ambient temperatures in an insulated container on the date of collection by overnight courier to the laboratory; analysis should

begin within 24 h of the time of collection. If analysis cannot begin within this time limit, the samples should be maintained between 3 and 30° C (ASTM 1996). All samples should be identified with unique identification numbers and handled following standard proper chain-of-custody procedures.

Samples of sludge and sediment are taken to determine the presence of culturable legionellae in any biofilm present. If the sludge and sediment are taken from areas in the absence of water, the data are not particularly meaningful. Such samples are also difficult to process and aliquots taken for culture may not be representative of the entire sample.

Culturable air sampling has been used in some outbreak investigations. A combination of an epidemiological study, air sampling for culturable legionellae and monoclonal antibody subtyping demonstrated that the source of one Legionnaires disease outbreak was aerosolized water from an evaporative condenser (Breiman *et al.* 1990). Positive air sampling results also corroborated identical monoclonal antibody subtype patterns found in patient and environmental samples in another outbreak investigation (Zingesser *et al.* 1990). While culturable air sampling can be a useful adjunct to epidemiological studies, this author does not recommend it because of the potential for false negatives resulting from environmental factors on survival, loss of viability during sampling stress and the fact that not all *Legionella* aerosol generators are continuous point sources (e.g. grocery mist generators).

Non-cultural analysis of bulk samples is carried out by direct fluorescent antibody (DFA) tests or PCR. As discussed earlier, these techniques have their advantages and disadvantages. The standard or primary method of detection and quantitation of legionellae is culture. Only competent laboratories with experience in isolating legionellae from environmental samples using APHA methods (APHA 1992) should be employed in culturing legionellae.

Table 5. Recommended sampling locations for *Legionella* in water systems.

Water system	Sampling location	Comments
Water storage tank and hot water heater	Water at bottom drain and at outlet pipes	
Water faucets and shower heads	Water from hot and cold faucets nearest, intermediate and most distant from water heaters	Collect the first water out and after a 2-min flush (or longer if necessary for temperature to stabilize)
Shower heads	Swab faucets and shower heads	
Water-cooled heat transfer equipment	Water from the make-up supply, locations distant from the make-up water outlet and where water enters sprayers or misters	

Humidifiers, spas, Water from tanks and reservoirs and
decorative their supply waters
fountains

Interpretation of data

This author is unaware of the existence of published standards or regulatory guidelines for interpretation of analytical data (e.g. the implication of 1 CFU ml⁻¹ of water). Various commercial laboratories have developed such guidelines (see Table 6).

Table 6. PathCon Laboratories criteria for interpretation of samples taken from areas occupied by low-risk individuals (Morris *et al.* 1994).

Legionellae ml⁻¹	Cooling tower	Domestic water
Detectable, but <1	A	B
1–9	B	C
10–99	C	D
100–999	D	E
1000+	E	E

KEY: A—Low level of concern; B—Legionellae count represents little concern, but indicates system is a potential amplifier; C—Low, but increased level of concern. Disinfection should be considered; D—Uncommonly high levels. Approaching levels that may cause outbreaks. Disinfect systems; E—Very high levels. Outbreak potential. Disinfect systems immediately.

Outbreak investigation

Investigation of a legionellosis outbreak starts with the identification of two or more infected individuals. The goal of the investigation is to identify the likely environmental source of those cases. Culture and DFA have been used to determine the presence of *Legionella* in environmental sources and specimens from patients. An enzyme-linked immunosorbent assay (ELISA) has also been used to assess the antibody titre in acute and convalescent clinical serum samples. Co-cultivation with amoebae has been proposed as particularly useful when indirect fluorescence antibody (IFA) results are positive and culture results are negative for environmental water samples (Fallon and Rowbotham 1990). An epidemiological link between the suspected source and case can be strengthened when the environmental and clinical isolates are of identical monoclonal antibody subtypes. The steps outlined below are recommended by CDC for carrying out an environmental investigation of nosocomial Legionnaires' disease.

1. Collect environmental samples.
2. Identify the source of contamination based on the epidemiological investigation and environmental sampling data.

3. Take action. Consider disinfecting the domestic water system, cooling towers, or other suspected sources, by heat shock or hyperchlorination treatment. Consider soliciting outside expertise from the local public health department or consultants.
4. Collect and re-test environmental samples one week after systems are disinfected.
5. Continue to take corrective action if the problem persists.
6. Continue to take environmental samples for several months.

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Chapter 3.5

RESPIRATORY TRACT INFECTIONS CAUSED BY INDOOR FUNGI

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RANGE OF FUNGI CAUSING MAMMALIAN RESPIRATORY INFECTIONS

Fungi causing respiratory tract infections in humans and other mammals can be broken down into three categories:

1. a small number of species that are capable of causing environmentally acquired, but not usually contagious, respiratory illness in the otherwise healthy, immunocompetent host: *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Coccidioides immitis*, *Cryptococcus neoformans* and, marginally, *Sporothrix schenckii* and *Penicillium marneffei*-
2. a single genus, *Pneumocystis*, capable of causing contagious respiratory illness, usually in the immunocompromised host; and
3. an ever-enlarging group of fungi unspecialized for vertebrate pathogenesis which can cause environmentally acquired respiratory disease only in the strongly immunocompromised patient, or rarely in the patient who is both debilitated and heavily exposed, or very rarely in the apparently otherwise normal patient.

Members of the first category are ordinarily referred to as “virulent fungal pathogens.” All except *C. neoformans* convert from a mould-like, filamentous state on environmental substrata, as well as on ordinary growth media, to a particulate state in the infected host and on special media incubated at 37°C *in vitro*. Species undergoing this conversion are called “dimorphic fungi,” or, more formally, “thermally dimorphic fungi”. Particulate states formed in infected hosts are diverse, and include budding yeasts (*B. dermatitidis*, *H. capsulatum*, *P. brasiliensis*, *S. schenckii*), spherules reproducing by means of internal cleavage into endoconidia (*C. immitis*) and fission yeasts or self-replicating arthroconidia (*P. marneffei*). *C. neoformans*, the sole member of the virulent fungal respiratory pathogens which is not ordinarily termed “dimorphic,” grows as a budding yeast both in the host and on ordinary laboratory media, and may also commonly occur in this state in nature as well. The species, however, is complex, and one variety, *C. neoformans* var. *gattii*, grows endophytically in certain *Eucalyptus* tree species in a dikaryotized, filamentous condition and reproduces with basidiomycetous teleomorphic structures as *Filobasidiella neoformans* var. *bacillospora* (Ellis and Pfeiffer 1990). In human

infections and in monosporous culture, this variety grows as a budding yeast very similar to that formed by the type variety. There has been speculation that the type variety may also have a natural habitat where it occurs in its mated, filamentous condition. A proportion of isolates within both varieties can be successfully mated and taken through the life cycle *in vitro*. Phylogenetically, *C. neoformans* is the only member of phylum Basidiomycota recognized as a virulent pathogen. Of the remaining virulent fungi mentioned above, all have affinities to the order Onygenales of the phylum Ascomycota except *P. marneffeii* and *S. schenckii*, which are related to the orders Eurotiales and Ophiostomatales of the same phylum, respectively.

Members of the second category of fungal respiratory pathogens, the *Pneumocystis* species, have traditionally been treated as protozoans. In confirmation of some earlier suggestions arising from ultrastructural and biochemical studies, they were recently decisively determined as fungi by means of DNA sequencing (Edman *et al.* 1988). These unique organisms, which appear to be remotely related phylogenetically to the Schizosaccharomycetales (fission yeasts) (Berbee and Taylor 1992) and to other phylogenetically basal fungi, reproduce *in vivo* by means of cysts, visually suggestive of yeast asci, which produce eight intracystic bodies. Whether meiosis occurs in these cysts or not is not definitively established: nuclear-associated organelles followed through the life cycle present a picture consistent with sexual conjugation and meiosis (Itatani 1996), whereas the most recent quantitative studies of 4',6 diamidino-2-phenylindole fluorescent-stained nuclei indicated an absence of apparent diploid DNA content in nuclei from all life cycle stages (Wyder *et al.* 1998).

Fungi in the third category of fungal respiratory pathogens are generally termed "opportunistic pathogens." The adjectival phrase "of the immunocompromised host" can accurately be added onto that basic term in the great majority of cases. A large and indefinitely expanding diversity of filamentous fungi and yeasts has been implicated in opportunistic respiratory pathogenesis (Table 1). The majority of respiratory opportunists are mould fungi with phylogenetic affinities to the phylum Ascomycota. For example, the two groups containing the most virulent of the filamentous fungal respiratory opportunists are the genus *Aspergillus*, a clade (group of organisms considered to have evolved from a common ancestor) of hyphomycetes with affinities to the ascomycete order Eurotiales, and a clade containing the ascomycete *Pseudallescheria boydii* and the related hyphomycete *Scedosporium prolificans*, both with affinities to the order Microascales. The great majority of *Aspergillus* infections are attributable to four species: *Aspergillus fumigatus*, *A. flavus*, *A. terreus* and *A. nidulans*.

Also important as opportunistic pathogens is a group of yeasts normally inhabiting the skin or the mucosal (oral, vaginal, lower intestinal) epithelia of humans and animals as commensal mycota. These yeasts have affinities in the ascomycetous class Endomycetes; *Candida albicans* is the most well known species. These yeasts will not be mentioned further in this review, as their connection to respiratory infections is very limited, mostly to a context of disseminated infection of the severely immuno-compromised patient, and is not significantly connected with inhalation of inoculum from environmental sources, indoors or elsewhere. Some members of the order Mucorales of the phylum Zygomycota are also agents of opportunistic respiratory disease of the immunocompromised host: the

genera most frequently implicated, in temperate parts of the world, are *Rhizopus* and *Absidia*. Finally, a few members of the phylum Basidiomycota rarely cause opportunistic respiratory infection. Yeasts in the anamorph genus *Trichosporon*, phylogenetically related to the order Filobasidiales along with the virulent *C. neoformans*, are perhaps the most frequently seen from significant cases. Occasionally, significant cases may be caused by a few members of the mushroom-forming order Agaricales, namely *Schizophyllum commune* and members of the genus *Coprinus* (which may be recorded under the name of a corresponding anamorph genus, *Hormographiella*).

ECOLOGY AND EPIDEMIOLOGY OF FUNGAL RESPIRATORY PATHOGENS IN RELATION TO HOMES AND INDOOR WORKPLACES

Virulent pathogens

Virulent fungal pathogens, fortunately, are strongly restricted to specific habitats and geographic areas. Each organism is distinctive in these respects and must be considered separately.

Table 1. Fungi other than endomycetous yeasts which have been confirmed as causing respiratory tract (pulmonary, bronchial) infection, sinusitis or disseminated infections potentially invading the lung in humans.

Species	Virulence category	Frequency of valid respiratory cases or sinusitis
<i>Pneumocystis carinii</i>	opportunistic	common
ZYGOMYCOTA		
<i>Absidia corymbifera</i>	opportunistic	uncommon but regular
<i>Apophysomyces elegans</i>	opportunistic	rare
<i>Conidiobolus incongruus</i>	opportunistic	rare
<i>Cunninghamella bertholletiae</i>	opportunistic	uncommon but regular
<i>Delacroixia coronata</i>	opportunistic	rare
<i>Rhizomucor pusillus</i>	opportunistic	uncommon
<i>Rhizopus microsporus</i> var. <i>rhizopodi-formis</i>	common	opportunistic
<i>Rhizopus oryzae</i>	opportunistic	common
<i>Saksenaia vasiformis</i>	?virulent	rare (subcutaneous primary infection)

ASCOMYCOTA (and
anamorphs)

<i>Acremonium kiliense</i>	opportunistic	rare
<i>Acremoniurn strictum</i>	opportunistic	rare
<i>Alternaria alternata sinusitis)</i>	opportunistic	common (especially
<i>Ascotricha chartarum</i>	opportunistic	rare
<i>Aspergillus flavus</i>	opportunistic	common
<i>Aspergillus fumigatus</i>	opportunistic	common
<i>Aspergillus nidulans</i>	opportunistic	uncmmon
<i>Aspergillus niger</i>	opportunistic	uncommon but regular
<i>Aspergillus ochraceus</i>	opportunistic	rare
<i>Aspergillus terreus</i>	opportunistic	uncommon but regular
<i>Aspergillus tetrazonus</i>	opportunistic	rare
<i>Aspergillus ustus</i>	opportunistic	rare
<i>Aureobasidium pullulans</i>	opportunistic	rare
<i>Blastomyces dermatidis</i>	virulent	endemic, common ^a
<i>Chaetomium globosum</i>	opportunistic	rare
<i>Cladophialophora devriesii</i>	opportunistic	rare
<i>Cladosporium cladosporioides</i>	opportunistic	rare
<i>Coccidioides immitis</i>	virulent	endemic, common
<i>Curvularia geniculata</i>	opportunistic	uncommon but regular
<i>Curvularia lunata</i>	opportunistic	common
<i>Curvularia pallescens</i>	opportunistic	rare
<i>Cylindrocarpon lichenicola</i>	opportunistic	rare
<i>Drechslera australiensis</i>	opportunistic	rare
<i>Drechslera hawaiiensis</i>	opportunistic	common
<i>Drechslera spicifera</i>	opportunistic	common
<i>Emmonsia parva</i>	opportunistic (obstructive, not invasive)	rare
<i>Epicoccum nigrum</i>	opportunistic	rare
<i>Eurotium amstelodami</i>	opportunistic	rare
<i>Exophiala dermatitidis</i>	opportunistic	uncommon
<i>Exophiala spinifera</i>	opportunistic	rare
<i>Exserohilum longirostratum</i>	opportunistic	rare

<i>Exserohilum mcginnisii</i>	opportunistic	rare
<i>Exserohilum rostratum</i>	opportunistic	rare
<i>Fonsacaea pedrosoi</i>	opportunistic	rare
<i>Fusarium dimerum</i>	opportunistic	rare
<i>Fusarium napiforme</i>	opportunistic	rare
<i>Fusarium oxysporum</i>	opportunistic	uncommon but regular
<i>Fusarium proliferatum</i>	opportunistic	uncommon but regular
<i>Fusarium solani</i>	opportunistic	uncommon but regular
<i>Fusarium verticillioides</i> (=F. moniliforme)	opportunistic	uncommon but regular
<i>Histoplasma capsulatum</i>	virulent	endemic, common
<i>Lecythophora hoffmanii</i>	opportunistic	rare
<i>Metarrhizium anisopliae</i>	opportunistic	rare
<i>Microascus cinereus</i>	opportunistic	rare
<i>Microascus cirrosus</i>	opportunistic	rare
<i>Myceliophthora thermophila</i>	opportunistic	rare
<i>Neosartorya pseudofischeri</i>	opportunistic	rare
<i>Nodulisporium</i> sp.	opportunistic	rare
<i>Ochroconis gallopava</i>	opportunistic	rare
<i>Paecilomyces lilacinus</i>	opportunistic	rare
<i>Paecilomyces variotii</i>	opportunistic	rare
<i>Paracoccidioides brasiliensis</i>	virulent	endemic, common
<i>Penicillium citrinum</i>	opportunistic	rare
<i>Penicillium decumbens</i>	opportunistic	rare
<i>Penicillium marneffeii</i>	virulent	endemic, common
<i>Penicillium purpurogenum</i>	opportunistic	rare
<i>Phaeoacremonium parasiticum</i>	opportunistic	rare
<i>Pleurophomopsis lignicola</i>	opportunistic	rare
<i>Pseudallescheria boydii</i>	opportunistic	common
<i>Scedosporium prolificans</i>	opportunistic	uncommon but regular
<i>Scopulariopsis brevicaulis</i>	opportunistic	rare
<i>Scopulariopsis brumptii</i>	opportunistic	rare
<i>Scopulariopsis Candida</i>	opportunistic	rare

<i>Sporothrix schenckii</i>	virulent	uncommon but regular
<i>Trichoderma longibrachiatum</i>	opportunistic	rare
BASIDIOMYCOTA (and anamorphs)		
<i>Cryptococcus neoformans</i>	virulent	common
<i>Rhodorula minuta</i>	opportunistic	rare
<i>Rhodotorula rubra</i>	opportunistic	rare
<i>Schizophyllum commune</i>	opportunistic	uncommon but regular
<i>Trichosporon asahii</i>	opportunistic	uncommon but regular
<i>Trichosporon inkin</i>	opportunistic	uncommon
<i>Trichosporon mucoides</i>	opportunistic	uncommon

a. "endemic, common"—common within endemic area

Coccidioides immitis, arguably the most virulent of these fungi, is restricted to desert soils in the "lower Sonoran life zone" (a phytogeographic zone tending to feature tall cactus species and creosote bush) in the southwestern USA and contiguous Mexico (Kwon-Chung and Bennett 1992) as well as scattered climatologically similar areas in South America.

Infection is usually acquired by inhalation of conidia from disturbed soil. Dust storms, construction activity, automobile dirt racing and so on are common activities that have resulted in outbreaks or prominent cases. Most cases resolve spontaneously after a few days of respiratory ailment resembling a common "chest cold", but a small proportion of persons experience more severe symptoms, ranging from pneumonia to erythema nodosum (an acute skin rash) to dis-seminated infection in which the organism spreads to cause skin abscesses, meningitis, and infections of other internal sites such as bones, kidneys, liver and epididymis (Kwon-Chung and Bennett 1992). Cases of dissemination are often fatal unless the disease is arrested by antifungal therapy. The fungus has complex interactions with host factors related to sex hormones and pigmentation, with the result that certain groups, such as women in the third trimester of pregnancy and people with high levels of dermal melanin (those of African ancestry, for example) are significantly more likely to acquire serious infection than others (Kwon-Chung and Bennett 1992). Adult males in general are about four times as likely as females to experience disseminated infection (Fiese 1958).

Because the fungus is strongly associated with outdoor soils, its connection with indoor environments is desultory, basically consisting of the possibility that outdoor spora entering buildings within the endemic region may include some infectious conidia. On rare occasions, a potted cactus directly lifted from the wild in endemic areas, or personal articles packed in an endemic area, may transport the fungus to a dwelling outside the endemic zone (Kwon-Chung and Bennett 1992). For example, my laboratory dealt with one case (unpublished) in which an immunocompromised Canadian woman,

who had never travelled to an endemic *Coccidioides* zone, became severely infected after receiving a visit from her sister who resided in Arizona. Workplaces have also rarely been affected after importing porous or fibrous material, such as cotton, which happens to have been exposed to sedimentation of *Coccidioides* inoculum in the endemic region (Kwon-Chung and Bennett 1992). Despite such occurrences, the outstanding risk factor for persons living outside the endemic region is travel to that region, particularly California's Death and San Joaquin valleys and certain parts of Arizona. As with tuberculosis, once a person has been exposed to *C. immitis*, a small residue of viable inoculum tends to persist indefinitely in a stable, calcified pulmonary granuloma (Rippon 1988), and immuno-suppression later in life is likely to cause the organism to re-emerge in the form of a severe infection. Tobacco smokers residing outside the endemic area also occasionally have such nodules misdiagnosed as carcinoma based on x-ray analysis, and may suffer biopsy or even erroneous surgical removal of the affected lung lobe.

Blastomyces dermatitidis and *Paracoccidioides brasiliensis* have very little connection with indoor environments, and therefore will not be discussed in detail here. The pathogenicity and ecology of the former fungus is summarized by Al-Doory and DiSalvo (1992). Both fungi are discussed in detail by Kwon-Chung and Bennett (1992) and Rippon (1988). In essence, *B. dermatitidis* is a riparian, nitrophilous fungus growing mainly on damp debris or soil near waterways in restricted, mostly mountainous and boreal endemic zones within eastern North America. The fungus also uncommonly occurs in parts of Africa and Asia. Very rarely, construction activity in cities in endemic regions has been tentatively linked to small disease outbreaks (Kitchen *et al* 1977). Otherwise, it is acquired outdoors or rarely in heavily animal-infested shacks or out-buildings (Bakerspigel *et al.* 1986). *P. brasiliensis* is restricted to parts of central and eastern South America and may be most closely associated with soils in zones in which coffee can be grown (Silva-Vergara *et al* 1998). Infection is generally acquired outdoors. *P. marneffeii* is also essentially linked to outdoor habitats in montane and high plateau areas of southeast Asia (especially northern Thailand, Burma, Laos, and Vietnam, as well as Yunnan, Szechuan, Guangxi, and Guangdong provinces of China) which are inhabited by the apparently associated bamboo rat *Rhizomys sinensis* (Kwon-Chung and Bennett 1992).

A dimorphic fungus with considerably more indoor significance is *Histoplasma capsulatum*. This species is found in various parts of the Americas, Africa and Asia, but is uncommon or rare in much of its range. It is, however, abundant in defined endemic areas of North and South America, including most of the watershed of the Mississippi river and its tributaries in the central USA (Kwon-Chung and Bennett 1992). It also has marginal endemic areas where it is locally common, such as parts of southeastern USA, as well as the parts of the lower St. Lawrence River valley in Canada and the northeastern USA. In its areas of greatest abundance, *H. capsulatum* may be common in outdoor soils, especially when these soils have been subject to nitrogen inputs from migratory and resident bird populations. In marginal areas it is probably seldom associated with such soils, but may be strongly associated with large depositions of bird and bat guano in buildings and caves. Similar sites in highly endemic areas also tend to be infested. Outbreaks frequently occur in groups of spelunkers in caves with resident bats, and in

renovation workers or householders cleaning up large depositions of bat or bird guano in attics or belfries which have housed long-established breeding colonies (Kwon-Chung and Bennett 1992). Exposure levels in such cases can be high, leading to relatively severe disease even in immunocompetent persons. Bat dung in particular may be similar to sawdust in texture, since it is mainly composed of parts of insect exoskeletons, and may sift through porous ceiling boards or be deposited in wall cavities at the edge of the attic, sometimes falling down wall interiors until it reaches lower floors of the building. In a recent Ontario outbreak investigated by the author's laboratory, workers infected by *H. capsulatum* had worked extensively in the attic, but the fungus was only isolated *in vitro* from bat dung deposits that had fallen into the basement.

Histoplasmosis, like coccidioidomycosis, most commonly manifests as an insignificant illness resembling a chest cold. In many cases, low-level exposure may lead to life-long seroconversion, with the seropositive individual remembering no corresponding illness. Such seroconversion, widely studied in the 1950's and 1960's using the histoplasmin skin test, is currently normally elucidated using immunodiffusion or complement fixation serological tests (Kwon-Chung and Bennett 1992). Persons who have seroconverted usually have a life-long immunity to the disease. As with coccidioidomycosis, however, the body's successful containment of the infection may leave behind one or more calcified pulmonary nodules. This entails the risk factors mentioned previously: recrudescence of the disease if the person becomes immunodepressed later in life, and misdiagnosis as cancer. Heavily exposed or immunocompromised individuals, including some persons with cellular immune deficits apparently not otherwise of great consequence, may contract a pneumonia-like illness. In immunocompetent persons this acute pulmonary histoplasmosis usually resolves spontaneously, but may continue as a chronic infection in some individuals, typically white, male smokers with pre-existing, incipient chronic bronchitis or emphysema (Goodwin *et al.* 1976; Latham *et al.* 1980). Obstruction of pulmonary blood vessels, bronchi, or the oesophagus as a result of swelling and granulomatous infection of chest lymph nodes is another possible complication, as is pericarditis. In a small proportion of cases, especially in immunocompromised or very young or old patients, the pulmonary infection may be succeeded by dissemination of the organism to internal organs such as the liver, spleen or meninges, as well as to mucous membranes or skin. In HIV-positive people with a CD4+T-cell count lower than 50 cells μL^{-1} , dissemination is common (Jung and Paauw 1998) and severe. Therapy with antifungal drugs is necessary in chronic pneumonia-like and disseminated infection, and may also be used in some cases of acute histoplasmosis. In a small proportion of cases, infection has been tentatively associated with ensuing visual problems in the otherwise completely recovered patient. This so-called "presumed ocular histoplasmosis syndrome" is controversial: since *H. capsulatum* is not detected in histopathology of the affected sites, the condition may involve a post-infection auto-immune effect provoked by an immunological cross-reaction (the sensitized human body attacking a self antigen which chemically resembles a fungal epitope), or it may be an epistemically reified coincidence of an unrelated ocular condition with prior *Histoplasma* exposure. The author has just become aware of a cluster of such diagnoses in the lower Fraser valley of British Columbia, Canada, an area where *Histoplasma* itself is unknown

and where at least some patients are sero-negative for this fungus.

Sporothrix schenckii is a fungus mainly associated with outdoor vegetative debris, particularly dried straw or straw-like materials (e.g. dried reeds) or dried *Sphagnum* peat moss (Rippon 1988). It is cosmopolitan, with certain neotropical areas such as parts of Mexico, Brazil, Argentina, Guatemala and Uruguay having unusually high exposure and being considered as endemic regions. The great majority of cases are not respiratory, but rather consist of subcutaneous abscesses caused by minor traumatic implantation, e.g. through a scratch, often accompanied by progressive infection and swelling of a series of proximal lymph nodes. Respiratory cases are very uncommon (Rippon noted about 150 recorded cases as of 1988) and a substantial proportion of cases are in chronic alcoholics. Presumably, in at least some of these cases, a significant inoculum load may be aspirated during extended contact with grass litter or soil while the patient is unconscious. Respiratory cases normally consist of a life-threatening cavitary pneumonia; a less common variant is an often spontaneously resolving infection mostly involving pulmonary lymph nodes (Rippon 1988). Because *S. schenckii* is strongly associated with poorly stored commercial peat moss (Dixon *et al.* 1991) and because this material is often used in potted plants, the rare to occasional occurrence of *S. schenckii* indoors is to be expected. The only recorded indoor isolations of *S. schenckii* are from potted plant soil incorporating oak/birch litter, not peat moss (Mariat 1968) and from the floor around an indoor swimming pool (Staib and Grosse 1983). Clearly, although there may be a small but appreciable risk of subcutaneous sporotrichosis in the indoor environment, especially when poorly stored peat moss is used in domestic cultivations, the probability of respiratory sporotrichosis connected with the presence of the fungus indoors is extremely low.

The ecology of *Cryptococcus neoformans* var. *gattii*, which appears not to intersect with indoor habitats, has been mentioned above. In contrast, *C. neoformans* var. *neoformans* is unique among the virulent fungi in at least potentially being more strongly associated with indoor than outdoor exposure. Like *H. capsulatum*, it is strongly associated with substantial deposits of bird or bat guano; the two species may occur together in the same deposits in histoplasmosis endemic zones. *C. neoformans*, however, as a cosmopolitan organism, is much more geographically widespread than *H. capsulatum*. It may also require lesser amounts of dung deposition to establish itself, and may infest pigeon dung accumulations on window ledges, bridge girders, and other such unexceptional human-constructed niches in urban areas. Fortunately, it is much less virulent than *H. capsulatum*, and a large exposure is usually (although not always) needed for an immunocompetent patient to become infected. On infrequent occasions, immunocompetent persons with no marked contact with guano may develop localized pulmonary lesions (Krajden *et al.* 1991). Persons, however, who expose themselves to high inoculum levels by disturbing large quantities of guano without using high efficiency particulate (HEPA) filter respiratory protection, may or may not develop discernible respiratory illness, but typically develop the very severe condition known as cryptococcal meningitis (Kwon-Chung and Bennett 1992). Not only is the meningeal infection severe, but the drug of choice for treatment, amphotericin B, has serious side effects in many patients, most notably rigors (severe stiffening muscle spasms involving

much of the body) after intravenous administration, and a risk of severe kidney damage potentially leading to a need for kidney transplant.

The worldwide HIV outbreak has enormously increased the number of cryptococcosis cases. In the U.S., for example, mortality due to cryptococcosis increased fourfold as a result of this outbreak (Selik *et al.* 1997). HIV-positive individuals with CD4⁺ T-helper lymphocyte counts below 50 cells μL^{-1} are at risk of developing cryptococcal pneumonia, meningitis, or disseminated infection (Arasteh *et al.* 1996). Unlike persons with intact cellular immune systems, such persons may be at risk of infection after inhaling a single cryptococcal cell or a small number of cells. In temperate and tropical areas, immunocompromised persons risk exposure to *C. neoformans* var. *neoformans* whenever they are exposed to bird or bat faecal deposits, regardless of the amount of material present. Aviaries, whether privately owned or in zoos, may have a high level of risk (Staib 1982; Staib 1985; Staib and Heissenhuber 1989). Persons with AIDS are advised to avoid birds (including domestic pets) when possible, or at the very least not to involve themselves in cleaning cages or other areas where dung might accumulate or where residual traces of dung may be present. In tropical areas, wounds on some tree species may also be reservoirs of *C. neoformans* var. *neoformans* in oculum (Lazera *et al.* 1996), but it is not clear how wide a range of trees may be involved.

Pneumocystis carinii

Although *P. carinii* has been detected in a wide range of mammalian species (Laakkonen 1998), molecular and attempted transfection studies have shown that strains are specific to individual host species (Stringer 1996). Also, the degree of genetic difference between strains from different animal species suggests a species-level separation (Stringer 1996). The study of *P. carinii* ecology and epidemiology has been greatly hindered by the organism's failure to grow in artificial culture. In recent years, however, molecular biological techniques have made studies of both infecting and environmental inoculum possible (Rabodonirina *et al.* 1997; Olsson *et al.* 1996; Bartlett *et al.* 1997). Pneumocystosis is normally only seen in patients with significant cellular immune deficiencies, and in "premature and marasmic infants between the tenth and twenty-fourth week of life" (Seed and Aikawa 1977). Although the disease may spread from the lungs to various parts of the body, initial infection is clearly respiratory (Hughes 1982). The host specificity of *Pneumocystis* types indicates a close host/pathogen adaptation, making growth and reproduction in the environment unlikely. *P. carinii* infection thus appears to be contracted from other infected conspecifics, and outbreaks typical of contagious disease have been reported in immunocompromised humans (Hennequin *et al.* 1995) and in experimental animals (Soulez *et al.* 1991). A study in which air from *Pneumocystis*-infected AIDS patients was passed through filters, which were then examined by nested PCR for *Pneumocystis* DNA, found that air from patients' rooms strongly tended to have *P. carinii* in it (Olsson *et al.* 1998). DNA typing by sequencing and allele-specific PCR showed that the strain detected by filtration in 4 of 5 patient rooms was identical to that found in the corresponding patient. Airborne inoculum thus appears to be shed by infected patients. Air filtering, however, also detected airborne *P. carinii* inoculum at sites distant from any person with diagnosed pneumocystosis (Bartlett

et al. 1997; Olsson *et al.* 1998). Immunocompetent hospital staff studied by serology and PCR showed no signs of becoming infected by *P. carinii* from infected patients (Lundgren *et al.* 1997). On the other hand, a survey of 77 HIV-negative, demonstrably immunocompetent patients with severe pulmonary disease revealed 5 patients infected with *P. carinii*. The infections were subclinical, giving rise to no significant symptoms, and the infected persons were tentatively classed as clinically silent carriers (Armbruster *et al.* 1997). Widespread infection has also been confirmed in immunocompetent rabbits raised in experimental colonies (Cere *et al.* 1997). Infection is probably transient: for example, in artificially immunosuppressed, Pneumocysti/s-infected rats allowed to recover from immunosuppression, inoculum detectable by histology and PCR steadily decreased, and was eliminated completely from over 75% of rats within one year (Vargas *et al.* 1995). Clinical evidence also suggests that patients are exogenously infected, rather than being infected by recrudescence of a primary infection acquired earlier in life (Latouche *et al.* 1994). Overall, it appears that although suitably immunocompromised patients are ubiquitously at risk of contracting serious *P. carinii* infection, immunocompetent persons are either not at risk or run a small risk of contracting a subclinical infection not producing discernible symptoms.

Opportunistic fungi

One matter of human and animal biology that the AIDS pandemic has helped to clarify is that the immune system is highly compartmentalized. Resistance to fungal infections appears to depend largely on the cellular component of the immune system (Levitz 1992). The humoral component, mainly consisting of antibodies and other soluble substances in serum, may be less influential than it is in other types of infection. Persons with a deficit of CD4⁺ T-helper cells, as seen in AIDS, become markedly susceptible to pneumocystosis as well as fulminant cases of infection caused by virulent fungi, such as coccidioidomycosis, cryptococcosis, histoplasmosis, sporotrichosis, and penicilliosis marneffei. In addition, they typically develop aggravated mucosal candidiases, particularly oropharyngeal, vaginal and oesophageal colonizations. They do not, however, generally develop systemic candidiasis or infections caused by opportunistic fungi (Levitz 1992). Only if they later, as a result of AIDS complications, suffer a strong attenuation of another cellular immune component, the neutrophil or granulocyte component, do they have a strongly elevated risk of aspergillosis and other opportunistic mycoses. The complications leading to such a neutropenic (neutrophil deficient) condition in persons with AIDS include B-cell lymphoma and treatment with certain antiviral drugs such as ganciclovir which have partial immunodepression as a side effect. Similarly, in people not affected by HIV, the condition generally conducive to opportunistic infection is neutropenia, and this condition is usually induced by (1) leukaemia, lymphoma, and related neoplasms; (2) immunosuppressive drugs used in the treatment of these or some other cancers; (3) immunodepressive therapies used in the prevention of rejection of organ transplants; or (4) certain congenital immunodeficiencies. Persons on prolonged, heavy corticosteroid therapy may manifest a partial suppression of neutrophil activity, resulting in an increased susceptibility to

opportunistic infections. The opportunistic fungi causing severe infection in neutropenic patients are normally harmless to non-neutropenic persons. A few unusual conditions, such as severe burns, may increase the risk of non-neutropenic patients to opportunistic fungal disease.

The above synopsis of conditions leading to opportunistic infection is mainly in reference to invasive conditions. The far less severe colonizing types of opportunistic infection have their own particular predisposing conditions. Allergic bronchopulmonary aspergillosis (ABPA), where the bronchial passages may become colonized with *A. fumigatus* or, rarely, other aspergilli (Summerbell 1998), generally affects persons who have had severe chronic asthma for many years (Varkey 1998) or persons with cystic fibrosis (Schwartz 1998). In both conditions, bronchial passages tend to manifest inflammation and to generate a large amount of mucous material. Long-term asthmatics or persons treated with long-term systemic corticosteroids may be at increased risk for allergic mycotic sinusitis, an allergically exacerbated fungal colonization of the sinus walls. Persons such as children or mentally handicapped individuals who place objects into their outer ear canals may be at elevated risk for otomycosis, fungal ear canal colonization.

A very large number of normally nonpathogenic fungi may cause infection of the cornea of the eye, but normally only after traumatic implantation, for example, when the eye is pierced by a flying piece of plant debris during agricultural processing. It is extremely rare for such events to occur in the indoor environment.

This explicit breakdown of predisposing conditions is given because members of the public are often confused about indoor moulds and opportunistic infection. Individuals whose homes or workplaces have been reported to contain potentially opportunistic moulds may fear systemic or ocular infections to an unrealistic degree. Also, persons with severe allergies or chemical sensitivities may purport that their immune systems are compromised or (as I have often been told) "completely destroyed." Various branches of alternative medicine inform essentially immunologically normal people that they are seriously immunocompromised, and a few even fallaciously assert that patients have yeasts or mould fungi growing in their blood. The conditions or pseudo-conditions diagnosed by these practitioners seldom bear any valid relationship to susceptibility to opportunistic mycosis. With the aid of precise information about predisposing conditions, confusion about susceptibility to fungal infections can be minimized.

Reactions of immunocompetent occupants of buildings to indoor mould proliferation almost invariably consist of a mixture of direct chemical irritation effects and allergic responses. These should not be confused with invasive or colonizing opportunistic infection, which are entirely different phenomena.

Many of the opportunistic mould fungi found in indoor environments are typical inhabitants of natural composts, tropical soils and other heated materials. Fungi such as *Aspergillus terreus*, *A. fumigatus*, *A. flavus*, *Rhizopus microsporus* var. *rhizopodiformis*, *R. oryzae* and *Rhizomucor pusillus* are thermotolerant, normally growing at temperatures above 45°C, as well as at lower temperatures (Domsch *et al.* 1993). Indoor sites particularly associated with these fungi therefore may be those where humid organic material is exposed to heat (Marsh *et al.* 1979), most notably within poorly maintained

heating ducts and attached humidifier structures, in soils of potted plants (Summerbell *et al.* 1989), especially those placed in warm locations (Staib *et al.* 1978), and in indoor composts. The thermotolerant aspergilli, however, are not uncommon in outdoor air, all-year-round in tropical areas (Christensen and Tuthill 1985) and seasonally in temperate areas (Kwon-Chung and Bennett 1992). Although they are generally quite uncommon in indoor proliferations in homes and non-agricultural workplaces, they are nonetheless a constant hazard for hospitals. Occasional instances of indoor proliferation, along with an association with pigeon dung on building surfaces (Mehta 1990), including areas near building air intakes (Burton *et al.* 1972), and a regular low-level presence as dormant propagules in dust combine to ensure that whenever renovation occurs and materials are disturbed, a significant influx of airborne propagules is likely to endanger hospitalized neutropenic patients (Arnow *et al.* 1978; Sarubbi *et al.* 1982; Nolard 1994). This risk can be significantly but not entirely controlled by using HEPA filters for air in areas with high-risk patients (Rose 1972; Rose and Hirsch 1979; Rosen and Sternberg 1976), along with other dust control techniques such as having incoming staff and visitors don sterile gowns and hair covers, and also shoe covers. In addition, high-risk patients can only be given food materials that have been suitably processed to inactivate fungal propagules. To be avoided in particular are substances such as spices which tend to have significant *Aspergillus* counts (De Bock *et al.* 1989). Likewise, flowers, tea leaves and other organic or potentially dusty environmental inputs are to be avoided (Nolard 1994). Air filters must be exchanged regularly; such filters may become hazardous sites of *Aspergillus* growth if they are allowed to accumulate sufficient organic material from the air (Arnow *et al.* 1991). Rigorous infection control measures which may be employed to minimize *Aspergillus* in bone marrow transplant units during construction include installation of HEPA filters, using paint amended with copper-8-quinolate on surfaces, installation of non-perforated ceiling tiles, sealing of windows, replacing potentially dusty horizontal-vened window blinds with vinyl roller shades, and regular cleaning of all surfaces (Loo *et al.* 1996). Where the source of contamination is outside the hospital building, both windows and strategically selected doors may be sealed; air handling systems are prepared where possible to filter incoming air and to draw incident air from an area away from the source of contamination or its downwind airstream (Streifel *et al.* 1983).

These precautions are not applicable to non-neutropenic patients, who have a very low risk of infection by thermotolerant aspergilli and zygomycetes. Many individuals, particularly agricultural workers, may be routinely exposed to such inoculum, putting them at risk of allergy, hypersensitivity reactions, or unfavourable chemical exposure, but not significantly at risk of invasive disease (Jappinen *et al.* 1987; Yoshida *et al.* 1993). A few idiopathic cases of invasive pulmonary aspergillosis in non-immunocompromised patients with no significant history of exposure (Karam and Griffith 1986) remain unexplained. Invasive mycotic sinusitis caused by *A. flavus* and other fungi may also occasionally occur in immunocompetent individuals (Hussain *et al.* 1995). As mentioned above, other predisposing conditions leave a few categories of individuals vulnerable to colonizing infections. In addition to the conditions mentioned above (ABPA, sinusitis, otomycosis), aspergilloma or *Aspergillus* fungus ball may occur. The possession of a pre-existing pulmonary cavity, e.g. one caused by previous tuberculosis or by a combat

wound, may predispose persons to acquiring this condition.

It is unclear whether patients with predispositions for colonizing infections are placed at particular risk by aggregations of *Aspergillus* inoculum beyond the background level. It is possible that there is sufficient thermotolerant *Aspergillus* inoculum present in normal ambient air and dust to effectively expose all such patients. A study by Radin *et al.* (1983), however, did find a correlation between exacerbations of ABPA and increases in total outdoor mould counts. Similarly, mouldy marijuana, bird dung, horticultural mulch and nearby outdoor dump sites have been apparently linked to initiation or exacerbation of ABPA (Summerbell 1998; Krasnick *et al.* 1995). In most situations, however, the rationale for rigorously eliminating amplifiers of thermotolerant aspergilli and other opportunistic moulds in homes and commercial buildings lies primarily in avoiding the allergenic, toxic and biochemical irritant effects of these fungi, and only to a lesser degree in avoiding risk of invasive or colonizing disease. At the same time, it should be pointed out that there is an increasing trend not to keep all neutropenic patients in hospital for the full duration of their immunodepression (Freifeld and Pizzo 1996), so a need may arise for certain non-hospital buildings to be as scrupulously clean of such fungi as is reasonably possible. In particular, patients may be sent home, and their homes should therefore be cleared of potted plants, indoor composters, birdcages, spice shakers and grinders, dried grains and other dried plant material, and other such notorious *Aspergillus* sources, during the time in which the patient is vulnerable (Summerbell *et al.* 1992; Summerbell *et al.* 1994). The patient is advised to avoid gardening and any construction, renovation or agricultural activities, or activities such as pigeon handling, for the duration of neutropenia. It would be ideal if the environment could be tested for the presence of opportunistic fungi before the patient is discharged to it.

Control of the opportunistic aspergilli is aided by knowing their normal growth sites. In general, most of this subgroup of aspergilli regularly grow in the following sites: composts; herbivore and bird dung accumulations; bird nests; tropical and subtropical soils, especially under cultivation; dead or parasitized insects; human and animal corpses, especially those in which bacterial de-composition is delayed by storage or environmental conditions; and stored seeds of all kinds, most notably spices, groundnuts and edible graminaceous grains such as wheat, barley, oats, sorghum, rice and maize (Domsch *et al.* 1993; Summerbell 1998; Millner *et al.* 1994). Most of these species degrade alkanes and aromatic compounds, and so may be found in association with decaying material adulterated with materials such as fuel oil, tar, organic solvents, phenolic compounds and paraffin (Domsch *et al.* 1993; Summerbell 1998).

Individual thermotolerant opportunists have distinct habitats in which they may become common within buildings. *A. fumigatus* is especially associated with potted plants, although *A. niger* is also common in these habitats (Staib *et al.* 1978; Summerbell *et al.* 1989; Thompson *et al.* 1994). Both these fungi are also strongly associated with poorly stored spices (Domsch *et al.* 1993; Nolard 1994). The recent popularity of home composting in parts of Europe has provided a new locus of *A. fumigatus* growth indoors (Staib 1996). *A. flavus* is particularly associated with poorly stored peanuts and other seeds (Domsch *et al.* 1993), but may be found in a variety of other indoor habitats, e.g. bird dung accumulations (Staib 1985) and food pellets for pet rabbits (Staib 1982). In a

recent case of indoor infestation, this species grew abundantly on moisture-damaged, tarred wooden floor tiles (Summerbell 1998), a finding consistent with its conjunction of hydrocarbon-degrading and cellulolytic abilities. It may also colonize various sites within hospitals (Arnou *et al.* 1991).

Rhizopus oryzae and another zygomycetous opportunistic fungus, *Absidia corymbifera*, have been found in potted plant soil in a haematology unit where a leukaemia patient developed invasive zygomycosis (Staib 1985). They were also abundantly represented in bird dung accumulations in an aviary (Staib 1985). In hospitals, wooden materials such as tongue depressors have repeatedly been a source of problematic *Rhizopus* inoculum (Mitchell *et al.* 1996; Verweij *et al.* 1997). Domsch *et al.* (1993) list isolations of *R. oryzae* from grains, onions, various nuts, and stored sweet potatoes.

Apart from the thermotolerant aspergilli and Zygomycetes mentioned above, a few other potentially opportunistic moulds are commonly associated with indoor habitats. *Paecilomyces variotii*, a fungus of much lower virulence than the aspergilli and Zygomycetes mentioned, is typically associated with sites high in oils, hydrocarbons, or substances such as chemical preservatives inhibitory to other fungi (Domsch *et al.* 1993). It has been detected in heavy growth in certain indoor situations such as commercial activated charcoal products (George *et al.* 1991), decaying urea formaldehyde foam insulation in walls (Bissett 1987) and deteriorated tarred, wooden flooring (Summerbell 1998). Domsch *et al.* (1993) note isolations from compost, peanuts, root vegetables, spoiled olives, decaying plant parts, leather, synthetic rubber, mouldy cigars and ink. The causation of respiratory infection by this fungus is possible but very unlikely in the neutropenic patient, and immunocompetent patients are certainly not at risk of such infections. It may, however, colonize sites where there are barrier breaks in the body integrity associated with medical implants, such as implanted catheter sites in chronic ambulatory peritoneal dialysis patients (Crompton *et al.* 1992).

Pseudallescheria boydii, a fungus most typically associated with compost, warm soil and dirty water (Domsch *et al.* 1993; Rippon 1988), has been isolated indoors in potted plants and in water from a canister collecting an air conditioner condensation drip (Summerbell *et al.* 1989). This fungus is capable of causing a range of infections similar to *A. fumigatus* (Kwon-Chung and Bennett 1992). Significantly less virulent, but still a serious hazard to the neutropenic patient, are *Fusarium oxysporum*, *F. solani*, *F. verticillioides* (= *F. moniliforme*) and *F. proliferatum* (Richardson *et al.* 1988). These species are associated with roots and lower stems of a variety of fresh vegetables, including lettuce and other greens as well as stored tubers and corms (Domsch *et al.* 1993). In addition, *F. solani* is common in fresh water and water pipes (Domsch *et al.* 1993) and hence with sinks and other sites connected to domestic plumbing. The author on the day of this writing examined an insulating duct lining from an industrial building and found the cellulose fabric of the interior surface heavily colonized by *F. solani*, in addition to several other fungal species. *F. verticillioides* is notoriously associated with decaying maize corn kernels (Domsch *et al.* 1993; Wicklow 1988).

Herpotrichellaceous black yeast anamorphs in the genus *Exophiala* are commonly found in association with sinks and drains indoors (Nishimura *et al.* 1987), a habitat

contiguous with their natural habitat in cool or cold, moist soils (Domsch *et al.* 1993). These fungi may cause ABPA-like colonizing infections in cystic fibrosis (Haase *et al.* 1991) and long-term asthmatic patients (unpublished data). An *E. dermatitidis* infection marked by recurrent haemoptysis occurred in a patient with a long history of bronchiectasis (Barenfanger *et al.* 1989). Aerosol splash connected with use of plumbing facilities is potentially a significant source of exposure. It should be noted that these same fungi are very common as contaminants of respiratory secretions and skin, and are among the most likely fungi to contaminate bronchioalveolar lavage (BAL) sampling equipment in hospitals.

Some additional marginally opportunistic fungi are very common in the environment, but have only rarely been implicated in causing primary pulmonary disease or invasive sinusitis of severely immunocompromised patients. Included in this group are species in the genus *Scopulariopsis* and a few members of *Acremonium* subgenus *Acremonium*. *Scopulariopsis brevicaulis*, a fungus commonly isolated from indoor dust, is one such fungus, as is the only slightly less environmentally common *S. Candida* (Krisel *et al.* 1994). *Acremonium kiliense* and *A. strictum*, which are readily isolated indoors, e.g. growing within amplifiers of *Stachybotrys* on very moist surfaces, also may cause pulmonary infection in the severely immunocompromised patient (Boltansky *et al.* 1984). *Paecilomyces lilacinus* is another common indoor dust fungus that may cause invasive mycotic sinusitis (Gucalp *et al.* 1996). *Alternaria alternata*, a fungus which is relatively common in proliferations on water-damaged wallboard, wallpaper (Miller and Holland 1981; Bemmam 1979), ceiling tiles (Ren *et al.* 1998) and other indoor substrates substantially consisting of finely divided cellulose (Simmons *et al.* 1997), is one of the more common agents of mycotic sinusitis (Morrison and Weisdorf 1993; Iwen *et al.* 1997), whether invasive or allergic.

Another group of opportunistic fungi which may become established in the indoor environment and which may rarely have a respiratory infection court consists of basidiomycetous yeast species in the genus *Trichosporon*. Recent molecular taxonomy studies have shown that the group of isolates formerly considered a single species, *T. beigeli*, actually contains several species, only a small number of which are regularly connected to human systemic invasive or colonizing disease (Guého *et al.* 1992). The most important of these are *T. asahii*, *T. mucoides* and *T. inkin*. Interestingly, *T. mucoides* and *T. asahii* are among the principal fungi colonizing tatami mats within Japanese style houses, and causing summer-type hypersensitivity pneumonitis in some immunocompetent individuals who have experienced prolonged inhalation of high quantities of inoculum (Nishiura *et al.* 1997). *Trichosporon* spp. are environmentally widespread, and the existence of some niches of proliferation within non-Japanese style housing can also be predicted.

There are some significant groups of opportunistic fungi which are not known to have significant proliferation loci indoors. Examples would include *Cochliobolus* anamorphs such as *Curvularia* and *Drechslera*, as well as diverse fungi such as the basidiomycete *Schizophyllum commune* and species in the zygomycete genus *Conidiobolus*. These will not be mentioned further in this review.

A significant factor in the epidemiology of all these opportunistic filamentous fungi is

their mode of dispersal. Hyphomycetes in general can be roughly broken down into species which produce dry conidia specialized for airborne dispersal, and species which produce mucoid conidia specialized for dispersal by water movement, splash aerosolization, arthropod vectors and water surface monolayer spread. The fungi in the former group may greatly predominate in hospital air (Summerbell *et al.* 1992). Members of the dry-conidial category, including aspergilli, *Paecilomyces*, *Scopulariopsis* and *Rhizopus*, pose the greatest risk to neutropenic patients both in hospital and at home. Many such fungi, including the groups named above, have conidia with diameters smaller than the 7 µm required for ready access to pulmonary alveoli. *A. fumigatus* in particular has conidia only 2.5–3 µm in diameter, and thus has easy access to deep pulmonary tissue upon inhalation. *A. flavus*, like some other aspergilli, has somewhat larger conidia to 4.5 µm in diameter; this may explain why it forms a much greater percentage of sinus aspergilloses than of pulmonary infections, where small conidial size is of greatest import (Chabra *et al.* 1996). Two potentially opportunistic *Fusarium* species, *F. verticillioides* and *F. proliferation*, although producing mucoid conidia under some conditions, may also produce aerial chains of small conidia under certain substrate conditions. The wet-conidial opportunistic fungi, such as the *Scedosporium* conidial state of *P. boydii*, *Absidia*, the black yeasts, *F. oxysporum* and *F. solani*, *Acremonium* and *Trichosporon*, may be rendered airborne by splash or by the eventual desiccation and fragmentation into dust of mucoid conidial aggregations. Splash aerosolization, a phenomenon most typically associated with raindrops in the outdoor environment, is probably mainly associated with sinks, showers and other aquatic washing facilities indoors. Shower heads, of course, may also directly aero-solize fungi, such as *F. solani*, that may be found in tap water.

The present author has observed that many (though by no means all) hospital infection control staff treat all problematic opportunistic fungi, e.g. in outbreaks, pseudo-outbreaks or contaminations of material, as if they were dry-conidial organisms. Thus, pseudo-outbreaks of *Exophiala* in BAL samples or *Acremonium* contaminations of virology sample transport medium may be responded to by deploying air samplers in a search for the source of contamination. Although wet-conidial fungi, for the reasons mentioned above, might derive from an airborne source, it is imperative that infection control staff also account for the reasonable possibility that such fungi have derived from an aqueous source. Such aqueous sources typically include not only bulk fluids but also biofilms attached to wet or frequently moistened surfaces, e.g. within equipment. In the case of BAL contamination, the equipment and fluids used to obtain the bronchial washing samples must be thoroughly tested for contamination, as must disinfection equipment (Hoffman *et al.* 1989). In all such cases of contamination by wet-conidial fungi, particular scrutiny should be given to stock solutions of all kinds containing antifungal drugs such as amphotericin B or other antifungal preservatives and inhibitors. *Acremonium*, *Fusarium*, *Pseudallescheria* and *Trichosporon* in particular may be insensitive to amphotericin B and most other known antifungals. Some dry-conidial fungi such as *P. variotii* and *P. lilacinus*, may also occur in similar situations. *Paecilomyces* species, especially *P. variotii*, are notoriously resistant to a wide array of noxious materials (Domsch *et al.* 1993) and may be expected in materials such as biomedical solutions or

cosmetics containing preservatives. The ability of some fungi such as certain *Paecilomyces* species to grow on certain types of plastics (Domsch *et al.* 1993; Roberts and Davidson 1986) must also be kept in mind. For example, a severe outbreak of *P. lilacinus* skin infection among neutropenic patients in a Swiss hospital was eventually traced to colonization of plastic skin lotion bottles prior to their being filled with the lotion (Orth *et al.* 1996).

SAMPLING METHODOLOGY FOR FUNGAL RESPIRATORY PATHOGENS

Only fungi which are potentially obtained from indoor growth sources (not incident outdoor dust) are treated below.

Virulent fungi

The dimorphic fungi corresponding or related to the teleomorph genus *Ajellomyces*, namely *Histoplasma capsulatum*, *Blastomyces dermatitidis* and *Paracoccidioides brasiliensis*, pose a unique problem in sampling. Only the first of these fungi is likely to proliferate indoors, but since sampling is similar for all these fungi, general comments will be made. In these fungi, either inoculum is normally extremely sparse even in problematic environmental materials, or the propagules of these fungi fare very poorly on isolation plates in competition with antibiotic-resistant bacteria and other fungi. Whatever the exact reason or reasons, it is certainly the case that these fungi are not successfully detected with standard culture tests relying on spreading of diluted environmental materials onto fungal growth medium (Ajello and Weeks 1983). Nor have they been detected in air samples. The sole proven isolation technique, as detailed by Ajello and Weeks, is the injection of suspensions of soil or other environmental material into experimental animals (usually mice), and the subsequent harvesting and culture plating of the livers and spleens onto systemic mycology isolation media. A raw enrichment-based *in vitro* direct isolation method for *B. dermatitidis* was reported by Baumgardner and Parestky (1999). Ordinarily, for these fungi, media supplemented with cycloheximide and enriched with high protein materials such as brain-heart infusion (BHI) and blood must be used for successful primary isolation from mammalian tissues (Larsh and Goodman 1980). Examples of such media include BHI sheep blood agar, BHI sheep blood agar plus 3% egg albumin (Chaturvedi *et al.* 1990), Sabhi agar, yeast extract agar, and plain BHI agar, all supplemented with cycloheximide, chloramphenicol and gentamicin. Incubation temperature is usually 28–32°C.

In cases where *H. capsulatum* is being sought in guano accumulations, it is useful to recall the high microbial competition and turnover which may occur in this substrate. There is no reason to believe *H. capsulatum* will be strongly persistent at its sites of growth. For this reason, nearby dust, e.g. on ledges or timbers, where *H. capsulatum* conidia may have settled out and remained dormant in relatively dry circumstances, away from the risk of immediate lysis by bacteria, should be sampled in addition to the guano

itself. It should be borne in mind that no number of negative sampling results is sufficient to demonstrate absolutely that a site is free of *Histoplasma*, since small or intermittent quantities, or quantities missed by chance alone (or through poor technique), may always be present. The majority of requests for such testing derive from persons wishing a precise assay of their health risk; it must be explained that this is not something this test can accurately provide. It is a "no false positives" type test, in which positive results are of value but negative results are of no practical significance, and its main functions are to confirm the local presence of *Histoplasma* in at least a proportion of suspected environmental foci, and to obtain isolates for possible epidemiological genetics or research-related testing. In endemic or marginal endemic zones, the most responsible approach to take is to assume that bird and bat guano deposits are likely to contain *Histoplasma*, and to proceed on that basis (Davies *et al.* 1995). In areas where *Histoplasma* has never been vicinally reported, this assumption is unnecessary; but the probability that *C. neoformans* or large amounts of *Aspergillus* may be present in guano must be borne in mind.

Molecular detection tests do not yet exist for *Histoplasma* or other dimorphic types in environmental material. They may, however, appear soon. The above caution about negative test results continues to apply to molecular testing, as it does to the conventional.

Cryptococcus neoformans, unlike *Histoplasma*, is readily isolated from environmental materials in ordinary dilution plating. Detection is facilitated by using Staib's phenol oxidase agar (*Guizotia abyssinica* birdseed agar or its refined derivatives such as caffeic acid agar) for detecting, through a brown colour reaction, the strong extracellular phenol oxidase activity peculiar to *C. neoformans* among yeasts (Staib 1962; Staib and Heissenkuber 1989; Salkin 1988). Occasional isolates of non-*C. neoformans* cryptococci may produce a similar reaction, but most do not. Also, some *Trichosporon* species may have similar phenol oxidase activity, but are easily distinguished in a microscopic mount. If this excellent medium is not available, the present author has observed that Littman oxgall (Difco Laboratories, Detroit, Mich.) streptomycin agar (Kane *et al.* 1997) incubated at 37°C readily grows *C. neoformans* from guano. Most non-*C. neoformans* cryptococci do not grow at this temperature, and a few which do grow poorly. Cryptococci are distinguished on Littman oxgall agar from any background *Candida* species because their colonies remain pale, whereas *Candida* spp. take up the methylene blue dye in the medium and become distinctly tinged with blue, or even bright blue.

Pneumocystis carinii

As mentioned above, *Pneumocystis* cannot be detected by culturing. Environmental detection is by filtering air through cellulose-ester filters, extracting DNA, and performing PCR with primers for *P. carinii* genes such as the ribosomal internal transcribed spacer (Bartlett *et al.* 1997; Olsson *et al.* 1998), the mitochondrial large subunit ribosomal RNA (Olsson *et al.* 1998) or the thymidylate synthase gene (Olsson *et al.* 1996). Either primers specific to *P. carinii*, or more general primers are used but resulting amplicons are verified as derived from *P. carinii* by rigorous analysis such as

sequencing.

Opportunistic fungi

In much modern work with indoor moulds, the historic focus on air sampling and airborne spora levels has been strongly diminished in favour of the physical detection of indoor mould amplification sites. These sites are then confirmed and analysed by direct microscopy, e.g. from scrapings of substrate material or from cellulose-acetate tape mounts (Samson 1985; Davies *et al.* 1995; Summerbell 1998). Even where reliably culturable moulds are concerned, this practice has the advantage of connecting instances of problematic mould proliferation with specific underlying causes related to building maintenance, and offers more precise analysis and control than air sampling alone does. Although finding indoor growth foci of opportunistic fungi in this way is ideal, and is appropriate when there are no severely immunocompromised persons in the environment, it is inadequate in any situation where neutropenic patients may be involved. The reason is that with neutropenic patients, even small amounts of inoculum, potentially connected not just with on-site proliferation but also with normal dust accumulation or even normal penetration of outdoor air, may be life-threatening. Also, in contrast to the situation where moulds are evaluated for allergenic or biochemical-irritant potential, in which case viability of propagules is not of major significance, in situations where invasive disease is a practical concern, only living propagules constitute a threat. This, then, necessitates a combination of volumetric sampling to monitor the air for culturable propagules, and dilution plating of any bulk materials potentially harbouring inoculum. The small conidial size of the most significant respirable pathogens prevents use of gravitational settle plate techniques for air sampling in connection with opportunistic infection, since these techniques underestimate the relative frequency of small propagules (Sayer *et al.* 1969; Solomon 1975). Settle plates have been demonstrated to miss *A. fumigatus* in haematology and bone-marrow transplant units when it is present at a frequency which is low but sufficient to cause new opportunistic infections (Iwen *et al.* 1994).

The tendency of almost all opportunistic fungi to grow at 37°C can be employed in selective isolation techniques on a variety of growth media. This selective effect can be used to advantage both in air sampling and in bulk sampling. Summerbell *et al.* (1989), for example, using Littman oxgall streptomycin agar incubated at 37°C, obtained significantly higher levels of *A. fumigatus* and *P. boydii* from potted plant soils than were obtained using the same medium incubated at room temperature. Streifel *et al.* (1983), using inhibitory mould agar incubated at 37°C, successfully monitored *A. fumigatus* concentrations before and after a hospital building demolition.

Black yeasts and related opportunistic fungi related to the family Herpotrichiellaceae, as well as virulent onygenalean fungi, *S. schenckii*, *C. albicans*, and several other medically important fungal groups, are noteworthy for their tolerance of cycloheximide. This attribute is routinely used in the design of selective isolation media for these fungi (Dabrowa *et al.* 1964; Summerbell *et al.* 1989). However, see comments above about special difficulties in isolating the *Ajellomyces* clade of the Onygenaceae. This technique is particularly important for *S. schenckii* and herpotrichiellaceous anamorphs, which may

grow very poorly at 37°C *in vitro*. Zhang and Andrews (1993) made an improved Sabouraud peptone glucose medium for environmental isolation of *S. schenckii* by adding an unusually high cycloheximide concentration (800 mg L⁻¹) in addition to 250 mg L⁻¹ chloramphenicol and 20 mg L⁻¹ of rifampicin. This medium has given very satisfactory results in the present author's laboratory routine for environmental *Sporothrix*.

The fungicide benomyl has excellent potential as a selective agent for isolation of certain opportunistic fungi (Summerbell 1993). It selects for fungi related to the family Microascaceae (e.g. *P. boydii*, *S. brevicaulis*), the orders Pleosporales (e.g. *Alternaria*, *Curvularia*), and Endomycetales (e.g. *Candida* spp.), and most members of the phylum Basidiomycota, while inhibiting the great majority of isolates of competing groups such as Eurotiales (e.g. *Aspergillus*, *Penicillium*), Hypocreales (e.g. *Fusarium*, *Acremonium*, *Trichoderma*), Herpotrichiellaceae (e.g. *Exophiala*) and Onygenales (e.g. *Chrysosporium*). Although it has been widely used in phytopathology and wood decay studies for decades, its use in environmental medical mycology is as yet underdeveloped.

Media with high solute concentrations can be used for the most efficient isolation of osmotolerant and osmophilic fungi. One of the most commonly used media for the efficient isolation of a wide range of fungi including osmotolerant species is dichloran-18% glycerol (DG18) medium (Pitt and Hocking 1985). Among the common respiratory opportunistic pathogens, members of the genera *Aspergillus* and *Fusarium* are the most likely to show improved isolation using such media. No study, however, appears yet to have been made with express reference to isolating a wide range of opportunistic fungi using this technique.

Specialized media have been designed for the selective isolation of *A. flavus* and the closely related *Aspergillus parasiticus* (Bothast and Fennell 1974; Pitt *et al.* 1983) and for the isolation of *Fusarium* spp. (Thrane 1996) from foods. These appear to have not to have been applied to the study of the same organisms as opportunists, but could well be.

To date, there has been relatively little work in the promising area of direct environmental detection of opportunistic fungi by means of molecular methodology. Sporik *et al.* (1993) sampled directly for the Asp f1 antigen, a molecule specific to mycelial but not conidial stages of *A. fumigatus*, in house dust and in mixed fungal cultures derived from house dust in an American and an English community. Finding the antigen, indicating active *A. fumigatus* growth in dust, was rare in dust extracts but common in dust culture extracts. This suggested the common presence of ungerminated *A. fumigatus* conidia in household dust. PCR primers for the associated Asp f1 gene have been prepared and have been used to detect *A. fumigatus* antigen in patient urine (Reddy *et al.* 1993), but do not appear to have been used on DNA extracts from environmental materials. The relative ease of selectively culturing *A. fumigatus* from environmental materials may largely obviate the development and use of such methods. In related fields where specific detection of target organisms in culture is more difficult, molecular detection is more advanced. For example, the detection of aflatoxigenic *A. flavus* via the specific PCR amplification of genes involved in aflatoxigenesis, has been developed for some food substrates (Shapira *et al.* 1996; Farber *et al.* 1997).

PREVENTION OF SIGNIFICANT EXPOSURE

In many areas, avoidance of significant exposure to indoor sources of virulent fungal pathogens means avoiding aspiration of material from gross accumulations of bird and bat guano. If a building is found to contain such a site, it should be well closed up and temporarily sealed, e.g. with tape around attic trap doors or plastic sheeting over vertical doors. Anyone entering the area is recommended to wear HEPA filter respiratory protection (not a conventional dust mask!) and suitable disposable protective clothing. Precautions normally used for asbestos cleanup and similar procedures should be followed, e.g. disposal of protective suit, showering while still wearing respirator mask until potentially adherent particulates have been washed from hair and skin, and so on. Disposable materials should be placed in suitable heat-resistant bags and autoclaved prior to being discarded as autoclaved biohazardous waste. For decontamination of source material, see below. All laboratory work should be done in a Class 2 biosafety cabinet.

Prevention of pneumocystosis in vulnerable patients has been accomplished only by the indefinitely prolonged administration of prophylactic drugs. Details about prophylaxis are beyond the scope of this chapter.

The now widespread recognition that construction or renovation activities in or adjacent to hospitals may pose a profound risk of aspergillosis to neutropenic patients has led to development of stringent infection control procedures. Representative procedures are outlined in the Ecology and Epidemiology section, above. Precautions for neutropenic individuals discharged to home environments are also discussed above. Prophylaxis is common in such patients, but as mentioned above, is beyond the scope of this paper. Immunocompetent individuals who are actively disturbing (e.g. renovating) indoor environments known to contain amplifiers (that is, documented *in situ* growth sites) of opportunistic respiratory pathogens, especially *A. fumigatus*, *A. flavus*, *A. terreus*, *A. nidulans*, *Neosartorya pseudofischeri*, *P. boydii*, *S. prolificans*, *R. oryzae*, *R. microsporus*, *A. corymbifera* or *R. pusillus*, would be at an advantage to wear HEPA filter respiratory protection. This would protect not only against the unlikely possibility of a respiratory infection, but also against the possibility of organic dust toxic syndrome, which may accompany high exposure levels to such fungi. Similarly, if amplifiers of these fungi have not been identified in connection with the building, but unusually high levels suggestive of *in situ* amplification are seen in fungal air samples, the same precautions apply. It should be noted that outdoor levels of *A. fumigatus* may not uncommonly exceed 10 colony forming units (CFU) m⁻³ air in some temperate areas and may seasonally approach 100 CFU m⁻³ (Mullins *et al.* 1976; Solomon and Burge 1975).

As these opportunistic fungi are relatively uncommon in indoor proliferations on building structural elements, except in decaying material associated with a heat source, they need not be assumed to be present in every situation where indoor mould amplification is found. In the vast majority of indoor proliferations, these fungi will be scarce or lacking.

DECONTAMINATION OF AMPLIFIERS AND ACCUMULATIONS

Detailed procedures for disinfecting guano, soil and building structural elements contaminated with *Histoplasma* and *C. neoformans* are given by Ajello and Weeks (1983). Briefly, the procedures involve drenching the contaminated materials with 5% formalin solution on each of three successive days at temperatures over 15°C. Regrettably, despite the tendency of formaldehyde to leave potentially noxious residues in many materials (Vink 1986), no tried alternative disinfection procedure exists for virulent fungal pathogens in environmental bulk substances. Adequate worker protection must be employed with regard to both pathogen and formaldehyde exposure (see section on prevention, above, and also Ajello and Weeks 1983). In addition, the most up-to-date state/provincial, national or international worker safety standards must always be obtained. Adverse residual formaldehyde effects on building occupants (Wieslander *et al.* 1997) should as far as is possible be minimized.

Opportunistic fungal pathogens growing in buildings can be disinfected in the same fashion as other indoor moulds (see Chapter 2.3), but adequate precautions for prevention of respiratory exposure (see section on prevention, above) should be taken.

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Chapter 3.6

AIRBORNE MICROORGANISMS AND DISEASE—RESIDENTIAL BUILDING RELATED ILLNESS: EPIDEMIOLOGIC AND CASE—RELATED EVIDENCE

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INTRODUCTION

The association of respiratory and other diseases with modern living environments is not restricted to those in the fully developed market economies. The World Bank Report Investing in Health (1994) has drawn attention to the significant loss of life from indoor air quality in the developing countries. These losses are mainly due to the burning of wood or coal indoors without proper ventilation for either the fire to burn or the smoke to leave the building. In contrast, the effects of residential indoor air quality in the developed market economies concern allergic diseases including asthma, increased respiratory infections and nonspecific symptoms such as headaches and general malaise. The keeping of domestic animals such as dogs, cats and birds increases disease. Cigarette smoking indoors causes much disease especially in children, including increasing response to allergens. With rare exceptions, the strongest health signals come from damp housing. Home dampness results in increased fungal burdens (Lawton *et al.* 1998), which probably increase numbers of insects, and possibly mites. The widespread use of fleecy surfaces such as carpets in modern housing traps both gaseous and particulate indoor and outdoor pollutants.

Approximately 30 studies have been conducted on the association of dampness, mould and respiratory health in Europe and North America in residential housing. Within the Harvard six cities programme, the respiratory health of 4600 children was examined in relation to mould and dampness in the homes. These measures were correlated to several respiratory symptoms as well as a number of non-respiratory symptoms. The effect was of similar dimension to parental smoking (Brunekreef *et al.* 1989). In 30 communities in Canada studied by Health Canada, two studies of 15,000 children and 18,000 adults came to similar conclusions. The authors suggested that a non-allergenic mechanism was involved. A dose-effect was also seen in that more visible mould yielded more symptoms. (Dales *et al.* 1991a,b).

Most of these cross-sectional studies report an association between self-reported symptoms and home dampness and mould. Similar health effects have been found both in children and adults and by different investigators using different questionnaires. Several studies that included objective measures of fungi also had positive results (Verhoeff and Burge 1997). However, the issue is deeply complicated because of the emerging evidence that the contaminants of underventilated energy-efficient residential housing in the fully developed market economies interact to produce their effects. The derivation of an attributable risk for each component eludes investigators. This review covers the principal contaminants of residential housing (Table 1), with some reference to studies in the non-industrial workplace.

INFECTIONS

Viral infections

Viral infections are among the most common illnesses worldwide (Dolin 1998) and the most common cause of acute respiratory illnesses and asthma exacerbations (Johnston *et al.* 1995). Indoor overcrowding and close contact increase the risk of infection because viruses are transmitted through direct contact and aerosolized respiratory secretions (Dolin 1998; Ijaz *et al.* 1985). Rhinovirus infection, the most common cause of “colds” alters the host response to allergens. Lemanske *et al.* (1989) experimentally induced “colds” by nasal inoculation with Rhinovirus 16. Compared to the uninfected state, infection increased airways responsiveness to both histamine and ragweed (*Ambrosia*) pollen. Furthermore, infection increased the likelihood of a late asthmatic reaction occurring. This observation suggests that not only are viral infections and allergens individually important but synergistic effects may also be relevant in the indoor setting. For example, one could postulate that a subclinical

Table 1. Mechanisms and agents proposed or proved as causing building-related illness.

Infections
1. Viral infections
2. Tuberculosis
3. Legionnaires’ disease
4. Fungal infections
5. Pet-associated illnesses
Allergic and other immune-mediated disease
1. Asthma
a. Dust mites

- b. Cockroaches
- c. Furred and feathered pets
- d. Indoor fungi

2. Extrinsic allergic alveolitis

Fungal toxins and irritants

- 1. Idiopathic pulmonary haemosiderosis
- 2. Nonspecific symptoms and indoor fungal contamination
- 3. Immunomodulation and indoor fungal contamination

Endotoxin

Non-biological irritants

health effect of an indoor biological contaminant might become apparent only during a respiratory viral infection.

Tuberculosis

It is predicted that tuberculosis will cause 30 million deaths worldwide in the next decade (McDermott and Glassroth 1997). Between 1953 and 1985, the incidence of the disease declined by 74%, but it has since risen in large part due to the increase in HIV infection (ATS 1990; McDermott and Glassroth 1997). As discussed more fully in Chapter 3.4, *Mycobacterium tuberculosis* is spread by aerosolized respiratory secretions. Coughing disperses particles throughout room air where the smaller particle, 1–5 μm in aerodynamic diameter, remain airborne for prolonged periods. Household contacts of active pulmonary tuberculosis are at increased risk of developing active disease and outbreaks have occurred in exposed hospital staff. Isolation of infectious cases and appropriate pharmacotherapy are most important to prevent disease spread. However, indoor environmental control measures can also reduce the risk of transmission, as Riley *et al.* (1962) demonstrated in an elegant experiment. Guinea pigs were exposed to air exhausted from a tuberculosis ward. The air was either untreated or treated with ultraviolet radiation before exposure. Tuberculosis developed in the guinea pigs exposed to untreated but not treated air. This elegant experiment demonstrates the transmissibility of serious disease in the indoor setting and the effectiveness of air treatment. Frequent air exchanges, ultraviolet radiation and high-efficiency particulate filtration all reduce the concentration of aerosolized bacilli (McDermott and Glassroth 1997; Mehta and Dutt 1997), whereas crowding and poor ventilation enhance the spread.

Legionnaires' disease

Legionella pneumophila was recognised as the cause of Legionnaire's disease after investigation of a 1976 outbreak of pneumonia among members of the American Legion who were meeting in a Philadelphia Hotel (Fraser *et al.* 1977; McDade *et al.* 1977). This previously unknown organism is an aerobic Gram-negative rod found in freshwater. Amplification of the bacterium occurs in badly designed or maintained cooling towers or domestic hot-water systems (Chapter 3.4). Infection occurs through inhalation of infected aerosols from sources which have included cooling towers, showers, whirlpool baths and mist machines used in grocery stores. It is not a rare disease. Sporadic cases are recognised as causing approximately 2% of community acquired pneumonias (Bohte *et al.* 1995). Pontiac fever, also caused by *Legionella* spp., is a febrile "flu-like" illness without pneumonia that lasts 2–5 days. The bacterial etiology is perhaps more difficult to recognise, since there is no pneumonia and it may mimic a viral flu-like illness.

Fungal infections

Clinical cases of confirmed acute histoplasmosis are well recognised (Harrison and Levitz 1996). The last case the senior author personally diagnosed was a young man who was renovating an abandoned farmhouse. He had cleared a chimney full of birds' nests and droppings, presumably the source of the fungus. His father who helped him also suffered fever with an acute pneumonia. Anderson *et al.* (1996) recently reported a nosocomial outbreak of aspergillosis. Of 148 children attending a pediatric oncology unit, six (4%) developed aspergillosis and no cases were identified in other areas of the hospital. The exhaust filter of the ward vacuum cleaner filter was contaminated with *Aspergillus fumigatus*, and air was found to seep from the waste disposal area into the patient areas due to a defective door. *A. fumigatus* was cultured from the door frame. No further cases of aspergillosis were identified in the 18-month period following the correction of the two identified problems.

Pet-associated illnesses

Zoonoses associated with the indoor environment receive little attention and are probably rare despite the prevalence of pet ownership, which is about 48% in homes across Canada, for example. Human infection with the dog hookworm can result from skin penetration in crawl spaces contaminated with *Ancylostoma caninum* (Elliot *et al.* 1985). Transmission of dog heartworm, *Dirofilaria immitis*, by mosquitoes can cause a solitary lung nodule in man. Ingestion of contaminated cat faeces, not impossible among young children, can transmit the tapeworm, *Dipylidium caninum*, and *Toxoplasma gondii*. The latter can infrequently cause serious illnesses such as ocular infection possibly leading to blindness, and congenital infection which may cause birth defects (Elliott *et al.* 1985). Psittacine birds (budgies, parrots, parakeets) infected with *Chlamydia psittaci* transmit this infection from their tissues and secretions. Inhalation by the pet-owner can cause an acute fever, headache, and pneumonia. Prevention of illness necessitates vaccination of

household pets and treatment for parasites. III pets should be seen by a veterinarian. Apart from infection, furry and feathered pets are a common cause of asthma and allergic rhinitis, and extrinsic allergic alveolitis can be caused by bird antigens (bird fancier's lung).

ALLERGIC AND OTHER IMMUNE-MEDIATED DISEASES

Asthma

Dust mites

Dust mites are small acarids that thrive in damp and warm environments, and eat skin scales. They are found in highest concentrations in mattresses, upholstered furniture and carpets. They are one of the most common known causes of asthma throughout the world, with the majority of asthmatic children being sensitized to dust mites (Platts-Mills *et al.* 1987). Threshold levels for sensitization ($>2 \mu\text{g mite antigen g}^{-1} \text{ dust}$) and acute symptoms ($>10 \mu\text{g g}^{-1}$) are commonly quoted. However, when Sporik *et al.* (1990) followed children from infancy to the age of 11 years, an inverse continuous relationship was demonstrated between the level of Der p 1 to which they were exposed during infancy and the age at which the first wheezing episode occurred. A recent study by Peat *et al.* (1996) reported a continuous dose-response relationship between the concentration of dust mites and the risk of developing childhood asthma, with relative risks being around 1 at concentrations of $2 \mu\text{g g}^{-1}$ of mite antigen in dust to c. 5 at concentrations $>10 \mu\text{g g}^{-1} \text{ dust}$. The argument for a causal association has been strengthened by older controlled clinical studies which have shown reductions in symptoms, and improved airways reactivity, following environmental clean-up (Murray and Ferguson 1983). Reflecting on these studies, and in the light of new information, one wonders if some component of the observed health effect of dust mites was due to coexisting bacterial endotoxins or fungi, both of which proliferate in damp environments and both of which cause disease. Michel *et al.* (1996) recently reported that at Der p 1 levels $>10 \mu\text{g g}^{-1}$ asthma control was related to endotoxin levels, but not to dust mite levels. Clearly future studies of dust mites must take into account these co-contaminants.

Cockroaches

Although always with us, cockroaches have recently come under the scrutiny of asthma epidemiologists (Kang *et al.* 1996, Sarpong *et al.* 1996). One of the most influential articles is the recent one by Rosenstreich *et al.* (1997). Some 476 children with asthma living in American inner cities underwent allergy skin testing and analysis of their house dust. At 37%, skin-test positivity was most prevalent for cockroach antigen, with dust mites being the next most common at 35%. Some 85% of bedrooms had detectable cockroach allergen, with 50% being above 8 units Bla g 1 allergen $\text{g}^{-1} \text{ dust}$, a level considered to be capable of inducing disease. Those children with positive skin tests to cockroach and high exposures had 50–100% more indicators of poor asthma control compared to those with negative skin tests or with low exposures. In light of the

identified interaction between dust mites and endotoxins, there may be similar synergistic effects between endotoxins and cockroach allergen.

Furred and feathered pets

The prevalence of asthma in animal handlers varies between 3 and 30% (Chan-Yeung and Lam 1986). In the residential setting, cats are considered one of the most allergenic animals. The major allergen Fel d I is produced primarily in cat sebaceous glands and skin epithelium. Unlike heavier dust mite particles which tend to sediment, cat allergen is carried on particles which can be $<2\ \mu\text{m}$ in diameter, resulting in detectable air concentrations even when the room is undisturbed (Platts-Mills *et al.* 1991). It has been estimated that cat-allergic persons would show significant bronchoconstriction if they stayed for 30 min in a $33\ \text{m}^2$ room with a cat. Short of banishing the cat from the house, some reduction in antigen levels may be achieved by minimizing carpets and upholstered furniture, by damp-mopping, by vacuum cleaning with a cleaner fitted with HEPA filters and by washing the cat regularly (Middleton 1991).

Indoor Fungi

The allergenic and asthmagenic potential of outdoor phylloplane fungi is well documented (See Chapter 3.1). *Alternaria* inhalation under laboratory controlled conditions produced acute bronchospasm (Hopkins *et al.* 1930), and sensitization to *Alternaria* is a risk factor for respiratory arrest in asthma (Hopkins *et al.* 1930). As far as fungi from indoor sources are concerned, allergic sensitization has been found for *Aspergillus*, *Penicillium* and *Wallemia* (Pross *et al.* 1987; Sakomoto *et al.* 1989), and Tarlo *et al.* (1988) reported that 14 of 26 allergic subjects (rhinitis, asthma), had positive skin prick tests to fungi in their homes. However, the potential of fungi with indoor sources to cause asthma is not well documented. Judging the allergenic potential of a fungus requires identification of the allergenic components. This is difficult because of the hundreds of species that can be found in homes, and also because a single species may have several allergenic components (Chapter 2.1). Until the allergens of a species can be identified it will not be possible to prepare extracts of known allergenic potency for human research. Nonetheless, Dekker *et al.* (1991) estimated that the maximum attributable risk for asthma related to reported mould exposures in Canada was c. 20%.

Hypersensitivity pneumonitis (extrinsic allergic alveolitis)

Salto *et al.* (1982) reported hypersensitivity pneumonitis in mouldy homes in Australia. Several case reports have documented the potentially adverse health effects associated with humidifier use. Banaszak *et al.* (1970) reported hypersensitivity pneumonitis (HP) in four of 27 office workers exposed to a contaminated humidification system. Symptoms included fever, chills, dyspnoea, impairment of pulmonary function, and chest X-ray infiltrate. A thermophilic actinomycete was cultured from the humidification system. Avoidance of exposure resulted in complete recovery. Fink *et al.* (1976) documented eight cases of HP due to contamination of home or office air conditioning systems. Hodges *et al.* (1974) recorded the first case caused by contamination of a cool-mist vaporizer, and Burke *et al.* (1977) reported on 2 patients who were exposed to home

humidifiers contaminated by thermophilic Actinomycetes. As in previous cases, exposure avoidance resulted in clinical improvement. These case reports are very few in relation to the large number of people exposed to humidifiers. They cannot address the magnitude of the risk of adverse health effects from humidifiers or guide clinical recommendations. One cross-sectional population survey did detect a health risk roughly equivalent to environmental tobacco smoke, but causality was not established (Dekker *et al.* 1991). Without random assignment to humidifier use, it was not possible to determine whether using a humidifier provoked asthma symptoms or whether the asthma symptoms prompted use of the humidifier. To address this issue, Dales *et al.* (1996) carried out one randomized controlled study of humidifier use on asthma. No effects were found, indicating that aerosolizing any inorganic particles (Highsmith *et al.* 1988) or biological contaminants in the average home humidifier has no acute effects on asthma control. This result in no way excludes the possibility of an important health effect arising from the exposure of a susceptible subject to an aerosol from a heavily contaminated humidifier.

FUNGAL TOXINS AND IRRITANTS

Idiopathic pulmonary haemosiderosis

In Cleveland, Ohio, paediatric pulmonologists at the Rainbow Babies' and Children's Hospital recognised that there had been an unusually high number of cases of idiopathic pulmonary haemosiderosis between January 1993 and December 1994. Ten were diagnosed during this time period, compared with three in the previous 10 years. They were found to be clustered within a 6-mile radius within an area that is prone to flooding because it receives water drainage from other parts of the city. This prompted a case-control study of ten cases diagnosed during their first 6 months of life and 30 age-matched controls living within 6 miles of the cases. Cases were mostly blacks with low incomes living in poorly maintained older homes with water damage (Montana *et al.* 1997, Etzel *et al.* 1998). Odds ratios were 9 for male sex and 16 for water damage; it was subsequently revealed that the odds ratio was 10 for airborne CFU *Stachybotrys* m³ indoor air. More than 30 cases of idiopathic pulmonary haemosiderosis have now been diagnosed, and ten infants have died. After hospital discharge, the risk of recurrence was five times more likely if the infant was exposed to environmental tobacco smoke. *Stachybotrys* as a causal factor is biologically plausible. This fungus produces trichothecenes, mycotoxins that can cause pulmonary haemorrhage in domestic animals and are present in mycelium and spores. In infancy, human pulmonary capillaries may be particularly susceptible to inhibition of protein synthesis caused by these mycotoxins. Questions remain, however. Why have endemic areas not been detected in other areas similarly prone to flooding? Why were cases not detected prior to 1993? Perhaps there is a synergistic effect of an unidentified agent. This is not unlikely, given the recently recognised interaction between ozone and allergen, and the possible interaction between endotoxin and allergen.

The idea that high levels of exposure to *S. chartarum* contributed to the development

of pulmonary haemosiderosis in the Cleveland infants, leading in some cases to death. (Dearborn *et al.* 1994, 1997, Etzel *et al.* 1998) has been challenged recently by the discovery that there were serious problems with the exposure assessments. However, the issue remains unresolved (Anon. 2000). There have been two other case reports on haemosiderosis in infants (Flappan *et al.* 1999, Novotny and Dixit 2000) and one in a child (Elidemir *et al.* 1999) associated with *Stachybotrys* or wet housing. In this last case report, viable *Stachybotrys* spores were recovered from lung lavage of the affected child (Eledemir *et al.* 1999).

Nonspecific symptoms and indoor fungal contamination

Description of symptoms

A large body of literature from several countries has confirmed the association between reported illness and reported dampness or fungal growth. A wide range of symptoms has been associated with home dampness and fungal growth. Symptoms suggesting mucosal irritation are eye, nose and throat discomfort. Among the lower respiratory symptoms—cough, wheeze, and self-reported asthma—the association with dampness is often strongest for cough. Other nonspecific complaints enumerated include depression, fatigue, malaise, backache, arthritis, headache, fever, flu-like symptoms, vomiting, diarrhoea, dermatitis, alopecia, sore throat, cough, rhinitis and epistaxis (Martin *et al.* 1987; Platt *et al.* 1989; Hyndman 1990; Johanning *et al.* 1996). This large number of reported symptoms would suggest either an over-reporting bias or the induction of a generalized malaise.

Objective health measures

The lack of association between objective measures of indoor fungal growth and objective measures of illness has weakened the argument for causality. In studies by Strachan (1988), Brunekreef *et al.* (1989), Hyndman (1990) and Brunekreef (1992), neither ventilatory function nor exercise-induced bronchoconstriction could be related to exposure. Possible explanations for this discordance include (1) the objective measurements of fungi not being accurate; (2) the objective measurements not being sensitive enough to detect a small decrement in lung function; (3) the mechanism of illness not being related to airway function; and (4) there being no real increase in illness, but rather subjects who are, or think that they are, in a contaminated environment being more likely to report symptoms. This would be consistent with the wide range of seemingly unrelated symptoms without easily detectable objective signs. Recently, however, two lines of research have provided evidence for objective health effects and the same time suggested pathogenetic mechanisms of disease. Severe pulmonary haemorrhage has been associated with a high degree of fungal contamination (Montana *et al.* 1997; Etzel *et al.* 1998). Secondly, two different studies have detected an association between fungal contamination and blood lymphocyte populations. These studies are discussed in the sections of this chapter dealing with idiopathic pulmonary haemosiderosis and immunomodulation.

Measures of Exposure

Symptoms are almost invariably associated with self-reported mould and/or dampness. As in the issues surrounding health measurement, self-reports may not be accurate and could be biased by the pre-existing symptom status of the subject. The objective measures of fungal exposure, required to strengthen the argument for causality, are still under development. Traditional measurements have several limitations. Intuitively, air samples seem more likely to represent inhalation exposure than are dust samples. However, air samples are usually taken over a few minutes although there are thousand-fold diurnal variations in air concentrations due to particles alternately settling and becoming resuspended. Collection of viable (or culturable) particles allows species identification, but set against this is the fact the non-viable (non-culturable) fractions, which are not measured by such methods, may comprise anything up to 99% of the air spora. The traditional quantitative sampling of dust and air in health studies was recently reviewed by Verhoeff and Burge (1997). The majority of studies found no association between health and total culturable fungi whether in dust or air. Although positive associations were reported in seven of nine studies, the exposure variables were not the same. They ranged from total airborne fungi to culturable fungi or different species. None of these studies used standardized methods such as those described by the American Industrial Hygiene Association (Dillon *et al.* 1996).

Apart from population studies, there are many reports of illness associated with indoor fungal contamination. Case reports of building-related symptoms (Croft *et al.* 1986; Mainville *et al.* 1988; Johanning *et al.* 1996) and the Cleveland cases of pulmonary haemorrhage (Montana *et al.* 1977; Etzel *et al.* 1998) were all associated with *Stachybotrys chartarum*. As mentioned in Chapter 2.1, this is a hydrophilic mould, and can produce highly toxic macrocyclic trichothecenes (Sorenson *et al.* 1988). Some *Penicillium* spp., also potentially toxigenic, have also been associated with nonspecific symptoms (Auger *et al.* 1994). Other adverse human health effects postulated to result from mycotoxin inhalation can be found in uncontrolled case-reports. These include renal failure (Di Paolo *et al.* 1993), tremorgenic encephalopathy (Gordon *et al.* 1993) and organic dust toxic syndrome (Yoshida *et al.* 1989).

In addition to the repeated incrimination of toxigenic fungi as causes of illness, another objective measure of fungal presence has been repeatedly related to symptoms. The polyglucose component (1 $\rightarrow$ 3)- β -D-glucan, of fungal cell walls and also contained in some bacteria and vegetable material, has been found in the air of waste composting facilities at geometric mean levels as high as 19 $\mu\text{g m}^{-3}$, and in house dusts at levels ranging from 750 ng g^{-1} mattress dust to >1,200 ng g^{-1} floor dust (Douwes *et al.* 1996). Rylander *et al.* (1989, 1992) have shown a relation between airborne β -glucan concentrations and upper respiratory tract irritation in both residential and day-care settings. More recently, Rylander (1997) demonstrated that renovation of a day-care centre resulted in a decrease of glucan from 11.4 to 1.4 ng m^{-3} air, and a reduction in the 1-sec forced expiratory volume (FEV1) response corresponding to that caused by 1.20 mg of methacholine. Cigarette smoke and glucan synergise to increase the amount of inflammatory cells in guinea pig lungs (Sjöstrand and Rylander 1997). This interactive effect needs to be tested in humans but such an effect would be consistent with the other

previously described interactive effects between allergens and ozone (Molfino *et al.* 1991), endotoxin (Michel *et al.* 1996) and viral infection (Lemanski *et al.* 1989).

Summary

Evidence supporting a causal relation between indoor fungal contamination and health is accumulating. Immune measures have provided objective measures of a health effect while the repeated presence of *Stachybotrys* and elevated (3-glucan are providing objective evidence of excessive fungal contamination. These objective measurements, however, have been associated with acute or subacute symptoms in highly exposed subjects. The mechanisms that might be responsible for the finding of increased symptoms in the general population that reports residential mould growth have not been established.

Immunomodulation and indoor fungal contamination

Johanning *et al.* (1996) reported lower CD4/CD8 ratios in the group of employees exposed to *Stachybotrys chartarum* compared to a control group. Among 49 New York employees exposed to a satratoxin H-producing strain of *Stachybotrys* in an office building ($>100,000$ CFU cm^{-3} in sheetrock walls), 43 underwent a health examination. Complaint involved the general areas of respiratory, fatigue, central nervous system, and skin and eye irritation. Immunoglobulin E specific for *Stachybotrys* was detected in four subjects. No control subjects were mentioned, making it impossible to determine if there was an exposure-health association in this descriptive study.

Dales *et al.* (1998) contrasted lymphocyte populations, measured by flow cytometry, between a group of children living in homes with more fungal contamination ($n=39$) and a group in less contaminated homes ($n=20$). Indicators of fungal biomass were viable fungi in house dust and airborne ergosterol (Chapter 4.1) in the child's bedroom. Living in more rather than less contaminated homes was associated with a larger number of CD3+ T-cells expressing CD45RO ($1.5 \times 10^9 \text{ L}^{-1}$ vs $1.1 \times 10^9 \text{ L}^{-1}$, $p=0.05$, two-tailed t-testing) and a reduced CD4/CD8 ratio (1.6 vs 1.8, $p=0.04$). The differences persisted over a 12-month period, and were not explained by the child's age or total serum IgE; dust mite antigens; or the presence of furry or feathered pets, or a humidifier.

ENDOTOXIN

As noted in Chapter 1.2, most bacteria indoors are Gram-positive species and are shed from humans. The sources of Gram-negative types include water reservoirs such as cooling towers, humidifiers and biofilms in toilets. Organic dusts, composed of animal, vegetable, or microbial material also promote the growth of Gramnegative bacteria and are consequently a source of endotoxins (Rylander 1995), which are lipopolysaccharide (LPS) components of the cell wall in these bacteria. In occupational settings, endotoxin inhalation has been implicated in organic dust toxic syndrome, bysinosis, and illness in swine-confinement workers. Acute symptoms include fever, chills, malaise, cough, dyspnoea and headache (Rylander 1994). Chronic health effects have also been reported.

Recently, Voelznag *et al.* (1998) detected an accelerated decline in lung function among 171 pig farmers followed for three years. The FEV1 fell by 73 ml per year at an exposure of 105 ng endotoxin m^{-3} air and a dose-response relationship was present. A doubling of endotoxin was associated with an FEV1 decline of 92 ml per year. The presence of a dose-response curve suggests that the lower levels of exposure found in homes may cause illness, albeit less dramatic than in the occupational settings. Michel *et al.* (1991) measured endotoxin concentrations in the house dust of 28 subjects with chronic stable asthma. Exposure to higher levels (>5.6 ng LPS ml^{-1}) was associated with poorer asthma control measured by symptoms, medication use and lung function. In a subsequent study, Michel *et al.* (1996) refined their previous findings in a group of 69 subjects with asthma who were sensitized to house dust mites. Asthma control was related to house dust endotoxin, averaging 2 ng mg^{-1} dust in those exposed to Der p I levels >10 $\mu\text{g g}^{-1}$ dust, but not in those exposed to lower levels of the major dust mite antigen. This suggests an interactive effect between injurious indoor agents: other examples will be discussed below.

NON-BIOLOGICAL IRRITANTS

Among non-biological irritants are allergens and irritants in cleaning agents, as well as dusts and fumes from hobbies. Tobacco smoke is the most important combustion product and the major source of fine particles indoors. PM3.5 are increased by 1 $\mu\text{g m}^{-3}$ air (averaged over a 24-h period) for each cigarette smoked (Wallace 1996). Approximately 40% of Canadian children are exposed at home to environmental tobacco smoke, which increases the risks of sudden infant death syndrome (SIDS), emergency visits and hospitalization for respiratory disease, middle ear disease and exacerbation of chronic respiratory symptoms and asthma. An increased risk of idiopathic pulmonary haemosiderosis or other toxic insults may be the latest addition to those diseases associated with tobacco smoke. Poorly installed, vented or maintained gas- and wood-burning units can significantly increase indoor levels of carbon monoxide, nitrogen dioxide and particles. One study found that indoor carbon monoxide levels were <5 ppm for airtight stoves, but up to 8 ppm for those that were not airtight. Maximum particle concentrations ranged from 71 $\mu\text{g m}^{-3}$ air with airtight to 650 $\mu\text{g m}^{-3}$ with non-airtight stoves (Traynot *et al.* 1987). Auto-mobile fumes have been observed to enter homes with attached garages.

Low-level exposure to ozone is an important factor in the frequency of respiratory admissions to hospitals (Burnett *et al.* 1994). Pre-exposure of house dust mite-sensitive asthmatics to ozone was found to reduce the amount of antigen required to induce a given response by approximately 3 times. Ozone-exposed subjects had increased eosinophils, neutrophils and eosinophilic cationic protein (Devlin *et al.* 1994).

Volatile organic compounds (VOC's), the gaseous chemicals released by numerous sources, such as paints, lacquers, glues, personal grooming agents (perfumes, hair sprays), cleaning agents and building materials, must also be considered. Volatile concentrations from consumer products in indoor air are typically 10 times but can be up

to 100 times those of outdoor air (Wallace *et al.* 1985). Unlike tobacco smoke, fine particles and ozone, it is not clear what role these may play in health (Mølhave *et al.* 1997). They may also affect response to biological contaminants.

The prevalence of asthma attributable to parental smoking has been estimated at 7.5% (Stoddard and Miller 1995). However, a longitudinal study in New Zealand demonstrated that asthma was triggered by exposures to allergens dust mites, cat and mould and that tobacco smoke acted to exacerbate the allergic response (Sears 1998). This is probably the same with other irritants such as endotoxin and (1→3)- β -D-glucan mentioned earlier in the chapter. This general feature of asthma, i.e. a hierarchy of contributing factors, has been reported in several studies (Fischer *et al.* 1998; Marbury *et al.* 1966). Non-biological irritants can play an important role in confounding patient response to the microbial contaminants discussed in this chapter.

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Chapter 4.

Microbiological investigation of indoor environments

Chapter 4.1

MYCOLOGICAL INVESTIGATIONS OF INDOOR ENVIRONMENTS

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INTRODUCTION

The firm recognition in the past few years that saprotrophic fungi and bacteria could have serious, even life-threatening, health consequences has revealed the need to investigate residential buildings and non-industrial workplaces for microbiological contamination. Investigations that are carried out for this purpose are however of uneven quality, because there are few people with the range of experience necessary to conduct sophisticated investigations, and because of the lack of standardized protocols for the microbiological sampling that might be necessary. The American Industrial Hygiene Association (AIHA) published a manual of consensus methods for microbiological sampling that has partially addressed this aspect of the problem (Dillon *et al.* 1996). The American Conference of Government Industrial Hygienists (ACGIH) has recently published a more comprehensive manual (Macher *et al.* 1999) on microbiological problems of buildings. This includes chapters on investigation and remediation and recognizes the AIHA manual. In the present chapter, an attempt will be made to put this information into a practical context and provide information on the limitations of the data obtained using different sampling methods. The sampling of *Legionella* has been covered in Chapter 3.4, and sampling for saprotrophic bacteria, endotoxin and peptidyl glycan (peptidoglycan) is discussed more fully in Dillon *et al.* (1996).

The goals of any investigation are to establish the nature and extent of fungal contamination, to minimize the possibility of false negatives and false positives and to determine the causes and degree of risk borne by the occupant population. At present, the degree of risk of exposure to saprotrophic microorganisms is frequently assessed by determining the area of contamination. This is partly a reductive position: current methods of sampling for saprotrophic microbes and endotoxin do not assess exposure in the strict sense. There are, however, positive reasons for this position. Dales *et al.* (1998) showed that children living in more, as opposed to less, mouldy homes had statistically and clinically significant (but small) changes in lymphocyte cell sub-populations. This biological difference was associated with the presence of visible mould on 1–5% of the area of exposed surfaces in the occupied space, the visible moulds in question being a mixture of toxigenic species often associated with gypsum board, or wallboard (Chapter 2.1). The principle that occupant risk is associated with the area of visible mould had

been articulated previously in the so-called New York Guidelines (Anon. 1993). The Canadian Federal-Provincial Committee on Occupational Safety and Health (Health Canada 1995) and the International Society for Indoor Air Quality and Climate (Flannigan and Morey 1996) have endorsed this.

INFORMED INSPECTION

The first step in investigating a building for microbial contaminants is an informed inspection, preferably by someone with a knowledge of engineering, architectural and moisture problems in buildings (Miller 1992). Contamination by saprotrophic bacteria is only likely to reach a level that may be of significance for health in stagnant water, such as is found in some humidifiers, drain pans and similar equipment. However, as has been discussed in Chapter 2.1, fungal contamination can develop because of water present on and in materials for a variety of reasons, including condensation, flooding and various types of leakage. Investigation of mould problems therefore requires a thorough knowledge of the design of the building envelope and of the types of failure that result in condensation and water leaks. Design issues have been discussed in detail in Trechsel (1994), and anyone carrying out an inspection should be aware of these. To take an example, where subtropical conditions prevail for part or most of the year, for instance in North America the climates of Southern Ontario and Louisiana, respectively, improper installation of the elements that control heat, air and moisture can lead to the growth of moderately xerophilic fungi such as *Aspergillus versicolor* on gypsum board or on the inner surface of the wall covering. These elements include air retarders (un-painted wallboard, spin-bonded polyolifin), vapour barriers (vinyl wallcovering, polyethylene sheeting) and insulating material. Since the non-insulated side of a layer that has been insulated on only one side is colder, measures must be taken to ensure that condensation does not occur at that surface. Chronic water leaks can lead to contamination by *Chaetomium* and *Stachybotrys* (Morey 1997).

Investigators should be aware that studies of buildings in the high humidity areas of USA have shown that uncontrolled airflow and rainwater barriers are the causes of most mould problems. Should the moisture status of buildings be negative in relation to that outside, moist air will be “pulled” into the wall cavity, leading to condensation and mould growth, regardless of whether the wall is correctly constructed or not. If rainwater leaks into the envelope of a building in which the moisture status is negative to the outside, problems will arise very quickly indeed. Perversely, mould growth—and the consequent damage and ill-health that can arise from it—may occur more frequently in expensive buildings with complex constructional requirements than in buildings of a more basic structure. Commonly, problems are the result of uncontrolled airflow due to leaking ducts and supply/exhaust imbalances bringing in warm wet air to cold surfaces (Odom and Du Bose 1996).

Failures in caulking, joints and other construction details, especially around windows, can lead to water leaks into wall cavities. This is a substantive problem in buildings. In Canada, a formal study of mould-damaged buildings in the Pacific Northwest revealed

that stucco was particularly likely to suffer from construction detail failures (Table 1). Poor installation of windows was the greatest cause of water leaks and mould growth in this set of buildings. Regardless of the type of cladding, the relative prevalence of water/mould damage problems was similar; detailing failures were for some reason more common in buildings with stucco cladding.

A detailed study of moisture and mould problems in 59 single-family dwellings in Southern Ontario found that objective measurements of mould and other biological contaminants could be accounted for by the degree of visible mould apparent during inspection. This was because much of the contamination was hidden in wall cavities. The greatest source of mould (Table 2) was on the envelope and was explained by condensation; a variety of other sources, including interior walls and ceilings of bathrooms, refrigerator drain pans and sumps, closely followed. Wicking of water into basements resulted in 30% of those surveyed showing mould growth (Lawton *et al.* 1998). As with buildings which have been discussed above, air flow in and out of buildings accounts for an appreciable percentage of water movement in residential housing. Evidence of air flows from patterns of dirt on the bottoms of closed interior doors, and other clues,

Table 1. Analysis of mould damaged buildings in the Pacific Northwest (British Columbia), after Anon. (1996).

Type of fault	Exterior finish		
	Stucco	Vinyl	Wood
Windows: no sealants at frame/cladding joints	9	1	
Windows: no sealants at corner mitre joints	10	1	1
Windows: poor flashing at head or sill	13	3	
Windows: poor paper installation	6	1	
Problems due to windows	27%	19%	16%
Poor deck/walkway/balcony waterproofing: field	12	3	1
Poor deck/walkway/balcony waterproofing: Junction with walls	16	1	
Problems due to deck/walkway/balcony	20%	19%	8%
Poor guardrail saddle joints	133	5	1
Poor guardrail cap flashings	8	1	4
Poor parapet cap flashings	7	1	
Problems due to horizontal surface flashings	20%	31%	36%
Poor base/transition/control joint flashings	14	1	
Poor roof/wall joint flashings	3	1	1

Poor concrete slab/wall joints	3	1	1
Poor dryer vents: leaking in wall	6	2	
Poor other details	8	2	1
Material/installation defects: cladding, sheathing	10	2	4
Problems due to other defects	33%	31%	40%

can shed light on the movement of air (Lstiburek and Carmody 1994).

A final category of mould and water damage in buildings which investigators should understand is the potentially catastrophic effect of water used to extinguish fires, and from hurricanes, earthquakes, floods or other natural disasters (Morey 1993; Miller *et al.* 2000).

The formal studies mentioned above all support the conclusions drawn from numerous case studies by investigators such as Morey (1997). As mentioned earlier, the physical investigation of moulds in both commercial and domestic buildings requires expert knowledge of the design, construction and operation of these structures. Microbiological sampling strategies should neither be designed nor executed unless a thorough building inspection is made either concurrently with or before the sampling. Sampling is typically carried out in order to document exposure and, importantly, to identify hidden contamination which only becomes evident on extensive destructive testing. After the results of sampling results are available, the data must be compared with the information obtained in physical inspections, including any new physical observations, which are prompted on examination of these sampling results. "Are the results plausible?" is a question that must always be asked and answered so that proper assessment can be made of the risk of false-negatives and false-positives for mould contamination. Additionally, documentation of the sources and nature of the contamination allows a failure analysis to be made for the building (or system); this will assist in developing further cost-effective investigation strategies and ultimately determine any remedial action that is necessary.

Table 2. Ascribed sources of water for visible mould growth in 59 single family dwellings in Southern Ontario, after Lawton *et al.* (1998).

Water source	Number of reports	Visible mould (%)
Condensation on envelope	95	36
Condensation on bathroom surfaces, refrigerator pans, sumps	53	34
Wicking from ground	12	16
Rain leaks	5	6
Plumbing accidents	8	5

DOCUMENTATION OF MOULD AREA

For both determining the sources and nature of mould contamination and estimating the degree of risk, documentation of the area of contamination is necessary. Within the informed inspection component of the overall investigation, detailed notes of the amounts of mould should be taken on the appropriate perspective of the building plans. The area of mould should be sketched on the plan, making the best effort to document the affected area. Video and photographic evidence can also be collected, but in many cases it is the written documentation and integration of the information that is most useful. Photographic evidence can be used to provide detailed information when failure analyses are being conducted.

Bulk samples (see following) should be taken from the visibly mouldy areas, and an equal number of samples should be taken from areas away from the visible contamination. Decisions can be made later as to whether to process these or not, but any decision should be made in the knowledge that such samples permit an evaluation of the mould just below that visible on the substrate. The colour of visibly mouldy material usually depends on the coloration and numbers of conidia present, since the vegetative mycelium is often non-pigmented. In the field, however, contamination can be substantial without the density of conidia being sufficient enough to make it visible to the naked eye, e.g. on gypsum board (see following).

Although a further 1 metre band of building material is removed from around the area visibly contaminated during remediation (depending on the circumstances), collection of bulk samples from areas not visibly mouldy will provide objective data on the true extent of contamination. In some circumstances, the processing of material that is not visibly mouldy removes the suggestion of bias from the investigation. Formal scientific studies necessarily involve “control” samples and field studies are not relieved of this burden, at least in principle.

Material from at least some bulk samples should be studied microscopically or plated on agar media in order to assess whether the colour on the building materials is indeed mould. Often, laboratory examination of samples of building material shows that mould is present although it has not been recorded under field conditions owing to, for example, poor light or the difficulty in seeing mould through a full-face respirator.

Where there is strong reason to believe that there is appreciable mould in hollow walls, estimations of the area contaminated by moulds should be made by removing the bottom 0.3 m from the inner side of walls. The real extent of contamination cannot be estimated without doing this, and it involves no more than using a keyhole saw and a boroscope (Miller *et al.* 2000). The cost of such destructive testing may ultimately be less than not doing it at all!

The field notes made should be accurate enough to permit the estimation of the area of contamination in square metres, and to allow faithful documentation of the points where

bulk samples were collected. An example of the kind of record that should be taken is found in Fig. 1, which maps mould-affected areas on interior walls.

Destructive testing of this type should be carried out within simple containments. Appropriate respiratory protection is required (ASHRAE 62-1989R; Macher *et al.* 1999). Polyethylene sheeting and duct tape can be used to make appropriately sized floor-to-ceiling containments (which also cover the floor) with a simple slit/door made from an additional piece of polyethylene sheet. Negative-air machines or a simple industrial vacuum cleaner with a HEPA filter should be used to make the air pressure in the containment negative to the occupied space.

In cases where these enclosures need to be large, other strategies can be used (Shaughnessy and Morey 1999). As personnel back out of containment areas, they should remove their coveralls and shoe covers, leaving them inside.

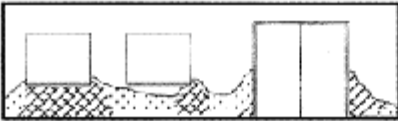
FIELD NOTES FOR INVESTIGATION AT: SAMPLE PROJECT

INTERIOR ELEVATIONS SCALE 1:100

Date: 12/12/00
Time: 10:15 AM
Bldg: _____
Unit: _____

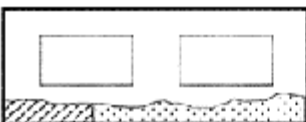
KEY TO MOLD OBSERVATIONS

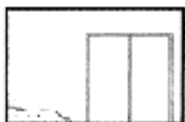
 CLEAN
 LIGHT
 MODERATE
 HEAVY

A

DINING/LIVING ROOM

D

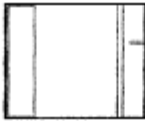
DINING

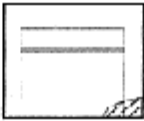
B

LIVING ROOM

C

LIVING ROOM

A

BEDROOM 1

B


C


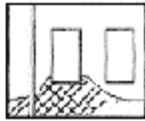
D


Fig. 1. Example of field note from an inspection for mould.

BULK SAMPLES

The term “bulk samples” refers to samples of building material which are physically removed by destructive methods, and also dust samples. There are two main methods of isolating fungi from bulks, including fabricated building material, viz. dilution plating and direct plating of fragments. Such cultural methods, particularly dilution plating techniques, are selective and usually fail to discriminate between actively growing and inactive organisms in the sample.

Dilution plating of bulk samples

A small, representative sample of a powdered material such as settled dust or ground-up gypsum board is suspended in an appropriate diluent and diluted in 10-fold steps. Aliquots are plated on agar media (in triplicate at least) and the suspension is evenly spread over the surface. The agar plates are incubated for a number of days, which depends on the medium and the incubation temperature, and the colonies that develop from propagules (living spores and hyphal fragments) associated with the dispersed material are then counted. An inoculum of mycelium or spores from these colonies is then transferred to appropriate agar media for identification of the species present. A good first approach is to transfer inoculum from all colonies on the isolation medium onto 2% malt extract agar (MEA) and Czapek-yeast extract agar (Pitt 1988). The aim of the whole procedure is to dilute to extinction the number of propagules from which colonies may arise, i.e. until there are no or very few colonies developing on the plates inoculated with aliquots from the highest dilution plated (Dillon *et al.* 1996). Interference competition between isolates (see following) is reduced, but not eliminated, by this technique. Based on their experience, sometimes commercial laboratories plate only one dilution, but choosing the wrong dilution risks having plates that are either too overcrowded with colonies to be counted or have so few colonies that only the most abundant species present are detected.

As mentioned earlier (Chapter 2.1), different species of microorganism have different growth requirements, so the use of any medium produces different “recoveries” or “recovery rates”. The spores of different species decline in viability with time, some remaining viable for years, but in other species the spores may only be viable for a few months at most. In general, the number of fungal propagules determined by cultural methods is substantially smaller than the total number of spores determined by direct (microscopical) methods. Counts of viable or culturable spores may be 1–50% of total counts, but this varies very much with species. This is mainly because dormant spores still respire and exhaust their endogenous reserves, and also because they dehydrate. The spores of *Stachybotrys chartarum*, for example, have a half-life of months, while those of *Penicillium* perhaps 10 years (Miller 1992). This means that when there are very large numbers of spores present it takes many years for all of them to die. At the same time, it means that when the percentage of viable spores is low it is possible to estimate how long it has been at a minimum since there was active growth. Another reason for differences in

recovery rate between species is that some species, e.g. *Trichoderma* spp., are very “aggressive” in culture and produce antifungal agents that affect the growth of others, i.e. there is what is termed interference competition (Taylor 1986).

To illustrate the relevance of this to assessment of contamination, the hypothetical outcome of an experiment in which spores of *Stachybotrys chartarum*, *Penicillium aurantiogriseum*, *Wallemia sebi* and *Trichoderma harzianum* are plated out is shown in Table 3.

The three species are plated separately on three media: MEA, corn meal agar (CMA) and dichloran-18% glycerol (DG18) agar (Dillon *et al.* 1996). Of the 1000 spores of *S. chartarum*, only 10 spores germinate on CMA, the best of the media for this species, i.e. 1% of the total plated.

Table 3. Model data from plating spores of four taxa on three types of agar: pure cultures.

Species	Spores plated	MEA	CMA	DG18
<i>S. chartarum</i>	1000	1	10	1
<i>P. aurantiogriseum</i>	40	10	20	25
<i>W. sebi</i>	20	1	10	20
<i>T. harzianum</i>	40	10	10	1

Recoveries of *S. chartarum* on the medium for moderately xerophilic fungi, DG18, are very low. In contrast, a high percentage of the moderately xerophilic species *P. aurantioghseum* are culturable on this optimal medium. For the xerophile *W. sebi*, good recoveries are obtained on DG18. Colonies of the hydrophilic, wood soft-rot species *T. harzianum* are difficult to count even under ideal circumstances, and the recovery on DC 18 is poor.

Table 4 illustrates the hypothetical response of plating a mixture of the species in Table 3. *S. chartarum* recoveries even on the optimal medium used, CMA, are much reduced because of the presence of *Trichoderma*. *W. sebi* does not appear at all and only the *Penicillium* species emerges “unscathed”.

These examples in Tables 3 and 4 demonstrate the point that counting propagules, or colony forming units (CFU), from dilution plating of powdered bulk or dust samples over-represents the xerotolerant *Penicillium* spp. with long-lived spores relative to species such as *S. chartarum* that have spores with a short half-life.

Table 4. Model data from plating spores of four taxa on three types of agar: mixture of all species.

Species Spores	plated	MEA	CMA	DG18
<i>S. chartarum</i>	1000	5	5	-
<i>P. aurantiogriseum</i>	40	20	10	20
<i>W. sebi</i>	20	-	-	-
<i>T. harzianum</i>	40	10	10	1

It has long been understood in soil mycology that dilution plating does not give a reliable indication of the species that are active in the ecosystem. In the upper half of Fig. 2, data are illustrated from dilution plating of soil samples taken over a period of about one year. The lower half shows measurements of hyphal growth in the soil (Kjoller and Struwe 1984).

As is typical for soil samples, not only are there no correlations between the two measures, hyphal growth being a direct measure of activity, but also 85% of the cultures found by dilution plating are of species that are not active in the ecosystem (Pugh 1980; Warcup 1965).

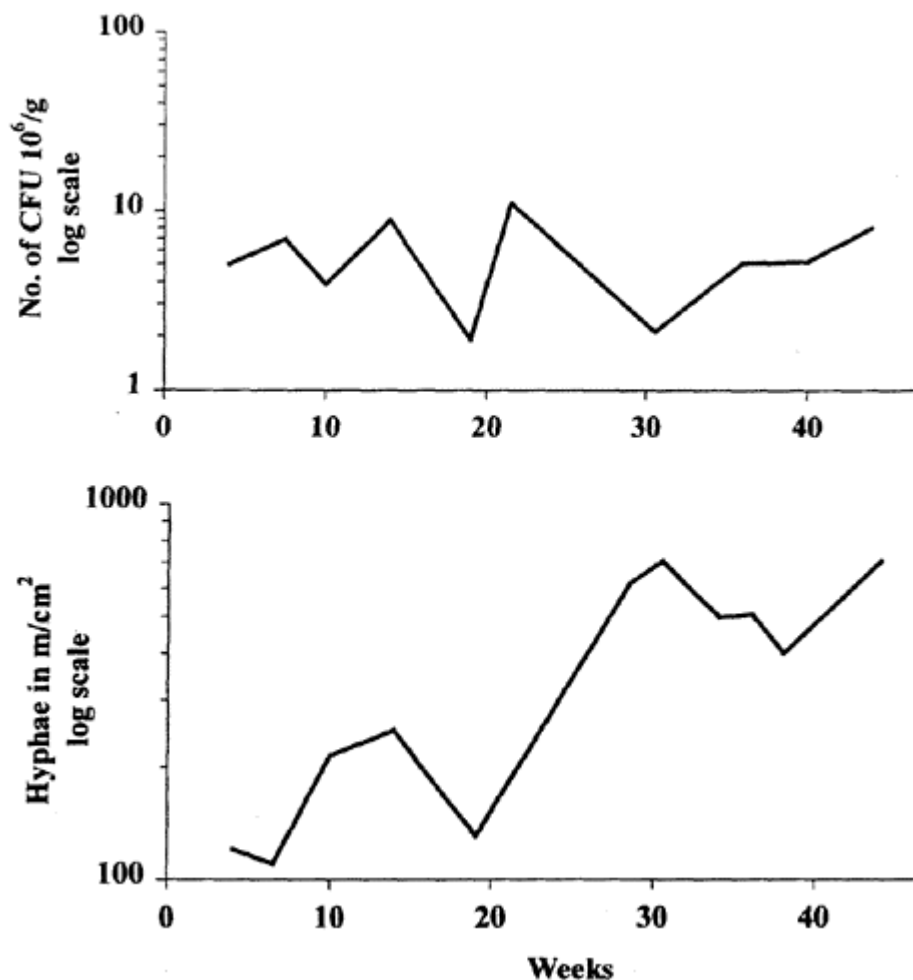


Fig. 2. Comparison of colony forming units (CFU) g^{-1} soil with *in situ* measurements of hyphal growth (after Kjoller and Struwe, 1982).

Among these are *Penicillium* spp., which because they are very heavily sporing fungi, may be particularly abundant on dilution plates. In the case of house dust samples, Miller *et al.* (1988) and Saraf *et al.* (1997) showed that ergosterol (an absolute measure of fungal biomass in the sample) and counts of CFU g^{-1} of settled dust were not well correlated. This means that the actual biomass present was not proportional to the numbers of spores detected. It is important to recognize that, in the context of bulk samples, CFU counts must be expressed in terms of an amount per gram per unit area OR must involve comparison of data for similar samples, e.g. carpet with carpet or hardwood floor with hardwood floor.

Other means of assessing least significant differences in CFU data also provide insight into their interpretability. In a study of Canadian houses (Lawton *et al.* 1998), a series of mould measurements for settled dust (CFU g⁻¹ dust plated on a medium for moderate xerophiles; tenfold increase in the proportion of moderate xerophiles) correlated weakly with moisture source strength (an estimation of water sources in a house) and were related to the size of the areas of mould growth estimated by inspectors. The ratio of counts of moderate xerophiles to those for phylloplane species in settled dust was a more sensitive indicator of fungal contamination. The least significant difference of CFU values appeared to be in the order of 100 (Lawton *et al.* 1998). Additionally, it was reported (Dales *et al.* 1997) that counts of *Aspergillus* and *Penicillium* in settled dust were higher for homes where occupants reported mould than where they did not. The higher mould counts in the dust were also associated with occupant reporting of mouldy odours, and with reported water damage, but again the association was weak.

Variations in dilution plating techniques, including wiping a sterile cloth (or cottonwool swab) over fixed areas of mouldy surfaces and plating dilutions of a suspension of microorganisms washed from it, all suffer from the technical problems described above. Counts obtained from dilution plating of bulk samples have no inherent quantitative value. Dilution plating has, however, considerable value in determining the total diversity present in a sample and there is some evidence that preparation of many replicates produces data that are related to the true value (Brewer *et al.* 1980). As observed in Pugh (1980), since these methods do not distinguish species present as hyphae from those in the form of dormant spores, they may be “dangerously liable to waste time and energy and lead to error”.

Direct plating of building material

The lower half of Fig. 2 illustrates the value of direct observation methods in reflecting activity (growth), but this method does not provide useful evidence of the taxa present. To do this, small fragments of the building material (for example, 0.5 g) are plated on different agar media. These are incubated and the colonies that grow out are enumerated and transferred for identification. When conducting mycological studies, inoculum is often taken from the leading edge of growing cultures. As the growth of hyphae is apical and the mycelium taken includes the hyphal tips, the hyphae there are assumed to be active. The notion about direct plating of material is the same as for the soil crumb technique (Warcup 1965; Pugh 1980), viz. the colonies that first emerge from a soil crumb or fragment of building material are on balance likely to reflect best those that are active in the ecosystem concerned

Table 5. Methods of determining fungal contamination of bulk samples.

Method	Characteristics
Dilution of sample	Over-represents long-lived, aggressive species, tempered by choice of

to extinction	medium, gives best picture of diversity, most taxa found probably not active
Single dilution	As above, reduced diversity, low precision
Soil crumb method	Most reflective of active species, low precision
Ergosterol	Reflects biomass, no qualitative information, good precision
(1→3)-β-D-glucan	Reflects biomass, no qualitative information, good precision, biologically active component

As suggested by the data presented in Fig. 2, the microscopical measurement and direct plating of bulk samples provide between them the most useful non-biochemical means of assessing fungal activity in samples, and thus minimize emphasis on species that are inactive in the system.

Samples of bulk materials that are plated directly onto agar should also always be mounted in lactophenol cotton blue and examined by microscopy for the presence of organisms that might not be culturable. This allows a comparison to be made between culturable and nonculturable components of the mycobiota of the bulk material.

Biochemical measures

As has been noted above and will be discussed more extensively later, ergosterol can be used to analyse fungal burdens in dust. Also, (1→3)-β-D-glucan has been shown to be a useful measure of the fungal burden in dust (Douwes *et al.* 1996). Immunological methods based on the presence of an antigen common to the two genera have been tested as measures of exposure to "*Aspergillus/Penicillium*" in dust and may be useful for these particular groups (Douwes *et al.* 1999), but are necessarily limited.

The strengths and weaknesses of the several methods discussed for processing bulk samples are summarized in Table 5.

Microscopical techniques

There are two basic techniques for the examination of mouldy surfaces by microscopy: tape samples and mounted scrapings of the mould-damaged area. In the field, tape samples are made by pressing good quality cellophane tape against the affected surface. On return to the laboratory, a marked area or around 2.5 cm² of the piece of tape is cut out and the adhesive side of this is rinsed with a few drops of 95% ethanol. After draining, the piece of tape is placed, sticky side down, on one or two drops of lactophenol cotton blue on a microscope slide. Another drop of lactophenol is put on top of the piece of tape and a glass coverslip laid on this. This preparation must be examined immediately and cannot be preserved. Where scrapings are available, they are mounted in lactophenol cotton blue on slides and examined. They can be preserved by sealing the coverslip.

As in all microscopical methods, large dark spores are easier to see and small hyaline spores are often overlooked, but this depends on the skill of the microscopist. The taxonomic information obtained is limited. Scrapings of mould-damaged areas are much

more useful than tape samples. The microscopy is better in the absence of dissolving cellulose tape, slides can be kept for other microscopists to examine or for the record, and if necessary the remainder of the collected scrapings can be plated to obtain better taxonomic information and viability data.

AIR SAMPLES

Variability in the air spora and its causes

Air samplers impacting particles on agar media provide information on culturable propagules. Samples impacted on agar media can greatly underestimate the total spore numbers present for the reasons discussed above. In general, the numbers of propagules determined by culture are substantially less (c. 1%) than those determined by direct methods (Flannigan *et al.* 1991, 1996). The variability of spore clouds in the air of a building with active mould growth is very large compared to the precision of available sampling methods. Spore concentrations often vary by several orders of magnitude; the precision of commercial samplers is around 50%.

The considerable variability in numbers of airborne propagules (See Fig. 4 in Nevalainen *et al.* 1992) was also illustrated by Stanevich and Petersen (1990), who reported that the variation between 1-min samples taken using an Andersen N6 sampler was more than six times greater than with 5-min samples. A variability of three orders of magnitude was found in weekly samples taken in Scottish houses (Hunter *et al.* 1988). Elsewhere, in a room studied for one day the hourly assessed airborne load varied by 4 orders of magnitude (Mouilleseaux and Squinazi 1991).

The reasons for the variability of spore concentrations include those related (1) to the spores and (2) to the building and its equipment. Spores produced by mould(s) growing on a particular surface such as a wall or HVAC duct will either fall off or be blown off by draughts into the air. However, regardless of airflow the ruling relative humidity affects the release of spores from a colony; the effect varies according to species. Variations in flow/relative humidity can be expected to induce variability in spore concentration (Pasanen *et al.* 1991). Once airborne, fungal spores have different sedimentation rates (Hunter *et al.* 1988; Flannigan 1993).

In large buildings, a common source of fungi is the HVAC system. Humidifiers, dirty filters, accumulated debris in ducts subject to condensation or leaks can all be sources of fungi. The distribution of fungi into the air is a function of airflow within the system. Accretions of material will eventually reach sufficient mass to be blown out of ducts in a periodic fashion, rather than continuously. Fungi are also released from contaminated wall cavities, faulty sewer drains, etc. Release from these sources will be affected by the air infiltration rates and pressure differentials, which are in turn affected by weather. Distribution of fungi from carpets or surface contamination is affected by activity in the room; the greater the activity, the greater the dispersion.

TYPES OF AIR SAMPLER

In addition to those samplers mentioned above in which spores and other particles impact on agar medium, there are several other types used for sampling airborne fungi. These include sticky-surface, filter and liquid impinger samplers.

Sticky-surface samplers

Sticky-surface samplers, such as Allergenco™, Burkard™ and Rotorod™ have been commonly used but there is little information on their quantitative and qualitative performance (Dillon *et al.* 1996; Heinsohn and Coss 1998). As with other non-cultural methods that depend on microscopy, a major limitation is the skill of the microscopist in counting fungal propagules in a field containing other materials and in recognizing small hyaline spores. These methods are mainly used for the rapid evaluation of the effectiveness of containments or occupational exposures during remediation of heavily contaminated buildings (Weber *et al.* 1995).

The performance of a new type of sticky surface sampler, the Zefon Air-O-Cell™, has been tested with *Penicillium brevicompactum* spores. Filters that must be purchased from the manufacturer are connected to a calibrated pump. This device traps the spore in a solid medium suitable for microscopic analysis. The 50% cut off had been reported as around 3 µm (Trakumas *et al.* 1998).

None of these methods is sufficiently quantitative to measure exposure as strictly defined, i.e. in terms of mg relevant agent m⁻³ air.

Filter sampling

Fungal spores can be collected on filters and numbers and type determined in several ways, including microscopy, after staining and plating out spores washed off the filters to obtain viable counts. The latter method is limited by the fact that the collection process results in desiccation and rapid reductions in viability (Dillon *et al.* 1996), depending on the air flow-rate through the filter and the susceptibility of the species to desiccation. The deposition of fungal spores on open-faced filters has been studied carefully in the field (Eduard *et al.* 1990) and under controlled conditions (Gao *et al.* 1997). Methods have been found to correct problems caused by aggregation of spores on the filters (Eduard and Aalen 1988). These data established that open-faced filter samples are reliable tools for collection of fungal spores. However, the estimations of spore numbers obtained are still dependent on the skill of the microscopist. Epifluorescence microscopy appears to be the best visualization technique (Eduard *et al.* 1990).

Several comparative studies have reported moderate to good correlations between data collected on agar media using impaction samplers and total fungal spores estimated by direct microscopy of microporous filter samples (Palmgren *et al.* 1986a,b; Miller *et al.* 1988; Flannigan 1992; Flannigan *et al.* 1996). Additionally, the distribution of fungal taxa in air samples impacted on agar media appears similar to that obtained on filters (Flannigan *et al.* 1996). Filters have the advantage that they can be run for a long time, even for days, to attempt to deal with the environmental variability.

The employment of filter samplers, if followed by careful microscopical assessment of all trapped microorganisms, viable and non-viable, probably gives useful measurements of exposure.

Impaction air samplers for culturable propagules

Under realistic conditions, all the proprietary air samplers for collection of culturable fungi by impaction are useful for assessing diversity (Laflamme and Miller 1992; Buttner and Stetzenbach 1993; Dillon *et al.* 1996). Dillon *et al.* (1996) provide detailed methods for the use of such samplers. However, it should be remembered that, as noted above, the variability of spore concentrations in air is vastly greater than variations in sampler performance.

Liquid impingers

These samplers have not been much used in building investigations and it is therefore difficult to evaluate their performance. Methods for the use of some liquid impinger samplers are found in Dillon *et al.* (1996).

Comparison of indoor air with outdoor air

Unless there are zero counts for fungi, in indoor air samples, it is necessary to compare the fungal species in indoor with outdoor samples. While numbers of CFU in air vary greatly, the ratios between the taxa present are more stable (Miller 1993; Flannigan and Miller 1994). Many of the problems that affect the analysis of dust samples described above apply to the analysis of culturable air samples. Depending on the taxa being collected, the growth of mixtures of fungi on semi-selective agar media (Tables 3 and 4) results in highly variable data. There is another very important problem that applies particularly to the taking of outdoor air samples or samples in air ducts, the movement of air over the sampling plane has profound effects on the recovery of spores from air (Nevalainen *et al.* 1992). Samples taken in air where there is substantial air movement cannot be directly compared with other samples. Precipitation also affects the data obtained.

The basis of the current methods for conducting sampling for culturable propagules is a comparison of the indoor value with outdoor air samples (Dillon *et al.* 1996; Health Canada 1995). Outdoor air samples must be taken at the air intake or well above ground level for naturally-ventilated buildings to avoid problems of collecting windblown soil particles, unless this is impossible.

A most detailed data set of fungal spores in outdoor air comes from Belgium (Nolard 1997). These data show that the 10-year average airborne concentrations of spores are low in January to March (see also Chapter 1.1). Starting in early spring, there is a changing array of fungal particles in the air as the season progresses. Order of magnitude changes occur within this qualitative change with average numbers occurring in July of approximately 20,000 particles m^{-3} , peaking at twice that value. In winter, *Aspergillus/Penicillium* (genera associated with soil particles) comprise 10% of the small numbers of fungi then present in outdoor air. For the rest of the year, the percentage is 0.1% of the much higher numbers of spores and species present (Coates *et al.* 1992;

Nolard 1997; Miller, unpublished data). In another study (Battarbee *et al.* 1997; Rose, personal communication), under dry conditions fungal spores were typically about 2% of particulate matter and the absolute values were estimated to be similar to the Belgian data. After rain, the percentage rose to 27% of particles as a result of a 3- or 4-fold rise in the numbers of spores and washout of larger inorganic particles. These data show that for several days after precipitation there are considerable numbers of particles of filamentous fungi among the fine particulate matter. The contribution of yeasts to this fine matter is also high, particularly after fog or rain; during fog yeast particles may increase to 100 times those in clear air, reaching 5000 m^{-3} air at 10°C (Fuzzi *et al.* 1997).

To accommodate these variations, data from a number of outdoor air samples must be pooled for making comparison between indoor and outdoor air. Percentages of the different taxa in the pool can then be used for such comparisons. With this approach, there is general agreement that properly conducted air sampling is useful in detecting the presence of fungal amplification sites indoors, but not for assessing exposure (Dillon *et al.* 1999). The numerical data obtained from culturable air samples have no value. In a study of these issues made by Miller *et al.* (2000) the data from extensive air sampling in an apartment building were compared with studies of mould damage after massive destructive inspection to examine wall cavities. Air samples were scored according to the guidelines in Dillon *et al.* (1996) and based on comparison with 50 pooled outdoor air samples taken over the life of the project. Approximately one-quarter of the data set is outlined in Tables 6 and 7, which are adapted from Miller *et al.* (2000).

As expected, descriptive statistics showed that recorded differences in viable count (CFU m^{-3}) between the two data sets were not statistically significant. However, the proportion of samples that failed the AIHA guidelines (Dillon *et al.* 1996) was different ($p < 0.005$). There was no correlation between CFU m^{-3} air and the area of visible mould revealed by the extensive destructive testing. In this partial data set (Miller *et al.* 2000), and in the full set, the proportion of "AIHA fails" correlated fairly well with mould area ($p < 0.11$).

Although it may indicate the occurrence of amplification sites within buildings, it is to be remembered that for reasons mentioned earlier sampling the air for culturable (viable) propagules by impaction or any other method does not measure exposure as defined previously, viz. mg agent m^{-3} air.

Biochemical measurement of airborne fungi

Because of the need for true exposure measures, research on the effects of microbes in indoor air have mainly focused on using indicator bio-chemicals that can be measured from particulate samples. For bacteria, these have included endotoxin and peptidyl glycan for Gram-negative and Gram-positive species, respectively. For fungi, these have included (1 $\rightarrow$ 3)- β -D-glucan and ergosterol. Methods for airborne endotoxin are well established, but for peptidyl glycan as yet immature (Dillon *et al.* 1996).

Table 6. Air sampling data from apartments with large areas of mould in wall cavities (after Miller *et al.* 2000).

<i>Apartment</i>	<i>AIHA "Fails"*</i>	<i>Mean culturable moulds (CFU m⁻³)</i>
1	4/6	664
2	3/4	142
3	4/4	109
4	4/4	172
5	4/4	203
6	4/4	277
7	3/4	778
8	4/6	146
9	5/6	74
10	5/6	192
11	5/6	201
12	2/4	1531
13	3/6	134
14	6/6	180
15	3/6	137
Mean	0.88±0.19	329±389

*AIHA "fails" registered when air samples differed from pooled outdoor air samples according to criteria in Dillon *et al.* (1996). Air samples (2 or 3, depending on the size of the apartment) were taken in the morning and afternoon in each room; number of air samples 76.

Table 7. Air sampling data from apartments with small areas of mould in wall cavities (after Miller *et al.* 2000).

<i>Apartment</i>	<i>AIHA "Fails"*</i>	<i>Mean culturable moulds (CFU m⁻³)</i>
1	1/4	130
2	0/4	153
3	1/4	332
4	0/4	889
5	0/6	204
6	0/4	194
7	0/4	164
8	0/6	132
9	1/4	128
10	1/4	50
11	0/6	19
12	0/4	214
13	1/4	172
14	0/6	229
15	0/6	197
Mean	0.08±0.12	214±201

* "Fails" registered and samples taken as in the morning and afternoon as in Table 6; number of air samples, 70.

Many measurements of fungi in air have been made using (1→3)-β-D-glucan (Rylander 1998), but, as noted by Miller and Young (1997), there have been no studies of the relative amount of glucan per spore. It appears, however, that the amounts of ergosterol and glucan in spores are similar. Therefore accurate glucan measurements should reflect ergosterol values and vice versa. There remains work to be done in assessing the analytical performance of glucan measurements (Dillon *et al.* 1996). Recent studies suggest that reliable analytical methods can be developed wherein the coefficient of variation approaches 25% (Douwes *et al.* 1996).

Altogether, perhaps 1,500 ergosterol measurements have been made of the fungal burden in indoor air samples. Ergosterol is the primary membrane sterol of filamentous fungi and environmental yeasts and, with rare exceptions, is not accumulated by other organisms. This fact has encouraged a great deal of research using ergosterol as a measure of biomass in ecosystems (Gessner and Newell 1997).

A method was developed for use in house dust (Miller *et al.* 1988) and the approach

has since been used by others (Axelsson *et al.* 1995, Saraf *et al.* 1997). Ergosterol content of spores is comparable to values for mycelium of the same fungi, and contents are similar in different species that commonly grow on building materials. The analytical performance of the method has been characterized by Miller and Young (1997). Given that, if properly used, open-face filters collect fungal spores quantitatively (see above), ergosterol provides unambiguous exposure measures as long as the spores are of similar size. The qualitative features of the exposure can be inferred from or known by analysing the fungal spores in dust or other means. A major innovation in the ergosterol method for air samples is microwave-assisted extraction, which permits the rapid processing of many samples at once. Fatty acids are also extracted during this process and can be analyzed by GC or GC/MS (Young 1995).

In the late 1970's, Dr. David White developed an approach for studying natural bacterial populations by biochemical means, called lipid signature profiling. It is based on different genera and some species within individual genera having characteristic patterns of cell lipids (White 1988). Recently, this technology has been extended for potential use in monitoring bacteria in spacecraft and other critical indoor environments (Macnaughton *et al.* 1997). Air samples are extracted and analyzed by GC/MS for signature lipids. This is followed by statistical treatments of the data to convert the analytical results to species profiles. The analysis provides information on the putative identities and biomass of dominant bacteria present in an air sample. Preliminary studies have shown that the lipid signature approach for bacteria gives biomass values which are "2–3 orders of magnitude" greater than those estimated by cultural techniques (Macnaughton *et al.* 1997). This accords very well with the experience described in the literature that culturable estimates of bacteria in indoor air represent less than 1% of the total (Flannigan *et al.* 1996).

Studies of aquatic fungi indicate that lipid signature profile methods should, in principle, work for analysing fungi in indoor air. Data of 21 known compounds present in mycelial extracts of four fungi (Table 8) enabled the Ascomycete (*Leptosphaeria oraemaris*) to be distinguished from the three other (Deuteromycete) species, and *Dendriphiella salina* from *Sphaerulina oraemaris* and *Monoditys pelagica*, using cluster analysis (Ludwig and Reynolds 1988). This demonstrates that, based on a single high resolution MS of a lipid extract followed by the statistical treatments described, the components of a mixture of these four fungi could be identified and their quantity estimated.

Relative scientific value of mould exposure measurements

As mentioned earlier, Lawton *et al.* (1998) found that measurements of mould in settled dust (CFU g⁻¹) in homes were correlated with moisture source strength (an estimation of water sources in a house) and related to estimations of mould area by inspectors, and higher mould counts were associated with occupant reports of mouldy odours, and with reported water damage.

Table 8. Fatty acids of four species of aquatic fungi (μg fatty acid g^{-1}), calculated from Miller *et al.* (1984).

Fatty acid	<i>M. pelagica</i>	<i>S. oraemaris</i>	<i>L. oraemaris</i>	<i>D. salina</i>
dodecanoic	0.6	0.5	1.6	2.6
tridecanoic	nd	0.35	0.8	1.3
12-M-tridecanoic	nd	nd	nd	1.3
tetradecanoic	10.5	15	12	107.9
12-M-tetradecanoic	nd	2	0.8	23.4
pentadecanoic	2.1	0.65	3.6	31.2
12-M-pentadecanoic	nd	0.5	nd	nd
hexadecanoic	127.2	211	42.0	517.4
11-hexadecanoic	15.3	38.5	nd	41.6
15-M-hexadecanoic	0.4	4	54	24.7
octadecanoic	21.6	64.5	141.6	nd
10-octadecanoic	53.4	4.5	12.8	289.9
hexadecanoic	0.9	4.0	1.6	13.0
octadecanoic	1.8	nd	3.2	nd
nonadecanoic	nd	0.5	nd	nd
eicosanoic	3.3	3	6.4	13
8,11-octadecanoic	14.1	12	8.8	32.5
11,14-octadecanoic	24	22	24.8	16.9
docosanoic	nd	2.5	8.8	16.9
tricosanoic	nd	nd	2.4	nd
tetracosanoic	nd	2	18.4	41.6

nd, not detected

Counts of *Aspergillus* spp. and *Penicillium* spp. in settled dust were higher when mould was reported by occupants than when not. Occupants who smoked or reported mould allergies under-and over-reported moulds compared to objective data (Dales *et al.* 1997). Because of the problems of using dilution plating data as a measure of exposure, which have been discussed above, these relationships are probably as good as they can be. Saraf *et al.* (1997) and Miller *et al.* (1988) showed that for settled dust ergosterol (an absolute measure of fungal biomass in the sample) and CFU g^{-1} were not well correlated. Douwes (1998) found that (1 $\rightarrow$ 3)- β -D-glucan (a measure similar to ergosterol) in settled dust and

immunologically based methods for assessing *Aspergillus* and *Penicillium* were useful predictors of some health outcomes.

Based on mycological research criteria, estimating fungal exposure in buildings is currently best assessed by accurately determining mould area with mycological analyses as described above. The next most useful measure is analysis of settled dust for moulds on a medium suitable for moderate xerophiles (DG18) together with a biochemical measure such as (1→3)- β -D-glucan or ergosterol. Properly conducted air sampling using the AIHA "pass/fail" criteria, spore-trap sampling, airborne ergosterol, (1→3)- β -D-glucan or total spores following epifluorescence microscopy are useful for establishing the presence of fungal amplifiers in buildings. Except for sampling via impaction on agar, all are probably useful as exposure measures.

The optimal technique, some measure of personal sampling, is beyond the reach of current methods because of lack of sensitivity. However, the existing methods can provide reliable information, with known uncertainties. The goal of all investigators must be to respect the difficulties and be very careful to make all tests with strict attention to detail.

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Chapter 4.2

LABORATORY ISOLATION AND IDENTIFICATION OF FUNGI

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INTRODUCTION

It is estimated that more than 75,000 species of fungi are known, but that our present knowledge includes only a fraction of 10–20 % of the species actually occurring in nature. In the indoor environment only a small portion of these fungi occur and it has often been indicated that this mycobiota is very similar to the common taxonomic groups, or taxa, found on food (Pitt and Hocking, 1997; Samson *et al.* 2000). In addition to microfungi, a number of Basidiomycetes can be found indoors, growing on wood in buildings. It is important to identify these species, because each has its own characteristics, and these are important for the control of wood rotting and renovation of wooden constructions in damaged buildings.

As described in Chapter 4.1, estimating fungal exposure in buildings is best assessed by accurately determining mould area and identifying the dominant species. Unfortunately, methods of isolation are still too often used which are based on those in bacteriology or medical mycology, rather than those which are more suitable for the environmental mycobiota.

In Section 5, the morphological features of the most common fungal species are illustrated, together with three species of the Actinomycetes that may be encountered indoors and are mentioned in a short segment on bacteria later in this chapter. The micrographs were prepared from cultures grown under optimum conditions or specimens and mounted in lactic acid or Shear's mounting fluid. The media used for cultivation, which differ according to species, are indicated in the legend.

ISOLATION AND EXAMINATION OF FUNGI FROM SAMPLES

Samples

Any material with suspected mould growth can serve as a sample for further examination and isolation. Samples should be transported and stored in dry conditions, since mould growth can continue to develop under moist conditions. Packaging in clean absorbent paper, such as Kleenex™, or newspaper is recommended, but *not* polyethylene bags.

Small plastic boxes or other containers are suitable, particularly in the case of very fragile material. Scrapings of suspected moulds from walls or other surfaces can be taken with a knife or scalpel.

A suitable method for sampling is to use transparent adhesive tape. After it has been pressed against a contaminated surface, the tape should be transferred onto a glass microscope slide for further microscopical examination (see also Chapter 4.1.).

Table 1. Surface sterilisation of building materials.

Surface	Particles are surface disinfected by vigorous shaking in freshly prepared sodium hypochlorite solution
disinfection	containing 0.4% chlorine (w/v) for 2 min. If a quantitative estimation is required a minimum of 100 particles should be disinfected. The solution must only be used once.
Rinsing	After pouring off the chlorine, rinse once in sterile distilled or deionised water.
Plating	As quickly as possible, transfer particles with a sterile forceps to previously poured and set plates, at the rate of 5–10 particles per plate.

Small pieces can also be cut from the tape bearing mould and transferred directly onto an isolation medium.

When sampling in dwellings and other buildings, one should be cautious where *Stachybotrys* and other toxic fungi are present. Protective clothing, gloves and respirators are recommended (See Chapter 2.3).

Isolation in the laboratory

For isolating fungi it is quite suitable to transfer small pieces of the (moulded) material to agar medium and then streak it out over the surface. Direct plating (see also Chapter 4.1.) is a technique which is commonly used to isolate fungi growing on food (Samson *et al.* 1992, 2000; Pitt and Hocking, 1997). In some cases, where the responsible mould contamination is hidden or overgrown by secondary invading species, surface sterilisation of the sample (brick, wall material, wood) can be performed. A procedure is described in Table 1.

Media

Many media are used for fungi (Table 2), but for isolation and detection the general purpose media dichloran-18% glycerol agar (DC 18 agar) and malt extract agar (MEA) are recommended. For the detection of moulds in wet environments water agar can be used, but it is always preferable to use two media to cover the complete mycobiota, including mesophilic and xerophilic species. Plate count agar and Sabouraud agar are not recommended because several species will not develop on these media. When media

which contain rose bengal are used, viz. rose bengal agar or dichloran rose bengal agar (DRBC) (King *et al.* 1979), it should be noted that they only detect mesophilic fungi. Furthermore, exposure to light should be avoided because in light rose bengal becomes increasingly toxic to some fungi, including *Rhizopus* and yeasts.

Many media can be used to isolate yeasts, but Samson *et al.* (1992) recommended malt extract agar for bathrooms and cooling water reservoirs in air-conditioning systems, where yeasts usually predominate. Chloramphenicol or oxytetracycline (100 mg L^{-1}) can be added, if necessary, to suppress bacteria. DRBC is preferable when yeasts must be enumerated in the presence of moulds.

Incubation

Plates should be incubated in an upright position at 25°C . For the detection of thermotolerant/thermophilic species, e.g. *Aspergillus fumigatus*, incubation should be at temperatures between 35° and 40°C . Plates can be incubated either in darkness or in diffuse light. When using rose bengal (see under media) plates or test strips should always be kept in the dark (see under Media).

Examining the cultures

For identification by microscopic examination it is essential to use appropriate and optimal light microscopic equipment. Good lenses, including $\times 100$ objectives will facilitate the investigation of the shape and surface ornamentation of fungal cells and spores. For the examination of colonies and samples with fungal contamination, a dissecting microscope with a total magnification of $\times 100$ – $\times 400$ and a good light source is recommended to use. A dissecting microscope is also helpful for making good microscopic slide preparations by enabling sporulating areas of a colony to be selected.

Table 2. Formulations of some mycological media for isolation of indoor moulds (for more media see Samson *et al.* 2000).

CMA Cornmeal agar (CBS)

Add 60g freshly ground cornmeal to 1 litre distilled water, heat to boiling and simmer gently for 1 h. Strain through cloth and sterilize at 121°C for 15 min. Fill up to 1 litre with water and add 15g agar and re-sterilize at 121°C for 15 min. Commercially available.

CYA Czapek yeast (autolysate) extract agar.

NaNO_3 3.0, K_2HPO_4 1.0, KCl 0.5, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01, Yeast extract 5.0, Sucrose 30.0, Agar 20.0, Distilled water 1000 ml. Final pH 6.0–6.5. NOTE: Addition of trace elements (see above) is recommended. Addition of mineral solution (MS), containing 5g KCL, 5g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ per 100ml water is also recommended in some media.

DG18 Dichloran 18% Glycerol agar;

Peptone 5.0g; Glucose 10.0g; KH_2PO_4 1.0g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5g; Dichloran (0.2% in

ethanol) 1.0ml; Glycerol 220.0g; Chloramphenicol 0.1g; Agar 15.0g; Distilled water 1 litre. Add minor ingredients and agar to ca. 800ml distilled water. Steam to dissolve agar, then make up to 1 litre with distilled water. Add 220g glycerol and sterilize by autoclaving at 121°C for 15 min. Final a_w is 0.955; final pH 5.6 ± 0.2 . Commercially available (Oxoid).

MEA Malt extract agar

Malt extract 20.0g; Mycological peptone 5.0g; Agar 15.0g; Distilled water 1 litre. Final pH 5.4 ± 0.2 . Sterilize by autoclaving at 115°C for 10 min.

OA Oatmeal agar (CBS)

Heat 30g oat flakes in 1 litre water to boiling and simmer gently for 2h. Filter through cloth and make up to 1 litre. Add 15g agar and autoclave at 121°C for 15 min. When using powdered oatmeal, filtering is superfluous. Sterile lupin stems may be placed in slants with oatmeal agar. Also commercially available.

PDA Potato dextrose agar

Add 200g scrubbed and diced potatoes to 1 litre water and boil for 1h. Pass through a fine sieve, add 15g agar and 20g dextrose and boil until dissolved. pH 5.6 ± 0.1 . Commercially available.

SNA Synthetischer Nährstoffarmer agar

KH_2PO_4 1.0, KNO_3 1.0, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5, KCl 0.5, Glucose 0.2, Saccharose 0.2, Agar 20.0, Distilled water 1000 ml. NOTE: Pieces of sterile filter paper may be placed on the agar. Recommended for the cultivation of *Fusarium*, but also for poorly sporulating Deuteromycetes.

WA Water agar 2%

Agar 20.0g; Distilled water 1 litre. Steam to dissolve agar and sterilize by autoclaving at 121°C for 15 min.

Table 3. Fungal flora with related water activities (from Samson *et al.* 1994, with slight modification).

Materials with a high water activity ($a_w > 0.90$ – 0.95): *Aspergillus fumigatus*, *Trichoderma*, *Exophiala*, *Stachybotrys*, *Phialophora*, *Fusarium*, *Ulocladium*, yeasts (*Rhodotorula*), Actinomycetes and Gramnegative bacteria (e.g. *Pseudomonas*).

Materials with a moderately high water activity ($a_w > 0.85$ – < 0.90): *A.versicolor*, *A.sydowii*, *Emericella nidulans*.

Materials with a lower water activity ($a_w < 0.85$): *Aspergillus versicolor*, *Eurotium*, *Wallemia*, *Penicillia* (e.g. *Penicillium chrysogenum*, *P.aurantiigriseum*).

Spore samples from indoors or outdoors, such as those collected on transparent adhesive tape or by Burkard or Rotorod samplers (See Chapters 1.1 and 4.1), can be directly examined by microscope. However, it should be emphasized again that, while species in some genera (e.g. *Alternaria*, *Epicoccum* and *Curvularia*) can be easily recognized by the

size, shape, septation and pigmentation of the spores, species in many other genera have very simple and similar spores (e.g. *Aspergillus* and *Penicillium*). Fig. 1 shows a mixture of spores as collected on a transparent tape preparation from wallpaper. A detailed examination of this preparation shows conidia, of *Alternaria*, *Cladosporium* and *Stachybotrys* and ascospores of *Chaetomium*. However, round hyaline spores which belong to either *Penicillium* or *Aspergillus* are also present. More precise identification can be made when structures of a fruiting body or conidophore can also be observed, as depicted in Fig. 2. Here, typical *Stachybotrys* conidiophores as well as conidia are present. Microscopic examination of preparations made from surfaces often also reveals structures which are not fungal, but may be crystals of salts (Fig. 3), textile fibres (Fig 4), or mite and insect faeces.

Indicator organisms

All species in the indoor environment have their own individual growth conditions and their presence gives good indications of the condition of the building. Samson *et al.* (1994) suggested that several species can be considered as indicator organisms where moisture and/or health problems are signalled (Table 3). For example the many species in the genus *Aspergillus* differ from each other in their growth requirements. Consequently, identification only to generic level is not sufficient; identification to species level is essential.

MITES AND OTHER LABORATORY CONTAMINANTS

Various species of mycophagous (fungus-eating) mites can be present on materials and surfaces where mould growth appears. These mites may act as vectors which spread the fungi and therefore it is important to take note of their presence. For experiments in the laboratory and maintenance of fungal cultures, mite infestation should be avoided, because it will hamper the isolation and cultivation of the moulds in pure culture.

When growing pure cultures in the laboratory, besides mites some microorganisms can also cause problems. *Chrysonilia* (*Monilia*) *crassa* is a very rapidly growing fungus, invading incubators, Petri dishes and even slant cultures. When this fungal contaminant is found, Petri dishes should be sealed immediately (with Parafilm or similar tape) and autoclaved. Furthermore, benches and incubators should be cleaned with alcohol and eventually disinfected with formaldehyde.

Fungi can produce volatile compounds and, although little is known about the health implications when individuals are exposed, health complaints are reported. It is recommended that, when fungal strains are being grown in the laboratory, the cultures should be in biosafety cabinets or under hoods with good ventilation.



Fig. 1. Transparent adhesive tape preparation showing various spores.

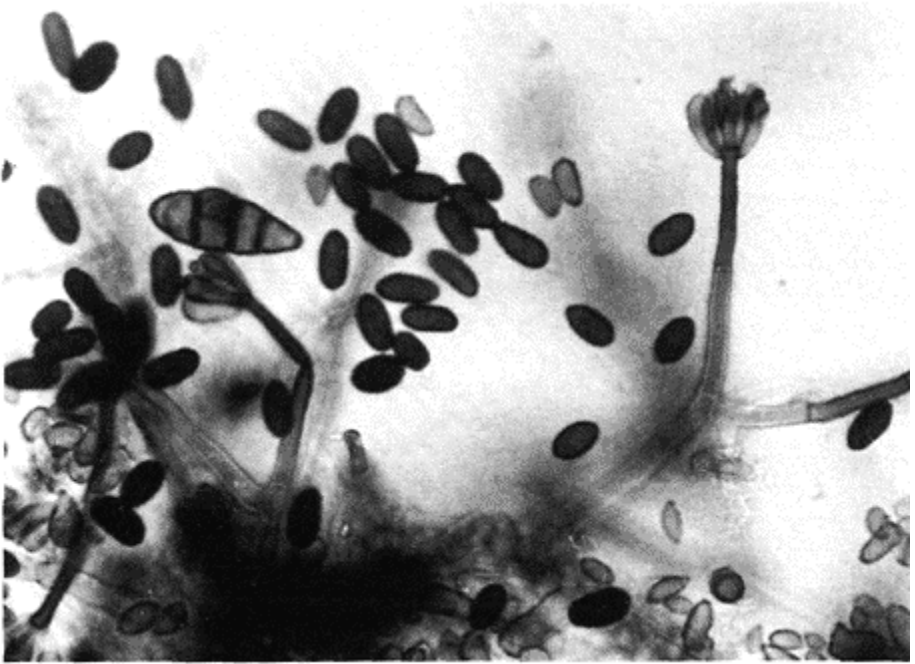


Fig. 2. Transparent adhesive tape preparation showing conidiophores and spores of *Stachybotrys chartarum* and spores of *Cladosporium* and *Alternaria*.



Fig. 3. Crystals of salts in a transparent adhesive tape preparation from a wall.

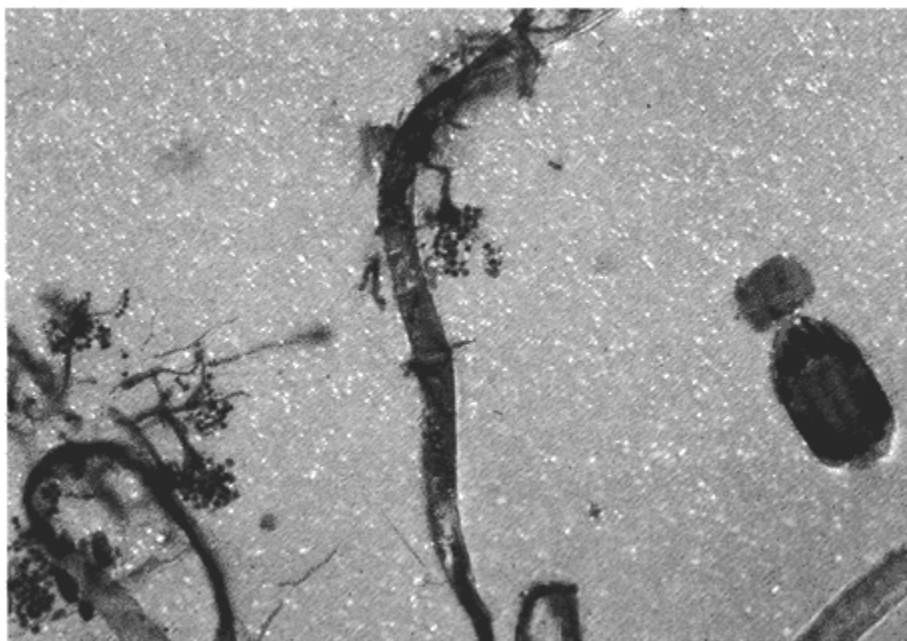


Fig. 4. Fungal structures belonging to *Aspergillus* with textile fibre and a mite.

NOMENCLATURE

The correct name of a taxon reflects the current state of taxonomic knowledge. An important problem which is associated with the identification of moulds is that much confusion exists about the naming of species. A well-known example is *Stachybotrys chartarum* (Ehrenb.) Hughes which is still, but incorrectly, named as *S. atra* Corda. The species *S. chartarum* was described in 1818 by a German mycologist, Ehrenberg, as *Stilbospora chartarum*. In 1958, Dr S. Hughes examined the type material collected by Ehrenberg and found that it belonged to *Stachybotrys* and therefore proposed the combination *S. chartarum* (Ehrenberg) Hughes. *S. atra*, which is identical to *S. chartarum*, but was described later by Corda in 1837, is therefore a synonym. Other incorrect names are *Penicillium frequentans* (correct name *P. glabrum*) and *Epicoccum purpurascens* (correct name *E. nigrum*).

A procedure for stabilising *Aspergillus* and *Penicillium* nomenclature has recently been developed to protect names in current use. In order to protect these currently used names from being threatened or displaced by names that are no longer in use, Pitt and Samson (1993) and Pitt *et al.* (2000) proposed a list of species names in current use for the family Trichocomaceae (Eurotiales). All published names and synonyms of *Aspergillus* and *Penicillium* are listed in Samson and Pitt (2000).

IDENTIFICATION

Identification of fungi is still primarily based on their physical appearance, i.e. their phenotypic characters. In yeasts, biochemical and physiological profiles are used and this facilitates the use of identification kits such as API and Biolog. Chemotaxonomic approaches such as profiles of secondary metabolites (including volatiles and mycotoxins) have been proposed for identification (for an overview see Frisvad *et al.* 1998) but have not yet gained any practical significance. Furthermore molecular techniques have been tested and will be introduced using identification kits. Depending on the validity of their databases, these may prove to be useful for rapid and objective identification.

IDENTIFICATION OF THE MAJOR FUNGAL GROUPS

When starting with an unknown isolate or specimen it is important to determine to which fungal group it belongs. For this the following key can be used:

KEY TO THE FUNGAL GROUPS

- 1 Colonies consisting of loose budding cells; pseudomycelium sometimes present but true
 - a. vegetative mycelium usually absent Yeasts
- 1 Colonies with abundant vegetative mycelium; conidia or spores borne in or on special
 - b. cells..... 2
- 2 Mycelium without, or with few septa, hyphae often broad; (mito)spores mostly formed
 - a. endogenously in sporangia Zygomycetes
- 2 Mycelium with regular septa; mitospores or meiospores not formed in sporangia..
 - b. 3
- 3 Meiospores formed in asci.
 - a. Ascomycetes
- 3 Meiospores not formed in asci..... 4
 - b.
- 4 Meiospores formed on basidia..
 - a. .Basidiomycetes
- 4 Mitospores (conidia) produced from special cells (conidiogenous cells)....
 - b. Deuteromycetes

Basidiomycetes

The Basidiomycetes is a large group mostly comprising species with typical fruiting bodies or basidiocarps which are mostly known as toadstools and bracket fungi. In the

indoor environment, some species which do not possess macroscopic fruiting bodies, such as *Sistotrema*, may occur in air samples. Typical basidiomycete species in buildings are the wood-rotting fungi, among which *Serpula lacrymans* is the most notorious species, causing dry rot. Airborne spores of *S. lacrymans* can be present in high concentrations and allergenic reactions are reported in the literature (Murphy *et al.* 1995). Table 4 lists the most common species found in North American and European buildings. Most basidiomycetes are difficult to grow on artificial media and they often do not form fruiting bodies which sporulate, but only sterile mycelium.

Table 4. Basidiomycetes occurring in buildings.

Dry rot fungus	<i>Serpula lacrymans</i>
Wet rot fungi	<i>Coniophora puteana</i>
	<i>Coniophora marmorata</i>
	<i>Donkioporia expansa</i>
	<i>Phellinus contiguus</i>
	<i>Antrodia xantha</i>
	<i>Antrodia (Fibroporia) vallantii</i>
	<i>Pleurotus ostreatus</i>
	<i>Sistotrema brinkmannii</i>
	<i>Schizophyllum commune</i>
	<i>Coprinus domesticum</i>

For identification of wood-rotting species in buildings the publications by Wagenführ and Steiger (1966), Coggins (1980), Bravery *et al.* (1987) and Hennebert and Balon (1996) are recommended.

Zygomycetes

Zygomycetes can be easily recognized because of their fast growth, which covers a Petri dish within 5 days. As mentioned in Chapter 1.2 and in the key to the major fungal groups above, the mycelium has no or few septa. Asexual reproduction occurs by means of sporangiospores produced endogenously in sporangia, or in merosporangia. Morphological features such as an apophysis (a swelling of the sporangiophore just below the sporangium) and stolons which adhere to the substrate by means of rhizoids (root-like structures) are present in some species but not in others and are therefore important in identification. Some species produce chlamydospores or oidia.

Sexual reproduction occurs by fusion of two multinucleate gametangia, resulting in a thick-walled, dark coloured zygospore, which is, how-ever, rarely found in isolates from

indoor environments. Several species, e.g. *Mucor plumbeus*, *Rhizopus stolonifer* and *Absidia corymbifera*, are frequently encountered in indoor environments, on surfaces and in air samples.

KEY TO THE COMMON ZYGOMYCETE GENERA IN THE INDOOR ENVIRONMENTS

- 1a. Sporangiospores formed in merosporangia which cover the swollen end of the sporangiophore *Syncephalastrum*
- 1b. Sporangiospores formed in globose or pyriform sporangia, with a columella 2
- 2a. Sporangia and sporangiophores usually dark pigmented, sporangiophores mostly unbranched, often occurring in groups. Sporangia vary from 50–360 µm in diam. Spores often striate *Rhizopus*
- 2b. Sporangia and sporangiophores not pigmented or only faintly coloured, often frequently branched. Sporangia never exceeding 100 µm in diam. Spores not striate 3
- 3a. Sporangia pyriform with a distinct apophysis, 10–40 µm in diam. (terminal sporangia up to 80 µm in diam.) *Absidia*
- 3b. Sporangia globose without an apophysis, mostly larger than 40 µm in diam.
..... *Mucor*

In hospitals and buildings in which there are environments at temperatures above 25° C, *Mucor* and *Rhizomucor* species are also sometimes encountered.

For more detailed descriptions and keys to the Zygomycetes the reader is referred to Zycha *et al.* (1969), Hanlin (1973), O'Donnell (1979) and von Arx (1981). Keys to the species of *Mucor* are in Schipper (1973, 1976, 1978), and the genus *Rhizopus* is covered by Schipper (1984) and Liou *et al.* (1990).

Ascomycetes

The Ascomycetes are the largest group in the Fungal Kingdom and most species can be found on plants and other substrates in nature. For the indoor environment most species belong to the Eurotiales with simple and small fruiting bodies. Species of *Chaetomium* are found on material containing cellulose. *Pyronema domesticum* is a species frequently found on fresh plaster in new and renovated buildings. On wood some species with distinct and large fruiting bodies can occur, such as *Peziza domiciliana*.

Asci usually develop in ascomata (ascocarps) and they contain mostly eight ascospores. The morphology of the fruit-bodies is important for a systematic division. The fruit-bodies may be cup-like apothecia; globose or subglobose cleistothecia, which are non-ostiolate (without a terminal pore), and more or less flask-shaped, mostly ostiolate perithecia. This ascus-producing state, the teleomorph, is often accompanied by one or more asexual reproductive states, the anamorph. The majority of the

Deuteromycetes are anamorphs in life-cycles of Ascomycetes.

Among ascomycetous fungi found in indoor environments, species of *Chaetomium* are notable on walls and cellulosic materials, as well as being frequently found as storage fungi on, for example, cereals or develop after heat-treatment of a product. For details of this genus the reader is referred to von Arx *et al.* (1986). As mentioned in Chapter 2.1, *Eurotium* spp. are the commonest members of the Ascomycetes encountered in indoor environments, on walls and other surfaces and in house dust. For optimal sporulation *Eurotium* should be grown on MEA or Czapek-Dox agar with additional sucrose (20–40%) or NaCl (10–30%). For isolation, DG18 agar can be used. Most of the ascomycetous species treated here mature after 10–14 days. *Chaetomium* grows well on oatmeal agar (OA).

KEY TO THE COMMON ASCOMYCETES

- 1a. Ascomata distinct with hairs or cupshaped, asci cylindrical or clavate 2
 - 1b. Ascomata small, mostly round without hairs asci small round 3
 - 2a. Ascomata (perithecia) covered with hairs, asci clavate
Chaetomium
 - 2b. Ascomata (apothecia) without hairs, asci cylindrical
Pyronema
 - 3a. Wall of the ascomata surrounded by thick-walled “Hülle cells”. Anamorph *Aspergillus nidulans* group *Emericella*
 - 3b. Wall consisting of one layer of flattened cells. Anamorph *Aspergillus glaucus* group ...
Eurotium
-

Table 5. Conidiogenesis of the common indoor moulds

Conidiogenesis	Genera
Phialides with dry conidia	<i>Aspergillus</i> , <i>Paecilomyces</i> , <i>Penicillium</i> , <i>Memnoniella</i> , <i>Wallemia</i>
Phialides with slimy conidia	<i>Acremonium</i> , <i>Clonostachys</i> , <i>Exophiala</i> , <i>Fusarium</i> , <i>Gliomastix</i> , <i>Phialophora</i> , <i>Stachybotrys</i> , <i>Trichoderma</i> , <i>Verticillium</i>
Phialides in pycnidia	<i>Phoma</i>
Annellides	<i>Scopulariopsis</i>
Thallic	<i>Geotrichum</i>
Blastic arthric	<i>Aureobasidium</i> , <i>Chrysonilia</i> , <i>Cladosporium</i> , <i>Geomyces</i> , <i>Oidiodendron</i>
Blastic single	<i>Botrytis</i> , <i>Epicoccum</i> , <i>Tritirachium</i>

Deuteromycetes

Most fungi found in air and in the indoor environment belong to the Deuteromycetes. As mentioned earlier, this group is not a formal Class in the Fungal Kingdom, but is composed of species without a teleomorph (sexual form). However, the species mostly belong *genetically* to the Ascomycetes, and some to the Basidiomycetes. The main criterion used in classifying fungi in the Deuteromycetes is the mode of conidium formation, or **conidiogenesis** (Table 5). For a more detailed account the reader is referred to Cole and Samson (1979). The specialized non-motile asexual propagules, the **conidia**, are not formed by cleavage like zygomycete sporangiospores. Their shape and colour vary widely and they may arise solitarily, synchronously, in chains or in slimy heads. They are borne on a specialized cell, the **conidiogenous cell**. Such cells can be borne directly in or from a vegetative hypha or on differentiated supporting structures (stipe and branches). For many genera in the indoor environment the special conidiogenous cell is called a **phialide**. Conidia are mostly produced in high numbers either in dry chains or in slimy heads.

Fig. 5 illustrates phialidic conidiogenesis in some common genera. The entire system of fertile hyphae is called the **conidiophore**. In Fig. 6 genera with annellides thallic and various blastic conidiogenesis are illustrated.

Building materials often provide optimum conditions for production of morphological structures by which these mitosporic fungi are identified, so that individual types can be rapidly recognized by using transparent adhesive tape and examining it by light microscopy. For some difficult genera such as *Aspergillus* and *Penicillium* identification to species level requires isolation and cultivation on special media. Most Deuteromycetes sporulate well on MEA (2%) and OA agar. For some genera, e.g. *Fusarium*, SNA agar with a piece of sterile filter paper is very suitable for achieving good sporulation.

Penicillium and *Aspergillus* require Czapek-Dox or Czapek-yeast extract agar (CYA) for correct identification (see also the description of each genus for culture methods).

Good microscopic mounts are required in order to examine conidiogenesis. The mounts are mostly made in lactic acid with some aniline blue. For fragile structures such as in *Botrytis*, *Oidiodendron*, *Geomyces* and *Cladosporium*, it is often helpful to prepare slides on which a small piece of adhesive tape or a cut-out square of transparent agar is mounted. The production of conidia in chains or slimy heads is best observed in the Petri dish under low power magnification. However, care must be taken to avoid inhalation of fungal products (volatiles) and conidia.

Table 5 lists the common genera of Deuteromycetes likely to be found in indoor environments. For the important genera a short description of characters is given below. Other genera are described in detail by Ellis (1971, 1976), Carmichael *et al.* (1980), Sutton (1980), Domsch *et al.* (1993) and Samson *et al.* (2000). A key to the genera commonly encountered indoors follows the descriptions (see pp. 262–264).

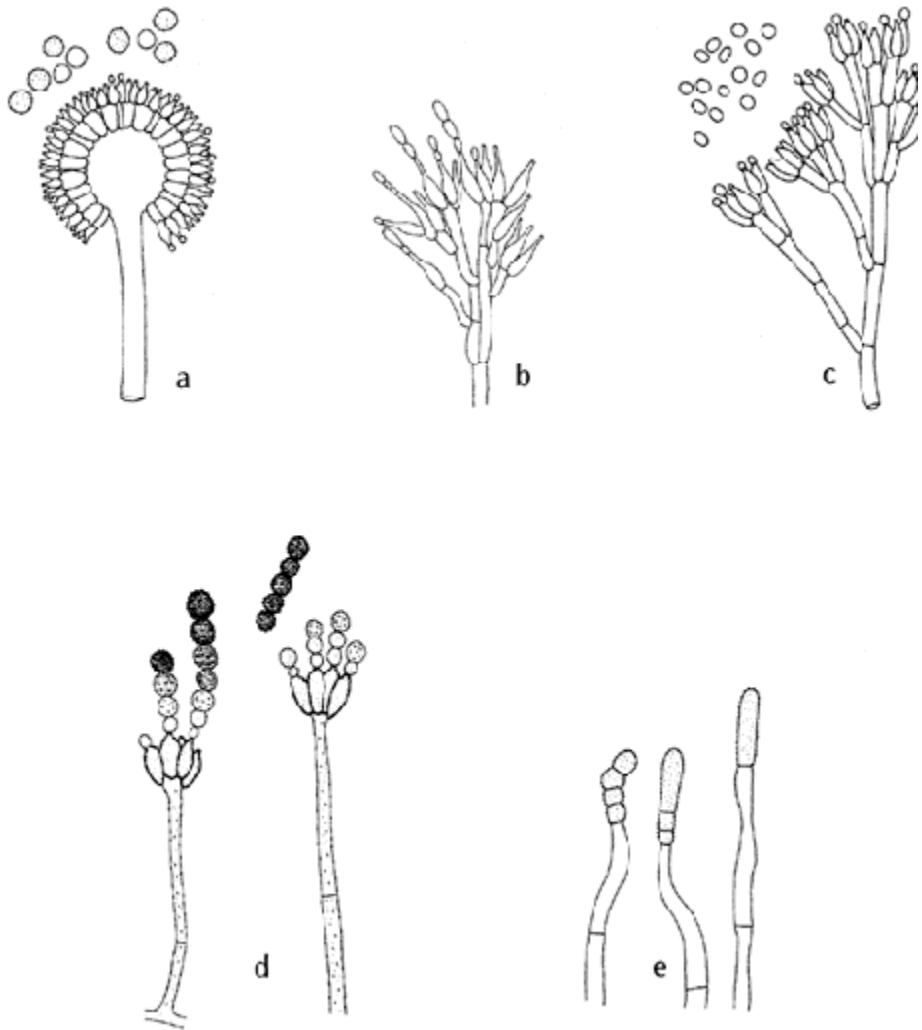


Fig. 5. Examples of phialidic Deuteromycetes with dry conidial chains: a. *Aspergillus*, b. *Paecilomyces*, c. *Penicillium*, d. *Memnoniella*, e. *Wallemia*.



Fig. 6. Examples of phialidic Deuteromycetes with wet or slimy conidial heads:
a. *Acremonium*, b. *Fusarium*, c. *Phialophora*, d. *Stachybotrys*, e.
Trichoderma, f. *Verticillium*.

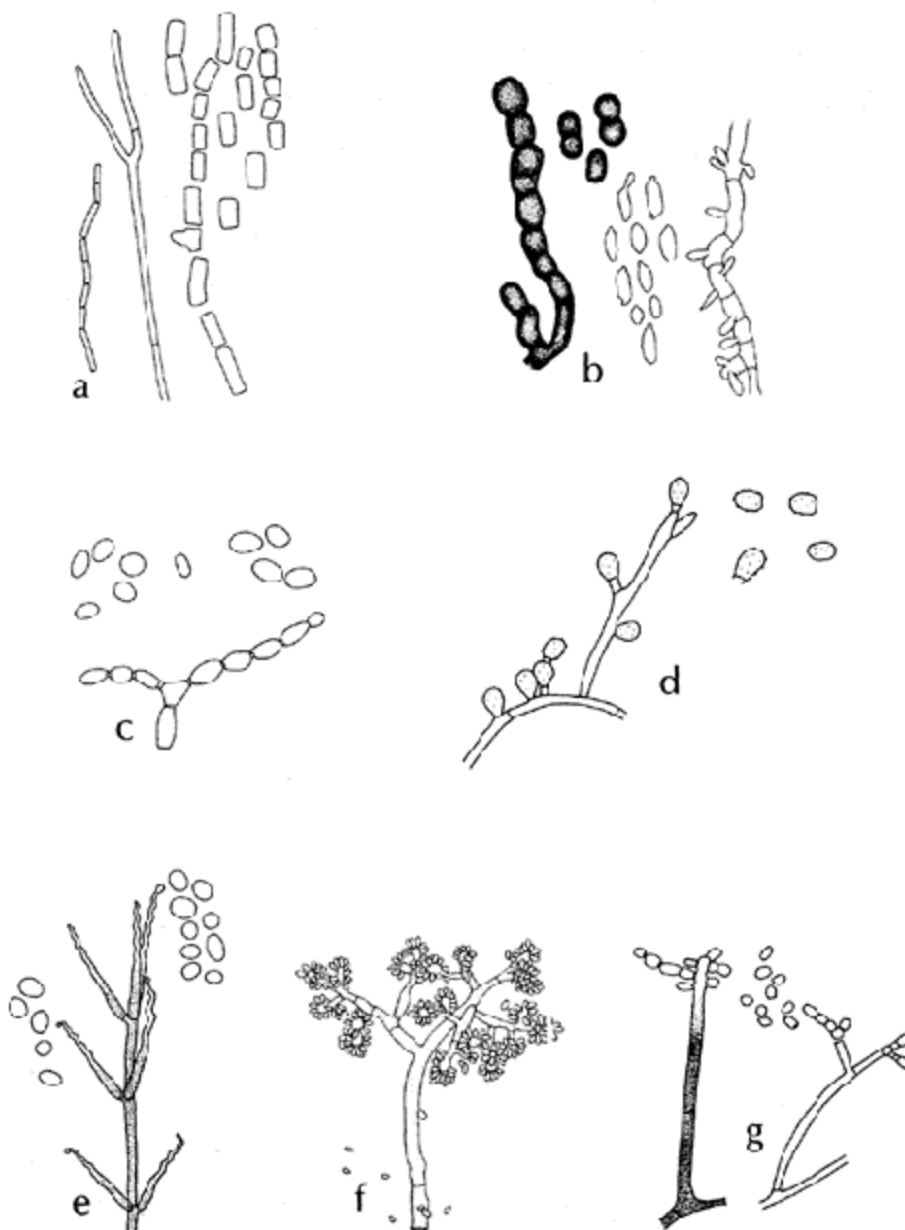


Fig. 7. Examples of thallic and blastic Deuteromycetes: a. *Geotrichum*, b. *Aureobasidium*, c. *Chrysonilia*, d. *Geomyces*, e. *Tritirachium*, f. *Botrytis*, g. *Oidiodendron*.

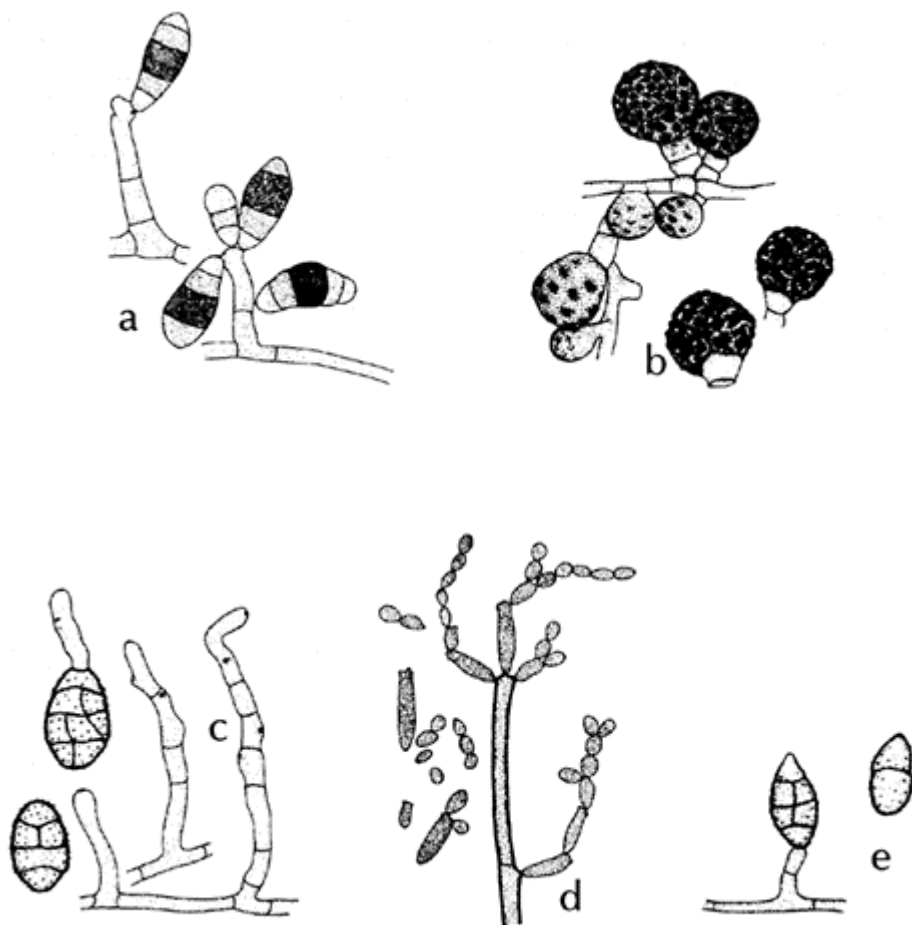


Fig. 8. Examples of blastic Deuteromycetes: a. *Curvularia*, b. *Epicoccum*, c. *Ulocladium*, d. *Cladosporium*, e. *Alternaria*.

Aspergillus

Aspergillus spp. are common contaminants on various substrates. In subtropical and tropical regions they occur more commonly than *Penicillium* spp.

Colonies usually growing rapidly, white, yellow, yellow-brown, brown to black or shades of green, mostly consisting of a dense felt of erect conidiophores. Conidiophores unbranched with a swollen apex (vesicle). Phialides borne directly on the vesicle (uniseriate) or on metulae (biseriate). Conidia in dry chains forming compact columns (columnar) or diverging (radiate), one-celled, smooth or ornamented, hyaline or pigmented. Species may produce Hülle cells (large, thick- and smooth-walled cells) or

sclerotia (firm, usually globose, masses of hyphae). Teleomorphs: *Eurotium*, *Emericella*, *Neosartorya* and other genera.

Raper and Fennell (1965) divided the genus into 18 groups and accepted 132 species with 18 varieties. Samson (1979, 1992, 1994a, b) has provided a compilation of the species and varieties described since 1965 with a critical review of the validity of the published taxa. The genus now contains more than 180 species, with about 70 named teleomorphs.

Practical laboratory guides for identification of common aspergilli which are primarily based on morphology and colony characters have been published by Pitt and Hocking (1997), Klich and Pitt (1988), Kozakowicz (1989) and Tzean *et al.* (1990)

Cladosporium

This genus has a world-wide distribution. Several species are plant pathogens or are saprophytic and more or less host-specific on old or dead plant material.

Colonies mostly olivaceous-brown to blackish-brown or with a greyish-olive appearance, velvety or floccose becoming powdery due to abundant conidia, and rather slow-growing. Conidiophores fragile, erect, straight or flexuous, unbranched or branched only in the apical region, with geniculate sympodial elongation in some species. Conidia in branched chains, one-celled, ellipsoidal, fusiform, ovoid, (sub)globose, often with distinct scars, pale to dark olivaceous-brown, smooth-walled, verrucose or echinulate; (blasto)conidia mostly formed on denticles in groups of 1–3 at the apex of the conidiophore, subapically below a septum or on the tip of previously formed conidia.

For more detailed descriptions and keys see De Vries (1952), Ellis (1971), Domsch *et al.* (1993).

Owing to the fragile conidiophore structure it is often difficult to obtain good microscope slides. It is helpful to use transparent adhesive tape and lightly touch the colonies.

Fusarium

Most *Fusarium* species occur in soil or on plants and have a world-wide distribution. The genus includes plant pathogens of crops widely grown around the globe, e.g. wheat and maize. A few fusaria can be found in indoor environments and are mostly associated with wet niches, including cooling units of air-conditioning systems. The current taxonomy of *Fusarium* is not clear, and molecular taxonomic data will probably change the classification and number of species radically. There are many monographs which can be used for identification. The following publications are recommended: Gerlach and Nirenberg (1982), Nelson *et al.* (1983), Marasas *et al.* (1984), and Nirenberg (1989; 1990), Thrane (1989) and Burgess *et al.* (1988).

Penicillium

Many species of *Penicillium* are common contaminants on a wide variety of substrates.

Colonies usually growing rapidly, in shades of green, mostly consisting of a dense felt of conidiophores, which arise from the substrate, aerial hyphae or from bundled hyphae. Conidiophores are hyaline, smooth- or rough-walled, single (mononematous) or bundled (synnematus). The penicillus consists of branches and metulae (penultimate branches which bear a whorl of phialides). The branching pattern is either one-stage branched (biverticillate), two-stage branched (terverticillate) or three- (quaterverticillate) to more-stage branched and is important in allocating the isolates to the various subsections. The phialides are mostly flask-shaped or lanceolate (= acerose; more or less narrow basal part tapering to a somewhat pointed apex) and produce long dry chains of conidia.

Because of the widespread occurrence of penicillia and their potential for producing mycotoxins, correct identification is important when studying possible *Penicillium* contamination. Purely morphological characteristics can be used for identification, but the isolates should be grown under standard conditions using malt extract agar and Czapek-yeast extract agar at 25°C for 7 days. For more recent detailed descriptions and keys to penicillia see also Pitt (1979, 2000). The most common *Penicillium* species are described in Samson *et al.* (2000).

For some species, odour and exudate production will help in recognition of the taxa, but it should be stressed that inhalation of conidia and volatiles may cause adverse health effects. However, as in *Aspergillus*, true human pathogenic species are rare in *Penicillium*, and limited to *P.marneffe* alone.

Scopulariopsis

Colonies vary from white, creamish, grey or buff to brown or even blackish, often darkening with age, but never in green like *Penicillium*. The conidiogenous cells are cylindrical or with a slightly swollen base, annellate (rings formed after conidium formation), single or borne on whorls. Conidia in chains with a broadly truncate base. For a more detailed account of descriptions and key see Morton and Smith (1963), and Domsch *et al.* (1993).

Stachybotrys

Species of *Stachybotrys* have a world-wide distribution and occur on paper, wallpaper, seeds (e.g. wheat, oats), soil, textiles and dead plant material. There are several species of *Stachybotrys* but the most well-known taxon is *S. chartarum*.

Colonies grow slowly, appearing blackish to blackish-green. *Memnoniella* resembles *Stachybotrys*, only differing in having dry conidia in chains, not in slimy heads like those in *Stachybotrys*. For detailed descriptions and keys see Ellis (1971) and Domsch *et al.* (1993). Exposure to this fungus on natural substrates or in culture should be avoided.

KEY TO THE COMMON INDOOR DEUTEROMYCETE GENERA (adapted from Samson et al. 2000).

- 1a. Conidia borne in pycnidia.....Phoma
- 1b. Conidia not borne in pycnidia, but on hyphae, conidiophores, sporodochia or synnemata.....2
- 2a. Conidia produced from special conidiogenous cells (phialides, annellides etc.) in chains or in heads, in basipetal succession 3
- 2b. Conidia not produced from special conidiogenous cells, but acropetally or by fragmentation of fertile hyphae, synchronously or single 14
- 3a. Conidia in dry chains 4
- 3b. Conidia in moist or slimy heads..... 10
- 4a. Conidia usually 2-celled, borne on filamentous conidiogenous cells, more or less obliquely inserted, arranged like a spike. Colonies pinkish....
..... *Trichothecium*
- 4b. Conidia always one-celled, borne on flask-shaped or cylindrical conidiogenous cells in straight chains. Colonies in various colours 5
- 5a. Colonies very restricted, reddish-brown Conidia formed (in a quartet) by division of a cylindrical verrucose fertile hypha, cubic, becoming (sub)-globose. Xerophilic...
..... *Wallemia*
- 5b. Colonies usually not restricted (except xerophilic *Aspergillus* species). Conidia not formed after division of fertile hyphae.... 6
- 6a. Conidiophores with a typical apical swelling
.....*Aspergillus*
- 6b. Conidiophores without an apical swelling.....7
- 7a. Conidiogenous cells annellidic. Conidia with a broad truncate base...
.....*Scopulariopsis*
- 7b. Conidiogenous cells phialidic. Conidia without a broad truncate base. 8
- 8a. Colonies dark grey to blackish. Phialides obovate (broadest at the top), conidia blackish.
..... *Memnoniella*
- 8b. Colonies not dark grey to blackish. Phialides flask-shaped or lanceolate (broadest at the base)..... 9
- 9a. Colonies yellow to brown. Phialides with a long neck.
.....*Paecilomyces*
- 9b. Colonies often greenish (some species whitish). Phialides with a short neck....
.....*Penicillium*
- 10a. Phialides long, awl-shaped, polyphialides absent.....

Acremonium

- 10b. Phialides more or less flask-shaped and/or polyphialides present, or phialides reduced..... 11
- 11 Colonies usually green (when grown in light)
- a. *Trichoderma*
- 11b. Colonies whitish, yellow, purple, violet, pinkish, brown or blackish 12
- 12a. Colonies white, yellowish pinkish, purplish, sometimes greenish. Septate banana-shaped conidia usually present..... *Fusarium*
- 12b. Colonies black, sometimes pinkish. Conidia not septate..... 13
- 13a. Phialides solitary or in loose whorls, flask-shaped with a conspicuous collarette or phialides reduced. Conidiophores not distinct
- Phialophora/*
- Lecytophora*
- 13b. Phialides in dense apical clusters, broadly clavate, widest near apex, without conspicuous collarette. Conidiophores distinct, with stipes *Stachybotrys*
- 14a. Colonies growing very fast, covering a 9 cm Petri-dish within a few days, loose, floccose, orange *Chrysosilia*
- 14b. Colonies not orange and not covering a 9 cm Petri-dish within a few days 15
- 15a. Conidia only arthric 16
- 15b. Conidia both arthric and blastic or only blastic 18
- 16a. Conidiophore stipes pigmented
Oidiodendron
- 16b. Conidiophore stipes hyaline or absent..... 17
- 17a. Conidia hyaline, smooth-walled, more or less cylindrical. Intercalary conidia absent *Geotrichu*
- 17b. Conidia (sub)hyaline becoming faintly coloured, and rough-walled with age. Intercalary conic present *Geomyces*
- 18a. Conidia formed in a quartet by division of a cylindrical fertile hypha.... *Wallen*
- 18b. Conidia not formed in a quartet..... 19
- 19a. Blastic conidia borne synchronously on hyphae, or from swollen cells or branches 20
- 19b. Blastic conidia not formed synchronously on hyphae or swollen cells or branches 21
- 20a. Conidia borne from denticles on terminally swollen conidiogenous cells. Conidiophores erect apically branched (tree-like). Colonies thin, greyish-brown *Botrytis*
- 20b. Conidia borne on hyphae or on almost completely swollen branches. Colonies yeast-like, creamish-yellow to light brown, pinkish-orange or blackish-

green	<i>Aureobasidium</i>
21a. Conidia formed singly on indistinct conidiophores, clustered and visible as black dots.....	<i>Epicoccum</i>
21 b. Conidia formed singly or in chains, conidiophores distinct, not clustered..	22
22a. Conidia rather thin-walled, mostly one-celled; basal conidia can be 2-celled, only with transverse septa.	<i>Cladosporium</i>
22b. Conidia septate with both transverse and longitudinal septa (muriform) or only with transverse septa	23
23a. Conidia with transverse septa, slightly curved, end cells paler than central cell	<i>Curvularia</i>
23b. Conidia with both transverse and longitudinal septa (muriform)	24
24a. Young conidia rounded at the base, mature conidia catenulate and/or rostrate	<i>Alternaria</i>
24b. Young conidia attenuated at the base, mature conidia single or in “false” short chains.....	<i>Ulocladium</i>

Yeasts

Yeasts mostly reproduce vegetatively by budding. Exceptions are species of the genus *Schizosaccharomyces*, which reproduces by a fission process, and of the genera *Sporobolomyces*, *Sterigmatomyces* and *Fellomyces*, which form buds on short stalks. The morphology of the unicellular yeast cell indicates the genus, but further identification should be performed using a number of physiological tests, including assimilation of a number of sugars. Commercial identification kits provide computer programs for processing the results obtained with them. These include the API 20C and API YEAST-IDENT™ kits in which growth tests are carried out in small wells on plastic strips. Biolog Inc. markets the Microlog™ system for yeasts which, by using the redox dye tetrazolium violet, detects whether a microorganism can utilize a given carbon source. For a recent review the reader is referred to Barnett *et al.* (2000a,b).

Bacteria

The isolation of bacteria from environmental samples is complicated by the fact that, while many bacteria will grow on a wide range of isolation media, others are extremely fastidious in their nutritional requirements. Where only a general picture of the variety of bacteria and the overall load is required, commercially available general purpose media such as plate count agar, nutrient agar, tryptone soya agar (TSA) or R2A agar can be used, and cycloheximide may be used as an antifungal supplement. Rather than the 37°C used in most bacteriological studies, it is preferable to incubate environmental plates at

25°C, although 15°C may be employed (Austwick *et al.* 1989). For isolation of particular bacteria, media in which their particular nutritional needs are met or selective media which retard or inhibit other bacteria are employed. For example, cetrimide agar or Pseudomonas agar are used for isolation of the pathogen *Pseudomonas aeruginosa*, and buffered charcoal yeast extract agar (Chapter 3.4) is used for *Legionella*.

For general isolation of thermophilic Actinomycetes, tryptone soya casein hydrolysate agar (TSC), i.e. half-strength TSA (Oxoid) supplemented with 0.2% casein hydrolysate, incubated at 55°C can be used. TSC can be made selective for *Thermomonospora* and *Saccharomonospora* by addition of 5 µg rifampicin ml⁻¹. For isolation of *Saccharopolyspora rectivirgula*, modified hippurate agar (HAX) can be used, and for *Thermoactinomyces* spp. Czapek yeast casein agar containing tyrosine and novobiocin (CYCTN), again incubated at 55°C (Lacey *et al.* 1992).

Identification of bacteria is highly specialized, and it is not intended to deal with this here. For further information on bacteria, the reader is referred to the Field Guide of the American Industrial Hygiene Association (Dillon *et al.* 1996). However, illustrated descriptions of selected thermophilic Actinomycetes are given in the final section of this book.

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Chapter 4.3

ANALYSIS OF MICROBIAL VOLATILE ORGANIC COMPOUNDS

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INTRODUCTION

An appreciable percentage of the biomass of the Earth is microbiological (Miller 1992). Microbes can be found everywhere—on all organic substrates and in all ecosystems, including deserts, forests, seas and lakes. The principal effect of their activity is to convert complex organic compounds into their constituents and thence into microbial biomass, free nutrients, water and energy. This is a very important process as 10^{17} g dry matter of plant production needs to be converted globally per annum. In this process, the microorganisms are responsible for 80–90% of these conversions. In the course of this metabolism, a wide variety of intermediary compounds is produced, depending on the type of compound being converted, the type of organism involved and environmental factors such as pH, temperature and oxygen availability. These intermediary compounds can be of high or low molecular weight, non-volatile or volatile, hydrophobic or hydrophilic, toxic or non-toxic. Man has taken advantage of the metabolic activities of microorganisms for the production of various by-products, antibiotics, organic acids, alcohol, etc.

As discussed in Chapter 2.1, buildings are subject to microbial colonization because of water infiltration resulting from a variety of causes. Whether the moisture occurs on surfaces or within the envelope, building materials are at high risk of being colonized by microorganisms. During microbial growth, the microbes present produce some of their by-products as a function of growth. These include toxins (e.g. Nielsen *et al.* 1998) and allergens, most of which are retained in the spores. Exposure to spores has been shown to affect the health of the building occupants (Flannigan and Miller 1994; Rylander and Etzel 1999). There have been numerous formal investigations that show the existing relationship between microbial growth in buildings and health problems (Norbäck and Widström 1988; Jaakkola *et al.* 1993; Rylander and Etzel 1999). Field investigators also report a similar relationship between ill-health and “sick” buildings.

The concentrations or species composition of the airborne microbiota in different investigations are similar, regardless of whether the microbial growth is visible or concealed within wall cavities (Miller *et al.* 2000). Experience has shown that analysis of the microbiota of the air is adequate when the ventilation system of the building is contaminated or if some mould-infested material is being handled within the building.

The difficulties of measuring microbial impact on the quality of indoor air have brought into focus the need to develop new analytical methods for the detection of microbial impact. We know today that the presence of microbial biomass on building surfaces or within the building construction affects the health of the users of the building in a negative way.

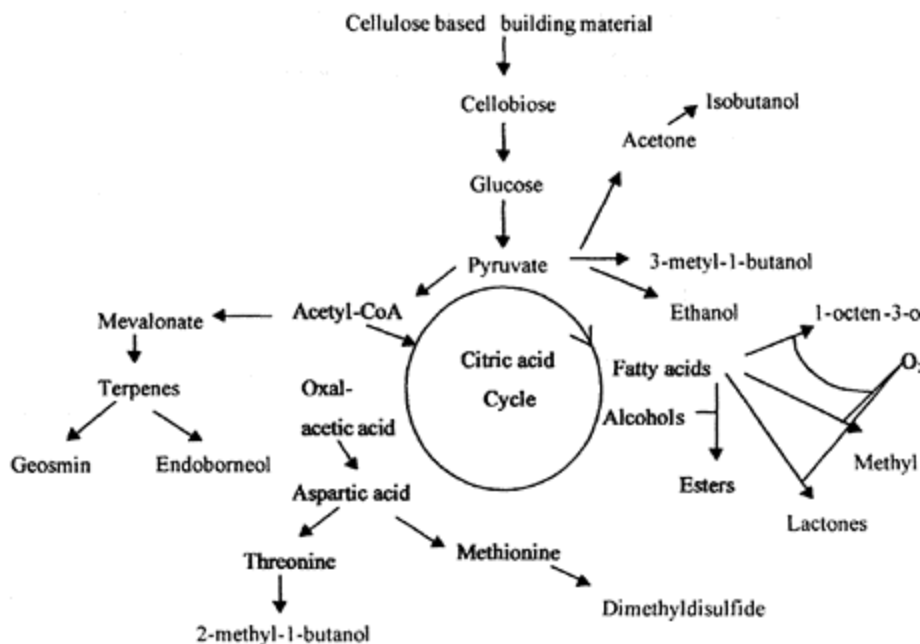


Fig. 1. Metabolic pathways leading to the production of some volatile metabolites by microorganisms growing on cellulose.

There is poor correlation between airborne spores or debris arising from microbial growth on building materials and the health status of building occupants. As mentioned in Chapter 4.1, this is generally seen as a limitation of the methods used. Some of the metabolites produced by microorganisms are volatile, and the microbial metabolic pathways that can lead to their production are shown in Fig. 1. It is possible that determination of microbial volatile organic compounds (VOC's) might be an alternative measure of exposure.

MICROBIAL VOLATILE ORGANIC COMPOUNDS IN BUILDINGS

It is known that fungal volatiles have antifungal and cytotoxic properties (Bruce *et al.* 1984; Miller *et al.* 1988), so that it is possible that under some circumstances microbial volatile organic compounds (MVOC's) may be partially responsible for complaints of ill-

health by building occupants (Samson 1985). MVOC concentrations are higher in damp buildings with occupant complaints than non-problem buildings (Ström *et al.* 1994; Smedje *et al.* 1996). In addition, MVOCs can pass through polyethylene sheeting (Smedje *et al.* 1996) and may therefore cause symptoms in the (unusual) absence of exposure to spores. However, although many of the known volatiles are cytotoxic, there is little direct evidence of an impact of MVOC's on human or animal health as yet (Flannigan and Miller 1994).

In a study of Canadian residential housing, Miller *et al.* (1988) found that MVOC's were present in houses with moisture problems, but measurement of MVOC's was less sensitive than visual observations of fungal growth. In addition, the majority of MVOC's in indoor air also have man-made or other sources. VOC's emanate from a wide range of solid materials (Table 1), as well as being present in water and air. Miller *et al.* (1988) therefore proposed the measurement of a smaller set of compounds that appeared more diagnostic for fungal growth in relation to other objective measures for fungal contamination. In addition to the complication of a proportion of MVOC's also having non-microbiological sources, a further problem is that these compounds are present in very low concentrations, some at picogram level.

Table 1. Classes of volatile organic compound and possible sources.

Compound	<i>Material/Source</i>								
	Wood	Linoleum	Paint	Plastics	PVC*	Glue	MO*	WP*	Putty
Terpenes	+	+				+	+		
Glycoether			+	+		+			+
Glycoether ester		+?	+	+	+	+			+
Aldehydes	+	+	+						
Alcohols		+	+	+	+	+	+		+
Ketones		+	+	+		+	+		
Esters			+	+	+	+	+		
Sulphides							+		
Furans	+	+					+		
Aliphatics			+	+		+			
Mono-aromatics			+	+	+	+			
Poly-aromatics									+
Chloro-aromatics									+

*MO, microorganisms; PVC, polyvinyl chloride floor covering; WP, wood preservatives

Irrespective of whether it is the room air or air from within the building fabric, such as

the floor, roof or wall, which is being sampled in a building, the analytical methods that are used to determine the presence and concentrations of VOC's are to a high degree the same. Air is actively sampled by drawing it by pump through an adsorbent, or is passively sampled by allowing it to diffuse into adsorbent. The sample is then extracted from the adsorbent either through thermal desorption (TD) or through wet extraction (WE). The extracted compounds are then separated in a gas chromatograph and identified in a mass spectrometer. While active sampling caters for environments in which concentrations of VOC's are low, diffusion sampling is more restricted in its application, being more suited to environments with high VOC concentrations. Another disadvantage of diffusion sampling is that it is more suitable for sampling single compounds, e.g. formaldehyde, than for a wide range of different compounds as is likely to be the case in sampling VOCs. Sampling with a pump is more reliable, as a known volume of air is applied to the adsorbent and all of the different compounds present will be collected at the same rate. During analysis, thermal desorption can be used to improve the sensitivity of the analysis to a broader array of volatiles with higher and lower boiling points. The alternative is extraction with a solvent such as pentane. However, this only permits analysis of compounds with a lower boiling point than the solvent employed. The thermal desorption method is only suitable for small air samples, while the solvent extraction method allows large volumes of air to be sampled. The choice of method can be crucial since some compounds appear at very low concentrations. The solvent extraction method also allows the compounds present to be derivatized, again increasing the sensitivity. As noted above, VOC's are present in a wide variety of products and it is very important to control these in order to identify and separate the MVOC's from the VOC's originating from building materials.

When VOC analysis is part of an investigation of indoor air quality it is very important to possess a library of key compounds that are associated with wood, linoleum, paint, glue, plaster, fungi, bacteria, plastics, etc. Independently of microbial growth, many building materials emit more and sometimes different volatiles when wet. Such emissions go on for long periods of time, even if the moisture has disappeared. Woody materials usually emit terpenes and aldehydes (Table 1). Linoleum floors generally emit compounds in low concentrations, with aldehydes dominating. Compounds more or less uniquely related to paint include glycolethers. Problems with glue can usually be traced through increased emissions of 1-butanol and 2-ethyl-1-hexanol, while microbial impact can be seen through the appearance of 3-methyl-1-butanol, 3-octanone, 1-octen-3-ol, etc. When the microorganisms present grow, they produce an MVOC-profile in which some VOC's can be regarded as common compounds and some that are unique to microorganisms. In some cases, the

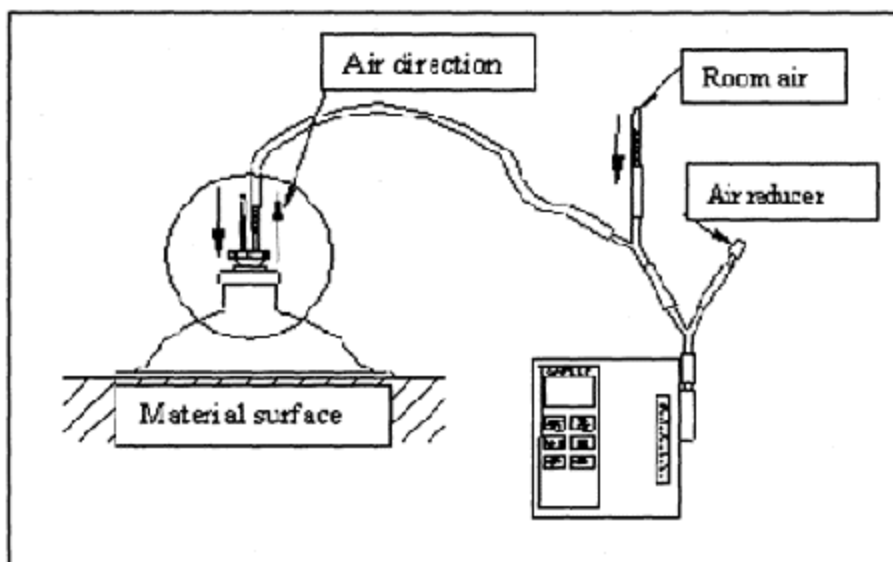


Fig. 2. Sampling the emission profile of the floor construction in an apartment of a “sick” building.

Table 2. The ratio of VOC to MVOC for some “problem” microorganisms.

Organism	VOC/MVOC ratio
<i>Aspergillus versicolor</i>	17:1
<i>Serpula lacrymans</i>	2–34:1
<i>Stachybotrys atra</i>	121:1
<i>Streptomyces griseus</i>	14:1

VOC's from building materials can exceed the amounts of MVOC's produced by a factor of 100 or more (Table 2).

METHODS FOR MVOC TESTING

Sampling

For sampling VOC's emanating from solid material we use of a desiccator lid as a sampling chamber (Fig. 2). Sampling performance can easily be extended by drilling a small hole (6 mm) in the wall, thereby sampling the trapped air. Our experience is that using the sampling chamber on surfaces it is possible to narrow down the areas that require remediation, thus avoiding costly renovation.

Samples are collected with portable constant flow pumps (500 ml air min⁻¹). In the laboratory environment, samples have been collected by passing air through glass tubes to adsorbent tubes containing 210 mg Anasorb 747 (beaded carbon). The adsorbent tubes are conditioned and sealed by the manufacturer (S.K.C., USA). The capacity of Anasorb 747 is high; it can retain more than 1 mg of VOC before breakthrough.

Desorption from adsorbents

In non-industrial environments, no breakthrough has been found when sampling air volumes up to 120 L over a period of 240 min. After the sampling period, the tubes are sealed with polytetrafluoroethylene caps and kept in a freezer cabinet until analysis. Each adsorbent tube is transferred to a 3.5 ml glass bottle and 1 ml dichloromethane is added. The glass bottles are placed on a shaker for 30 min. After desorption, the solvent is transferred to a 2 ml glass vial with a Pasteur pipette and sealed using a crimp-top seal with rubber liners.

Chromatography

Analysis is carried out employing a Hewlett-Packard HP 5890 gas chromatograph equipped with a HP 5971 mass selective detector (MSD). The analytical method has been adapted from Wessen and Schoeps (1996). For each hydrocarbon, a mass spectrum is selected, together with the retention time. The selective ion monitoring (SIM) ion values within 0.1 amu are determined to get the highest response. Three ions are selected for each compound. The SIM method increases the detection level for specific compounds by two to three orders of magnitude.

Selected indicator compounds

Indicator compounds and their commercial sources are: 3-methyl furan (Tokyo Kasei Kogyo Co., Tokyo, Japan), 2-methyl-1-propanol (Merck, Schuchardt, Germany), 1-butanol (Merck), 3-methyl-1-butanol (Merck), 2-methyl-1-butanol (Merck), dimethyl disulphide (Merck), 2-pentanol (Merck), 2-hexanone (Merck), 2-heptanone (Merck), 1-octen-3-ol (Merck), 3-octanone (Merck), 2-pentyl furan (Merck), iso-butyl-acetate (Merck).

Other studies

Similar methods have been used by Morey *et al.* (1997). This group has used HP MSD with single ion monitoring from solvent extracts of Anasorb 747 through which 48 L

room air are typically passed. They report detection limits of 0.5 ng per sample. The indicator compounds used by Morey *et al.* (1997) were 3-methyl furan, 3-methyl-1-butanol, 3-methyl-2-butanol, 2-pentanol, 2-hexanone, 2-heptanone, 3-octanone, 3-octanol, 1-octen-3-ol, 2-octen-1-ol, 2-methylisoborneol, geosmin and 2-isopropyl-3-methoxypyrazine.

Miller *et al.* (1998) have collected 200-L air samples on Tenax GC in residential housing and used thermal desorption followed by high resolution mass spectroscopy to determine the volatiles present. They determined 21 compounds known to come from 7 fungi common in damp homes, and used 3-methyl-1-butanol, 2-hexanone and 2-heptanone as indicators of fungal growth.

LESSONS FROM CASE STUDIES

In a block of rented apartments south of Stockholm with obvious dampness problems, a large number of the occupants experienced health problems, reporting mainly respiratory symptoms. During the physical investigation of the buildings it was shown that the floors, roofs and walls were damp. The VOC profiles (Fig. 3) revealed key compounds indicative of microbial growth, including 3-methyl furan, dimethyl disulphide and 3-methyl-1-butanol.

The presence of 3-methyl-1-butanol suggested the possibility of microbial growth in some cellulose-based material. This led to further sampling, and the construction space in the floor, wall and roof was sampled, with the indoor air serving as a reference. The sampling procedure involved drilling 6 mm holes, inserting the adsorbent tubes in these holes and sampling with the pumps. The samples were then analyzed for MVOC (Fig. 4). The results raised suspicions of a major microbial source located somewhere in the floor construction. This was later confirmed through microbiological analysis of the wood chips in the floor construction. This microbiological analysis was a combination of direct microscopical (Hobbie *et al.* 1977) and viable count (Jensen 1962) methods. The importance of using direct microscopy must be emphasized since inactive microorganisms may be the source of VOC emissions. If only methods based on culture are used MVOC sources sometimes cannot be confirmed.

When the microbiological source has been located it has to be removed, as has any damaged building material. At this stage, one has to be aware of the characteristics of the VOC's detected. Since some VOC's have a low boiling point they readily vaporize, while others have a high boiling point and consequently vaporize slowly. A further complication is that different compounds have different sink effects, i.e. the degree to which they adsorb to surrounding building material(s) varies. The effect of this is that compounds like 1-octen-3-ol and 2-hexanone will remain in the building for a long period of time after remedial action (Fig. 6), while compounds like 3-methylfuran and dimethyl disulphide will disappear more quickly (Wessen and Schoeps 1995). The fact that different compounds do not disappear simultaneously may mean that the total MVOC declines only slowly to normal levels. As a result of MVOCs being liberated when remediation involves opening up the construction and replacing the damaged

building material with new material, there may in fact be a build-up for a short period before the slow decay in concentration (Fig. 6).

In analysing for MVOCs in 119 indoor air samples, Morey *et al.* (1997) found that 1-octen-3-ol and 2-octen-1-ol were indicative of past water damage and consequent fungal growth. They found that the presence of these compounds in indoor air was a better indicator of chronic moisture problems than sampling for airborne culturable fungi (Morey 1997).

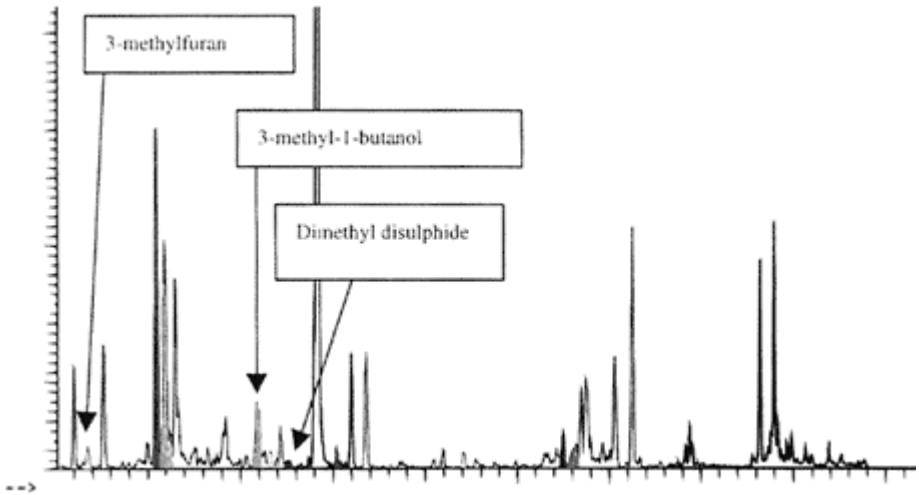


Fig.3. Gas chromatograph trace showing the VOC profile for air in a problem building.

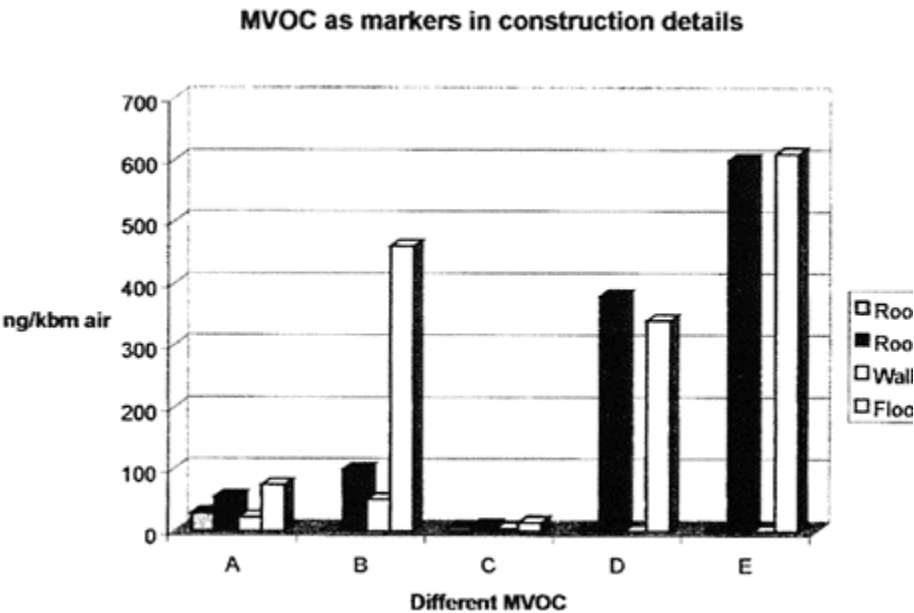


Fig.4. The impact of emissions from constructional spaces on MVOC content of room air: A, 3-methyl furan; B, 1-octen-3-ol; C, dimethyl sulphide; D, 2-pentanol; E, 3-methyl-1-butanol.

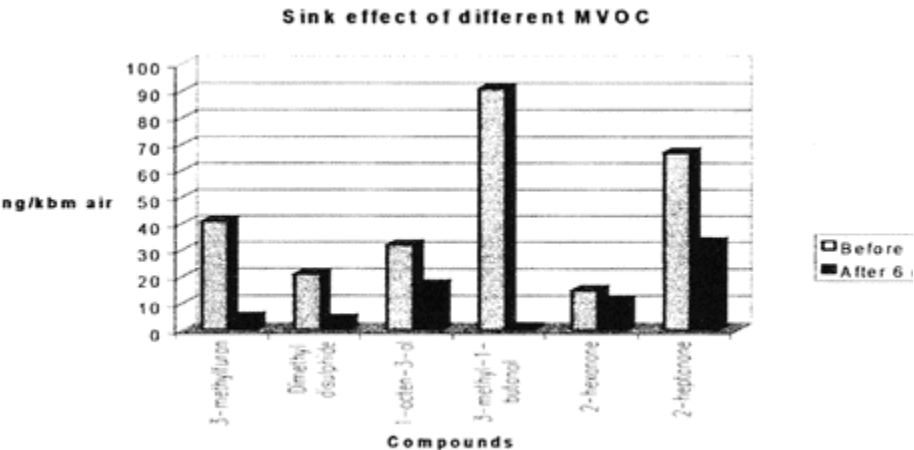


Fig.5. Concentration of selected MVOCs in indoor air before and after remedial treatment in a building.

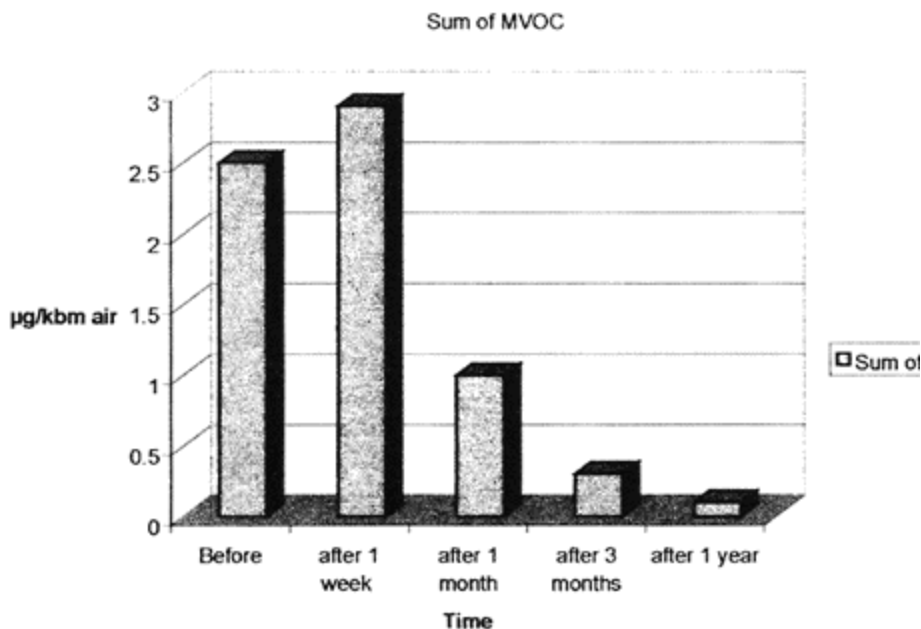


Fig.6. Example of changes in total MVOC content of air with time after remediation.

However, at present there are few data comparing MVOCs with other objective measures of fungal contamination. As further studies are carried out and the database is expanded, the utility of MVOC analysis as an investigative tool in different situations should become apparent.

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Chapter 4.4

MICROBIOLOGICAL INVESTIGATIONS OF INDOOR ENVIRONMENTS: INTERPRETING SAMPLING DATA- SELECTED CASE STUDIES

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INTRODUCTION

Interpreting microbial sampling data is challenging because there may occur in buildings a diversity of contaminants, including many different culturable and non-culturable fungi and bacteria and their toxins, e.g. endotoxins, mycotoxins and β -(1 $\rightarrow$ 3)-glucans. Methods of sampling and analysis for microorganisms and microbial products are reviewed in this volume (Chapters 4.1, 4.3 and 4.5) and elsewhere (ECA 1993; Health Canada 1995; AIHA 1996; ISIAQ 1996; ASTM 1998; ACGIH 1999). Past reviews have emphasized specific microorganisms (Health Canada 1995; ASTM 1998), certain kinds of buildings (ECA 1993; ISIAQ 1996), sampling and analytical methodology (AIHA 1996), and general principles of microbial evaluations (ACGIH 1999). This chapter aims to provide examples of practical case studies where the interpretation of sampling data has been a useful component of the building evaluation.

INTERPRETATION OF SAMPLING DATA DURING BUILDING EVALUATION

Sampling must be performed and analytical data must be interpreted in the context of an understanding of how the building performs or operates. For this purpose information on operation of any heating, ventilation and air-conditioning (HVAC) system present, the construction of the building and its envelope, and occupant susceptibilities must be understood prior to the development of the sampling plan. For example, when the sampling objective is to document exposure to *Stachybotrys* or its mycotoxins it is essential to know the location of pre-existing water damage in building systems and the specific location of water-damaged cellulosic materials. In a medical centre where the intention in sampling is to estimate risk of potential infection by *Legionella*, an understanding of configuration and operation of potable water services and cooling tower systems is essential for the development of the sampling plan.

The limitations of the sampling and analytical method must be understood by the investigator at the beginning of the evaluation. The collection of a single sample, or a few samples, in a building seldom adequately characterizes environmental microbiological conditions (AIHA 1996; ACGIH 1999). In addition, sampling data cannot be used to prove that a building is “safe” or that a microbial agent does not exist. Thus, the inability to detect culturable *Legionella* in a cooling-tower water sample does not mean that these bacteria were absent at any time in the past, or that currently *Legionellae* are absent in unsampled biofilms that may be present on surfaces in water systems.

Investigators must always be aware of limitations in sampling and analytical methodology that affect data interpretation. If the aim of sampling is to determine that *Stachybotrys* is present, then exclusive use of culture-based methods may overlook non-culturable spores detectable only by direct microscopical methods, e.g. cellulose tape and spore-trap sampling (see Chapter 4.1). In evaluations where it is intended to document past flooding of interior surfaces by sampling for culturable fungi such as *Chaetomium*, *Phoma* or *Trichoderma*, use of isolation media for hydrophilic fungi (e.g. malt extract agar) rather than for xerophilic species (e.g. DG-18) is essential for appropriate data interpretation.

Sampling in buildings must be preceded by a clear evaluation pathway that outlines how analytical data will be interpreted. For example, when the objective of air sampling is to determine whether exposure conditions in a building are “normal” after clean-up of visible fungal colonization is completed, collection of samples at many locations and at various times indoors, as well as concurrently in the outdoor environment, is the minimum required for data interpretation. In addition, comparison of air sampling data obtained both before and after clean-up, as well as knowledge that moisture problems have been eradicated and that visually mouldy materials have been physically removed (Chapter 2.3) add to the strength of possible data interpretations.

CASE STUDIES WITH INTERPRETATIONS

The case studies described here are categorized as “broad”, “indicative” or “focused” (ACGIH 1999) according to the methodology used for sample collection and analysis. When the objective of the evaluation is general, such as to determine if a normal distribution of phylloplane fungi occur indoors, sampling and analytical methodology which can potentially identify a wide range of taxa are used. Analysis of collected air or source samples on a medium such as malt extract agar can be expected to provide information on a broad range of fungi that may be present.

Indicator methods are used in sampling where the information is sought about a group of organisms or agents that can be used as an indicator of certain environmental conditions. Sampling for *Stachybotrys* and *Chaetomium* can be used as an indicator of soaking wet conditions on cellulosic materials. Sampling for endotoxin can be used as a surrogate indicator for the presence of Gramnegative bacteria.

Focused methods are used when information on specific microorganisms known to cause disease is sought. Sampling for *Aspergillus fumigatus* and *Legionella pneumophila*

in a medical centre following the occurrence of infection among patients is a highly focused building evaluation.

In the case studies that follow broad (Buildings 1–3), indicative (Buildings 4–9) and focused (Buildings 10–11) approaches to interpretation of sampling data are illustrated.

Building 1

Air sampling was performed in classrooms in a school where the envelope walls (mostly brick, concrete block, and hard plaster) had been chronically wet. At the time of sample collection the moisture problem had been remedied. Sampling was undertaken to determine whether airborne fungi in classrooms were similar to those present in the outdoor air or not. The medium used in the culture plate sampler (2% malt extract agar; ECA 1993) is supportive of the growth of a wide range of fungi.

Analytical results showed a general similarity of fungi collected indoors and outdoors with *Cladosporium cladosporioides*, *Epicoccum nigrum*, yeasts and non-sporulating fungi accounting for at least 80% of the taxa found (Table 1). The sampling results suggested that the environment in the classrooms was mycologically “normal”. The presence of the hydrophilic species *Ulocladium chartarum*, in the classrooms but not outdoors might have indicated the presence of water damaged building materials (Table 1). Concurrent inspection of classrooms and building structural systems did not, however, reveal water damage or visible fungal growth.

Table 1. Air sampling for a broad range of culturable fungi in a building with a past history of chronic roof and window leaks.

Fungi in outdoor air (Ranked taxa; % of total)*

Cladosporium cladosporioides (71)

Epicoccum nigrum (14)

Yeasts (5)

Non-sporulating fungi (4)

Penicillium brevicompactum (2)

Cladosporium herbarum (1)

(*Aureobasidium pullulans*, *P. simplicissimum* ≤0.5%)

Fungi in indoor air (Ranked taxa, % of total)*

Cladosporium cladosporioides (56)

Non-sporulating fungi (16)

Epicoccum nigrum (10)

Ulocladium chartarum (8)

Yeasts (3)

Arthrographis sp. (2)

Penicillium brevicompactum (1)

Tritirachium sp. (1)

(*Aureobasidium pullulans*, *Pithomyces chartarum* $\leq 0.5\%$)

* Average total concentration of fungi in outdoor air = 350 CFU m⁻³ air; Average total concentration of fungi in indoor air = 200 CFU m⁻³; MEA medium; N=6 samples indoors and outdoors.

In conjunction with the results of the building inspection, the data in Table 1 suggested that exposure to fungi in classrooms was broadly typical of that expected in non-problem schools and outdoor air.

Building 2

Surface sampling of supply air vent (diffuser) louvres was performed because several occupants claimed that fungi present in dusts on louvres were the cause of sick building syndrome complaints. While sampling cannot alone prove the presence or absence of building-related symptoms, the data from sampling can provide a general indication of the presence of fungal growth in a building. Surface sampling of air vent louvres for culturable fungi was performed at multiple locations of the portion of the building where complainants worked, and in multiple locations in the same building and in other control buildings nearby that had not been visited by the complainants. The objective in sampling was to determine whether the kinds of fungi on louvres in locations where complainants worked were suggestive of chronically damp or wet conditions. An association between chronic dampness and fungal growth on interior surfaces and the occurrence of allergic respiratory symptoms has been reported (Health Canada 1995).

Results of surface sampling showed that *Cladosporium* dominated the fungi recovered from both complainant and control locations (data from one complainant and two control locations are shown in Table 2). Sampling data also showed that the concentration of culturable fungi varied by up to three orders of magnitude between clean and dirty (dusty) louver areas of the same air vent. In addition, concurrent inspection of occupied spaces and the HVAC system of both symptomatic and control building areas showed an absence of water damage or visible fungal growth on interior surfaces.

Table 2. Surface sampling for a broad range of culturable fungi on louvres of air supply vents in three rooms.

Location and Description	CFU cm ⁻² surface*	Fungal taxa
Room #1 (symptomatic occupant work area)		
Visibly dirtiest louvre	280	<i>Cladosporium</i> spp. (86%), <i>Penicillium</i> spp. (9%), <i>P. corylophilum</i> (6%)
Visibly cleanest louvre	<5	
Room #2 (control area)		
Visibly dirtiest louvre	2×10 ⁴	<i>Cladosporium</i> spp. (95%), <i>Penicillium</i> spp. (5%), <i>Penicillium</i> spp.
Visibly cleanest louvre	5	
Room #3 (control area)		
Visibly dirtiest louvre	1×10 ⁵	<i>Cladosporium</i> spp. (98%), <i>Penicillium</i> spp. (2%) <i>Cladosporium</i> spp. (67%), <i>P. corylophilum</i> (33%)
Visibly cleanest louvre	1.5×10 ³	

Sterile 1×5 cm template applied to louvre; surface dirt removed by sterile wet cotton swab; dirt eluted in sterile water and plated on DG-18 agar medium. Limit of detection 5 CFU cm⁻².

Table 3. Air sampling for a broad spectrum of cultivable fungi in a residence with a past moisture problem.

Fungi in outdoor air (Ranked taxa, %)*
<i>Cladosporium cladosporioides</i> (92)
<i>Penicillium brevicompactum</i> (2)
<i>C. sphaerospermum</i> (1)
<i>P. implicatum</i> (1)
<i>P. sclerotiorum</i> (1)
Yeast (1)
<i>Aspergillus niger</i> (0.5)
(<i>P. citrinum</i> , <i>Rhodotorula</i> sp., non-sporulating fungi at ≤0.5%)

Fungi in indoor air (Ranked taxa, %)**A. versicolor* (24)*P. corylophilum* (21)*C. cladosporioides* (14)*P. citrinum* (8)

Non-sporulating fungi (8)

Wallemia sebi (7)*A. sydowii* (7)*Eurotium herbariorum* (2)*P. sclerotiorum* (2)*(P. brevicompactum, P. implicatum, A. niger, A. flavus, Rhodotorula spp., C. sphaerospermum at ≤1%)*

*Average total concentration of fungi in outdoor air=2,000 CFU m⁻³; mean total concentration of fungi in indoor air= 340 CFU m⁻³; DC-18 agar medium; N=3 indoors and outdoors.

Together with the building inspection in this study, the results of sampling suggested that complaints were unrelated both to fungi in surface dust on air supply vents and to the mycological conditions in the indoor environment.

Building 3

Rupture of a pipe resulted in wetting of carpet in several rooms of a residence. Fungi were observed growing on portions of the wet carpet. All carpet, which had been wet, was removed and replaced with new carpet. Carpet throughout the rest of the house was cleaned with a low efficiency vacuum cleaner. Occupants who had previously vacated the residence after the rupture reported the onset of mucous membrane and upper respiratory tract irritation after returning. The air was sampled for culturable fungi in the residence and also outdoors in order to see if the broad range of taxa indoors indicated a possible hidden mould problem or cleaning that was inadequate. Concurrent visual inspection of the occupied space and HVAC system did not reveal water damage or visible fungal colonization either on interior surfaces or in the HVAC system.

Cladosporium cladosporioides accounted for more than 90% of the fungi recovered in the outdoor air at grade level around the residence (Table 3). Minor amounts of *Penicillium* and *Aspergillus* were also present in the outdoor air. The rank order of taxa present in indoor air was distinct from that outdoors, with *A. versicolor* and *P. corylophilum* recovered as predominant fungi. The isolation of these fungi, and *Aspergillus sydowii* and *Wallemia sebi*, in the indoor air suggested hidden dampness in the building infrastructure or the presence of reservoirs of fungal spores not adequately removed during previous clean-up activities. While air sampling data in this case cannot be used to explain occupant complaints directly, the dominating occurrence of non-phyloplane *Penicillium*, *Aspergillus*, and *Wallemia* indoors is suggestive of chronic

interior dampness which has in other studies been associated with allergic respiratory symptoms (Flannigan and Miller 1994; Health Canada 1995).

Building 4

Air sampling was performed during simulated use (opening and closing) of books and files to determine if these materials could be taken into a clean, newly constructed building. The books and files had been stored in a facility where extensive growth of fungi had occurred on the surface of the interior walls and ceiling. Books and files were neither water-damaged nor visibly mouldy. However, at the time of sampling they were being stored temporarily in a building with noticeable roof leaks and water stained ceiling tiles.

Sampling was initially performed during simulated use of books and files in the water-damaged storage room of the temporary building. The objective of sampling was to determine if fungi such as *Aspergillus versicolor*, that would indicate if reservoirs of spores accumulated during past storage under mouldy conditions were still present on the books and files. Sampling in the temporary storage room showed that *Penicillium* was dominant in room air both before and during simulated use of books and files (Table 4).

Table 4. Air aampling for non phylloplane culturable fungi during simulated use of books and files that had been stored in a mouldy building.

Sample Description	Culturable Fungi (CFU m ⁻³)*			
	Total	<i>Cladosporium</i> spp.	<i>Penicillium</i> spp.	<i>Aspergillus</i> <i>versicolor</i>
Quiescent indoors	520	150	230	4
Simulated use of books indoors	500	155	210	6
Outdoor air	1160	340	70	nd
Simulated use of books outdoors	1130	345	35	2

DG-18 agar medium; N=4-7; Nd, not detected

From this data alone it could not be determined whether the recovered fungi originated from the books and files or from the water damaged temporary storage facility itself.

A repetition of air sampling during simulated use of books and files outdoors showed that concentrations of *Penicillium* and *A. versicolor* did not rise above background levels. *Cladosporium* was the dominant identifiable fungus in all outdoor samples, in which there were many non-sporulating fungi. Air sampling thus indicated that books and files were unlikely to contain strong reservoirs of non-phyloplane spores associated with past storage in water damaged or mouldy buildings. The books and files could be moved into the clean building.

Building 5

Air sampling by spore trap was performed in a new building that had suffered water damage. Large areas ($>1000\text{ m}^2$) of visibly mouldy wallpaper, paper-fibre gypsum board, cellulosic fire insulation and floor coverings had previously been removed. At the time of air sampling, water leaks had been repaired and the building infrastructure had been dried. However, there were considerable amounts of settled dust associated with past clean-up activities on floors and other surfaces. The object of air sampling was to determine whether or not respiratory protection was still needed by clean-up and construction personnel working in the building.

Sampling in the outdoor air showed that the concentration of *Cladosporium* spores was approximately twice that of *Penicillium/Aspergillus* (Table 5). Air sampling indoors under quiescent conditions (dust not actively being stirred-up by indoor activities) showed that while *Cladosporium* levels were similar to those outdoors, *Penicillium/Aspergillus* concentrations exceeded 10^4 CFU m^{-3} air. In addition, settled dusts collected from floors and other horizontal surfaces in the building were analyzed for the presence of culturable fungi. Results indicated that *Penicillium/Aspergillus/Eurotium* accounted for $>95\%$ of culturable fungi in settled dusts, the total concentration being approximately 10^8 CFU g^{-1} dust (Table 5).

The dominance of *Penicillium* and *Aspergillus* in samples of both air and settled dusts indicated that strong reservoirs of fungi associated with past water damage were still present in the building. Sampling data also showed that it was likely all persons working in the building would be exposed to *Penicillium/Aspergillus* concentrations approaching those typically found during handling of mouldy agricultural materials. Consequently, use of personal protective equipment such as P-100 respirators (Morey and Sawyer 1999) by all restoration personnel was required.

Table 5. Air sampling for fungal spores in a dusty building with past moisture problems.

Sample Description	Spores m^{-3} air	
	<i>Cladosporium</i>	<i>Penicillium/Aspergillus</i>
Outdoors	120	55
Indoors †	120	11500

Spore trap operating at $0.01\text{ m}^3\text{ min}^{-1}$ used to perform air sampling, † Composite sample of settled dusts from floors contains about 10^8 CFU g^{-1} ($>95\%$ *Penicillium*, *Aspergillus* and *Eurotium*) on DG-18 agar medium.

Table 6. Air sampling for culturable phylloplane and non-phylloplane fungi as an indication of effectiveness of remediation.

Location of Samples	Culturable Fungi (CFU m ⁻³)*			
	Total	AV only	P/Asp/E	C/Alt /Epi
Before Clean-up				
Floor 1 (N=10)	650	140	560	45
Floor 2 (N=20)	120	6	60	30
After Clean-up				
Floor 1 (N=10)	450	4	75	300
Floor 2 (N=30)	730	2	120	400

* Cellulose Medium. AV, *Aspergillus versicolor*, P, *Penicillium*; Asp, *Aspergillus*; E, *Eurotium*; Alt, *Alternaria*; Epi, *Epicoccum*

Building 6

Chronic moisture problems and extensive (>30 m²) visible mould growth occurred in two rooms on the lower floor of a two-storey building. *Aspergillus versicolor* and other non-phylloplane species were identified as dominant fungi on colonized surfaces in rooms. Visibly mouldy materials were removed from the building using full containment remediation techniques (see Chapter 2.3; ACGIH 1999; Morey and Sawyer 1999).

Air was sampled for culturable fungi before and after remediation to provide an indication of whether or not restoration activities had shifted the profile of fungi from domination by non-phylloplane to phylloplane species. For this purpose phylloplane fungi were defined as the total of all *Cladosporium*, *Epicoccum* and *Alternaria* CFU, while non-phylloplane fungi were defined as the total of all *Penicillium*, *Aspergillus* and *Eurotium*.

Air sampling prior to clean-up on the lower floor showed that *Aspergillus versicolor* was present at an average concentration of approximately 140 CFU m⁻³ air, or around 22% of the total (Table 6). On the lower floor, the combined *Penicillium*/*Aspergillus*/*Eurotium* concentration was about 12 times greater than that of *Cladosporium*/*Alternaria*/*Epicoccum*. Non-phylloplane species also dominated the airborne fungi on the upper floor.

When sampling was performed on the lower floor several weeks after the completion of cleanup activities, the *A. versicolor* concentration was only 4 CFU m⁻³ air, or about 1% of the total (Table 6). Phylloplane species were now the dominant microbiota, with *Cladosporium*/*Alternaria*/*Epicoccum* accounting for 67% of the fungal count.

The sampling data from this building indicated that restoration activities had successfully changed the profile of airborne fungi from non-phylloplane to phylloplane species. The presence of low (<1 %) concentrations of *A. versicolor* after clean-up (this

species was not detected in outdoor air) can be interpreted as indicating the need for additional vacuum cleaning to remove traces of residual dust overlooked in restoration of the building.

Building 7

Inspection of a new building revealed that paper-fibre gypsum board was visually mouldy and spray-on fire insulation which contained some cellulosic constituents was discoloured. A dispute arose between parties involved in construction as to the primary cause of visual damage, viz. whether it was flooding resulting from roof leaks or the inability of the building operator to control indoor relative humidity and dampness.

Pieces of discoloured fire insulation and visibly mouldy paper fibre gypsum board were analyzed respectively by serial dilution for culturable fungi and by direct microscopical observation (cellulose tape lifts; AIHA 1996) to prove insight into the nature of the moisture history. The dominating presence of hydrophilic fungi would indicate soaking wet conditions (Flannigan and Miller 1994). If damage to building materials was associated with chronically damp conditions then xerotolerant fungi should predominate in samples.

Fig. 1 shows fungi directly observable on a representative gypsum board surface. Perithecial hairs and *Chaetomium* spores were predominant on this and other gypsum board surfaces. *Chaetomium* characteristically grows well on wet cel-lulosic surfaces. *Stachybotrys chartarum* was the dominant fungus found by plating of serial dilutions of pieces of fire insulation on malt extract agar (Table 7). *S. chartarum*, and yeasts and *Fusarium* present as minor contaminants in samples (Table 7), are characteristically found in water-logged substrates.

The results in Table 7 and Fig. 1 indicated that physical water leaks (flooding), not damp operating conditions, were associated with mould growth and discoloration. This information was used to assign responsibility for damage among the parties involved in building construction and building commissioning.

Building 8

A sewage flood occurred on the lower floor of a multistorey building. Sewage-backflow waters and water-damaged porous finishing materials (e.g. carpet) were removed; affected surfaces were disinfected by repeated application of bleach; and construction and finishing materials were dried with the use of dehumidifiers. Restoration efforts notwithstanding, occupants were concerned about possible exposure to harmful organisms, e.g. *Escherichia coli*, that still might be present on surfaces previously contaminated by the sewage-backflow water.

Table 7. Presence of cultivable *Stachybotrys chartarum* in fire insulation indicates previous flooding conditions.

Sample Description	Fungal taxa present (CFU g ⁻¹)*
Composite sample of fire insulation near surface facing return air plenum	6×10 ⁴ ; 95% <i>S. chartarum</i> ; 5% yeasts
Composite sample of fire insulation adjacent to ceiling slab	1×10 ⁴ ; 90% <i>S. chartarum</i> ; 5% yeast; 3% <i>Fusarium</i>

* Malt extract agar; serial dilution.

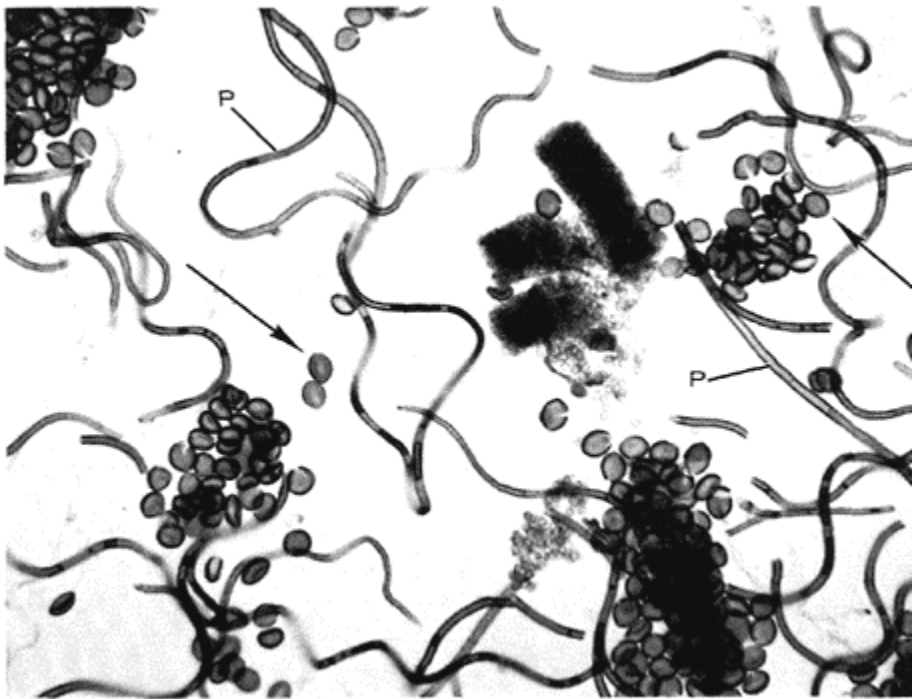


Fig. 1. Direct microscopical observation of fungi present on surface of paper fibre gypsum board in a cellulose tape sample. Spores (arrows) and hairs (P) from sexual reproductive structure (perithecium) of the ascomycete, *Chaetomium*, are abundant. This photomicrograph is indicative of past soaking wet conditions on the paper-fibre surface of the gypsum board.

Air was sampled for endotoxin both in rooms previously affected by backflow of

sewage and in upper-floor rooms which were unaffected by the backflow. Since Gram-negative bacteria such as *E. coli* are predominant in sewage waters and all Gram-negative bacteria contain endotoxin, it was hypothesized that if clean-up was successful levels of endotoxin throughout the entire building would be similar, and that levels of endotoxin indoors would be no higher than those outdoors. Endotoxin sampling was thus performed as a surrogate indicator for the presence of residual sewage organisms.

Sampling results showed that the average amount of endotoxin in the area of sewage-affected rooms was about 3 endotoxin units (EU) m^{-3} while concentrations in upper-floor rooms and in the outdoor air were about 0.5 EU m^{-3} (Table 8). Relative limit values (RLV) of 10–100 times the background outdoor level have recently been suggested for airborne endotoxin (Milton 1999). Adverse health effects such as fatigue, cough, and acute airflow obstruction become frequent when measured endotoxin levels approach or exceed RLV's (Milton 1999).

Table 8. Post-remediation sampling to indicate if clean-up following a sewage backflow had been adequate.

Sample Location	Airborne Endotoxin (EU m^{-3} air)*
Outdoor air; (N=2)	0.5
Rooms previously affected by backflow†; (N=4)	3.1
Rooms unaffected by backflow†; (N=3)	0.5

† Floor surfaces tested for presence of the following culturable microorganisms: *Aeromonas*, *Escherichia coli* O157/H7, *Salmonella*, *Shigella*, *Vibrio* and *Streptococcus faecalis*. A 30×30 cm floor surface at the point of junction with a wall was repeatedly swabbed with a sterile gauze pad saturated with water. The gauze pad was transported at 4°C to the laboratory, mixed with additional sterile water to a volume of 50 ml. Aliquots of eluate were plated on various culture media including MacConkey agar with and without sorbitol; xylose-lysine-desoxycholate agar; thiosulphate-citrate-bile-sucrose agar; and Columbia-nalidixic acid agar. Culturable bacteria characteristic of sewage waters were not detected.

* *Limulus* assay (Milton *et al.* 1992); EU, Endotoxin Unit.

While the average concentration of endotoxin in sewage affected areas was below the suggested RLV, occupants remained unconvinced over the effectiveness of disinfection and cleanup activities. Consequently additional testing was undertaken for bacteria commonly associated with sewage, such as *E. coli* O157/H7, *Streptococcus faecalis* and others listed in the footnote to Table 8. A wet-swab method (Table 8) was used to extract dusts from floors in sewage-affected and sewage-unaffected rooms. Bacteria characteristic of sewage were not detected by culture in any samples (Table 8). Collectively, the data indicated that disinfection and cleaning had probably removed pathogenic bacteria from rooms previously affected by the sewage backflow.

Building 9

Workers in one area in an industrial building reported a sporadic flu-like illness associated with exposure to water mist. The mist originated from a reservoir containing water that was continuously recirculated with minimum addition of make-up water. Air was sampled for endotoxin in misty and non-misty indoor areas, as well as outdoors, to indicate whether or not the water reservoir was an amplifier for Gram-negative bacteria and other microorganisms.

Sampling results showed that the endotoxin concentration in the misty area was at least 100 times greater than in non-misty areas or in the outdoor air (Table 9). Thus, the airborne endotoxin level in the misty area exceeded the RLV's suggested by the ACGIH (1999). The data in Table 9 indicated that amplification of Gram-negative bacteria was occurring in the water reservoir from which mists originated.

Table 9. Air sampling for endotoxin in a misty area of a building where occupants reported a flu-like illness.

Sample Location	Endotoxin (EU m ⁻³ air)*
Outdoor air; (N=4)	23
Indoors; misty zone (N=4)	3200
Indoors; dry zone (N=2)	8

**Limulus* assay (Milton *et al.*, 1992); EU=Endotoxin Unit.

Table 10. Detection of culturable *Legionella* in water systems in a building where occupants developed legionnaires' disease.

Sample Locations	<i>Legionella</i> (per ml)	Species and Serotype*
Hot water tank and piping; (N=10)	10–1000	<i>L. pneumophila</i> serogroup No. 1
Water spray nozzle at end of piping; (N=3)	10–100	<i>L. pneumophila</i> serogroup No. 1
Hot water tank and piping in occupants' residences; (N=6)	none detected (LOD=0.5)	—

Method: culture on buffered charcoal yeast extract agar (AIHA, 1996).

Additional sampling showed that *Pseudomonas*, *Flavobacterium* and other Gram-negative bacteria were the dominant culturable organisms in both the water reservoir and water-mist droplets. A follow-up medical and environmental study suggested that some

of the flu-like illness reported by workers was associated with the toxic effects of high levels of endotoxin exposure (Milton *et al.* 1995).

Building 10

Two workers and one office employee in a small industrial facility developed pneumonia and required hospitalization. Laboratory tests performed independently by private physicians showed elevated levels of *Legionella pneumophila* antibodies (serotype not determined) in sera. Sampling focused on detection of Legionellae in both the workplace and residential environment was initiated after the management at the facility realized that the three cases had occurred within a relatively short time (several months).

An inspection of the facility showed that water sprays were used to suppress dusts in and around buildings. Water for industrial sprays and for potable hot and cold services originated from wells on the property. The water from the wells was initially stored in outdoor tanks above ground and was consequently subject to insolation, the temperature of water in tanks being about 30–40°C.

Culture analysis of water samples from spray nozzles and from hot water tanks and piping showed the presence of *L. pneumophila* serogroup 1 at concentrations of 10 to >100 CFU ml⁻¹ (Table 10).

This, together with the absence of *Legionella* spp. (detection limit 0.5 CFU ml⁻¹) in potable water systems in the employees' homes, suggested that pneumonia was likely due to legionellae in the workplace environment. The sampling evaluation in this building differed from that in Buildings 1–9 because pre-existing medical data focused sampling to one culturable bacterial species, *Legionella pneumophila*.

Building 11

Three bone-marrow transplant patients who had sequentially occupied one suite in a medical centre developed aspergillosis. According to the pathology reports, infection was caused by *Aspergillus fumigatus* and/or *A. flavus*. A sampling investigation was performed to determine whether these *Aspergillus* spp. were present in the patient suite or in the HVAC system serving this area. The evaluation was focused on the recovery of thermotolerant (37°C) *A. fumigatus* and *A. flavus* in air and dust samples which if present would suggest that infections were hospital-acquired.

Three air samples were collected quiescently in the former patient suite (Table 11). Thermotolerant fungi were only detected in one sample, which contained a total fungal concentration of 15 CFU m⁻³ air (*A. fumigatus*, 9 CFU m⁻³; *A. flavus*, 6 CFU m⁻³). Settled dust collected from the floor and from the return register grille in the suite contained *A. fumigatus* at a level of about 3,000 CFU m⁻³ (Table 11).

All five dust samples collected from the air-handling unit and supply ductwork, including the final filter serving the patient suite contained culturable *A. fumigatus*, but only two contained *A. flavus* (Table 11).

The collective sampling results showed that patient infections were hospital-acquired, and that the HVAC system was the source of thermotolerant fungi causing infections.

Table 11. Air sampling for thermotolerant *Aspergillus* in hospital where several immunocompromised patients developed aspergillosis.

Location and description of samples	Culturable thermotolerant (37°C) fungi*
Air samples in room that had been occupied by affected patients	One sample with <i>A. fumigatus</i> (9 CFU m ⁻³) and <i>A. flavus</i> (6 CFU m ⁻³); two samples without detectable fungi
Settled dust in room	Dust contains <i>A. fumigatus</i> (approximately 3,000 CFU g ⁻¹)
Adherent/settled dust in air-handling	unit and ductwork providing air to room
5 dust samples collected; all contained <i>A. fumigatus</i> ; 2 contained <i>A. flavus</i> ; Concentration of <i>Aspergillus</i> spp. in samples varied from 100 to 10,000 CFU g ⁻¹	

*Malt extract agar.

The focused study in Building 11 provides a rare example where environmental organisms (*A. fumigatus* and *A. flavus*) in a building were shown to be the agents causing patient disease (aspergillosis).

CONCLUSION

The cases described above illustrate that microbial sampling should be performed in the context of the objective of the overall building investigation. An understanding of building operation or non-operation established by an informed inspection is a prerequisite for sampling. The objective of sampling, including an understanding of how data will be interpreted, should be established prior to sample collection. The investigator must select analytical methods appropriate to the study objective and be aware of limitations in sampling methodology. Interpretation of analytical results should be made in the context that sampling is just one component of the informed building evaluation.

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Chapter 5.
Common and important
species of fungi and
Actinomycetes in indoor
environments

Chapter 5

COMMON AND IMPORTANT SPECIES OF FUNGI AND ACTINOMYCETES IN INDOOR ENVIRONMENTS

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INTRODUCTION

In the following chapter the most common fungi and Actinomycetes are illustrated, together with comments on their occurrence and medical implications.

The micrographs of these microorganisms were made from typical cultures isolated from indoor environments. The microscope slides were prepared in lactic acid, sometimes with the addition of aniline blue. Isolates of the species are in the CBS culture collection (<http://www.cbs.knaw.nl>).

The health implications of exposure to moulds is discussed below and hazard levels classified and coded. For each of the alphabetically arranged species, the comments on its medical implications include the coded hazard category. Where there have been well established reports of species being allergenic, a note of their allergenicity has been made. However, it should be remembered that all fungi contain proteins and glycoproteins to which humans can be allergic. Therefore, all of the species described (and others not described), whether labelled as allergenic or not, should be regarded as potentially allergenic. Information on relevant mycotoxins produced by toxigenic species occurring indoors is also provided.

A short list of references is given at the end of this introductory section of the chapter, but additional information on, and references to, the species may be found in the preceding chapters.

HEALTH IMPLICATIONS

Definition of normal mould exposure

Moulds may have effects on human health in various different ways—through allergenicity, through direct biochemical effects (irritation, non-allergic immunoactivation or toxicity), or through invasive or colonizing disease. In this section,

some perspectives are given about the invasive and colonizing disease hazards posed by moulds. Allergenic and direct biochemical effects are dealt with elsewhere in this volume.

The list of mould species defined as agents of “opportunistic infection” has rapidly expanded in recent years, giving the appearance that humans risk exposure to hundreds of dangerous, disease-causing mould species. This appearance is mostly, although not entirely, illusory. The perspective that is occasionally lost is that opportunistic disease mainly affects limited groups of people with severe immune deficiencies. Of course, such people are important, and their protection is strongly encouraged, but the problems that moulds pose for them are best managed in light of a full knowledge of the ubiquity of most of the organisms involved.

Whereas allergenic and toxic mould hazards are always dependent on the *quantity* of material present, infectious disease hazards may depend merely on the *presence* of an infectious organism, regardless of quantity. That is because the more virulent an infecting organism is, the more it has the ability to multiply in the body and increase its population levels after the infected human has been exposed to it, making the actual level of initial exposure relatively unimportant. There is a “grey area,” however, in that some infectious disease hazards may also depend on quantity: that is, some organisms that may seldom or never cause disease when contacted in small inoculum quantities may cause disease on heavy exposure to them. This is especially true for partially immunocompromised persons, e.g., persons taking high doses of corticosteroids, or those with HIV infection and reduced CD4⁺ T-lymphocyte counts. Both the discrete (presence/absence) and the inoculum-dependent effects seen in mould infections are taken into account below.

The above suggestion that many moulds labelled as opportunistic lack the wherewithal to cause disease in most people may be of significant comfort to many persons who, for one reason or another, see lists of moulds isolated or detected at their homes or workplaces. Owners and managers of workplaces, public buildings and reted accommodation, however, should not take similar comfort. Individuals generally know whether they themselves are immunocompromised, since this condition nearly always derives from medical treatment, or from serious genetic or retroviral disease. Building managers, however, must realize that they cannot know whether or not users of their facilities belonged to various categories of immunocompromised patients, and also there is there no effective or legitimate means of excluding such people. People who have no immunocompromising condition today may develop one later; and, especially with HIV infection, some persons may discover only belatedly that they are immunocompromised. From the building management perspective, therefore, indoor colonization by known, opportunistically invasive moulds are best managed as if immunocompromised persons were present and at risk of exposure; this prudence takes precedence over the knowledge that the great majority of people will suffer only allergic or, at worst, toxic effects from most indoor mould colonization. Precise management in the case of indoor mould colonization may include a request for a building’s occupants to disclose any serious medical condition which they have that might predispose them to mould infection. It should always be remembered, however, that persons have no obligation to reveal their confidential medical details in such situations, and in most legal systems they would not

be considered to have relieved managers of the burden of responsibility by failing to disclose these details. In any event, they may be insufficiently informed about their condition or its possible relation to mould infection.

After the above practicalities have been taken into account, the actual invasive disease risks posed by moulds can be considered in detail. Some fungi never cause human or animal disease, but many species, if they are able to grow at 37° C, may cause opportunistic infection in humans and animals with compromised immune systems. How alarmed should people be by such fungi? Clearly, since serious fungal infections are quite uncommon in otherwise healthy people, we are not under much threat from the usual moulds found in our clean outdoor air and our tap water. Therefore, it is useful to define a normal mould that people may routinely be exposed to, referred to here as a “tabletop mould” (TTM), in terms of what may commonly be found throughout the world in such clean, non-hazardous materials.

The definition of TTM excludes geographically limited “virulent” fungi such as *Coccidioides immitis* that cause mild to severe disease in most typical, immunocompetent people who are exposed to them. These fungi may occasionally be carried to indoor environments in their endemic areas, e.g. deserts in southwest USA in the case of *Coccidioides*, and may also be encountered in “clean” outdoor air in these regions. Clearly, however, any mould that usually causes infections in most people that encounter it (even if these infections are often mild) must be considered hazardous. Likewise, *Cryptococcus neoformans* (not a mould, but a yeast) must be considered to pose a hazard beyond the TTM level. It is common in typical urban outdoor air and poses a negligible hazard to most persons who inhale it daily, but predictably causes severe disease in immunologically normal persons who inhale high inoculum levels, e.g. when breathing heavy dust while cleaning large quantities of pigeon guano from attics. In this case, however, the hazard is dependent on the inoculum level contacted. The fungi defined in this writing as TTMs neither predictably infect immunocompetent people nor pose a known or strongly suspected inoculum-dependent hazard to the normal person. To summarize then, the virulent fungi such as *Coccidioides* are not considered to be everyday, ubiquitous components of outdoor air, while the fungi posing an inoculum-dependent hazard may well be common in typical air, but the inoculum levels that are hazardous are not normally found in outdoor or indoor air. Thus, although both virulent and inoculum-dependent, invasive fungi intersect with normal life to some degree; the hazards they pose cannot be fully synonymized with the hazards usually posed by the common environments and materials of human existence.

TTMs, on the other hand, are very much a part of normal life everywhere. A good illustration of the contrast between the low hazard associated with most TTMs and the seemingly threatening labels they are sometimes given is found in *Aspergillus fumigatus*. Although it is often referred to as the most virulent “opportunistic mould” and is the subject of much medical literature based on cases in immunocompromised patients, it is a common outdoor air fungus all year round in many tropical areas, and becomes common in clean air during the late summer and autumn in most temperate parts of the world. The reason for this is that in the natural environment it colonizes warm piles of decaying vegetation, including fallen leaves and matted grass remains. It is also found in

essentially all barns and sheds where plant material is stored on farms, and is present in the soils of most indoor potted plants, where it is often one of the dominant members of the fungal community. There are many other common habitats and materials with relatively high levels of this organism, e.g. composts, small accumulations of bird dung and dried marijuana. Without question, it is therefore a TTM, and everyone on this planet is routinely exposed to it. Although years of constant heavy exposure to it in barns may induce severe allergies in some farmers, this fungus does not usually cause invasive disease even when high inoculum quantities are encountered. Only for certain types of immunocompromised patients does it pose a predictable hazard, and for these patients outdoor air and also typical indoor air are hazardous. Very rare cases of serious disease caused by this organism may be found in seemingly otherwise healthy patients, but such persons may well have an undetected immunodeficiency; and in any case these persons most often appear to have acquired their unusual infections from exposures to completely normal, everyday conditions. Whatever factors occasion infection by such opportunistic fungi, they clearly do not affect the overwhelming majority of other people with comparable exposures. In the category of fungi commonly found in water, *Fusarium oxysporum* and *Fusarium solani* are greatly feared as opportunistic fungi in hospital bone marrow transplant wards, but are normally found in tap water as well as in essentially all fresh plant material, including vegetables. Probably nobody normally goes through a day without ingesting a significant quantity of these fungi.

Even persons with AIDS, unless the AIDS is complicated by certain cancers or use of immunosuppressive antiviral drugs such as ganciclovir, are not sufficiently immunocompromised to be significantly threatened by most nominally opportunistic moulds. This extreme example illustrates the extent to which the distinction between a typical environmental mould exposure and a more hazardous mould exposure is biologically realistic. Humans, like other mammals, are highly adapted to resist infection arising from our normal environmental mould exposures; only a few specialized moulds are able to overcome our adaptive defences, and even certain major immune system handicaps are insufficient to make us vulnerable to them.

Despite this degree of immune defence, we have some minor, common vulnerabilities. For example, some ubiquitous TTMs may cause painful ear canal colonizations, especially in small children who put objects into their ears, or in farmers exposed to heavy plant dust. The same fungi or others may cause non-invasive nasal sinus infestations in persons with chronic allergic conditions. Again, these hazards are completely attributable to materials found in normal environments: common small objects, typical agricultural materials and normal air. Thus, fungi found in these medical conditions cannot be ascribed an exceptional degree of hazard. Indeed, many are among the most common and widely distributed environmental moulds, guaranteed to be found on every tabletop in the world. For example, one of the major agents of non-invasive fungal sinusitis, *Alternaria alternata*, is the second-most common fungus in almost all surveys of normal outdoor air in temperate latitudes. The most common fungus in such surveys is *Cladosporium herbarum*, which cannot grow at or near body temperature and so never causes infections. We inhale *A. alternata* with each breath we take; exposure to it is thus generally no more hazardous than breathing itself is.

The moulds in this list have been classified with regard to whether they pose any kind of an invasive or colonizing disease hazard. In the former category of hazard, the fungus invades human or other mammalian tissue; in the latter, the fungus grows non-invasively on surfaces within the body cavities such as sinuses, and causes irritation and inflammation. The more hazardous fungi are those that may potentially cause a disease not associated with common, normal TTM exposures. The diseases are categorized according to how they may possibly be acquired, e.g. by inhalation or introduction of fungus under the skin (into the dermis). Secondly, within the hazard levels posed by TTMs, and thus by normal air, water and surfaces, moulds that may cause problems in immunocompromised patients are similarly categorized. It should be noted that so-called “barrier breaks” are regarded as a kind of immunocompromising condition; these are perforations of the body so that fungi can enter into areas that are normally never exposed to such organisms, even in typical cuts and scrapes. Thus, a classification is made for TTMs that can become hazardous if introduced into the body via perforations into the interior of the eye, the peritoneum, the major blood vessels and similar recognized sites of immunologically consequential barrier rupture. Most such perforations occur as part of medical procedures, but other possibilities are deep perforations in accidents, and use of microbiologically unclean injection materials such as street drugs. In the case of the cornea, a deep scratch caused by a piece of sharp plant material or metal may be sufficient to bring about a significant barrier break and introduce a TTM, converting it from innocuous to potentially hazardous.

Finally, some categories are made for TTMs that have never been reported as infecting any kind of human patient or warm-blooded animal, no matter how deeply immunocompromised.

Definition of fungal disease hazards as calibrated to take normal exposure into consideration.

Below are listed the TTM-calibrated hazard categories for fungi, from most to least hazardous. Some of the more serious hazard categories may not be represented by any fungus on the list in this book. *Coccidioides immitis*, a virulent fungus discussed above, would be categorized as ABC. The C is attached to the self-explanatory AB because the severity of inhalation-derived infection is known to be increased in heavily exposed persons, e.g. laboratory workers exposed accidentally to pure cultures. *Penicillium citrinum*, as a contrasting example, is one of the most common indoor/outdoor moulds, and is normally completely harmless. Since, however, it is known from rare eye infections after corneal perforation, plus two opportunistic infections of severely immunocompromised cancer patients and a non-invasive bladder colonization of an otherwise healthy woman (reported in 1951, with no similar cases reported since), it is categorized as EGI. *Aspergillus fumigatus* is categorized EFG(?D). The (?D) is attached because *A. fumigatus* very rarely is reported to cause the subcutaneous infection mycetoma, and it is not clear whether the persons contracting such infections are immunodeficient or merely that their deep skin layer has been inoculated with an unusually heavy quantity of material bearing *A. fumigatus*. *Pseudallescheria boydii*, a

fungus which is often considered similar in medical impact to *A. fumigatus*, but which regularly causes mycetoma and which may cause fatal disease in otherwise-healthy persons who have inhaled large quantities of water-borne inoculum in near-drowning experiences, can be categorized as BCG. One might raise the query “Should the B in that categorization be downgraded to an F because *P. boydii* may commonly be found in typical warm, dirty water, and thus potentially on tabletops or in outdoor air, making its hazard categories by definition fall within the scope of TTM hazards?” The answer to this query is a firm “No”, since there is no indication that *P. boydii* mycetoma can be regularly acquired from common materials; in population terms, cases are very rare, whereas *P. boydii* is a relatively common organism (A full discussion of the known virulence gradations in this complex species and the epidemiological probabilities of a healthy person acquiring a serious infection are beyond the scope of this writing). In contrast, neutropenic patients could very readily acquire lethal *A. fumigatus* infection by inhaling typical room dust or being exposed to normal outdoor air. The standard of common, normal life exposures thus excludes perforations inducing *P. boydii* mycetoma, even though the fungus may be commonly contacted, but includes exposure to the microbial conditions that cause opportunistic aspergillosis in neutropenic patients. Compare the above discussion of *C. neoformans*, classed as CDG(?B). As a final example, *Cladosporium herbarum*, an abundant outdoor air species with a maximum temperature for growth of 32° C, is categorized as HI. Like all common contaminant fungi, it has been the subject of occasional erroneous and dubious disease attributions in medical literature, e.g. de Hoog and Guarro (1995); such reports only demonstrate that the fungus can be isolated from medical specimens and misinterpreted, or that other disease-causing fungi may be misidentified and given the name of a nonpathogenic fungus.

Hazard levels above TTM/normal outdoor air, material surface and tap water level.

- A. Poses an inhalation hazard beyond the hazard conditions typical of normal, clean materials.
- B. Poses a deep skin (dermal) inoculation hazard beyond the hazard conditions typical of normal, clean materials.
- C. Poses a known or likely inoculum-dependent inhalation hazard beyond the hazard conditions typical of normal, clean materials.
- D. Poses a known or likely inoculum-dependent deep skin (dermal) inoculation hazard beyond the hazard conditions typical of normal, clean materials.

Hazard levels within TTM/normal outdoor air, material surface and tap water level.

- E. Poses an inhalation hazard (e.g., for immunocompromised persons) *within* the hazard conditions typical of normal, clean materials.
- F. Poses a deep skin (dermal) inoculation hazard (e.g., for immunocompromised persons) *within* the hazard conditions typical of normal, clean materials.
- G. Poses a hazard related to a major barrier break (e.g., corneal perforation, major surgery, peritoneal or venous catheter presence, injection drug use) *within* the hazard conditions typical of normal, clean materials.

Categories describing absence of known risk.

- H. Poses no known inhalation hazard whatsoever (some highly dubious or demonstrably false reports may be found in medical literature).
- I. Poses no known dermal inoculation hazard whatsoever (some highly dubious or demonstrably false reports may be found in medical literature).

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List of species

SPECIES	PAGE	SPECIES	PAGE
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<i>Aspergillus candidus</i>	302	<i>Penicillium brevicompactum</i>	394
<i>Aspergillus clavatus</i>	304	<i>Penicillium chrysogenum</i>	396
<i>Aspergillus flavus</i>	306	<i>Penicillium citrinum</i>	398
<i>Aspergillus flavipes</i>	308	<i>Penicillium commune</i>	400
<i>Aspergillus fumigatus</i>	310	<i>Penicillium corylophilum</i>	402
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<i>Aspergillus ochraceus</i>	314	<i>Penicillium glabrum</i>	406
<i>Aspergillus penicillioides</i>	316	<i>Penicillium janthinellium</i>	408
<i>Aspergillus restrictus</i>	318	<i>Penicillium olsonii</i>	410
<i>Aspergillus sydowii</i>	320	<i>Penicillium rugulosum</i>	412
<i>Aspergillus terreus</i>	322	<i>Penicillium simplicissimum</i>	414
<i>Aspergillus versicolor</i>	324	<i>Penicillium spinulosum</i>	416
<i>Aureobasidium pullulans</i>	326	<i>Penicillium variable</i>	418
<i>Botrytis cinerea</i>	328	<i>Phialophora fastigiata</i>	420
<i>Candida peltata</i>	330	<i>Phialophora verrucosa</i>	422
<i>Chaetomium aureum</i>	332	<i>Phoma glomerata</i>	424
<i>Chaetomium globosum</i>	334	<i>Phoma macrostoma</i>	426
<i>Chaetomium indicum</i>	336	<i>Pithomyces chartarum</i>	428
<i>Chrysonillia sitophila</i>	338	<i>Pyronema domesticum</i>	430
<i>Cladosporium cladosporioides</i>	340	<i>Rhizopus stolonifer</i>	432
<i>Cladosporium herbarum</i>	342	<i>Rhodotorula mucilaginoso</i>	434
<i>Cladosporium sphaerospermum</i>	344	<i>Schizophyllum commune</i>	436
<i>Clonostachys rosea</i>	346	<i>Scopulariopsis brevicaulis</i>	438
<i>Coprinus cordisporus</i>	348	<i>Scopulariopsis Candida</i>	440

<i>Cryptococcus laurentii</i>	350	<i>Scopulariopsis fusca</i>	442
<i>Curvularia lunata</i>	352	<i>Serpula lacrymans</i>	444
<i>Emericella nidulans</i>	354	<i>Sistotrema brinkmanii</i>	446
<i>Epicoccum nigrum</i>	356	<i>Sporobolomyces roseus</i>	448
<i>Eurotium amstelodami</i>	358	<i>Stachybotrys chartarum</i>	450
<i>Eurotium chevalieri</i>	360	<i>Syncephalastrum racemosum</i>	452
<i>Eurotium herbariorum</i>	362	<i>Trichoderma harzianum</i>	454
<i>Exophiala dermatitidis</i>	364	<i>Trichoderma koningii</i>	456
<i>Fusarium culmorum</i>	366	<i>Trichoderma viride</i>	458
<i>Fusarium solani</i>	368	<i>Tritirachium oryzae</i>	460
<i>Fusarium verticil lioides</i>	370	<i>Ulocladium chartarum</i>	462
<i>Geomyces pannorum</i>	372	<i>Verticillium lecanii</i>	464
<i>Geotrichum candidum</i>	374	<i>Wallemia sebi</i>	466
<i>Memnoniella echinata</i>	376		
<i>Mucor hiemalis</i>	378	Actinomycetes	
<i>Mucor plumbeus</i>	380	<i>Micropolyspora faeni</i>	468
<i>Mucor racemosus</i>	382	<i>Streptomyces griseus</i>	470
<i>Oidiodendron griseum</i>	384	<i>Thermoactinomyces vulgaris</i>	472

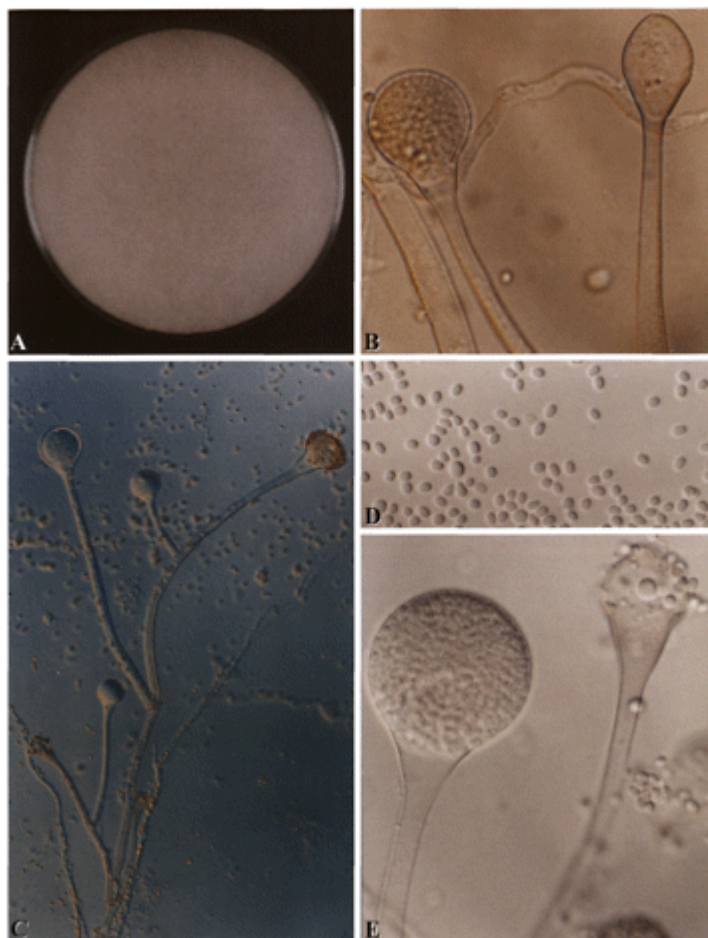
Absidia corymbifera (Cohn) Sacc. & Trotter

=*Absidia ramosa* (Lindt) Lendner

A. Colony on MEA after one week; B. sporangiophore with columella and remnant of sporangium wall after dehiscence, $\times 920$; C. sporangiophores bearing sporangia, $\times 230$; D. sporangiospores, $\times 920$; E. sporangiophore with sporangium (left), sporangiophore with columella (right), $\times 920$.

Occurrence: Worldwide, in soil, decaying plant matter and composts. Found in carpet and mattress dust. Also in hay, cereals, flour; potted plant soil; accrued bird droppings.

Medical significance: EFG.

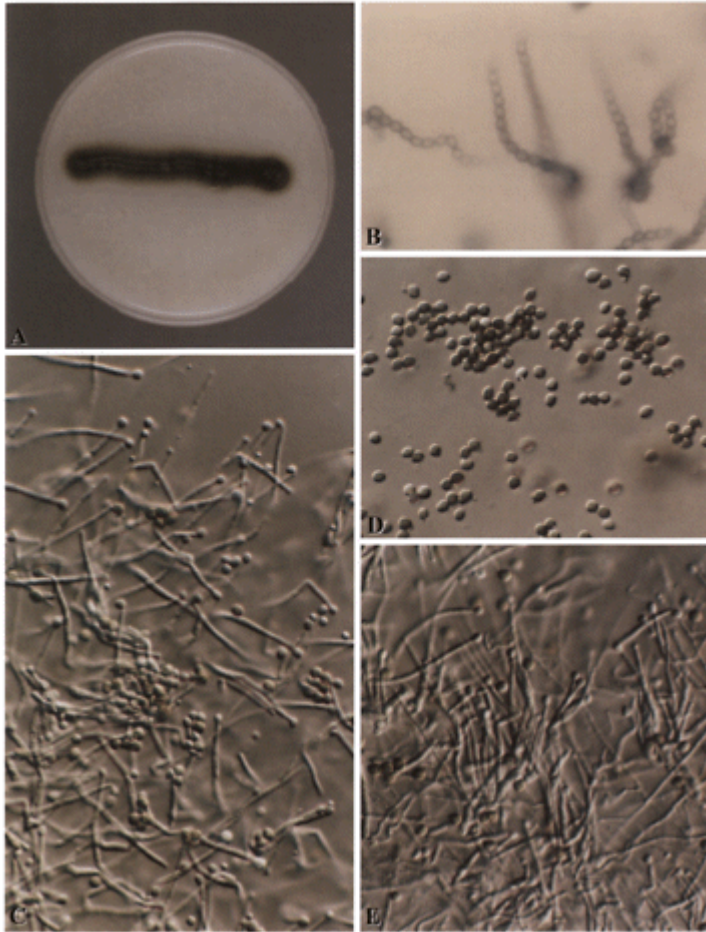


Acremonium murorum (Corda) Gams
 =*Gliomastix murorum* (Corda) Hughes

A. Colony on OA after one week, B. conidiophores with conidia in chains, in Petri-dish, $\times 920$; C. conidiophores with conidia, $\times 920$; D. conidia, $\times 920$; E. conidiophores with conidia, $\times 920$.

Occurrence: Isolated from cellar wall, swimming pool floor; cereals.

Medical significance: HI.



Acremonium strictum W.Gams

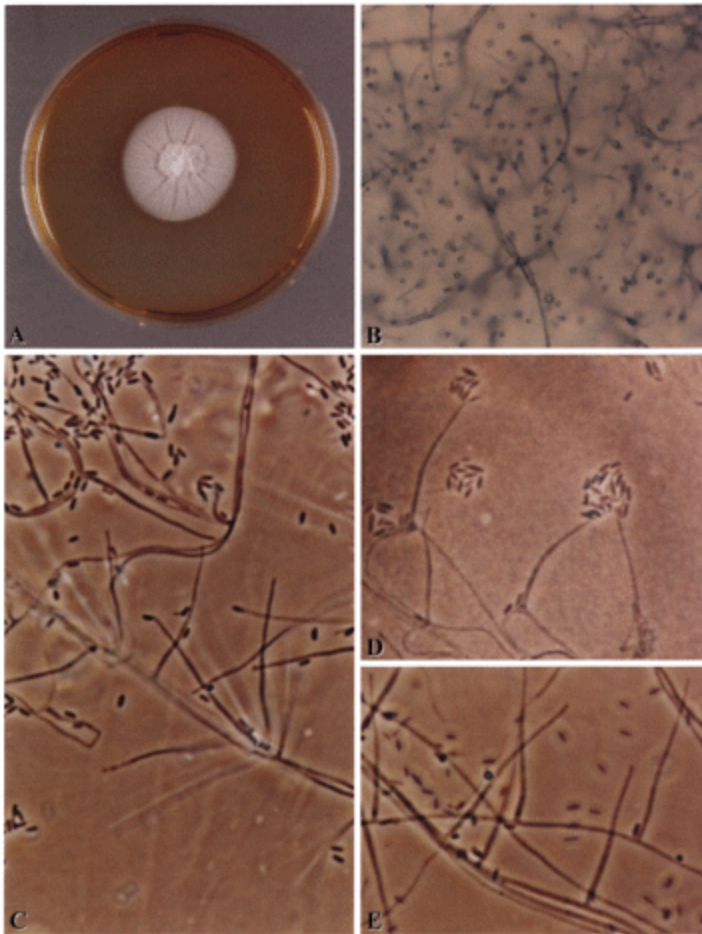
A. Colony on MEA after one week; B. conidiophores with conidia in slimy heads, in Petri-dish, $\times 230$; C-E. conidia, conidiophores and phialides, $\times 920$.

Occurrence:

Found in carpet and mattress dust, and present on damp or wet (acrylic painted) walls, gypsum board, wallpaper.

Also isolated from humidifier water; exposed wood.

Medical significance: EFG.



Alternaria alternata (Fries) von Keissler

=*Alternaria tenuis* Nees

Teleomorphs: *Clathrospora diplospora* (Ellis & Everh.) Wehm., *Clathrospora elynae* Rabenh., *Leptosphaeria heterospora* (de Not.) Niessl.

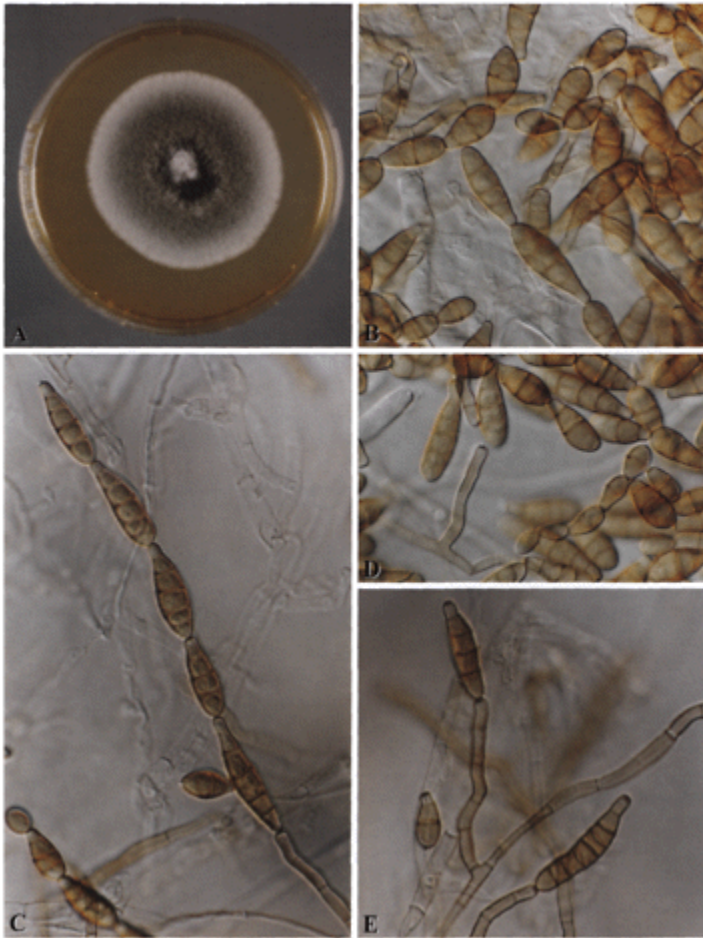
A. Colony on MEA after one week; B-E. conidia and conidiophores, $\times 920$.

Occurrence: Worldwide phylloplane species on a broad range of plants, and in soil.

Common in floor, carpet and mattress dust; less common on damp walls, gypsum board and wallpaper. Cause of soft rot and blue stain of wet joinery. Also isolated from UFFI, window putty, (acrylic latex) paint; humidifier water; textiles; hay, cereals, fruit.

Medical significance: EFG. Characterized allergens causing rhinitis, asthma, atopic allergic dermatitis, allergic sinusitis (see Chapter 3.1).

Toxicity: Some strains produce tenuazonic acid, alternariol and other metabolites of low animal toxicity (Visconti 1994; Nielsen *et al.* 1999).



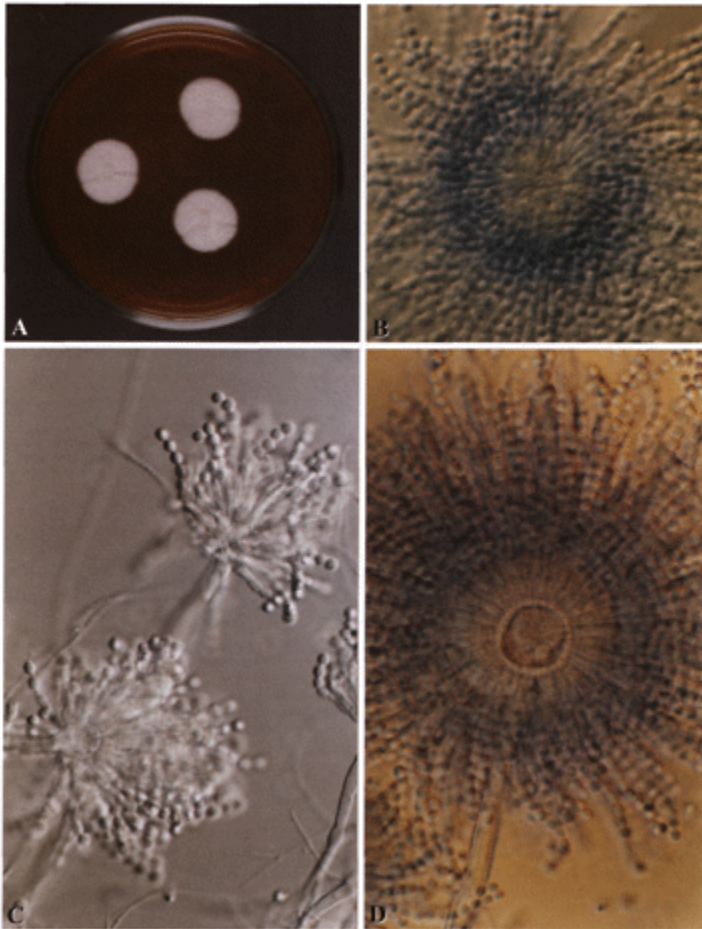
***Aspergillus candidus* Link**

A. Colonies on MEA after one week; B. conidial head, $\times 920$; C. atypical reduced conidial head, $\times 920$; D. conidial head, $\times 920$.

Occurrence: Common in soil, dung and crops, predominantly in tropical and subtropical regions, and frequently present in stored products.

Commonly in floor and carpet and mattress dust. Also isolated from HVAC system air; weaving mill; hay, cereals, flour.

Medical significance: EFG.



Aspergillus clavatus Desmazières

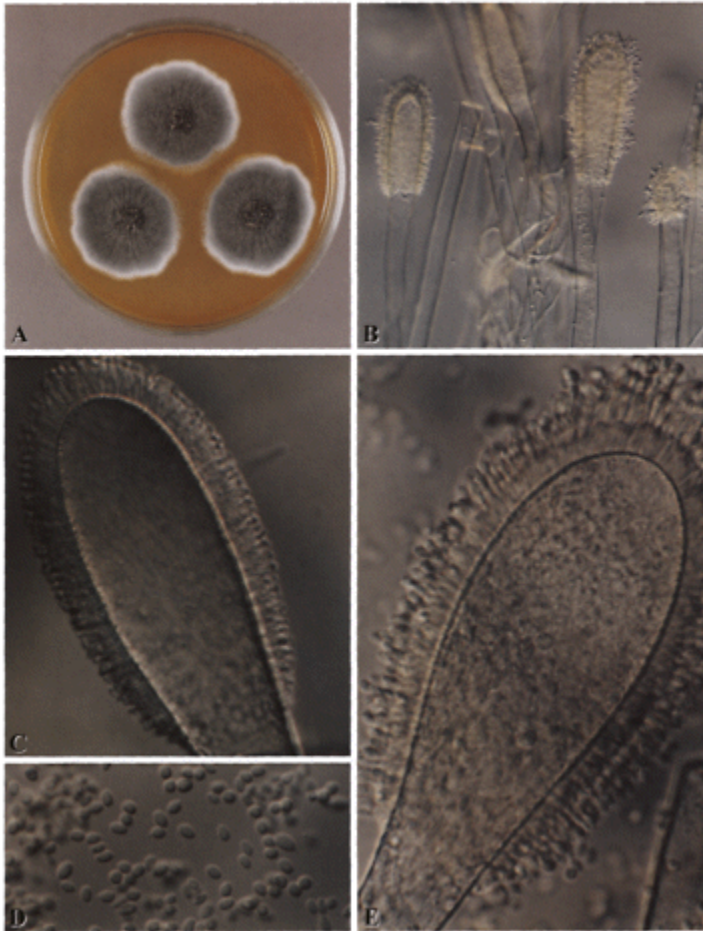
A. Colonies on MEA after one week; B. conidial heads and tip of conidiophore, $\times 230$; C. conidial head, $\times 920$; D. conidia, $\times 920$; E. conidial head, $\times 920$.

Occurrence: Widely distributed in soil and composts.

Uncommon in non-industrial indoor environments, but has been isolated from mattress dust, window sill, and rarely from indoor air in homes. Associated with bakeries and particularly maltings. Also isolated from cereals, and flour, and also fish meal.

Medical significance: [E?] IG. Allergenic—occupational hypersensitivity pneumonitis known as malt worker’s lung (Flannigan and Pearce, 1994).

Toxicity: Produces patulin, tryptoquivalines and cytochalasins; mycotoxicosis in farm stock fed on malting by-products (Flannigan and Pearce, 1994).



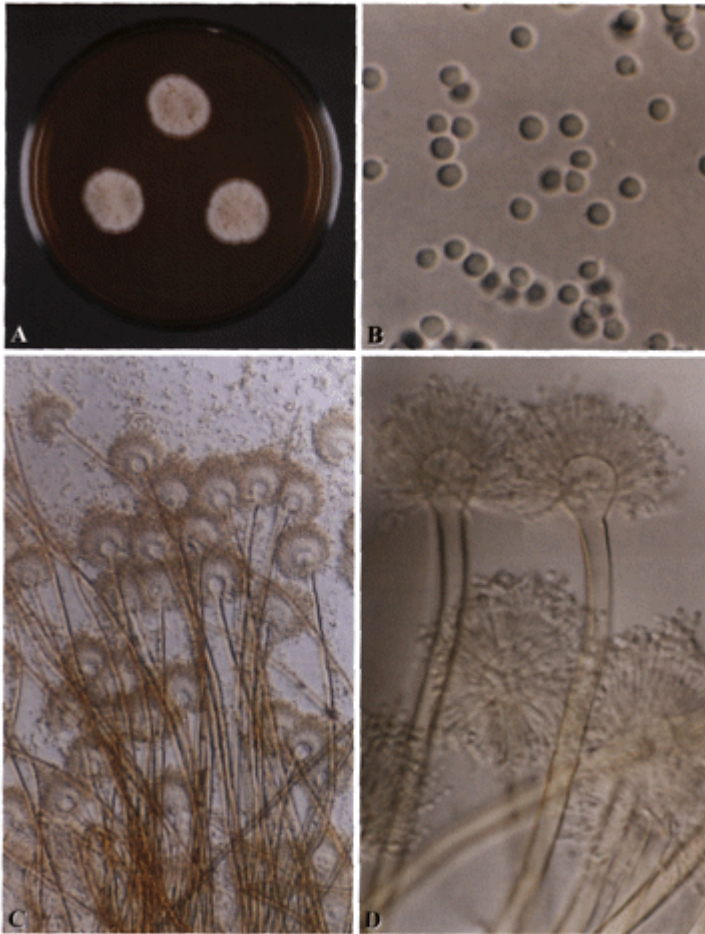
Aspergillus flavipes (Bainier & Sartory) Thorn &
Teleomorph: *Fennellia flavipes* B.J.Wiley & E.G.Simmons

A. Colonies on MEA after one week; B. conidia, $\times 2330$; C. conidial heads and tip of conidiophore, $\times 230$; D. conidial heads and tip of conidiophore, $\times 920$.

Occurrence: Mainly a soil-borne species.

Isolated from upholstered furniture; hospital air; coal-mine dust.

Medical significance: EIG



***Aspergillus flavus* Link**

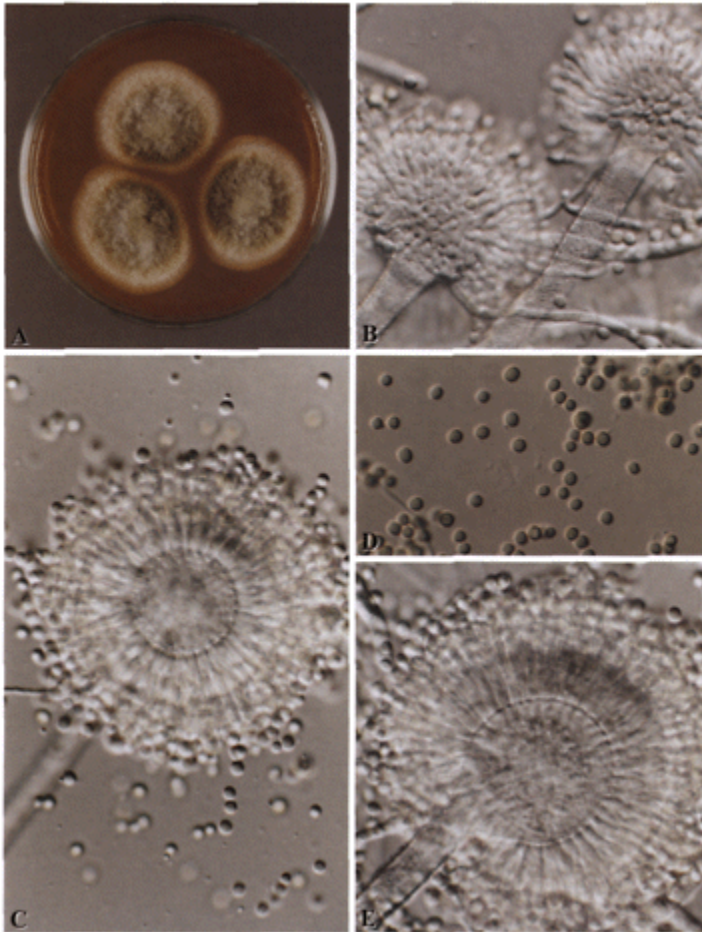
A. Colonies on MEA after one week; B,C conidial heads, $\times 920$; D. conidia, $\times 920$; E. conidial head, $\times 920$.

Occurrence: Widely distributed in soil, particularly in tropical and subtropical areas, particularly associated with maize, nuts and insects in the field and a vast array of commodities in storage in warm climates (Miller 1999).

Relatively rarely growing on building materials, but may be found on damp walls, wallpaper, and glues or plastic cements. Also isolated from floor and carpet dust; tarred wooden flooring, polyurethane foam; particle board frames of filters, humidifiers and HVAC fans; bakeries; shoes, leather, cotton yarn; hay, cereals, flour, bread; bakeries; cosmetics; rabbit food pellets, accrued bird droppings.

Medical significance: EFG. Relatively common cause of aspergillosis (Summerbell 1998).

Toxicity: Strains of *A. flavus* may produce aflatoxin, an IARC Class 1 carcinogen. Occupational inhalation exposure mainly to spores containing aflatoxin in feed mills where grain dusts are tightly managed results in serum aflatoxin adducts associated with increased relative risks of liver cancer (Autrup *et al.* 1993).



***Aspergillus fumigatus* Fresenius**

A. Colonies on MEA after one week; B. detail of colony showing columnar conidial heads, $\times 44$; C, D. conidial heads, $\times 920$; E. conidia, $\times 2330$.

Occurrence: A common and extremely versatile decay fungus associated with composting vegetation, wood chips, garbage and other materials at temperatures above ambient (Lacey and Dutkiewicz 1994).

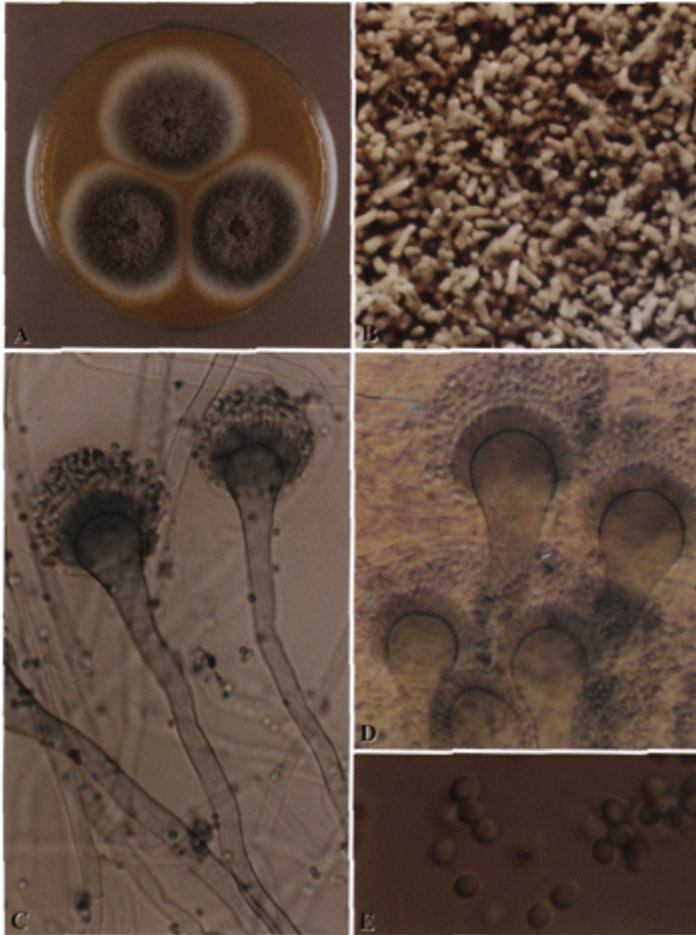
Often seen in dust infiltrating from outdoor air, particularly when cleaning standards are low, and hence carpet and mattress dust. Can grow on warm, wet building and finishing materials, e.g. wallpaper. Also isolated from polyurethane foam, plastic; particle

board; HVAC insulation, fans, filters, humidifier water; air/dust in bakeries, makings, sawmills; mushroom compost, sawdust, wood chips, bagasse; hay, cereals; potted plant soil.

Medical significance: EFG[D?]. Cause of aspergillosis. Known allergens (Day 1996; Summerbell 1998).

Toxicity: Produces various mycotoxins, including the potent toxin gliotoxin; some have been measured in air (Fischer *et al.* 1998).

Note: This species can be easily recognized by its rapid growth, grey blue colonies and the columnar conidial heads



Aspergillus niger van Tieghem

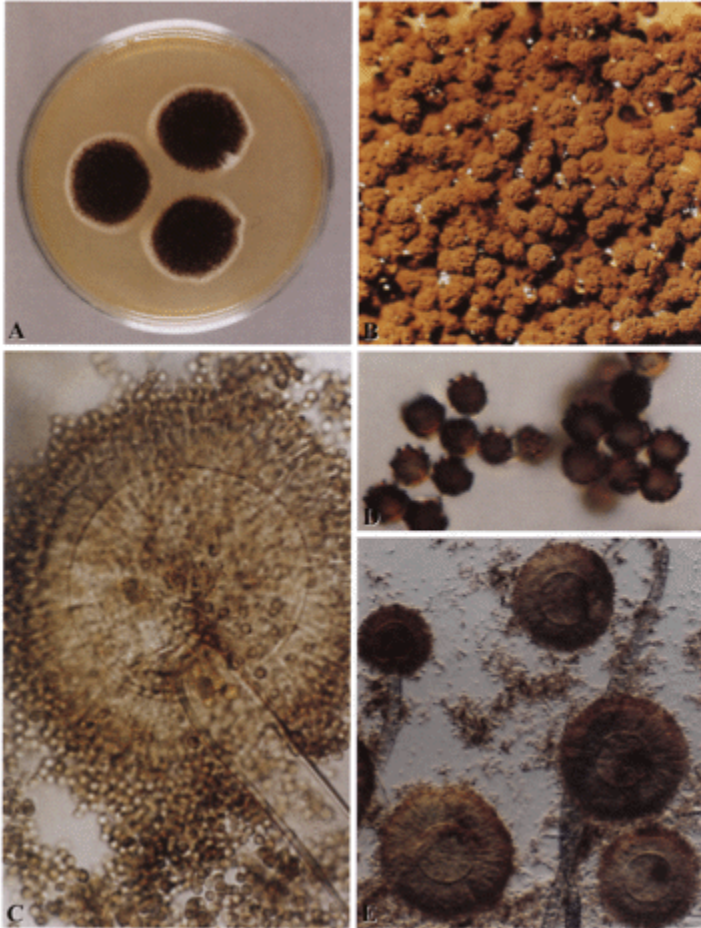
A. Colonies on ME A after one week; B. detail of colony showing radiate conidial heads, $\times 44$; C. conidial head, $\times 920$; D. conidia, $\times 2330$; E. conidial heads and tip of conidiophore, $\times 230$.

Occurrence: Worldwide in soil and plant litter, particularly notable as a contaminant of spices and other sun-dried plant products.

Often seen in indoor environments, but not generally associated with contaminated building materials. Found in floor, carpet and mattress dust; acrylic paint, UFFI, polyester polyurethane foam; polyurethane footwear, leather, cosmetics, wood chips; HVAC filters and fans; bakeries, cotton mills, cotton yarn; hay, cereals, cotton seed; potted plant soil.

Medical significance: EFG. An uncommon cause of aspergillosis, but does cause ear, nose and lung infections especially in immune compromised people (Summerbell 1998).

Toxic metabolites: Produces metabolites of low animal toxicity (Nielsen *et al.* 1999).



***Aspergillus ochraceus* Wilhelm**

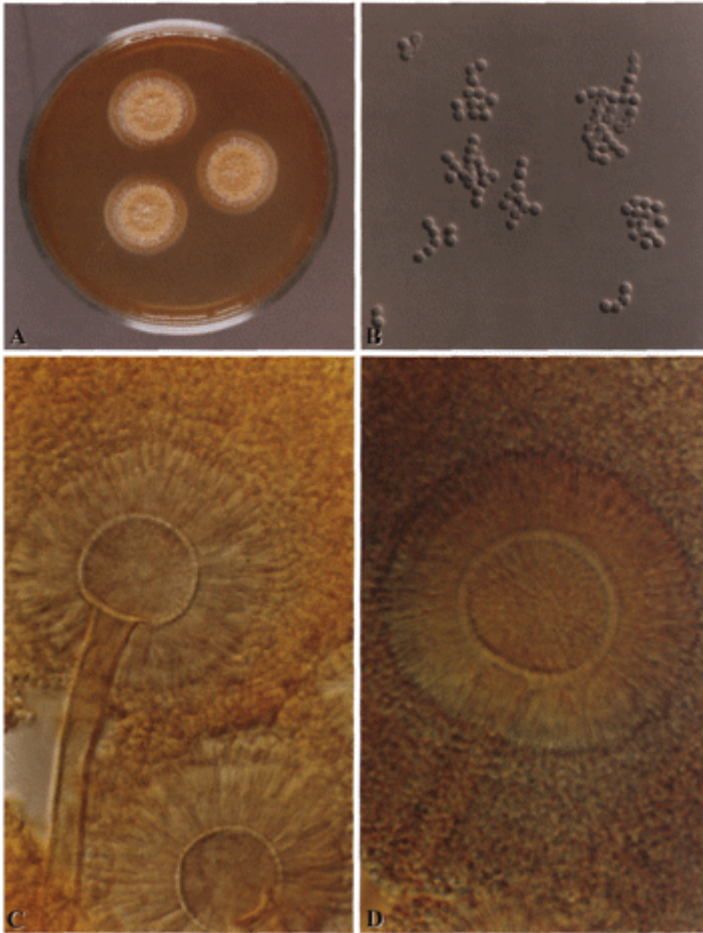
A. Colonies on MEA after one week; B. conidia, $\times 920$; C, D. conidial heads, $\times 920$.

Occurrence: A soil-borne species commonly associated with stored seeds and other foodstuffs.

May often be seen in indoor environments, but uncommonly associated with building materials, being found in carpet and mattress dust. Also isolated from cotton yarn, talcum powder, wheat flour; HVAC filter; bakeries.

Medical significance: [E?]IG

Toxicity: Produces ochratoxin, which has been measured on mouldy pet food in air ducts (Richards *et al.* 1999).



***Aspergillus penicillioides* Spegazzini**

A. Colonies on MEA+20% sucrose after one week; B. detail of colony showing radiate conidial heads, $\times 44$; C. conidial heads, $\times 920$; D. conidia, $\times 2330$; E. conidial heads, $\times 920$.

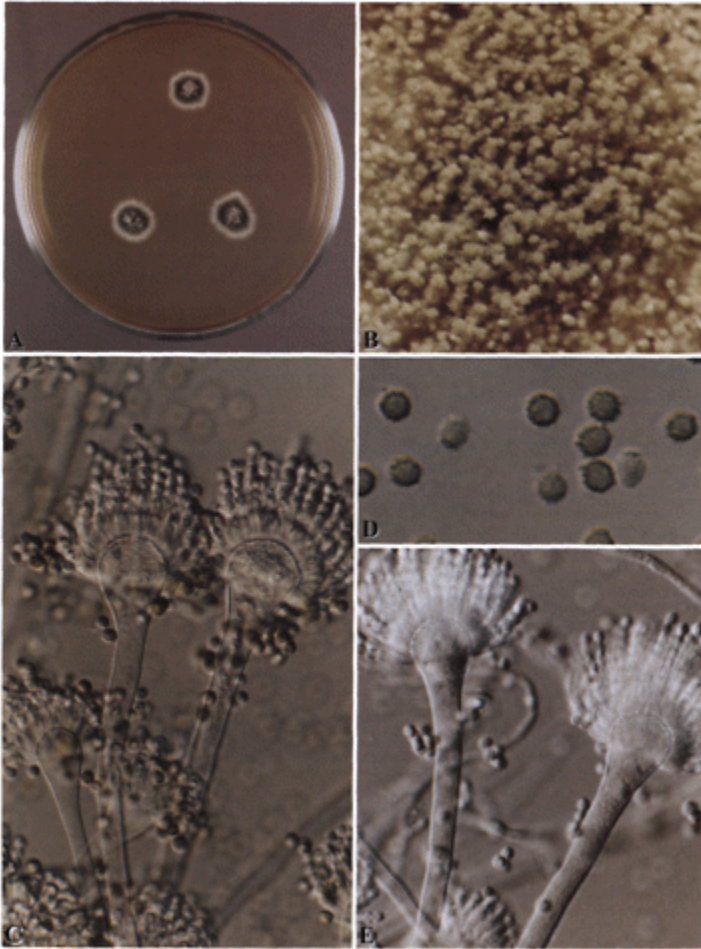
Occurrence: A slow growing xerophile that has been isolated from soil, dried foods and air.

Found in floor, carpet and mattress dust Also isolated from glass lens, electronic meter;

air from air-conditioning system; dried fish, cereals.

Medical significance: HI.

Note: This species can only be detected when using appropriate low wateractivity media such as MEA+ 20 % sucrose.



***Aspergillus restrictus* Smith**

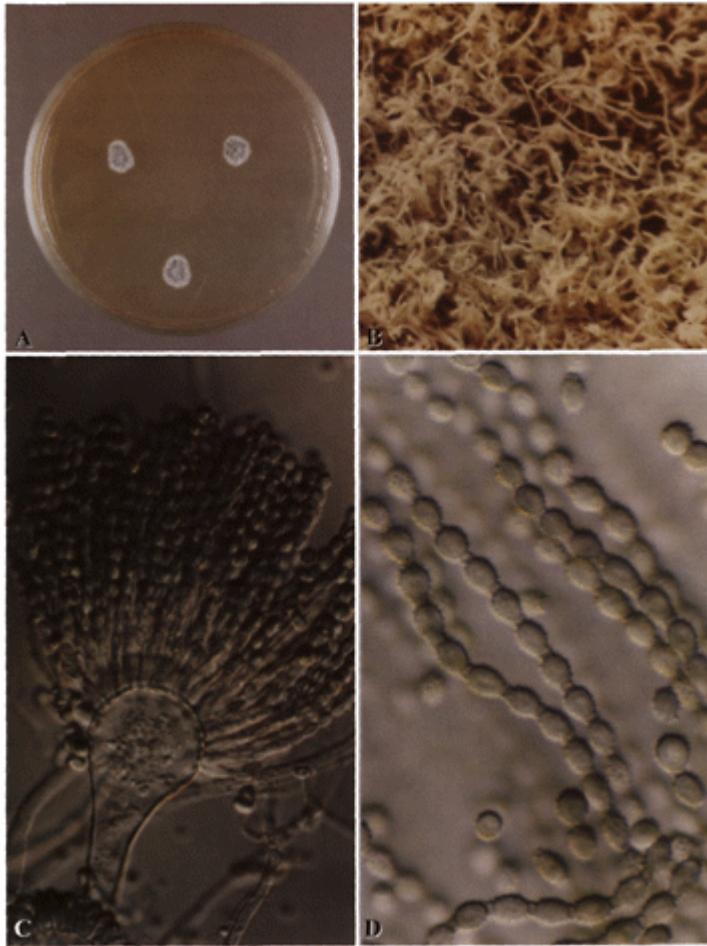
A. Colonies on MEA+20% sucrose after one week; B. detail of colony showing columnar conidial heads, $\times 44$; C. conidial head, $\times 920$; D. conidia, $\times 2330$.

Occurrence: A slow growing xerophile that has been isolated from soil, seeds, fruit juices and air.

Found in floor and carpet, mattress and upholstered-furniture dust. Also isolated from damp walls; leather armchair, cotton fabric, cloth; air from air-conditioning system; cereals.

Medical significance: HI (Many medical reports are based on misidentification).

Note: This species is xerophilic and only be detected using appropriate low wateractivity media such as MEA+20 % sucrose.



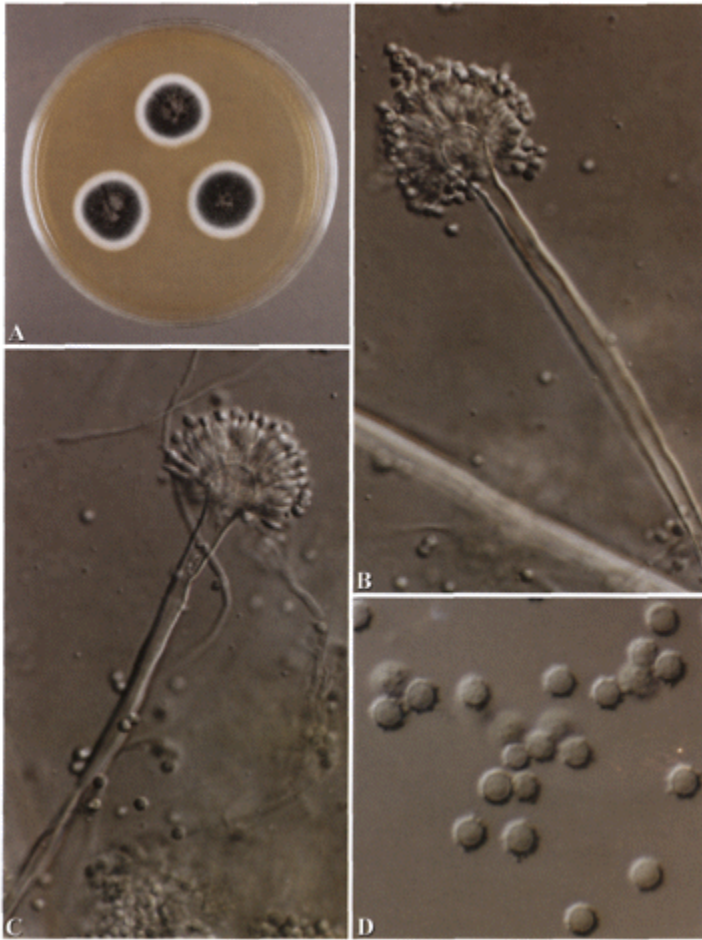
Aspergillus sydowii (Bainier & Sartory) Thorn & Church

A. Colonies on MEA after one week; B, C. conidial heads with tip of conidiophore, $\times 920$; D. conidia, $\times 2330$.

Occurrence: Soil fungus with a worldwide distribution.

Very common on mouldy gypsum board and associated wallpaper and paint, and found in floor, carpet and mattress dust. Also isolated from UFFI; cotton yarn, talcum powder; cereals, wheat flour; air in makings.

Medical significance: HI[G?].



Aspergillus terreus Thom

A. Colonies on MEA after one week; B. detail of colony showing columnar conidial heads, $\times 44$; C. conidial heads with tip of conidiophore, $\times 920$; D. conidia, $\times 2330$.

Occurrence: Worldwide but more abundant in tropical and subtropical areas, this soil-borne species is frequently associated with stored crops.

Uncommonly occurs on building materials, but found in floor, carpet and mattress dust. Also isolated from PVC/paper wallcovering, plastic, decomposing wood; cotton, cotton yarn, cloth; hospital air, bakery; coal-mine dust; hay, cereals, flour.

Medical significance: EFG. Uncommon cause of aspergillosis and fungal growths, especially in immunocompromised individuals (Summerbell 1998).



Aspergillus versicolor (Vuill.) Tiraboschi

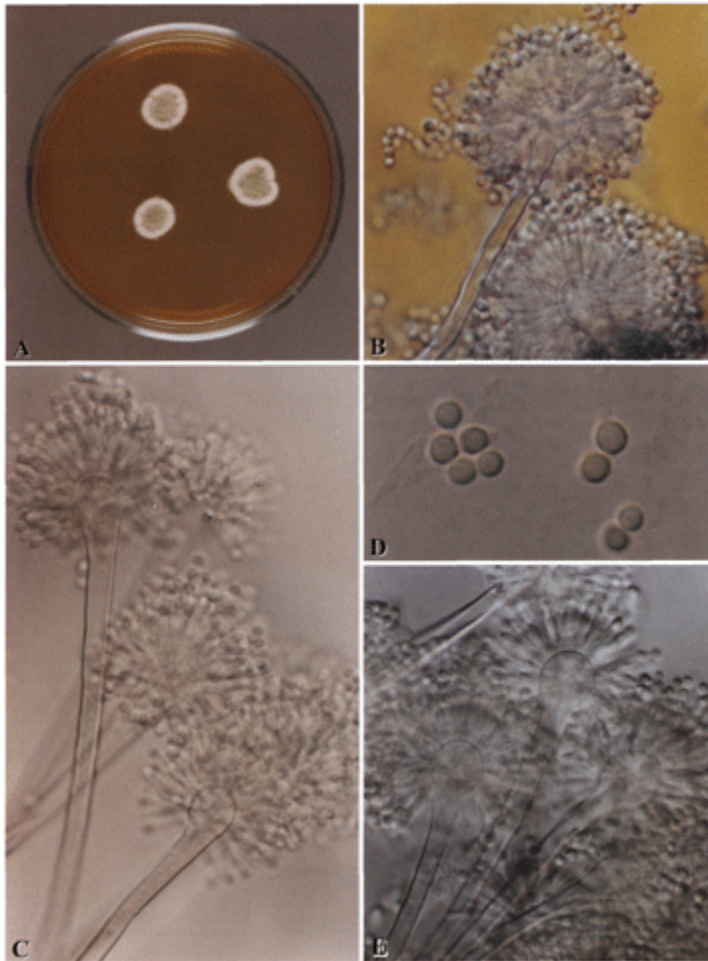
A. Colonies on MEA after one week, B, C. conidial heads with tip of conidiophore, $\times 920$; D. conidia, $\times 2330$; E. conidial heads, $\times 920$.

Occurrence: Occurs in soils worldwide, and present in many stored food products of plant origin.

Very common on gypsum board and other mouldy building materials, and in floor, carpet, mattress and upholstered-furniture dust; damp walls, painted metal; rock wool/glass fibre insulation, UFFI, polyester polurethane foam; swimming pool floor, tobacco, paraffin wax, talcum powder, cellophane, cotton yarn, cloth; hay, cereals, flour.

Medical significance: [E?]IG (Some erroneous medical reports exist).

Toxicity: On building materials produces the potent mycotoxin sterigmatocystin, an IARC Class 2A carcinogen (Nielsen *et al.* 1999).



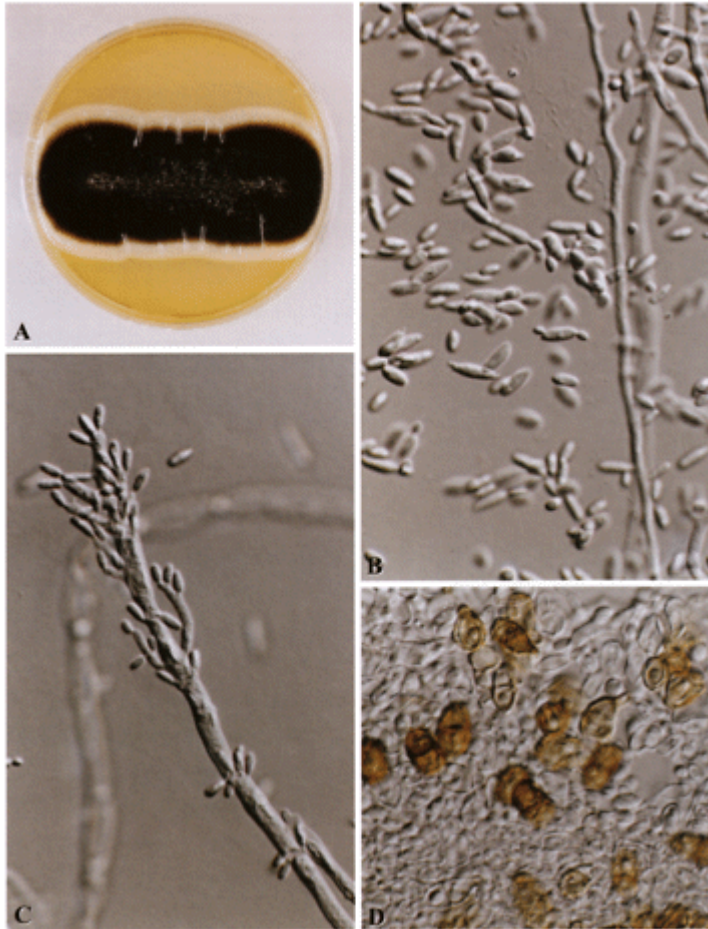
Aureobasidium pullulans (De Bary) Arnaud

A. Colony on MEA after one week; B. conidia, $\times 920$; C. conidiogenous cells producing (blastic) conidia synchronously, $\times 920$; D. arthroconidia, $\times 920$.

Occurrence: Common soil and phylloplane species, also found on both fresh and dried plant foodstuffs.

Grows on very wet wood and window frames, and hence common in floor, carpet and mattress dust. Also isolated from damp walls, acrylic paint, painted and preservative treated wood, wood chips; humidifier water; cereals, fruit, vegetables.

Medical significance: EIG. A very rare cause of home-associated hypersensitivity pneumonitis (Flannigan *et al.* 1991), but associated with occupational disease in wood processing (Lacey and Dutkiewicz 1994).



***Botrytis cinerea* Pers.**

A. Colony on MEA after one week, B. conidia, $\times 920$; C, D. conidiophore producing conidia simultaneously from swollen conidiogenous cells, C. $\times 230$, D. $\times 920$.

Occurrence: Soil-borne facultative parasite of a broad range of plants, known as “grey mould”.

Found in floor, carpet and mattress dust. Also isolated from mouldy cardboard; hay, cereals, carrots, tomatoes, strawberries.

Medical significance: HI.

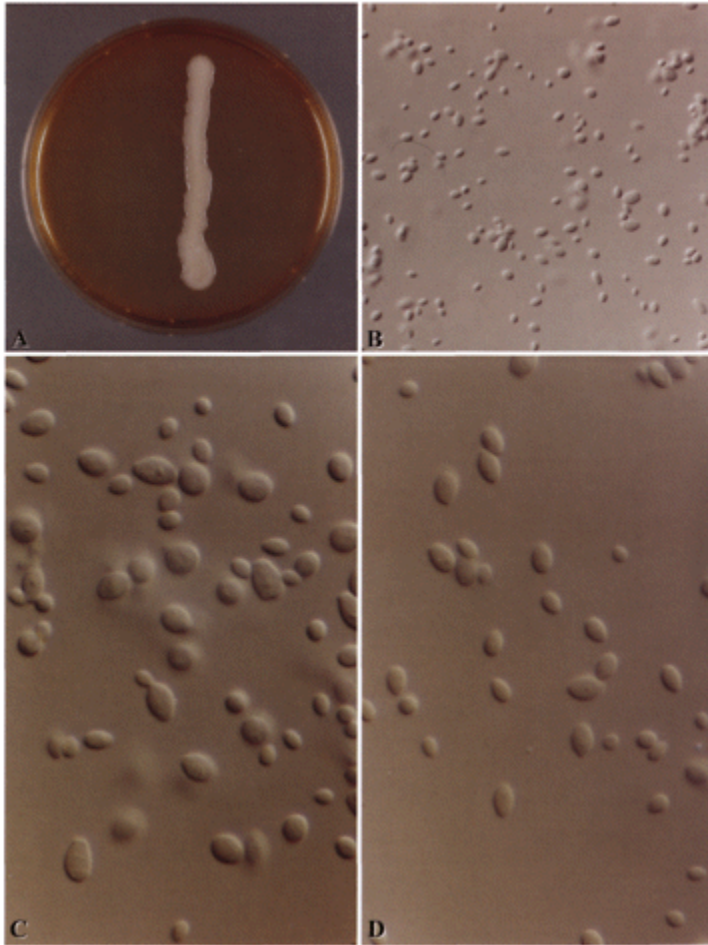


Candida peltata (Yarrow) Meyer & Ahearn

A. Colony on MEA after 5 days; B. cells, $\times 920$; C, D. cells, $\times 2330$.

Occurrence: air, milk

Medical significance: HI.



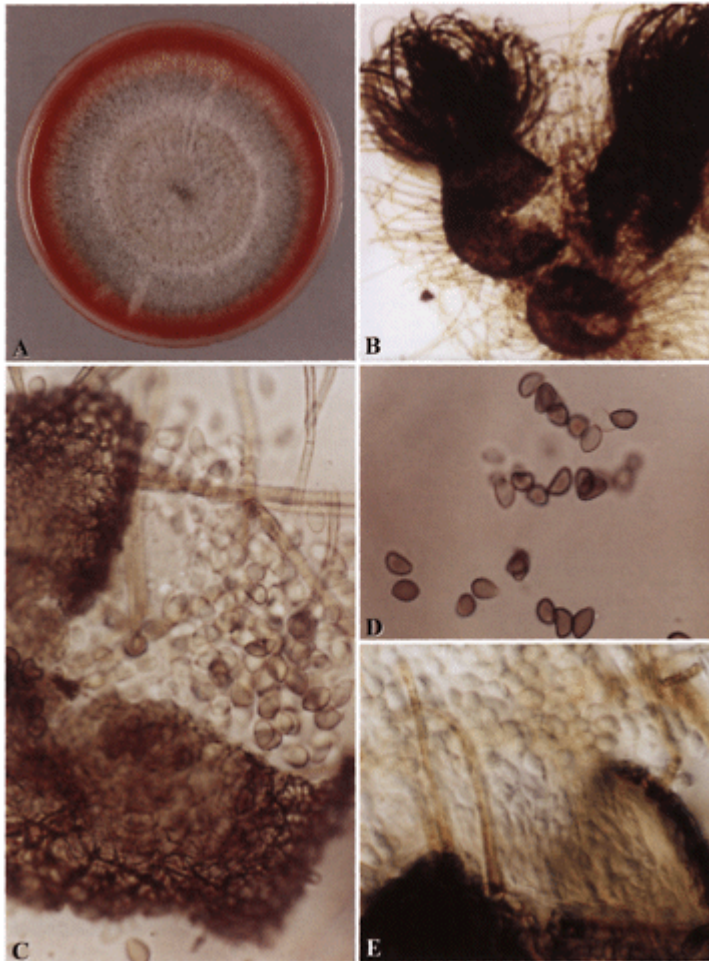
Chaetomium aureum Chivers

A. Colony on MEA after two weeks; B. two perithecia, $\times 230$; C. perithecia with ascospores, $\times 920$; D. ascospores, $\times 920$; E. perithecia with ascospores, $\times 920$.

Occurrence: Soil, cellulosic materials

Compost, tobacco

Medical significance: HI.



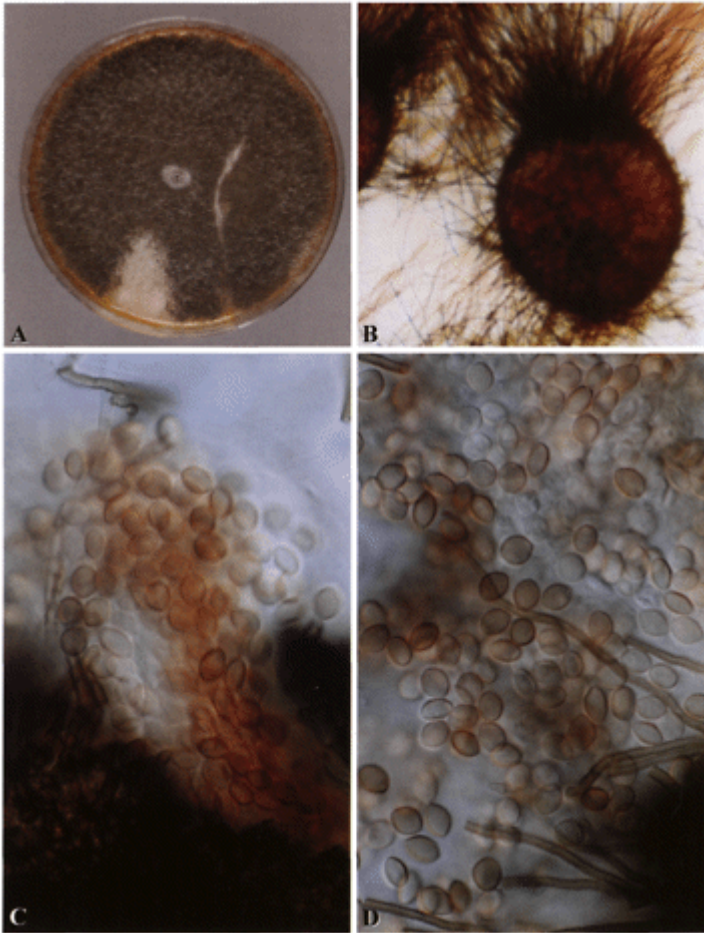
***Chaetomium globosum* Kunze ex Steud.**

A. Colony on MEA after two weeks; B. perithecium, $\times 230$; C, D. ascospores, $\times 920$.

Occurrence: A potent cellulytic species found on various cellulosic material e.g. wall paper.

Very common on mouldy building materials that have been very wet, including gypsum board, cellulose board and wood. Also isolated from mattress dust; cardboard, paper, polyurethane foam; cotton, cotton yarn, polyurethane coated fabric; cereals.

Medical significance: EI[G?].

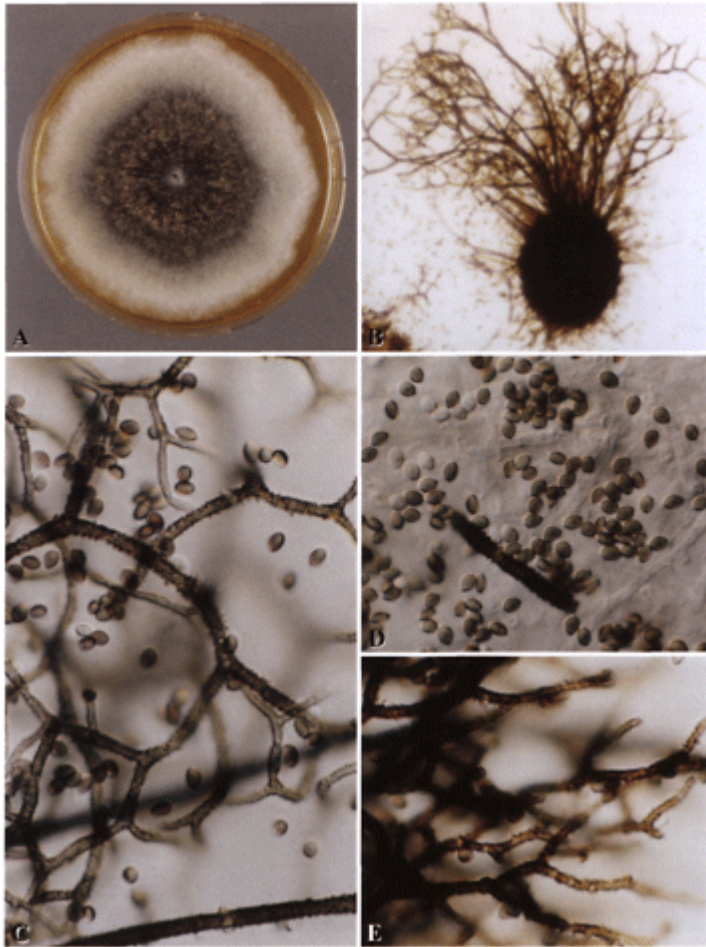


Chaetomium indicum Corda

A. Colony on MEA after two weeks; B. perithecial, $\times 230$; C. ascospores with dichotomously branched hairs, $\times 920$; D. ascospores, $\times 920$; E. dichotomously branched hairs, $\times 920$.

Occurrence: Isolated from mattress dust; hay, cereals.

Medical significance: HI.



Chrysonilia sitophila (Mont.) v. Arx

=*Monilia sitophila* (Mont.) Sacc.

=*Penicillium sitophilum* Mont.

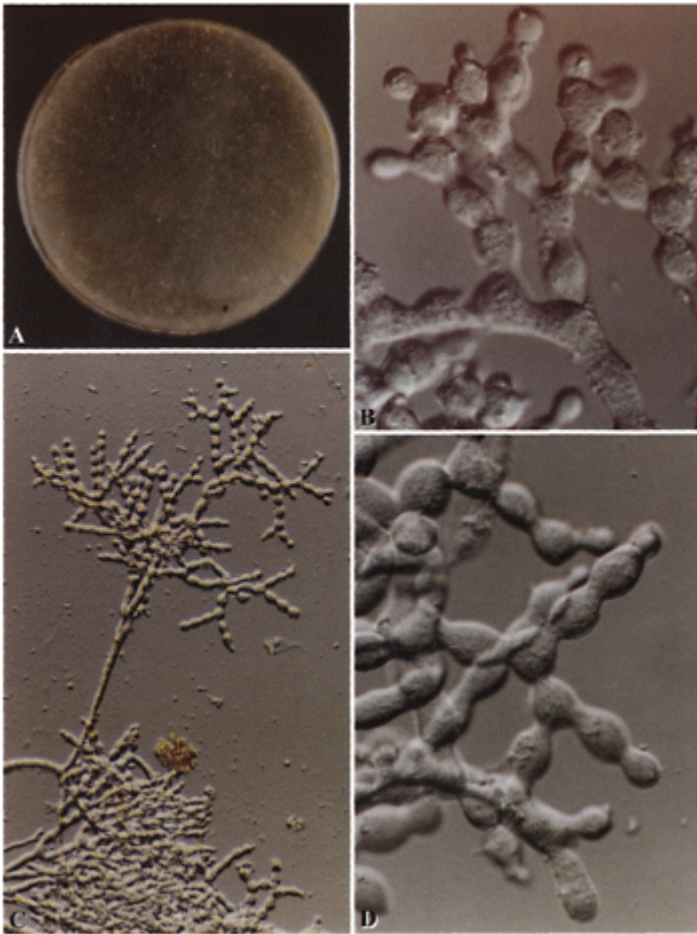
Teleomorph: *Neurospora sitophila* Shear & Dodge.

A. Colony on MEA after 5 days; B-D. conidiophores, each branch forming chains of conidia, B.×920, C. ×230, D.×920.

Occurrence: This “red bread mould” has a wide distribution, and is found on bakery goods and causing a storage rot of soft fruits.

Isolated from carpet and mattress dust; bakeries; burnt wood.

Medical significance: HIG



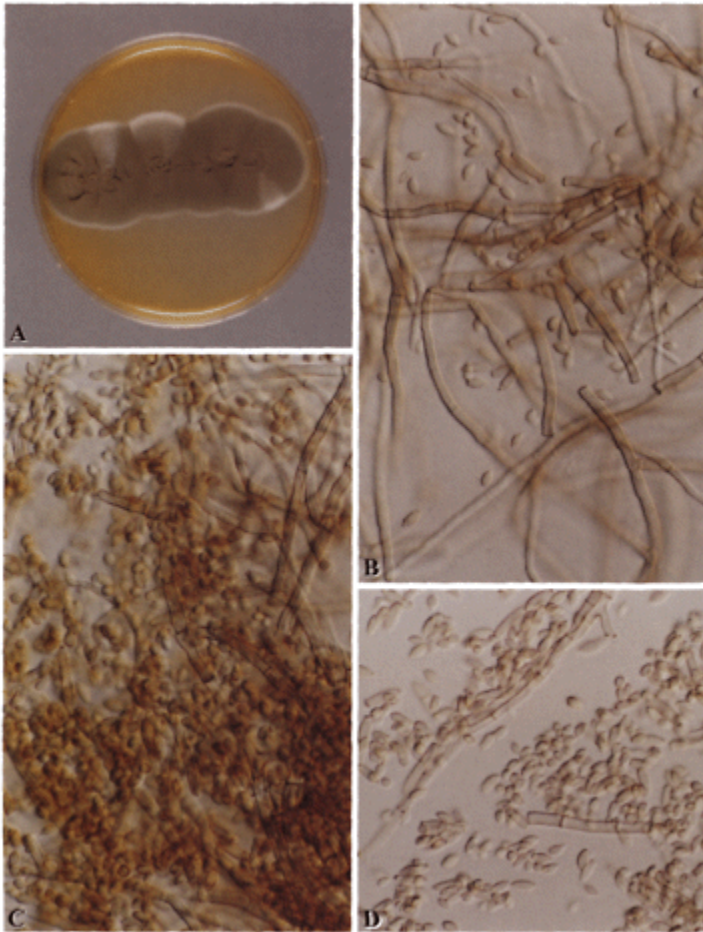
***Cladosporium cladosporioides* (Fres.) de Vries**

A. Colony on MEA after one week; B-D. conidia, $\times 920$.

Occurrence: With a worldwide distribution, this common phylloplane species occurs also in soil and on exposed textiles, and in summer is abundant in air.

Although this species is claimed to *grow* on building materials, it is not always clear whether such strains are truly *C. cladosporioides*, an undescribed species or variants of *C. sphaerospermum*, which does grow on wet building materials. Isolated from floor, carpet and mattress dust; damp PVA emulsion, acrylic painted walls, wallpaper; UFFI; bakery dust; cereals and other foodstuffs.

Medical significance: [E?]IG. Allergenic (Day 1996).



***Cladosporium herbarum* (Pers.) Link**

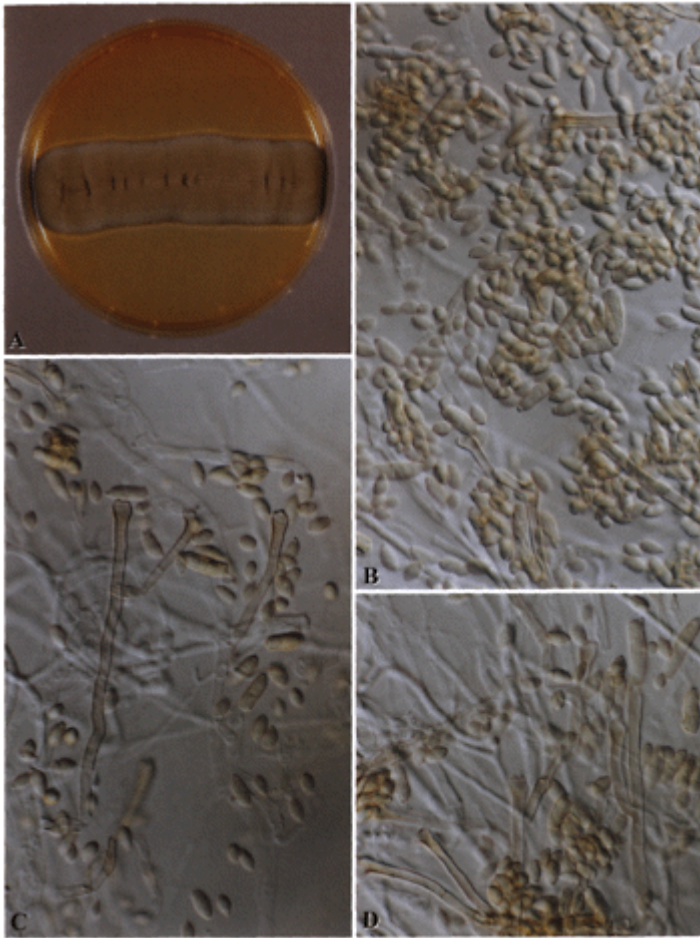
Teleomorph: *Mycosphaerella tassiana* (de Not.) Johanson

A. Colony on MEA after one week; B-D. conidiophores with conidia, $\times 920$.

Occurrence: Similar to *C. cladosporioides*, and on senescent or dead plant matter.

Less common than *C. cladosporioides* in indoor environments. Isolated from floor, carpet and mattress dust; damp acrylic painted walls, wallpaper; talcum powder; HVAC insulation, filters and fans; hospital air; cereals.

Medical significance: HI. Allergenic (Day 1996).



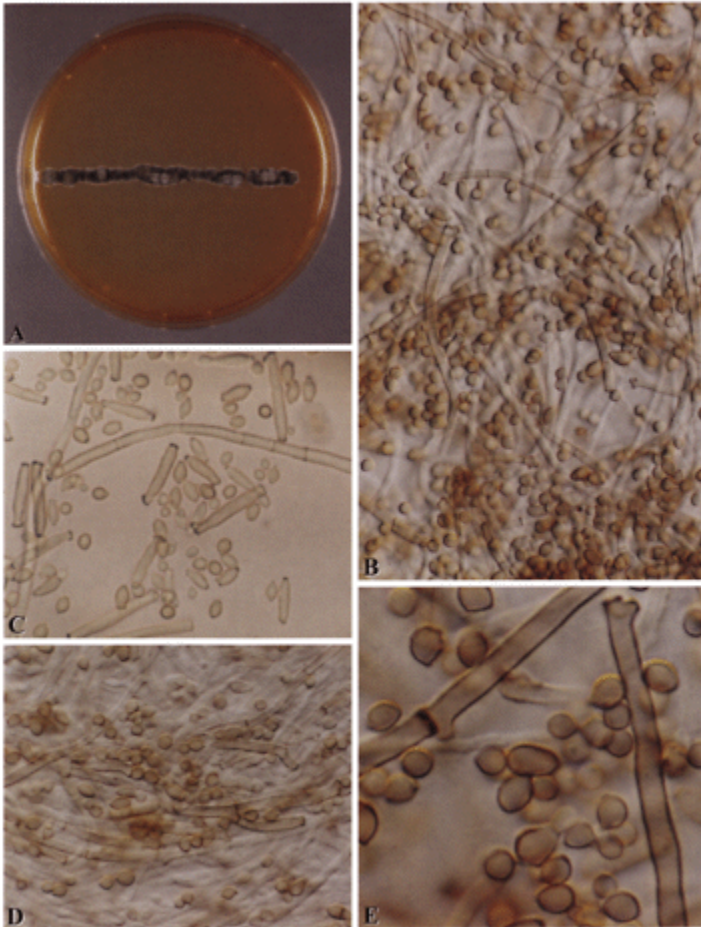
***Cladosporium sphaerospermum* Penzig**

A. Colony on MEA after one week; B-D. conidiophores with conidia, $\times 920$; E conidiophore with conidia, $\times 2330$.

Occurrence: Similar to *C. cladosporioides*.

Common on wet building elements such as gypsum board, acrylic and oil painted walls, painted wood, and wallpaper. Also isolated from carpet and mattress dust; bakeries; HVAC fans, wet insulation in mechanical cooling units; cloth, pharmaceutical cream; meat (in cold store), bread.

Medical significance: HI. Allergenic (Day 1996).



Clonostachys rosea (Link:Fr.) Schroers *et al.*

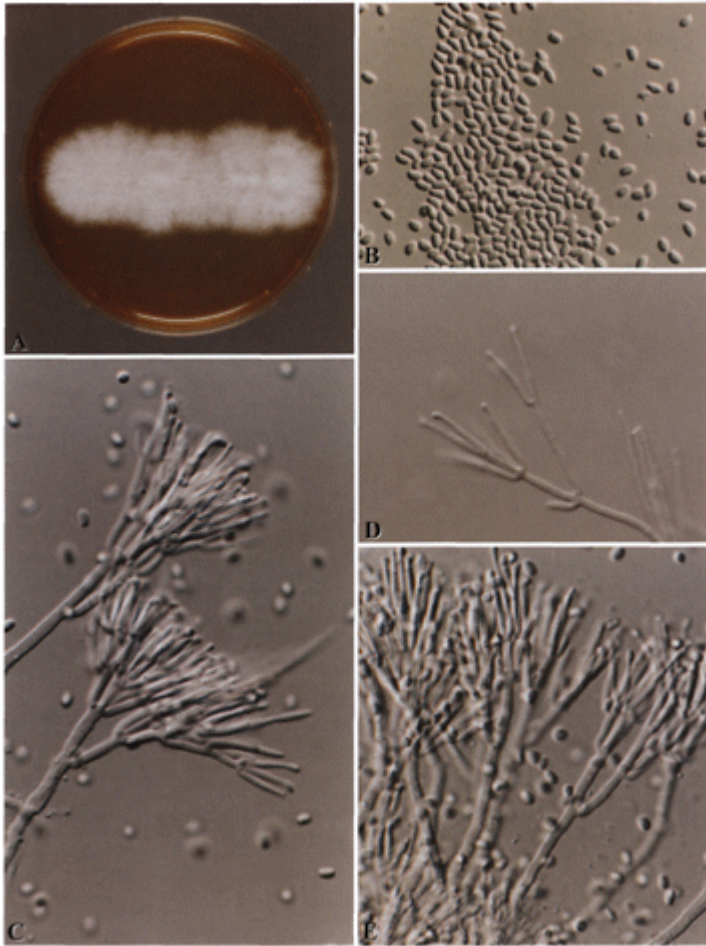
=*Gliocladium roseum* (Link) Thorn

Teleomorph: *Bionectria ochroleuca* (Schw.) Schroers & Samuels

A. Colony on MEA after one week; B. conidia, $\times 920$; C-E. conidiophores with conidia, $\times 920$.

Occurrence: isolated from housedust; cooling tower slats, gymnasium floor; polyurethanes; greenhouse dusts; cereals.

Medical significance: HI.



Coprinus cordisporus Gibbs

A-C. Fruiting bodies of different age; D. basidiospores, $\times 2330$.

Occurrence: wood, often in crawling spaces

Medical significance: HI.

Note: Various species of *Coprinus* can occur indoors. The species depicted here is merely an illustration of the morphological features



Cryptoeoccus laurentii (Kufferath) Skinner

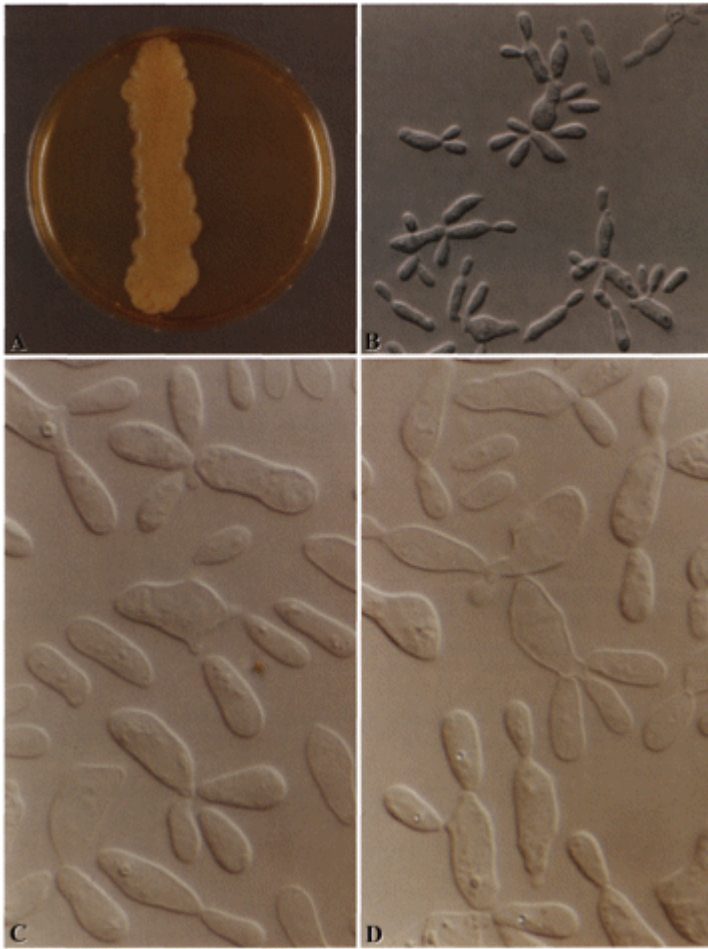
Colony on MEA after 5 days; B. cells, $\times 920$; C, D. cells, $\times 2330$.

Occurrence: indoor air

Also found in food and beverages, wheat, maize, soil, litter, plants,

Medical significance: [E/][F?][G?].

Many dubious or erroneous medical reports based on incorrect attribution of infection to probable contaminants.



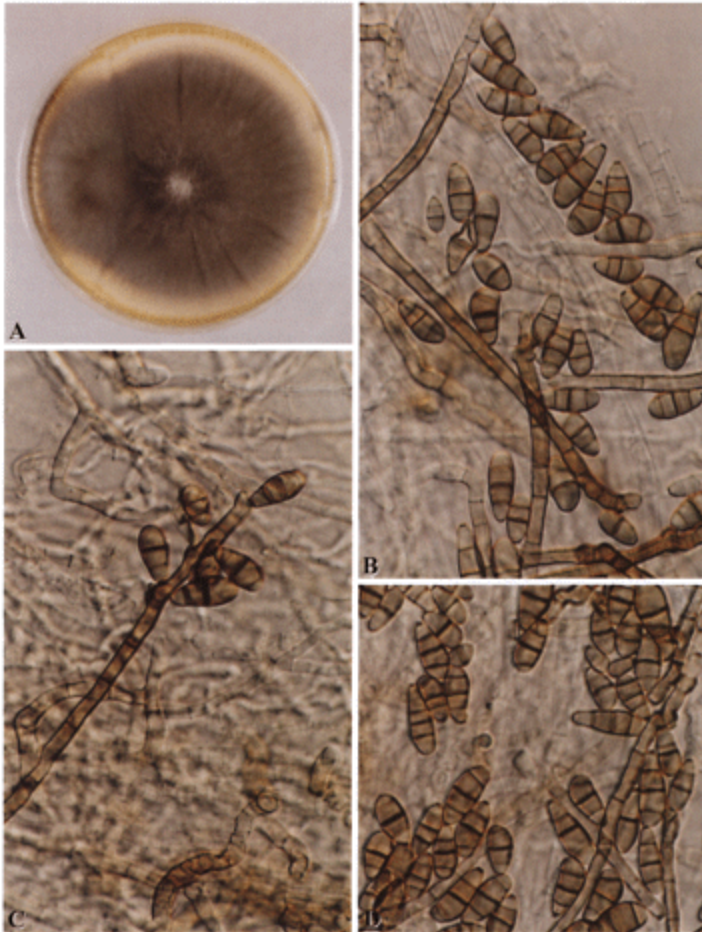
Curvularia lunata (Wakker) Boedijn

A. Colony on MEA after one week; B-D. conidia and conidiophores, B.×920, C.×920, D.×920.

Occurrence: A widely distributed contaminant of seed crops.

Isolated from floor and mattress dust; wallpaper, painted wood; cereals.

Medical significance: EFG.



***Emericella nidulans* (Eidam) Vuill.**

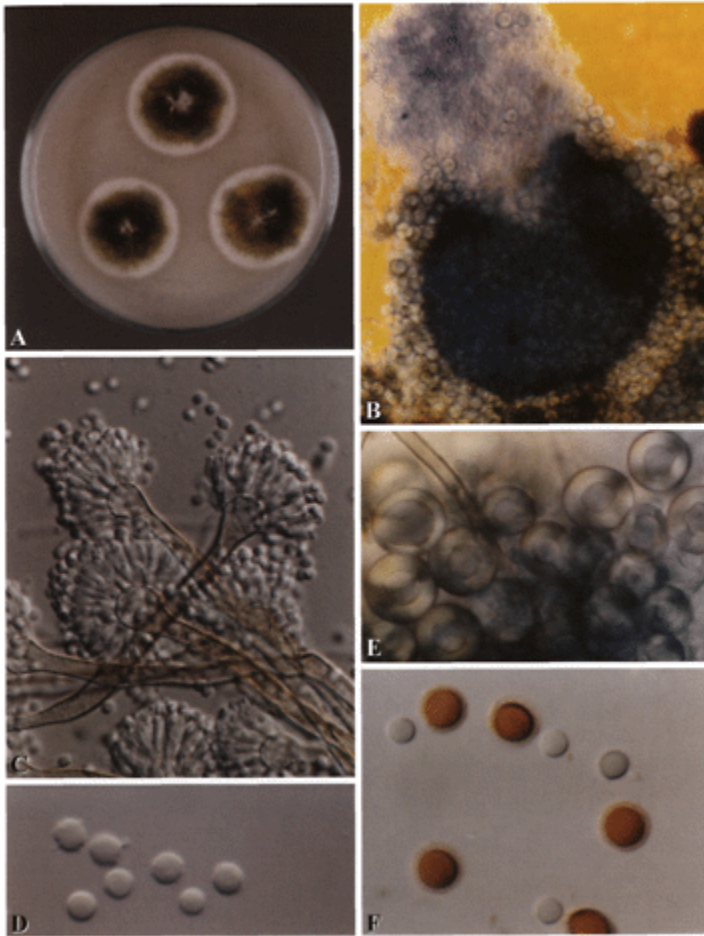
Anamorph: *Aspergillus nidulans* (Eidam) Wint.

A. Colonies on OA after one week; B. ascoma surrounded covered by Hülle cells, $\times 230$; C. conidiophores of *Aspergillus nidulans*, $\times 920$; D. conidia of *Aspergillus nidulans*, $\times 2330$; E. Hülle cells, $\times 920$; F. ascospores and conidia, $\times 2330$.

Occurrence: A widespread soil fungus, most common in tropical and subtropical regions, also associated with seeds.

Isolated from carpet and mattress dust; cotton lint, cotton yarn, decomposing wood; hay, cereals. Often in air samples, particularly from compost plants.

Medical significance: EFG.



***Epicoccum nigrum* Link**

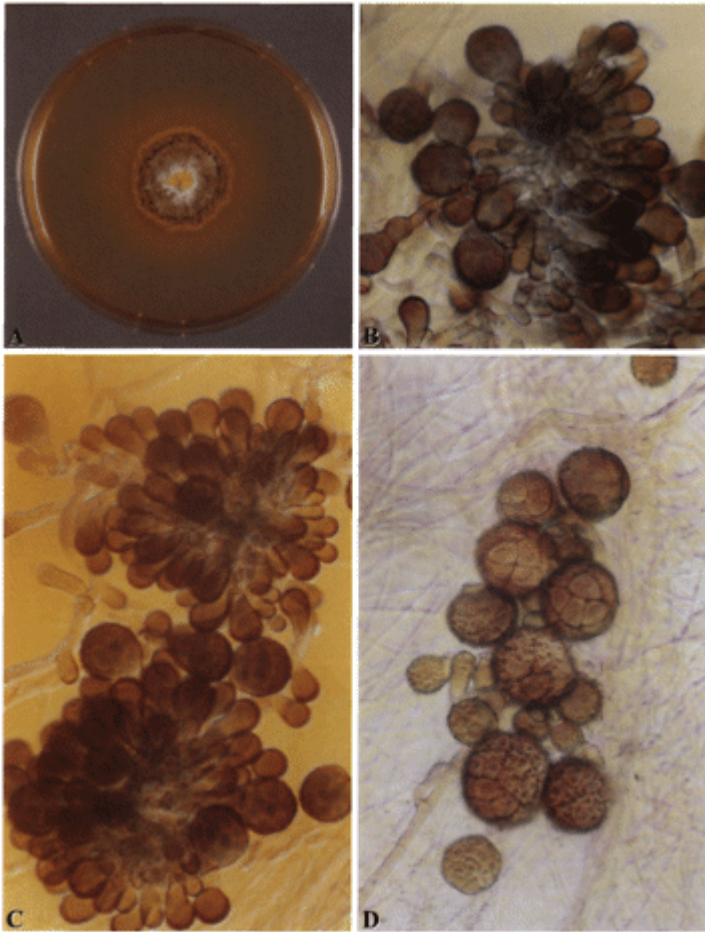
=*Epicoccum purpurascens* Ehrenb.

A. Colony on MEA after one week; B, C. sporodochia with septate (blasto)conidia, $\times 920$; conidiogenous cells with septate (blasto) conidia, $\times 920$.

Occurrence: Universally distributed, this colonizer of senescent and dead plants also occurs in soil and is prominent in freshly harvested seeds.

Isolated from floor, carpet and mattress dust; UFFI; hospital air; exposed acrylic paint, canvas; cereals, beans.

Medical significance: [E?]I.



***Eurotium amstelodami* Mangin**

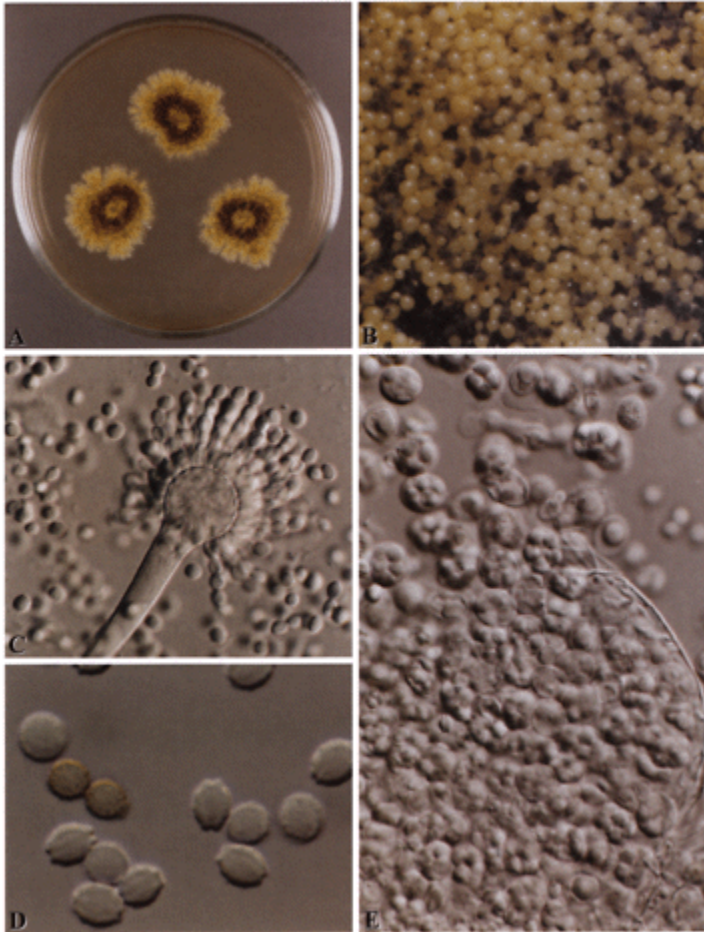
Anamorph: *Aspergillus amstelodami* (Mangin) Thorn & Church

A. Colonies on MEA+20% sucrose after two weeks; B. ascomata, $\times 40$; C. conidia and conidiophore of *Aspergillus amstelodami*, $\times 920$; D. ascospores and conidia, $\times 2330$; E. portion of ascoma with asci, $\times 920$.

Occurrence: In nature, this xerophile is more common in tropical and subtropical soils and is commonly isolated from dried or concentrated food materials.

Found in floor, carpet and mattress dust. Also isolated from hospital air, cloth, shoes; hay, cereals, nuts, fruit juices.

Medical significance: EIG.



***Eurotium chevalieri*!** Mangin

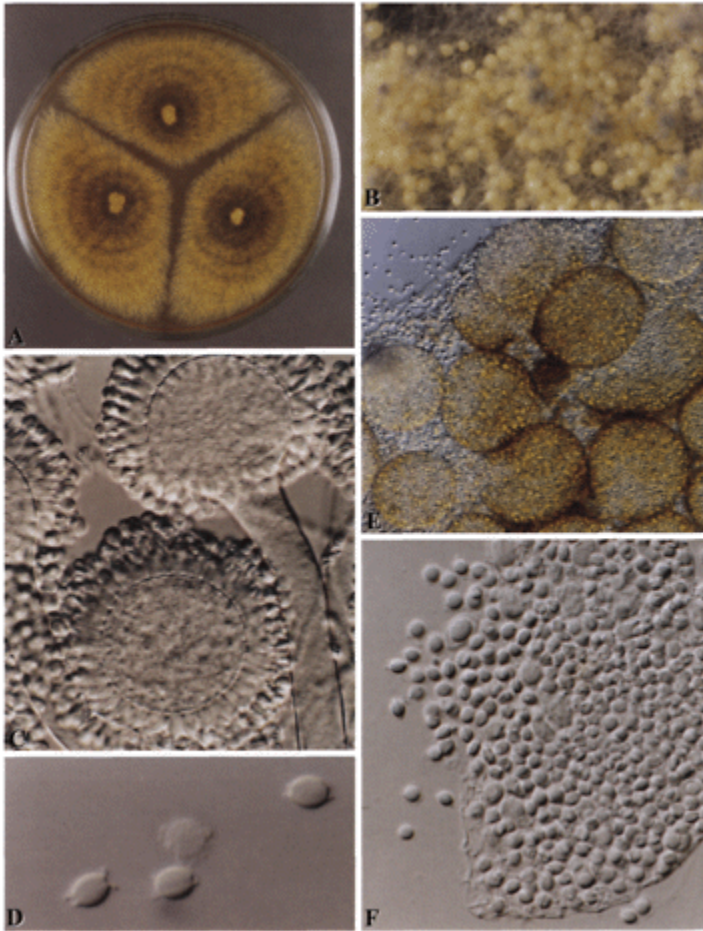
Anamorph: *Aspergillus chevalieri*! (Mangin) Thorn & Church

A. Colonies on MEA+20% sucrose after two weeks; B. ascomata, $\times 40$; C. conidiophores of *Aspergillus chevalieri*, $\times 920$; D. ascospores, $\times 2330$; E. ascoma, $\times 230$; F. portion of ascoma with asci and ascospores, $\times 920$.

Occurrence: With a distribution similar to *E. amstelodami*, this ubiquitous soil-borne xerophile is associated with leather, cotton, dried seeds and milled foodstuffs.

Found in floor, carpet and mattress dust. Also isolated from HVAC system air; hay, cereals, flour.

Medical significance: EIG



Eurotium herbariorum (Wiggers) Link

Anamorph: *Aspergillus glaucus* Link

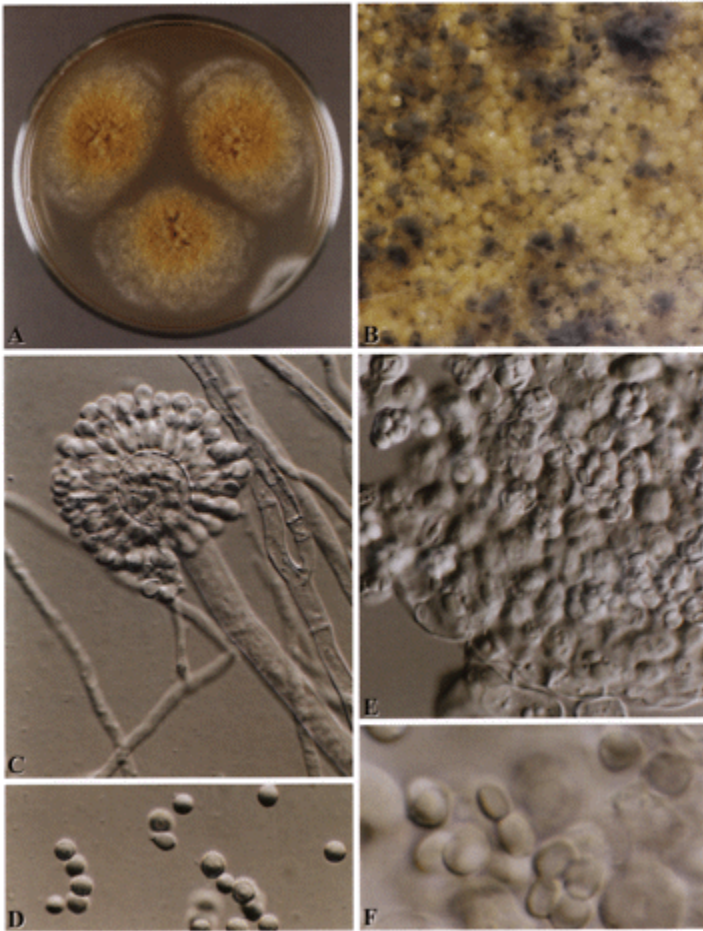
A. Colonies on MEA+20% sucrose after two weeks; B. ascomata, $\times 40$; C. conidiophore of *Aspergillus glaucus*, $\times 920$; D. conidia of *Aspergillus glaucus*, $\times 920$; E. portion of ascoma with asci, $\times 920$; F. ascospores, $\times 2330$.

Occurrence: This xerophile also has a distribution similar to *E. amstelodami*, and is associated with meat products as well as dried plant products.

Occurs on damp gypsum board and other building materials, and in floor, carpet and mattress dust. Also isolated from HVAC insulation; food store dust, weaving shed wall; hay, cereals, flour, desiccated coconut, nuts.

Medical significance: HI[G?]. Many dubious medical reports based on misidentification or incorrect attribution of disease causation to contaminants.

Note: *Eurotium repens* de Bary is a similar species with the same distribution.



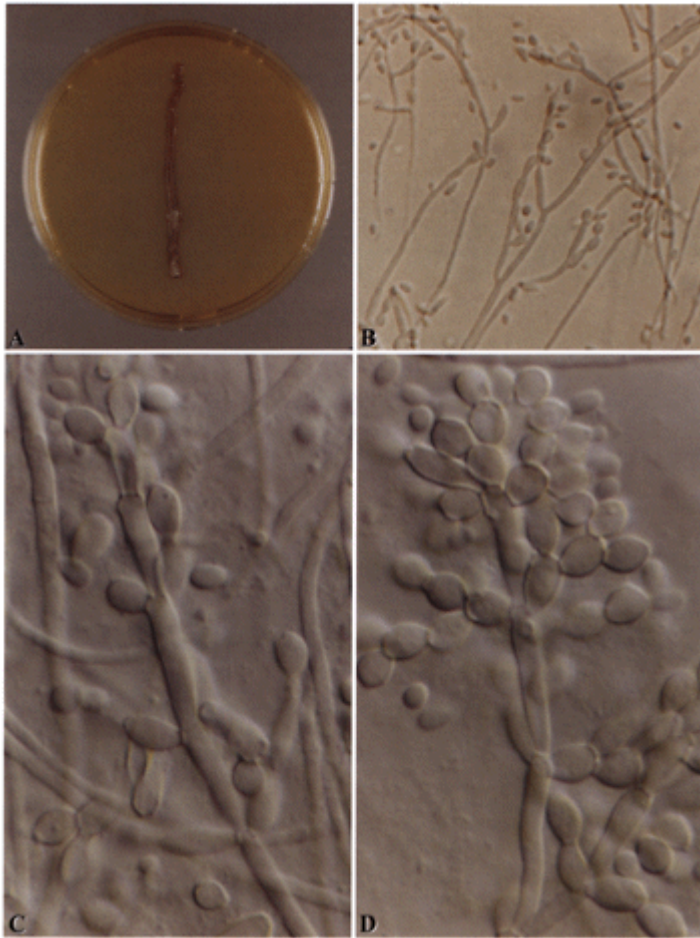
***Exophiala dermatitidis* (Kano) de Hoog**

A. Colony on MEA after one week; B-D. conidiogenous cells with conidia, B.×920, C, D.×2330.

Occurrence: An inhabitant of cool moist soils.

Commonly found in sinks and drains. Also isolated from bronchioalveolar lavage sampling equipment.

Medical significance: CDG.



Fusarium culmorum (W.G.Smith) Sacc.

A. Colony on MEA after one week; B-F. conidiophores and macro-conidia, B. in Petri-dish, $\times 230$, C-F. $\times 920$.

Occurrence: With a worldwide distribution, this soil-borne grass and cereal pathogen is more prevalent in temperate regions.

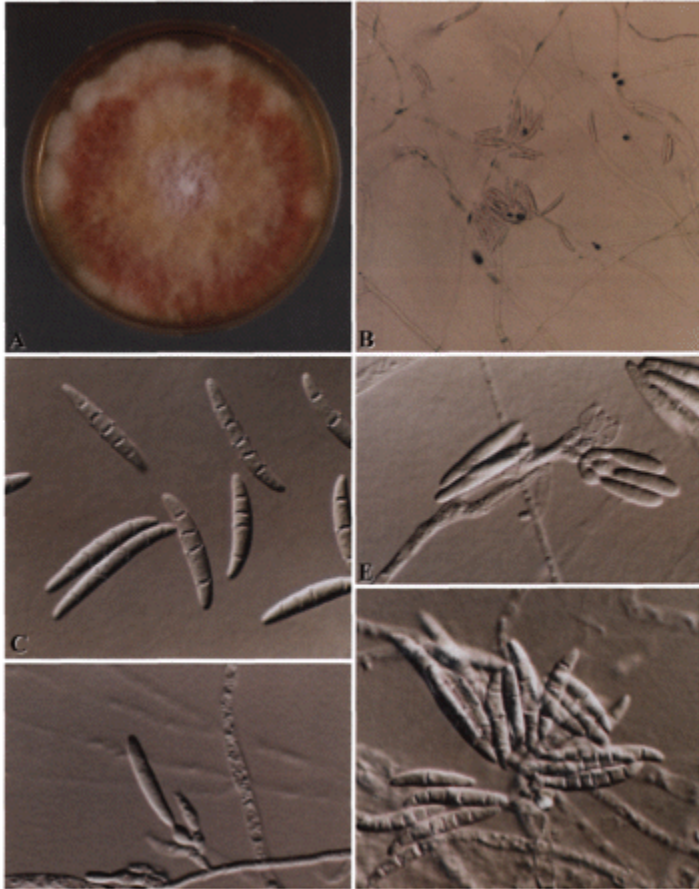
Isolated from floor, carpet and mattress dust; damp wall; polyurethane foam; cereals, apples, potatoes.

Medical significance: HI. Allergenic (Franklin 1983).

Toxicity: Produces zearalenone and deoxynivalenol.

Note: In many areas the related species *F. graminearum* is much commoner. Ascospores of its teleomorph, *Gibberella zeae*, may be common in outdoor air during the growing season where there is a lot of green space and/or wheat or maize fields, but does not grow on building materials. It also produces deoxynivalenol and zearalenone

(Miller 1999) and is allergenic (Franklin 1983).



***Fusarium solani* (Mart.) Sacc.**

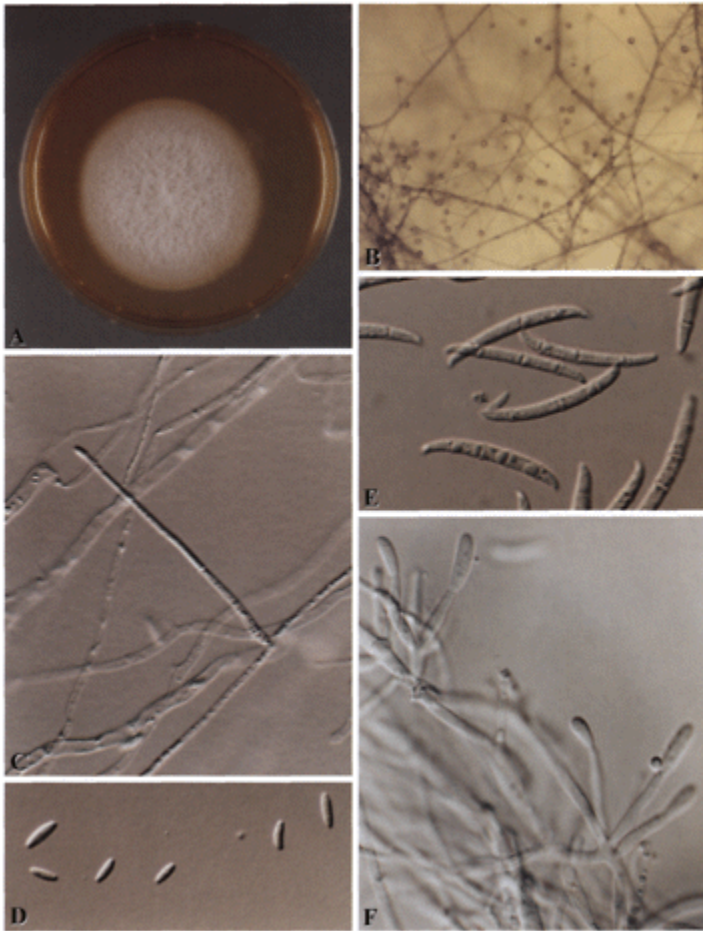
Teleomorph: *Nectria haematococca* Berk. & Br. var. *brevicona* (Wollenw.) Gerlach

A. Colony on MEA after one week; B. conidiophores with conidia in slimy heads, in Petri-dish, ×230; C. elongated conidiophores and phialides forming micro-conidia. ×920; D. micro-conidia, ×920; E. macroconidia, ×920; F. conidiophores forming macroconidia, ×920.

Occurrence: A cosmopolitan soil-borne pathogen of a wide range of crop plants. Also common in fresh water.

Isolated from carpet and mattress dust; damp walls, wallpaper; polyester polyurethane foam; cotton of insulating duct liner; water pipes, humidifier water; metal working fluid, coolant; treated wood.

Medical significance: EFG. Keratitis in man; associated with wounds; isolated from eyes and finger nails.



***Fusarium verticillioides* (Sacc.) Nirenb.**

=*Fusarium moniliforme* Sheld. *sensu stricta*

Teleomorph: *Gibberella moniliformis* Winel.

A. Colony on MEA after one week; B. catenulate micro-conidia, in Petri-dish, $\times 230$; C. conidiophores, $\times 920$; D. micro-conidia, $\times 920$; E. macro-conidia, $\times 920$.

Occurrence: Soil-borne pathogen (in North America formerly called one of the mating types of *Fusarium moniliforme* Sheld.) of maize and a wide range of other crops in tropical and subtropical areas, but rare in temperate area, except under glass.

Can grow in badly-maintained humidifier pans and other areas where stagnant water occurs in HVAC systems; such isolates produce fumonisin. Also isolated from mattress dust; damp walls; raw cotton; cereals and other seeds.

Medical significance: EFG. Keratitis in man, and an increasingly observed cause of invasive mycoses in immune compromised individuals (Nelson *et al.* 1994).

Toxicity: Produces the agriculturally important toxin fumonisin (Nelson *et al.* 1991, Miller 1999).



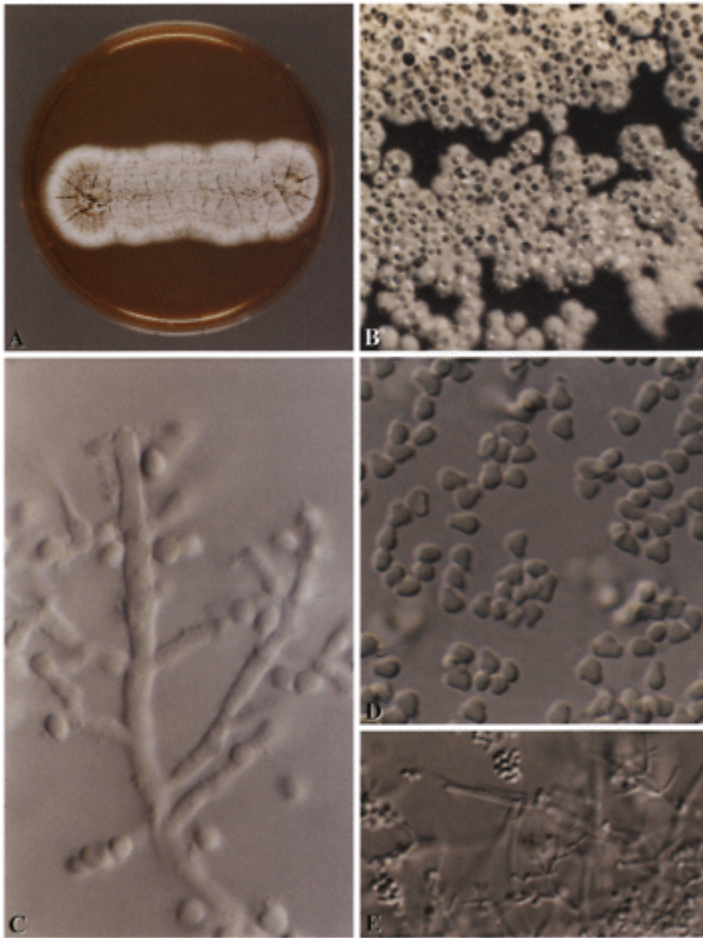
Geomyces pannorum (Link) Sigler & Carmichael

A. Colony on MEA after one week; B. detail of colony, $\times 40$; C. conidiophore and conidia, $\times 2330$; D. conidia, $\times 2330$; E. conidiophores with conidia, $\times 920$.

Occurrence: Isolated from mattress dust; damp walls; soil floor in building, laboratory contaminant, swimming pool floor, gymnasium floor; meat, cod, gelatin, flour.

Often found on paper in archives and libraries

Medical significance: HI (Some erroneous medical reports exist).



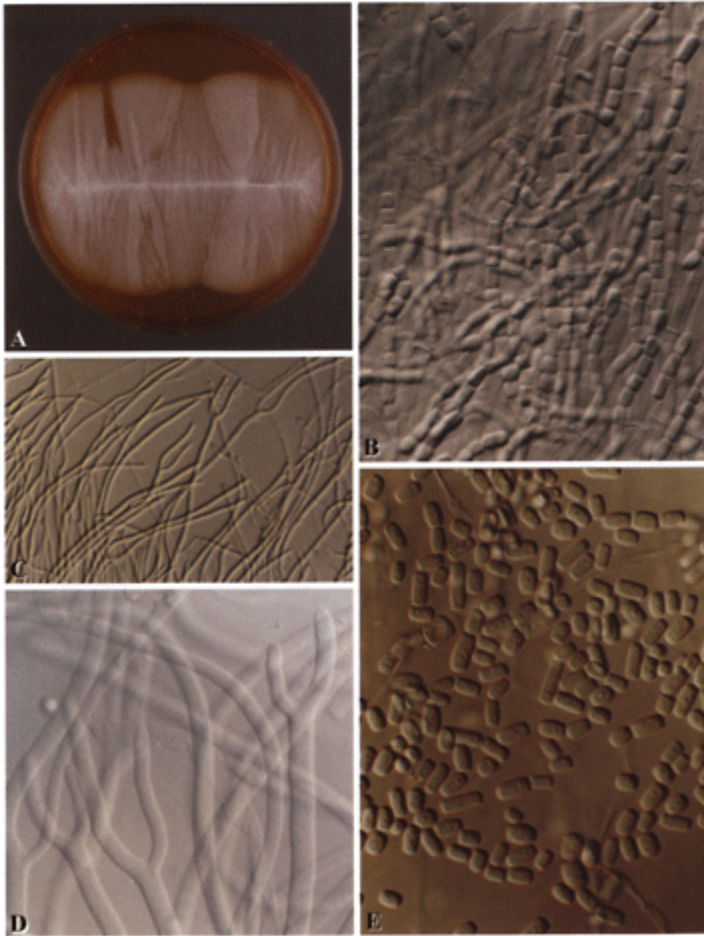
***Geotrichum candidum* Link**

A. Colony on MEA after one week; B. disarticulation of fertile hyphae into (arthro) conidia, $\times 920$; C, D. dichotomously branched (forked) hyphae, C. $\times 230$, D. $\times 920$; E. (arthro)conidia, $\times 920$.

Occurrence: A cosmopolitan species in soil, water and air, associated with a wide range of seeds and fruits.

Isolated from carpet dust; damp walls; swimming pool floor, gymnasium floor; bakeries.

Medical significance: EIG.



Memnoniella echinulata (Rivolta) Galloway

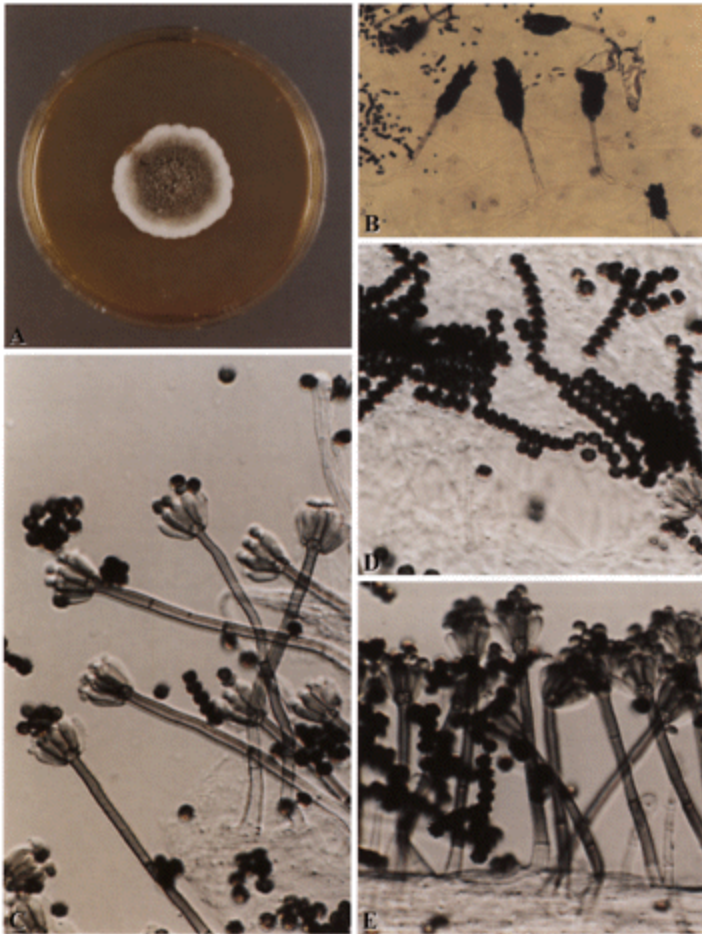
A. Colony on MEA after one week; B. conidiophore with conidia in columns, $\times 230$; C. conidiophores with conidia, $\times 920$; D. conidia, $\times 920$; E. conidiophores with conidia, $\times 920$.

Occurrence: A cellulolytic species common on exposed canvas and other cotton fabrics in subtropical and tropical regions.

A common contaminant of very wet gypsum board, particularly in warmer climates.

Medical significance: HI.

Toxicity: Produces griseofulvins, trichodermin and trichodermol (Jarvis *et al.* 1996).



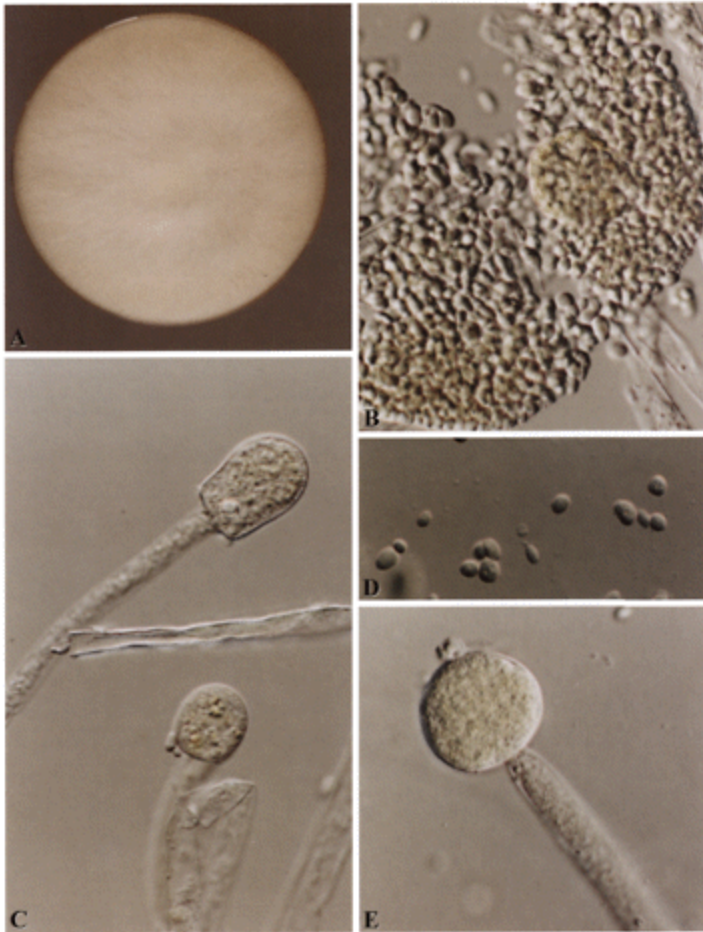
Mucor hiemalis Wehmer

A. Colony on MEA after one week; B. sporangiophore with columella and sporangiospores, $\times 920$; C. sporangiophore with columella, $\times 920$; D. sporangiospores, $\times 920$; E. sporangium containing sporangiospores, $\times 920$.

Occurrence: Cosmopolitan species which is one of the commonest of soil fungi, and associated with a wide range of plant produce, fresh and dried.

Isolated from floor, carpet and mattress dust; bakery dust; hay, cereals.

Medical significance: HI.



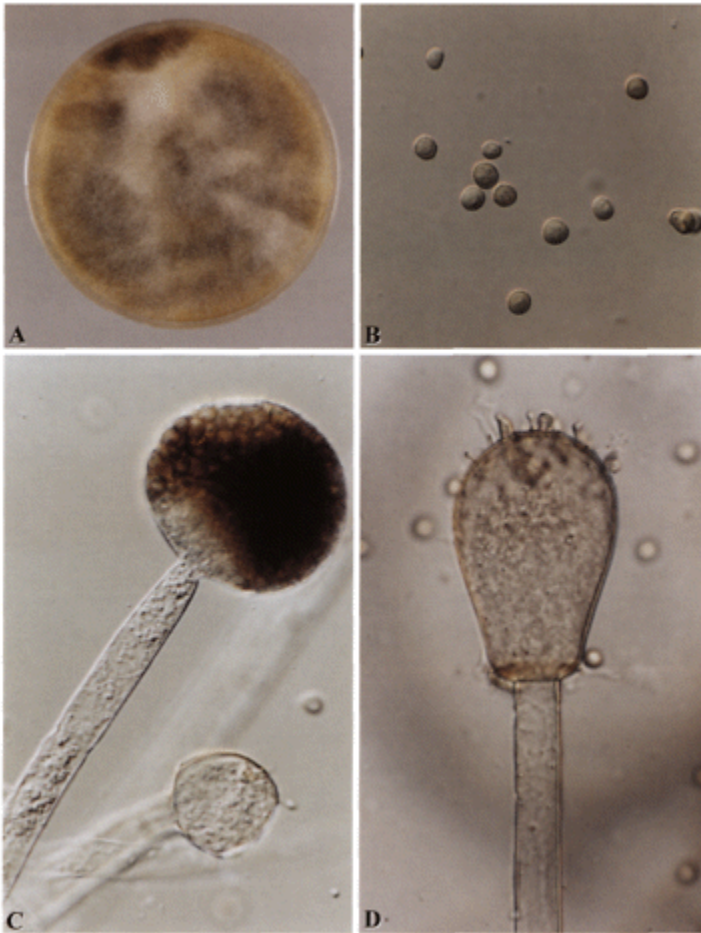
***Mucor plumbeus* Bon.**

A. Colony on MEA after one week; B. sporangiospores, $\times 920$; C. sporangium containing sporangiospores, $\times 920$; D. sporangiophore with columella, $\times 920$.

Occurrence: Soil-borne species with a worldwide distribution, associated with stored plant produce.

Isolated from wood, polyurethane coated fabric; HVAC filter; hospital air; bakeries, cork factories; hay and cereals.

Medical significance: HI.



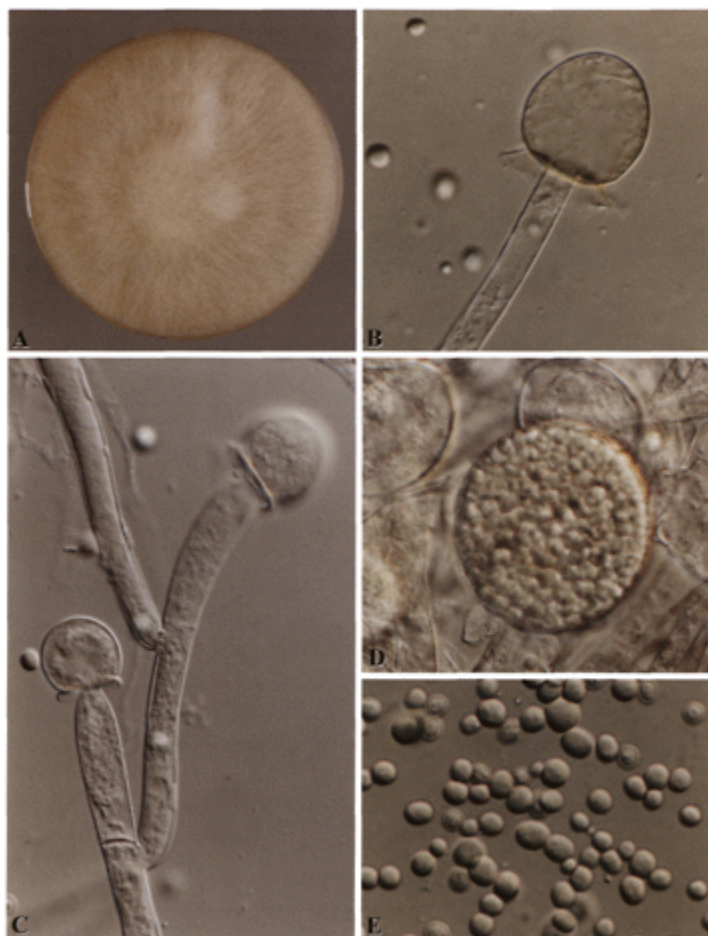
***Mucor racemosus* Fres.**

A. Colony on MEA after one week; B. sporangiophore with columella and part of sporangium wall, $\times 920$; C. sporangiophore with columellae, $\times 920$; D. sporangium, $\times 920$; E. sporangiospores, $\times 920$.

Occurrence: Cosmopolitan soil organism, associated with cereals and other stored foodstuffs.

Isolated from mattress dust; polyurethane coated fabric; bakeries; wood shavings.

Medical significance: HI.



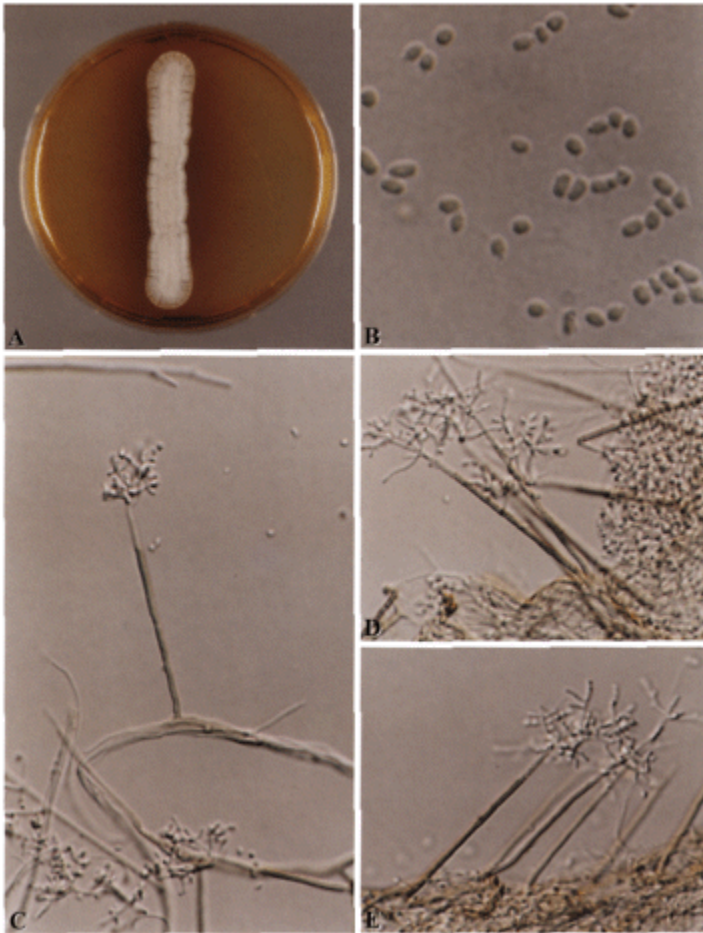
***Oidiodendron griseum* Robak**

A. Colony on MEA after one week; B. conidia, $\times 2330$; C-E. conidiophores, $\times 920$.

Occurrence: wood, air and sputum man

Isolated from wood pulp; soil.

Medical significance: HI.

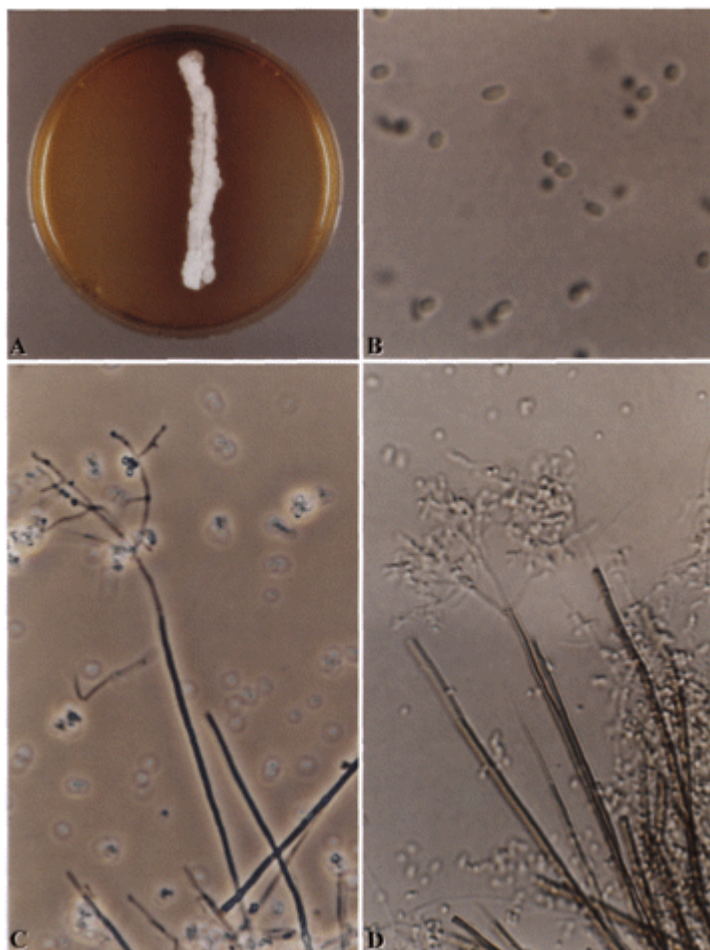


***Oidiodendron rhodogenum* Robak**

A. Colony on MEA after one week; B. conidia, $\times 2330$; C,D. conidiophores, $\times 920$.

Occurrence: Isolated from air in cork factory; straw, bottle cork; decaying furniture and sputum man

Medical significance: HI.



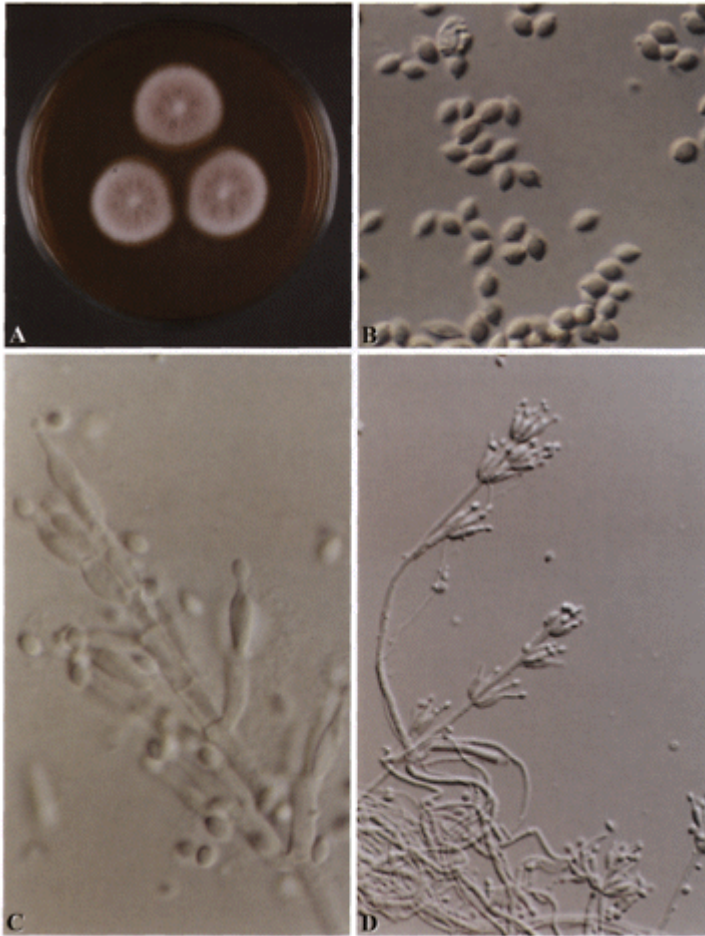
Paecilomyces lilacinus (Thorn) Samson

A. Colonies on MEA after one week; B. conidia, $\times 340$; C. conidiophore with conidia, $\times 2330$; D. conidiophores, $\times 920$.

Occurrence: Common in house dust.

Also isolated from humidifier water; swimming pool floor; cotton drill; synthetic rubber, plastic bottles; stored foods.

Medical significance: EFG.



***Paecilomyces variotii* Bain.**

A. Colonies on MEA after one week; B-D. conidiophores with conidia, ×920.

Occurrence: A species associated with composts and other self heated materials, common in air.

Occurs on damp walls, wet plaster work and in carpet dust. Also isolated from wood, wood chips, tarred wooden flooring, UFFI, synthetic rubber, leather, library paste; HVAC fan; air in bakery; salami, margarine; pectin, hay, grain. Sometimes occurring as a heat-resistant spoilage agent in food.

Medical significance: EFG. Can cause invasive mycoses in compromised individuals (See <http://www.nfid.org/publications/clinicalupdates/fungal/noso.html>).

Toxicity: Produces metabolites of moderate toxicity (Nielsen *et al.* 1999).



***Penicillium aurantiogriseum* Dierckx**

A. Colonies on MEA after one week; B. conidia, $\times 2330$; C,D. conidiophores, $\times 920$.

Occurrence: Cosmopolitan species very common on maturing and drying cereals, but relatively rarely isolated from soil.

Common on damp building/finishing materials such as gypsum board wallpaper. Found in floor, carpet and mattress dust. Also isolated from UFFI; gelatin, cheese, groundnuts, cereals, flour; factory air.

Medical significance: HI.

Toxicity: Produces a number of metabolites of moderate toxicity (Frisvad and Filtenborg 1989).

Note: This species belongs to the *Penicillium aurantiogriseum* complex with similar taxa e.g. *P. polonicum*, *P. commune*, *P. viridicatum* and *P. cyclopium*. It is not related to *P. verrucosum*, which does not occur indoors but mostly on wheat.



***Penicillium brevicompactum* Dierckx**

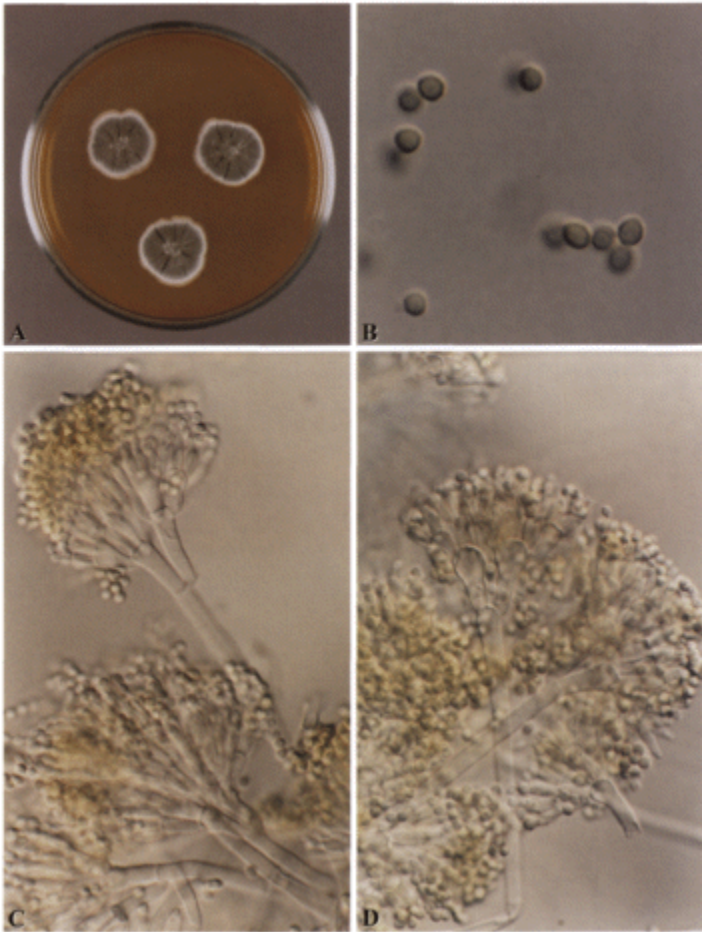
A. Colonies on MEA after one week; B. conidia, $\times 2330$; C,D. conidiophores, $\times 920$.

Occurrence: Widespread in nature, in soil and decaying vegetation.

Common on damp walls and building materials such as gypsum board. Present in floor, carpet, mattress and upholstered furniture dust. Also isolated from UFFI, red lead paint; cotton yarn; hay, cereals; mushroom compost.

Medical significance: HI.

Toxicity: Produces a number of metabolites of moderate toxicity (Nielsen *et al.* 1999).



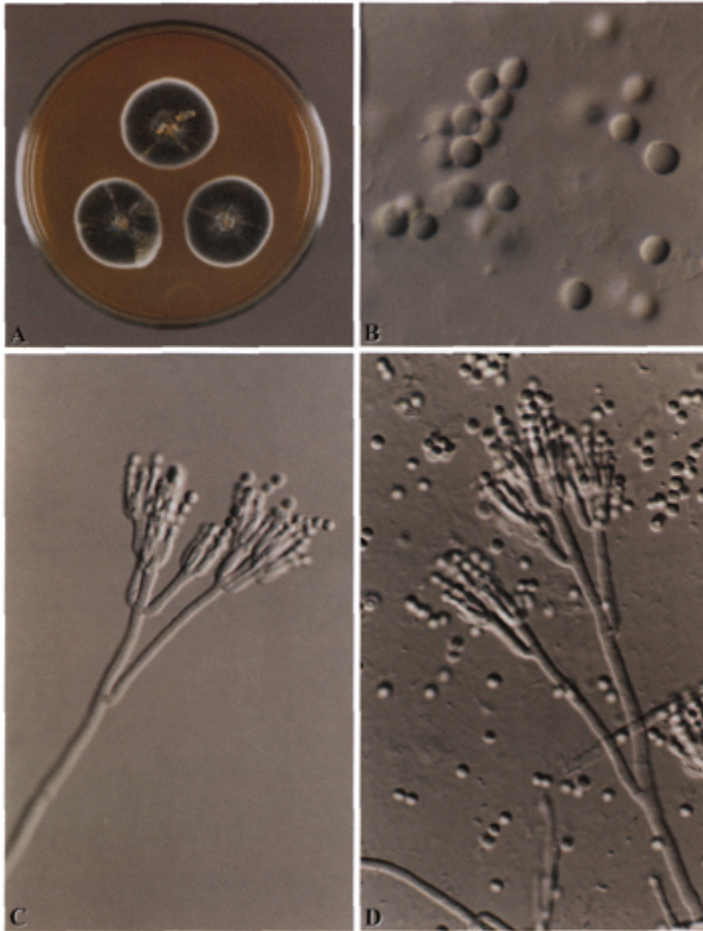
***Penicillium chrysogenum* Thom**

A. Colonies on MEA after one week; B. conidia, $\times 2330$; C,D. conidiophores, $\times 920$.

Occurrence: A cosmopolitan species found in a wide range of habitats from soils to foodstuffs. Extremely common on damp building materials, walls and wallpaper and in floor, carpet, mattress and upholstered furniture dust. Also isolated from PVA emulsion paint, oil paint, UFFI; polyester polyurethane foam, polyurethane coated fabric, paper, talcum powder; school ceiling, fruit store air.

Medical significance: EIG

Toxicity: Produces a number of metabolites of moderate toxicity (Nielsen *et al.* 1999).



***Penicillium citrinum* Thom**

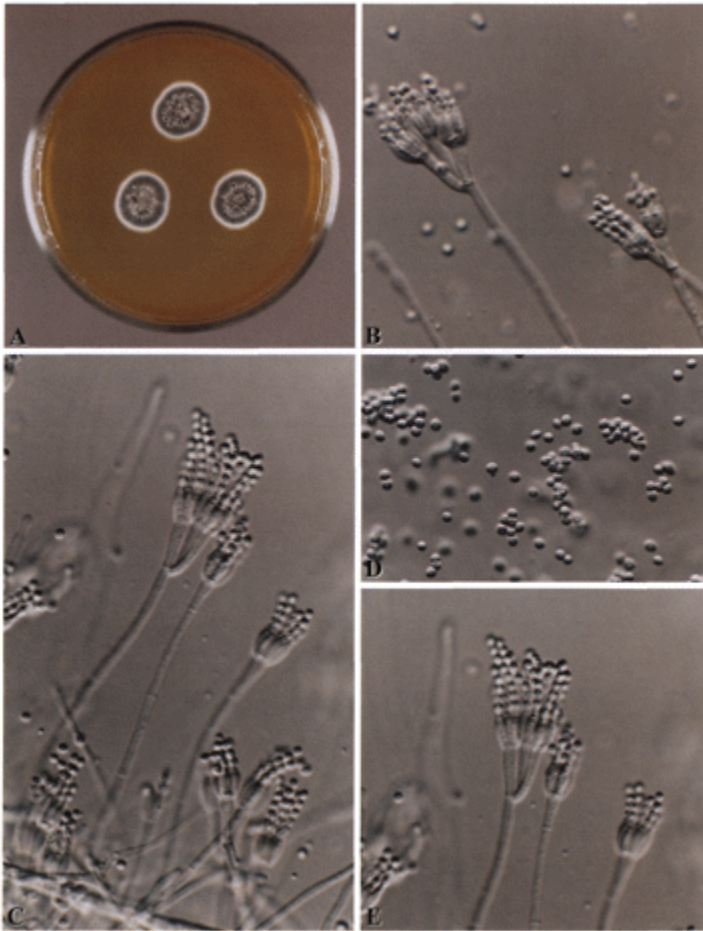
A. Colonies on MEA after one week; B ,C. conidiophores, $\times 920$; D. conidia, $\times 920$; E. conidiophores, $\times 920$.

Occurrence: Ubiquitous; in soil, decaying vegetation and the air, and an active deteriogen.

Common on damp building materials such as gypsum board, especially in warmer climates, and in settled dust. Also isolated from cotton fabric, textiles; flour.

Medical significance: EIG.

Toxicity: Produces a number of metabolites of moderate toxicity (Frisvad 1987).



Penicillium commune Thom

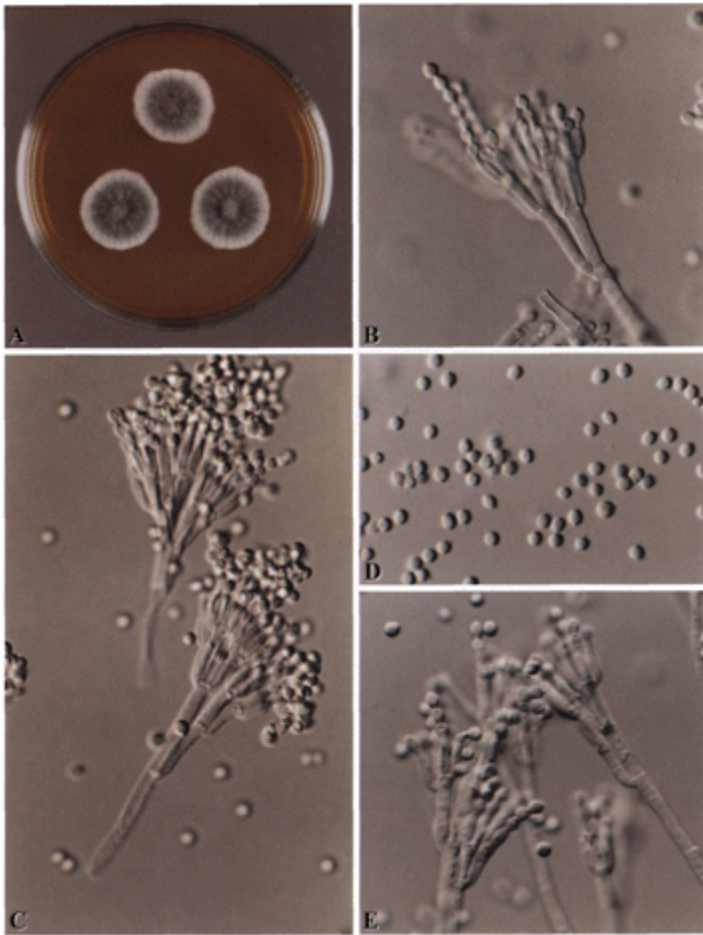
A. Colonies on MEA after one week; B, C. conidiophores, $\times 920$; D. conidia, $\times 920$; E. conidiophores, $\times 920$.

Occurrence: Common on foodstuffs.

Found on damp building materials such as gypsum board, and in house dust. Also isolated from artificial leather cloth.

Medical significance: HI.

Toxicity: Produces a number of metabolites of moderate toxicity (Frisvad and Filtenborg 1989).



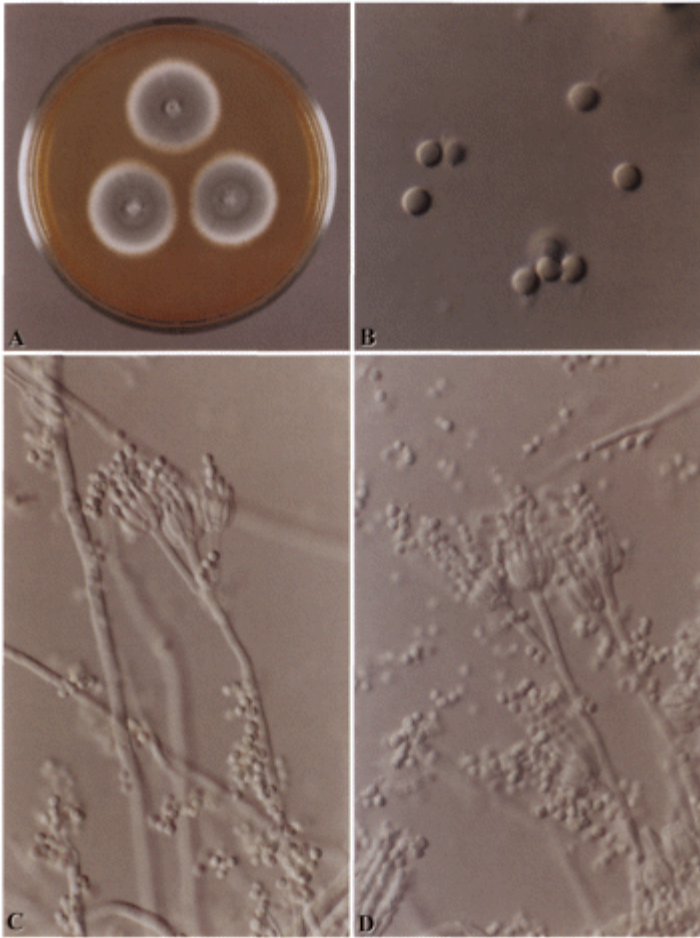
Penicillium corylophilum Dierckx

A. Colonies on MEA after one week; B. conidia, $\times 2330$; C, D. conidiophores, $\times 920$.

Occurrence: In soil and decaying vegetation, and common on cereals and a wide range of other foods.

Found in floor and mattress dust. Also isolated from silk screen, painted wood.

Medical significance: HI.

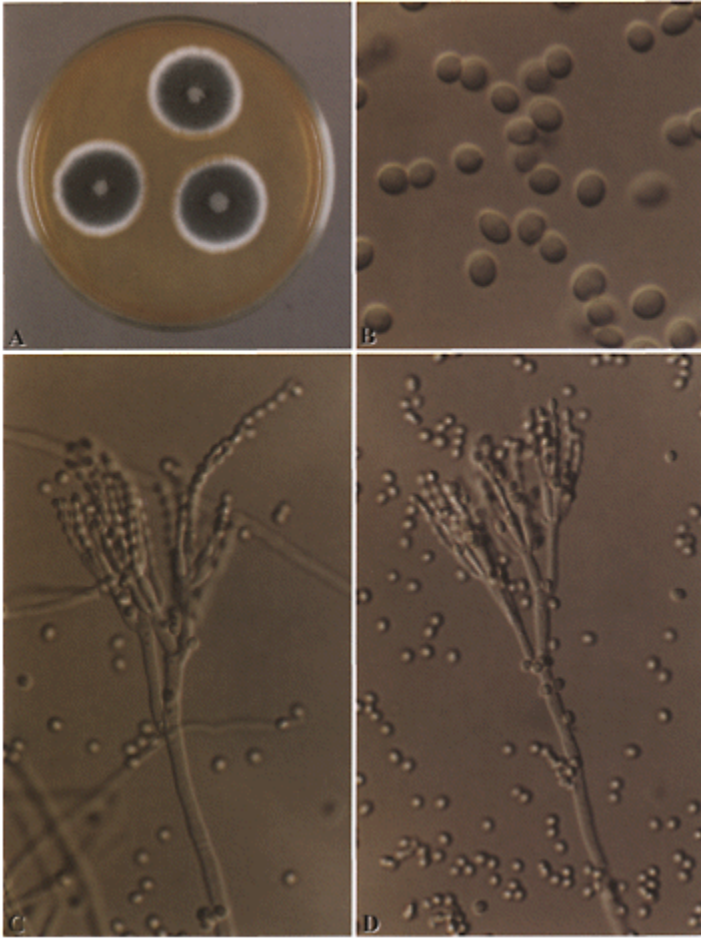


***Penicillium expansum* Link**

A. Colonies on MEA after one week; B. conidia, $\times 2330$; C,D. conidiophores, $\times 920$.

Occurrence: Particularly associated with pomeaceous fruits, such as apple
House dust; polyurethane foam, red lead paint; HVAC filter; cereals, flour, cake, beer.

Medical significance: HI.



***Penicillium glabrum* (Wehmer) Westling**

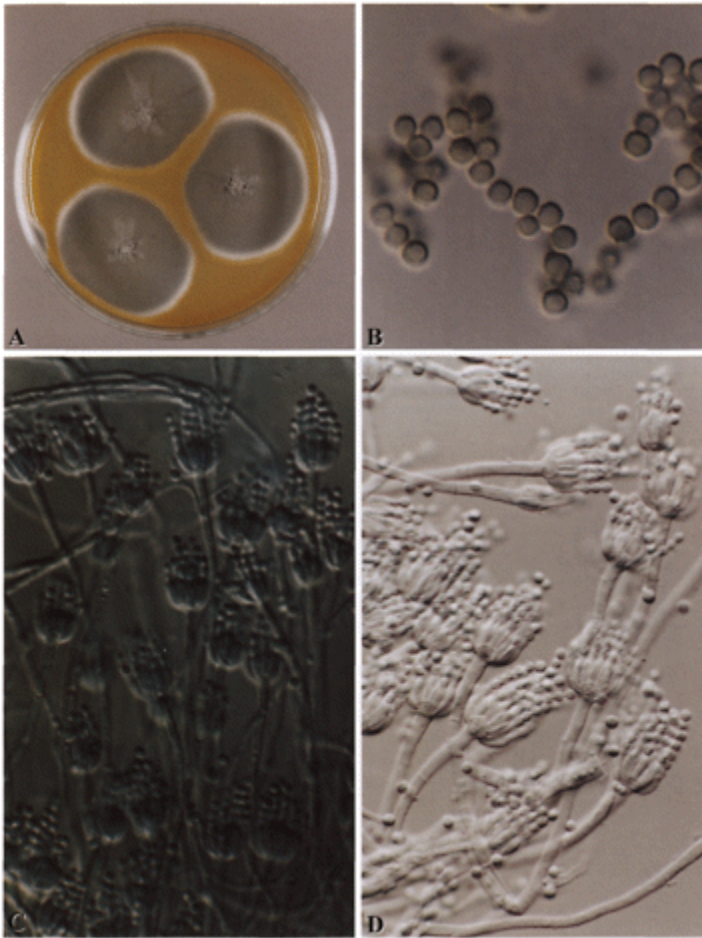
=*Penicillium frequentans* Westling

A. Colonies on MEA after one week; B. conidia, $\times 2330$; C, D. conidiophores, $\times 920$.

Occurrence: An important deteriogen widely distributed in soils

Very common species in floor dust, and also in mattress dust. Present on damp walls, and also isolated from starch paste adhesive; UFFI; wood pulp, cork factories.

Medical significance: HI. Associated with occupational respiratory disease in the cork and wood industries (Lacey and Dutkiewicz 1994).



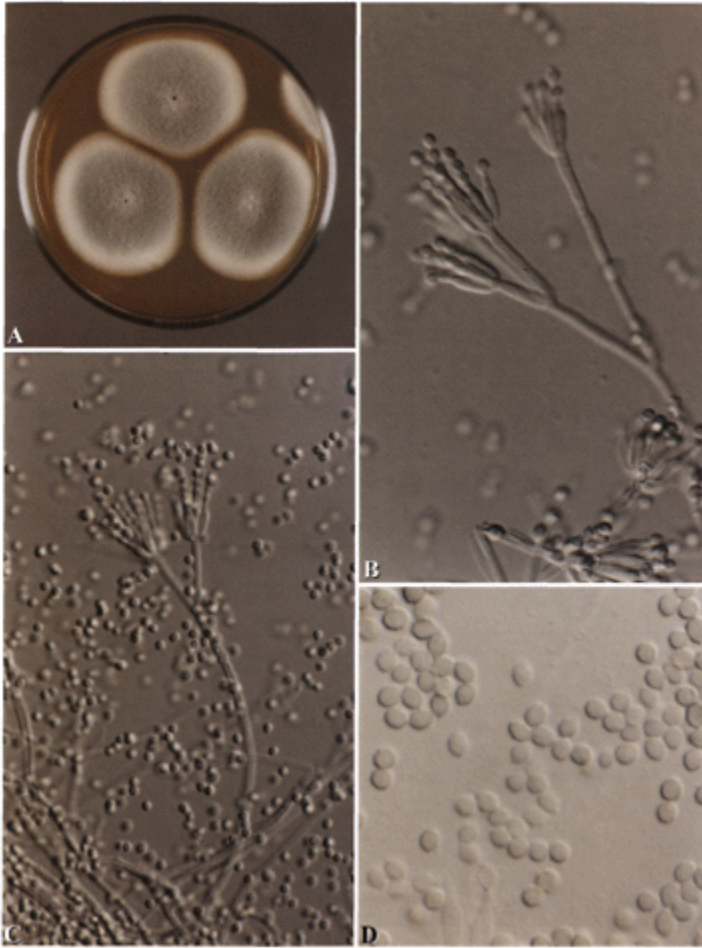
Penicillium janthinellum Biourge

A. Colonies on MEA after one week; B, C. conidiophores, $\times 920$, D. conidia, $\times 2330$.

Occurrence: A rather variable, ubiquitous soil species.

Found on damp building and finishing materials, e.g. PVC/paper wallcovering, UFFI, and in house dust. Also isolated from cereals.

Medical significance: HI. A rare cause of invasive mycoses (Bates *et al.* 1997).



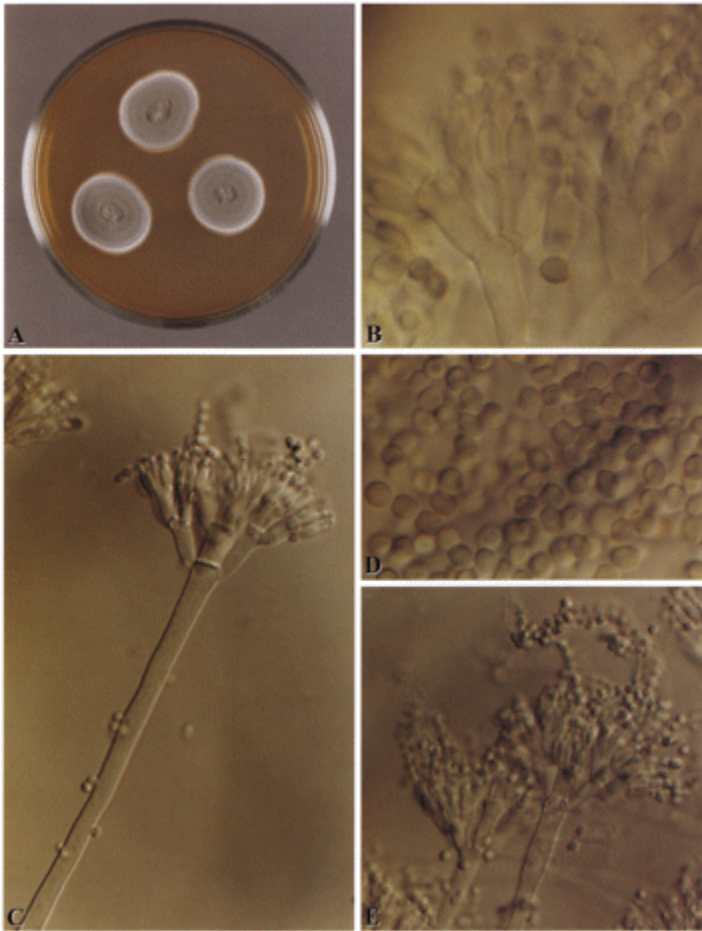
Penicillium olsonii Bainier & Sartory

A. Colonies on MEA after one week; B, C. conidiophores, B. $\times 2330$, C. $\times 920$; D. conidia, $\times 2330$; E. conidiophores, $\times 920$.

Occurrence: A relatively uncommon species, in soil and water.

Found on damp building materials and in carpet dust. Also isolated from straw, bread, jam.

Medical significance: HI.



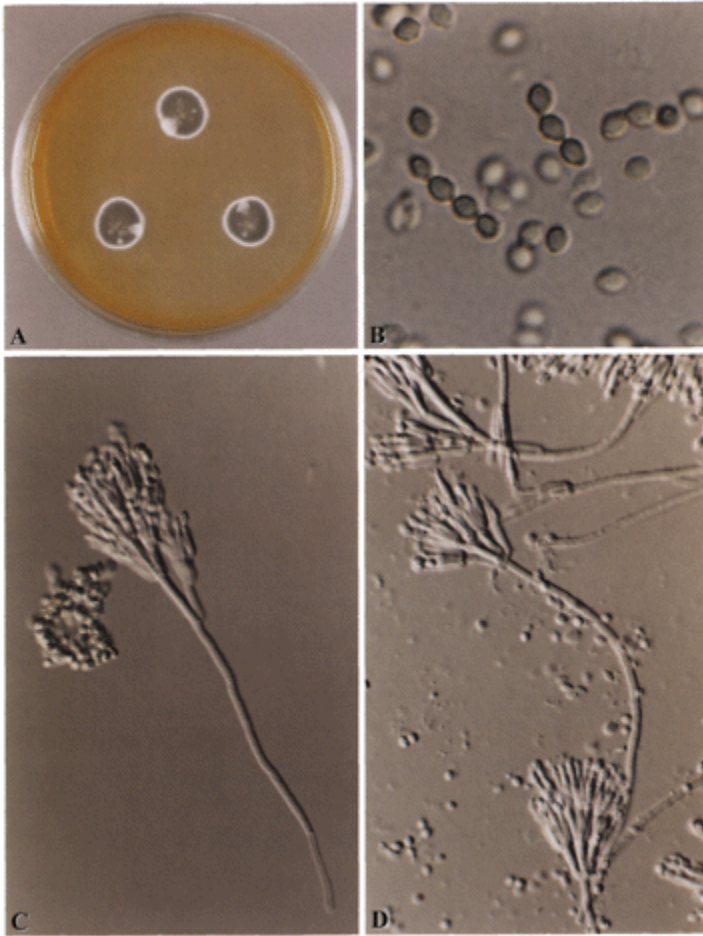
Penicillium rugulosum Thom

A. Colonies on MEA after one week; B. conidia, $\times 2330$; C, D. conidiophores, $\times 920$.

Occurrence: A slow growing soil-born species thought to be commoner than records of isolations would indicate.

Occurs on damp building materials and walls, and in carpet and mattress dust. Also isolated from outdoor acrylic paint.

Medical significance: HI.



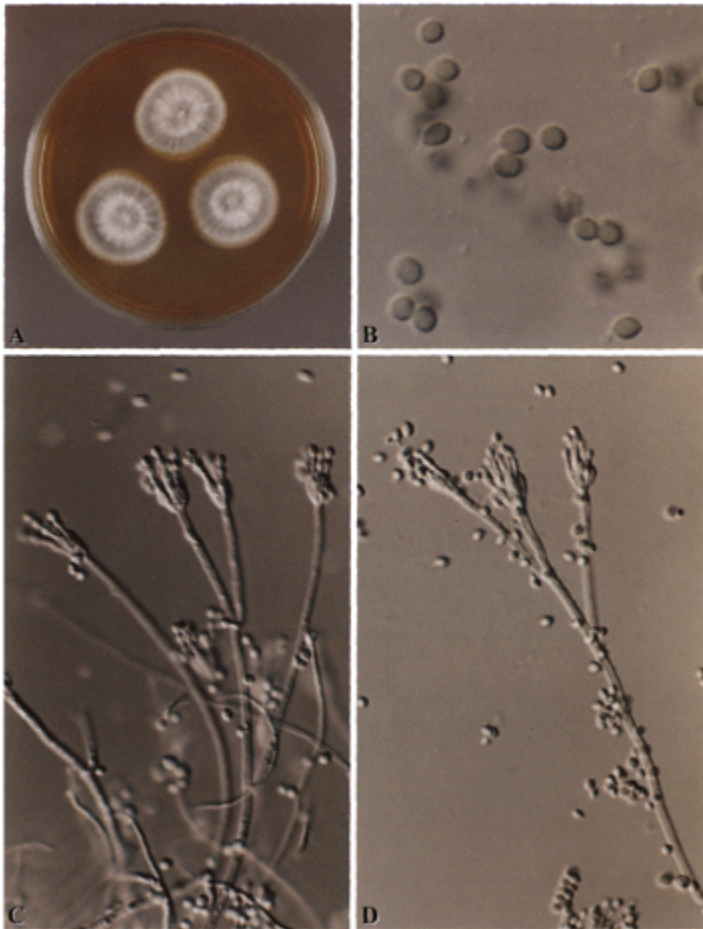
Penicillium simplicissimum (Oudemans) Thom

A. Colonies on MEA after one week; B. conidia, $\times 2330$; C, D. conidiophores, $\times 920$.

Occurrence: One of the more commonly isolated penicillia in soil and decaying materials, an active degradative agent.

Found on damp building materials. Also isolated from polyether polyurethane foam, polyester polyurethane foam.

Medical significance: HI.

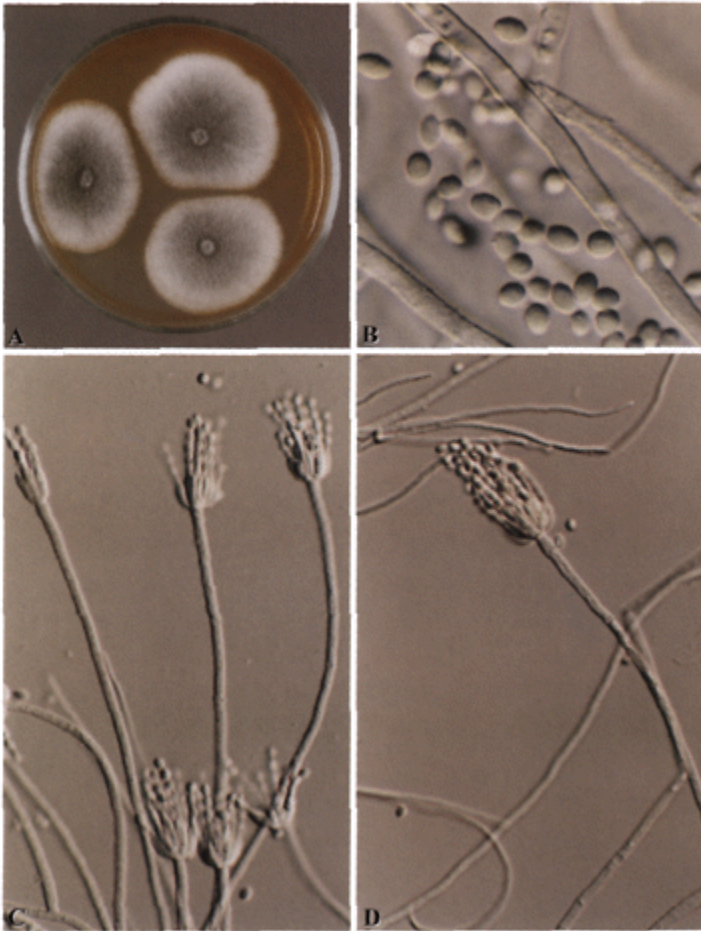


***Penicillium spinulosum* Thom**

A. Colonies on MEA after one week; B. conidia, $\times 2330$; C, D. conidiophores, $\times 920$. Occurrence: A ubiquitous species common in soil, decaying vegetation, foods and also textiles, appears to be tolerant of tannins and heavy metals.

Found on damp walls, vinyl wallcovering and other building/finishing materials, including chromate-treated emulsion paint. Very common in house dust. Also isolated from UFFI; distilled water; tanning solutions, chrome tanned leather.

Medical significance: HI (Several incorrect medical reports exist). Associated with occupational respiratory disease in the cork and wood industries (Lacey and Dutkiewicz 1994).



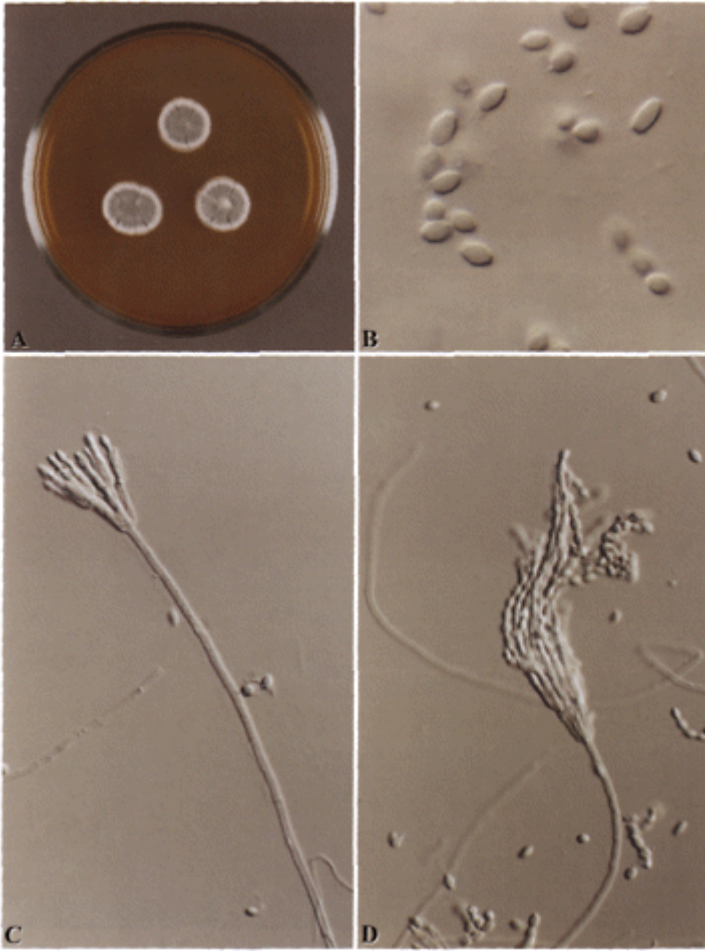
Penicillium variable Sopp

A. Colonies on MEA after one week; B. conidia, $\times 2330$; C, D. conidiophores, $\times 920$.

Occurrence: A ubiquitous species frequently isolated from soils.

Found on damp building materials and in house dust. Also isolated from coconut matting; paper pulp.

Medical significance: HI.



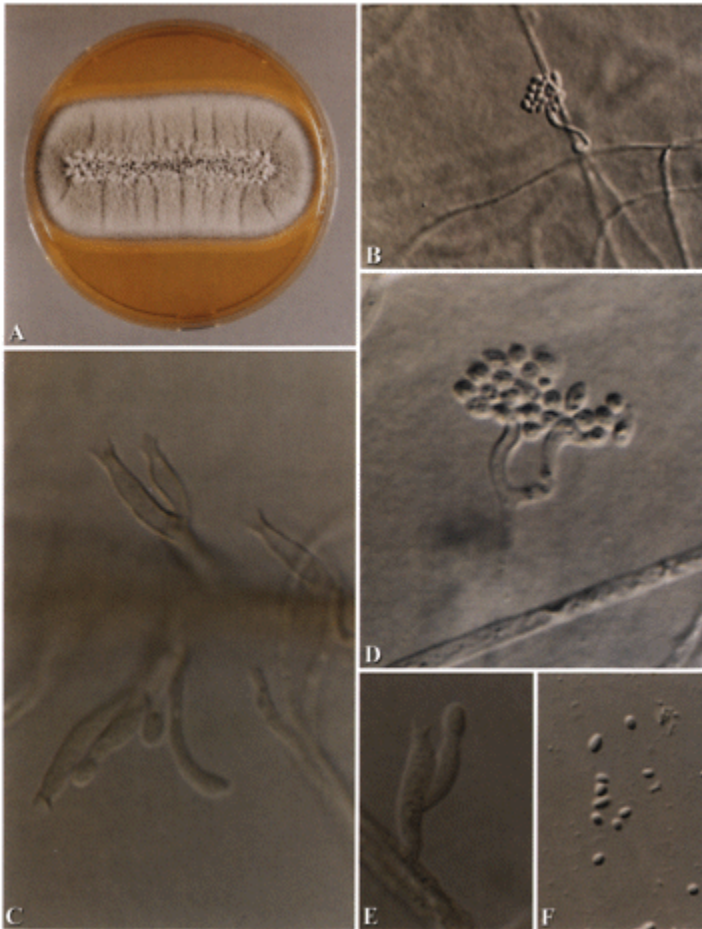
Phialophora fastigiata (Lagerb. & Melin) Conant

A. Colonies on MEA after one week; B. conidiophores, $\times 920$; C. phialides with collarettes, $\times 2330$; D. conidiophores, $\times 2330$; E. phialides with collarettes, $\times 2330$; F. conidia, $\times 2330$.

Occurrence: Found in soil, decaying wood and some cereal and grass seed in various regions, including Europe, N. America and the Antipodes.

Associated with very wet wood (Ellis 1971), including blue stain wood. Also isolated from humidifier water; paper pulp.

Medical significance: HI.



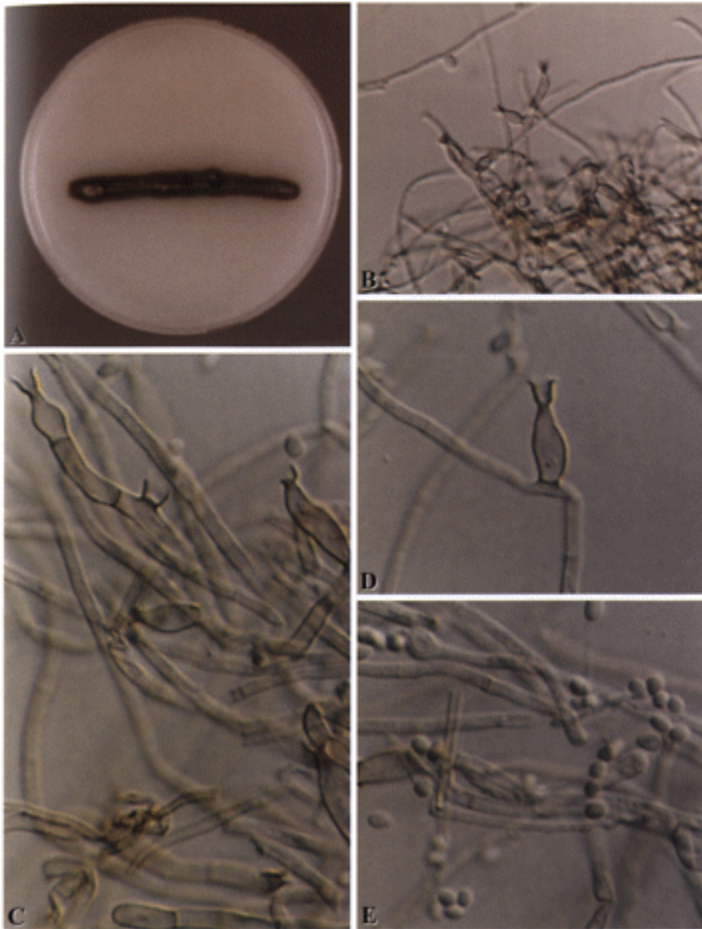
Phialophora verrucosa Medlar

A. Colony on OA after one week; B. conidiophores, $\times 920$; C, D. phialides with collarettes, $\times 2330$; E. conidia, $\times 2330$.

Occurrence: in cooling water of airconditioning systems, bath-rooms, sometimes in air.

Associated with very wet wood (Ellis 1971), e.g. decaying lumber and pulpwood chips.

Medical significance: BEG.

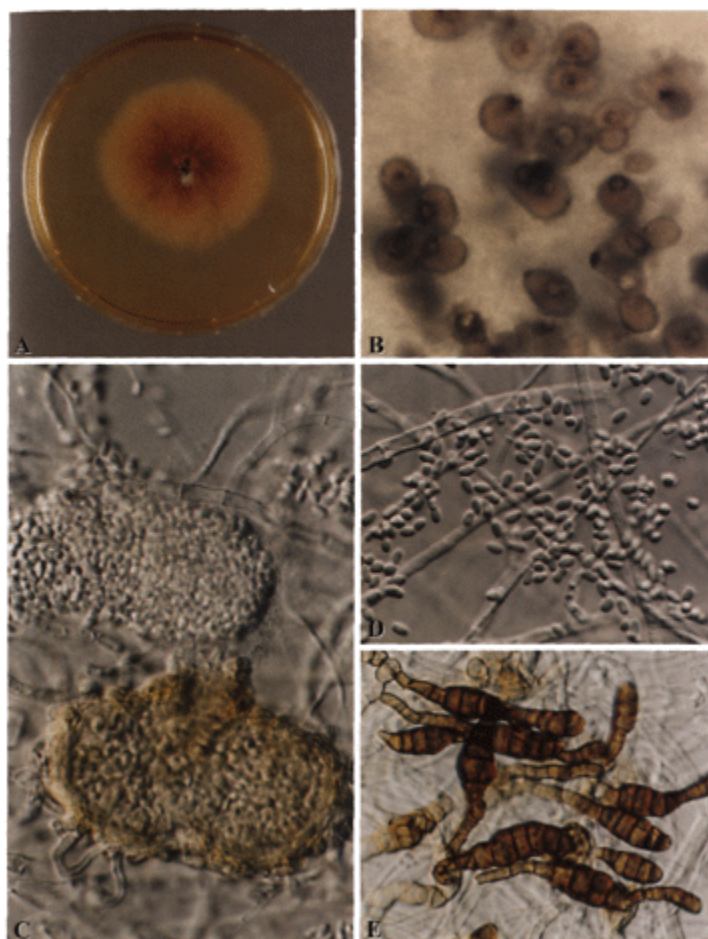


***Phoma glomerata* (Corda) Wollenweber & Hochapfel**

A. Colony on MEA after one week; B. pycnidia with ostioles, in Petri-dish, $\times 230$; C. pycnidia with ostioles, $\times 920$; D. conidia, $\times 920$; E. dictyochlamydospores, $\times 920$.

Occurrence: Cosmopolitan in soil and a broad range of plants and plant materials. Wood, cement, oil paint; wool.

Medical significance: HI.



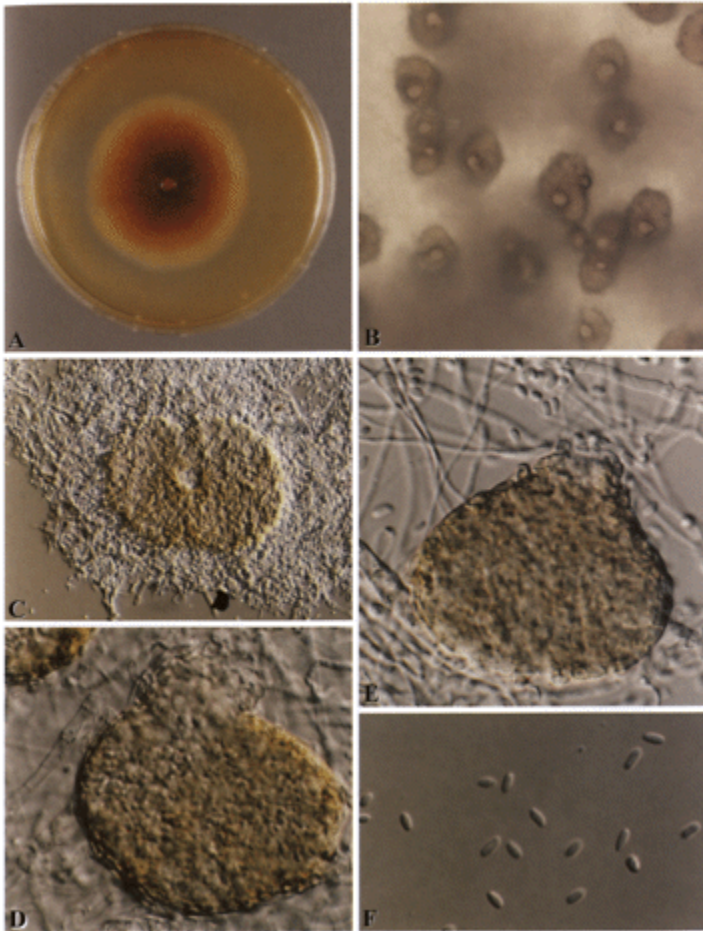
Phoma macrostoma Montagne

A. Colony on MEA after one week; B. pycnidia with ostioles, in Petri-dish, $\times 230$; C -E. pycnidia, C. $\times 230$, D. $\times 920$, E. $\times 920$; F. conidia, $\times 920$.

Occurrence: wet environments, bathrooms, kitchen

Know as a plantpathogen

Medical significance: HI.



Pithomyces chartarum (Berkeley & Curtis) M.B.Ellis

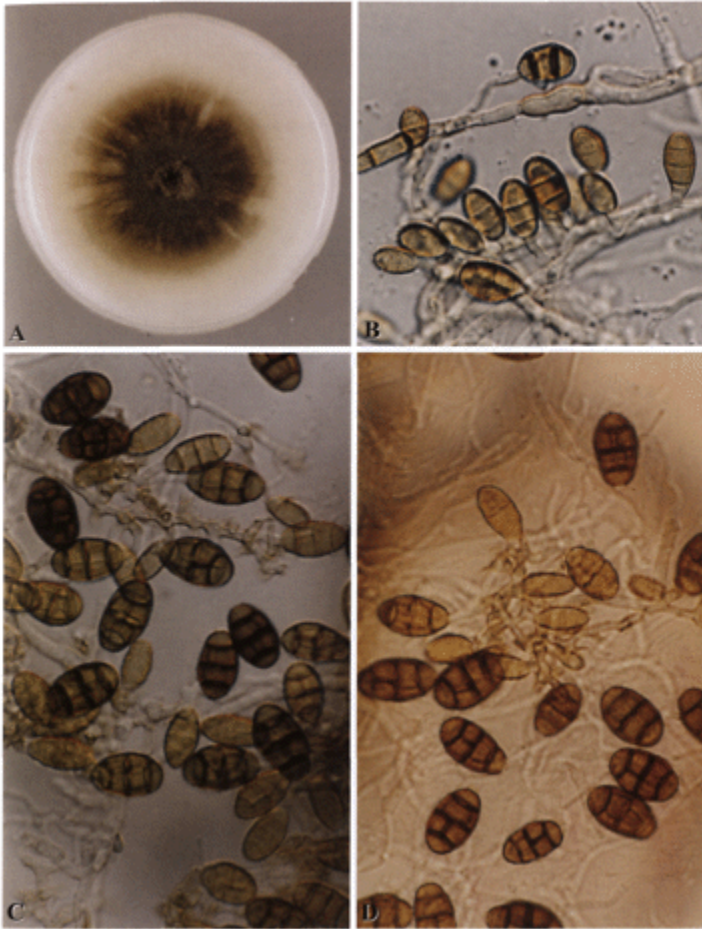
A. Colony on OA after one week; B-D. conidiophores with conidia, $\times 920$.

Occurrence: A soil-borne toxigenic species associated with forage grasses, particularly in New Zealand.

Found occasionally on paper in indoor environments (Ellis 1971), and also reported on ceiling tiles. May be present in carpet and mattress dust. Also isolated from UFFI; factory air.

Medical significance: HI.

Toxicity: Produces potent toxins (Leigh and Taylor 1976).

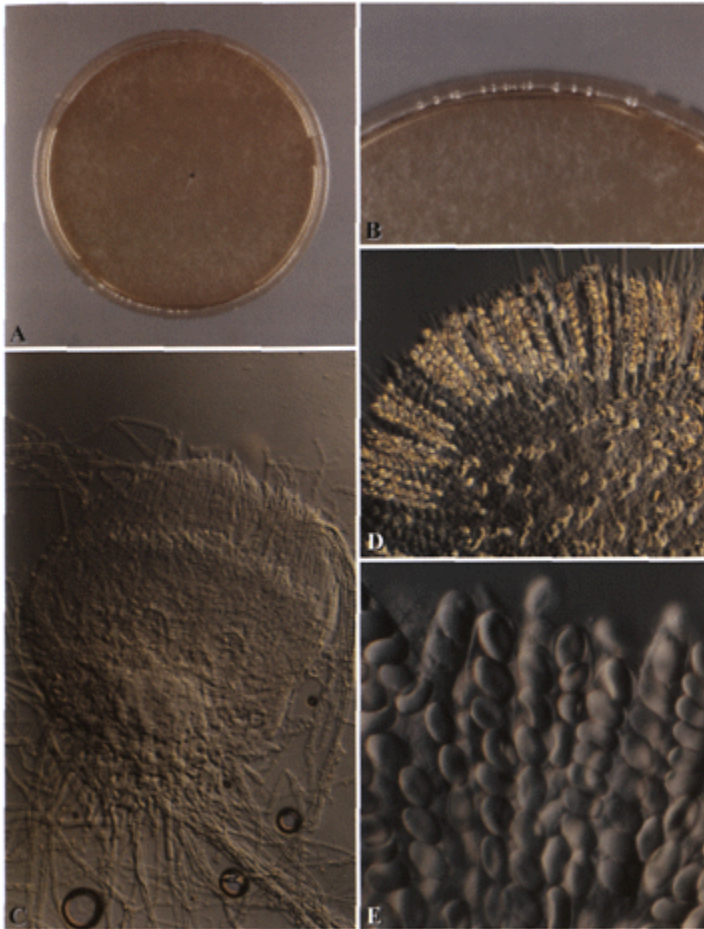


Pyronema domesticum (Sowerby: Fries) Saccardo

A. Colony on MEA after two weeks; B. detail of colony showing apothecia; C. apothecium, $\times 92$; D. detail of apothecium, $\times 230$; E. asci with ascospores, $\times 920$.

Occurrence: Wet plasterwork.

Medical significance: HI.



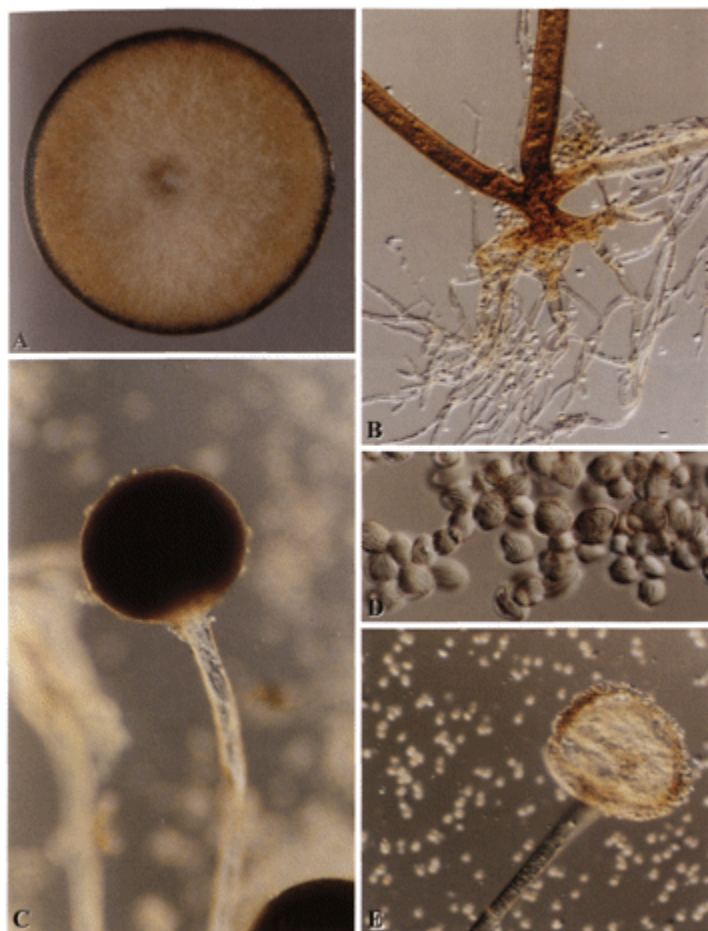
Rhizopus stolonifer (Ehrenb.) Lind.

A. Colony on MEA after one week; B. rhizoid, $\times 230$; C. sporangiophore with sporangium, $\times 230$; D. sporangiospores, $\times 920$; E. sporangiophore with columella, $\times 230$.

Occurrence: A cosmopolitan airborne and soil-borne contaminant of food, exposed surfaces and materials.

Found in carpet and mattress dust. Also isolated from polyurethane coated fabric; water filter; HVAC filter; bakeries.

Medical significance: HI.

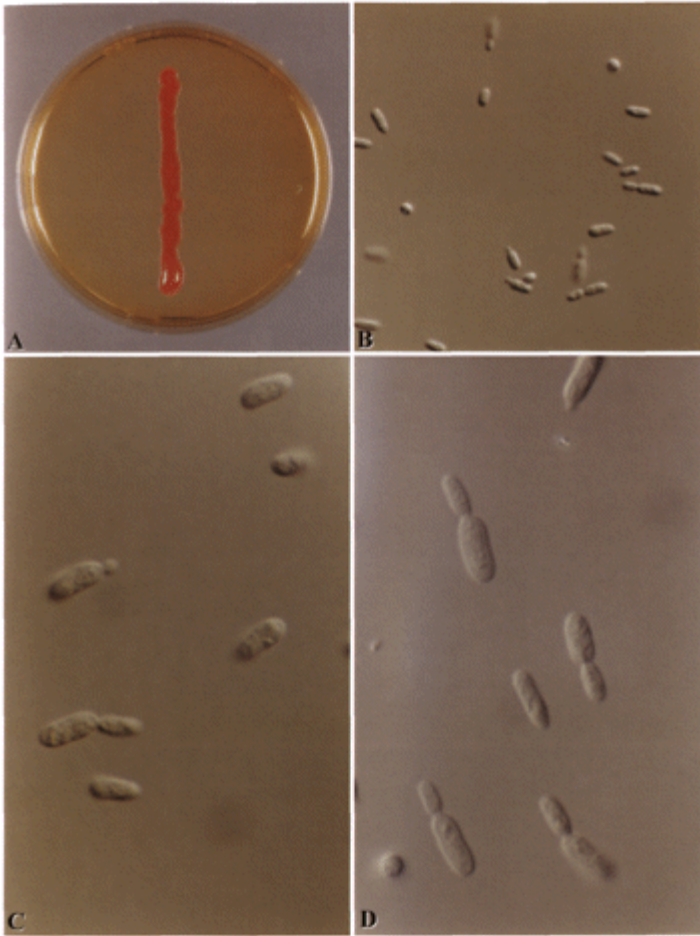


Rhodotorula mucilaginosa (Jørgensen) Harrison

A. Colony on MEA after 5 days; B. cells, $\times 920$; C, D. cells, $\times 2330$.

Occurrence: food, air

Medical significance: EIG.

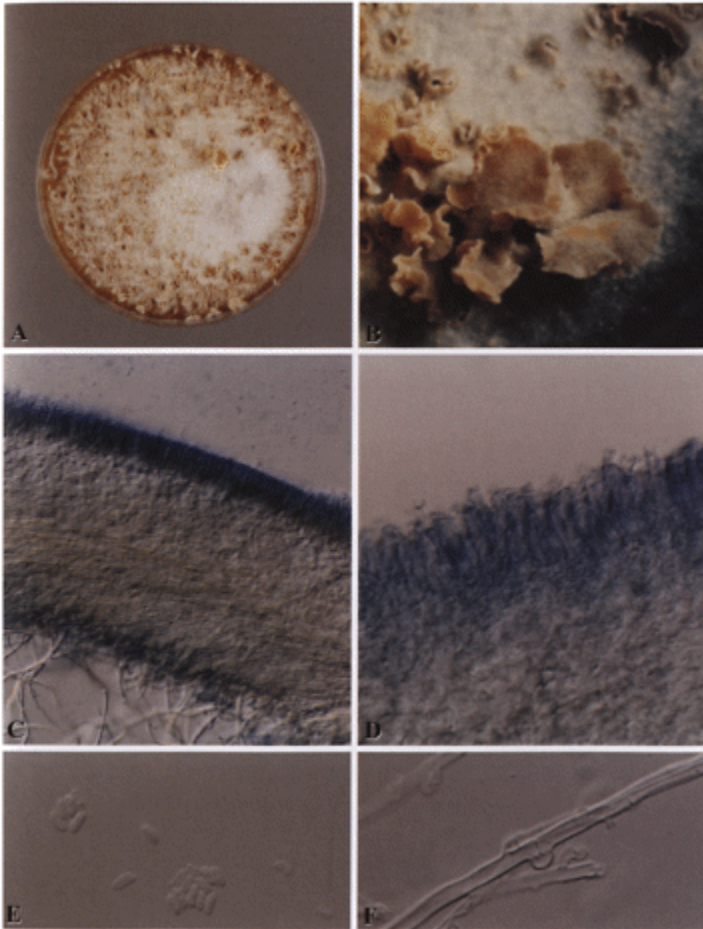


Schizophyllum commune Fries

A. Colony on MEA after 3 weeks; B. detail of colony showing fruit body; C. section through fruit-body showing divided inrolled gills, $\times 230$; D. basidiospores, $\times 920$; E. detail of fruit-body, $\times 920$; F. hyphae with clamp, $\times 2330$.

Occurrence: Isolated from (mahogany) wood.

Medical significance: EIG.



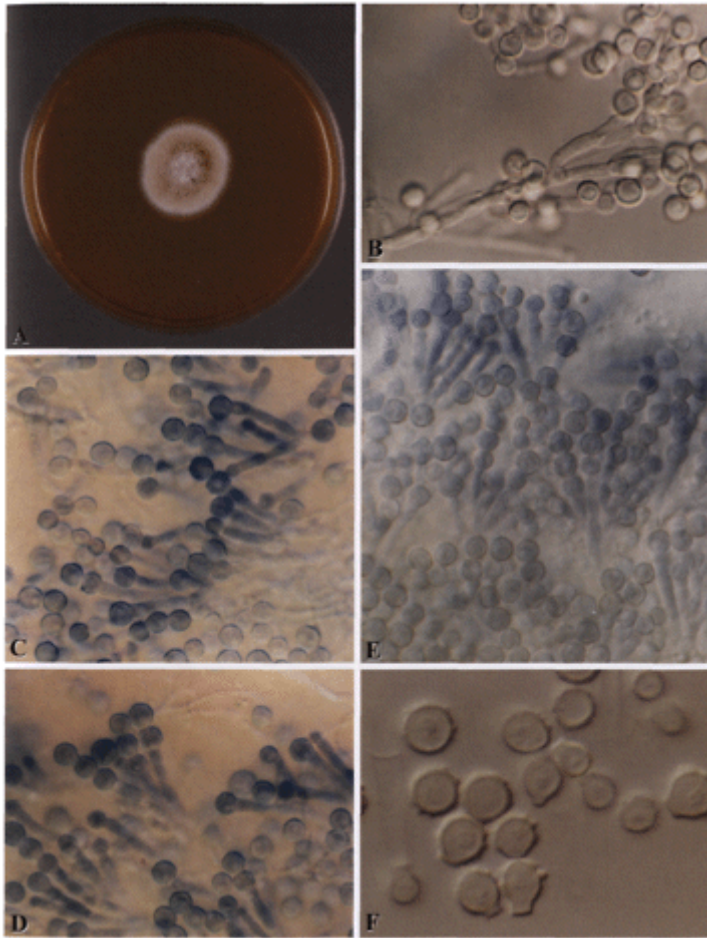
***Scopulariopsis brevicaulis* (Sacc.) Bain.**

A. Colony on MEA after two weeks; B-E. conidiophores with conidia, $\times 920$; F. conidia, with a truncate base, $\times 2330$.

Occurrence: A cosmopolitan species found in soil, and decaying wood, and various other plant and animal products.

Found on damp walls, cellulose board and wallpaper, as well as wood, and common in floor and mattress dust. Also isolated from metal working fluid; straw, cereals, apples, groundnuts, meat, cheese, butter.

Medical significance: EIG. Skin lesions.



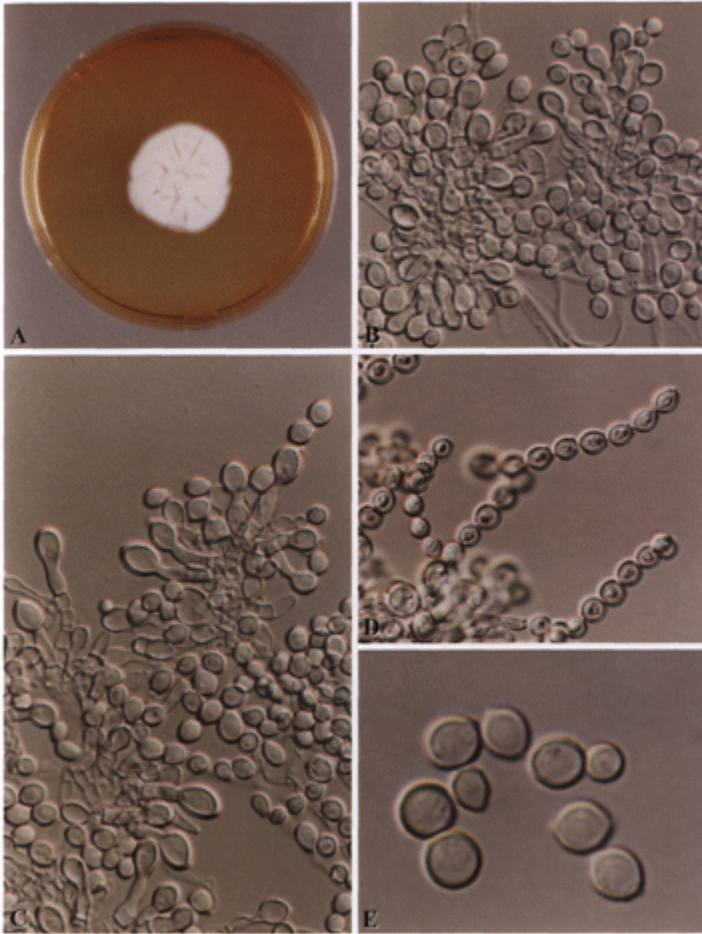
Scopulariopsis Candida (Guéguen) Vuill.

A. Colony on MEA after two week; B-D. conidiophores with conidia, $\times 920$; E. conidia, $\times 2330$.

Occurrence: A soil-borne fungus also found on plant matter.

Carpet and mattress dust; hospital floor, swimming pool; wooden food packing, shoes; wood pulp; hay, cereals, flour, cheese.

Medical significance: EIG. Skin and nails

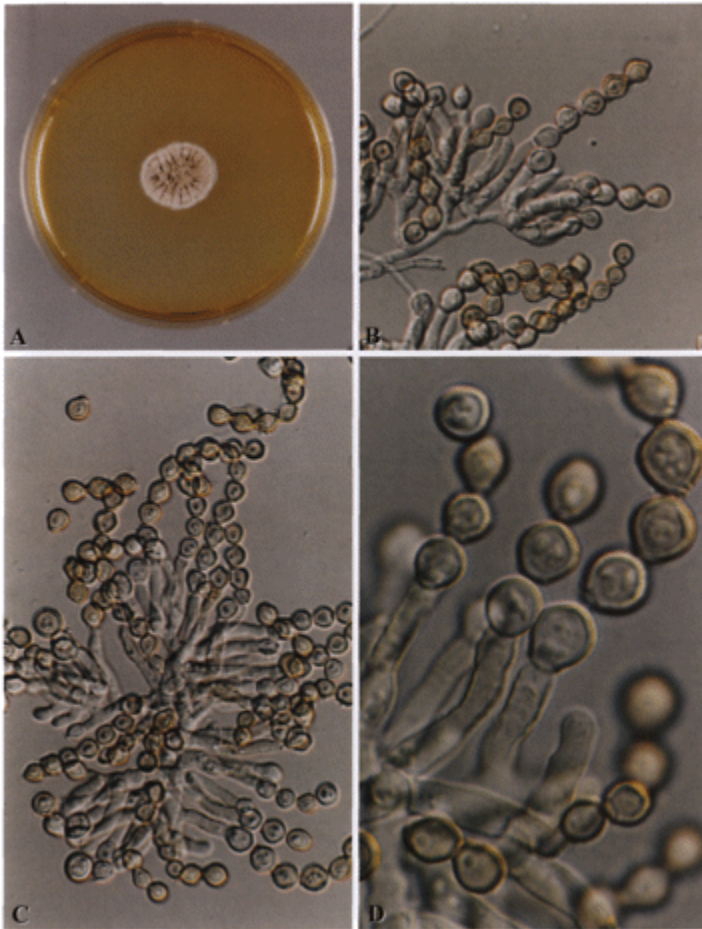


A. Colony on MEA after two week; B, C. conidiophores with conidia, $\times 920$; D. annellate conidiogenous cells with conidia, $\times 2330$,

Occurrence: A soil-borne species also associated with decaying plant matter.

Found in mattress dust. Also isolated from straw packing, wooden food packing; cheese, eggs.

Medical significance: [E?], [G?]. Onychomycosis, dermatomycosis.



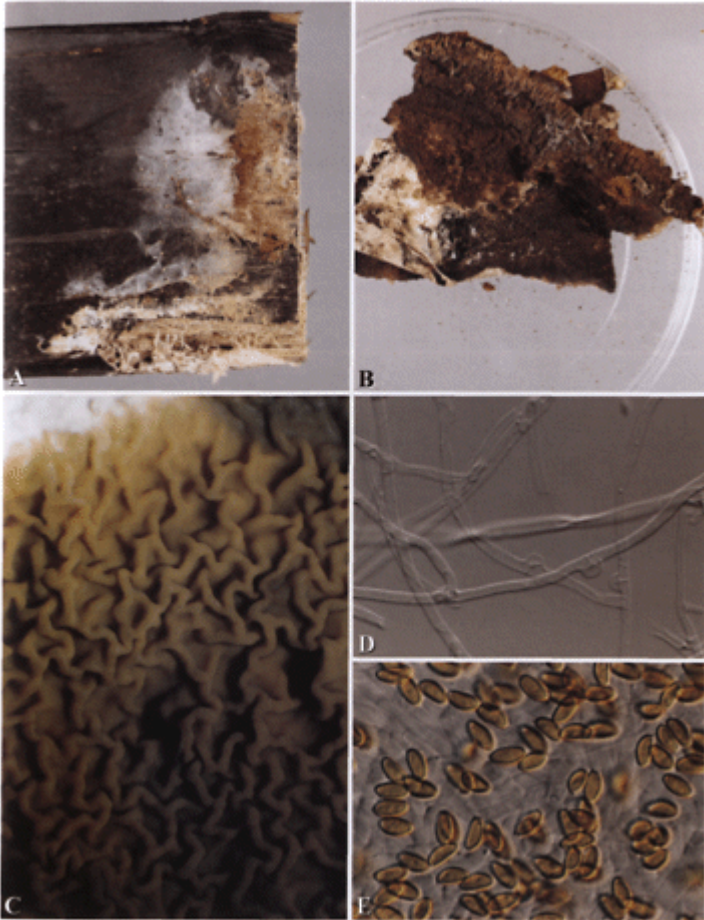
Serpula lacrymans (Wulfen: Fries) Schroeter

Dry rot fungus.

A. Piece of wood with *S. lacrymans*; B. fruiting body; C. detail of fruiting body; D. hyphae with clamp connections, $\times 2330$; E. basidiospores, $\times 2330$.

Occurrence: Most often on softwood in contact with or embedded in wet brickwork where ventilation is poor and natural drying is impeded.

Medical significance: HI. Allergenic—asthma, hypersensitivity pneumonitis.



Sistotrema brinkmanii (Bresadola) Eriksson

A. Colony on MEA after 3 weeks; B. piece of wood with *S. brinkmanii*; C. detail of colony; D. hyphae with clamp, $\times 2330$; E. detail of piece of wood with *S. brinkmanii*.

Occurrence: Commonly isolated from wet, decaying window/door joinery.

Medical significance: HI.



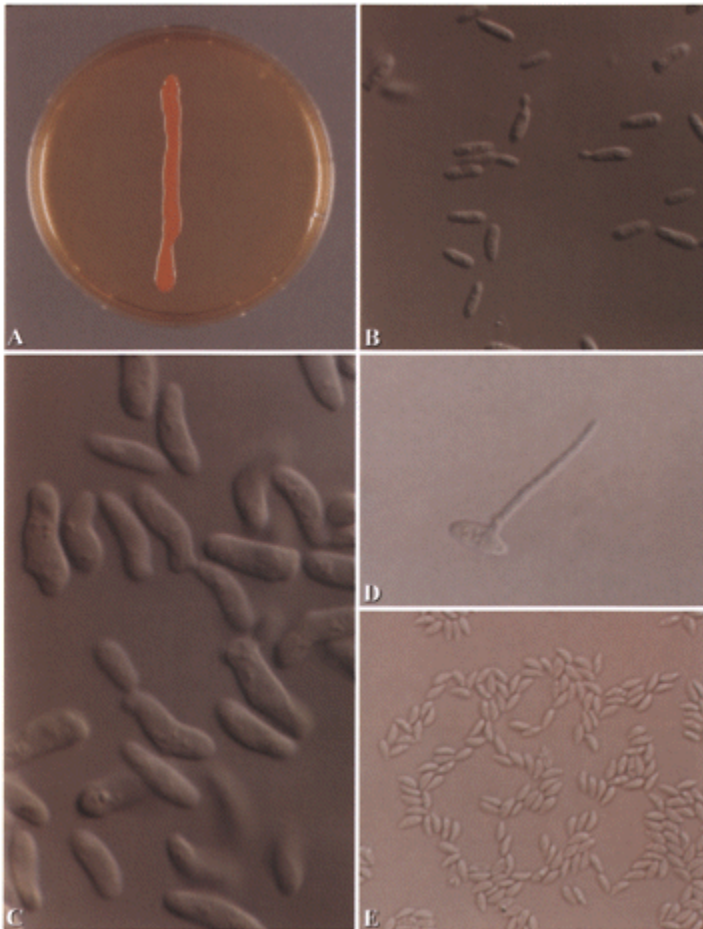
Sporobolomyces roseus Kluyver & Niel

A. Colony on MEA after one week; B. cells, $\times 920$; C. cells, $\times 2330$; D. sterigmata, $\times 2330$; E. ballistoconidia, $\times 2330$.

Occurrence: air (indoor and outdoor)

Isolated from damp walls; *S. salmonicolor* from air-conditioning humidifier water.

Medical significance: EIG.



***Stachybotrys chartarum* (Ehrenb.) Hughes**

=*Stachybotrys atra* Corda

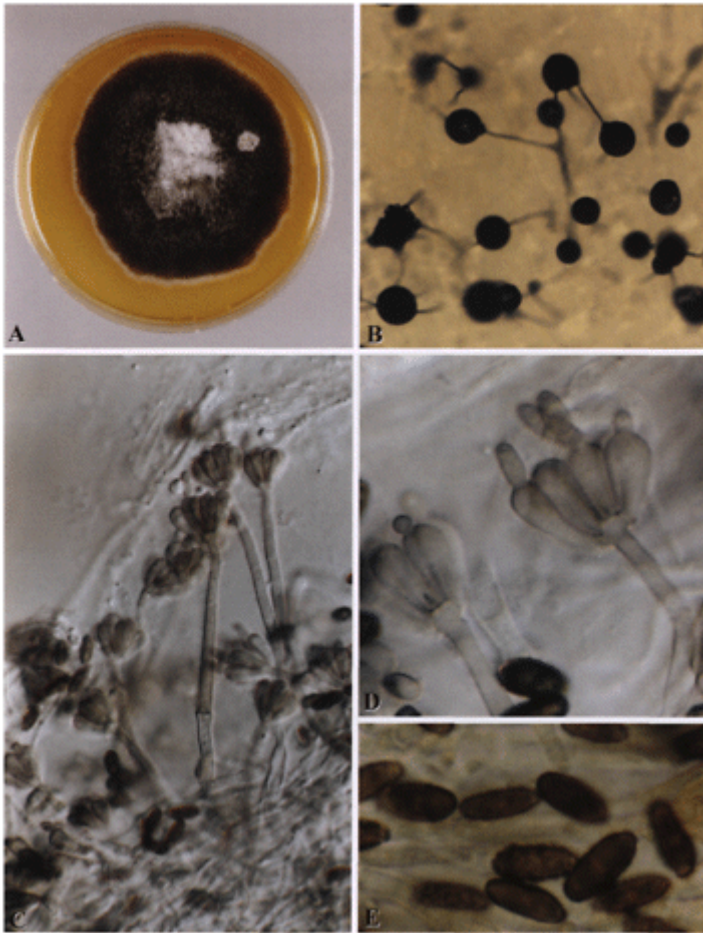
A. Colony on MEA after one week; B. conidiophores with conidia in slimy heads, in Petri-dish, $\times 90$; C-D. conidiophores, $\times 920$; E. conidia, $\times 2330$.

Occurrence: A cosmopolitan cellulolytic species isolated from soil and mouldering plant matter, e.g. damp straw.

Commonly on very wet gypsum board/walls and wallpaper, and on cotton fabrics/textiles such as canvas. Also isolated from asbestos building substitute, UFFI; HVAC humidifier water and fan; hospital/factory air.

Medical significance: HI.

Note: In indoor environments also other species of *Stachybotrys* occur, which are recognized by a different conidium size and ornamentation.



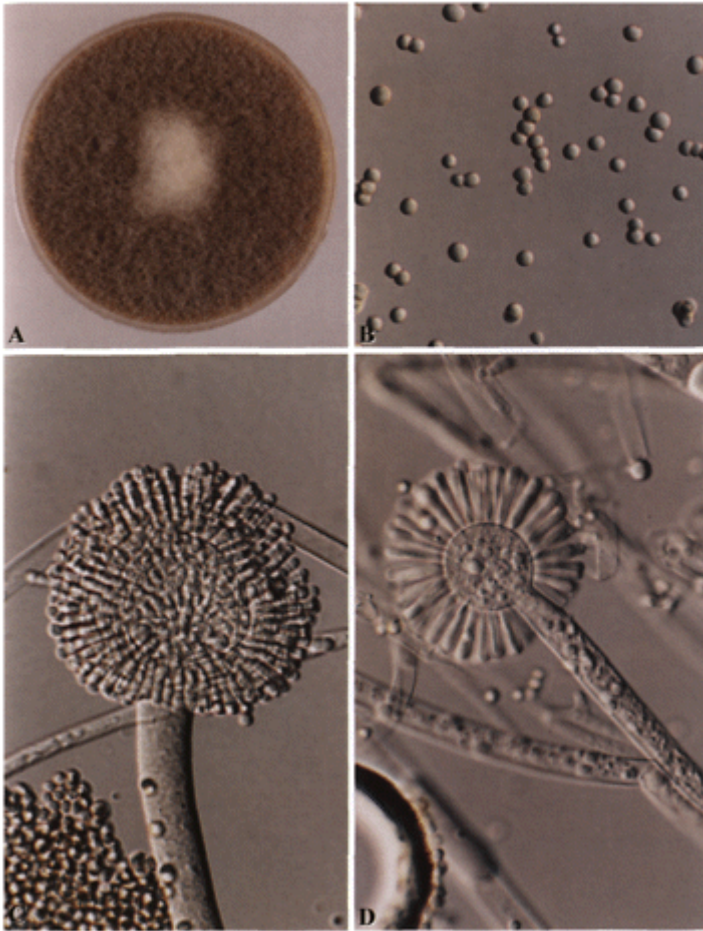
***Syncephalastrum racemosum* Cohn**

A. Colony on MEA after one week; B. merospores, $\times 920$; C, D. merosporangia with merospores, $\times 920$.

Occurrence: A cosmopolitan species found in soil, dung, cereals and other foodproducts.

Found in mattress dust. Also isolated from cloth, cured tobacco, toilet soap, bottle cork; air-conditioning filter.

Medical significance: EFG.



Trichoderma harzianum Rifai

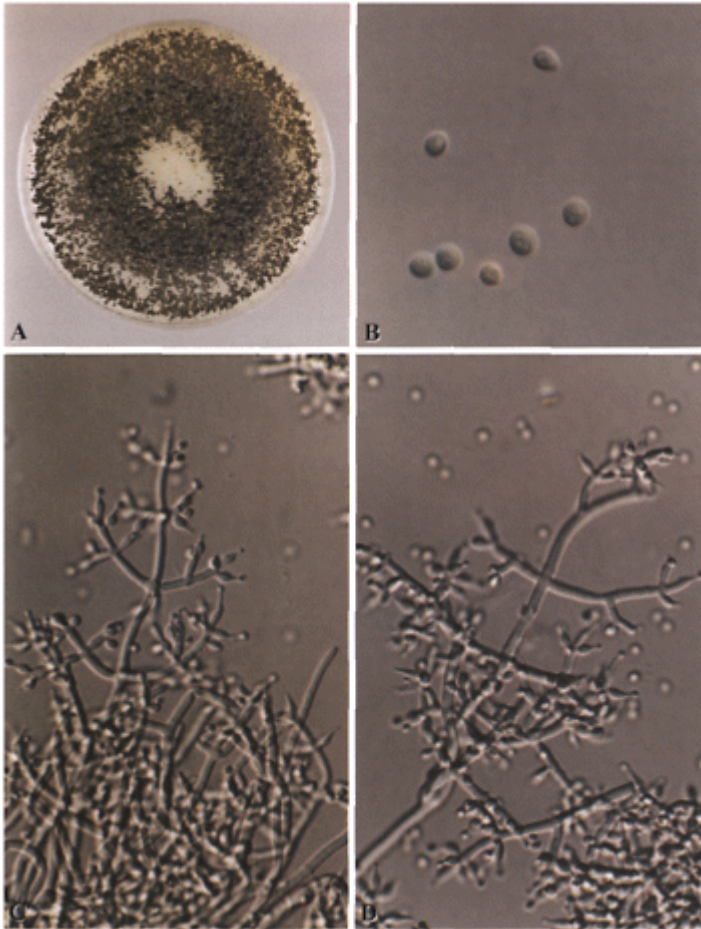
A. Colony on MEA after one week; B. conidia, $\times 2330$; C, D. conidiophores, $\times 920$.

Occurrence: A cellulolytic soil species with a worldwide distribution, also found in cereals and textiles.

Commonly occurs on gypsum board and water-saturated wood (Lubeck *et al.* 2000). Found also in carpet and mattress dust, and isolated from UFFI, polycaprolactone polyurethane, polyester polyurethane foam, polyurethane footwear; air-conditioning filter; bakery, mushroom farms.

Medical significance: HI.

Toxicity: This species produces potent toxins (Taylor 1986).



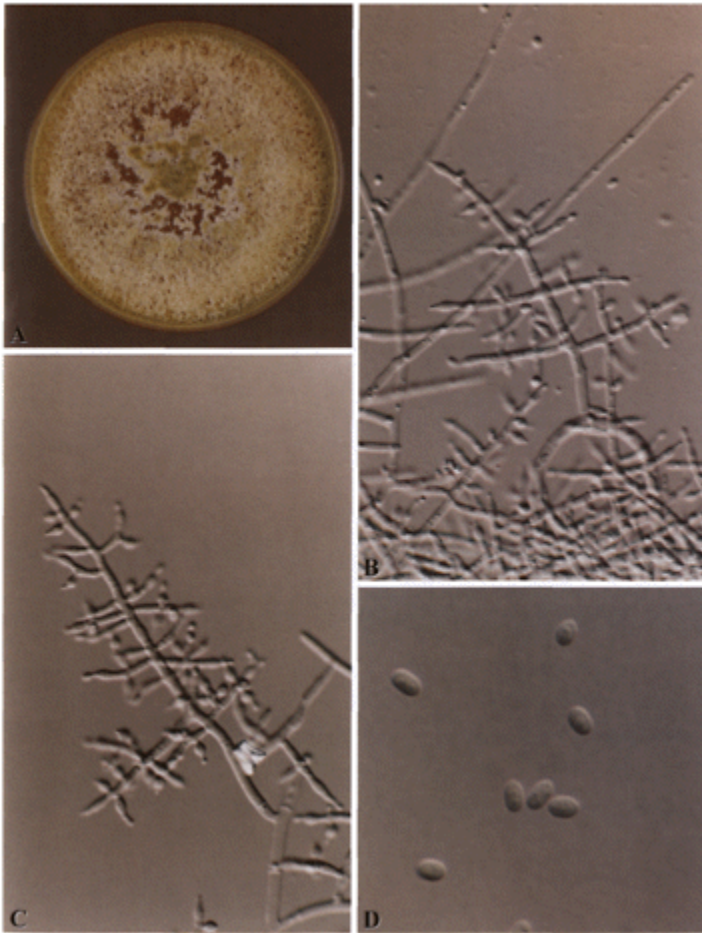
Trichoderma koningii Oudemans

A. Colony on MEA after one week; B, C. conidiophores, $\times 920$; D. conidia, $\times 2330$.

Occurrence: Found in carpet dust.

Also in mushroom spawn/compost, household waste.

Medical significance: HI.



***Trichoderma viride* Pers.**

A. Colony on MEA after one week; B, C. conidiophores, $\times 920$; D. rough-walled conidia, $\times 2330$.

Occurrence: Cosmopolitan soil fungus, this cellulolytic species is also found in decaying wood, stored cereals, sweet potatoes and other plant foodstuffs

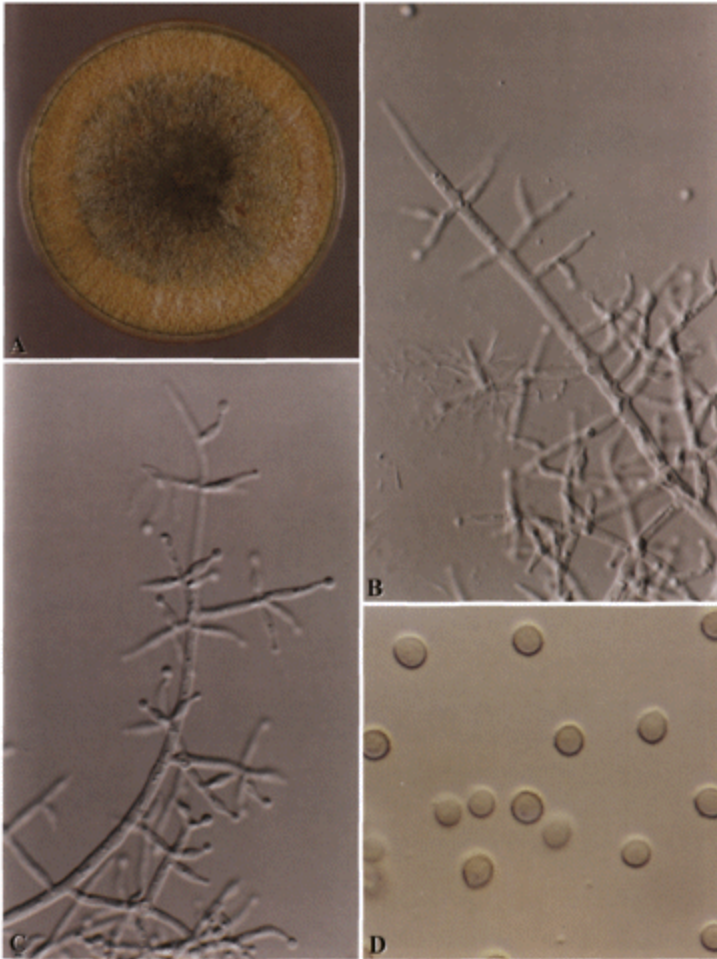
Commonly occurs on gypsum board and water-saturated wood (Lubeck *et al.* 2000). Found in floor, carpet and mattress dust. Also isolated from paint, UFFI; domestic water supply, air from HVAC system; wine cellar, grain store dust, mushroom compost, factory air; sawmills; preservative-treated timber.

Medical significance: HI.

Toxicity: Produces potent toxins (Taylor 1986).

Note The difficult-to-distinguish species *T. citreoviride* and *T. longibrachiatum* are probably also common, but more associated with insulation and roofing material than

gypsum wallboard (Lubeck *et al.* 2000). These latter species are associated with invasive mycoses in immune compromised individuals.

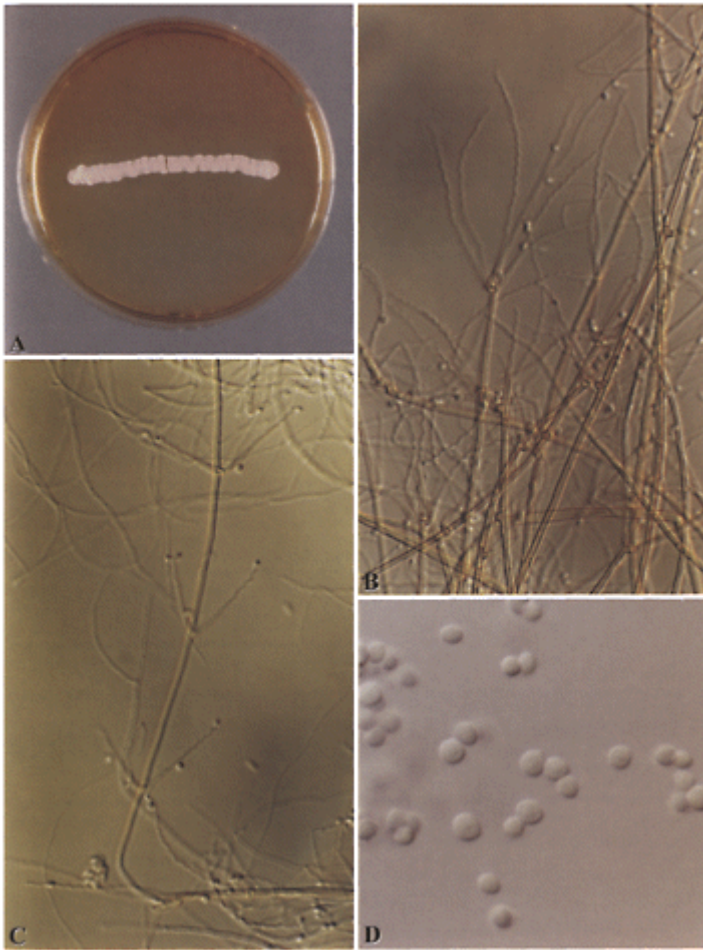


Tritirachium oryzae (Vincens) de Hoog

A. Colony on MEA after one week; B, C. conidiophores with conidia, $\times 920$; D. conidia, $\times 2330$.

Occurrence: air and isolated from nylon, soil.

Medical significance: HI.



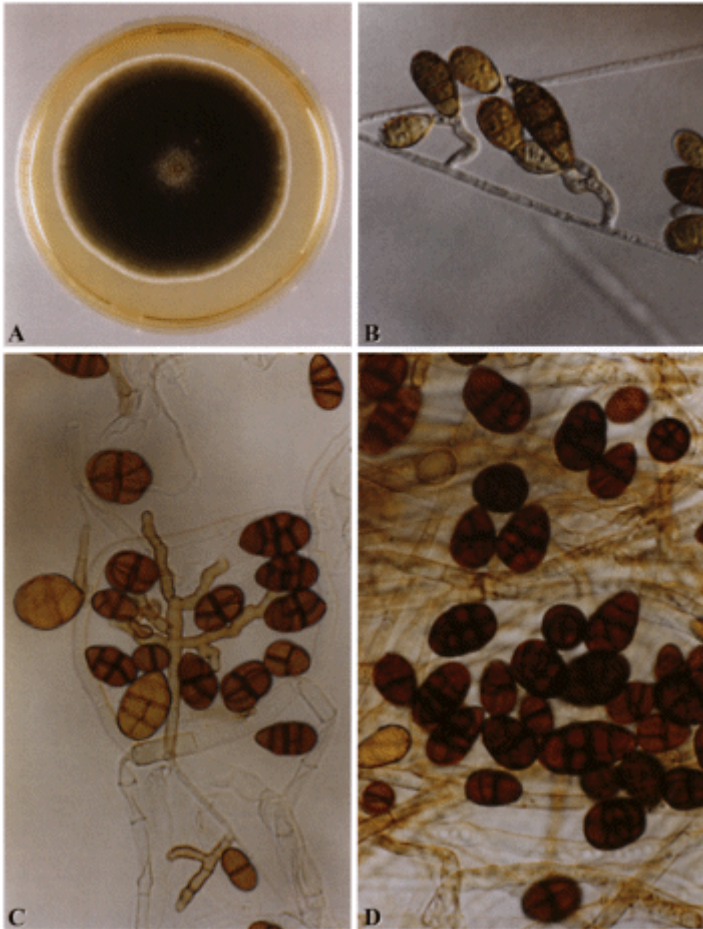
Ulocladium chartarum (Preuss) Simmons

A. Colony on MEA after one week; B-D. septate conidia, $\times 920$.

Occurrence: A widespread species found in soil, dung, plant litter and wood.

Occurs along with *Cladosporium sphaerospermum* on painted surfaces; on damp walls finished with wallpaper or water-based emulsion paint. Found in floor and mattress dust. Also isolated from UFFI, manila fibre, plywood.

Medical significance: [E?] F [G?].



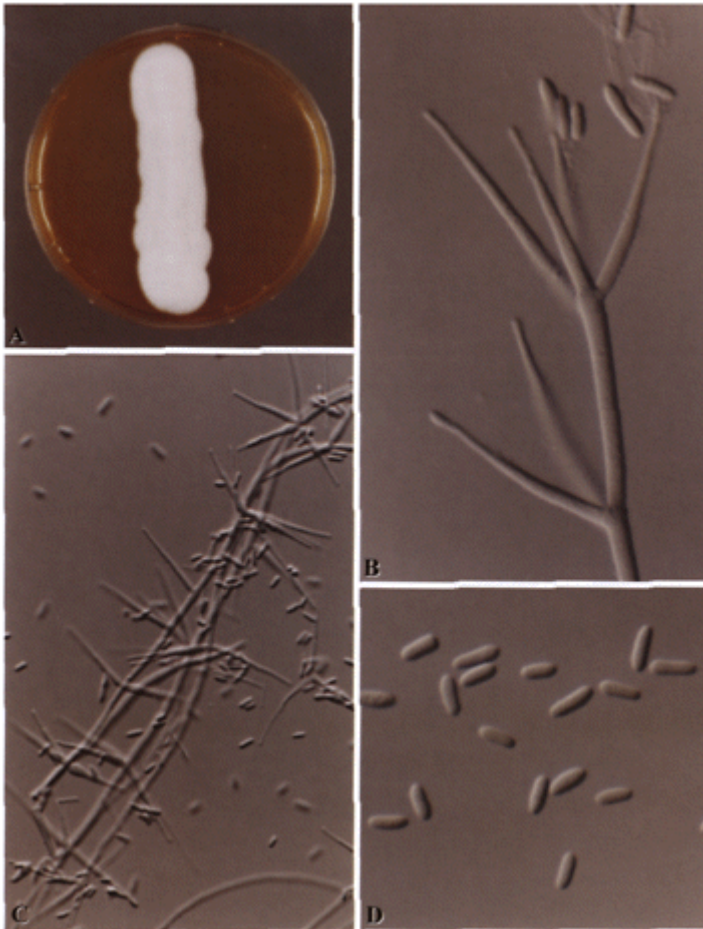
Verticillium lecanii (Zimmermann) A.W.Viégas

A. Colony on MEA after one week; B, C. verticillate phialides, B.×2330, C.×920; D. conidia, ×2330.

Occurrence: Often associated with cereals, hyper-parasitic on other moulds, used in control of insect pests.

Isolated from air in greenhouses, glyphosate solution; contact lens.

Medical significance: HI.



***Wallemia sebi* (Fries) von Arx**

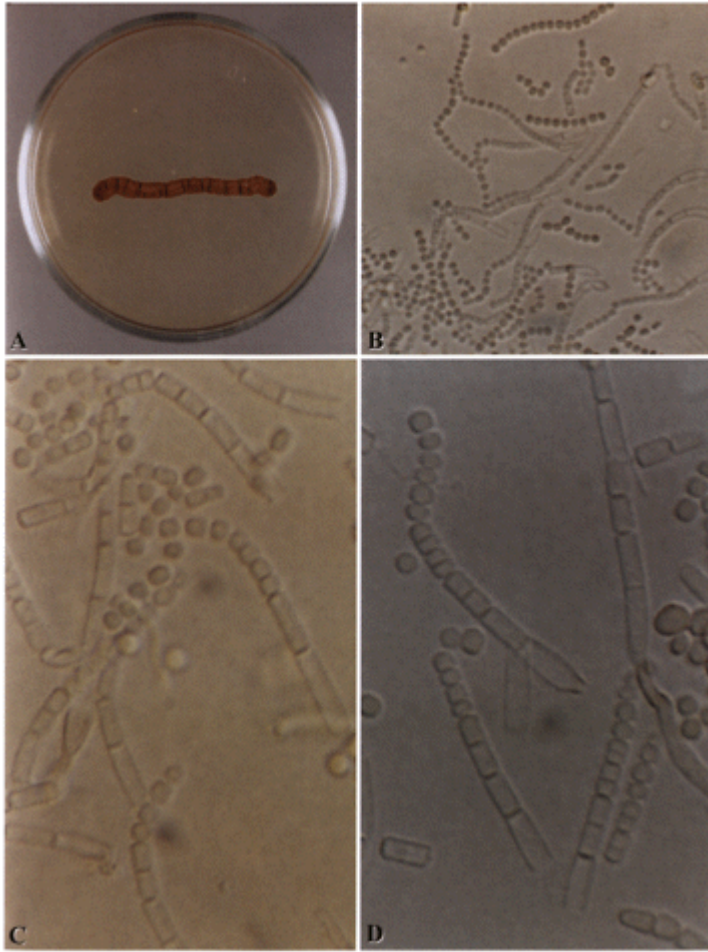
A. Colony on MEA+20% sucrose after one week; B-D. conidiophores each with an apical fertile, cylindrical hyphae which separates into 4 conidia, maturing in basipetal succession, B.×230, C.×960, D.×960.

Occurrence: A cosmopolitan xerophile, present in soil and air, and particularly associated with dried foodstuffs.

Found in floor, carpet, mattress and upholstered furniture dust; radiator; hay, cereals, cake, dates, salted fish.

Medical significance: HI. Allergenic (Sakamoto *et al.* 1989).

Note: This xerophilic species is common and can only be detected by using appropriate low water activity media such as MEA+20 % sucrose.



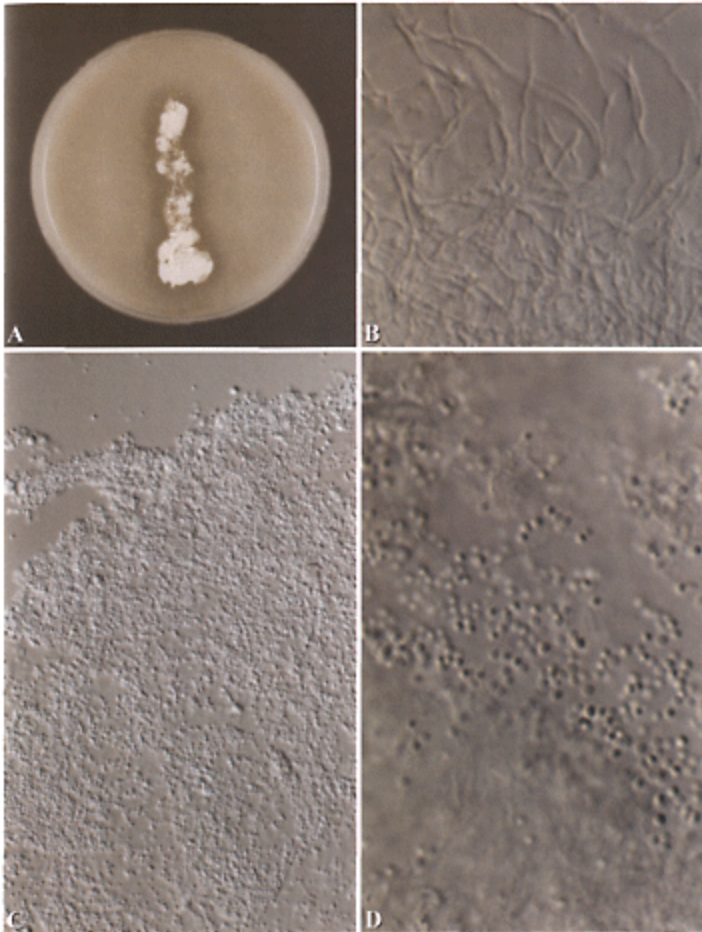
Saccharopolyspora rectivirgula (Krassilnikov and Agre) Korn-Wendisch *et al.*
(*Faenia rectivirgula*, *Micropolyspora faeni*)

A. Colony on OA after one week at 55° C; B. hyphae, ×2330; C, D. spores, C.×920, D.×2330.

Occurrence: Cosmopolitan thermophilic species usually associated with composts and self-heating agricultural materials.

Isolated from air-conditioning systems with water spray cooling, furnace dust, humidifiers; hay, cereals; mushroom farms, cotton and sugar cane mills.

Medical significance: Allergens implicated in occupational hypersensitivity pneumonitis known as farmer's lung, but role in bagassosis and mushroom worker's lung in question (See Chapter 3.2).

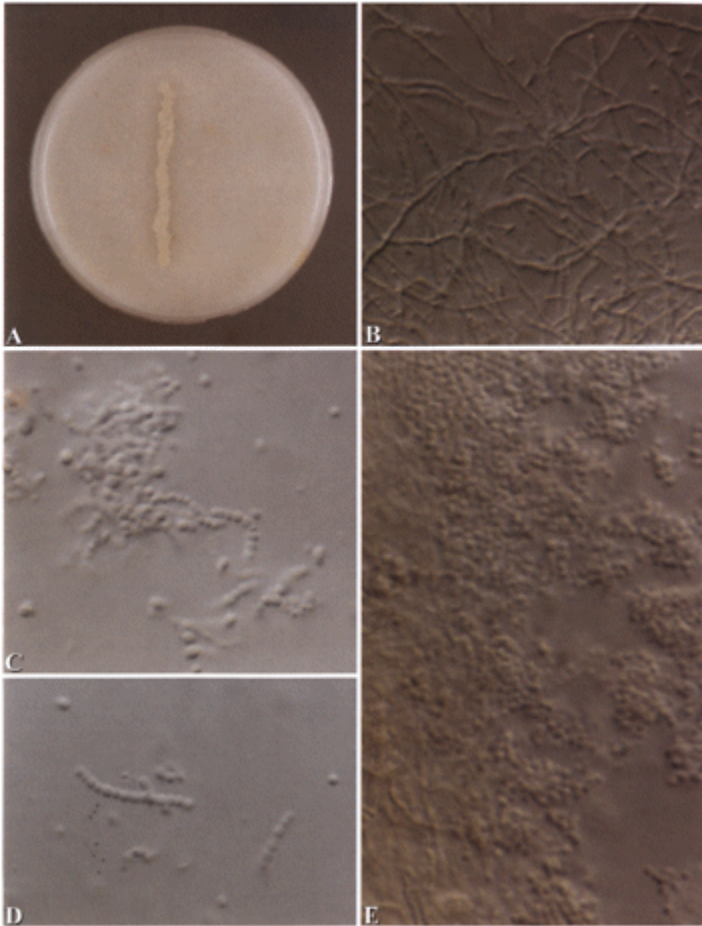


Streptomyces griseus (Krainsky) Waksman and Henrici

A. Colony on OA after one week at 24° C; B. hyphae, ×2330; C-E. spores, ×2330.

Occurrence: A cosmopolitan streptomycin producing member of a genus associated with, and imparting the characteristic smell (geosmin) to, soil.

Isolated from cork sheeting and other constructional materials; hay, cereals.



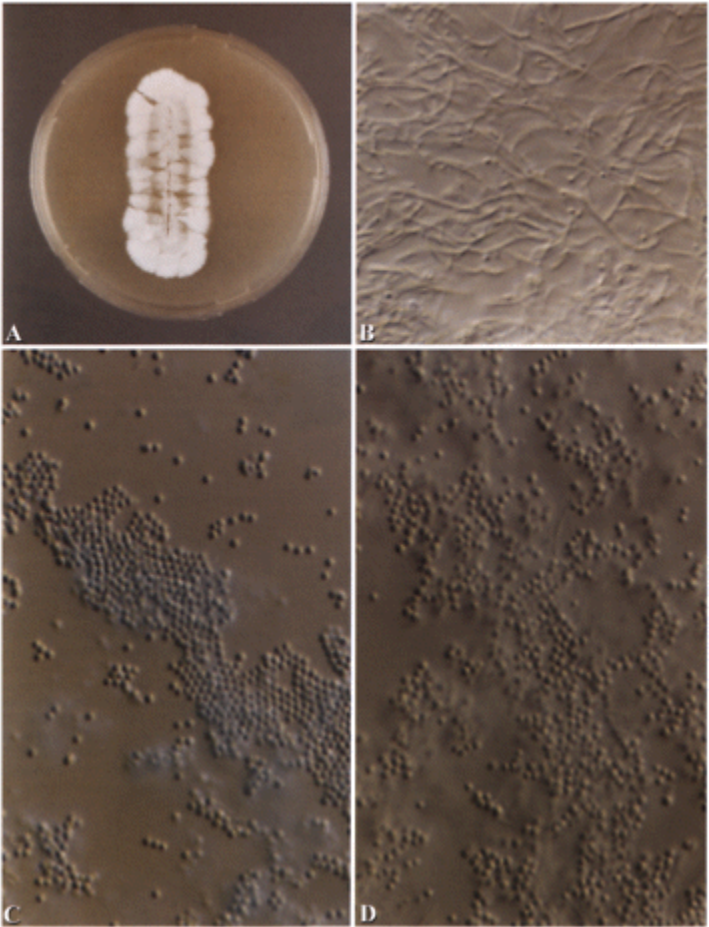
***Thermoactinomyces* vulgaris** Tsilinsky

A. Colony on V8 after one week at 55° C; B. hyphae, ×2330; C, D. spores, ×2330.

Occurrence: Cosmopolitan thermophilic species usually associated with composts and self-heating agricultural materials.

Isolated from humidifiers; wood chips; hay, cereals, bagasse; mushroom farms, cotton and sugar cane mills.

Medical significance: Allergens implicated in occupational hypersensitivity pneumonitis known as farmer's lung, but role in bagassosis and mushroom worker's lung in question (See chapter 3.2).



APPENDIX

GLOSSARY OF MYCOLOGICAL TERMS

The following glossary is slightly modified from Samson *et al.*, (2000). For a more detailed dictionary of mycological terms see Hawksworth *et al.* (1995). Ainsworth and Bisby's Dictionary of the Fungi, 8th Ed. CAB International, Wallingford.

aceroze: needlelike and stiff (like a pine needle)

acervulus: a lens- or cup-shaped fruiting body containing conidiophores and conidia embedded in the host plant.

acropetal: describes the succession of conidia arising in chains with the youngest conidium at the top (apex) of the chain

anamorph: imperfect (or asexual state) conidial form of sporulation

annellide: a conidiogenous cell which forms blastoconidia in basipetal succession, each conidium is produced through the same opening of the previously formed conidium and leaves a ringlike band (annellation) at the fertile apex after seceding. The conidiogenous cell elongates during conidiogenesis (progressive)

apophysis: a swelling at the top of the sporangiophore just below the sporangium in Mucorales (e.g. *Absidia*)

apothecium: a cup- or saucer-like ascoma

arthric: (of conidiogenesis), thallic conidiogenesis. Cells are separated from an undifferentiated part of a hypha, and transformed into conidia (see also arthroconidia)

arthroconidia: (=thalloconidia) conidia resulting from breaking up of a hypha into separate cells and transformed into conidia, e.g. in *Geotrichum*

ascigerous: producing asci

ascoma (pl. **ascomata**) (=ascocarp): a fruiting body in Ascomycetes containing asci and ascospores

ascospores: sexual spores produced in an ascus ascus: a sac-like organ in which the ascospores are formed after karyogamy and meiosis

aseptate: without a cross-wall

ballistoconidia: see ballistospore

ballistospore: a basidiospore which is forcibly propelled from the sterigma bearing it

basidium: the organ of the Basidiomycetes which bears the basidiospores after karyogamy and meiosis

basipetal: describes the succession of conidia in which the youngest conidium is at the base, e.g. of the chain

basitonous: with branches on main axis confined to the lower part of the axis

biseriate: in two series. In *Aspergillus*: with phialides not formed directly on the vesicle, but on metulae

- blastoconidium:** conidium in which the wall of the conidiogenous cell has bulged out apically to form the conidial wall. Conidia can be produced solitarily or in acropetal chains, i.e. each new conidium can bud at its tip.
- budding:** a process of vegetative multiplication in which there is a development of a “daughter” cell from a small outgrowth of a “mother” cell
- chlamydo-spore:** a thick-walled, thallic, terminal or intercalary asexual resting spore, mostly for longtime survival; usually nondeciduous
- cleistothecium:** an ascoma enclosing the asci, but lacking a special opening (ostiole)
- collarette:** a cup-shaped structure at the apex of a phialide
- colony:** (of mycelial fungi) a group of hyphae (with or without conidia), which arise from one spore or cell. Colony appearance varies, e.g. velvety, floccose (cottony), funiculose (hyphae aggregated into strands), fasciculate (in little groups or bundles), granulose, powdery, or synnematosus (compact groups of erect and sometimes fused conidiophores)
- columella:** an usually swollen sterile central axis within a sporangium, e.g. in Mucorales
- conidiogenesis:** process of conidium formation
- conidiogenous cell:** the fertile cell from or within which conidia are directly produced. Conidiogenous cells may be morphologically identical with or differentiated from vegetative cells (vegetative hyphae)
- conidioma: (pl. conidiomata);** any structure which bears conidia, e.g. separate conidiophores, synnema, acervulus, pycnidium, sporodochium etc.
- conidiophore:** specialized hypha, simple or branched, on which conidiogenous cells are born
- conidium:** asexual, vegetative, non-motile propagule, not formed by cleavage (as in sporangiospores). In Deuteromycetes the term “conidium” is recommended and the term “spore” is reserved for zoo-, sporangio-, basidio- and ascospores
- diploid:** having $2n$ chromosomes
- echinulate:** with small pointed processes or spines on surface, e.g. of conidia or conidiogenous cells
- endoconidium:** conidium formed inside a hypha
- exudate:** droplets excreted by the mycelium. Can be characteristic of a species, e.g. *Penicillium chrysogenum*
- fasciculate:** hyphae and/or conidiophores in bundles
- floccose:** cottony
- foot cell:** basal part of the conidiophore (e.g. in *Aspergillus*) or basal cell of conidium in *Fusarium*
- fruiting body (=fruit-body or fructification):** a general term for spore-bearing organs, applicable to micro-and macrofungi, but more often applied to the latter
- funiculose:** hyphae/conidiophores aggregated into strands
- conjugation:** fusion
- gametangium:** a meiosporangium in which generative (sexual) cells (gametes) are formed
- geniculate:** bent like a knee
- hilum:** a mark or scar, especially that on a spore at the point of attachment to a

conidiophore or sterigma

Hülle cells: terminal or intercalary thick-walled cells surrounding the ascomata (in some *Aspergillus* species)

hyaline: transparent or nearly so

hypha (pl. hyphae): (vegetative) filament of a mycelium, without or with crosswalls

intercalary: between two cells, between the apex and base (of cells, spores etc.)

lateral: at the side

macroconidia: the larger conidia of a fungus, usually multicellular, e.g. in *Fusarium*

microconidia: small conidia, usually one-celled

meiosis: that part of a life cycle in which a diploid nucleus ($2n$) undergoes reduction and becomes haploid (n)

meristem: growing zone

merosporangium: (of Mucorales) a cylindrical outgrowth from a swollen end of the sporangioophore, in which asexual merospores are produced in a linear row

merospores: asexual spores formed in a merosporangium by cytoplasmic cleavage

mesoconidia: in *Fusarium*, conidia intermediate between micro- and macroconidia.

Usually one- to several-celled but smaller in size and distinguishable in shape from the macroconidia

metabolite: any substance produced during metabolism

metula (pl. metulae): apical branch(es) of conidiophore bearing phialides, e.g. *Penicillium* and *Aspergillus*

mononematous: with hyphae or conidiophores arising singly from the substrate

monopodial: a way of branching, in which the persistent main axis gives off branches

multinucleate: containing many nuclei

muriform: having longitudinal and transverse septa

mycelium: (vegetative) mass of hyphae; thallus or vegetative body of the fungus

mycotoxins: fungal secondary metabolites which in low concentration are toxic to vertebrates and other animals when introduced via a natural route.

osmophilic: growing under conditions of high osmotic pressure (e.g. on substrate containing 20–40% sucrose or NaCl)

ostiole: any pore by which conidia or spores are freed from a fruiting body, e.g. pycnidium or perithecium

pedicel: a small stalk

penicillate: like a little brush

perithecium: a globose or flask-shaped ostiolate ascoma within which asci are produced

phialide: a conidiogenous cell which produces conidia (phialoconidia) in basipetal succession, without an increase in length of the phialide itself

pionnotes: a spore mass with a fat or grease-like appearance (in *Fusarium*)

polyphialide: conidiogenous cell with more than one opening from each of which conidia are produced in basipetal succession; the new conidiogenous loci develop by sympodial proliferation of the fertile cell

poroconidia: (=tretic conidia) dark conidiogenous cells with an apical, pigmented wall thickening. The conidium is pierced through, after dehiscence a pore is visible

pseudomycelium: the formation of a filamentous structure consisting of cells which arise

exclusively by budding

pseudothecium: an ascoma with asci developing in unwallled cavities (loculi) in a stroma or arising from cushion-like structures in loculi

pycnidium: flask-shaped to globose fruiting body, usually with one apical opening (=ostiole), containing conidiogenous cells

racemose: a way of branching, in which the main axis produces side branches which are shorter than the main axis.

rachis: conidium-bearing extension of a conidiogenous cell (resulting from sympodial development, zigzag geniculate extension)

ramoconidium: (**pl. ramoconidia**) a fertile apical branch of a conidiophore which produces conidia and later secedes and functions as a conidium itself, e.g. in *Cladosporium*

retrogressive: describes mechanism of conidiogenous cell development in which the fertile cell shortens during basipetal conidium formation, e.g. in *Trichothecium*

rhizoid: hyphal root; a root-like structure, acting as a feeding organ and/or holdfast, e.g. in *Rhizopus*

rostrate: having a beak

sclerotium: a resting body, usually globose, produced by aggregation of hyphae into a firm mass with or without host tissue, normally sterile

septum: a cross-wall

spine: a narrow process with a sharp point

spinulose: delicately spiny

sporangiole: small, usually globose sporangium with a reduced columella and containing one or a few spores

sporangiophore: a specialized hyphal branch, which supports one or more sporangia

sporangiospore: a spore produced within a sporangium

sporangium: asexual reproductive structure, in which spores are produced by cytoplasmic cleavage

spore: a general term for a reproductive structure in fungi, bacteria, and cryptogams. In fungi the term "spore" is used in several combinations, e.g. chlamydospore, ascospore, zoospore or basidiospore

sporodochium: a cushion-like mass of conidiophores; conidia and conidiogenous cells produced above the substrate

sterigma: a conical or elongated process which bears the basidiospore

stipe: stalk

stolon: a "runner" or arching aerial hypha which touches the substratum at intervals, e.g. in *Rhizopus*

striate: marked with lines, grooves or ridges

stroma: a mass or matrix of vegetative hyphae, with or without tissue of the substrate, in or on which fructifications can be produced

substrate: the material on or in which an organism is living

suspensor: a hypha which supports a gamete, gametangium or zygospore

sympodial: describes a mechanism of conidiogenous cell proliferation in which each new growing point appears just behind and to one side of the previous apex, often

giving a geniculate cell configuration; of conidiophores characterized by continued growth after the main axis has produced a terminal spore, or by development of a succession of apices each of which originates below and to one side of the previous apex.

synnema: (pl. **synnemata**) bundles of erect hyphae and conidiogenous cells bearing conidia (in some Hyphomycetes)

teleomorph: perfect (sexual) state of a fungus; ascigerous or basidial form of sporulation

teliospore (teleutospore): one of the spore forms (commonly resting spore) of the Uredinales (rust fungi) or Ustilaginales (smut fungi) from which the basidium is produced

thallic: (of conidiogenesis), a segment of a fertile hypha differentiates by conversion into a conidium (see arthric)

tretic: (see poroconidia)

truncate: ending abruptly, as if cut straight across

uniseriate: in one series. In *Aspergillus* phialides arise directly from the vesicle

velutinous: velvety

verrucose: having small rounded processes (warts)

verruculose: delicately verrucose

verticil: whorl vesicle: a bladder-like sac; the swollen apex of the conidiophore in *Aspergillus*

zygospore: a sexual spore produced by Zygomycetes, thick-walled, often ornamented and darkly pigmented, formed by fusion of a pair of gametangia

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